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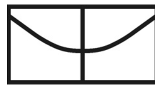
Proceedings

First International Meeting on Cassava Breeding, Biotechnology and Ecology



University of Brasília

Brasília, 2007
Brazil

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Dedication
To Dr. Sang Ki Hahn
To CTCRI, India
In recognition for their great contribution to cassava growers and consumers worldwide.
First International Meeting on Cassava Breeding, Biotechnology and Ecology.
Brasilia, 2007

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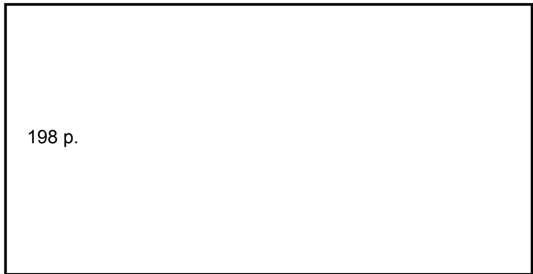
Editing supervision
Daniele Yasmim

Electronic editing
Daniel Dino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Cassava Genetic Resources: Wild species and indigenous cultivars and their utilization for breeding of the crop

Nagib M. A. Nassar
Departamento de Genética e Morfologia,
Universidade de Brasília, Brasília 70919, Brazil

Abstract

Cassava wild relatives are perennial and vary in growth pattern from nearly acaulescent subshrubs to small trees. They have been used as a source of useful characters such as high protein content, apomixis, resistance to mealybug and mosaic disease and tolerance to drought. Indigenous clones are potential source of b-carotene and lycopene. Apomixis genes have been transferred successfully through interspecific hybridization to the crop, and apomictic clones arising from these hybrids are being now grown at the Universidade de Brasilia. Interspecific hybrids produced early have been polyploidized and have their fertility restored. Different useful types of chimera were also produced.

Wild Manihot species – a botanical review

Wild cassava relatives are perennials and vary in growth pattern from nearly acaulescent subshrubs to small trees. Procumbent, semiherbaceous subshrubs, shrubs, and trees are found in the genus (Figs 1-7). The branching pattern is typically dichotomous or trichotomous, having at the branching point a terminal inflorescence. Bark of the woody species is generally smooth. Many of the species are lacticiferous, and some species particularly *M. glaziovii* (ceara rubber) are cultivated in Brazil and in some countries (Nigeria) for rubber production (Rogers 1965, Rogers and Appan 1973) This species was used by Storey and Nichols in the 1930s in Tanzania (Formerly Tanganyka) to transfer resistance to mosaic disease. Many species such as group *tripartita*

have their stems adapted to dry periods; die back to a root crown regularly and shed their leaves during the dry season. Majority of *Manihot* species are found on limestone derived and well drained soils.

All *Manihot* species are monoecious and a few are dioecious, which make them obligate out-crossers. In many species, they are protogeneous; i.e., pistillate flowers open before staminate flowers of the same inflorescence. Pollination is by insects to whose bodies the sticky pollen adheres. This cross pollination phenomena leads to formation of extremely heterozygous gene pools. Being allopolyploid species, partially apomictic, and having week barriers in addition to the allogamous nature, have led to the rapid speciation of this group and formation of the large number of species (Nassar 1999, 2000, 2001).

All species of the genus *Manihot* are native to South America (particularly Brazil). The only species found in other tropical regions of the world are those that have been introduced since Columbus voyages to the American continent. The species of *Manihot* are all rather sporadic in their distribution and rarely become dominant of the local vegetation. Majority of these species are found in relatively dry regions, and only a few are found in rain forest regions. Their typical habitats are openings in the forest as the case of *M. anomala*. So they are typically heliophiles that grow only in the absence of shading. Many of these species such as *M. pohlii, M.zehntneri and M.grahamii* are weedy types capable of invading new agitated areas, frequently are found on limestone derived and well drained soils. All of the species are damaged by frost with exception of a few such as *M. grahamii* and *M.neusana*, whose

Fig. 1 – Manihot falcata

Fig. 2 – Manihot oligantha

Fig. 3 – Manihot stipularis Pax

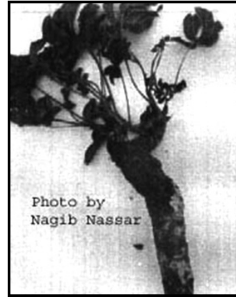

Fig. 4 – M. nana Muell

Fig. 5 – Manihot neusana

Fig. 6 – Manihot glaziovii

Fig.7 – Manihot pseudoglaziovii

According to Rogers and Appan (1973), 98 *Manihot* species have been recognized. Only one species, *Manihotoides pauciflora,* is known in the closest related genus *Manihotoides.* Several of its attributes are not found in any *Manihot* species, including mono-flower inflorescences, which is a primitive characteristic compared with the multi-flowered inflorescence in

Manihot, and leaves born at the apex of short, condensed stems arising from branchlets. Such evidences suggest this species as a probable origin of all *Manihot* group. Unfortunately this species is on the verge of extinction, and may be eventually extinct (Nassar 1999).

Rogers and Appan (1973) classified *Manihot* species into 19 sections, varying from trees in the section *Glazioviannae* to sub-shrubs, nearly acaulescent, in the section *Stipularis*. The species in this latter section are also characterized by being more dioecious than monoecious, a condition reversed in all other *Manihot* species. Other sections, such as *Tripartitae* and *Graciles,* are perennial sub-shrubs with large woody roots; their stems frequently die back to the root crown in response to dry periods or fires.

All *Manihot* species are native to tropical regions of the New World, particularly Brazil and México. Nassar (1978d) defined four centers of diversity for these species: Mexico and northeast, central, and southwest Brazil. Microcenters of diversity of these species exist within central Brazil, where large numbers of species are concentrated in small areas (< 50 km in diameter) (Nassar 1978 b, c, d, e, f, 1979 a, b, 1980 a, b, 1982, 1984, 1985, 1986). These microcenters arose to the frequent hybridization between species and the heterogenic topography of their habitats, which help isolate fragmented gene pools that lead to speciation. Tree-like species, such as *M. glaziovii* and *M. pseudoglaziovii*, are found in northeastern Brazil, whereas short species and sub-shrubs are found in central Brazil.

Wild Manihot species and their interspecific hybrids

Wild *Manihot* species have been used by this author through hybridizing them with cassava. Probably the most impressive case is the interspecific hybrid of *M. oligantha* with cassava. This hybrid had high protein content, which reached 4% of peeled roots; i.e., double of protein content in common cassava, combined by low HCN content of 90 mgm per kg. (Nassar and Dorea 1982). Recently, genes for apomixis from the wild species *M. neusana* were transferred successfully (Nassar 2000, Nassar *et al.* 2000). Probably the most important utilization of wild *Manihot* species is the discovery of resistance to mealybug in *M. glaziovii*, and its transfer to cassava gene pool through interespecific hybridization (Nassar 1996). This interespecific hybrid could be polyploidized and its fertility restored (Nassar 2004).

Natural hybridization occurs between wild *Manihot* species and between them and cassava (Nassar 1984, 1989). Barriers within the genus appear to be weak due to recent evolution of the group. All wild *Manihot* species examined cytogenetically have a chromosome number of $2n = 36$ (Nassar 1978a). Despite this high chromosome number, *Manihot* species behave meiotically as diploids. Therefore, they are believed to be allopolyploids and this seems to have anticipated the emergence of the whole group and is responsible for their rapid speciation and their weak interespecific barriers. All these factors have led to frequent natural interespecific hybridization, the formation of heterozygous gene pool leading to rapid speciation.

Nassar (1980a) reported frequent hybridization between *M. reptans* and *M. alutacea* in sympatric natural habitats where their population boundaries overlap (Fig. 8). Morphological markers such as leaf color and bract size were used to identify this interespecific hybridization (Fig. 9). The range of *M. reptans* has expanded during the past 100 years (Nassar 1984) and this is attributed to the continuing gene introgression of *Manihot* species. Introgression of *M. reptans* with germplasm from other species allowed its ecotypes to penetrate and colonize areas where *M. reptans* (pure) had previously been unable to do so. This phenomenon was also noted in other species such as *M. cearulescens* (Nassar 1980a). From a plant breeding viewpoint, the value of these hybrids lies in their ability to cross with the cultigen.

Fig.8 – Natural hybrid (right) of Manihot alutecea (left) with M. reptans (medium)

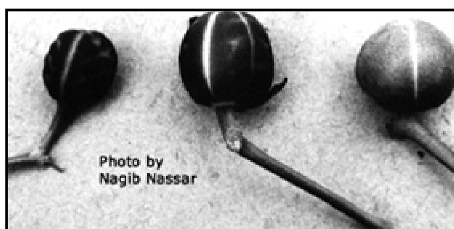

Fig. 9 – Fruit shape and color as markers in the hybrid

Morphological markers for lobe shape, the presence of stem nodes, flower disc color, fruit color, and fruit shape (Figs. 9, 10) were discovered in controlled crosses between cassava and *wild Manihot* species, as well as in natural hybrids between cassava and different species. These genes were used to identify hybridization. Interespecific hybrids of cassava with *M. glaziovii*, *M. pseudoglaziovii*, *M. aesculifolia*, *M. pilosa*, *M. dichotoma*, *M. pohlii*, *M. neusana* and *M. anomala* were obtained through controlled crosses, although their frequency was low. The meiotic behavior of several hybrids (cassava with *M. neusana* and *M. pseudoglaziovii*) was studied by Nassar (1992), and results indicated low hybrid fertility between these species and cassava.

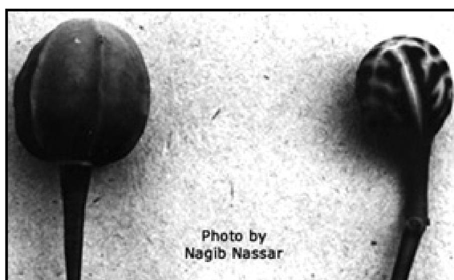

Fig. 10 – Morphological markers winged fruit (left) and variegated fruit (right)

Production of cassava cultivars by interspecific hybridization

Cassava cultivars are deficient in many economic characteristics such as resistance to insects, diseases, and drought and have low protein content (Nassar and Dorea 1982, Nassar and Grattapaglia 1986). This can be attributed to the mode of evolution in 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 interespecific hybridization with wild relatives which possess these genes (Nassar and Grattapaglia 1986). Wild species of cultivated crops have frequently been used as an important source of genetic diversity and have been employed effectively in a variety of breeding programs (Stalker 1980).There are interespecific barriers to hybridization but these are weak and can be broken in different ways (Nassar *et al.* 1995).

Nassar, (1978, 1980, 1989, 1994) reported production of interespecific hybrids of several *Manihot* species with cassava through controlled crosses by insect vectors (Figs. 11 and 12) The following morphological markers were used to identify interespecific hybrids: variegated color of fruit dominant to smooth, red color of flower disk dominant to yellow, setaceous bracteole dominant to foliaceous, and nodded stem dominant to smooth. Observations of growth habit, height, stem texture, and tuber formation were also recorded. Other characters provided indirect evidence of hybridization. The hybrid plants exhibited dominant phenotypes from cassava, namely, ribbed fruit, red color of the flower disk, nodded stem, and tuberous root (Table 1). These results show that glabrous stem, setaceous-foliaceous bracteoles, red-creamy color of flower disks, variegated-green color of fruit, and ribbed – no ribbed fruit are simple morphological markers that can be used to recognize interespecific hybridization. It is evident that interespecific barriers between *Manihot* species can be broken by the use of an abundant diversity of pollinator gametes transmitted by insect vectors. Interespecific crosses were difficult to fertilize manually in the present and in previous crosses (Nassar 1980a). This evidence suggests that barriers between cassava and other *Manihot* species are weak and recently evolved. It seems they have arisen not as a primary isolating event but rather secondarily after geographic isolation. Nassar (1978b) postulated that cassava is an interespecific hybrid that appeared by domestication approximately 2000 years ago or less.

Fig. 11 – Hybrid of M. pseudoglaziovii

Fig. 12 – Clone UnB 120 selected from the interespecific hybrid M.cearulescens x cassava progeny

Table 1. Comparison of morphological characteristics of *M. neusana*, cassava and their hybrid

Characteristic	*M. neusana*	Cassava`	Hybrid
Growth habit	Procumbent shrub (1.5-2m)	Erect shrub (1.5-2m)	Erect shrub (1.5 – 2 m)
Young stem texture	Hairy	Glabrous	Hairy
Bracteoles	Foliaceous	Setaceous	Setaceous
Fruits	Globose, without ribs, variegated	Ovoid, ribbed, green	Ovoid, ribbed, variegated
Tuber formation	None	Forms tubers	Forms tubers
Growth habit	Erect shrub (2-2.5m)	Erect shrub (1.5-2 m)	Erect shrub (1.5 – 2m)
Young stem texture	Hairy	Glabrous	Hairy
Bracteoles	Semi-foliaceous	Setaceous	Setaceous
Flower disk color	Anomala	Lobed; 5 lobes	Anomala
Fruit	Globose, without ribs	Ovoid, ribbed	Ovoid, ribbed
Tuber formation	Scarcely forms tuberous roots	Forms tuberous roots	Forms tuberous roots

Interespecific hybrids of cassava with *M. glaziovii* and *M. pseudo-glaziovii* were produced (Nassar, 1996), and propagated by cuttings and planted alternately with clone Sonora. This clone was chosen because of its high resistance to bacterial wilt. Seeds were collected, planted, and whole plants were grown. In March 1994, these plants were reproduced by cuttings; six of each were planted for assessing yield and survival during the drought season of June to October (5 months). In November 1994, surviving plants were evaluated for root formation. Ten clones were selected and given to the Semi-Arid Centre at Pernambuco for propagation and cultivation under semiarid conditions of northeastern Brazil. The selected clones were characterized morphologically according to Rogers and Appan (1973), and Nassar and Grattapaglia (1986). This characterization was aimed at detecting the association of different morphological characters with tolerance to drought

Morphological characterization showed that certain characters were associated with tolerance to drought. All selected clones have a notable brown, thick, and rough superficial epiderm. It seems that the brown-colored thick epiderm is associated with tolerance to drought because of its isolative nature, which impedes evaporation. All the wild species investigated by this author have fibrous roots with brown external color and their epidermic layer is thick. This character may be therefore inherited from the wild. Graner (1942) reported that this character behaves dominant to white. Anatomically, the distinct portion of the enlarged root is composed of three sections. Firstly, a layer referred to as the phelloderm which is generally composed of the previously mentioned epidermis, a subepidermis, and a thicker inner layer. The phelloderm is thick and easily separated from the next inner layer. Secondly, a layer of parenchymatous cells that constitutes the bulk of the root and is the carbohydrate storage region. Thirdly, a portion called the cortex of flesh at the center of the root is a well-defined central vascular core. As noted previously, the outer epidermis is so thin that it is difficult to measure, but it is possible to evaluate its thickness using the naked eye. It is about 0.5 mm in the thickest types.

The second interesting case in selected clones is the prominence of leaf scars on stems. All selected clones have a prominent enlarged leaf scar. This character which seems to be well associated with enlarged root formation in the hybrid progeny apparently was inherited from cassava. All wild species studied by this author have a smooth stem without any leaf scar. All selected clones gave deep fibrous roots in addition to enlarged ones. Is seems

that this character is inherited from the wild. This appears to be a mechanism of cassavas to tolerate drought since they are capable of capturing water from long distance. Both wild species and their interespecific hybrids produced long, deep roots from the fourth month onwards, reaching 4 or 5 m when the plants were one year old.

Another mechanism of tolerance to drought is the thick epiderm layer, probably because of its structure, it impedes evaporation. The dieback of the vegetative parts to the crown in dry season was the third common character shown by all the selected clones. Presumably this habit helps plants to reduce respiration and consumption of carbohydrate deposits. From this study, it is obvious that breeders can make use of morphological characterization as a criterion to detect the association of morphological characters and drought tolerance and consequently selection of genotypes complying with this objective.

The transfer of apomixis genes from *Manihot* species to cassava

Apomixis means seed formation without fertilization. In cassava, it is an alternative to reproduction by cuttings which normally is practiced by farmers. The latter type of propagation leads to accumulation of viral and bacterial diseases that reduce productivity and may cause extinction of superior genotypes. Thus, by the use of apomictic plant in propagation, systemic pathogens could be avoided, and the genetic segregation in the progeny is exduded. Plant-produced stems through apomixis from a contaminated clone will be free from viral and bacterial germs and can begin a new cycle of clone life in place of its extinction. If apomixis was found or had been introduced into the excellent Brazilian clones such as Guaxupe and Vassourinha, they would not have been extinct, and had been preserved for a longer time. The use of apomixis in preserving superior genotypes and filtering the bacterial contamination provides benefits to international cassava programs that export routinely their germplasm. It is sufficient in this case, for the destination country to produce only one plant and further reproduce it vegetatively to maintain the original superior genotype.

Facultative apomixis was discovered in the wild cassavas *M. dichotoma* and *M. glaziovii* (Nassar 1994, Nassar *et al.* 1998). It was noted earlier in *M. neusana* – a species characterized by extreme resistance to bacterial wilt and stem borers (Nassar 1985). Interspecific hybridization was carried out to transfer

these useful genes to the cultigen (Nassar 1989). The clearing method was used to detect apomixis (Nassar *et al.* 1996). The anatomical studies of ovules showed that the embryo was formed by apospory from a somatic cell in the nucellus. The megasporogenesis in ovules with aposporous development proceeds normally up to a certain moment when nucellar cells enlarge and the nuclei divides to form aposporous embryo sacs (Figs. 13a, 13b). These aposporous embryo sacs appear to develop faster than sexual embryo sacs, probably because they are not delayed by meiotic division (Asker 1979, Nogler 1984). In some cases, development of apospory embryo sacs from cells within the sexual one was noted. Both the aposporous and sexual embryo grew in parallel and finally coexisted.

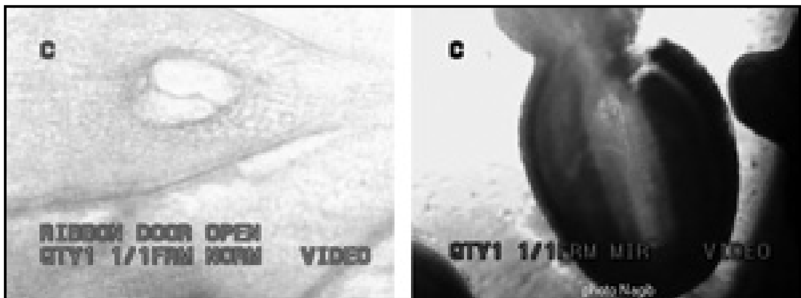

Fig. 13 – Multiembryos in an aposporic ovule

This observation confirms results from a previous study (Nassar 1995), where two seedlings grew side by side; one of which was apomictic and one of sexual origin. Nogler (1984) reported that aposporous and sexual processes coexisted in one individual ovule producing several embryos that of *Potentilla,*. This study documents the survival of two aposporous embryo sacs beside a sexual one, all of them in a developed stage in the ovule (Figs. 13a, 13b).

The cytogenetical study showed that out of the 25 individuals examined, 13 plants were sterile; the percentage of pollen viability ranging from 4 to 15 % (Table2). Two plants had 2n + 1 while the rest were 2n. The other 12 plants were highly fertile with pollen viability ranging from 92 to 97 %. Their chromosome number was 2n.

The embryonic study revealed that all of the sterile plants were partially apomictic while the fertile plants were sexual. Sterility apparent1y leads to apomixis. Sterility is caused by consistent defects of meioses due to lack of pairing. All of these sterile plants showed asynapsis in meiotic metaphase. Formation of univalents ranged from 4 to 6 per cell. The irregu-

lar chromosome -segregation in these sporocytes must lead to geneticalIy unbalanced and aborted gametes. It seems that this sterility triggers certain genes of apomixis to act. Apomixis will function and be established in such genotypes since it's favored by natural selection as it offers an escape from lethality, providing a perpetuation of the current genotype.

Table 2. Chromosome constitution in relation to pollen viability and apospory in apomictic progeny

Clone	Pollen viability %	Apospory %	Chromosome number
1	9.2	1.2	2n
2	6.8	1.5	2n + 1
3	4.9	1.7	2n
4	2.0	1.6	2n + 1
5	4.7	1.1	2n
6	6.1	1.2	2n
7	15.6	1.3	2n
8	4.1	1.4	2n
9	8.3	1.7	2n
10	5.6	1.3	2n
11	4.1	1.8	2n
12	4.6	1.4	2n
13	4.7	1.3	2n

In summary, the nature of apomixis in cassava is different from other types found in other crops since it is present at very low leve1s (1-2 %). It depends on meiotic irregularity that often causes sterility in plants. This sterility triggers a certain gene in cassava that activates a number of somatic cells in the nucellus or in the sexual embryo sac to form aposporic embryo sacs. Natural selection favors this apomictic genetic system since it is an escape from extinction and a mode of perpetuation for the current genotype.

Polyploidization of the interspecific hybrids

Four interespecific hybrids between cassava and wild *Manihot* species, obtained earlier by this author, were used for polyploidization. These hybrids are *M. neusana* x *M. esculenta, M. glaziovii* x *M. esculenta; M. aesculifolia* x *M. esculenta*, and *M. pohlii* x *M. esculenta.* Twenty vegetative buds of stem cuttings of each hybrid were soaked in 0.2% colchicine aqueous solution for 24 h. Sprouting shoots were examined for leaf shape. Pollen viability of the pollen was estimated and pollen mother cells [PMCs] were studied to determine chromosome number.

To study PMCs at meiotic division (Fig. 14), the buds were fixed in absolute alcohol-glacial acetic acid, smeared and stained with acetocarmin. Pollen viability was estimated by acetocarmine-iodine mixture. Five hundreds pollen grains were examined in each cross. To distinguish different types of chimeras and tetraploid tissue, stomata and guard cells on leaf surface and leaf shape were examined on both sides of the emerging stem after colchicine treatment.

The colchicine treatment resulted in production of both complete and chimeral tetraploid stems with tissues having different ploidy levels growing side by side on the same stem. This result is due to the stratified arrangement of cells in the meristem treated by the colchicines, and derivation of mature tissue from these layers. The derivative cells of the outermost layer of the tunica form epidermis. The second layer LlI forms the subepidermal tissues. LlII form the pith and vascular tissue. The chimeras were distinguished to sectorial and periclinal. The frequency of polylpoids obtained from 20 buds treated is given in Table 3.

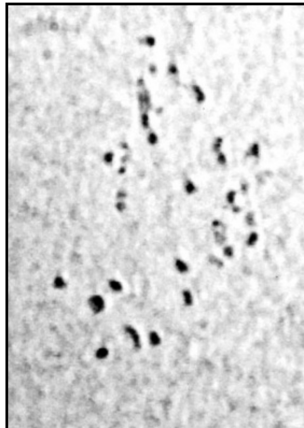

Fig. 14 – Metaphase interespecific I of polyploidizedhybrid M. glaziovii x cassava

Table 3. Frequency of polyploids obtained in four interespecific hybrids between cassava and wild *Manihot*

Hybrid Total polyploids	Chimera Total	tetra ploids	of polyploids%	Frequency
M. esculenta x *M. neusana*	**1**	**3**	**4**	**20**
M. esculenta x *M. glaziovii*	**5**	**-**	**5**	**25**
M. esculenta x *M. pohlii*	**2**	**2**	**4**	**20**
M. esculenta x *M. aesculifolia*	**-**	**3**	**-**	**15**

Identification of chimera It was possible to identity the chimera tissues on the basis of pollen grain viability, leaf shape and stem anatomy. The polyploid section of the stem in sectorial chimeras had the had and short leaves while the diploid side developed narrow and longer diploid form In case of periclinal chimera, pollen viability, leaf shape and stomata size were used as a selection criterion. Pollen formed from LII layer while leaves are differentiated form the LI layer. In periclinal chimeras, leaf is different and stomata enlarged and pollen viability is much higher than diploid plants. Ali the chimeras in the interespecific hybrid *M. esculenta* x *M. neusana*, and *M. esculenta* x *M. glaziovii* were sectorial while two sectorial, one periclinal chimeras were obtained in the cross cassava x *M. aesculifolia.* In sectorial chimeras, pollen grain viability on one side was low as in a diploid while on the other side it was as high as observed in the tetraploids. The size of pollen grains was notably larger in the later part. The pollen grain size in the periclinal chimeras reflects the ploidy level of this layer (Table 4).

Table 4. Pollen viability in diploid and tetraploid sectors

Interespecific hybrid Diploid tissue	Pollen viability % Tetraploid	tissue
Cassava x *M. neusana*	**18**	**92**
Cassava x *M. glaziovii*	**11**	**90**
Cassava x *M. pohlii*	**13**	**93**
Cassava x *M. aesculifolia*	**15**	**91**

Instability of chimeras

Little information is available about production of chimera in root crops and much less in cassava, but the use of polyploidy in cassava breeding is frequently reported by Indian breeders. Probably, the most interesting reports came from the Indian team at Thiruvanthpuram (Sreekumari *et al.* 1999). Their article reports production of total tetraploids in cassava but it does not mention chimera induction. Since appearance of chimera is a frequent phenomenon after colchicines treatment, it is possible that the resulting chimeras were overlooked or simply ignored in the above research. Due to stratification of the shoot apex, cytochimeras with different ploidy levels appear in each layer of tissue and their derivatives when the buds are trated by colchicine (Fig. 15). A competition between tetraploid and diploid tissues in chimeras was reported earlier (Stewart et al. 1974). This competition leads to the loss of desired traits. Only the chimeras in LlI (periclinal) layer have a chance of transmitting desirable characteristic to their progeny. In the chimeral sectors observed by us, the stem exhibited diploid phenotype after about six months growth restoring the normal leaf shape that became narrow, and pollen viability was that of a diploid. It seems that the .growth rate of tetraploid tissue is slower than that of the diploid, and tetraploid tissue is often overgrown by diploid tissue. However it is possible to propagate tetraploid tissue through somatic selection. This was done by cutting the apical buds of the chimeral stem, followed by removal of the lateral shoots growing from the diploid sector and allowing only the tetraploid side branches to grow.

One of the striking features of polyploidization is the restoration of the fertility of the interespecific hybrids. Yet a very small portion of unviable pollen formed in the polyploids due to formation of 3% multivalents in the polyploidized tissue. Quadrivalent formation occurs in cassava itself. Fertility restoration in the interespecific hybrids through polyploidization improves chances of using the wild species for crop improvement. This means the creation of new tetraploid species with high fertility and capable of self- reproduction maintaining their unique characteristics with a new closed gene pool in every interespecific hybrid (Nassar 2002, Sreerkumari and Abraham 1997). This technique allows to incorporate desirable genes in further crosses. The strategy involves backcrossing the polyploidized interespecific hybrids with cassava followed by selection for the desirable traits in the progeny. Preferential autosyndetic pairing between chromosomes of cassava may result into

elimination of the majority of chromosomes of the wild species during meiotic segregation. Even selfing of a fertile hybrid may produce useful recombination between wild *Manihot* species and cassava. One interesting approach in utilizing the induced polyploid types could be to cross them with the facultative apomictic clones, which may lead to the production of apomictic triploid clones that combine both heterosis and polyplody.

Amino-acids in cassava and the interspecific hybrid ICB 300

Cassava roots are poor source of protein in spite of its quality and the proportion of amino acids therein. Methionine and lysine are however limiting amino-acids in the root. If cultivars can be bred with more quantity of these amino acids it would enhance the value of cassava as a food or feed. Only about 60 % of total nitrogen in cassava is derived from amino acids and about 1 % of it is in the form of nitrates and hydrocyanic acid.. The remaining 38 to 40 % of total nitrogen remains unidentified (Diasolua *et al.* 2002, 2003).

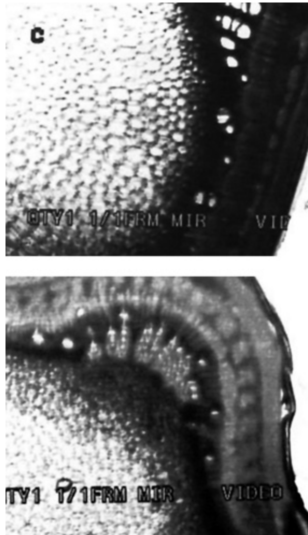

Fig. 15 – Section in diploid stem (above), and chimera stem (below), respectively

Cassava proteins are comparable to rice protein in digestibility. The biological value (Block and Michell equivalent) of the total protein is 48 %. The crude protein content of roots appears to be relatively stable and constant with maturity of the plant. According to Close *et al.* (1953) the protein of processed cassava includes the highest percentage of glutamic acid and the lowest of methionine (1%). Osuntokun *et al.* (1968) reported that both cystine and cysteine are involved in cyanide detoxification. Cyanide is produced when the glycoside linamarine is hydrolysed by linase.

Powder sample from a cassava cultivar (UnB 01), an interespecific hybrid between cassava and *M. oligantha* (ICB 300 Diploid), and its offpring (ICB 300 Progênese 4, ICB 300 Pregênese 9, ICB 300, Progênese 3 and ICB 300 Progenese 10) were analyzed. For this analysis, 500 mg samples of cassava root powder were extracted with 1 mL of 10 mM HCI for 4 h, at 25ºC, under agitation at 1,200 rpm in Thermemixer (Eppenderf, Hamburg, Germany). The suspensions were then centrifuged for 4 min at 6,000 rpm in a bench centrifuge. The supernatant (800µL), called acid extract, and the remaining powder were dried down in a SpeedVac vaccum centrifuge (Savant, New York, USA). The dried powder was extracted in the same way with 1 mL of 10m NH_4OH producing an alkaline extract. The vial with acid extract was resuspended with 750 of 10 mM HCI, washed with extra 750 µL of the same dilute acid, and added the 750 pL Df the alkaline extract. The total extract was exhaustively dialyzed against MilliQ water and vaccum-dried in a SpeedVad centrifuge. Aliquetes of 150 µg of each extract were dissolved in 75 µL of 100 mM HCI, respectively. Acid hydrolysis of the samples was performed in 6 M HCI under vacuum for 24 h at 109°C. After acid hydrolysis, the hydrolyzed samples were solubilized in 75(.11 of 100 mM HCI, and 50 µL were injected into an amine acid analyzer Hitachi l:8500 (Tokyo, Japan). The analyses for determination of amino acid compositions were performed in triplicates. The total protein contents of the samples were calculated by summing up the amounts of the amino acids.

Amino acid compositions from *Manihot* proteins were determined by analyzing sample extracts which were dialyzed against water to remove free amino acids, salts, monosaccharide and other small molecules. Tryptophan could not be analyzed since it is degraded upon acid hydrolysis. By adding the amounts of the analyzed amino acids, it was possible to determine the protein content for each sample. Among the six samples analyzed in this study (Table 5), interespecific hybrid ICB 300 offspring 3 Raiz showed the

highest amount of protein (1.654 g 100 g^{-1} of sample powder), followed by ICB 300 Diploid (1.454 g 100 g^{-1}), ICB 300 Progenese 9 (0.922 g 100 g^{-1}). The other samples (ICB 300 Progenese 10 Raiz, ICB 300 Progenese 4 and UnB 01 Raiz) were poorer in protein contents (0.350 g 100 g^{-1}). Essential amino acids were more concentrated also in Progenese 3 Raiz (His, Leu, Lys, Met, Phe and Val) and ICB 300 Diploide (lIe and Thr), with low or undetectable amounts in the other cultivars. Thus, Progênese 3 Raiz and ICB 300 Diploid would be more interesting for human consumption based on such nutritional characteristics.

Progênese 3 and 9 showed equal amounts protein; i.e., double of common cassava indicating high heritability of this character and possibility of selecting high protein cassava. Essential amino acids in cassava are arginine, histidine, isoleucine, leucine, phenylalanine, threonine, tryptophan and valine whereas methionine and tryptophan are lacking. The essential amino acids profile of cassava seems to be deficient in sulphur-containing amino acids (methionine, cystine and cysteine) (Bailey 1961). Osuntokun *et aI.* (1968) pointed out that both cysteine and cystine are involved in the cyanide detoxication (cyanide is produced when cyanogenic glucoside - linamarine present in cassava is hydrolyzed by linamarinase or by acid). The cysteine is mainly detoxificated by conversion to thiocyanate, in the process of which it reacts with cysteine and cystine. Excessive detoxication may be responsible for the low concentration of sulphur-containing amino acids.

Action was taken before to increase protein content of cassava roots by interespecific hybridization with wild species –namely *M. saxicola* and *M. mefanobasis.* Over a period of 10 years beginning 1932 and ending with Japanese occupation of Java in 1942, Bolhuis (1953) carried out a program of cassava breeding for increased protein content in roots. Crosses with *M. saxicola* yielded a few seedlings with as much as 2 % protein in fresh roots. In the clones he propagated from these seedlings protein content fell back to typical levels: Jennings (1957) reported that the roots of the F_1 progeny of *M. esculenta* x *M. melanobasis* possessed approximately twice as much protein as their cassava parent. The offspring were lost and not cultivated anywhere, probably because of poor root yield.

Barros and Bressani (1967) and researchers at Centro Internacional de Agricultura Tropical (CIAT) reported cultivars with high protein content (7%). It is however doubtful if the nitrogen referred to in such cultivars was protein or the breakdown of cyanogenic glucosides. It is therefore not

unlikely that the reported cultivars of high nitrogen content turn out to be nothing but bitter cultivars with high glucoside content. Another interfering factor for assessing protein as total nitrogen humidity while drying the material Excessive drying of the root powder may increase drastically percentage of nitrogen by 3-fold. Thus, it is important evaluating protein content as amino acids jointly with the evaluation as nitrogenous matter.

Table 5. Amino acid (AA) profile in peeled roots of cassava cultivar UnB, its inter-specific hybrid with *Manihot oligantha* –namely ICB 300 Diplóide, and ICB 300 Diplóide offspring (Progênese 3, Progênese 10, Progênese 4, Progênese 9 and Diplóide)

Sample mass (g per 100 g)						
AA	UnB 01	Progênese 3	Progênese 10	Progênese 4	Progênese 9	ICB 300 Diplóide
Ala	0.020	0.093	0.017	0.019	0.040	0.098
Arg	0.037	0.261	0.061	0.082	0.320	0.108
Asp	0.016	0.146	0.023	0.033	0.052	0.137
Cys	0.027	0.029	0.026	0.025	0.026	0.025
Glu	0.039	0.222	0.044	0.065	0.151	0.221
Gly	0.012	0.078	0.012	0.015	0.037	0.075
His	0.000	0.038	0.010	0.010	0.027	0.036
Ile	0.008	0.068	0.010	0.010	0.018	0.069
Leu	0.016	0.131	0.013	0.000	0.041	0.127
Lys	0.010	0.098	0.020	0.019	0.034	0.079
Met	0.014	0.041	0.004	0.000	0.019	0.037
Phe	0.016	0.129	0.058	0.000	0.065	0.120
Pro	0.000	0.054	0.000	0.000	0.000	0.066
Ser	0.012	0.088	0.013	0.018	0.033	0.078
Thr	0.008	0.061	0.007	0.013	0.022	0.066
Tyr	0.000	0.000	0.000	0.000	0.000	0.000
Val	0.019	0.115	0.027	0.025	0.039	0.112
Total	0.254	1.654	0.344	0.336	0.922	1.454

Indigenous cassava cultivars as a source of carotinoides

Vitamin A deficiency results in progressive eye damage. It is a serious problem in northern and northerneast Brazil (Simmons 1976, Flores and Araújo 1984, Dricot-d'Ans *et al.* 1988) and many other areas of the country (Desai *et al.* 1980, Wilson and Nery 1983, Favaro *et al.* 1986, Gonçalves-

Carvalho *et al.* 1995). Pro-vitamin A carotenoids are a cheap source since they are found abundantly in plants. Since cassava is one of the most important sources of food in northeast Brazil, selecting high carotene content clones may contribute significantly to solve vitamin A deficiency in poor countries (FAO 2003).

The average requirement of β-carotene recommended by WHO for adults ranges between 2.4 mg to 3.5 mg. The range of carotene content in cassava was assessed to select clones rich in carotene and good palatability. To evaluate carotenoides in cassava cultivars and hybrids, 10 g of mature roots and 5 g of leaves were extracted three times with acetone (5 ml per g). The filtered acetone extract was added in separation funnel containing petroleum ether, distilled water and ethylic ether (100:100:0.3 v:v:v). The aqueous fraction was discarded, and the organic fraction was submitted to saponification. Saponification was preferred since it removes accompanying lipids and chlorophylls.

The vitamin A values were calculated according to the conversion factor given by NAS-NRC (NAS-NRC 1989) whereas 6 μg of *trans*-β-carotene correspond to 1 μg of retinol equivalent (RE), and the activities are related as follows: 100% for *trans*-β-carotene, 50% and for *trans*-α-carotene and *cis*-β-carotene (Britton 1995). All the cassava clones presented the same major carotenoids in different concentrations. Lutein, *trans*-α-carotene and *trans*-β-carotene were separated and identified according to Davies (1976) and Britton (1995).

The colorimetric method for cassava clones characterization in terms of parenchyma color proved useful and is pragmatically used to detect if the variation exists. Among the cassava clones studied, the most impressing one was UnB-400 with 236 μg/g of lutein viz. a viz. zero in other cultivars. This antioxidant material is extremely important for health conditions of poor people. The same clone has a reasonable quantity (2.2 μg/g), which is considered by WHO sufficient for daily requirements of adults considering that a person consumes normally 500 g of cassava daily. The presence of lutein with this immense quantity adds to the valuable importance of this cultivar. In palatability tests, this clone was one of the most outstanding. It was easy cooked within 5 to 10 min maximum turning to a very soft mass like a cream. The clones showed very low HCN content, as judged by its taste.

Fig. 16 – Selected indigenous clone 401 **Fig. 17** – Selected indigenous clone

The most striking result is the content of both *trans*-β-carotene and lutein in leaves of clones UnB-400 and ICB-300 (Fig. 16). The *trans*-β-carotene reached 27.40 μg/g in the former becoming this clone one of the richest sources for this precursor of vitamin A available for poor people. ICB-300 had almost 20 μg/g of this type of carotene, making it also a very rich source. This clone (ICB-300) is a hybrid ensuing from crossing cassava with the wild relative *M. oligantha*. It has 5 % protein compared to 1.5 to 2 % in common cassava (Nassar and Dorea 1982). The amazing result was the amount of lutein in UnB-400 and ICB-300: 3081 and 9108 μg, respectively. Such quantities mean are about 4 to 12 times more carotenoid content than normal clones. Apparently the hybrid ICB 300 having 4% protein in the roots, 20 μg/g *trans*-β-carotene, 9108 μg/g of lutein in the leaves is an excellent source of these important components. UnB-400 is a very good source of precursor of vitamin A, considering its excellent palatability. ICB 300 clone is a very good ingredient to be added to wheat flour forming bread considering its high protein and carotene content. Brazilian government is looking now to mix cassava flour with wheat to reduce importations of the latter. An obstacle though is the low level of protein in common cassava compared to 7% in wheat. Using the flour of the hybrid ICB 300 may resolve this problem. Being rich in vitamin A precursor in its leaves adds an advantage to its future use. These results show the need for assessing cassava interespecific hybrids for carotene content because previous research showed that a cassava hybrid ensuing from crosses with *M. dichotoma* had double carotene content (22 mg kg^{-1}) viz. a viz. that (13 mg kg^{-1}) of cassava (Nassar *et al.* 2004).

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Indigenous cassava clones as a new source of lycopene

Osmindo Rodrigues Pires Junior
Carla Simone Vizzotto
***Carlos Alberto Schwartz*[1]**
***Nagib Nassar*[2]**
[1]Departamento de Ciências Fisiológicas, Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, DF, 70910-900, Brazil
[2]Departamento de Genética, Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, DF, 70910-900, Brazil

Abstract

Indigenous cassava clones acquired through their domestication a large diversity in relation to many economic traits such as high content of carotenoids and excellent palatability among other characters. One of these clones, which has been grown by indigenous Brazilian farmers and now being maintained in the Univ. of Brasilia gene bank, showed a high level of lycopene content (5 mgm kg^{-1} viz. a viz. zero in common cultivars, and 12 to 20 mgm kg^{-1} in tomato, a lycopene- rich). This is the first report of a cassava clone rich in lycopene.

Introduction

Cassava is the most important crop in the tropics and a staple food for more than 800 poor people. Cassava is also the principal food for about 60 million people living in northeast Brazil. The majority of cassava clones grown and consumed in this region are known to be free of carotenoids, which leads to many health problems to inhabitants of this region. One of the approaches to develop cassava clones rich in carotenoids is to screen indigenous clones for this substance. This concept is based on the fact that indigenous clones of this crop accumulated desirable mutations that were further selected by indigenous farmers in their long history of cultivation.

The nutritive importance of carotenoids is attributed to its conversion to vitamin A as it is the case of β-carotene, and to its antioxidant property and ability to quench singlet oxygen as in the case of lycopene. Lycopene interacts with free radicals eliminating their poisonous effect (Palozza and Kirinsky 1992). Early screening for carotenoid contents in these indigenous clones revealed one of the most striking features of this crop domestication: the clone UnB 400 showed 4 mgm kg^{-1} of b-carotene (Nassar *et. al.* 2005). The clone UnB 401 also attracted our attention due to its red root flesh which may be regarded as an indication of lycopene.

Materials and methods

The clone UnB 401, an indigenous cassava clone grown by in the Amazon, and maintained at Univ. of Brasilia living *Manihot* species collection was analyzed for lycopene content. This clone has a stem gray, 1.5 m height, scars largely raised, 2 to 3 branches. Its leaves are 7-lobed, and the leaf lobe is linear, with the margins slightly sinuous, whereas the medium lobe shows 10 to 12 cm length. The leaf has a green petiole, and the young foliage is reddish. The inflorescence is a 3 to 6 cm glabrous panicle. Bracts and bracteoles are inconspicuous and caduceus. Flowers are monoecious; showing the pistillate flowers a basal opening, whereas the staminate apical opening occurs 3 weeks later. Fruits are green and winged and the roots are conic, with a rough, pink-brownish surface. The root flesh is slightly red and turns to dark red after cooking (Photo1).

Photo 1. Cooked roots of clone UnB 400

Extraction for lycopene analysis 10 grams of mature roots were extracted three times with acetone (5 ml per gram). The filtered acetone extract was added in separation funnel containing petroleum ether, and distilled water. The aqueous fraction was discarded, and the organic fraction was submitted to saponification. Saponification was preferred since it removes accompanying lipids. In our work, the optimal conditions for mild saponification were achieved with 10% methanolic potassium hydroxide solution (100 ml) overnight at room temperature. After saponification the aqueous fraction was discarded and the organic fraction was dried with anhydrous sodium sulphate. The organic fraction was evaporated to dryness at 30 °C, re-suspended in 1000 μl of ethyl acetate and methanol (v,v; 50,50) and submitted to a HPLC system.

Equipment Carotenoid analyses were performed by Shimadzu LC-10A HPLC equipped with a photodiode array detector SPD MXA-10 and a Rheodyne injection valve with 20 μL loop. The separation was carried out on a C18 Vydac 218TP54 column 250 x 4.6 mm i.d. (5 μm particle size) with 100% MeOH as mobile phase at a flow rate of 1 mL/min at temperature of 15°C. The chromatograms were processed at wavelengths of maximum absorption (450 nm). The identification of carotenoids was achieved by retention time (TR) comparisons with those of the standard compounds and using the wavelength of maximal absorption (λmax) and the shape of the spectrum between 300 to 600 nm compared with data available in the literature (Davies 1976).

Quantification The calibration curves for lycopene were purchased from Sigma Inc., and purified from tomato, while for trans-β-carotene (purchased from Sigma Inc.), and for α-carotene (purified from "alfafa") were constructed with a minimum of the concentration levels thrice. All curves showed a good relation of area and concentration achieving a coefficient of determination (R^2) of 0.96, 1.00 and 0.98, for lycopene, trans-β-carotene and trans-α-carotene, respectively. The cis isomer of lycopene was quantified using the calibration curve of lycopene.

Results

The chromatogram file of lycopene isolated from tomato and the cassava clone extract are shown in Fig. 1A and Fig. 1B, respective. Lycopene showed to be the major catotenoid, although α-carotene and cis-lycopenes

were also found. The identification and characterization of the peaks are given in Table 1. Others carotenoids were not able to be identified in the cassava clone, which shows a concentration of 5 mg of lycopene per gram of wet root weight.

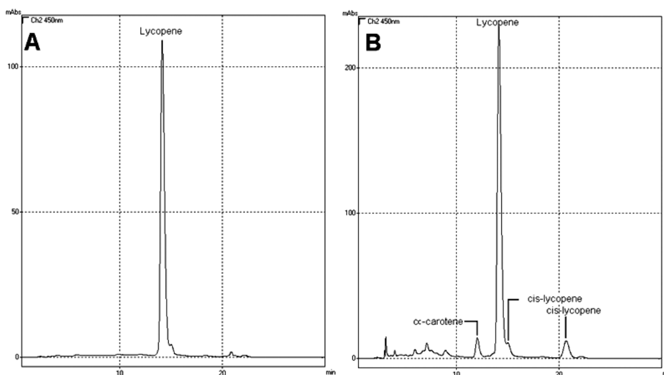

Fig. 1. Chromatogram profile of (A) lycopene and (B) cassava clone, showing peaks of trans-α-carotene, lycopene and cis lycopene. HPLC analysis conditions: RP column C18 Vydac 218TP54 column 250 x 4.6 mm, mobile phase 100% MeOH, flow 1 ml min^{-1}

The retention time in HPLC system, and the similarity of the spectrogram profile 300-600nm in photo-diode array of 0.98 (Fig. 2) confirms the presence of lycopene in this cassava clone.

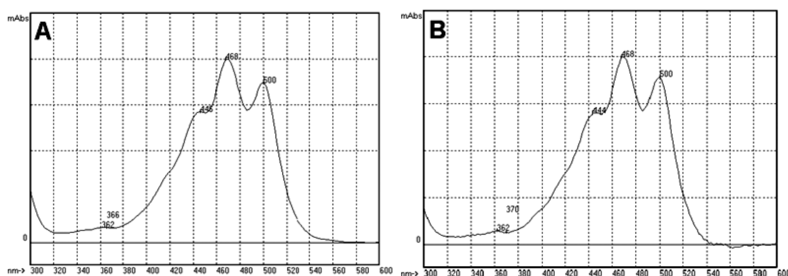

Fig. 2. Spectrogram profile (range 300-600nm) of peak referent to lycopene (A) isolated from tomato and (B) of cassava clone roots, showing a similarity of 0.98. RP column C18 Vydac 218TP54 column 250 x 4.6 mm, mobile phase 100% MeOH, flow 1 ml min^{-1}

Disussion

The most striking result of this research was the lycopene content in this cassava clone that was not previously reported in this crop species to the best of the authors' knowledge. This research provides means of better understanding cassava domestication and further breeding by indigenous Amazon farmers. The clone is grown in the Amazon, and from there it was brought to the State of Sao Paulo, where it was further grown by a few farmers. This clone could originate from a gene mutation that breaks the sequence of β-carotene formation, then adopted by Amazon's farmers, who used it probably for ritual or cultural ceremonies. This clone forms few roots compared to other improved cultivars. However increasing its root yield appears feasible by crossing with another clone possessing high combining ability for root yield.

Lycopene occur in tomato, guava, watermelon and pink grapefruit, and lycopene appears to be associated with reduced degenerative diseases. Other potential human health benefits include a possible role in the fight against digestive tract, breast and prostate cancer (Di Mascio *et al.* 1989, Handelman 2001). Other researchers have also emphasized lycopene's protection against lung, stomach, and prostrate cancer (Gerster 1997, Sies and Stahl 1998, Stahl and Sies 1996). Epidemiological studies have shown that high intake of vegetables containing lycopene is inversely associated with the incidence of certain types of cancer. For example, habitual intake of tomato products has been inversely associated with the risk of cancer of the digestive tract. Lycopene is a precursor of β-carotene, whose synthesis includes an enzymatic cycle in the chain-end (Krinsky 1994). The high lycopene level found in this cassava clone may indicate a dysfunction in the biosynthesis of β-carotene. The lycopene accumulation in this cultivar may therefore be the result of a deficiency in the β-carotene synthesis due to a mutation.

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Recent Trends in Cassava Breeding in India

S G. Nair* and M. Unnikrishnan
Central Tuber Crops Research Institute,
Trivandrum, India.

Introduction

Cassava along with maize, sugarcane and rice, constitute the most important sources of energy in the diet of tropical countries in the world. Cassava is rapidly emerging as a crop of considerable importance in India. Latin America has been reported as the place of origin, where the indigenous population for at least 4,000 years has grown it. After the discovery of America, Europeans soon recognized the advantages of the crop and took the crop to Africa as a potentially useful food crop, later to Asia also to be grown as a food security crop as for the extraction of starch (Howler, 2004). Cassava was either introduced into Sri Lanka and India by the Portuguese during the 17th century, or it was directly introduced from South America to India in 1840 (Abraham 1956). Kerala and Tamilnadu account for about 80% of the total acreage of the crop in India. India possesses the highest national tuberous root yield in the world (27.6 t ha^{-1}). It is cultivated in an area of 0.2 million ha producing 5.5 million t of tuberous roots. Cassava has the capacity to produce large amount of food calories per unit area, ability to adopt to erratic climatic conditions, resistance to locusts and several pests and diseases. Easy culture, low labor requirement, and cost of production are among some of its unique features that further encourage the spread of its culture to several regions of the country. Besides being important in human diet in Kerala, cassava provides cheap nutritious feed for livestock as well. Its tuberous roots have innumerable industrial uses also, particularly for starch extraction (Magoon 1967).

Globally, cassava is grown in an area of 18.5 million ha producing 202.6 million t with a productivity of 10.95 t ha^{-1} (FAO 2005). All the major

cassava growing countries in the Asian continent have the productivity of more than the world average productivity. Indonesia, Thailand and India are the major countries growing cassava in Asia. In the 19th century cassava became an important crop in southern India. It is a crop of food security in Kerala. By virtue of its diversified uses, it has become an important commercial crop in the agricultural economy of the states like Tamil Nadu and Andra Pradesh. The diverse use of cassava is the reason for the sustainability of the crop in contrast to increased income and standard of living of the people (Sreenivas and Anantharaman 2005).

Though cassava is under cultivation in India for more than one and a half century, systematic research in this crop was lagging until about 1940 when certain research projects were started in the Department of Botany, University of Travancore (Koshy 1947). In 1951, research was considerably expanded under a scheme jointly funded by the Indian Council of Agricultural Research and the Government of Travancore-Cochin (Abraham 1956). During the third 5-year plan, the Tuber Crops Research Institute was established (in 1963) by the Government of India for intensification of research on the improvement of root and tuber crops. The approaches to cassava breeding at Central Tuber Crops Research Institute (CTCRI) in Trivandrum involved the use of familiar tools of introduction, assay, selection, intervarietal, and interspecific hybridization, production of chromosomal races, genome approach, mutation breeding, tissue culture, and a diverse improvement program keeping in view the requirement of farmer, consumer and industry is actively underway on this root crop at the Institute.

Cultivar improvement

Genetic variability is the essence for any plant-breeding program. An assembly of diverse genetic stocks of any crop is the raw material from which a new cultivar can be molded to suit the requirement of farmers. Considerable amount of variability exists in the crop in India and much more is available in other cassava growing regions particularly in tropical South America. A genebank including wild relatives from within and outside the country was built up. Starting from a meager collection of 56 cultivars by Koshy (1947), the Central Tuber Crops Research Institute had launched a program on the collection of genetic stocks on cassava soon after its inception in 1963. Currently, the Institute maintains a cassava germplasm collection of nearly 1650 genetic stock of indigenous and exotic origin, which is the largest assemblage

in Asia. The important sources of exotic genetic stocks are Colombia, Madagascar, Nigeria, Thailand, Ghana, Uganda, Malaysia, Indonesia, Sri Lanka, Senegal and Gabon. In addition to such genetic resources, eight species of *Manihot* were also collected. The germplasm accessions have been evaluated based on the morphological and biochemical characters.

Many of the cassava types under cultivation in Kerala and other states of India are either chance seedling or bud mutations selected for desirable characteristics and maintained by vegetative propagation. Cultivars best suited for the requirements imposed by the local environments are generally adopted and popularized in the various cassava growing locations. The majority of types have the native name which generally indicates one of the striking features of the plant, like for instance, 'Anakomban' meaning the tuberous roots are white and long similar to an elephant's tusk. The maturity period of the different indigenous cultivars varies from 6 to 12 months. Cultivars with lesser toxicity are generally preferred in many areas.

Significant diversity still exists for this crop in India, which can be used as sources of parents for intervarietal hybridization or released, after selection, for general cultivation. The evaluation of the early introduction from exotic sources resulted in the identification of two promising cultivars M4 and M6 from Malaya (Abraham 1956). An indigenous selection S-856, high yielding cultivars with early harvest ability (7-8 months) was released for general cultivation in 1987 under the name Sree Prakash (Nair *et al.* 1988).

Intervarietal hybridization

An extensive intervarietal hybridization program having varied objectives is also underway. Several cultivars are distinctively better combiners than some others. Testing of indigenous and exotic cassava cultivars for their combining ability has been a regular activity of the cassava breeding program. A large number of intervarietal hybrids have thus been obtained and they are continuously being included in yield trials, followed by critical selection based on yield and several other criteria. Intervarietal hybridization in cassava, if carried out on a large scale, offers great combination of characters. Being highly heterozygous, such crosses can be expected to give a wide segregation and allow considerable scope for selection even in the first generation. An extensive intervarietal hybridization program launched by CTCRI culminated in the evolution of promising hybrids H 97, H 165, H 226, H 1687, H 2304 (Magoon *et al.* 1970, Jos *et al.* 1980). Of these, H 97, H 226 and H 165 were

released for general cultivation in 1971 and H 1687 and H 2304 in 1977 under the name Sree Vishakam and Sree Sahya, respectively. One of the hybrids H 3641 developed at CTCRI has been identified as high yielding and was released in Tamil Nadu as cultivars Mulluvadi as CO3 (Nayar and Joseph 1994). The tuberous yield of the cultivar was 30 to 35 t ha^{-1} under the recommended package of practices while the local check recorded an average of 20 t ha^{-1}. The Kerala Agricultural University has released a short duration cultivars called 'Nidhi' which can be harvested in 5.5 to 6 months and which has been recommended for paddy fallows. The Tamil Nadu Agricultural University has released CO1 and CO2 as high yielding cultivars, which recorded tuberous root yield 30-35 t ha^{-1}. CO1 is a selection from local collection, while CO2 is a clonal selection from the seedling progeny of a local clone.

Combining ability in cassava

The common breeding approach in cassava is through hybridization among appropriate parents. Seedlings with desirable attributes are selected and subsequently propagated clonally. However, selection of parents based on their direct performance may not always be dependable due to the type of gene action involved for the trait and diverse genetic structure of the parent. Hence, it is necessary to estimate the combining ability of parents before they are used in hybridization program (Rajendran 1989).

The line x tester analysis gives useful information regarding the choice of parents and elucidates the nature and magnitude of various types of gene action involved in the expression of quantitative traits. Recurrent selection and other procedures currently used in cassava also emphasize the importance of general combining ability and additive gene action (Byrne, 1984).. CTCRI has developed successive generations of inbred lines of cassava. Both additive and non-additive gene action were involved in the expression of all the characters (Eswari Amma *et al.* 1999). A straight forward hybridization program followed by phenotypic selection, as suggested by Kawano (1985), may be effective in creating desirable recombinants for traits with predominant additive gene action.

Heterosis breeding

Koshy (1947) and Abraham (1957) suggested that evolving homozygous lines in cassava for the purpose of exploiting hybrid vigor offers the

most promising line of work in the improvement of cassava. Considering the highly heterozygous nature of cultivated cassava types, perpetuated through years of asexual propagation, selfing undoubtedly offers very good scope for exposing locked up variability for selection (Magoon 1967). After selfing, inbred lines up to fourth generation were produced. Inbreeding depression was evident in morphological characters, field survival and above all yield of tuberous roots and components. The seed germination, in general, was less drastic due to inbreeding. Selected S_1 and S_2 progenies were top crossed with Sree Vishakam-a high yielding released cultivar. The top cross hybrids were significantly superior to the inbred parents and some were superior to or equal to Sree Vishakam in yield. A few hybrids exceeded the released cultivars in cooking qualities, dry matter (DM), starch content and harvest index, reveals that top-crossing is effective in bringing about population improved in inbred lines. Seven elite top-cross selection (TCH1, TCH2, TCH3, TCH4, TCH5, TCH6 and TCH7) showed high tuberous root yield (37-44 t ha^{-1}), high harvest index (61.6-74.9 %), high starch content (26.7-34.3 %), low levels of HCN (53-89 ppm on fresh weight basis), and excellent cooking qualities. These selections were evaluated in multi-location trials and two promising top-cross hybrids TCH1 and TCH2 were released under the name Sree Rekha and Sree Prabha, respectively.

Breeding for early maturity

A majority of cultivated cassava cultivars take about ten months for maturity and thus occupy land for a longer period. Greater attention will have to be paid in developing early maturing cultivars, so that they can be effectively utilized in crop rotation program now in vogue in the country. At CTCRI, these early maturing clones *viz.* CI 649, CI 731 and CI 732 were identified from the locally adapted germplasm. The clones had recorded a mean tuberous root yield of 25 t ha^{-1} at six months stage. Based on the evaluation trials and multilocation trials, two promising lines CI 649 and CI 731 were identified and released for general cultivation under the names of Sree Jaya and Sree Vijaya.

Use of triploidy in cassava improvement

Another approach for cassava improvement, besides hybridization and analytical methods of breeding, which warrants investigation, is the production

of colchiploids as well as triploids. Garner (1941) and Abraham *et al.* (1964) described colchicine induced tetraploids of cassava. Triploids (2n = 3*x* = 54) were also obtained later by other authors after crossing induced tetraploids with some of the cassava cultivars. They are found to be superior to colchiploids in yield and sometimes out-yielded 'diploids' (2n = 2*x* =36).

Polyploidy breeding has unique advantages in cassava, because the economically useful product is a vegetative part. Commercial cultivation is through clonal propagation, while the crop is amenable to hybridization. As stated earlier, all cassava cultivars posses 36 chromosomes. Therefore with a view to test the yield potential and adaptability of colchiploids, tetraploidy has been successfully induced through colchicine treatment in a few agronomically superior cassava cultivars. The colchiploids possess 72 chromosomes. These induced tetraploids are also being crossed with some of the selected diploid cultivars to produce triploids (Magoon *et al.* 1969, Jos *et al.* 1970). The adoption of the diploid clone as female parent was found to be more successful than the reciprocal. The high recovery of triploid plants in a particular cross combination, suggests that meiosis in the tetraploid plants results, as expected, in a large number of diploid pollen. The leaf thickness was found to be a reliable parameter for the preliminary screening of the population of triploids. Most of the triploids were equal or better than the better parent for tuberous root yield, and root dry matter content and they had invariably compact plant types. The compact plant types and high harvest index prevalent in a number of triploids indicates the possibility of increasing planting density, thereby facilitating higher yields.

The dry matter and starch content of triploids from specific cross combinations were higher than that of the diploids. An array of such clones have been identified of which two best selections (76/9 and 2/14) had particularly higher yield and dry matter content. These clones are ideal for the starch industry: 2/14 recorded an average yield of 35 to 40 t ha^{-1} in multi-location trials. This selection was later released by the State Variety Release Committee for use in Industrial areas under the name of Sree Harsha.

Interspecific hybridization

Interspecific hybridization and genome analysis carried out on different crops have opened up new avenues of improvement of crop plants and have successfully contributed to the development of radically new and

better types. However, as compared to other crops, cassava breeders have not yet scratched the surface of utilizing the genetic variability occurring within the species in nature. Added to the genes in the cultivated types are the vast arrays of genes in the related 'species' which possess reservoirs of unexplored genetic characters, incorporation of which into cultivars would appear to be of precise importance in any modern cassava breeding program. The transfer of characters from one taxa to another is not only of great practical importance, but is of considerable genetic interest as well.

All species of the large genus *Manihot* are confined as wild plants to the American tropics; no native species are found in the old world (Rogers 1965). Very few species have been used in the breeding programs probably due to the non-availability of extensive specific collections at various Indian research centers. However some useful work relating to interspecific crossing for breeding improved cassava cultivars have been reported. There is an urgent need for international cooperation for collection, maintenance and proper evaluation of this vast diversity for effective screening and full exploitation of sources of this genetic diversity in improvement work of this crop.

Cassava is not found in wild state but known to share a common gene pool with other species, which make the harnessing of the other desirable wild genes rather more important. However the preservation of wild species is rather difficult even though different methods of propagation are suggested (Nassar 1978). The survival of wild species under the climatic conditions of the Indian sub-continent is also poor. Although eight species are currently maintained at CTCRI, some of them have the desirable genes hitherto not identified at inter-specific level of cassava. Transfer of disease resistance into cultivated cassava was attempted by hybridizing ceara rubber (*Manihot glaziovii*) on selected clones of cassava. Repeated backcrossing of the resulting inter-specific hybrid was carried out but appropriate root quality was not achieved (Abraham, 1972). Earlier attempts to introgress the cassava mosaic disease (CMD) resistant genes from *Manihot glaziavii* into cassava (Magoon *et al.* 1966, Vijaya Bai *et al.* 1972) did not result in high levels of resistance into the backcross offspring. Intensive work is in progress to utilize the desirable wild species genes. The interspecific hybrids of *M. caerulescens* and the backcross (BC) generation of the interspecific hybrid showed resistance to CMD. CMD resistant cultivars with the edible normal tuberous roots and high yield were obtained in the BC4 generation of the inter-specific hybrid of *M. caerulescens* with cassava.

Manihot caerulescens: A new source of resistance to CMD

CMD is the most important problem of the crop in India leading to 16 to 80% yield loss. Wild *Manihot* species has been used as source of many useful traits in cassava. At CTCRI, 37 accessions of wild *Manihot* species, comprising of *M. glaziovii, M. pseudoglazovii, M. caerulescens, M. tristis, M. peruviana and M. flabellifolia*, were screened for resistance to CMD through wedge grafting. All accessions of *M. caerulescens* exhibited high level of resistance and were used as donor parents for transferring resistance to elite Indian cultivars. Resistant interspecific crosses were graft inoculated reciprocally with highly susceptible cultivars to confirm resistance. This research suggests the need for characterization and utilization of this novel source of CMD resistance with other tools, including DNA markers,

Production of carotene rich cassava

Cassava is an important food crop grown in humid tropics and one of the most important sources of starch used in several industries. Besides, being important in human diet, it provides cheap nutritive feed for livestock. Even though the crop provides high energy, it is considered as a poor food in view of lack of nutrients other than carbohydrates. In most of the clones, the flesh, the edible portion of the tuberous root is white and devoid of any carotene, the precursor of vitamin A. Malverhas (1964) reported yellow flesh cassava cultivars from the Amazon that contain about 800 IU β-carotene. Cassava leaves are also rich in carotene: up to 16,000 IU (Van Veen 1975). A total of 21 clones, available in CTCRI, have yellow flesh of different intensities, and with β-carotene content ranging from 65 to 670 IU/100g on fresh weight. An attempt was made to elevate the carotene levels through gene pool development from the existing gene resources. The carotene content could be enhanced to 1500 IU/100g in the first cycle and later to 2200 IU/100g in the second cycle through gene pool development (Jos *et al.* 1990). In the third cycle, yellow flesh clones had a carotene range of 1016 to 2983 IU/100g while the orange flesh clones with carotene ranging from 1024 to 3217 IU/100g were noticed. Such levels hitherto never achieved in cassava (Nair and Santha Pillai 1999). This wide variation indicated that there is room for cultivar screening, selection, and gene manipulation to improve the carotenoid content of cassava tuberous roots, and thereby enhance its nutritive value (McDowell and Oduro 1983). By simple recurrent

selection, the carotene content was enhanced to 1500 IU in the first cycle, to 2200 IU in the second, to 3217 IU in the third and to 3983 IU/100g in the fourth cycle.

Cassava true seed program

Cassava has enormous potential in India for poverty alleviation and food security due to its ability to grow well in marginal and wastelands under poor management and its capability to yield well even under such unfavorable conditions. The slow multiplication rate under clonal multiplication, bulk of seed materials and the dreaded CMD are the major impediments that prevent the rapid spread of the crop in far-flung poverty stricken areas of the country (Rajendran *et al.* 2005). The propagation of cassava through true (sexual) seeds rather than by clones is a promising option due to its manifold advantages such as enhancing the multiplication rates, keeping the dreaded CMD under check, longer seed viability, ease of storage and transport. The high genetic heterogeneity and consequent variation in the seedling is the major stumbling block in sexual propagation. The rate of sexual propagation could be more than 20-fold over the traditional clonal propagation. Removal of taproot of seedling while transplanting enhanced tuberous root development. Seed treatment with 1% KNO_3 or 300ppm GA promoted uniform seed germination and seedling vigor and reduced transplanting period. Tuberous root yields of first clone were significantly superior to that of the seedlings. The dry matter content and starch output of the seedling and the first clone were comparable to that of commercial cultivars. Similarly cyanide (HCN) and the cooking quality of the seedling and first clone were at acceptable levels. Further research indicates that a cassava true seed program has potential in industrial areas due to its high multiplication rate, ease in covering extensive areas at lower cost and low transmission of cassava mosaic disease.

Evaluation of CIAT seedlings

Cassava seeds received from the Centro of Agricultura Tropical (CIAT) were initially screened at the regional center, Bhubaneswar (Orissa). During the period from 1989-1996, five sets of cassava botanical seeds comprising 119 accessions were introduced from CIAT directly to CTCRI. The seedlings were evaluated preliminarily for yield characters and their host plant resis-

tance to CMD, which was not noticed at the Regional Center in Bhubaneswar. However, when these promising selections were transferred to the germplasm bank at CTCRI, clear CMD symptoms were noticed in all the clones. Tissue culture materials were multiplied and evaluated in CTCRI. Out of these, one cultivar, MNga-1 showed resistance to CMD. The evaluation trials conducted at the institute recorded an average tuberous root yield of 25 28 t ha^{-1}. The plant type is semi-spreading and tuberous root skin is white, long and cylindrical. Multi-location trials were conducted in the industrial areas of Tamil Nadu and showed resistance to CMD. It recorded an average tuberous root yield of 25 t ha^{-1} with 22 to 25% of starch. This cultivar is being proposed for release in the industrial belt of Tamil Nadu for general cultivation.

Area, production and yield

Globally cassava is grown in an area of 18.5 million ha producing 202.58 million t with a productivity of 10.95 t ha^{-1} (Table. 1).

Table 1. Area, production and productivity of cassava in major cassava growing countries of Asia

Country	Area (million ha)	Global area (%)	Production (Million t)	Global (production %)	Productivity (t ha^{-1})
World	18.50	100.00	202.58	100.00	10.95
Total Asia	3.52	19.00	58.92	29.09	16.76
Indonesia	1.27	6.85	19.26	9.51	15.20
Thailand	1.05	5.67	20.40	10.07	19.43
Vietnam	0.38	2.07	5.69	2.81	14.83
Sri Lanka	0.26	0.14	0.23	0.11	8.64
China	0.25	1.35	4.20	2.07	16.80
India	0.24	1.30	6.70	3.31	27.92
Philippines	0.21	1.11	1.64	0.81	7.99
Malaysia	0.04	0.22	0.43	0.21	10.49

Source: FAO (2005)

The crop is grown n 102 countries around the world. The African continent ranks first with 66.2% of cassava growing area, and producing 52.4% of world cassava production and it is staple in many of the African countries. Even though the area is more in Africa, its production is low

due to low productivity (8.8 t ha^{-1}), which is lower than the world average productivity (Sreenivas and Anantharaman 2005). Though rice and wheat form a major part of the staple food of Asians, it is heartening to note that Asian continent is the second largest in terms of area and production of cassava with a productivity of 16.8 t ha^{-1}. South America has 13.4% growing areas worldwide, and producing 16.9% of the world cassava. Nigeria has the largest area under cassava (22.25%) among all the cassava growing countries in the world with an annual output of 38.2 million t. Democratic Republic of Congo ranks second for area and accounts for 10% of the world production, followed by Brazil in terms of area, which ranks second for worldwide production.

All the major cassava growing countries in the Asian continent have the productivity more than the world average productivity (Table.1). Indonesia, Thailand and India are the major cassava growing countries in Asia. India acquires significant position in the global cassava scenario due to its highest productivity in the world (27.9 t ha^{-1}). It is cultivated in 0.24 million ha producing 6.7 million t. It is a crop of food security in Kerala. By virtue of its diverse uses, it has become an important commercial crop in the agricultural economy of the states like Tamil Nadu and Andra Pradesh. A diverse use of cassava is the major reason for the sustainability of the crop in the country in the context of increased income and standard of living of the people.

An analysis of cassava planted area and production in India shows an enormous increase up to 1974-1975 (0.39 million ha) after which it started declining fast until 1985-1996 (0.27 million ha) followed by a slower decrease thereafter (Table 2). Kerala where the crop was first introduced, accounts for 50% of the area under cassava (0.13 million ha), Tamil Nadu for 32% (0.08 million ha), and Andhra Pradesh for 9% The production area in Kerala during the last four years (1999-2003) is showing a slight decreasing trend (Tables 3, 4 and 5). The major factor accounting for the decline is the shift in cropping pattern in Kerala, where plantation crops are starting to dominate the agricultural economy. Even though the production area of cassava is showing a decreasing trend, the production of cassava is almost same in every year or showing-increasing trend in 1999-2001. The productivity of cassava was more or less same up to 1986 (16-18 t ha^{-1}) and after which it started an increasing trend and during 2000-2002 achieved 27 to 28 t ha^{-1}.

Table 2. Area, production and yield of cassava in India (1973-1974 to 2003-2004)

Year	Area (`000 ha)	Production (`000 t)	Yield (kg ha^{-1})
1973-74	368.2	6420.9	17439
1974-75	387.6	6325.9	16321
1975-76	392.0	6638.3	16934
1976-77	385.8	6375.0	16524
1977-78	658.3	5688.3	15876
1978-79	361.5	6050.1	16736
1979-80	351.9	5845.3	16611
1980-81	346.2	5868.0	16950
1981-82	323.0	5292.0	16384
1982-83	302.0	5341.0	17685
1983-84	319.0	5886.0	18451
1984-85	305.3	5662.1	18546
1985-86	275.7	4884.3	17716
1986-87	265.3	4814.4	18147
1987-88	268.4	5416.5	20181
1988-89	249.0	4833.0	19410
1989-90	242.0	4962.0	20504
1990-91	243.0	5111.0	21033
1991-92	250.9	5832.5	23246
1992-93	234.9	5412.8	23043
1993-94	245.8	6028.9	24528
1994-95	242.8	5929.3	24420
1995-96	228.2	5443.2	23853
1996-97	256.1	5662.8	22112
1997-98	264.3	6681.9	25282
1998-99	243.4	5830.4	23954
1999-2000	223.5	6014.1	26909
2000-01	251.8	7123.8	28292
2001-02	247.6	6834.0	27601
2002-03	207.0	5426.2	26214
2003-04		5500.0	

Source: Agriculture, Center for monitoring Indian Economy.

Table 3. Major states by cassava area (000 ha) in India. (1995-96 to 2002-2003)

	1995 -1996	1996 -1997	1997 19-98	1998-1999	1999 -2000	2000 -2001	2001 -2002	2002 -2003
India	**228.2**	**256.1**	**264.3**	**243.4**	**223.5**	**251.8**	**247.6**	**207.0**
Kerala	**118.7**	**120.4**	**132.9**	**129.9**	**109.3**	**114.6**	**109.3**	
Tamilnadu	**77.6**	**82.2**	**103.7**	**80.9**	**85.3**	**104.8**	**102.0**	**68.0**
Andhra Pradesh	**20.5**	**22.0**	**17.7**	**22.0**	**18.1**	**21.5**	**17.7**	**13.1**
Meghalaya	**3.9**	**3.9**	**4.0**	**4.0**	**4.0**	**4.1**	**4.0**	
Assam	**2.6**	**2.4**	**2.5**	**2.7**	**2.8**	**2.9**	**2.9**	
Karnataka	**0.9**	**0.9**	**0.9**	**1.0**	**1.0**	**1.0**	**0.9**	
Nagaland	**0.7**	**0.8**	**0.8**	**0.8**	**0.8**	**1.3**	**1.3**	
Sikkim	**1.6**	**1.6**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	
Mizoram	**0.5**	**0.4**	**0.5**	**0.5**	**0.2**	**0.4**	**0.3**	
Rajasthan	**0.2**	**0.2**	**0.2**	**0.1**	**0.1**	**0.1**		

Source: Agriculture, Center for monitoring Indian Economy.

Table 4. Major states by cassava production (000 t) in India (1995-1996 to 2003-2004)

	1995 -1996	1996 -1997	1997 -1998	1998 -1999	1999 -2000	2000 -2001	2001 -2002	2002 -2003	2003 -2004
India	5443.2	5662.8	6681.9	5830.4	6014.1	7123.8	6834.0	5426.2	5500.0
Kerala	2406.0	2588.3	2841.8	2810.9	2563.5	2586.9	2471.8		
Tamilnadu	2763.8	2819.9	3889.3	2804.7	3266.4	4295.5	3834.6	2146.5	
Andhra Pradesh	207.2	174.5	192.3	132.0	111.3	166.1	353.9	79.5	
Meghalaya	21.5	21.5	21.1	21.3	21.5	21.9	20.6		
Assam	12.1	11.5	11.7	13.4	13.4	13.5	13.7		
Karnataka	7.3	7.1	7.4	8.5	8.1	8.0	7.2		
Nagaland	1.4	1.3	15.8	15.8	15.8	16.0	16.0		
Sikkim	1.0	1.3	1.2	1.2	1.2	1.2	1.2		
Mizoram	6.6	6.7	7.0	2.5	0.2	2.0	1.5		
Rajasthan	0.3	0.3	0.3	0.4	0.4	0.2			

Source: Agriculture, Center for monitoring Indian Economy.

Table 5: Major states by cassava yield (kg ha^{-1}) in India (1995-1996 to 2002-2003)

	1995 -1996	1996 -1997	1997 -1998	1998 -1999	1999 -2000	2000 -2001	2001 -2002	2002 -2003
India	23853	22112	25282	23954	26909	28292	27260	26214
Kerala	20270	21500	21383	21639	23454	22572	22615	
Tamilnadu	35605	34291	37512	34654	38283	40988	37594	31589
Andhra Pradesh	10127	7920	10874	6000	6158	7724	20000	6049
Meghalaya	5513	5513	5275	5325	5375	5342	5150	
Assam	4654	4792	4680	4963	4786	4655	4724	
Karnataka	8111	7889	8222	8120	7951	7784	8021	
Nagaland	2000	1625	19750	19750	19750	12308	12308	
Sikkim	625	812	2400	2400	2400	2400	2400	
Mizoram	13200	16750	14000	5000	1000	5000	5000	
Rajasthan	1500	1500	1500	4000	4000	2000		

Source: Agriculture, Center for monitoring Indian Economy.

Cassava production statistics for 2001-2002 reveals that Tamil Nadu, the predominant state where cassava is grown as an industrial crop, the area, production and productivity increased (Tables 3, 4 and 5). The growing cassava area in Tamil Nadu increased from 0.08 million ha in 1995 to 0.1 million ha in 2001, with the production increasing from 2.8 million t to 3.8 million t in 2001. The productivity also showed a steady increase from 1995 (35.6 t ha^{-1}) to 2001 (37.5 t ha^{-1}). The productivity in 2000 was 40.1 t ha^{-1}. The increased productivity was due to the popularization of the high yielding intervarietal cassava hybrids of CTCRI. The intervarietal hybrids H-165 and H-226 occupy more than 2/3 of the cassava area in Tamil Nadu. Similarly in Andhra Pradesh, another predominant cassava growing area for industrial products, the same two intervarietal cassava hybrids are gaining popularity and are grown in an area of about 17,700 ha in 2001-2002, with a total production 0.35 million t due to its productivity of about 20 t ha^{-1}. Presently, there is a preference in the cultivation of short duration cassava (Sree Jaya) so that the plant can be harvested in six months, thereby utilizing the monsoon season more effectively in Andhra Pradesh. Cassava yields in Tamil Nadu can reach up to 40.9 t ha^{-1}, which is the highest recorded worldwide.

Even though the overall national trend in area and production is declining, India's current cassava yields of 28.2 t ha^{-1} (2000) or 27.6 t ha^{-1} (2001) are the world's highest and more than double of the world average

($10.95\ t\ ha^{-1}$). The foregoing trend in cassava cultivation and its utilization as an industrial crop for starch and value added products, amply explains the quantum leap that India had registered in record productivity of the crop in recent years.

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Selection of Cassava Parents by morPHological and Agronomic TRAITS, and Genetic Divergence Analysis

Alejandra S. Albuquerque
EMBRAPA-Amazônia Oriental,
Belém-Pará, CEP 66.095-100, Brazil

Introduction

Brazil has been suggested as the center of origin and domestication of cassava (Allem 1987, Olsen and Schaal 1999, 2001). In the Amazon region, cassava is mainly grown as a subsistence crop by small farmers, thanks to its ease of cultivation, cheap production and its ability to tolerate poor soils. Moreover it suffers from few serious pests and diseases and it is avaible throughout the year. Although cassava suffers from fewer diseases than other major food plants, in northeast Pará State, Brazil, *Phytophthora drechsleri* root rot (Fig. 1) can cause significant damage in crop and loss can reach 80 to 100% when susceptible cultivars are grown.

Fig. 1 – Phytophthora drechsleri *root rot*

Significant cassava genetic improvement only can be achieved for resistance to biotic factors, root quality and good productivity through the intensive selection of complementary parents, and transfer of improved gene pools to breeding programs. The aim of this research was to group elite genotypes by genetic divergence analysis on agronomic traits to indicate what accessions can be used more intensively as parents in crossing blocks.

Materials and Methods

The preliminary trial held at the EMBRAPA – Amazônia Oriental, included 10 flour purpose cassava genotypes showing more than 25% starch storage root content and fresh root yield above 5 kg per plant. The accessions were 01-Pacajá, 34-Pretinha, 51-Caravela 1, BGM 911-59, BGM 019, BGM 844-2, Maranhense 2, Milagrosa 20, Taxi-Mo and Vermelhão. Therefore, these elite genotypes were evaluated, at 12 months after planting, for other seven morphological and agronomic features (Fukuda and Guevara 1998) such as plant height (m), first ramification height (m), total number of roots (per plant), number of rot roots (per plant), root length (cm), root diameter (cm) and shoot mass (kg plant^{-1}). Ten plants for each elite genotype were measured for these features and genetic diversity was evaluated by Tocher`s method. Standardized average Euclidean distance was used as dissimilarity measure.

Results and Discussion

Morphological and agronomic averages for elite genotypes held at the EMBRAPA – Amazônia Oriental are given in Table 1.

Table 1. Morphological and agronomic averages for elite genotypes

Elite genotypes	Plant height (m)	First ramification height (m)	Total number of roots (per plant)	Number of rot roots (per plant)	Root length (cm)	Root diameter (cm)	Shoot mass (Kg/plant)	Root mass (Kg/plant)	Starch storage root (%)
01-Pacajá	2,45	0,85	10,80	0,60	25,60	3,56	3,40	6,84	29,71
34-Pretinha	2,50	1,45	7,20	0,20	28,60	6,18	2,40	5,60	30,00
51-Caravela 1	2,10	0,80	8,00	0,20	25,10	5,79	1,70	5,28	27,00
BGM 911-59	2,42	0,71	6,20	0,20	29,20	6,40	3,04	5,72	28,58
BGM 019	2,67	1,21	9,40	1,40	26,50	5,20	2,88	5,20	27,46
BGM 844-2	1,98	0,65	7,24	0,25	29,90	5,00	4,50	5,50	29,99
Maranhense 2	2,47	0,33	4,40	0,80	31,50	6,62	5,00	7,50	28,02
Milagrosa 20	3,32	0,43	13,80	0,80	22,20	5,24	3,64	7,00	29,00
Taxi - Mo	2,11	0,29	10,80	1,80	25,70	5,60	2,00	5,20	28,92
Vermelhão	2,91	0,86	7,40	0,40	28,80	4,51	5,24	9,60	25,48

The flour purpose elite cassava genotypes 01-Pacajá, 34-Pretinha, 51-Caravela 1, BGM 911-59, BGM 019, BGM 844-2, Maranhense 2, Milagrosa 20, Taxi-Mo and Vermelhão were grouped into three similar clusters according to Tocher´s method (Table 2).

Table 2. Elite cassava genotypeclusters according to Tocher´s method

Cluster	Elite genotypes
Group 1	34-Pretinha, BGM 911-59, 51-Caravela 1, BGM 844-2, BGM 019, 01-Pacajá, Taxi-M
Group 2	Maranhense 2, Vermelhão
Group 3	Milagrosa 20

The best accession in each Tocher's cluster for their starch storage root content 34-Pretinha in Group 1 and Maranhense 2 in Group, whereas Milagrosa 20 was the only accession in Group 3. Since accessions in a Tocher`s cluster are similar to each other, the best in each cluster for starch storage root content will be further used as parents in crossing blocks receiving pollen from root rot tolerant genotypes..This breeding approach should therefore exploit the great genetic variability in the segregant population derived from high yielding, disease tolerant parents.

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Cassava Cooking Time

Lilian Azevedo Miranda[1],
Adelaide del Pino Beleia[2]
and Nelson Fonseca Jr.[3]
[1]Sítio Mirantro, Londrina-PR, [2]UEL, [3]IAPAR, Brazil

Abstract

Cassava roots are used at home mainly cooked, or fried after cooked. It is therefore important to have a trustful and simple way to estimate the cooking time. We built an instrument, based on Matheson's to measure peas cooking time. It consists of two aluminum supports and 15 stalks (90g) each with a needle on its end. We worked with six different cultivars, cultivated for two consecutive years. The roots were harvested from 8 to 20 months after planting. We analyzed 3 plants for each cultivar using the median part of each root with a 80 g weight. They were cooked with de-ionized water. Cooking time was settled when 8 needles entered completely the root. The coefficient of variation ranged 2 to 7 %, and cooking time varied from 19 to 35 min. It was possible to statistically distinguish the different cultivars with different ages. Our instrument may help cassava breeding for home uses.

Introduction

Cassava is a plant of the Euphorbiaceae family, and its center of origin is the Amazon region. In Brazil cassava is used as human food. Beyond the popular cassava flour, the roots can be consumed by simply cooking them (known also as "bread of Brazil"), or they can be an ingredient of some recipes as a substitute of potato (Pereira *et al.* 1983, Silva 1996). However consumption of cassava roots reduced by 50% in the late 1990s - from 120 kg of cassava per capita per year in 1970 (Transformações no padrão de consumo alimentar 1999). This reduction occurred because the roots generally are dirty and have to be peeled; i.e., consuming time of the house owner. The

supply of cassava after minimum processing (peeled, washed and packed in plastic bags) to the food markets can increase its use because consumers search for foods with easy handling. The value added through minimum processing contributes also to quality life improvement for the producers and, consequently, for their setting in the agricultural sector.

The main desirable attributes for table cassava roots, beyond high yield, are fast cooking and the pleasant flavor and texture. The irregularity of the culinary quality of the roots is another factor that has restricted the increase of the consumption of cassava. Research carried by cassava breeders has proven difficult for distinguishing between cultivars with distinct cooking times. This trait shows significant variation among plants of the same cultivar, as well as between roots of the same plant, and of parts of the same root (Pereira *et al*. 1985). Fukuda *et al.* (1988) evaluated 22 cassava cultivars for cooking time 11 months after planting. Their technique consisted of kneading the roots cooked with fork and pressuring with the fingers the mass against the palm of the hand. The cooking times varied from 18.3 min to 59.7 min. The coefficient of variation for their assessments ranged between 5 and 20.5 %. Lorenzi (1994) assessed the texture of cassava roots with a fork, and observed variation in cooking time (16-26 min) in samples of the same root, among roots from the same plant (28-60 min), and .between different cultivars (32-39 min on average). Safo-Kantanka and Owusu-Nipah (1992) evaluated cooking time of two cassava cultivars 10 to 11 months after planting at two planting dates using the method of Fukuda and Borges (1988). Their research showed that the environment and the genotype influenced the texture of cooked cassava roots. Wickham and Wilson (1988) assessed the effect of storage on three cooking time for cassava cultivars 11 months after planting. About 20 min after cooking, slices from 15 roots of each cassava cultivar were pressed with a fork, and differences between cultivars were recorded. The objective of our research was therefore to identify cassava cultivars with desired cooking time for a minimum processing.

Materials and methods

Experimental design The most popular cassava cultivars of the region of Londrina [Yellow Catherine (CA), White Catherine (CB), Mato Grosso (MT) and Pretona (PR)] along with IAPAR-l9-Pioneer (PI) –selected by the Agronomic Institute of Paraná (IAPAR) as a fast cooking cultivars,

and IAC-576-70 –recommended by the Agronomic Institute of Campinas for the State of São Paulo, were included in this experiment. Cultivars were planted in Londrina, Paraná (23º19' S, 51º12' W), in a Hapludult soil during two years (September1996 and September 1997).The field layout was a randomized block design with four replications. Each cultivar was grown in a experimental plot consisting of six rows of 8 m length, 1 m between row, and 1 m between plants within the row. Manure was not applied and when necessary manual weddings were undertaken. There were seven bimonthly harvests in each year, starting 8 months after planting. At each time 3 competitive plants of each block were harvested; thus 12 plants for each cultivar at each time of harvest were assessed.

Cooking Time Evaluation To evaluate the cooking time, an adaptation of the device of Mattson, which was developed to determine the cooking time of peas (Mattson 1946), was made. This device was used for determining ooking time of beans and soybeans (Jackson and Varriano-Martson 1981). The adaptation consisted of a support formed by two parallel grades, and each supporting 15 cylindrical aluminum connecting rods of 90 g contained in the tip a bronze needle. Five healthy roots were selected from each harvested plant, peeled and cut in sections in their central part. The pieces were washed, drained, weighed (80 g), conditioned in polyethylene bags, identified, and placed in cold chamber at 5°C, prior to their cooking in the following day. Since 3 plants per plot were harvested, 15 root pieces were used.

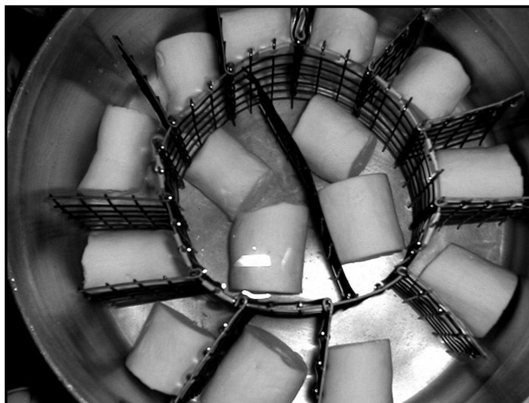


Fig. 1 – Cassava roots distributed inside the pan

In a pan 7 L of de-ionized water were placed, and warmed until boiling. The pieces to be cooked were separated in an aluminum screen within the pan (Fig. 1). A chronometer was used to record the cooking time. Supported in the edge of the pan, the support with the rods was placed (Fig. 2), with utmost care to support the tip of the needle of each rod (Figs. 3 and 4) on of the central part of each piece of the cassava root. The cooking time was recorded after total penetration of eight needles of the rods in the roots.

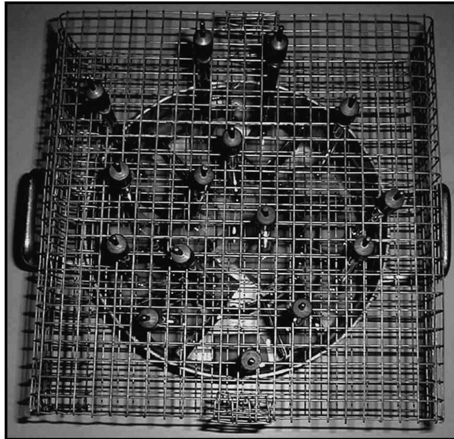


Fig. 2 – Pan showing the support with the rods

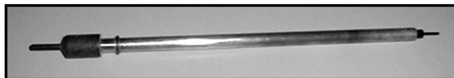
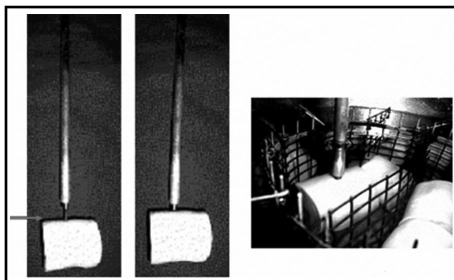


Fig. 3 – Rod used to determine when the root is cooked, with a needle on its right end



crude **cooked** **cooked, inside the pan**

Fig. 4 – Details of the penetration of the needle, when the root is cooked

Analysis of the data Cooking time data were transformed to natural logarithms, which allows a normal distribution, prior to the analyzes of variance (ANOVA) (Steel and Torrie 1980). SAS® 6.12 software was used for the ANOVA, which follows a split-plot treatment design. Graphs were drawn using program STATISTICA 5.0.

Results and Discussion

The average cooking time for the seven harvests on the first planting was about 20 min. IAPAR 19-Pioneer was a fast cooking cultivar followed by Catherine Branca. All cultivars showed acceptable cooking time (< 25 minutes) 12 months after planting. Yellow Catherine, Mato Grosso and Pretona showed the longest cooking time on the two years. About 16 months after planting, Yellow Catherine needed about 41 min for cooking, Mato Grosso 46 min, and Pretona 40 min (Table 1). These cultivars are therefore not suitable as table cassava when harvest occurs 12 months after planting. The coefficients of variation in our experiments were very low: (Tables 1 and 2), which indicated that the methodology used was appropriate for assessing cooking time of roots among cassava cultivars.

Table 1. Cooking time among six cassava cultivars at seven times of harvest (months after planting, MAP) (UEL, Londrina 1996 planting)

Cultivars	8 MAP	10 MAP	12 MAP	14 MAP	16 MAP	18 MAP	20 MAP	Average
CA	21 a C	238 a CD	24.4 a CD	25 bc CD	41.4 ab A	27.9 a BC	29.1 ab B	27.5 a
CB	211 a B	212 ab B	22.9 a B	23.3 c B	31.9 abc A	19.3 b B	19.3 b B	22.7 b
MT	18.2 ab D	234 a CD	25.4 a CD	34.1 a B	45.7 a A	28.2 a C	33.2 a B	29.8 a
PI	15 b C	18 b B	18.4 b B	23 c AB	25.2 c A	19.4 b B	22.6 ab AB	20.2 b
PT	224 a D	21.4 ab D	25.1 a CD	32 ab B	40 abc A	22.4 ab D	28.4 ab CD	27/4 a
IAC	17.4 ab C	20 ab C	24.4 a B	29.5 abc A	26 bc AB	20 b C	26.7 ab AB	23.4 b
CV (%)	4	3	6.1	2	3.9	5.2	4.8	4.1

Values followed by same small letters within each column are non-significantly different according to Tukey's text at 5%. Same capital letters indicate that times of harvest were non-significantly different according to Tukey's text at 5%.

CA: Yellow Catherine, CB: Catherine Branca, TM: Mato Grosso, PI: IAPAR 19-Pioneer, PT: Pretona, IAC: IAC576-70

Table 2. Cooking time among six cassava cultivars at seven times of harvest (months after planting, MAP) (UEL, Londrina 1997 planting)

Cultivars	8 MAP	10 MAP	12 MAP	14 MAP	16 MAP	18 MAP	20 MAP	Averages
CA	20.2 b B	23.3 a B	23.1 a B	32.1 a A	30.6 a A	28.4 a A	29.2 a A	26.7 a
CB	23.3ab AB	21.3ab AB	21.2 ab B	21.8 bc AB	25.2 b A	23 a AB	22.6 ab AB	22.6 ab
MT	23.8 ab AB	20.5 ab B	21.9 ab B	25.1b AB	28.1 ab A	27.8 a A	27.4 ab A	24.9 ab
PI	19.5 b AB	15.3 b B	15.9 b B	17.7 c B	25.6 ab A	26.2 a A	22.6 b AB	20.4 b
PT	25.7 a A	22.6 a AB	21.9 ab B	24.9 b A	26.1ab A	25.6 a A	24.9 ab A	24.5 ab
IAC	20.5 b B	19.4 ab B	21.4 ab B	33.4 a A	33.4 a A	25.7 a AB	28.4 a A	26. a
CV (%)	5	6.3	5.3	7.5	5.4	5.5	6.2	3

Values followed by same small letters within each column are non-significantly different according to Tukey's text at 5%. Same capital letters indicate that times of harvest were non-significantly different according to Tukey's text at 5%.
CA: Yellow Catherine, CB: Catherine Branca, TM: Mato Grosso, PI: IAPAR 19-Pioneer, PT: Pretona, IAC: IAC576-70

In conclusion, our research shows the suitability of using medium parts of the roots for assessing cooking time of cassava cultivars with the modified device of Mattson. The cooking time of cassava roots depend on the genotype and therefore breeders can select for, e.g. IAPAR 19-Pioneer can be used as a parental source in crossing blocks due to its fast cooking trait.

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Biotechnology and Mutagenesis in Genetic Improvement of Cassava

S. Mohan Jain
Department of Applied Biology, University of Helsinki, PL-27, Helsinki, Finland

Introduction

Cassava is a perennial tropical root crop, cultivated for its starchy tuberous roots as a valuable source of calories, and is planted on about 16 million ha. It belongs to the family Euphorbiaceae and requires at least 8 months of warm weather to produce a crop. The crop has broad adaptability to a variety of soil and climatic conditions, drought tolerance and ability to grown depleted and marginal soil (Mathews *et al***.** 1993). The total annual cassava root production worldwide is 184 million t, out of which 50% production is in Africa, 30% in Asia and 20% in Latin America. The average yield varies widely, e.g. 7 to 10 t ha^{-1} in Ghana, which is far below that of India (26 t ha^{-1}) and Thailand (37 t ha^{-1}). The low yield in cassava growing countries is probably due to: poor fertilization, drought, heavily infected of planting material with African cassava mosaic virus (ACMV; Fig. 1), East African cassava mosaic virus (EACMV), and newly identified virus named South African cassava mosaic virus (SACMV) (Pita et al 2001), and diseases, and poor quality cultivars. There is a synergism between ACMV and ESCMV infecting cassava (Fondong et al, 2000), and that could result in a greater DNA accumulation and consequently inducing severe symptoms (Pita *et al.* 2001). Cassava crop is an important source of carbohydrate in adverse climatic conditions. The crop is valued in many areas for food security. In Ghana, the President's Special Initiative (PSI), under which the government will promote cassava starch, was launched. The cassava project will create ready market for 25,000 farmers, and about 70,000 jobs would be created (The Daily Graphic 17th August 2001). In addition, the tuberous roots of cassava can be left in the ground for several years prior to harvest, providing security against famine (Ihemere *et al.* 2006). Cassava also has the highest rate of CO_2 assimilation into sucrose

of any plant measured, and has a great potential for enhancing carbohydrate allocation to sink tissues. It is also increasingly being used in processed food and fodder products and by the chemical, pharmaceutical, paper and textile industries (Balagopalan 1998). There is a lack of proper supply of improved cassava planting material to the growers. The crop is faced with major constraints, responsible for poor yield and threatens food security. Among the most important are viral and bacterial disease, weeds and abiotic constraints, poor shelf-life of cassava roots after harvesting – less than three days, which can render it unpalatable and unmarketable.

In this article, we will discuss different tools, such as mutagenesis and biotechnology, which can be used to genetically improve cassava by addressing constraints facing cassava production and consumption, and to identify potential uses of the crop, e.g. bio-ethanol production.

Cassava nutrition

Cassava is poor in providing sufficient nutrition to the consumers. The tuberous roots are the main source of carbohydrates (35%), and provides negligible amount of proteins. Fresh leaves have much higher amount of proteins (7%) as compared to tuber flesh (0.5-1.5%) (Table 1). Starch is the main carbohydrate source in tuberous roots, and its amount is very low in fresh leaves (Table 1). Future efforts are needed to improve cassava nutrition both in tuberous roots and fresh leaves by using mutagenesis and latest tools ensuing from molecular biology. The selection of appropriate genetic material should be made from the natural and induced germplasm for the development of new cassava cultivars high in nutritional values so that malnutrition and related diseases, e.g. Konzo could be addressed. Konzo is a neurological disorder and leads to spastic paralysis of the legs; and is attributed to high dietary cyanide in cassava (Diasolua Ngudi 2006).

Fig. 1 – Cassava infected with African cassava mosaic virus (ACMV) in Ghana (right). Healthy cassava is in the experimental station (left). Picture was taken in BNRI, Accra, Ghana

Table 1. Differences in nutrition components between cassava tuberous root flesh and fresh leaves

Cassava nutrition components (%)	Tuberous root flesh	Fresh leaves
Water	62	80
Carbohydrates	35	7
Proteins	0.5-1.5	6
Fat	0.3	1
Fibre	1-2	2.5
Mineral matter	1	2.7

The peel of cassava roots is much richer in proteins.

Biotechnology

Plant tissue culture refers to growing and multiplication of cells, tissues and organs of plants on defined solid or liquid media under aseptic and controlled environment. Micro-propagation technique for rapid shoot proliferation is primarily achieved from any part of the plant such as shoot tip, tiny stem cuttings, roots, and auxiliary buds. The process of micro-propagation is usually divided into several stages: pre-propagation, initiation of explants, subculture of explants for proliferation, shooting and rooting, and hardening. It is critical to select proper genotypes and grow mother plants under the controlled environment, and determine the maximum number of subcultures before initiating new fresh cultures. By failing to do so, *in vitro* grown plants will show somaclonal variation in the field. It could become a major economic problem for the cassava growers. Normally, commercial companies use extensively micro-propagation in large-scale plant multiplication. However, the high cost of *in vitro* plant production, low volumes produce, labour intensive, and somaclonal variation hinder the rise in the profits of commercial enterprises, and therefore it is highly desirable to modify the techniques to overcome these problems for the supply of high quality planting material to small and commercial cassava growers. Cassava plants can be regenerated via organogenesis from different type of explants which are immature zygotic embryos (Fregene *et al.* 1999), shoot meristem culture for virus free plant production (Biotechnology and Nuclear Research Institute, Accra, Ghana), cotyledons of somatic embryos (Li *et al.* 1998), axillary buds and nodal explants (Konan *et al.* 1994, 1997), leaf explant (Mussio *et al.* 1998), and through improvement of shoot organogenesis with silver nitrate (Zhang *et al.* 2001).

Somatic embryogenesis This technique is ideal for clonal propagation of woody and fruit plants (Jain and Gupta 2005) and genetic gain can now be captured through it. Cassava somatic embryogenesis has been induced from floral tissue (Woodward and Puonti-Kaerlas 2001), young leaf lobes (Joseph *et al.* 2001, Groll *et al.* 2001), immature leaves (Ma and Xu 2002), leaf segments of *in vitro* plantlets (Takahashi *et al.* 2000), zygotic embryos (Raemakers 1993, Stamp and Henshaw 1982, Szabados *et al.* 1987), and friable embryogenic callus and cell suspension cultures (Taylor *et al.* 1996).

Formation of somatic embryos from somatic cells by a process of resembling zygotic embryogenesis in one of the most features of plants and offers a potentially large-scale propagation system for superior clones. Normally, the initiation of embryogenic cultures is done by culturing immature zygotic embryos, or sometimes with mature zygotic embryos, and off shoots. The maintenance of embryogenic cultures is critical for preventing somaclonal variation, and therefore, regular subcultures are done. Likewise, it is critical to cryopreserve immediately after embryogenic cultures are initiated to prevent variation and preservation of elite germplasm. Abscisic acid (ABA) is added in the culture medium for the maturation of somatic embryos, which look like zygotic embryos. Well developed somatic embryos are germinated to regenerate plants (somatic seedlings), and finally are acclimatised and transfer to the field. In conifers, somatic emblings (somatic embryo-derived plantlets) are being field tested by few commercial companies in North America. Somatic embryogenesis is highly genotypic dependent, and it would be useful to modify the culture medium accordingly. For large-scale production of somatic embryos, the 'bioreactor' system works well, e.g. 'temporary immersion system' (RITA bioreactor; http://www.cirad.fr/products/rita/en/fonction.htm). The low cost of production of somatic embryos and high germination rate are highly desirable for large-scale production in a bioreactor. This system has not yet been tried in cassava.

In cassava, secondary somatic embryogenesis has been induced (Groll *et al.* 2001). It is the phenomenon whereby new somatic embryos are initiated from primary somatic embryos (Raemakers *et al.* 1995). They have several advantages over primary somatic embryos, such as a high multiplication rate, independence of an explant source and repeatability. Furthermore embryogenic property can be maintained for prolonged period of time by repeated cycles of secondary embryogenesis (Raemakers *et al.* 1995), and has a potential applications in practical plant breeding when vigorous for-

mation of shoots and roots. In order to achieve it, the quality of secondary embryos is highly desirable.

Somatic embryogenesis has both advantages and disadvantages (Table 2). The major problem with this system is highly genotypic dependence and that is one of the reasons that it has not been adopted commercially in most of the crop species. In conifers, Douglas fir somatic embryogenesis system is well adopted and is being routinely used by Weyerhaeuser Paper Company in the USA.

Table 2. Advantages and disadvantages of somatic embryogenesis

Advantages	Disadvantages
Cost effective clonal propagation	Low number of field-plant able plantlets
Both shoot and root meristem development in the same step of the process	Highly genotypic dependent
Quick and easy to scale-up in liquid cultures, e.g. bioreactors	Inability to produce somatic embryos from mature seeds in many plant species
Long-term storage via cryopreservation	Gradual fluctuation and eventual decline in embryogenic culture productivity
Establishment of genebank	Somatic embryogenic cultures from seeds or seedlings have unproven genetic value
Production of somatic seeds by encapsulation of mature somatic embryos (Danso and Ford-Lloyd 2003)	Long life cycle may show genetic variability or new mutations at the later stage of development
Somatic seedlings may be rejuvenated	
Genetic transformation	
Automation of somatic embryo production	
Somatic seedlings are virus-free	
Mutation induction	

Doubled haploid production Since the discovery of haploid production by anther culture in *Datura* (Guha and Maheshwari 1964) this technology has come a long way to apply in plant breeding for crop improvement. It is routinely used in doubled haploid production of barley, brassica, wheat, rice and oats (Jain *et al.* 1996, Maluszynski *et al.* 2003, Palmer *et al.* 2005, Tenhola. Roininen *et al.* 2006). Anther culture is an ideal way to produce homozygous pure lines, which otherwise takes 8 to 10 years by conventional backcross breeding. Cassava is highly heterozygous crop and cannot be bred to homozygosity easily. The production of haploids will be of great help in producing doubled haploids

and mutation induction. Ceballos *et al.* (2004) described opportunities and challenges in cassava breeding. They indicated that the use of doubled haploids in cassava breeding for selection among doubled haploids (DH) would not be affected by dominance effects, additive effects among DH are twice as large as in the current array of evaluated genotypes, homozygous lines are genetically fixed –thus their genetic superiority (as progenitors) can be better exploited than genetically unstable heterozygous parents, and germplasm exchange based on botanical seed is much easier than that of vegetative cuttings. Furthermore, Ceballos *et al.* (2004) advocated the use of DH because back crossing is not feasible in cassava due to constant heterozygous state throughout the breeding process, and since the crop is highly heterozygous, dominance effects are likely to play role in the performance of the selected material. These authors argued that genetic improvement of cassava could benefit by inbreeding process but it would take 9 to 10 years to attain acceptable level of homozygosity. Hence, haploid production is the answer for the production of homozygous lines by anther or microspore culture. Other advantages are that cleaning planting stocks from viral or other pathogens could be achieved without meristem culture, mutation breeding would more easily implemented, identification of useful recessive mutants would be greatly facilitated, and DH would allow designing better performing hybrids compared to less efficient systems based on heterozygous parents. To the best of our knowledge, there is no available written report on doubled haploid production in cassava. A research group at Centro International de Agricultura Tropical (CIAT, Cali, Colombia) started haploid production in cassava. They have yet to see the success. There are not many groups involved in anther culture of cassava. This area needs attention for haploid production, mutagenesis, and functional genomic studies.

Genetic engineering Transgenics is an idealistic approach in introducing a trait specific gene in a plant by using methods such as *Agrobacterium*-mediated transformation or biolistic. There are several essential factors for the success of genetic transformation (Puonti-Kaerlas 2001). They include an efficient and reliable *in vitro* plant regeneration from multi-cellular explant or a single or a group cells (cell clumps, 2-4 cells per clump), an efficient method for gene transfer, stable integration of transgene into the plant genomic DNA, an excellent selection marker system for the identification of transformed cells –e.g. kanamycin, GUS, molecular marker analysis of transgenic plants to determine the integration of transformed gene, and stable integration of transgene in primary transgenic plants and stably transmitted to their progeny.

In vegetatively propagated crops such as cassava, primary transgenic plants can either be multiplied either by traditional vegetative propagation or micro-propagation. Before incorporation of vegetatively propagated transgenic plants, it is essential to test the stability of transgene in the subsequent progenies. Cassava is susceptible to *Agrobacterium*, however the rate of infection is very much dependent on bacterial strain and the cassava genotype. There were three independent, first reports on regeneration of transgenic cassava in 1996 (Puonti-Kaerlas 2001). Raemakers *et al.* (2001) used cassava friable embryogenic callus (FEC) to obtain transgenic plants using particle bombardment, electroporation, and *Agrobacterium tumefaciens*. FEC cultures have been obtained in 6 of the10 tested genotypes. In all genotypes FEC could be regenerated into plants however, the efficiency differed between the genotypes. Only recently transgenic plants have been regenerated with enhanced agronomic traits (Ihemere *et al.* 2006). At the International Institute of Tropical Agriculture (IITA, Ibadan, Nigeria), Hankoua *et al.* (2006) produced first transgenic cassava via direct shoot organogenesis from friable embryogenic callus and germination of mature somatic embryos. They suggested the potential of transgenic approach to improve cassava in Africa.

Zhang *et al.* (2003) reported the expression of an artificial storage protein (*ASP1*) gene in cassava leaves and roots; however, its expression had little effect on the overall amino acid composition of leaf proteins. More recently, Siritunga *et al*. (2004) reported the over-expression of hydroxynitrile lyase in roots, leading to accelerated cyanogen removal and food detoxification. Cassava plants having altered amylose and amylopectin starch ratios have also been generated by RNAi silencing of the gene encoding granular bound starch synthase. Table 3 indicates a recent list of transgenes transferred in cassava.

Table 3. A recent list of transgenes transferred in cassava with enhanced agronomic traits

Transgene	Trait	Reference
ADP-glucose pyrophosphorylase (AGPase)	Starch synthesis	Ihemere et al. 2006
Artificial storage protein (ASP1) gene	Storage protein rich in essential amino acids	Zhang et al. 2003
Antisense expression of CYP79D1/D2	Inhibition of cyanogenic glycoside synthesis	Siritunga and Sayre 2004
sbeI and sbeII	Starch branching enzymes I and II	Baguma et al. 2003.
Bar gene	Herbicide resistant	Sarria et al. 2000

There is a great further scope to improve cassava with transgenic approach due to readily *in vitro* plant regeneration. Its successful implementation is very much dependent on the availability of trait specific transgene, and also a close collaboration with cassava breeders, and the acceptance of genetically modified cassava by consumers.

Nuclear techniques for mutagenesis

Nuclear applications in food and agriculture have contributed greatly in enhancing agriculture production of seed and vegetatively propagated crops (Jain 2005). Even though nuclear technology has benefited greatly agriculture, it has still a great potential in genetic improvement of cassava and other crops. More than 2300 mutant varieties have officially been released in many countries (http://www-mvd.iaea.org). Both chemical and physical mutagens are used to induce mutations. Among them, gamma rays (Fig. 2) and ethyl-methane sulphonate (EMS) are widely used for mutation induction. Fine embryogenic cell suspension cultures are most suitable for inducing mutations by transferring to the filter paper and plated on the agar-solidified culture medium for gamma irradiation. Initially LD_{50} dose is determined, which is used as an optimal dose for mutation induction. Irradiated cells are further cultured to the fresh medium for the development, maturation, and germination of mutated somatic embryos. This approach provides mutated somatic seedlings in a short period and also prevents chimeras problem which otherwise requires to multiply plants up to $M1V_4$ generation for chimera dissociation. Alternatively, shoot tip or bud wood can be irradiated and multiply plants up to $M1V_4$ generation for producing pure mutants by dissociation of chimeras.

Sung and Somerville (USA), working on *Arabidopsis thaliana*, have discovered a mutation, called "pickle" in plants that mimics what happens in seeds, which typically are accumulating and storing proteins and oils. This mutation in plants that makes the tap root accumulates large amounts of oils, proteins, and starch. This finding could also make possible the creation of more nutritious root crops with better balance of oil, protein, and starch, *e.g.* in cassava and other root crops (www.sciencedaily.com/releases/1997/07/970707211536.htm)

Fig. 2 – Gamma irradiator for irradiation of experimental material for inducing mutations

Differences between transgenic and mutant plants Induced mutations and transgenesis have important roles to play in cassava improvement programs. Both approaches have their own merits and disadvantages (Table 4). Transgenic research is more precise, and a known trait specific gene (from any source) is transferred in plants. The expected results are transgenic plants with the specific introduced trait, even though insertion of transgene is at random in plant genome. Mutagenesis process is random, modifies the genes of an organism, requires an excellent selection system, and a large population for mutant selection. Both transgenesis and mutagenesis have not yet delivered most wanted traits in cassava, even though there is a great potential of both approaches in cassava improvement. The transgenic approach should be applied when conventional breeding fails to solve the problem in cassava improvement. Mutation-assisted breeding has been quite effective in crop improvement worldwide (http://www-mvd.iaea.org). Cassava breeders should interact with genetic engineers and mutation-assisted breeders to work on addressing major problems facing cassava improvement such as nutrition or viruses.

Cassava mutants induced with gamma radiation A mutant cassava cultivar 'Tek Bankye' has been released by the Ghanaians (IAEA-AFRA 2000). The mutant cultivar was developed in 1988 by radiation treatment (25 Gy) of cassava cuttings from a segregate of Isunikakiyan –a local Nigerian cassava cultivar, introduced from IITA in 1984) (Asare *et al.* 1997). The cultivar was officially released in Kumasi in November 1997. This cultivar has an excellent cooking quality and good pundability. It also has very high dry matter content of about 40%, and very popular among cassava growers. However, it is susceptible to ACMV. Ahiabu *et al.* (1997) irradiated Ghana-

ian cassava cultivar ‘Bosom nsia’ with gamma radiation and obtained two ACMV tolerant mutants –VT1 and VT2. These mutants were healthy after infection with virus and did not show any sign of infection. Since this report, there is no available report on cassava mutant resistant to ACMV and requires utmost attention on the development of disease resistant mutants.

Table 4. Differences between transgenic and mutant plants

Transgenic plants	Mutant plants
Uses tools of molecular biology to isolate, clone and incorporate genes into plants	Mutation is a random event
It is a more precise technique than mutation	Requires large population for screening the best desired mutants
Transgene integrates into plant genome randomly	It cannot be directed to make changes at the DNA
Changes at both DNA and protein are well understood.	Mutants possibly can carry other changes in their DNA
It can add new gene/genes in plants. Gene(s) can be from any source including animals, insect, plants, bacteria	Mutagenesis can only modify the genes of an organism. Mutagen treatment fragments DNA first followed by DNA repair mechanism, and that results in mutations
It requires tissue culture protocols for transgenic plant regeneration	In vitro mutagenesis also requires tissue culture for plant regeneration of mutants
Consumer’s lack of confidence towards transgenic food	Consumers accept food derived from mutagenesis
Transgenic plants has less probability to carry other changes in their DNA	Selection system is placed for agronomic desirable trait mutant selection
Molecular techniques are used to confirm the integration of transgene in plant	TILLING (Target induced local lesions in genomes), a new strategy for reverse genetics
Costly research. Developing countries may have problem to support it.	Cheaper. International Atomic Energy Agency (IAEA, Vienna, Austria) provides free services of gamma irradiation of experimental material
Most transgenes are not readily available to anyone interested in using them	Does not concern

Joseph *et al.* (2004) used globular somatic embryos of cassava variety PRC 60a and irradiated with 50Gy gamma radiation dose for the induction of mutations. During the field trials, more than 50% of the regenerated mutant lines varied morphologically from wild-type plants. Among the different mutant lines obtained, S14 and S15 showed large morphological variation. Ten-month-old S14 and S15 mutant lines showed 17-fold and 60-fold, respectively reduction in storage root yield as compared to wild type plants.

The storage roots of S15 mutant plants also exhibited nearly 50% decrease in starch content and a significant reduction (30%) in amylase content.

Space breeding concept Space conditions can induce mutations of plant seeds, and can be helpful to accelerate the crop breeding. It may be possible to obtain rare mutants that may make a great breakthrough in important economic characters of the crop, such as yield and quality, which are difficult to get using the other breeding methods on ground. The plant seeds are sent in the space on a space rocket, and when the rocket is back on earth, plant seeds or *in vitro* shoot cultures or microspores are assessed to determine the influence of cosmic rays in generating new mutants. There are only few countries involved in this type of work, e.g. China. Since 1987, 13 recoverable satellites have been used by Chinese scientists and researchers to carry more than 80 kg of plant seeds belonging to over 70 species, involving main cereal, fiber, oil, vegetable, and fruit crops. Through ground planting and selecting experiments by breeders in more than 50 research units covering more than 20 provinces, cities and regions in China, good achievements have been made. More than 20 mutant cultivars were developed and officially released. In rice, a new cultivar –EYH No.1, has been released due to its grain yield (14.5 t ha^{-1}). Space breeding involves big investment and good technological support. The chances of conducting the space experiment are very limited. It is important to make ground simulation on space factors to conduct research work for revealing the mechanism of space-induced mutation and applying it for plant breeding. In cassava, somatic embryogenic cultures could be used for cosmic radiation treatment at the ground simulation facilities.

Cassava as a bio-fuel crop

Cassava has a great potential as a food, feed and bio-energy crop. In Brazil, sugarcane is a major bio-energy crop and has made this country a world leader in bio-ethanol production. Cassava has a potential to become another major bio-energy crop together with sugarcane. It is an attractive fuel crop because it can give high yields of starch and total dry matter in spite of limited drought and poor soil. Energy inputs of cassava represent only 5 to 6% of the final energy content of the total biomass, showing an energy profit of 95%, assuming complete utilisation of the energy content of the total biomass. Alcohol production from cassava has an overall efficiency of 32%. Cassava could become an industrial crop by developing cultivars with different starch composition. Useful variations in native starch quality-altering

the proportion of amylase to amylopectin, for instance, which changes the physiochemical properties of the polymer-could open new market niches at better prices (Marie *et al.* 1998). Joseph *et al.* (2004) suggested that different mutants of cassava, varying in starch yield and composition would be a suitable material to study the mechanism of starch biosynthesis. Furthermore, it would be easier to identify with molecular techniques genes responsible for starch synthesis. Dr. Li research group (Beijing, China) has developed a new sweet sorghum mutant cultivar Yuantian No.1 by seed irradiation with gamma radiation. This cultivar has 20% more sugar than the parental lines and is an excellent source both as feed and bio-energy crop. In Thailand, Charoenrath *et al.* (2006) reported the official release of a new cassava cultivar (Rayong 9) with improved starch and ethanol yields. This cultivar also has good plant type, producing good quality stakes with a high rate of germination as well as large number of stakes from each plant. In Brazil, Joaquim *et al.* (2004) identified a new cassava type with a storage root showing unusual free sugar accumulation and novel starch. Twenty-seven clones high in free sugar were identified under cultivation in primitive rural community areas. Clones of CAS36 accumulate over 100 times more free sugar (mainly glucose) than commercial cultivars. Hill *et al.* (2006) suggested that transportation bio-fuels such as synfuel hydrocarbons or cellulosic ethanol, if produced from low-input biomass grown on agriculturally marginal land or from waste biomass, could provide much greater supplies and environmental benefits than food-based bio-fuels. They found that ethanol produced from corn yields 25% more energy than the energy invested in its production whereas bio-diesel produced from soybeans, yields 93% more. Cassava can grow under harsher climatic conditions, and would be an ideal for transport biofuel.

Outlook

Cassava mutants could be developed to produce value added biomass for cost effective production of bio-ethanol. The use of this crop as a source of bio-energy would generate employment, enhance economic status of growers, protect environment, and most likely cut consumption of fossil fuel. Arable land for growing cassava may have to increase for bio-energy production, and that would also create more job opportunities and enhance export of bio-ethanol in energy hungry countries such as China, India. This can be achieved with biotechnology (Jain 2001), and mutation induction, and also by exploiting cassava genetic resources and its genetic variation for breeding (Vollmann *et al.* 2004).

Tissue culture for plant regeneration via somatic embryogenesis and organogenesis has already been achieved. Somatic embryogenic cell suspension culture is an ideal system for mutation induction either with physical or chemical mutagens. Even though there are few cassava induced mutants, mutagenesis has a great potential in cassava enhancing and utilisation of germplasm for developing useful mutant lines. This can be achieved provided there is a close collaboration between plant breeders and biotechnologists for proper germplasm utilization to develop new value-added cassava cultivars. Biotechnology is an additional tool to assist plant breeders, and can be helpful in reducing time for developing a cultivar, e.g. doubled haploid breeding in combination with mutation-assisted breeding. In the past, lack of interaction between plant breeders and biotechnologist could not deliver goods to crop improvement even though traditional breeding is faced with maintaining sustainable crop production under the climatic change and ever-growing human population growth.

TILLING (Target induced local lesions in genomes) is a new strategy for reverse genetics (Henikoff *et al.* 2004). In TILLING, traditional chemical mutagenesis is followed by high-through put screening for point mutations. Furthermore, TILLING does not involve transgenic modifications or cell culture manipulations. It produced an allelic series of mutations including hypomorphic alleles that are useful for genetic analysis.

The following aspects may be considered for cassava improvement as a crop to food security, nutrition, and marketing:

- Meristem tissue culture
  - Rapid plant multiplication, including mutants
  - Disease-free plant production and dissemination world-wide
  - Germless conservation
- Low-cyanide cultivars with desired yield and quality
- Salinity and drought resistant
- Virus resistant, e.g. African cassava mosaic virus (ACMV)
- High yielding polyploid cultivars, e.g. triploids
- Apomictic lines – for commercial cultivation from cassava true seed
- Early-maturing types
- Excellent cooking quality
- Starch quality
- Bio-energy
- Improvement of protein content in tubers
- Improvement of post harvest storage of tubers- shelf-life

It is highly desirable to develop a mutant data base of available cassava mutants, which can be characterised with molecular techniques, e.g. amplified fragment length polymorphisms [AFLP] (Sanchez *et al.* 1999), microsatellite-primed PCR markers (Carvalho and Schaal 2001), quantitative trait loci analysis (Jorge *et al.* 2001), and identify useful genes and determine their functions. This research will lead to functional genomics-led cassava breeding. Mutants are needed to improve cassava nutrition as well as cooking quality without compromising total crop yield.

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Amylose Content Variation of Indonesian Cassava Genotypes and its Correlation with RAPD and AFLP Markers

E. Sudarmonowati, N.S. Hartati,
Hartati and L. Sukmarini
Research Centre for Biotechnology-LIPI, Jl. Raya Bogor Km. 46,
Cibinong 16911, Indonesia

Abstract

A total of 20 random decamer primers and 6 primer pairs of RAPD (Random Amplified Polymorphic DNA) and AFLP (Amplified Fragment Length Polymorphism), respectively were screened to analyse over 150 accession numbers of cassava mainly collected in various parts of Indonesia which amylose content ranged between 22.65%-32.53%. Only seven primers (OPE-5, OPE-15, OPF-4, OPF-13, OPH-17, OPE 20, OPB-10) were used for further study and three of them were used to analyse 60-97 genotypes. By correlating the obtained bands with amylose content, five candidate RAPD markers of high amylose i.e. OPE-15 H, OPE-15 I, OPE-15K , OPE-15 L and OPE-15O, and that of high amylopectin i.e. OPE-15-B, OPE-15F and OPE-15I were obtained although further confirmation were needed. Dendogram constructed based on a combination of three RAPD primers i.e. OPE-15, OPF-4 dan OPF-13 indicated that no distinct pattern between genotypes possessing low amylose with the high amylose one although some genotypes with the same characters were grouped in the same cluster or subclusters. This results were also confirmed with the results obtained using AFLP. These amylose content variations was also compared with the morphological variation. Effort to obtain genotypes with extreme amylose content is underway through genetic engineering and irradiation with or without crossing combination.

Introduction

In Indonesia, cassava is mostly planted by farmers in a small-scale in marginal lands. The ability of cassava to tolerate drought and low soil fertility facilitates its growth by farmers in dry areas of Indonesia for daily subsistence and small-scale trading. Despite low nutrition content and relatively high cyanide in its tuberous root, and short tuberous root storage, cassava starch industry –as an alternative to potato starch– developed rapidly both nationally and internationally. According to the Indonesian Agrochemical and Forestry Product Directorate General of the Ministry of Industry and Trade, cassava area in 2002 was 1,276,533 ha –a slight decrease from 2001, and could not cope with demand that increased –from 544,439 t in 2001 to 575,433 t in 2002. Ironically, despite the large area and the environment suits to grow cassava, Indonesia became a net importer country for cassava starch –export in 2002 was 20,082 t while the import was 25,754 t. SThe demand of cassava roots increased 0.9% per year while the production increase was 1.6 % annually due to 2.1 % productivity gains. The growth of plantation area was negative; i.e., -0.5 %, which indicates that the area used for cassava plantation was converted to other crops. Sadly, the production of cassava starch in Indonesia has been decreasing since 1991 despite a high demand of starch.

Starch is the principal carbon-reserve polysaccharide in plants, and the major source of carbohydrates in the diets of both humans and animals. The great economic importance of starches has stimulated much interest in the potential to modify their properties through genetic engineering. This requires a thorough understanding of starch biosynthesis, particularly as it relates to starch granule formation and the formation of amylose and amylopectin, which are key determinants of the industrial properties of starch. Biochemistry, molecular biology and molecular genetics will expand knowledge on cassava starch biosynthesis. Such knowledge will facilitate the use of genetic engineering in the near future to produce novel starches with altered starch properties, which are tailored to specific nutritional, industrial and other end-user requirements. Starch is an important raw material for industrial applications, such as in the paper, textile, plastics, food and pharmaceutical industry. It is currently being used in the production of biodegradable packing materials and in the development of biodegradable plastics, which is becoming an increasingly attractive alternative to petroleum-based products. There is considerable interest from the industry in the prospect of diversifying the structure of starch polymers. Improvement of starch properties for industrial uses can be achieved by chemical or physical modification after isolation, but also through the *in planta* modification of starch.

The amylose-free potato starches can be used in frozen foods to improve the freeze-printing properties. High amylose starches also have numerous industrial applications; e.g. in fried snack products to create crisp, and in the production of gum candies due to their rapid setting properties.

The need of cassava as raw materials in developing countries has been increasing each year by 2.0% for food and 1.6% for feed, while total production based on current production level is projected to reach 168 million t in 2020. Cassava starch has a wide range of applications in both food-related and nonfood-related industries. Cassava starch could be converted to maltotriose, maltose, and glucose and other modified sugar and organic acid. Starch hidrolysate has been widely used as additive compound in food industries (candies, bread, canned food and frozen food). Certain industries require very low amylose for paper filling and other industries require very low amylopectin. In certain countries like Thailand, the majority of cassava starch (~80%) is used in the production of MSG and lysine. Significant quantities are also used to produce sugars (glucose and fructose), sugar alcohol (sorbitol), and citric acid. It is also used as a binding or thickening in various food products, such as ice cream, noodles and puddings. Outside of the food industry, cassava starch is used in the manufacture of paper, textiles and plywood, and more recently in the production of ethanol and biodegradable polymers.

Among starch producing plants, cassava has been considered as the highest producer (25%-40% higher than rice and maize) and as the most efficient (the highest) converter from solar energy to carbohydrate per unit area. Increased demand for cassava starch has spurred interest in the development of new cassava cultivars with greater yields and higher starch contents. Traditionally, cassava varietal improvement has been achieved through conventional breeding. Since most elite cassava cultivars are vegetatively propagated hybrids, a major drawback of this approach is that some of the favorable allelic combinations occurring in the parental generation are lost in the subsequent filial generations. Most of research has focused on starch production in maize, wheat, potato and rice. By contrast, only a handful of starch biosynthetic genes have thus far been characterized in cassava.

Despite several decades of intensive research on the biosynthesis of storage starch, the process is still incompletely understood. However, in the last decade significant progress toward understanding of starch biosynthesis has been made through the use of molecular biology and genetic engineering. Molecular characterization of the genes encoding starch synthetic enzymes has thus far revealed important information on starch biosynthesis in plants.

In cassava, however, limited information of starch biosynthesis is currently available and a few numbers of the genes participating in starch biosynthesis of cassava have been characterized. Information ensuing from the isolation and characterization of genes involved in both pathways will lead to a better comprehension of how the root system of cassava generates its starch-filled tuberous roots. Various functional analysis techniques, such as RNA interference (RNAi) and gene over-expression, are also being used to determine the precise effects of individual genes on the properties of starch produced. The knowledge gained from these experiments will greatly facilitate the effort to obtain cassava cultivars with enhanced starch characteristics and yield through such techniques as marker-assisted selection and genetic engineering.

Although Indonesian is not the center of origin of cassava, the crops shows wide variation for leaf, petiole, stem and tuberous root color, as well as tuberous root shape and texture. Genetic marker-aided research on starch composition of the tuberous roots will be of interest and will assist the improvement of this trait by the breeding program.

Materials and methods

DNA Extraction Cassava DNA was extracted using the procedure of Gillies (1997) with a slight modification. The extraction process was either manually or using RETSCH grinder apparatus.

Primers and RAPD analysis conditions Twenty primers of kit B, E, F and H were screened which yielded six suitable primers for further analysis. The six primers were OPE-5, OPE-15, OPE-20, OPF-04, OPF-13, OPH-17. PCR conditions were pre-PCR 94°C for 3 min followed by 40 cycles of denaturation at 94°C for 12 min, annealing at 35°C for 24 sec, and extension at 72°C for 2 min, then followed by post-PCR at 72°C for 7 min. DNA fragments yielded from the amplification were separated at 1.5% agarose gel using 1x TAE buffer. A UV trans-illuminator was used for visualization of the amplification products. Documentation was conducted using a camera and Polaroid film.

Primers and AFLP analysis Two enzyme restrictions were tried (MseI-PstI and MseI-EcoRI). The analysis was performed following the protocol of Life Technologies Inc. involving pre-amplification and amplification using PCR, which involved the use of restriction enzymes and ligation process.

Data analysis based on bands visualized on agarose Data were analyzed using software POPGENE version 1.0 and NTSYS, which are based

on the presence and absence of DNA bands on agarose gel. Dendograms were constructed based on each RAPD or AFLP primers and compared with several primer combinations.

Starch extraction This process was made manually using grinder and cloth for filtering the grinded roots. Drying was conducted naturally under the sun. Starch content analysis was based on international standard procedure developed by Horwitz (1990).

Results and Discussion

Amylose content variation The content of amylose ranged from 22.65% to 32.4% among the 160 cassava genotypes. Groupings based on amylose content were as follows: Group I with very low (22.6%–22.8%). Group II with low (23.8%–24.9%), Group III with medium (25.4%–29.6%) Group IV with high (30%–31.2%), and Group V with very high (32.4%–32.5%) contents. The lowest amylose content cultivars were genotypes L-89-26, Selengan (22.6–23.3%) while the highest amylase content cultivar was Iding (32.53%). As the amylose content of the cassava collection was not extremely low or high, efforts to reduce or increase the content were initiated by inducing mutations through γ-irradiation and genetic engineering.

RAPD analysis The total number of genotypes that DNA could be amplified by each primer is given in Table 1. Fig. 1 shows DNA bands amplified by OPE-15, OPF- 4 and OPH-17 primers.

Table 1. RAPD primers and number of genotypes assessed

Primers (decamer)	Number of genotypes
OPE-5	16
OPE-15	96
OPE-20	9
OPF-4	31
OPF-13	35
OPH-17	35
OPE-15, OPF-4	28
OPE-15, OPF-13	6
OPE-15, OPH-17	8
OPE-15, OPF-4, OPH-17	12
OPE-15, OPF-4, OPF-13	4

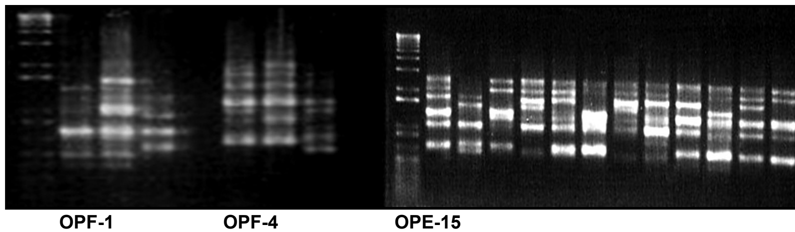

Fig. 1 – Banding pattern of several Indonesian genotypes generated from three RAPD Primers (OPE-15, OPF-1 and OPF-4)

The dendogram based on OPE-15 primer or those based on OPH-17 primer alone, or from a combination of OPE-15, OPF-4, and OPF-13 primers (Figs. 2 and 3) shows two main clusters, although the genotypes grouping in each cluster were not the same. Dendograms based on OPE-15 primer showed that cluster 2 only consists of one genotype (Malang 1), while cluster 1 consists of two sub-clusters, which differed in the number of genotypes. Sub-cluster 2 consists of Iding and Kabu-kabu and was separated from other genotypes in sub-cluster 1, which consists of Adira 1, Adira 4 , Malang 2, Malang 4, and Darul Hidayah that are cultivars released by the Indonesian Government. These genotypes were also in the same sub-cluster with Thailand and Kasetsart, which are very popular among farmers in the largest production center of Lampung. The last two genotypes are regarded as having high starch content and early maturity. Although, the dendogram did not clearly differentiate between genotypes containing high or low amylose, Iding, which is considered as high amylose content genotype, was separated from sub-cluster 1 that included L89-26, Dayo V, 10067, Gebang and Selengan, whose amylose contents were very low to low. The genetic distance of genotypes with low amylose content was close, e.g. between 10067 and Dayo V.

The similarity among genotypes assessed by OPE-15 primer ranged between 56% and 100%, which revealed high genetic variation. The result also differentiated genotypes based on source of collection, e.g. Dayo V, Pahauman and Kalbar I from West Kalimantan Province in Kalimantan (Borneo) Island. This primer also grouped genotypes with low amylose content such as 10044, 10067, Dayo IV.

A RAPD marker appears to correlate with amylose and amylopectin content. Five candidate RAPD markers of high amylose were initially selected: OPE-15 H, OPE-15 I, OPE-15K , OPE-15 L and OPE-15O, and other

three for high amylopectin: OPE-15-B, OPE-15F and OPE-15I, although further research to verify this assertion will be needed.

Discriminant analysis on 59 genotypes revealed five groups for amylose content: very low, low, medium, high and very high, which confirmed the separation of the highest amylose content genotype (Iding, indicated by number 5 in Fig. 3) from theothers. Similarly, the lowest amylose content genotypes were separated from the others as shown in Fig. 4 as number 5.

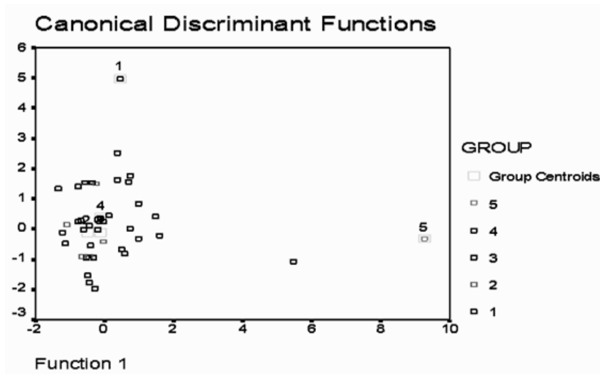

Fig. 4 – Discriminant analysis showing distribution of genotypes based on amylase content [1 = very high, 2 = high, 3 = medium, 4 = low, 5 = very low]

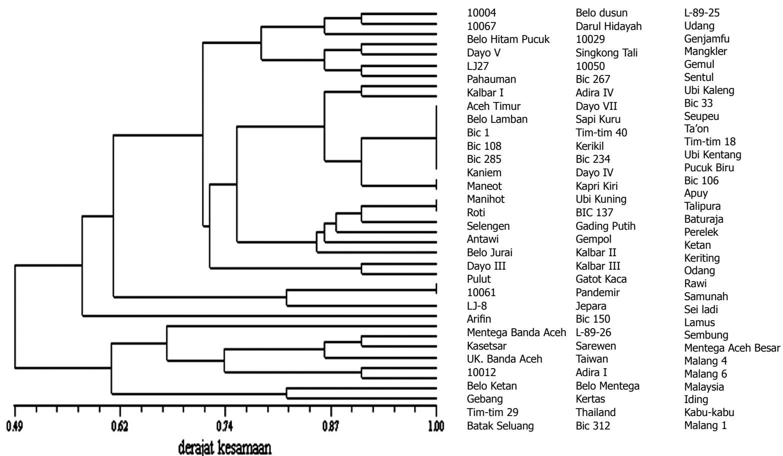

Fig. 2 – Dendogram constructed based on OPE-15 primer of various Indonesian cassava genotypes

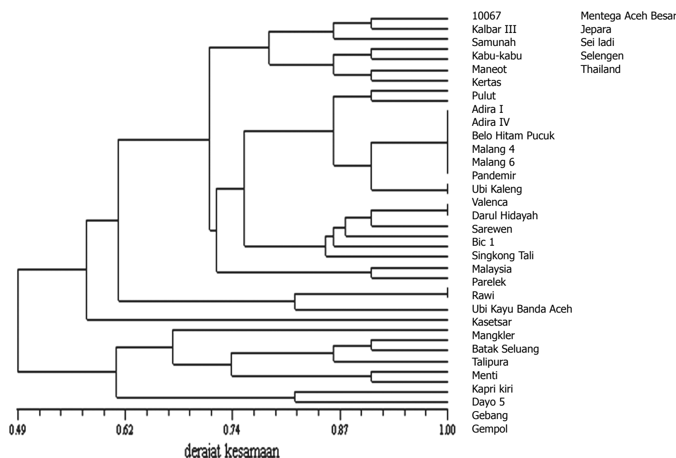

Fig. 3 – Dendogram constructed based on OPH-17 primer of various Indonesian cassava genotypes

The dendogram based on OPH-17 primer consisted of two clusters but the number of genotypes in cluster 2 was smaller than that of cluster 1. Ap per OPE-15 primer, Gebang and Selengan were also in one cluster, and that cluster 1 consisted of most Indonesian improved cultivars (Adira 1, Adira 4, Malang 2, Malang 4 and Darul Hidayah). Rawi and Menti, which are high yielding and have high starch content, were also grouped in this cluster. However, Kasetsart was not in the same cluster as Thailand (Fig. 3). The dendogram ensuing from a combination of three primers (OPE-15, OPF-4 and OPH-17) consisted of two clusters, Adira 1 with Adira 4 in same cluster and separated from cluster 1 (Fig. 5).

AFLP analysis The number of primer pairs was 13 and 6 after using MseI-PstI and MseI-EcorI enzyme restrictions, respectively. A total of 48 and 16 genotypes were assessed using PAA-MCAC (P11-700/MSe-48) and PGT-MCAC (P22-800/MSe-48) primer pairs, respectively. Of 13 primer pairs tried, only two could amplify DNA and gave a neat resolution in most genotypes analyzed (Table 2). The result also showed that MseI-PstI was the best restriction enzyme tried so far.

Table 2. AFLP primer pairs tried and result of amplification

No.	Primer pairs	Amplification
1.	PAA-MCAC (P11-700/Mse-48)	++
2.	PGT-MCAC (P22-800/Mse-48)	++
3.	PAT-MCAC (P14-800/Mse-48)	++
4.	MCT-MCAG (P10-700/Mse-49)	+ ("smear")
5.	PAA-MCAG (P11-700/Mse-49)	+ ("smear")
6.	EACG-MCAG (E37-700/Mse-49)	-
7.	EACG-MCAG (E37-700/Mse-48)	-
8.	EACG-MCAG (E37-700/Mse-49)	-
9.	EAGA-MCAG (E38-700/Mse-49)	-
10.	EAGA-MCAG (E37-700/Mse-48)	-
11.	EAGA-MCAG (E38-800/Mse-49)	-
12.	EAAC-MCAC (E32-700/Mse-48)	-
13.	EATG-MCAC (E45-800/Mse-48)	-

The DNA bands from PAA-MCAC (P11-700/MSe-48) and PAT-MCAC (P14-800/Mse 48) primer pairs were used for constructing dendograms (Figs. 6 and 7). Both primer pairs resulted in two main clusters although the grouping of the two was different. P11-700/Mse-48 primer pair separated Adira 1 from Adira 4 in distinct clusters. As per with RAPD primers several genotypes that are of high amylose content, such as Taon and Kapri Kiri, were grouped in one cluster. The dendogram ensuing from P4-800/Mse-48 grouped Adira 1, Adira 4, Darul Hidayah and Iding in the same cluster. However, the two primers were similar in terms of separating cluster 1 with another cluster consisting of Taon, Genjamfu and Kapri Kiri. When the dendograms were compared with that ensuing from using RAPD primers, some alikeness was noted. For example, both OPE-15 and PAT-MCAC (P14-800/Mse-48) grouped Maneot and Manihot in one cluster and the similarity index was very close; i.e., 1, which suggests that the two could be the same cultivar but with two given names. The results indicated therefore that the two DNA markers systems are complimentary and to each other.

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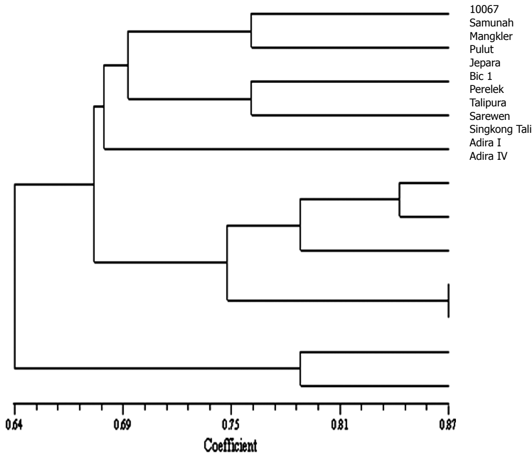

Fig. 5 – Dendogram constructed based on a combination of three primers (OPE-15, OPF-4 and OPH-17) of various Indonesian cassava genotypes and cultivars

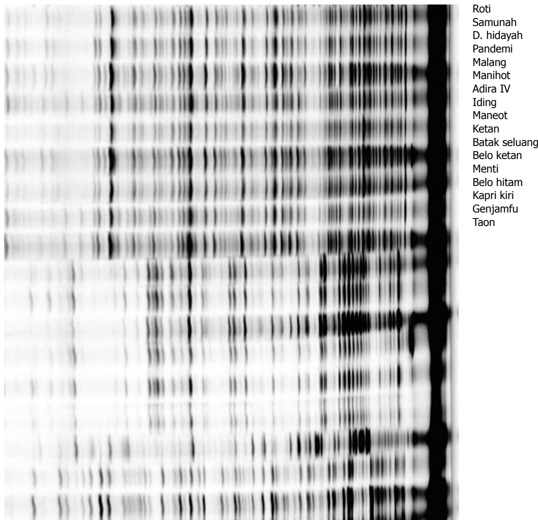

Fig. 6 – DNA bands of Indonesian cassava generated by AFLP primer (Licor/ M48-P11700- MCAC- PAA)

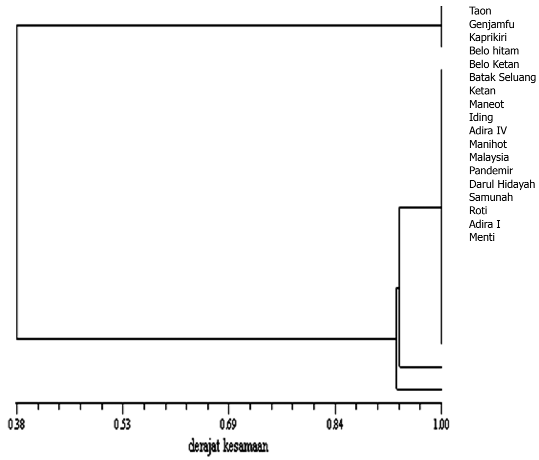

Fig. 7 – Dendogram constructed based on
P14-800 AFLP primer of Indonesian cassava genotypes

In Vitro Tuberization in Genotypes of Cassava

C.M. Fogaça**, F.L. Finger,
W.C. Otoni, D.C. Cordeiro,
T.D. Correia, M.P. Lauriano
and A.O. Ferreira
Laboratório de Progênies de Hortaliças,
Departamento de Fitotecnia, Universidade Federal de Viçosa,
36570-000, Viçosa, MG, Brazil

Abstract

The objective of this research was to induce *in vitro* tuberization in two cassava genotypes grown in liquid media with addition of 6-bezylaminopurine (BAP) and α-naphthaleneacetic acid (NAA) in different concentration of sucrose. Nodal segments (3 cm height) from the cultivars Mantiqueira and Parazinha were used as explants and kept *in vitro* MS media containing also mio-inositol (100 mg L^{-1}) and sucrose (30 g L^{-1}). The pH of the media was adjusted to 5.7 ± 0.1 before autoclaving. The root inducing media was composed by MS media plus 30 g L^{-1} sucrose (T1), MS plus 0.4 µM BAP + 1.6 µM NAA, in different concentrations of sucrose including 30 (T2), 60 (T3) and 80 g L^{-1} (T4), and MS media supplemented with 22 µM BAP, grown in 30 (T5), 60 (T6), 80 g L^{-1} sucrose (T7). The experiments were undertaken at 50 to 60 µmol light irradiance for eight hours at 27 ± 1°C. Number, length and pattern of the induced tuber roots were recorded. The length and number of roots were higher for T1, especially in Mantiqueira, but no formation of tuberous roots was observed. Tuber roots was observed after using T4 in both cultivars.

Resumo

O presente trabalho teve como objetivo a indução *in vitro* de raízes tuberiformes de dois genótipos de mandioca cultivadas em meio líquido, sob a ação de 6-benzilaminopurina (BAP) e ácido α-naftalenoacético (ANA), e diferentes

concentrações de sacarose. Como explantes foram utilizados segmentos nodais, com cerca de 3 cm de comprimento, excisados de plantas de mandioca das cultivares Mantiqueira e Parazinha, mantidas *in vitro* mediante subcultivos em meio constituído dos sais de MS, mio-inositol (100 mg L^{-1}) e sacarose (30 g L^{-1}). O pH dos meios foi corrigido para 5,7 ± 0,1, antes da autoclavagem. Os meios de indução constaram do meio MS com 30 g L^{-1} de sacarose (T1), MS suplementado com 0,4 µM BAP + 1,6 µM NAA, sob diferentes concentrações de sacarose 30 (T2), 60 (T3), 80 g L^{-1} (T4), e MS com 22 µM de BAP também sob diferentes concentrações de sacarose 30 (T5), 60 (T6) e 80 g L^{-1} (T7). Os experimentos conduzidos sob regime luminoso de 16 horas escuro e 8 horas diárias de luz à temperatura de 27 ± 1°C e irradiância de 50-60 µmol m^{-2} s^{-1}. Foram avaliados para a indução das raízes tuberiformes o número, comprimento e do padrão de desenvolvimento das raízes. O T1 foi o melhor tratamento para as características comprimento da raiz (CR) e número de raízes (NR), principalmente para a cultivar Mantiqueira, no entanto, não foi constatada microtuberização. Já no T4, o desenvolvimento das raízes tuberiformes foi mais eficiente, apresentando uniformidade tanto no aspecto, como na textura em ambas as cultivares.

Introduction

Cassava is a perennial plant originated from South America, which stores starch in a storage taproot. This dicotyledonous plant, belong to the family Euphorbiaceae, and the genus *Manihot* has about 200 species grown mainly in regions with tropical and subtropical climate (Figueiredo 1996). Although the cassava plant cannot be considered a crop with all needed human nutritional requirements, its storage roots are among the most important sources of cheap carbohydrate for developing countries (Junqueira 2001). Cassava is the third most common source of carbohydrate in the tropics, after rice and maize. Brazil is the world-leading producer, with an annual production of 25 million t (FAO, 2000). Cassava cropping is widespread in most of the Brazilian territory due to its high rusticity and well adapted to different soils and climates. The crop is usually grown by small farmers. In most of the growing regions the productivity is still low, mainly due to the low technological level of cultural practices adopted by farmers (Reis 1987).

The cassava is crop restricted to tropical and subtropical regions, which may account for the scanty available literature regarding environmental and endogenous factors involved in the control of the tuberization process. Most of the information available about tuberization was from potato

(*Solanum tuberosum* L.). It is well known that the tuberization is a complex process, which is coordinated, by the environment conditions, hormonal control, nutrition and genetics (Villafranca *et al.* 1998). Potato tuberization is characterized by anatomical modifications, hormone and physiological changes. The use of *in vitro* growth of plants for production of microtuber has the advantage of higher control of the different factors that might affect the tuber formation, compared to plants grown in soil (Veramendi *et al.* 1999). Furthermore, by using microtubers it is possible to maintain gene-bank accessions in a much smaller space, and to remove virus- infection in asexually propagated species (Ranalli *et al.* 1994, Zobayed *et al.* 2001). Another advantage of *in vitro* propagation viz. a viz. conventional methods is the possibility to produce plantlets of asexual propagated plants in a much large scale in a shorter period, without being affected by field seasonality for producing tubers (Hussey and Stacey 1984, Debon *et al.* 1998).

Previous potato research focused on the influence of growth regulators and photoperiod on the induction of tuberization (Hussey and Stacey 1984, Nowak and Colborne 1989, Villafranca *et al.* 1998, Silva *et al.* 2001, Lopez-Delgado and Scott 1997). Short day and sugars influence potato tuberization (Xu *et al.* 1998, Omokolo *et al.* 2003). The addition of carbohydrate on the growing media shifts the source/sink ratio affecting the development of shoots and tuber (Peres *et al.* 2005). Additionally, other factors such as temperature and nitrogen influence tuberization induction of potato (Hussey and Stacey 1984, Ulloa *et al.* 1997). The objective of this research was therefore to investigate the role of benzylaminopurine, naphthaleneacetic acid and sucrose on the development of tuberous roots in two commercial cassava cultivars grown *in vitro* using liquid media.

Material and Methods

Nodal segments (3 cm height) were used as explants. They were taken from plants of cv. Mantiqueira, micropropagated in the Tissue Culture Laboratory of UFV/MG. The cv. Parazinha used in the experiment was donated by the EMBRAPA/CENARGEN. All the cultivars were micro-propagated in three consecutive sub-growths for 42 days each. We used MS media (Murashige and Skoog 1962) containing 100 mg L^{-1} mio-inositol, vitamin complex for the MS media (0.2 g L^{-1} glycine, 0.05 g L^{-1} nicotinic acid, 0.05 g L^{-1} pyrodoxin–HCl and 0.01 g L^{-1} thiamine-HCl), and 30 g L^{-1} sucrose in solidified 2.3 g L^{-1} Fitagel (Sigma, USA). The pH of the media was adjusted to 5.7 ±1 before placing in

autoclave at 115 °C for 30 min. For the tuberization liquid media it was used the MS composition supplemented with 30 g L^{-1} sucrose (T1), MS media plus 0.4 µM de BAP and 1,6 µM NAA (Figueiredo 1996), with different concentrations of sucrose as follow: 30 (T2), 60 (T3) e 80 g L^{-1} (T4). A MS media with 22 µM de BAP (Silva *et al.* 2001) plus 30 (T5), 60 (T6) e 80 g L^{-1} sucrose (T7) was also tested. The experiments were conducted at light regime conditions of 16 h under light and 8 h at dark in a room illuminated with white fluorescent light kept at 27 ±1°C with 50 to 60 µmol m^{-2} s^{-1} of irradiance.

After 45 days of growth in the tuberization media, the length of aerial portion (LAP), number of roots (NR), length of the largest roots (LR), and pattern of the roots development were recorded. The experimental design was completely random with eight replications per treatment, and each experimental unit was one flask containing two plantlets each. The data were subjected to the analysis of variance using the software SAEG/UFV (Euclydes 1983), and the mean separation was performed using the Tukey test at $P \leq 0.05$.

Results and Discussion

The different treatments, under the light regime for tuberization induction resulted in distinct morphological changes in the plantlets grown after 45 days in liquid media. The treatment with MS0 + 30 g L^{-1} sucrose (T1) resulted in longer aerial part of the plantlets (LAP), length of the longer roots (LR) as well as number of roots (NR) compared to the remaining treatments, containing BAP, NAA or higher levels of sucrose in the media (Tables 1 and 2).

Table 1. Influence of the media composition on the length of aerial portion (LAP), length of largest roots (LR), and number of roots (NR) 45 days after growth in the tuberization liquid media of cv. Mantiqueira

Treatments	LAP	LR	NR
T1= MS0 + 30 g L^{-1} sucrose	25.37 a	14.62 a	21.50 a
T2= 0.4 µM BAP + 1.6 µM ANA + 30 g L^{-1} sucrose	11.00 b	5.12 b	7.25 bc
T3= 0.4 µM BAP + 1.6 µM ANA + 60 g L^{-1} sucrose	6.12 c	4.75 b	12.50 b
T4= 0.4 µM BAP + 1.6 µM ANA + 80 g L^{-1} sucrose	6.25 c	3.50 b	4.50 bc
T5= 22 µM BAP + 30 g L^{-1} sucrose	3.37 c	4.00 b	4.62 bc
T6= 22 µM BAP + 60 g L^{-1} sucrose	4.50 c	4.37 b	3.75 c
T7= 22 µM BAP + 80 g L^{-1} sucrose	3.87 c	5.12 b	5.25 bc

Means separation within the columns was done by the Tukey's studentized range test at $P \leq 0.05$.

Table 2. Influence of the media composition on the length of aerial portion (LAP), length of largest roots (LR), and number of roots (NR) 45 days after growth in the tuberization liquid media of cv. Parazinha

Treatments	CPA	LR	NR
T1 = MS0 + 30 g L^{-1} sucrose	12.82 a	5.29 a	7.37 a
T2 = 0.4 μM BAP + 1.6 μM NAA + 30 g L^{-1} sucrose	8.57 bc	0.76 b	3.12 ab
T3 = 0.4 μM BAP + 1.6 μM NAA + 60 g L^{-1} sucrose	8.21 bc	0.47 b	1.25 b
T4 = 0.4 μM BAP + 1.6 μM NAA + 80 g L^{-1} sucrose	9.21 ab	1.52 b	2.37 b
T5 = 22 μM BAP + 30 g L^{-1} sucrose	5.04 c	1.82 b	3.12 ab
T6 = 22 μM BAP + 60 g L^{-1} sucrose	6.35 bc	1.86 b	4.62 ab
T7 = 22 μM BAP + 80 g L^{-1} sucrose	5.09 c	2.05 b	5.62 ab

Means separation within the columns was done by the Tukey's studentized range test at $P \leq 0.05$.

The MS0 + 30 g L^{-1} sucrose (T1), which favored mostly leaf and root growth, did not induce any tuberization in either cultivar, and influenced mainly the vegetative growth without affecting the secondary growth in the roots. Furthermore, these effects were higher in Mantiqueira than in Parazinha (Table 3). The former cultivar was affected by the MS0 + 30 g L^{-1} sucrose (T1) in extending the length of the leaves and roots, and the number of roots (Table 3).

Table 3. Development of cassava cvs. Mantinqueira and Parazinha grown in MS media suplemented with 30 g L^{-1} sucrose

Cultivars	LAP	LR	NR
Mantiqueira	25.37 a	14.62 a	21.50 a
Parazinha	12.82 b	5.29 b	7.37 b

Means separation within the columns was done by the Tukey's studentized range test at $P \leq 0.05$.
LAP: length of the aerial portion; LG: length of the largest roots; NR: number of roots.

The treatment containing 0.4 µM BAP + 1.6 µM ANA + 80 g L^{-1} sucrose was the most effective to induce tuberous roots in cassava, which was well differentiated after 45 days of growth in the inducing media, and observed only in Parazinha (Fig. 1). This cultivar changed its pattern of growth compared to Mantiqueira. The former had significant smaller growth of aerial portion and roots (Table 3), which led to the initiation of tuberous roots 20 to 30 days after being under tuber inducing conditions. These favor-

able conditions for tuberous root initiation included exposure to cytokinin, auxin, high content of sucrose, and in a short dark environment. The most affective treatment for cassava had a small amount of BAP, and requires NAA, but with a similar high concentration of sucrose (Fig. 1). Similar results showing the interaction between cytokinin, auxin and sucrose were observed by Estrada *et al.* (1986) working with the induction of microtubers of potato. Most of the literature indicates that high sucrose levels are needed for potato tuberization (Hussey and Stacey 1984, Ranalli *et al.* 1994). Cytokinins play an important role in creating the sink during plant development, and through regulating the expression of a gene involved in the partition of assimilates towards the stolons as observed in potato (Prat 2004).

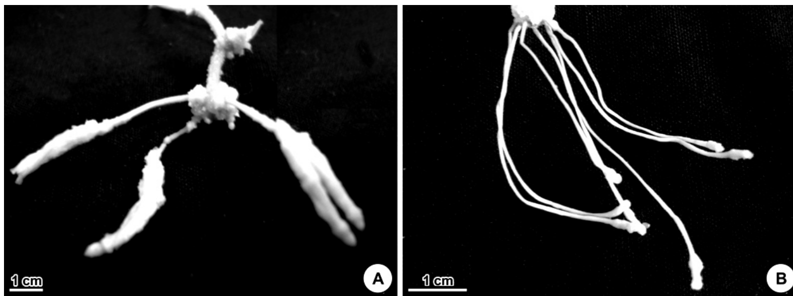

Fig. 1 – Root development in cvs. Parazinha (A) and Mantiqueira (B) cultivated in tuberization inducing media MS supplemented with 0.4 μM BAP + 1.6 μM NAA + 80 g L^{-1} sucrose 45 days after growth

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Environmental and Genotypic Effects on the Growth Rate of in Vitro Cassava Plantlet

Sunday E. Aladele
National Centre for Genetic Resources and Biotechnology, PMB 5382, Moor Plantation, Ibadan, Nigeria

Abstract

Two cassava cassava were evaluated *in vitro* in the screen house and culture room using five different media treatments. Each treatment was replicated 12 times and observed for 5 weeks before sub-culturing. There were significant differences in the growth rate of plantlets in different media, which suggests an interaction between treatments and environments. The survival of TMS 188/00106 was significantly different from TMS 083/00125 in the culture room than in the screen house. The study confirms that tissue culture derived plantlets can be raised in the screen house but suggests that plantlets should be allowed to survive in the culture room before transferring to the screen house for further growth.

Introduction

Tissue culture offers a unique opportunity to mass propagate plant materials especially, disease free plantlets. Vegetative propagation through tissue culture has played significantly in the mass production of vegetative propagating materials (Ajithukumar and Seemi 1998). It is faster and requires less space than that required for conventional methods of preparing cassava cuttings. The provision of electricity in the laboratory and the cost of maintaining a generating plant for regular power supply are very high in Nigeria and other developing countries. Even if the government can afford it, the private laboratories and seed companies may not be able to break even.

Cassava has just entered the international market in Nigeria. The need to rapidly produce disease-free planting materials to meet the growing local and international demand was a propelling factor for this investigation. Secondly, the space in many laboratories cannot accommodate the commercialization of major crops as demanded by big time farmers, hence the need to look beyond the laboratory environment. *In vitro* culture in the screen house will not only reduce the cost of production, it will also enhance quick acclimatization. There is rapid growth rate due to high temperature in the screen house than when in the laboratory. This study aims therefore to assess the use of screen houses to maintain cultures and rapidly propagate important crops with less contamination at a reduced cost.

Materials and Methods

Two genotypes of cassava (TMS 188/00106 and TMS 083/00125) were obtained from the International Institute of Tropical Agriculture (IITA, Ibadan, Nigeria), while five different medium were prepared using Murashige and Socoth (1962) with minor adjustments as follows:

- Treatment 1 (T1) - Liquid only
- Treatment 2 (T2) - Liquid with 50% normal agar (2 g L^{-1})
- Treatment 3 (T3) - Liquid media with filter paper embedded
- Treatment 4 (T4) - Media with normal Agar (4 g L^{-1})
- Treatment 5 (T5) - Liquid media with filter paper projecting out

The pH was measured and dispensing was done at the rate of 3 ml before autoclaving. The sub-culturing was done the following day. A total of 120 test tubes were used for each cultivar with 12 test tubes per treatment. A complete set of 60 test tubes with 5 treatments of 12 replicates was placed in the laboratory while the second set was placed in the screen house in the first day for TMS 188/00106. The same procedure was adopted the following day for TMS 083/00125. Data were recorded weekly for 5 weeks before sub-culturing. The second generation was observed for only two weeks to ensure the repeatability of the data recorded during the first generation. The data records include explants survival, shoot development, root development, nodal formation, leaf growth and increase in height. Each of the records were scored on a 0–3 scale [0: dead, 1: alive, but not growing, 2: growing slowly, and 3 growing very well). Survival rate was recorded

for two weeks only while the other five traits were scored continuously for three weeks consecutively. Only the screen house explants were subculture after 5 weeks to ensure the repeatability of the findings. The subculture materials from the screen house explants were also placed in both screen house and culture room (laboratory). The same recording was undertaken for responses of the explants to the culture medium and environment as in the first generation explants.

Results and Discussion

The two environments were not affecting significantly shoot, root, node, leaf and height development (Figs. 1–2). The laboratory plantlets grows better in liquid, and liquid with filter paper embedded media than when placed in the screen house, which might be due to high temperature recorded at the time of placement (32 -36°C viz. a viz. to 22-25°C in the laboratory).

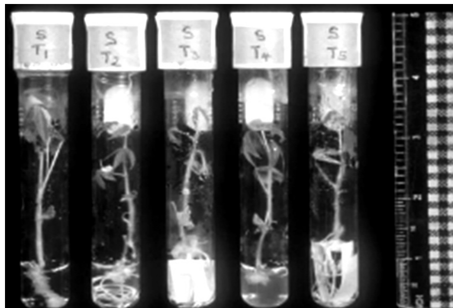

Fig. 1 – Screen house performance of the 5 treatments. T1: liquid only, T2: liquid with 50 % normal agar (2 g L^{-1}), T3: liquid media with filter paper embedded, T4: media with normal agar (4g g L^{-1}), and T5: liquid media with filter paper projecting out.

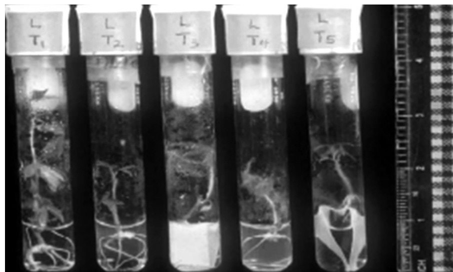

Fig. 2 – Laboratory performance of the 5 treatments T1: liquid only, T2: liquid with 50 % normal agar (2 g L^{-1}), T3: liquid media with filter paper embedded, T4: media with normal agar (4g g L^{-1}), and T5: liquid media with filter paper projecting out.

There was a major difference in growth rate of plantlets (Table 1), which indicates an interaction between treatment and environment. This result suggests that for long storage, the laboratory may be ideal while for short storage, screen house can be adopted. TMS 188/00106 survived better in liquid media than TMS 083/00125 (Table 2). The two genotypes grew equally in the media except on survival in liquid media only (Figs. 3–7).

Table 1. Environmental effect on *in vitro* cassava survival, shoot root node leaf height

Survival		Shoot		Root		Node		Leaves		Height	
SH	LAB	SH	LAB	SH	LAB	SH	LAB	SH	LAB	SH	LAB
1.77	2.41	1.92	1.93	0.73	1.55	2.00	1.93	1.92	1.93	1.92	1.96
1.67	1.44	1.56	1.42	1.67	1.33	1.86	1.63	1.67	1.46	1.61	1.33
1.02	1.81	1.21	2.04	0.46	1.42	1.46	2.17	1.29	2.00	1.21	2.04
1.73	1.27	1.83	1.00	1.00	0.75	2.04	1.29	2.00	0.96	1.79	1.00
1.73	1.27	1.88	1.13	1.63	1.04	2.13	1.29	1.79	1.13	1.92	1.17
CV 42%		*49%*		*89%*		*48%*		*51%*		*48%*	
Lsd 0.38.4		*0.4470*		*0.2987*		*0.4889*		*0.470*		*0.439*	
Std 0.193		*0.2266*		*0.5892*		*0.2479*		*0.2383*		*0.2228*	

SH = Screen House LAB = Culture room in the Laboratory

The survival was significantly different according to the media used (Table 3), which suggests that before the explants can be transferred to the screen house, one needs to ensure their survival in the laboratory. It can therefore be concluded that when the need arises, *in vitro* plantlets of cassava can be raised adequately in the screen house and even be raised faster than in the laboratory.

Table 2. Genotypic effect on *in vitro* growth rate of cassava survival, shoot Root node, leaf height

	Survival		Shoot		Root		Node		Leaves		Height	
	G_1	G_2	G_1	G_2	G_1	G_2	G_1	G_2	G_1	G_2	G_1	G_2
I	2.37	1.51	2.25	1.34	1.29	0.77	2.29	1.39	2.29	1.30	2.21	1.46
II	1.67	1.37	1.46	1.53	1.79	1.05	1.67	1.84	1.54	1.60	1.46	1.47
III	1.13	1.71	1.21	2.04	0.71	1.17	1.25	2.38	1.13	1.17	1.29	1.96
IV	1.54	1.46	1.33	1.50	1.00	0.75	1.79	1.54	1.54	1.42	1.42	1.38
V	1.54	1.46	1.58	1.42	1.00	1.67	1.63	1.79	1.42	1.5	1.54	1.54
CV	*43%*		*50%*		*92%*		*48%*		*52%*		*51%*	
Lsd	*0.3898*		*0.4494*		*0.5889*		*0.4791*		*0.4684*		*0.4600*	
Sed	*0.1976*		*0.2278*		*0.2985*		*0.2429*		*0.2375*		*0.2332*	

G1 = TMS 188/00106 G2 = TMS 083/00125

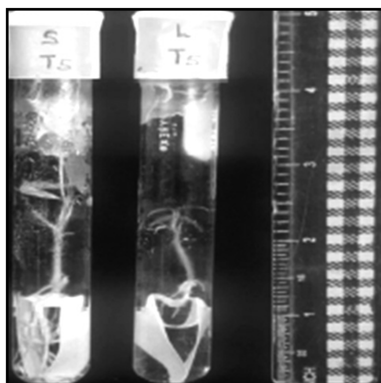

Fig. 3 – Comparison of treatment 1 [liquid media] in two environments [S = Screen house L = Laboratory]

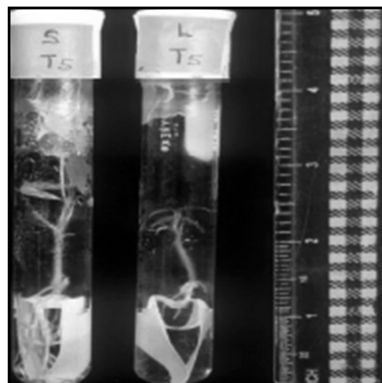

Fig. 4 – Comparison of treatment 2 [liquid with 50 % normal agar (2 g L^{-1})] in two environments [S = Screen house L = Laboratory]

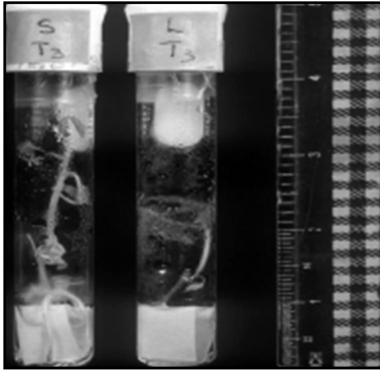

Fig. 5 – Comparison of treatment 3 [liquid media with filter paper embedded] in two environments [S = Screen house L = Laboratory]

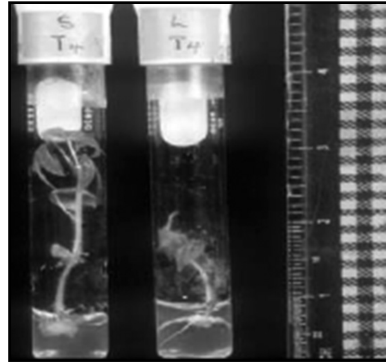

Fig. 6 – Comparison of treatment 4 [media with normal agar (4g g L^{-1})] in two environments [S = Screen house L = Laboratory]

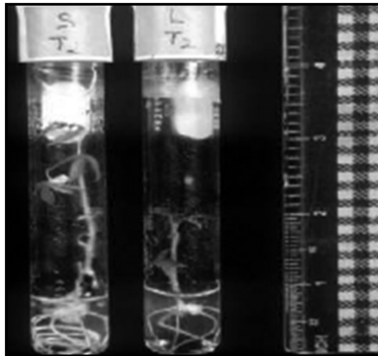

Fig. 7 – Comparison of treatment 5 [liquid media with filter paper projecting out] in two environments [S = Screen house L = Laboratory]

Table 3. Analysis of variance of the effect on five treatment media on *in vitro* growth rate of cassava survival, shoot Root node, leaf height

Parameters	DF	Sum of square	Mean square	Variation Ratio	F. Probability
Survival	4	14.1374	3.5343	7.87	<001
Shoot	4	7.7280	1.9320	3.12	0.008
Root	4	13.349	3.337	3.12	0.160
Node	4	2.6084	0.6521	0888	0.474
Leaves	4	75502	1.8875	2.77	0.029
Height	4	8.4901	2.1225	3.56	0.008

Acknowledgment

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Development of Transgenic Cassava Cultivars from Northeastern Brazil

A.B. Ibrahim[1, 2], F.F. Heredia[1], C.P. Barbosa[1], T.F. Machado[3] and F.A.P. Campos[1]

Abstract

Following the establishment of a cyclic system of somatic embryogenesis, in excess of 20 independent friable embryogenic callus (FEC) lines originating from different embryogenic cycles were established when fragments of somatic embryos were cultured on GD medium containing 2% sucrose supplemented with 50μM picloram. Histodifferentiation of these embryogenic structures was achieved when the tissues were transferred to MS medium devoid of growth regulators or to a medium supplemented with NAA or 2, 4-D. Differentiation of the tissues demonstrated that somatic embryos can be recovered at the rate of 450 embryos per gram of FEC tissues over a period of six weeks. Histodifferentiation of the FEC resulted in malformed cotyledon-stage embryos, which when subjected to maturation, germination and organogenesis using three different strategies did not regenerate into plants. Histological cuttings of the embryogenic tissues confirmed the malformation and absence of meristem. Up to 50mg L^{-1} of kanamycin is required to arrest embryogenic potential of green cotyledons from somatic embryos. While 50 to 60mg L^{-1} of paromomycin arrested the proliferation and histodifferentiation of FEC, 5mg L^{-1} of the antibiotic was sufficient to arrest the proliferation of the cells in liquid medium. Particle bombardment

1 Universidade Federal do Ceará, Departamento de Bioquímica e Biologia Molecular, Caixa Postal 6039, CEP 60455-900, Fortaleza, CE, Brazil.
2 Jigawa Research Institute, Biotechnology Laboratory, P.M.B. 5015, Kazaure, Nigeria.
3 Embrapa Agroindústria Tropical, Fortaleza, CE, Brazil

of somatic cotyledons and FEC or FEC-derived embryogenic suspensions using the plasmid pBI426, showed that helium pressures of 900psi and 1,200psi can be used effectively to transform the tissues. Optimal conditions for transient expression of the gus visual marker gene were achieved when tissues were pre-cultured in a medium containing an equimolar mixture of mannitol and sorbitol at 0.2M and bombarded using tungsten particle.

Introduction

In recent times, the increasing economic importance of cassava in most of the northeastern states of Brazil has been brought to focus. This is due, largely to the increasing demand for the crop in the poultry and bakery industries due to high prices of maize and wheat flour, coupled with its traditional use as food and fodder (Machado 2004). Globally, dependence on the crop is expected to increase with a projected 60% increase in production by 2020 (Scott et al. 2000) especially with the crop's potential in the starch industries (Aerni 2006).

The need to increase production to match this demand underscores the corresponding need for more genotypes that are adapted to the prevailing agro-ecological settings in the northeastern states of Brazil. Until recently, tissue culture techniques (meristem culture, axillary proliferation, somatic embryogenesis, organogenesis) and genetic transformation protocols for the cassava genotypes presently used in northeastern Brazil, have not been developed. In order to meet this need, we have made attempts towards developing protocols for the regeneration of cassava plants through somatic embryogenesis and organogenesis of 10 major cassava genotypes. We demonstrate transient expression of reporter genes in a number of these cultivars through Agrobacterium mediated transformation and particle bombardment of embryogenic tissues. Here, we report the progress made in our laboratory on the development of transgenic cassava cultivars with improved tolerance to drought stress.

Cyclic Somatic Embryogenesis

An important prerequisite for the development of genetic transformation system is the availability of morphogenic culture that can easily be used in gene transfer techniques (Taylor et al. 1996, 2004). In cassava, the most

efficient way of developing this culture is through somatic embryogenesis. Defined as a process by which a haploid or somatic cell gives rise to a plant while passing through the embryogenic phases similar to zygotic embryos (globular, heart-shaped, torpedo and cotyledon) from non-zygotic cells, without the fusion of male and female gametes (Emons 1994), the process was first reported in cassava by Stamp and Henshaw (1982) from zygotic cotyledons and clonal leaf materials. As in many other plant species, the general strategy for the development of an embryogenic culture in cassava is to induce the formation of somatic embryos from explants on a MS medium (Murashige and Skoog 1962) medium supplemented with auxin from which immature leaf lobes or shoot apexes can be used to regenerate plants that are clones of the mature parent material, while preserving all the traits of the given germplasm.

In all the cassava cultivars from northeastern Brazil that were subjected to somatic embryogenesis, primary somatic embryos were induced by culturing shoot apex isolated either from in vitro plants or from mature plants grown under field conditions using cassava induction medium (CIM), which was composed of MS salts (Murashige and Skoog 1962) supplemented with MS vitamins, 2%(w/v) sucrose, 0.5 mg L^{-1} $CuSO_4$ and 8 mg L^{-1} picloram and incubated in the dark. Maturation of embryos was achieved by transferring the primary embryos into MS based medium supplemented with vitamins, 2% sucrose, 0.5mg L^{-1} $CuSO_4$ and 0.1mg L^{-1} BAP (CMM) and incubated in 16 h photoperiod. Through this means, a cyclic system of somatic embryogenesis was established by subjecting cut pieces of cotyledons from mature somatic embryos CIM and sub-cultured every 4 weeks and kept as stock.

Induction and Establishment Friable Embryogenic Callus Lines

We demonstrate transient expression of the reporter gene gus (uidA) in somatic embryos regenerated from transformed cut pieces of somatic cotyledons. However, with the growing concern about the multi-cellular origin of these structures, focus has shifted to a new kind of embryogenic culture based on the production of friable embryogenic callus (FEC). Since its discovery in cassava (Taylor et al. 1996), FEC system has been applied in a number of cassava genotypes of different geographical origins (Raemakers et al. 1993a, b, 1997a, b, Sofiari et al. 1997, Taylor et al. 2001). The system

provides an alternative embryogenic culture in which embryogenic tissues which proliferate in an uncoordinated manner produce friable callus in which the large majority of the cells are totipotent (Taylor et al. 2001). These cells have been identified as ideal targets for use in gene transfer due to their putative unicellular origin (Taylor et al. 1996). The general strategy for obtaining FEC is to culture somatic embryos on GD medium (Gresshoff and Doy 1974) supplemented with 2% sucrose and 33 to 50uM picloram. Following a period of incubation, highly friable and pale-yellowish tissues can be seen emerging from the surface of the embryos. Once induced, maintenance of FEC is relatively simple and suspension cultures can be obtained by transferring a given amount of FEC into SH medium (Schenck and Hilderbrandt 1972) supplemented with 6% sucrose and 50μM picloram. Through this means, gram quantities of the FEC can be obtained and used as target for transgene insertion and prior regeneration of embryos which will subsequently give plants (Taylor et al. 1996, 2001, Schreuder et al. 2001).

We have succeeded in inducing and establishing FEC lines by transferring fragments of somatic embryos from CIM to GD medium with 2% sucrose and 50μM picloram (GD2P50) incubated in a 16 h photoperiod. The initial day of FEC appearance was recorded for each line obtained and percentage of somatic embryos that produced FEC was counted at the end of every cycle of 3 weeks. From our experiments, the FEC production capacity of the embryo fragments lies within the second cycle of the GD treatment. Lines of FEC from different explants originating from different embryogenic cycles were created in order to evaluate the possible existence of morphological differences among the FEC thus isolated. Scoring the friability of each different line showed that all the lines thrived well even after the date of evaluation and no apparent morphological difference was observed. FEC produced were continuously selected and transferred to fresh GD2P50 for proliferation while what remained of the fragment was further subcultured. Through this means, we evaluated the FEC production capacity of somatic embryos with time. Over 33% of 10 explants of embryo clusters per Petri dish produced FEC over a period of two cycles, giving rise to homogenous and highly friable FEC culture. By the 4th cycle, FEC production of individual embryo fragment decreased (Fig. 1), by which time the entire tissue must have turned into FEC or died up altogether.

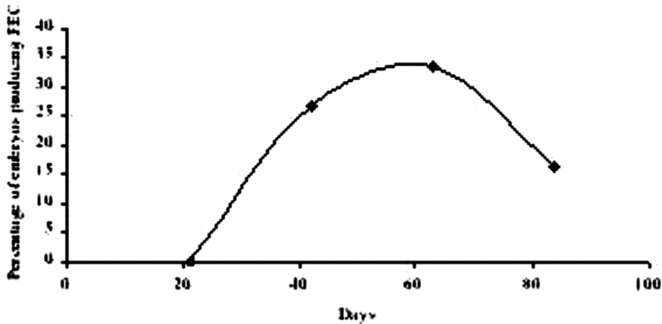


Fig. 1- FEC production capacity of somatic embryos on GD2P50. The optimal FEC production capacity of the embryo fragments lies within the second cycle of 28 days subculture

Histodifferentiation of Embryogenic Structures

Having thus established a protocol for the production of FEC, we set out to test the ability of the tissues to regenerate in to complete plants through histodifferentiation and germination experiments. Regeneration of the embryogenic tissue into a plant often involves the transfer of the tissue into an MS based medium devoid of growth regulators or supplemented with NAA (Taylor et al. 1996, Schopke et al. 1996, Gonzalez et al. 1998, Taylor et al. 2001). In our work, although cotyledon stage embryos were obtained both on MS medium containing 2% sucrose and 5µM NAA (MS2NAA5) and on MS medium with 2% sucrose (MS2), with an average production of over 450 embryos per gram tissue of FEC over a period of 42 days, all the embryos generated were malformed with fused cotyledons (Fig. 2.). Reports exist in which malformed embryos from FEC were obtained (Taylor et al. 2001, Raemakers et al. 2001). It has been recommended that young FEC tissues should be used to regenerate plants as old ones tend to be exhibit low regeneration capacity (Raemakers et al. 2001). However, when we subjected 2 months and 1 year old FEC tissues, similar pattern of histodifferentiation with accompanying production of malformed embryos was observed. Embryogenic suspension produced embryos only in media devoid of growth regulators (MS2) and took much longer time (approximately 30 days) to emerge as against the two weeks observed in FEC tissues transferred to MS2NAA5. This was expected, because embryogenic suspension has higher contact with auxin in the liquid medium and would therefore require longer time to eliminate auxin, the removal of which from the medium has been implicated in the triggering of embryogenic

competence (Emons 1994). When subjected to serial sieving, the fraction of embryogenic suspension retained by 40 mesh sieve proved to show higher embryo conversion than other fractions.

Plant Regeneration

Efforts to germinate the embryos on BAP rich medium met with failure, even when the level of auxin was almost eliminated through desiccation in a medium containing 0.5% activated charcoal (Mathews et al. 1993). We tested three different strategies of regeneration of mature plants from cotyledon-stage embryos emerging from histodifferentiating embryogenic callus. In the first strategy, individual embryos were cultured in an MS2 medium supplemented with 4.4μM BAP, 5.0μMBAP and 2.0μM BAP. Half of these were kept in the dark while the other half were kept in a 16 h photoperiod. Although none of the embryos regenerated into a plant, active cell division with evidence of foliose structures can be observed in all the treatments. Embryos in the medium containing 4.4μMBAP gave relatively more organized and highly enlarged vegetative structures which were erect and sometimes with the emergence of root. Embryos kept in the dark turned pale with no evidence of shoot growth. In the second strategy, the embryos were first inoculated in an MS2 medium containing 0.5% activated charcoal for 1 week, before transfer to the 3 different mediums described above. Similar morphological responses were observed. In the third strategy, cut pieces of green cotyledons emerging from FEC were inoculated in two different organogenesis medium one composed of MS2 with 1μM BA and 2.5μMIBA (Puonti- Kaerlas et al. 1997) and the other composed of MS2 with 1μM BAP, 0.5μM IBA, 0.5mg L^{-1} $CuSO_4$ (Machado 2004) and incubated in the dark for 20 days. The cotyledons produced callus and sometimes embryo-like structures but never shoot primordium. Currently, efforts seem to be shifting towards the induction of organogenesis directly from FEC (Hankoua et al. 2006). It is however, subject to experimentation, whether the system is reproducible in other cultivars of cassava.

Histological Analysis

As part of our effort check the "inregenarability" of the embryos, we made an attempt to evaluate the underlying cellular changes that accompany the embryogenic development. Histological cuttings obtained not only con-

firmed the malformed nature of the embryos, but also indicated the absence of meristem in the apparently bipolar structures obtained (Fig. 2.). A question has arisen as to whether these deformities were as a result of mutation or were induced at certain stage of embryogenic development and elicited by auxin. Our result underscores the need for further investigations with a view to acquiring thorough knowledge of the morphological development, cellular and molecular basis of FEC induction and histodifferentiation.

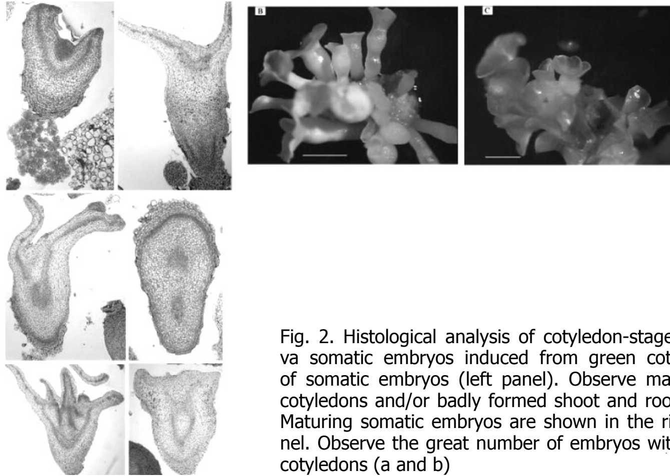


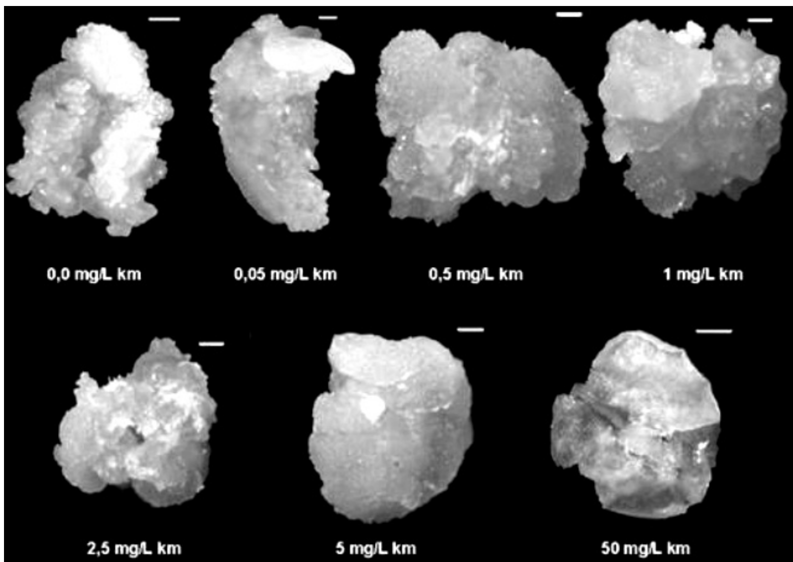
Fig. 2. Histological analysis of cotyledon-stage cassava somatic embryos induced from green cotyledons of somatic embryos (left panel). Observe malformed cotyledons and/or badly formed shoot and root poles. Maturing somatic embryos are shown in the right panel. Observe the great number of embryos with fused cotyledons (a and b)

Since the conversion of globular embryos appears to be the limiting step for the regeneration of plants from FEC (Puonti-Kaerlas 2001), it follows that the success of any regeneration system of FEC must depend on strategies that will take advantage of the underlying competence of these structures. We are inclined to believe that the malformed structures obtained in our regeneration experiments might have been caused by a certain aberration in the expression of morphogenic competence of the cells.

Determination of Phytotoxic Levels of Antibiotics for Selection of Putative Transgenic Tissues

To meet a part of the requirements for the development of transformation systems in cassava cultivars from northeastern Brazil, we subjected both somatic embryos and FEC or embryogenic suspension to a series of

killing curve experiments using kanamycin and paromomycin. Increasing the concentration of kanamycin in CIM cultured with cut pieces of cotyledons led to decrease in their embryogenic potential as evidenced by reduction in the frequency of embryogenesis and the number of embryos produced (Fig. 3. upper plate). There was also a dramatic increase in the production of callus with increasing kanamycin during embryo maturation except in the treatment with 50 mg L^{-1}, where the explants died completely. The decrease in somatic embryogenesis and concomitant increase in callus with increasing concentration of kanamycin may be due to hormonal imbalance mediated by cellular degradation of the antibiotic (Lin et al. 1995).Although our investigations show that 50 mg L^{-1} of kanamycin efficiently selects for putative transgenic embryos, slightly lower concentrations are recommended. At 10 mg L^{-1}, kanamycin inhibited up to 93% of embryos maturation. Callus production was discreet at concentrations below 5 mg L^{-1} with slight increase through 10mg L^{-1}. At 25mg L^{-1} and above there was evidence of tissue oxidation (Fig. 3. lower plate).We recommend that 10 mg L^{-1} of kanamycin is sufficient to select for embryos during maturation.



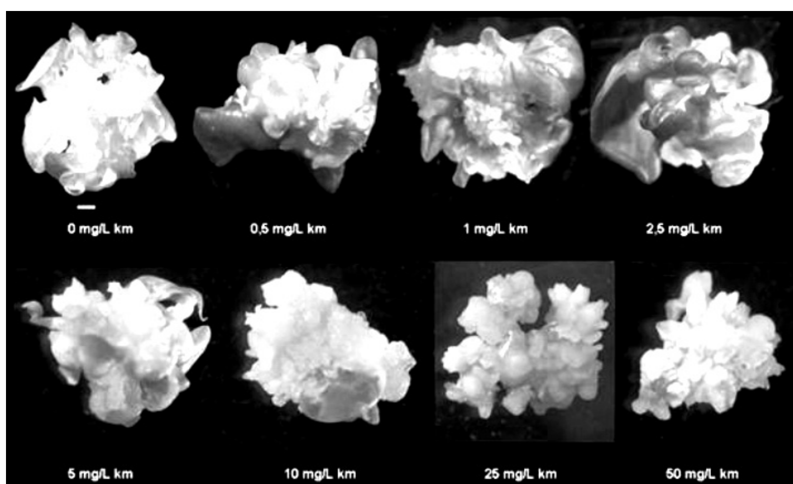
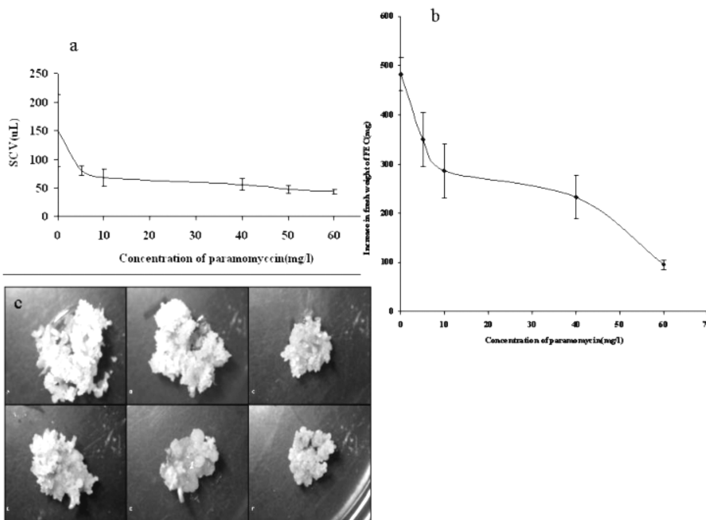


Fig. 3. Effect of kanamycin on somatic embryogenesis demonstrating the production of callus accompanied by decrease in embryogenic potential (upper plate) and arrest of embryo maturation (lower plate)

A killing curve using different concentrations of paromomycin showed that 100mg of FEC proliferates and increases in fresh weight on GD2P50 medium supplemented with up to 45mg L^{-1} paromomycin. At 60mg L^{-1}, there was an arrest of FEC proliferation and loss of friability (Fig. 4b). This is true for both embryogenic suspension in liquid media and for FEC subjected to histodifferentiation (Fig. 4a and 4c, respectively). While as little as 5mg L^{-1} of the antibiotic was sufficient to arrest the growth of and kill majority of the cellular suspensions as evidenced by the reduction in the settled cell volume (SCV) and viability test (result not shown), 60mg L^{-1} promptly killed the cells with accompanying disintegration of the same. A phenomenon observed in both proliferating and histodiffrentiating FEC is that, at lower concentration (usually 5mg L^{-1}), there appeared to be a pronounced growth of different types of callus which were often lighter in weight than homogenous FEC (Fig. 4c). It is probable that lower concentrations of paromomycin also tend to interfere with hormonal balance leading to the production of large amount of callus.



Optimization of Parameters for Transient Expression of GUS

Results from Agrobacterium-mediated transformation experiments using three different strains indicate that higher co-culture time reduces of embryogenic potential of the explants (results not shown). We are presently screening a collection of Agrobacterium tumefaciens strains in order to identify more suitable ones for use in future transformation events. Due to the comparative advantage of genetic transformation via particle bombardment (Altpeter et al. 2005), we used embryogenic tissues to carry out a preliminary evaluation of working parameters in particle bombardment of cassava. The interdependence among different bombardment parameters such as plasmid type, cultivars and pressure has long been established as crucial point in developing a transformation system for any species. In our experiments, the highest frequency of transient expression as well as the number of blue spots on mature somatic embryos was observed when tungsten particle with size 0.5μm (M5) was used along with 900psi helium pressure against the combination of M10 and 1,200psi. The explants presented small but well defined blue spots with higher distribution in (Fig. 5a.). The combination of bombardment particles with small size and relatively low pressure has been shown to have a positive effect in generating high frequencies of transient expression in different plant species (Yang et al. 1999, Devi and Sticklen 2002, Tee and Maziah 2005).

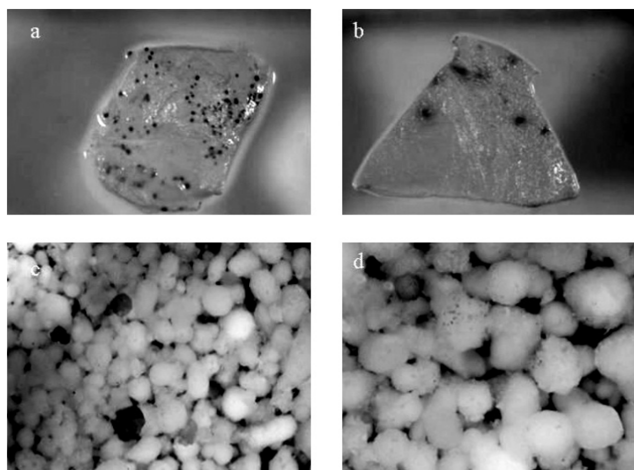


Figure 5. Transient expression of GUS in cotyledons from somatic embryos and FEC 24hrs. Higher expression was observed in the cotyledons with the combination of M5 and 900psi (a) against M10 with 900psi (b). Bombardment of plasmolysed FEC with 1,200psi gave higher expression when M5 (c) particle was used against M10 (d)

Similar experiment was carried out using FEC/embryogenic suspension. It has been demonstrated that plasmolysis treatment of embryogenic tissues with 0.2M mixture of mannitol and sorbitol before and after bombardment are beneficial in particle bombardment of FEC (Schopke et al. 1997). Using a helium pressure of 1,200psi, we studied the effect of plasmolysis and particle size on the transient expression of GUS from bombarded FEC. Histochemical analysis of FEC tissues and embryogenic suspension was carried out 24 h after bombardment. Although the overall frequency of transformation in both treatments seems to be extremely low, the relatively higher level of blue spots observed in the plasmolysed tissues shows that this treatment has a positive effect on the frequency transformation. The optimal condition for transient expression was achieved when tissues were treated with 0.2M mannitol and sorbitol using tungsten particle M5 (Fig. 5a). Following this, large scale experiments were carried out in which, two kinds of embryogenic tissues (FEC that was proliferating in solid medium and embryogenic suspension from liquid medium) were bombarded with pBI426 (Datla et al. 1991) using M5 particle and a pressure of 1,200psi. 24 h after bombardment, 1.6 ± 0.13924% of intact FEC units gave positive GUS test while 1.142742 ± 0.242142% embryogenic suspension gave positive GUS test. Selection regime with paromomycin was started one week after bombardment.

Reviewing the progress made in FEC transformation of cassava, Raemakers et al. (2001) highlighted such limitations as the amount of tissue that can be used per bombardment and the number of bombardments necessary to produce desired transgenic plants. Such factors depend on the type and stringency of selection and the characteristics of the traits introduced, which in turn depend on the genotype. These parameters underscore the need to carry out preliminary evaluation of bombardment conditions on any genotype to be transformed.

Selection of Bombarded Embryogenic Structures

Selection of the bombarded tissues using paromomycin was started one week after bombardment. Different reports used paromomycin (Schopke et al. 1996, Gonzalez et al. 1998, Taylor et al. 2001) as a selective agent following the bombarded of embryogenic tissue. Although no embryos emerged from the tissues placed in the histodifferentiation medium (from both types of tissues), when the transient assay was repeated 5 to 6 weeks after bombardment, an average of 3.6% for embryogenic suspension and 3.5% for FEC was observed. It has been reported that the use of paromomycin selection reduces the regenerative potential of the transgenic material (Puonti-Kaerlas 2001). Reasons such as this are often given in defense of selection systems based on combining antibiotic selection using phosphinotricin and luciferase (Raemakers et al. 1996). Although the use of luciferase may sometimes allow the development of both transformed and non-transformed maturing embryos from bombarded FEC, screening could be used to exclude escapes, while inclusion of the antibiotic during embryo maturation could help in regenerating a number of transgenic lines. However, the use of luciferase detection method may not be practicable despite its non-destructive nature, because it requires access to costly equipment (Puonti-Kaerlas 2001). An alternative system which combines the visual GUS assay with positive selection using mannose seems promising (Zhang and Puonti-Kaerlas 2000), but will only be beneficial if it ensures the elimination of non-transformed tissues without compromising the regenerative potential of the putative transgenic tissues

In summary, the results from our work will form the basis for the transfer of drought resistance and other agronomically important genes in cassava cultivars of northeastern Brazil. Since the low regeneration rates or regeneration of abnormal plants reduces the transformation efficiencies

obtained after transformation (Taylor et al. 1996, Raemakers et al. 1997, Schopke et al. 1997, Munyikwa et al. 1998, Zhang et al. 2000) for any transgenic program in cassava to be successful, the target must be the development of a reproducible protocol that will allow for reduction in the length of time required for tissue culture before plant regeneration while ensuring single insertion of the transgene. Efforts to develop new protocols of plant regeneration based on combining the now usual pathway of histodifferentiation and organogenesis(Hankoua et al. 2006) as well as the employment of strategies that will create conditions similar to seed development (Schmidt et al. 2005) seem promising in improving this.

Acknowledgements

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Improving Cassava for Enhancing Yield, Minimizing Pest Losses and Creating Wealth in Sub-Saharan Africa

Rodomiro Ortiz

Abstract

Cassava was brought to Africa by the Portuguese at the end of the 16th Century and today should be regarded among the two top staples of the continent. However the crop faces many challenges because it may be affected by biotic stresses and end-user demands. Cassava can be a source of income by adding-value through domestic agro-processing or as raw material for the local industry. This article provides an overview of cassava improvement through crop breeding, especially for cassava mosaic disease and bacterial blight, and biological control of pests such as cassava mealy-bug and green mite. The achievements in the genetic enhancement of the crop or its eco-friendly plant health management result from using genetic resources of the crop or biological control agents brought from the South American center of origin of cassava. Without these research-for-development successes brought cassava output cassava production would be 50% or less in Africa; i.e., over 13 million t year^{-1} of dry cassava, enough to meet the calorie requirements of 65 million people in sub-Saharan Africa. In recent years, cassava changed rural landscapes, and this poor farmer's crop became a pacesetter of African rural development. Cassava post-harvest processing may be a major vehicle for job creation and poverty reduction in rural areas. The accomplishments of cassava research-for-development in, and for Africa ensued from a strategy that considers producing locally, minimizing risks and creating wealth.

Introduction

Cassava became the most important food crop in sub-Saharan Africa, which accounts for most of the root harvest worldwide, followed by Asia and Latin America –the center of origin for Manihot species. The Portuguese brought cassava to Africa in the late years of the16th Century (Jones 1959). The crop became widespread in Africa, especially in locations with high population density, such as southern Nigeria, western Democratic Republic of Congo, and eastern and northern Tanzania, where the crop remains an important staple for their inhabitants' food security (IITA 1992).

Due to its resilience in marginal environments, cassava can be grown in drought-prone locations, or in acid soils, which explain the role of this crop in alleviating faming, especially among the resource poor. In Africa and Latin America cassava is mostly used for human consumption, while in Asia and parts of Latin America it is also used commercially for the production of animal feed and starch-based products. Roots are processed into granules, pastes and flours or eaten freshly -boiled or raw. A dry cassava leaf contains up to 40% of crude protein but it may vary across cultivars (Lancaster and Brook 1983). The leaves are therefore eaten in Africa and some Asian locations as a green vegetable, which provides protein and vitamins A and B.

This article provides an overview on cassava research in sub-Saharan Africa considering that an effective means to alleviate poverty, and in turn its inseparable partner hunger, is through agriculture and the production of more nutritious and profitable food. As pointed out by the Director General of the International Institute of Tropical Agriculture (IITA) Hartmann (2004), "a successful approach cannot only be about agriculture —it has to recognize the vital role it plays in the bigger picture. The strength of the IITA approach of local production, wealth creation, and risk reduction, is its embrace of strategies that recognize that the issues that contribute to poverty are intertwined. The degree of impact from this approach depends on several factors, not least of which is investor and implementing entity choices. The choices investors make in how activities are financed may be as important as how much. Equally, the choices made by development institutions such as IITA and its national and regional partners on problem definitions and research-for-development methods, are also critical."

Producing locally

The argument for local production can be viewed from several perspectives. Hartmann (2004) argues for local production because it is the most stable way to improve livelihoods, increase food security and contribute to long-term and broad-based economic growth. By taking this approach, any research-for-development undertaking also addresses food security issues, which are directly related to poverty. As Hartman (2004) points out that "focusing on local production is also needed because the alternative is food imports and that is not without limitations because such an approach does not fully accommodate nuances of geopolitics, climate, food preferences, global and regional trade, availability of foreign currency as well as available information and infrastructure. More importantly, the import approach does not address what is desired by the developing world and what is available from the industrialized world."

It was therefore not surprising that cassava research in Africa started in the 1930 by focusing on two central constraints to local production, namely African cassava mosaic virus (ACMV), and low yield of cassava, to which ACMW contributed. ACMV (later known as CMD or cassava mosaic disease) spreads by an insect vector and further distributed by infected plant cuttings, whereas low yield results from the use of poor planting materials and lack of access by farmer to bred-cultivars (IITA 1992). Breeding for ACMV began in Ghana, Kenya and Tanzania in the 1930s, and some hybrid clones ensue from crosses between cassava and M. glaziovii (Nassar and Ortiz 2007). Clones such as Gold Coast Hybrid 7 (GCH-7 bred in Ghana) and 5318/34 (bred at Amani, Tanzania) were brought to the Moor Plantation (Ibadan, Nigeria) in the 1940s and 1950s as source material for further cassava breeding, particularly for host plant resistance to ACMV. One of the clones selected in Nigeria was 58308 – an important source of new hybrids bred in the 1970s by IITA such as TMS 30572 and TMS 4(2)142, which are still widely grown in the Nigerian cassava belt and other African locations.

The overall rationale, in the mind of IITA founders, for establishing this Institute as an international high quality research organization in sub-Saharan Africa, was to find ways to enhance yields and quality of tropical food crops such as cassava. The research domain included all aspects that allow increasing and improving the quality of food. In the early years (1970s), IITA's agenda was organized into four programs; being one of them the Root and Tuber Improvement Program that was led by Dr. Sang Ki Hahn (Ortiz

2004). When Hahn arrived in Ibadan in 1971 to establish this program at IITA, he rightly saw that no amount of research effort would increase cassava yields until the problem of CMD was solved.

**Source population (up to 100 000 seedlings)
after crossing selected parents**

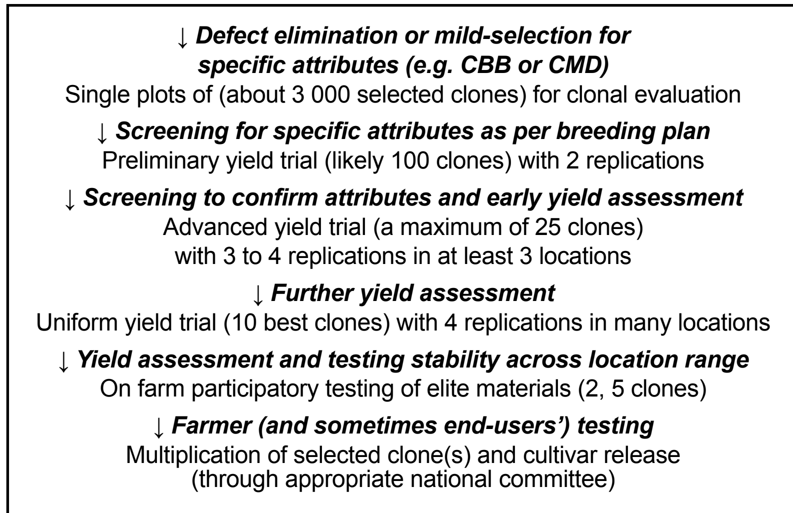

Fig. 1. Conventional breeding scheme for cassava Sources: Jennings and Iglesias (2002), Ortiz (2004b). CBB = cassava bacterial blight, CMD = cassava mosaic disease

Hahn recognized the enormous implications of an endemic disease aggravated by humans through the use of diseased cuttings, and he focused cassava research tightly on this disease problem (Hahn et al. 1989). With Eugene Terry as pathologist, Hahn began the arduous task of searching the germplasm for resistance characters and then combining those characters with lines having desirable yield and quality factors (Hahn et al. 1980a). Fortunately, Hahn had access to the mosaic resistant families developed from A.J. Storey's work in East Africa nearly 30 years before and that of Brian Beck at Moor Plantation in the 1950s. However, these families had very poor root yields. He also brought cassava germplasm from Asia and South America. The latter incorporated wild Manihot genes that were initially bred by Prof. Nagib Nassar (Univ. of Brasilia) and other researchers in Brazil. It remained the IITA team's task ably assisted by Audrey Howland, an outstanding breeding research associate, to cross, select, clone, challenge,

rogue, and select, beginning each season with up to 10,000 seedlings, until the desired level of resistance was incorporated into "elite" IITA cassava breeding materials (Fig 1).

In the early 1970s, cassava bacterial blight (CBB) was reported in Nigeria, and this 'black disease', as known by Nigerian farmers –particularly in eastern and mid-western states, caused huge crop losses because the best available cultivars (60444 and 60447) proved to be very susceptible (IITA 1992). Afterwards CBB epidemics were observed in a dozen African countries because their local cultivars were also susceptible to this disease. The clone 58308 –with low cyanide potential and resistance to ACMV, was also used as a source of resistance to CBB. An important genetic enhancement research finding was that CBB resistance derived from M. glaziovii was associated to with resistance to CMD (Hahn et al. 1980b). Furthermore, this IITA breeding endeavor led to new hybrid clones with resistance to CBB and ACMV, plus high yield and acceptable quality traits (Hahn et al. 1989). This breeding success ensued from the use of cassava clones brought from other continents, which were included in crossing blocks along with IITA disease-resistant clones, local African landraces, and the strong partnership research with the National Root Crops Research Institute (NRCRI) at Umudike, southeastern Nigeria (IITA 1992). The Centro Internacional de Agricultura Tropical (CIAT, Cali Colombia) also facilitated the acquisition by IITA of new parental materials, especially those grown in South America or suitable for dryland areas.

In summary, the target of this cassava breeding strategy was broadly based breeding populations that would be further selected by national researchers and local partners according to their needs (Jennings and Iglesias 2002). Hence, crosses among local cultivars was high in the Institute's breeding agenda as well as incorporating judiciously exotic germplasm into the desired gene complexes, but minimizing inbreeding and restoring heterozygosity to escape from inbreeding depression (Ortiz et al. 2006). The improved cassava germplasm was sent for testing across African locations through in vitro methods for elite genotypes, or as seed for half-sib and full-sib recombinant breeding populations. Furthermore, as indicated by Robinson (1995), the concept of farmer participatory schemes for plant breeding was initiated by Hahn and co-workers, who enlisted the help of small country schools in many West African locations with whom they shared some seeds of their promising materials.

Minimizing risk

Producers face risks that need to be managed. The poorer the farmers, the more limited their ability to deal with these risks. Addressing them, Hartmann (2004) says, is an important strategy for poverty reduction. He further indicates that “like anyone else, farmers, rural families, and the poor try to avoid or reduce their risks. Poor farmers consistently attempt to diversify their sources of income by working part time. Knowledgeable about climatic risks, they not only grow different crops, but also grow them in different locations. Unfortunately farmers’ excellent strategies often let them down because the tools at their disposal and their ability to respond to risks are limited. Farmers are confined to certain localities and have limited purchasing power and very low asset base. Droughts, for example, affect not only the different locations where crops are grown, but also other agricultural activities in the area. The result is that alternative employment opportunities are reduced at the very time farmers need them most. So in spite of remarkable levels of knowledge and creativity, farmers’ responses to risks are limited. Thus the second line of this approach is devoted to supplementing risk management efforts. Here is a critical point where investor choices determine options.” The risks faced by producers and rural communities fall into four broad groups: biological, commercial, natural and political. In the decision process under a risk-minimizing agenda should give preference to research-for-development methods that are less dependent on policies, inputs, costly government programs and services.

In this regard, cassava appears as an important crop option for marginal environment (e.g. drought-prone locations), where cereals and other species do not grow well, and it also grows well in poor soil (Ortiz and Hartmann 2003, Nassar and Ortiz 2006). Under drought stress, the cassava plant reduces water use by following an avoidance strategy of stomatal closure and leaf area reduction. After the stress, recovery of cassava leaf area occurs, which, of course, influences root yield in cassava depending on the developmental stage of the crop and the environment where it grows. Because cassava roots can be stored in the ground for up to 24 months, and some cultivars for up to 36 months, harvest may be delayed until market, processing, or other conditions are favorable.

The successful biological control of crop pests in cassava is another example of this approach for minimizing risk in the cropping systems of sub-Saharan Africa (Neuenschwander 2004). The prerequisite for the suc-

cess of such very knowledge-intensive programs is the nature of investor support and financing. It is difficult to implement biological control options successfully without long-term commitment to knowledge generation.

Cassava mealybug was one of the most serious pests for this crop, especially during the 1970s and 1980s because it destroyed producing fields and local sources of planting materials to such an extent that production practically came to a halt (IITA 1992). Led by Drs. Hans R. Herren and Peter Neuenschwander, entomologist and insect ecologist respectively, the IITA biological control team went well beyond the scope of most biological control projects in formulating the scientific explanation for the behavior of the host plant (cassava), the enemy (mealybug) and the control agent (beneficial parasite or predator). Several significant contributions to the body of scientific knowledge were made by this IITA team (Neuenschwander 2001). These contributions include: the identification of the cassava mealybug as a newly introduced pest into Africa; the location of the same mealybug species in its area of origin in South America (in collaboration with CIAT entomologist Anthony Bellotti); the successful rearing of cassava mealybugs as well as their imported natural enemies in the laboratory at IITA; the migration and dispersal data assembled after the release of the beneficial parasite Anagyrus (Apoanagyrus, Epidinocarsis) lopezi –a predator wasp, the impact studies (rarely attempted exclusion experiments and population dynamics and biological data on both pest and parasite or predator); and the development of a simulation model showing plant/pest/predator-parasite interactions (Herren and Neuenschwander 1991). The introduced parasitoid A. lopezi dispersed, and controlled the cassava mealybug wherever it was released. For control stability, a complex of natural enemies is more desirable than a single species. The IITA team gathered considerable data on several mealybug predators, but none has shown the survival and dispersal qualities of A. lopezi. As a result of this effort cassava mealybug is now held in check across most of the African's cassava producing areas (IITA 1992). The spectacular control of the cassava mealybug was the first of many successes in the history of the Biological Control Center for Africa set up by IITA at Cotonou (Benin Republic) in the 1980s.

After cassava mealybug was brought under control by the introduced predator wasp A. lopezi, IITA researchers undertook the biological control of cassava green mite (Dixon et al. 2003). This pest was accidentally introduced into Africa in the 1970s and within a decade spread across Africa's cassava belt (Yaninek and Herren 1988). The major achievements in the cassava green mite research until the late1980s was the establishment of

the mite's true identity, its behavior within the cassava ecosystem, and its damage to the cassava plant (Mégevand et al. 1987). It was in the second half of the 1990s that IITA researchers J. Steve Yaninek and Rachid Hanna succeeded with classical biological control of cassava green mite by identifying, introducing and establishing predatory mites (Typhlodromalus aripo and T. manihoti) and later an acaropathogenic fungus (Neozygites tanajoae) from climatically similar areas of Brazil. Where the predatory mites have been continuously present for at least two years, cassava green mite density declined by 30 to 60% and cassava yields increased between 15 and 35%, with two cases of 62 and 85% increase in yield. The addition of the fungus N. tanajoae has led to further 25% decline in cassava green mite populations. This cassava green mite biocontrol campaign is continuing in Central, Eastern and Southern Africa with the addition of strains of predatory mites adapted to mid-altitude agro-ecologies, and the emphasis on the integration of cassava cultivars suitable to predatory mites (a relatively nascent dimension of biological control in general), owing to the sensitivity of T. aripo to specific morphological characteristics of the cassava apex where the predators reside during much of the daylight hours.

IITA successes on biological control of cassava pests rely on the strong partnership with national programs in sub-Saharan Africa. National capability was enhanced in the last 2.5 decades by intensive training of crop protection workers, after creating awareness among decision makers that there are alternatives to the use of agrochemicals for control of crop pests. The philosophy of this IITA capacity building approach for ecologically sound pest management considers that the ultimate goal is to strengthen national capability in biological control and not just secure resources for a single control campaign (IITA 1992). In this endeavor, IITA engaged the United Nations Development Program and the Food and Agriculture Organization as well as other investors of international aid to develop national biological control units in Africa and their continuous support by providing materials, financing and technical assistance.

Creating Wealth

The wealth creation concept according to Hartmann (2004) "is to take what farmers already produce and use it to earn more income. They can be helped to sell it at the next rung on the ladder." If farmers increase crop production, a sound research-for-development agenda should create

outlets for their produce. Simple agro-processing of crops such as cassava can double or even triple incomes (Dixon et al. 2003). Similarly dual-purpose use for food and feed leads commodities into other users and places, which provides another powerful poverty reduction concept: the expansion of markets through the creation of new outlets contributes to price stabilization without the need for costly government programs.

In view of the importance of cassava as a major source of calorie for Nigerians and potential source of large scale agro-industry uses in the country, the Federal Government of Nigeria asked IITA in 2002 to implement a cassava mega-project to preempt an outbreak of a more virulent strain of CMD (Dixon et al. 2003). This call was the result of a timely warning by IITA researchers on the potential attack by new strains of CMD in Nigeria, which can combine to form a more destructive strain of the virus. IITA, therefore, took immediate preventative action to avoid a repetition of the devastation that occurred in Uganda during the 1990s. Since the launching of this mega-project, IITA has been producing thousands of new, disease-resistant, cassava plantlets and cuttings and delivers them to Nigerian farmers. The improved plants will not only resist the disease but will also slow its spread to non-resistant cultivars, acting as a barrier to the advance of CMD. IITA-bred cultivars also produce more cassava per plant and their distribution should lead to increases in total Nigerian production. As part of the unique preemptive strategy, IITA and its partners from both the Nigerian public and private sectors are working to establish value-added industries and post-harvest processing to ensure markets for the increased production that is expected. Hence, this Nigerian Presidential Cassava Initiative, which in 2003 brought the strong funding commitment of a Global Development Alliance between the largest oil company in the country and the major aid investor of the CGIAR, improves technology transfer to address CMD and to develop cassava processing that will provide greater incomes to farmers in 11 states, mostly in the southern “cassava belt”. It will also help identify further commercial markets for cassava, such as ethanol production, livestock feed and use in baking. In addition to being a staple food, starch from cassava is already used in other industries including textile manufacturing. Similarly and encouraged by IITA research impacts on the crop, the Integrated Action Program for Cassava Starch Production and Export was launched by the President of the Republic of Ghana for developing the cassava starch industry in this country as a major vehicle for job creation and poverty reduction in rural areas.

Impacts

Maredia and Raitzer (2006) indicate that the largest development impact of the Consultative Group on International Agricultural Research (CGIAR) in sub-Saharan Africa came via support of long-term crop improvement and integrated pest management research dealing with biological risks. Indeed, research to increase yields in a broad range of agro-ecological zones and cultivation systems, to suit a wide variety of consumer preference, was launched by IITA through the deployment of bred-cassava cultivars in many African locations. There were about 206 releases of cassava cultivars in 20 African nations. In the 1990s African programs incorporated IITA bred-materials in 80% of their cassava bred-germplasm that led to 50% gains in cassava yields on average (Manyong et al. 1999). Such cassava cultivars represent an important contribution to Africa's food security, especially among the poor (Nweke et al. 2002) because the improved cultivars raised per capita output by 10% continent-wide, benefiting 14 million farmers. For example, the total benefits from the cassava multiplication partnership project between the National Agriculture Research Organization (NARO, Uganda) and IITA to combat the cassava mosaic disease pandemic in six districts was approximately US $ 36 million over four years (1998-2001) for an initial investment of US $ 0.8 million (Dixon et al. 2003). The success of cassava genetic enhancement in sub-Saharan Africa points out the benefits of having an eco-regional center such as IITA doing crop breeding, and together with many continental partners delivering the new seeds that impact on African livelihoods (Ortiz and Hartmann 2003). Clearly, there are some circumstances where national programs are already sufficiency developed to fulfill this role. In such cases (and the list will hopefully be rapidly expanding), international centers have a duty to rapidly devolve these activities to the national partners through technology and skills exchange (Ortiz and Crouch 2007).

The spectacular biocontrol of the cassava mealybug yielded economic returns of 200:1, with minimum benefits of US $ 2.2 billion from a total expenditure of US $ 14.8 million (Norgaard 1988, Zeddies et al. 2000). One could easily surmise that, without biological control, the mealybug would have destroyed most of the cassava grown across Africa. This project was unique in its geographic scale, organization and level of documentation, and has become a classic textbook example (Neuenschwander 2004). Likewise, in West Africa alone, where cassava green mite biological control was first achieved, economic benefits have reached a hundred fold – US $ 100 in return for each US $ 1 invested in the program (Dixon et al. 2003). It was not

surprising therefore that the most recent impact report to the CGIAR Science Council indicates that about 80% of the US $ 17 billion estimated impact of the CGIAR in sub-Saharan Africa result from the biological control of pests by IITA and national partners across the region (Maredia and Raitzer 2006). This is a truly awesome success and as such it is appropriate that one asks the question "what factors and players were important for the agenda and priority setting process that led to this long-term endeavor" (Ortiz and Crouch 2006). Not surprisingly the answer is complex, since a broad range of actors can be acknowledged: scientists who were on target with their ideas and translating them into research undertakings, stakeholders and clients who guided their priority setting, managers who supported the scientists and sought the resources for implementing their research, and donors who were convinced by the arguments from managers and scientists and were then willing to invest in this research-for-development agenda that relies on producing locally, minimizing risks and creating wealth in sub-Saharan Africa.

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An Innovative Ratooning Technique for Rapid Propagation of Cassava in CÔTE D'IVOIRE

Boni N'zue, Sékou Doumbia, Abdourahamane Sangare, Goli Pierre Zohouri and Christophe Kouame

Centre National de Recherche Agronomique (CNRA)

Abstract

Cassava is grown on approximately 80 % of the territory of Côte d'Ivoire. It constitutes the second food crop after yam with a total production estimated at 1.5 million t. It becomes both a subsistence and cash crop for farmers. The low rate of current propagation of cassava limits its extension and the on farm diffusion of new cultivars. To overcome this constraint, researchers carried out an experiment during two consecutive years in Côte d'Ivoire. It consisted in taking cuttings on growing plants at 10 cm, 35 cm and 60 cm from the soil of three cassava cultivars 7 months after planting. Control was non-ratoon plants. Plants and tuberous roots were harvested 8 month after ratooning. Results showed that at 35 and 60 cm from soil, the loss in dry matter was significant. However, at 10 cm from soil, the rates of loss in yield and dry matter of tuberous roots were low and were estimated at less than 3.5%. Moreover, at that level, the quantity of cuttings was the highest. Applying this technique, farmers will solve the lack of available on farm planting material.

Introduction

Cassava is mainly grown for its tuberous roots, which constitute an important source of energy for consumers. Leaves are sometimes used as vegetable. Cassava is vegetatively propagated by cuttings and sexual reproduction is applied in research stations for cultivar improvement. The

low rate of current field propagation (10) prompted the development of new techniques of rapid multiplication, namely, micro-propagation by in vitro culture and mini-propagation. These techniques significantly increase the rate of multiplication. However, these innovations require financial means and technical skills, which limit their adoption. Moreover, they do not permit farmers to have high quantity of quality tuberous roots.

In Côte d'Ivoire, the lack of planting material constitutes a constraint to the adoption of cassava crop by farmers. Cuttings are sold in form of small faggots whose cost can exceed FCFA 20000 (US$ 40) for 1 ha of planting (Ndabalishye 1995). Experimentation was therefore conducted at research station during two successive years in Côte d'Ivoire to determining levels of ratooning of cassava plants, which permit to obtain cuttings before root harvest without significantly deteriorating the essential traits related to tuberous roots.

Material and methods

The plant materials included one landrace (Yacé) and two local improved cultivars (84/701 and 89/130) (Table 1). Experiments were carried out at a research station at Bouaké in the Center of Côte d'Ivoire from 1996 to 1998. The experimental design was a split-plot arrangement with four replications. The principal factor and the secondary factor were cultivar and ratooning respectively. Four levels of ratooning were defined, namely (i) control (non- ratoon), (ii) ratooning at 60 cm from soil, (iii) ratooning at 35 cm from soil, and (iv) ratooning at 10 cm from soil. Each useful plot comprised 21 plants in 21 m² with 1 m between rows and 1 m between plants. Cuttings were horizontally planted in ploughed soil. Tuberous roots were harvested 15 months after planting.

Table 1. Traits of cassava cultivars used in this experiment

Cultivar	Origin	Yield (t ha^{-1})	Dry matter (%)	Resistanc To mosaic	Resistance to mealybugs	Resistance to mites
Yacé	On farm (CI)	20	40	weak	weak	weak
84/701	IDESSA (CI)	30	35	moderate	good	good
89/130	IDESSA (CI)	28	40	moderate	moderate	moderate

CI: Côte d'Ivoire. IDESSA: Institut des Savanes

The observations and measurements on plants were as follows:

• Number of cuttings At ratooning, 7 months after planting, the number of suitable cuttings that can provide the ratoon plants was recorded. The control was not taken into account in the analysis of variance since it remained intact.

• Yield in fresh tuberous root At harvest all tuberous roots per plot were weighed and yield was noted.

• Rate of dry matter At harvest samples of tuberous roots were taken in plots. The roots were peeled and cut into small washers of approximately 2 g each. Samples of 200 g were spread out over aluminium foil and introduced into a drying oven at 90 °C during 24 hours. Root dry matter was weighed.

Statistical analysis Data were subjected to an analysis of variance using GLM (General Linear Models Procedure) and means tested through the least significant difference at 5 % level.

Results and Discussion

First year (1996/1997) Number of cuttings There was a significant difference between the three cutting levels. The ratooning at 10 cm from soil could provide the greatest number of suitable cuttings with an average of 4.7 cuttings per plant whereas the weakest average (1.5 cuttings per plant) was recorded at 60 cm from the soil (Table 2). For a density of 10,000 plants ha^{-1} and a ratooning at 10 cm from the soil, it is possible to obtain 47,000 cuttings, which can be planted on a surface of at least 4 ha. A ratooning at 60 cm produces only 15,000 cuttings, i.e., being able for planting 1.5 ha. The clone 89/130 provided the greatest number of cuttings with 3.6 cuttings per plant (Table 3).

Table 2. Number of cuttings from cassava plants at 3 ratooning level 7 months after planting

Ratooning levels	Number of cuttings per plant		
	1996/1997	1997/1998	1996/1998
60 cm	1.5	1.0	1.2
35 cm	3.2	2.7	3.0
10 cm	4.7	4.9	4.8
Least significant difference (5%)	0.7	0.5	0.4
Mean	3.1	2.8	3.0
Coefficient of variation (%)	27	19	23

Yield The yields did not vary significantly across rationing levels neither if compared with the control, although losses of about 6.5%, 6.3% and 0.1% were recorded for the ratoon plants at 10, 35 and 60 cm from the soil, respectively (Table 4). There were significant differences amongst cultivars: the clone 84/701 recorded the best yield with 20.87 t ha^{-1} viz. a viz. 17.38 t ha^{-1} for clone 89/130 and 13.87 t ha^{-1} for the landrace Yacé.

Table 3. Number of cuttings obtained from 3 cassava cultivars 7 months after planting

	Number of cuttings per plant		
Cultivar	1996/1997	1997/1998	1996/1998
Yacé	2.7	2.9	2.8
84/701	3.0	3.2	3.1
89/130	3.6	2.4	3.0
Least significant difference (5%)	0.7	0.5	0.4
Mean	3.1	2.8	3.0

Table 4. Influence of ratooning levels on cassava traits

Ratooning levels	**WTRP (kg $plant^{-1}$)**	**WTR (kg per tuberous roots)**	**Yield (t ha^{-1})**	**IRY[1]**	**Dry matter (%)**	**IRM[2] (%)**
Control	2.04	0.51	17.96	—	38.3	—
60 cm	1.88	0.48	17.94	- 0.1 %	36.9	- 3.7
35 cm	1.89	0.44	16.82	- 6.3 %	36.1	- 5.7
10 cm	1.96	0.52	16.79	- 6.5 %	36.2	- 5.5
LSD (5%)	0.27	0.06	2.82	—	2.5	—
Mean	1.94	0.49	17.37	—	36.9	—
Coefficient of variation (%)	17	14	19	—	8	—

LSD: Least significant difference. WTRP: weight of tuberous roots per plant. WTR: weight of tuberous roots
(1) IRY: increase rate of yield compared with control. (2) IRM: increase rate of dry matter compared with control

Rate of dry matter The stem cutting on plants did not present a significant effect on dry matter in spite of losses of about 3.7 %, 5.7 % and 5.5 % for the ratoon plants at 60, 35 and 10 cm from the soil, respectively (Table 4). These results did not agree with those reported by Osiname and Landu (1992), who showed that leaf harvest significantly increased dry matter

content of tuberous roots of cultivars Kinuani and Mpelo-longi; but it did not have a significant effect on cultivar F100. The non-significant losses of dry matter could be explained by the fact that the ratoon plants could compensate the losses during the 8-month period from rationing to harvest due to the regeneration of new organs such as leaves or stems. Dry matter of Yacé and 89/130 were almost identical and significantly higher than that of variety 84/701 (Table 5). The dry matter rates can be comparable to those shown in Table 1; i.e., without ratooning.

Table 5. Agronomic traits of 3 cassava cultivars 15 months after planting

	Yield (t ha^{-1})			Rate of dry matter (%)		
Cultivar	1996/1997	1997/1998	1996/1998	1996/1997	1997/1998	1996/1998
Yacé	13.87	21.43	17.65	38.5	34.8	36.6
84/701	20.87	32.53	26.70	34.8	37.2	36.0
89/130	17.38	27.98	22.68	37.3	41.4	39.3
LSD (5%)	2.44	1.70	1.45	2.2	1.3	1.2
General mean	17.37	27.31	22.34	36.9	37.8	37.3

LSD: Least significant difference

Second year (1997/1998) Number of cuttings The number of cuttings significantly varied through ratooning levels. The highest number of cuttings (4.9 cuttings per plant) was obtained when the plants were ratooned at 10 cm from the soil. The ratooning at 60 cm can only provide an average of one cutting per plant (Table 2).

Yield Non-significant losses of yield were recorded; about 3.6% for 60 cm and 5.2 % for 35 cm viz a viz. the control. Ratooning at 10 cm increased, although non-significantly, yield –about 2.6 % versus the control (Table 6). The superior yield of cuttings at 10 cm could be explained by the repeated defoliation of control plants caused by locusts, and by the combined action of mealybugs, mites and mosaic disease, which were less on ratoon plants at 10 cm than on ratoon plants at 60 cm and 35 cm. The cultivar 84/701 recorded the best yield with 32.53 t ha^{-1} versus 27.98 t ha^{-1} of cultivar 89/130 and 21.43 t ha^{-1} for landrace Yacé (Table 5).

Rate of dry matter Ratooning did not affect significantly dry matter, although losses of 3.6, 1.8 and 0.5 % were recorded respectively for cut-

tings at 60, 35 and 10 cm from the soil (Table 6). The small coefficient of variation (5 %) indicates the reliability of the data collected. Dry matter of landrace Yacé fell significantly to 34.8 % versus the initial 40 % 7 months after planting (Table 1), and became the weakest (Table 5). This fall could result from the use of a part of starch stored in the tuberous roots for the synthesis of new leaves further to the repeated devastation of organs caused preferentially by locusts on the landrace Yacé.

Combined over two years (1996-1998) Number of cuttings There were 4.8, 3 and 1.2 cuttings per plant at 10, 35 and 60 cm ratooning from the soil, respectively. The analysis of variance indicated that numbers of cuttings were significantly different according to rationing levels (Table 2). The number of cuttings did not however vary significantly among cultivars. The general average was 3 cuttings per plant (Table 3).

Yield The ratooning did not have a significant effect on tuberous root yield and average weight. Non-significant losses of 2.2%, 5.5% and 0.1% were recorded for cuttings at 60, 35 and 10 cm from the soil, respectively (Table 7). Previous research by Dahniya (1981), Osiname and Landu (1992), and Lutete et al. (1992) revealed that leaf harvest significantly reduced tuberous root yield. Dahniya (1981) showed that the harvest of leaves at 1-, 2- or 3-month intervals reduced yield by 56 to 76%, 34 to 62 %, and 15 to 32 %, respectively. Lutete et al. (1992) reported 49.2% yield loss if three successive harvests were carried out. In our research, the non-significant yield losses could result from the increased photosynthetic activity of leaves regenerated after ratooning. For the period from cutting to root harvest, this activity would favour the plants and compensate carbohydrate reserves, which were used for the synthesis of new organs (e.g. leaves, stems). There was a significant difference between yield of the two cultivars and the landrace. The clone 84/701 was the highest yielding (26.7 t ha^{-1}) followed by 89/130 (22.68 t ha^{-1}) and Yacé (17.65 t ha^{-1}) respectively (Table 5).

Table 6. Influence of rationing levels on cassava traits

Ratooning levels	WTRP (kg plant^{-1})	WTR (kg per tuberous roots)	Yield (t ha^{-1})	IRY [1] (%)	Dry matter (%)	IRM [2] (%)
Control	3.26	0.57	27.74	—	38.4	—
60 cm	3.18	0.59	26.75	- 3.6	37.0	- 3.6
35 cm	3.23	0.58	26.31	- 5.2	37.7	- 1.8
10 cm	3.42	0.59	28.45	2.6	38.2	- 0.5
LSD (5 %)	0.30	0.05	1.97	—	1.5	—
Mean	3.27	0.58	27.31	—	37.8	—
Coefficient of variation (%)	11	10	9	—	5	—

LSD: Least significant difference. WTRP: weight of tuberous roots per plant. WTR: weight of tuberous roots
[1] IRY: increase rate of yield compared with control. [2] IRM: increase rate of dry matter compared with control

Table 7. Influence of rationing levels on cassava trait across two years (1996-1998)

Ratooning levels	WTRP (kg plant^{-1})	WTR (kg per tuberous roots)	Yield (t ha^{-1})	IRY [1] (%)	Dry matter (%)	IRM [2] (%)
Control	2.65	0.54	22.85	-	38.4	-
60 cm	2.57	0.55	22.34	- 2.2	36.9	- 3.9
35 cm	2.56	0.51	21.56	- 5.6	36.9	- 3.9
10 cm	2.65	0.54	22.62	- 0.1	37.2	- 3.1
LSD (5 %)	0.20	0.04	1.68	-	1.4	-
General mean	2.61	0.53	22.34	-	37.3	-
Coefficient of variation	13 %	12 %	13 %	-	7 %	-

LSD : Least significant difference. WTRP : weight of tuberous roots per plant. WTR : weight of tuberous roots
[1] IRY: increase rate of yield compared with control. [2] IRM: increase rate of dry matter compared with control

In summary, this research showed that it is possible to ratoon cassava plants at 10 cm from the soil surface 7 months after planting without affecting yield and dry matter in tuberous roots. Ratooning of plants at 60 and 35 cm caused however a significant loss of dry matter (3.9%). At 10 cm cutting, the number of cuttings was the highest and losses of yield and dry matter were non-significant. On poor soils, the ratoon plants at 10 cm could however need increased fertilizer requirements to improve growth and root yield.

Acknowledgments

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Small-Scale Cassava Processing and Vertical Integration Into the Cassava Sub-Sector in Uganda

Jane Nabawanuka-Oputa and J.A. Agona
National Post Harvest Program,
Kawanda Agricultural Research Institute,
PO Box 7065 Kampala, Uganda

Introduction

Uganda is a landlocked country astride the equator, about 800 km inland from the Indian Ocean (Fig. 1). It lies on the northwestern shores of Lake Victoria. The area of Uganda is about 241,551 km^2 where about 26.8 million people live. The agricultural sector dominates the Ugandan economy and most industries and services in the country depend on the agricultural sector. The contribution of agriculture to total GDP was about 33% in 2004.

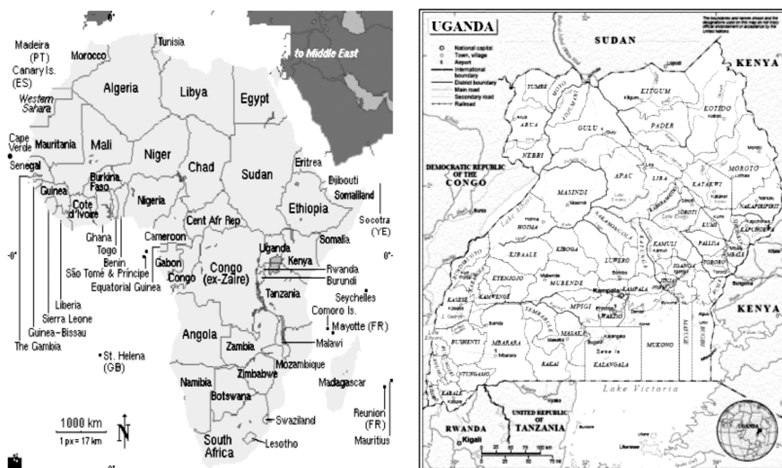

Fig. 1 Map of Africa and Uganda

Cassava in Uganda

Cassava is becoming an important food item as well as an important industrial raw material in Uganda. In the early 1990s, a new and virulent strain of the mosaic virus — named the Uganda variant of African Cassava Mosaic Germinivirus (ACMV) — attacked 80% of the country's 500,000 hectares under cassava cultivation. By 1994 researchers at the Namulonge Animal and Agricultural Research Institute developed three new cultivars. Farmers evaluated these new cultivars for their taste, color and texture, in addition to their ACMV resistance.

Small-scale cassava processing and vertical integration into sub-sector in Uganda

The overall goal of the project is to enable the development of cassava products as widely traded commodities that contribute to the economic growth of cassava growing countries in Southern and Eastern African and to strengthen the cassava sub-sector with sustained links between suppliers and users of cassava products. We aim to developing the income generating potential of cassava as a cash crop by providing simple market-oriented technologies to small holder farmers and enabling them to transform highly perishable cassava into stable market grade intermediate products like chips or flour. Our main objectives are to conduct sub-sector analysis and identify promising cassava products, their marketing infrastructure, the location of pilot project sites and the stakeholders; to develop, on a pilot scale, an appropriate village-level processing system for the supply of high quality cassava products (HQCPs) to the identified markets, to establish systems for ensuring the sufficient & timely supply of raw materials for the operation of the processing systems at pilot sites, to establish or identify the most appropriate organisational structures to facilitate the participation of farmers and other related groups and institutions to facilitate the delivery of HQCPs; to build capacity of farmers and other participants to contribute to the strengthening of the cassava sub-sector; to provide access to other interested parties knowledge and experience gained from the pilot projects; and to ensure uninterrupted and efficient implementation of the project activities, assess the progress made and the impact generated by this project. The project partners are listed in Table 1.

Table 1. Project partners for small-cassava processing

Institution	Role
National Post-Harvest Program Kawanda Agricultural Research Institute	Post-harvest technologies (e.g. for processing), farmer training , coordination of project activities
Namulonge Animal and Agricultural Research Institute (NAARI) EARRNET	Provision of planting material and production technologies
Private sector (Millers)	Market outlet for quality dried chips
Private sector (Tonnet Enterprises)	Fabrication of equipment; e.g. chippers
Non-governmental organization (Africa 2000 Network)	Mobilization of farmer groups
Farmer groups	Production of quality cassava chips
Microfinance institutions	Provide credit facilities to farmers

Project activities

Cassava Sub-sector Analysis The project aims to identify, products, producers and processors, buyers, location, quantities demanded. It also selects pilot site and participants and will be developing a marketing strategy.

Processing Technology Available technologies are being assessed by the project, which also selects and introduces appropriate technologies, and organizes pilot operations.

Fresh Cassava Production The project established a system for supplying raw material to the industry, and assists in multiplication sites by supplying improved planting materials.

Organization of farmers, processors and other institutions Stakeholder meetings, appropriate organizational structures for the mobilization of farmers, processors and end-users, and the coordination structure have been set by the project.

Training Training need assessment was undertook on the basis of the pilot operations. The formulation and further implementation of a training program was also among the project undertakings.

Dissemination of projects output An information dissemination strategy was developed. We also compile experience and lessons learned and prepared dissemination materials, which were further distributed. We organize workshops for prospective participants in project activities

Project implementation, monitoring, evaluation and impact assessment The project has established a reference data for sub-sector analysis. It

also undertakes research to determine the factors influencing the adoption of introduced cassava production, processing and utilization technology, and will assess their impacts.

Opportunities

At the project level dried cassava chips for integration into animal feed industry was the target product due to the annual demand for feed; i.e., about 80,000 t in Uganda. The integration of 10 to 25% of dried cassava chips into animal feeds produces good results, which suggest between 8,000 to 20,000 t of chips for animal feeding. The price of cassava chips was about 75% that of maize (Fig. 2).

Setting up of pilot sites

Traditional practices are used for manual peeling, slicing, drying and milling. Farmgates and local markets are the sale points for cassava and its based products.

Effects of traditional practices We are researching issues such as use of simple tools and occupational hazards, drying of cassava chips (which takes 5 to 7 days depending on the weather), the reasons for poor quality chips (Fig. 3), potential mycotoxin contamination (e.g. aflatoxin levels >20 ppb). The quality of traditionally processed dried cassava chips could be significantly affected by mycotoxins Small quantities are processed and sold by individual processing and marketing. The price of dried cassava chips is about UShs 180 kg^{-1}.

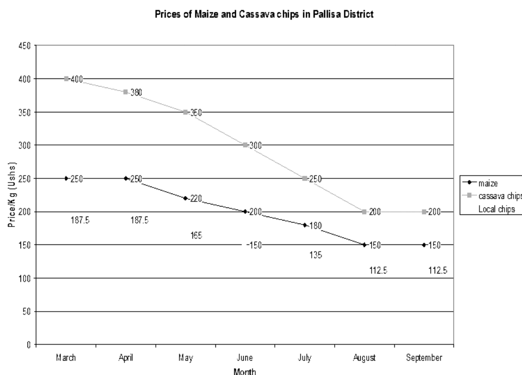

Fig. 2. Maize and cassava chip prices in Pallisa district

Fig. 3. Traditionally processed cassava chips

Project interventions Pilot sites were selected considering the supply of raw materials (cassava roots) for processing, and the presence of organized farmer groups (participants) Sensitisation campaigns created awareness on the opportunities in cassava production, processing and marketing. We provided hands-on training cassava processing and quality maintenance, and facilitated farmers work with chippers (motorized or manual), enhanced their drying technologies using raised racks, biomass dryer, polyethylene sheets, improved store, water containers Weighing scales and improved local ovens were also given to the project participants. The drying period of processed chips guaranteed within less than 2 days in the hot dry season. The project facilitated participating farmer groups to form an association marketing named Kibuku cassava processors association (KICAPA). Farmers were trained in processing quality cassava chips, group dynamics, machine operation and maintenance, processing cassava- based snacks (bread with 21% cassava flour, cakes, cookies), business skills (business environment, business management, record keeping, marketing). Feed millers were also trained in utilization of cassava in feed formulation.

Achievements

Aflatoxin contamination levels were significantly reduced (0-0.5%) and the price increased (USh 250-400 kg^{-1}) as a result of this project, which provided means for producing high quality cassava chips that were processed using appropriate methods (Fig. 4).. The new technology adopted includes the peeling of cassava skins prior processing and the use of cassava clones such as ‘TME14’ and ‘2691’. The farmers and processors are now operation in groups, and farmers are now able to access input credits. The dried cassava chip for

food strategy was adopted whereas the feed strategy was dropped due to low price and poor commitment by buyers because of small volumes available.

Fig. 4. High quality cassava chips processed using appropriate methods

Conclusion

Cassava could contribute to increased agricultural transformation and economic growth in developing countries but it has to become more competitive in domestic and international markets Cassava has a high income generating potential and can enable resource poor smallholder producers to improve livelihoods once they adopt and use appropriate production, processing and marketing opportunities as prescribed and provided by this project. The policies in Uganda should favour industrial utilization of cassava to encourage uptake as raw material, and hence stimulate increased production.

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Production of Cassava Bred Using Indigeneous Micro-flora and Improved Cultivars

Edema Mojisola Olayinka and Abiola Kehinde
Microbiology Department, University of Agriculture, PMB 2240, Abeokuta, Ogun State, Nigeria

Abstract

An important commitment to eliminate hunger and malnutrition in developing countries is the development of methods for utilizing local agricultural resources to design new food products. This research involved the production of a bread specialty from 100% cassava flour using starter cultures selected from dominant epiphytic micro-flora in spontaneously fermented cassava flour, genetic modification of cassava to produce low cyanide, and high flour yielding, quality protein cassava cultivars for bread-making in Nigeria.

Introduction

Microorganisms necessary in food fermentations may be added as pure single or mixed cultures. Although in some instances, no cultures may be added if the desired microorganisms are known to be abundantly enough in the original raw material. Even then, research shows it is better to use starter cultures as there are usually other numerous, sometimes undesirable and competing microorganisms. The use controlled starter cultures drastically reduces the period of fermentation, sometimes more than half depending on the amount of starter used and its viability. Research on the use of starter cultures also shows that microorganisms develop niches where they thrive and to transplant an organism from one natural environment to another is not a good formula for success.

Bread is an important staple food in Nigeria. Its consumption is steadily increasing. It is however, relatively expensive; being made from imported wheat that is not grown extensively in the country for climatic reasons. In time past, there has been research on the use of composite flours for bread-making taking into consideration the natural resources of the geographical region involved (Yañez et al. 1981, Chauhan et al. 1992, Hounhouigan et al. 1993, Shalini and Sudesh 2004). Materials grown in the tropics include cereals (maize, sorghum and millet), starchy tubers (cassava, sweet potato and yam), while oil seeds (from soybean, bean/cowpea and groundnut) can be used as protein quality improvers. The use of cassava flour for bread making will reduce the import of wheat, and increases the production of cassava in the country. More than 35 million t of cassava, which is basically a carbohydrate food, is produced in Nigeria annually.

Many African foods are fermented before consumption and a variety of microorganisms are widely used as starter organisms in these food fermentations because they convert sugars into organic acids, ethanol, aldehydes, ketones, diacetyl and others, thus improving the organoleptic and rheological properties of the products. It is known that fermentation may enrich foods in protein by removing part of the fermentable carbohydrate as documented in fermented foods made from cassava such as gari and foofoo (Okafor 1977, Oyewole and Odunfa 1990). This work was therefore aimed at producing cassava bread from 100% cassava flour using indigenous micro-flora.

Materials and Methods

Collection and processing of samples Flour was produced from two cassava clones grown for 12 to 15 months, which were selected as most suitable for bread-making by the Root and Tuber Improvement Program of the International Institute for Tropical Agriculture (IITA, Ibadan, Nigeria). The low cyanide, high flour yielding cassava clones were processed into flour using the method described and illustrated by IITA (1990).

Microbiological analyses Cassava flour was prepared for natural fermentation by mixing equal amounts of each cassava flour type with sterile tap water (to avoid inoculating other organisms besides those originating from the flour) and allowing the mixture to ferment at room temperature for 48 h or until the pH fell to a stabilized level. From appropriate 10-fold dilutions, the pour plate technique was used to isolate microorganisms by the

method of Meynell and Meynell (1970). Colony-forming units (cfu) were determined on the following media, temperatures and incubation periods: de Mann Rogosa Sharpe (MRS) medium for lactic acid bacteria (Oxoid, U.K.) at 37°C for 48 h, Saboraud dextrose agar (SDA) medium for yeast and moulds (LAB M, idg plc,U.K) at 30°C for 72 h, plate count Agar (PCA) medium for total bacterial counts (Oxoid, U.K.) at 37°C for 72 h. MRS and one set of PCA plates were incubated anaerobically in anaerobic jars using Oxoid gas generating kit. Starter cultures were then selected from the purified and characterized isolates and used for cassava bread-making.

Analysis of fermented cassava flour The functional properties of the cassava flours [physico-chemical and visco-elastic (by the automatic visco-analyzer, AVA)] were determined by standard procedures.

Baking procedure The two flour samples were used in baking cassava bread. The amounts of other ingredients per 100g of cassava flour were: 10 g baking fat, 30 g sugar, 0.5 g salt, 0.1 g ascorbic acid, 1 ml starter culture (containing 10^6 to 10^7 cells per ml) and 120 ml water. All ingredients were weighed in a bowl and mixed (Philips hand mixer Type HR 1453) for 10 min. at high speed. The mixture was allowed to stand for 4 h at room temperature for batter development with gentle mixing for another 5 min, after which the batter was scaled (batter weight = 150g) into greased baking pans. Baking was at 160°C to 180°C for 35 min in a Moulinex OPTICHEF Oven Model BH5 with timer. After baking, the loaves were left for about 10 min in the oven. They were then quickly removed from the pans, arranged in trays and returned to the oven for 1 h before for analysis. Analyses were carried out after the baked loaves had attained room temperature or internal crumb temperature was about 35±2°C. The effect of using indigenous micro-flora as starter cultures for cassava bread was assessed with bakers' yeast- leavened bread serving as control.

Results and Discussion

Two cassava flour samples from different clones were tested for cassava bread making using indigenous micro-flora as inoculum. The final pH of the spontaneously fermented cassava flour was 4.2 and 3.7 for the flours A and B respectively. These values were lower than values obtained by previous workers (Assanvo et al. 2006). The visco-elastic properties of the flours are shown in Table 1 while the physico-chemical properties are given in Table 2.

Table 1. Visco-elastic properties of fermented cassava flours (The units are in RVA except where otherwise stated)

Flour	peak	trough	breakdown	Final viscosity	Setback	Pasting time (min)	Pasting temperature (°C)
A	294.92	127.58	167.33	164.17	36.58	4.47	76.75
B	169.00	128.25	40.75	169.25	41.00	5.27	78.05

Table 2. Physico-chemical properties of fermented cassava flours

Flour	Amylose (%)	WAC (%)	Swelling power (%)	Solubility (%)	Flour protein (%)
A	19.43	83.74	7.85	6.92	27.4
B	18.33	72.58	7.35	6.58	29.2

WAC – Water Absorption Capacity

It was observed that the pasting temperatures observed in this research were higher than those observed by Eggleston et al. (1993), whereas final viscosity, swelling power and solubility values were comparatively lower in this research. Total plate counts were in the order of 10^5 to 10^7 cfu g^{-1} of flour (Table 3).

Table 3, Total counts (cfu g^{-1}) of microbial groups in fermented cassava flours

Flour	PCA Counts	MRS Counts	SDA Counts
A	5.8×10^7	3.6×10^6	3.5×10^5
B	5.5×10^7	4.1×10^6	3.7×10^5

The range was quite comparable to those observed by previous workers (Eggleston et al. 1993; Assanvo et al. 2006). Yeast, lactic acid bacteria, aerobic and micro-aerophilic bacilli and other bacteria were isolated. This was not surprising as the trend for the fermentation of cassava products is generally the same. However, only yeast and lactic acid bacteria were selected and tested as starter cultures for cassava bread-making. Lactobacillus plantarum, L. brevis, Leuconostoc dextranicum, Saccharomyces cerevisiae, Candida tropicalis and Schizosaccharomyces pombe were selected and used as starters for cassava bread-making while bakers' yeast leavened bread

served as control. The bread samples from lactic acid bacteria starters were sour in taste but quite acceptable to many of the consumers (51%) used for the acceptability test, though they were not considered to be bread but acceptable as a new snack in Nigeria. The yeast starters gave products comparable with the bakers' yeast leavened bread sample and were also acceptable (46%). A few of the consumers were indifferent to the bread samples (3%). It appears that more work is required to effectively determine which starter (s) will be best for this new bread specialty. This research has however shown that the baking quality of cassava flour was improved by using starter cultures selected from indigenous micro-flora of the cassava flour.

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Cyanogenic Potential of Cassava Cultivars Grown Under Varying Levels of Potassium Nutrition In Southwestern Ethiopia

Solomon Endris
Ethiopian Institute of Agricultural Research (EIAR), Jimma Center, PO Box 192, Jimma

Abstract

Three cassava cultivars (NR-44/72, NW-45/72 and OY-44/72) were grown at 0, 50, 100, 150, 200 and 250 kg K_2O ha^{-1} in a field experiment at Jimma Agricultural Research Center. The experiment was carried out to determine the effect of potassium nutrition on root cyanide (HCN) content of cassava cultivars. The three cultivars investigated in this experiment were found to have inherently high HCN content; however, there was a significant variety by potassium interaction in which the lowest root HCN content (50.65 ppm) was recorded for cultivar NR-44/72 at a potassium rate of 250 kg K_2O ha^{-1}, while the highest root HCN (108.37 ppm) was found in cultivar OY-44/72 at the control (no potassium). A significant cultivar difference for HCN content was also observed. Cultivar OY-44/72 had the highest level of HCN (80.59 ppm), while cultivar NR-44/72 contained the lowest level of HCN (71.76 ppm) in its root.

Introduction

Cassava is widely cultivated in many parts of the world. The carbohydrate rich but low in protein storage roots represent an important energy source and are a staple foodstuff for more than 500 million people throughout tropical Africa, Latin America and parts of Asia (Yeoh et al. 1998). Cassava can grow under poor soil conditions and can withstand drought. It is therefore

usually considered as an important famine reserve crop in countries with unreliable rainfall. Although cassava can grow on poor soils, adequate levels of nitrogen (N) and potassium (K) are required for optimum top growth and tuber yields (Obigbesan and Fayemi 1976, Howeler 1991). Hence, soils that have low N (< 0.10 % total N) and K (< 0.15 meq per 100g) will require fertilization for optimum tuberous root yield (Kang and Okeke 1991).

Cassava contains the potentially toxic compounds cyanogenic glucosides. If present in sufficient quantities, these compounds can cause acute cyanide poisoning and death in humans and animals when consumed. At concentrations less than 50 ppm, cassava products are considered harmless. Consumption of such non-toxic cassava over long periods of time results however in chronic toxicity (Food Safety Network 2005). There are over 5000 known phenotypically distinctive cassava cultivars (Best and Hargrove 1993). All contain varying concentrations of the cyanogenic glucosides linamarin and lotaustralin, which are hydrolyzed to hydrogen cyanide (HCN) by endogenous linamarase when the tissue is damaged (Haque and Bradbury 2003, Wilson, 2003). The cyanogenic potential of known cassava cultivars ranges from less than 10 mg kg^{-1} as HCN fresh weight basis to more than 500 mg kg^{-1} as HCN fresh weight basis (O'Brien et al. 1994).

Consumption of cassava and its products is thought to cause cyanide poisoning with symptoms of vomiting, nausea, dizziness, stomach pains, weakness, headache and diarrhea and occasionally death (Mling et al. 1992, Akintonwa et al. 1994). Moreover, high dietary cyanogen exposure from poorly processed cassava roots may be associated with the occurrence of the neurological disorder konzo –an irreversible paralysis of the legs (Ernesto et al. 2002). It is therefore crucial to characterize cassava cultivars based on their cyanogenic potential and assess factors affecting level of HCN in cassava roots such as growing conditions and plant nutrients so that cultivars for household consumption and industrial use can easily be identified and better strategies to reduce HCN content in cassava can be devised.

One strategy to reduce the cyanide content of processed cassava is to improve processing methods used for conversion of roots to storable cassava products such as flour. The major methods of flour making in Africa involve sun drying of peeled roots followed by crushing in a pestle and mortar and sieving. This method retains 25 to 33% of the original linamarin present (Cardoso et al. 2005). Bradbury (2004) also indicated other methods (such as heap fermentation), which are known to remove twice as much linamarin

as does sun drying, but still 12.5 to 16.5 % of linamarin is retained because of the lack of intimate contact between the linamarin that is located inside each tiny cell and the hydrolyzing enzyme linamarase that is located in each cell wall. He further described that in order to produce cassava flour with 10 mg HCN equivalents per kg of flour (ppm) –i.e., the WHO safe level, one would need to use sweet cassava roots containing not greater than 32 ppm linamarin. It is therefore clear that attempts made to reduce the HCN content of cassava roots and products to safe levels by using processing techniques alone are not successful. Hence, manipulation of the growing conditions such as moisture and mineral nutrients is also very crucial.

There seems to be some irregularity in results obtained from experiments involving the relationships between some nutrients especially potassium (K) and the content of cyanogens in cassava roots. Many authors have reported significant reduction in the hydrocyanic acid (HCN) content of cassava tubers in response to potassium fertilization (Susan John et al. 2005, El-Sharkawy and Cadavid 2000, Tandon and Sekhon 1988). Attalla et al. (2001) described however results of a field experiment where high HCN level in tuber tissues of cassava was noticed with increasing rates of potassium fertilizer (K_2SO_4). Hence, the objective of this research was to evaluate the effect of potassium fertilization (as muriate of potash, KCl) on the cyanogenic potential of three cassava cultivars under the prevailing conditions of southwestern Ethiopia.

Materials and Methods

Description of the study area The experiment was carried out at Jimma Agricultural Research Centre located 343 km southwest of Addis Ababa at an altitude of 1753 meters above sea level, 7°46'N and 36°E. The area receives an average annual rainfall of 1595 mm. The mean minimum and maximum temperatures of the area are 11.3 and 25.9 °C respectively. The soil is slightly acidic (Table 1), and it is classified as Nitosol (Paulos and Tesfaye 2000).

Field experiment A factorial combination of three cassava cultivars (NR–44/72, NW–45/72 and OY – 44/72) at six potassium rates (0, 50, 100, 150, 200, and 250 kg K_2O ha^{-1}) were investigated in a randomized complete block design with three replications. A composite soil sample was taken initially (before treatment application) for analysis. Soil and plant tissue

samples were also taken at harvesting. The experimental site was uniform and previously under cassava cultivation with out any fertilization.

Table 1 Soil nutrient composition of the trial site obtained from 15 cm soil depth

Soil properties	
pH	5.3
Sand (%)	30
Silt (%)	10
Clay (%)	60
OC (%)	4.2
Total N (%)	0.20
Available P (ppm)	4.43
Extractable K (ppm)	34

HCN Analysis Analysis of total cyanogens was made using the simple picrate paper kit developed by Bradbury et al. (1999). The visual comparison with the color chart was done in addition to the more accurate absorbency method. The absorbance of the solution produced by immersing the exposed picrate papers to the sample in 5.0 ml H_2O for 30 min was measured at 510 nm using a spectrophotometer (Spectronic 1201) against a blank solution obtained by immersing picrate paper with out exposure to the sample. The content of HCN (ppm) was finally calculated using the following formula:
Total cyanogens (ppm) = Absorbance x 369

Data analysis was done using MSTATC statistical software.

Results and Discussion

Analysis of tissue samples for HCN indicated that the three cultivars exhibited inherently high and significant root HCN content ($P < 0.05$) (Table 1). The cultivar OY – 44/72 had the highest HCN (80.59 ppm) while NR – 44/72 had the lowest HCN content (71.3 ppm).

Table 1. Mean cultivar distribution of hydrogen cyanide (HCN) in cassava (N = 3)

Cassava cultivars	HCN (ppm)
NR – 44/72	71.76
NW – 45/72	71.99
OY – 44/72	80.59
Standard error	2.91
Coefficient of variation (%)	14.7

The data indicated existence of cultivar difference for HCN content in the three cassava cultivars investigated in this experiment (Table 1). The results are therefore in line with previous reports that the amount of HCN in cassava varies strongly according to cultivars, growing conditions and even different parts of the same plant (Bradbury et al. 1999, Food Safety Network 2005).

Cultivar by potassium interaction was highly significant ($P < 0.01$) (Table 2). Hence, the cyanide level in the roots of variety NR-44/72 supplied with the highest potassium rate (250 kg ha^{-1} K_2O) was 50.65 ppm whereas cultivar OY-44/72 –grown without potassium (control), had a root HCN content of 108.37 ppm. In general, the level of HCN content exhibited a consistent trend of reduction with increasing rates of potassium especially at the higher rates (Fig.1). The results obtained in this experiment are therefore in harmony with results reported by various authors (Howeler 2002, Food Safety Network 2005; Tandon and Sekhon 1988). El-Sharkawy and Cadavid (2000) also found reduced root HCN levels in cassava plants grown with potassium fertilizer. Similarly, from a long term fertilizer experiment with cassava, Susan John et al. (2005) reported low HCN content of roots in cassava plants supplied with ash and crop residues which are known to have high content of K, Ca and Mg.

Table2. Hydrogen Cyanide content of cassava roots affected by the interaction between cultivar and potassium fertilization

Variety	Potassium (kg ha^{-1} K_2O)						Variety mean
	0	50	100	150	200	250	
NR-44/72	66.45	61.28	103.22	78.80	70.17	50.65	71.76
NW-45/72	58.63	88.77	76.65	78.65	66.20	63.08	71.99
OY-44/72	108.37	85.33	81.58	81.37	65.43	61.48	80.59
K means	77.82	78.46	87.15	79.61	67.27	58.40	
Coefficient of variation (%) = 14.68							
$LSD_{0.05}$ = 18.21							

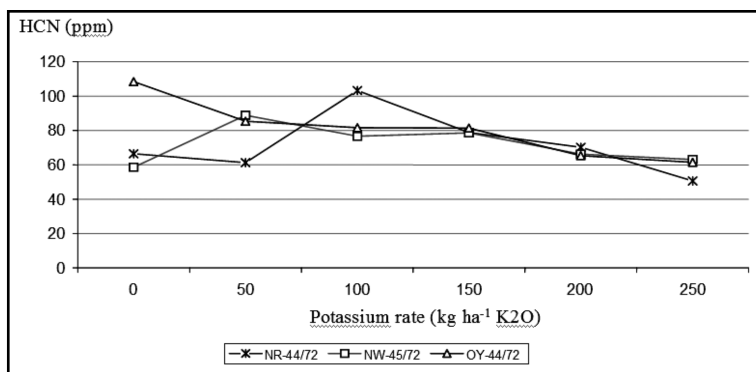

Fig. 1. The effect of potassium application on cyanide (HCN) content of different cassava cultivars

The presence of adequate level of K nutrition promotes CO_2 assimilation and the translocation of carbohydrates from the leaves to the tubers of potatoes, which is the reason why the starch content of tubers is high in potatoes when well supplied with potassium (Lachover and Arnon 1966 cited in Mengel and Kirkby 1982, Howeler 2002). Likewise, tubers or tuberous roots of crops where carbohydrates are the main storage material, such as sweet potato, cassava, yam and others respond in a similar way to nutrition (Mengel and Kirkby 1982). Obigbesan (1973) also indicated that in cassava tuberous roots not only was the starch content enhanced by potassium, but the content of the poisonous cyanide also decreased.

In summary, the potentially toxic HCN content in cassava roots was significantly reduced by potassium application. At lower doses of potassium application root HCN content was relatively high. It substantially decreased at higher rates of potassium, which indicates the need for further experimentation with more cultivars and other sources of potassium. Although potassium is found important in reducing the HCN content of cassava roots, other locally available and cheap sources of potassium such as wood ash can alternatively be used by the mainly subsistent farmers who usually cultivate the crop

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"Bitter" Cassava: Toxicity and Detoxification

Darna L. Dufour
Department of Anthropology, University of Colorado, Boulder, Colorado, 80309 USA

Abstract

Cassava is an interesting food crop because both the roots and leaves contain cyanogenic glucosides (primarily linamarin) which can yield hydrogen cyanide (HCN), a potent toxin. All cassava cultivars contain cyanogenic glucosides but amounts vary greatly depending on both genotype and the effects of local edaphic conditions. Cultivars with high levels of cyanogens are potentially toxic and commonly referred to as being "bitter." The processing of bitter cassava roots and leaves for food typically reduces, but does not eliminate the presence of cyanogens. The manner in which cassava is processed varies widely. In South America the roots are typically macerated, dewatered and baked as flat bread, or water soaked, macerated and dry toasted into farinha (a coarse meal). In Africa the roots are commonly processed into gari, a product similar to farinha, a fermented paste, or sun dried and pounded into a flour. In Africa, but not in South America, health problems, such as acute toxicity, konzo (a neurological disorder) and tropical ataxic neuropathy, have been associated with the residual cyanogens in cassava-based foods. In many instances these health problems have been linked to conditions of nutritional vulnerability such as drought. In addition, dietary exposure to cyanogens has been linked to iodine deficiency disorders in populations with low dietary iodine intake.

Introduction

Cassava is a perennial woody shrub with edible roots and leaves native to the neotropics. It is propagated vegetatively and the species as a whole is composed of many different clones which show considerable diversity in a

wide range of characteristics (Lozano 1985). It is also a crop of many names. In English both the plant and the edible roots are referred to as cassava or manioc, terms most likely derived from native peoples. The term cassava is probably derived from Arawak word for bread, casavi, or cazabi (Gade 2003), and manioc from the Tupi word maniot which French speaking explorers converted to manioc (Gade 2003). In Portuguese the plant is referred to as mandioca, a term thought to have been derived from the Tupi-Guarani word mandiog (Gade 2003). In Spanish speaking countries of Latin America, the term is yuca, a Taino Indian word from Hispanola (Gade 2003).

Toxicity of cassava

Cassava is an interesting food crop because it is cyanogenic and capable of releasing cyanide in quantities that are potentially toxic to humans. Both the roots and leaves contain cyanogenic glucosides, primarily as linamarin, which liberate hydrogen cyanide (HCN) upon hydrolysis (White et al. 1998). Cyanogenesis proceeds in two steps. In the first linamarin is converted to acetone cyanohydrin. This is initiated when the plant tissue is damaged and linamarin in the vacuole is brought into contact with the endogenous enzyme linamarase in the plant cell walls. In the second step acetone cyanohydrin is decomposed to yield hydrogen cyanide (HCN) and acetone, both of which are volatile. Cyanohydrins are relatively unstable and decompose spontaneously at pH > 5 or temperatures > 35°C. In leaves, but not in roots, the decomposition of cyanohydrins is catalyzed by hydroxynitrile lyase (HNL) (White et al. 1998). The cyanide containing compounds, cyanogenic glucosides, acetone cyanohydrin and HCN, are referred to collectively as cyanogens. Cyanogen content is usually reported on a dry matter basis in units of mg per kg, parts per million (ppm) or mg HCN equivalents per kg.

All cassava cultivars contain cyanogenic glucosides. The glucoside content of leaves in all cultivars is similar, but the glucoside content of roots varies with cultivar (Mkong et al. 1990, Cock 1985) and edaphoclimatic conditions (Coursey 1973, Bruijn 1983, Sinha and Nair 1968, Bokanga 1994). The total cyanogen content of roots ranges from < 20 to > 4000 mg per kg of dry matter with a mean of 314 ± 416.8 (range is < 10 to about 1000 on a fresh weight basis) (Wheatley et al. 1993). The distribution skewed with more cultivars of low cyanogenic potential (Bokanga 1994). The factors responsible for this variation are not well understood (McMahon et al. 1995). In general roots with a cyanogen content of < 100 mg per kg of

fresh weight are referred to a low in cyanogenic potential and the reminder as high in cyanogenic potential. The breakpoint of 100 between low- and high-cyanogenic cultivars on a fresh weigh basis is based on a scale originally proposed by Koch (cited in Bolhuis 1954) which was based on total hydrolysable cyanogens in peeled roots measured as HCN.

In all parts of the world where cassava is an important subsistence crop people distinguish the low- from high-cyanogenic cultivars, and sometimes treat the two as different crops. This distinction is coded in language. In English the terms are sweet cassava and bitter cassava, in Portuguese the distinction is between mandioca mansa (also macaxeira or aipim) and mandioca brava, in Spanish it is yuca dulce and yuca brava, in Tukano (northwest Amazon region) the distinction is makasera and kii (Dufour 1988), and in Tonga (Malawi) it is vyakuzizira and vyakubaba (Chiwona-Karlton et al. 2004). At the time of first European contact with Brazil, the native Tupi-Guarani distinguished aypi and maniot (Metraux 1948, Gade 2003).

Both the bitter and sweet distinction made ethnographically and Koch's categories refer to the cyanogen content of the edible portion of the root; i.e., the peeled root or pulp. In low-cyanogenic cultivars the linamarin concentration of the peel is considerably higher than the pulp whereas in high-cyanogenic cultivars it is distributed more evenly throughout the entire root (Gomez et al. 1985, Dufour 1988). There is little correlation between the cyanogen content of the peeled root and the leaves. The latter tend to be high in cyanogens in both low- and high-cyanogenic cultivars (Gomez et al. 1985).

Processing and Detoxification

At the village level, cassava roots are typically processed into some type of food before being consumed. Among traditional peoples in the Amazon region of South America this food is typically a flat bread known as casabe, or a toasted granular product known as farinha de agua. In Africa some of the better known cassava-based foods are gari (a granular product similar to farinha de agua), baton de manioc (a fermented paste) and flour that can be used to make the stiff porridge known as foo foo or ungali.

Processing serves to produce foods with culinary qualities of interest, as well as to reduce cyanogen levels. The effectiveness of the cyanogen reduction achieved in the preparation of these foods varies considerably. In order to understand why it is useful to look at the techniques more closely.

The following six examples illustrate key aspects of the village-level processing techniques in reducing cyanogens.

Casabe The flat bread known as casabe is the dietary staple of Tukanoan Indians and other indigenous groups in the Amazon region. To prepare casabe the roots are scraped to remove the bark (periderm), but not the inner peel, washed and grated into a fine mash. The grated mash is separated into three fractions: liquids, starch and fiber by washing (with water and extracted juices) and squeezing the mash in a basketry strainer. The starch is allowed to settle out of the wash water, and both starch and fiber are allowed to ferment, at least overnight, but preferably for 48 h. The fiber is then dewatered and lightly toasted, mixed with the starch, and the mixture cooked on a griddle as a flat bread.

The efficacy of the Tukanoan casabe-making technique in reducing cyanogen levels was studied by Dufour (1989), who found that total cyanogens dropped rapidly with the grating and washing (Fig. 1a, 0-4 h). The grating causes extensive disintegration of the plant tissues and hence contact between the cyanogenic glucoside, linamarin, and the endogenous enzyme, linamarase. This results in decomposition of the glucoside and release of HCN, which can volatilize off. The fact that the inner peel is grated along with the pulp probably facilitates hydrolysis of the glucoside because enzymatic activity in the peel is higher in the pulp (Bruijn 1983, Nambisan and Sundaresan 1985). The washing of the grated mash also helps to remove cyanogens. The de-watering of mash probably removes both cyanohydrins and HCN, and the cooking should further reduce levels of both. The experiments described by Dufour (1989) demonstrated that the Tukanoan casabe-making technique can achieve a reduction of total cyanogens (linamarin, acetone cyanohydrin and HCN) of 97 % in two days (approximately 48 h).

Gari The dry toasted meal, gari, is the basis of the diet in much of Nigeria and other areas of West Africa. To make gari the roots are peeled and grated. The mash is allowed to ferment for a several days, dewatered and then toasted on a dry griddle while being stirred to prevent clumping. Vasconcelos et al. (1990) found that the initial grating (Fig. 1b, probably 0-2 h) was the most important stage in detoxification as it resulted in tissue disruption and hence contact between linamarin and the endogenous enzyme linamarase, and hence hydrolysis of the former. The last processing step, roasting or garification (Fig.1b, 100 h), was also important in reducing levels

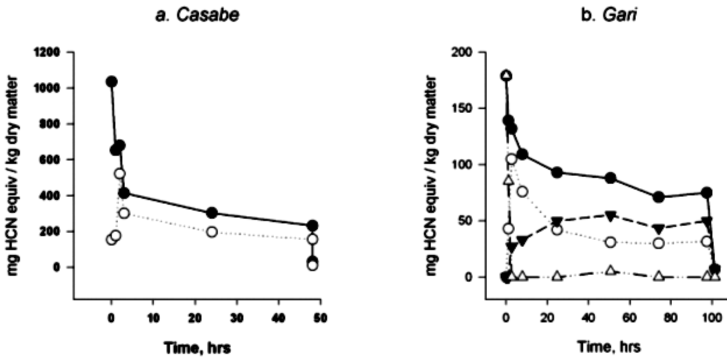
of residual cyanohydrins and HCN. Fermentation by lactic acid bacteria played a role in flavor development but not in detoxification. The farinha of Brazil is made by a similar process, and one which is slightly different one than that used for farinha d'agua (see below). As Vasconcelos et al. (1990) demonstrated, processing for gari removes 93 % of the total cyanogens and can be accomplished in four days.

Baton de manioc This is a fermented cooked paste commonly served with soups, stews or sauces in Central Africa. To make it the roots are peeled, chopped, soaked in water for two or more days, de-watered, pounded or ground to a fine paste, wrapped in leaves and boiled. Cyanogen loss was studied by O'Brien et al. (1992) in Cameroon. They found that the greatest loss of cyanogens occurred with the initial step of soaking (Fig. 1c, about 0-48 h) presumably because both the chopping and plant cell disintegration resulting from soaking led to contact between the glucoside substrate and the endogenous enzyme, linamarase, and hence decomposition of the former. They also found that the final cooking was important in removing residual cyanogens. Prior to cooking most of the cyanogen content was in the form of cyanohydrins which are relatively stable in the more acidic conditions of fermentation, but readily decomposes at temperatures > 35°C. The processing described by O'Brien et al. (1992) removed > 99 % of the cyanogens and took four days.

Farinha d'agua This is a dry toasted meal common in the diets of both indigenous and non-indigenous peoples in the Amazon region. It is very similar to gari and farinha in appearance but the preparation is slightly different. To prepare farinha d'agua the whole roots are first soaked in water for several days, then grated and the mash allowed to ferment for several days. The mash is then dewatered and toasted on a dry griddle while being stirred to prevent clumping. In the processing of farinha d'agua studied by Dufour (1989) the loss of total cyanogens was gradual in the first 72 h of soaking (Fig. 1d), accelerated between 72 and 96 h and with the grating (about 96 h). Little further change occurred while the mash fermented (> 96 h). Presumably, the plant cell disintegration resulting from soaking and then grating led to enzymatic hydrolysis of linamarin. The rate of cyanogen loss in soaking was slower than that in the processing experiment for baton de manioc described above. This is probably because the roots were soaked whole rather than chopped, and chopping leads to more rapid enzymatic hydrolysis (Okafur et al. 1984). The processing for farinha d'agua described by Dufour (1989) reduced total cyanogens by 99 % and took about 8 days.

Cassava flour prepared as a stiff porridge is the common starchy accompaniment to soups, stews and sauces in many parts of sub-Saharan Africa. It is referred as ugali, fufu, and wide variety of local names. The processing techniques used to make cassava flour commonly rely on sun-drying as a first step.

Heap fermented flour In some parts of Uganda the flour is produced though a process known as heap-fermentation. In this process the roots are peeled, sun-dried for 3 to 4 days, piled in a heap and covered for several days, scraped clean of mold, crushed, sun dried for 2 to 3 days, and then pounded and sieved into a flour. Essers et al. (1995) reported that the sun drying phase serves to inhibit bacterial growth, and that the heap phase (Fig. 1e, about 30-96 h) maintains moisture levels, which in turn extends the time for enzymatic degradation of cells and hydrolysis of linamarin. They also found that the heap phase produces conditions favorable to the growth of micro flora that might also play a role in the reduction of cyanogen levels. Further, they found that pH levels in the heap phase were optimum for both the activity of linamarase and the breakdown of cyanohydrins to volatile HCN. The processing experiment described by Essers et al. (1995) reduced total cyanogens by 95 % and took about 5 days. The authors note that in the villages the processing normally takes 5 to 9 days, depending on local weather conditions.



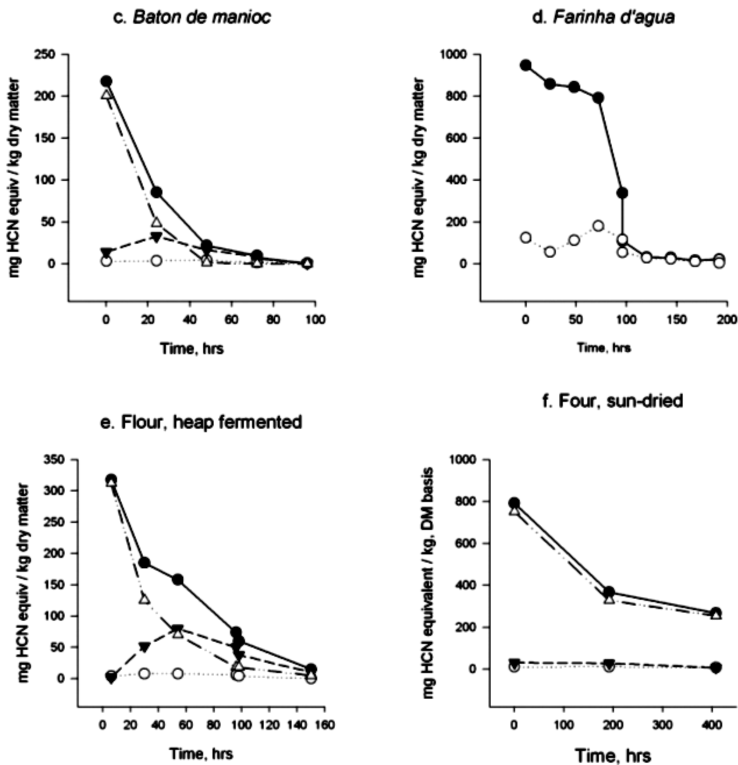


Fig. 1. Loss of cyanogens during village processing of cassava roots into casabe, gari, farinha d'agua, baton de manioc, and flour. Data for casabe and farinha d'agua from Dufour (1989), data for baton de manioc from Table 2 (part a) in O'Brien et al. (1992), and data for sun-dried flour from Table 3 in Milingi and Bambridge (1994). Data for gari replotted from Fig. 1a in Vasconcelos et al. (1990), and data for heap-fermented flour replotted from Fig. 2 in Essers et al. (1995).

Sun dried flour In Tanzania sun dried roots are referred to as makopa, and are the basis of ugali, the stiff porridge that is a staple of the diet. To make makopa the roots are peeled, and sometimes split longitudinally, and set in the sun to dry for a number of days. The relative effectiveness of this technique on the removal of cyanogens was assessed by Mlingi and Bainbridge (1994). They found that the reduction in linamarin depended on gradual root disintegration and enzymatic hydrolysis, and that it virtually ceased at a moisture level of about 13 %. Both cyanohydrins and HCN disappeared rapidly with sun-drying. The processing for flour they described removed 66 % of the total cyanogens in 17 days. Of the cyanogens remaining, 95 % were linamarin (Fig.1f)

Table 1 summarizes the efficacy of the six processing techniques described above. With the exception of sun drying, all were effective in reducing total cyanogens by 93 % or more. The reduction was only 66 % for sun-drying. The fact that almost of all the residual cyanogens were linamarin indicates that sun drying is not an effective process for the enzymatic hydrolysis of linamarin. However, sun-drying with a heap fermentation phase is quite effective in reducing total cyanogens because the heap condition maintains moisture levels adequate for the enzymatic hydrolysis of linamarin. The processing techniques that involve grating as a first step are the ones that most rapidly reduce cyanogen levels. The Tukanoan casabe processing technique which follows grating with a washing step is the most rapid. Techniques that rely on soaking as a first step take longer, and sun-drying on its own takes the longest amount of time. The time needed to process cassava is an important consideration when cassava is the basis of the diet because time constraints can lead to short-cuts in processing and less adequate detoxification (Essers et al. 1992, Banea et al. 1992).

Although the processing techniques described above were all, except for sun-drying, very effective in reducing cyanogen levels, residual cyanogens were found in all six types of foods. Residual cyanogens reflect both the effectiveness of the processing technique and the starting level of cyanogens in the fresh root. For example, the processing for casabe is effective in removing 97 % of the cyanogens, but given the highly-cyanogenic roots used (> 1000 mg HCN equivalents per kg of dry matter), the finished product still contained 32 mg HCN equivalents per kg of dry matter. The cyanogen content of the fresh roots used in the farinha d'agua and sun-drying experiments were also high. The level of residual cyanogens in casabe, and both heap fermented and sun-dried flours exceeded the FAO/WHO (1991) safe level of 10 ppm total cyanogens in foods. Only the sun-dried flour exceeded the standard of 40 ppm, which has been adopted in Indonesia (Damardjati et al. 1993). Since, the actual cyanogen content of cassava foods produced at the village level can vary considerably (Cardoso et al. 2005, Damardjati et al. 1993), the residual cyanogen content of the foods made by six techniques described above should be considered only as examples.

Table 1. Summary of efficacy of six processing techniques

Food	Processing steps	Days	Cyanogens removed (%)	Cyanogens (mg HCN equiv per kg dry matter)
Casabe	Scrape, grate, wash to separate starch, ferment, dewater, cook	3	97	32
Gari	Peel, grate, ferment/ dewater, toast	4	93	6
Farinha d'agua	Peel, soak, grate, ferment, dewater, toast	8	99	8
Baton de manioc	Peel, chop, soak, dewater, grate/pound, wrap, boil	5	>99	< 1
Flour (heap fermented)	Sun-dry, heap ferment, crush, sun-dry, pound, sieve	6	95	15
Flour (sun-dried)	Sun-dry, pound, sieve	17	66	267

Cassava related disorders

In Africa, but not in South America, health problems have been associated with cassava consumption and the residual cyanogens in cassava-based foods. These problems include acute toxicity and two chronic neurological diseases, konzo and TAN (tropical ataxic neuropathy). In addition, dietary exposure to cyanogens has been linked to iodine deficiency diseases of goiter and cretinism.

Acute toxicity. Well documented cases of acute toxicity (Akintowa et al. 1994) are the clearest examples of the negative impacts of residual cyanogens on health. Cyanide is toxic because it interferes with the action of cytochrome oxidase, a key enzyme in the energy conversion process in the body. The lethal dose for an adult depends on body weight and is between 30 and 210 mg of HCN. Sometimes these limits are exceeded by persons eating a cassava meal and deaths occur due to cyanide poisoning (Mlingi et al. 1992). Fatalities, however, are very rare in cassava-consuming communities, most likely because of low levels of residual cyanogens in processed foods (Oluwole and Onabolu 2003). Non-fatal doses cause symptoms of dizziness, headache, stomach pains, nausea, vomiting and diarrhea. These symptoms are common in some communities heavily dependent on bitter cassava, especially under circumstances that compromise food availability, like drought or war (Cliff et al. 1999).

Konzo is a disease characterized by an abrupt onset of symmetric spastic paraparesis (weakness of the legs), which is non-progressive and irreversible (WHO 1996). It is most common in children and women of child bearing age.

Konzo was first described in the late 1930s in the Democratic Republic of Congo and continues to occur there (Ngudi 2005). In recent years it has also been reported in Cameroon, Mozambique, Tanzania, and the Central African Republic (Oluwole and Onabolu 2003). The neuropathy of konzo is not fully understood, and the role of cassava borne cyanide in causing the disorder is not clear (Oluwole and Onabolu 2003). However, konzo is only found in very poor rural areas where bitter cassava is the staple crop and dominates the diet, and the intake of high quality proteins from animal foods is low (Ngudi 2005). In addition, it tends to occur during the dry season and especially during periods of drought and civil unrest, when the diet is largely restricted to cassava (Banea-Mayambu et al. 1997, Cliff et al. 1997, Ernesto et al. 2002).

Tropical Ataxic Neuropathy (TAN; or ataxic polyneuropathy) is a neurological disease characterized by a gradual onset of gait ataxia (incoordination), optic atrophy, and neurosensory deafness (Oluwole and Onabolu 2003). It is a chronic condition and commonest amongst poor males and females in their 50's and 60's. The disease is probably best known from Nigeria, although it also occurs in other African countries. Like konzo, TAN is a disease associated with extreme poverty and a high cassava diet. Although cassava-borne cyanide has long been thought to be the causal agent in the disease, recent studies have cast doubt on this hypothesis (Oluwole et al. 2002).

The iodine deficiency disorders of goiter and cretinism have been linked to cassava consumption, but only in areas where dietary iodine intake is low. Here, the mechanism is understood: ingested cyanide is metabolically detoxified in the body to thiocyanate, which inhibits iodine uptake (Delange et al. 1994). Since thiocyanate is a normal constituent of body fluids, the metabolic detoxification of cyanogens simply increases the thiocyanate load in the body. This increased load, in combination with a dietary intake of iodine below recommendations, leads to an increased the risk of goiter and cretinism (Delange et al. 1994).

In summary, cassava is a cyanogenic crop, the edible roots of which can release potentially toxic amounts of HCN. The processing of roots into culturally acceptable foods at the village level can be very effective in reducing cyanogens to physiological tolerable levels, but effectiveness varies with processing technique. The presence of residual cyanogens in cassava-based foods has been linked to health problems in Africa, but only in instances where dietary intake is less than satisfactory. Instances of acute toxicity are probably an exception.

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Monitoring of Malnutrition in Weaning Tribal Infants by Introducing Cassava Made Indigenous Semisolid Diet

Nagesh S. Tekale
Nutritional Research Lab, Bandodkar College of Science, Thane (W) 400 601 M.S. India

Abstract

Early marriages of girls, inadequate breast feeding to new born and shifting children directly on plane solid diet are the regular traditional practices in the tribal belt of western India, which leads to an early triggering of grade IV malnutrition and mortality of the infants. The interventions were carried out to introduce cassava-made semisolid diet for weaning children for six months before shifting them to solid diet. Results show complete eradication of malnutrition and control over the diarrhea.

Introduction

Inhabitants of tribal belts are the indigenous people constituting about 8% of Indian population. Their habitat is in hilly areas surrounded by forests which are usually inaccessible to various nutritional programs and health facilities offered by the Government. Disturbance in biodiversity, dependence on forest and its produce, low or no intake of oil and milk products due to taboos, non-availability of animal flesh when desired, high intake of fiber and low traditional agricultural produces rich in anti-nutritional factors has triggered micronutrient deficiencies, specifically vitamin A, zinc and iron. The main sufferers are expectants, lactating mothers and growing five-year old or younger children. Although there are more than 300 tribal groups in India, many of them are socio-economically and nutritionally backwards

carrying disease burden on them. Early marriages of girls, inadequate breast feeding to new born and then shifting children directly on plane solid diet are often the traditional practices in tribes inhabiting western India that lead to early triggering of grade IV malnutrition and terminate into diarrhea-prone mortality of one-year old or younger children.

Tribal people are fond of underground modifications of roots and stems. Although forests in western India are still rich in varieties of corms, tubers and rhizomes, cassava appears to be critically endangered because of earlier heavy exploitation of this crop for its edible starchy tuber. The general project objectives were to introduce cassava-made semisolid food as a substitute to dry bread for weaning babies –especially those deprived of the right of breast feed at early stage, reduce the malnutrition and diarrhea among weaning children, educate the mothers on the importance of semisolid diet after discontinuation of breast feeding and role of nutrient-rich cassava, support and sustainability for traditional low productive agricultural practices in tribal belt by promoting large scale genetically improved cassava cultivars, and a conservation strategy for cassava in endangered areas.

Materials and Methods

The nutritional survey was undertaken between May 2004 and December 2004 in two hamlets located in the interior, inaccessible tribal belt of western India, where there was prevalence of grade III and IV malnutrition and frequent occurrence of diarrhea. Two group of weaning subjects constituting control and experimental between the six-month and one-year old were selected. The pre-weaning weights and malnutrition status of each baby was recorded using Gomez' classification by weight per age. The pooled data are given in Table 1.

Table1. Prevalence of malnutrition and diarrhea in weaning tribal infants between six-month to one-year old (N = 95)

Nutritional Status	Grade (%)	Diarrhea cases (%)
Grade I (moderate malnutrition)	10	15
Grade II (noticeable malnutrition)	46	28
Grade III (severe malnutrition	36	60
Grade IV (total malnutrition)	8	100

From the onset of the weaning process, he indigenous cassava based semisolid diet -including all six nutrient parameters and providing 1800 cal per day, was given to each of the children thrice a day under perfect hygienic conditions for six months until they were shifted on traditional cereal based solid diet. The health and malnutrition status of children were monitored regularly. The results were compared with a control group.

Results

The introduction of cassava-based weaning food made from boiled tuberous roots supplemented with green leafy powder as a source of beta carotene had decreased the malnutrition and malnutrition-linked infectious diseases significantly: up to 92% compare to control, in which all the children were malnourished with diarrhea infections (Table 2).

Table 2. Comparison of groups given cassava tuber diet in semi-solid form with control after six-month trial (N = 20)

Nutritional status	Experimental Group		Control Group	
	Grade (%)	Diarrhea (%)	Grade (%)	Diarrhea (%)
Normal	85	—	—	—
Grade I (moderate malnutrition)	15	30	—	—
Grade II (noticeable malnutrition)	—	—	65	40
Grade III (severe malnutrition)	—	—	30	85
Grade IV (total malnutrition)	—	—	5	100

In experimental group we noticed increase in body weight and height with MAC. Children were seen in active by giving response to scientific toys. which were used to enhance their learning abilities.

Discussion

The introduction for the first time in this tribal belt of India of 80% of pre-boiled cassava tuberous roots meshed with flour of black gram as a source of protein, and appropriate amount of green leafy vegetable powder, as a source of micronutrient, in weaning food form helped to reduce malnutrition-linked mortality in tribal infants. This concept was totally missing in this area because of which every year thousands of malnourished tribal infants were true victims of diarrhea. Tribes love and affection for under ground starchy food helped us to popularize this indigenous zero-cost supplementary baby food in tribal belt. The weaning baby food prepared from cassava appears

to be very promising source of nutrition to the children to eradicate the root cause of malnutrition. The results of this experiment were observed and confirmed by many lactating tribal mothers on the experimental site. This experiment gave boost to the community nutritional program of the Government and charity organization which are actively participating in eradication malnutrition and diarrhea in this tribal belt of western India.

Cassava is an endangered crop in this tribal belt. Hence, there is an urgent need to introduce large scale cultivation of genetically improved cassava in this nutritionally affected belt to make this nutritional program sustainable. Such action will definitely support the traditional agricultural cropping system opening the gates for a permanent solution to eradicate child malnutrition in this tribal belt by giving their population balanced-nutritious diet.

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Physical Properties of Cassava Starch Films Containing Glycerol

P.V.A. Bergo, P.J.A. Sobral, J.M. Prison
Food Engineering Dept., FZEA, University of São Paulo,
PO Box 23, 13635-900 Pirassununga (SP) Brazil

Abstract

The aim of this work was to investigate some physical properties of cassava starch films containing glycerol. Films were prepared from film-forming solutions with 2 g cassava starch per 100 g water plus 0, 15, 30 and 45 g glycerol per 100 g starch, and analyzed to determine its mechanical properties by tensile tests, the glass transition temperature (Tg) by differential scanning calorimetry, the crystallinity by X-ray diffraction, and moisture content by a new, fast and non-destructive microwave methods. The infrared spectra of the films were also recorded. The resistance values of the films decreased while those of the elasticity increased with the increase of the glycerol concentration, due to plasticizer effect of glycerol, which was also observed in DSC curves. The Tg of the films prepared decreased with the glycerol content. However, for samples with 30 and 45 g glycerol per 100 g starch, two Tg curves were observed, probably due to a phase separation phenomenon. According to the XRD diffractograms, films with 0 and 15 g glycerol per 100 g starch presented an amorphous character, but some tendency to show crystalline peaks was observed for films with 30 and 45 g glycerol per 100 g starch. The results obtained with FTIR corroborated these observations. Microwave measurements were sensitive to the moisture on films.

1
2 Energy and Nuclear Research Institute, Po. Box. 11049, 05315-970, SP, Brazil

Introduction

Edible films are thin materials based on biopolymers, such as polysaccharides. Starch is a polysaccharide composed by the amylose, a linear or sparsely branched polymer, and the amylopectin, a highly branched polymer (Mali et al. 2005, Famá et al. 2005). This biopolymer could be interesting in the edible film technology because it is produced abundantly around the world, and it could be considered as inexpensive.

An important starch source is cassava, which is a tropical root crop. Cassava starch is able to form transparent coatings (Vicentini and Cereda 1999) and flexible films (Vicentini et al. 2005) without any previous chemical treatment, neither plasticizer addition. However, for the production of edible films with good workability, a plasticizer such as the glycerol is usually used. The plasticizer modifies the interactions between the macromolecules, resulting in an increase in the chains mobility and consequently, causing a reduction in the glass transition temperature of the system (Sobral et al. 2001). Thus, plasticizers may affect all physical properties of films. The aim of this work was to investigate some selected physical properties of cassava starch films containing different glycerol content, by means of mechanical analysis, X-ray diffraction, differential scanning calorimetry, microwave methods and Fourier-transform infrared.

Material and methods

Cassava starch, supplied by a local industry (Flor de Lotus Co., Brazil), had the following characteristics (Vicentini et al. 2005): 14.9% moisture; 16% amylose, 0.21% soluble total sugars, 0.23% ash, 0.39% fiber, 0.24% total nitrogen, and 0.15% lipids.

For edible films production, film-forming solutions (FFS) were previously prepared with 2g of starch per 100g of water and 0, 15, 30 and 45g of glycerol per 100g of starch. The starch gelatinization was undertaken by thermal treatment at 70ºC for 1 min. in a water bath (Tecnal, TE 184). The FFS was poured in an acrylic plate and dehydrated in an oven with air circulation and renewal (Marconi, MA037), at 30ºC for 18 to 24 h. Thus, transparent and flexible films with 0.07 0± 0.002 mm thickness of were obtained.

Mechanical properties were determined by tensile tests, using a Texturometer (TA.XT2i, SMS), following the method suggested by Paschoalik et al. (2003). Each test was done in quadruplicate. These tests were performed at

room temperature (22-25ºC) with samples previously conditioned at 25ºC and 58% of relative humidity (NaBr saturated solution), for one week. However, for the subsequent analysis, samples were conditioned over silica gel. The glass transition temperature of the films were measured using a differential scanning calorimeter (DSC-2010, TA Instruments), in duplicate. The samples were weighted (± 0.01 mg, Analytical Plus, Ohaus) in aluminum pans, hermetically sealed and heated at a rate of 5ºC per min (Sobral et al. 2001). The reference was a void pan. The crystallinity of films was studied with an X-ray diffratometer (Rigaku), with Cu source, operating at room temperature, 40kV and 30mA. Rectangular samples were analyzed between 2θ = 10º and 2θ = 30º with a step size 2θ = 4º. Also, infrared spectra (FTIR) of films were recorded between 4000 and 600 cm^{-1} at 4 cm^{-1} of resolution, with a Spectrum One (Perkin Elmer) spectrometer, supplied with a universal attenuated total reflectance (UATR) accessory, as suggested by Vicentini et al. (2005). For each spectrum, 25 scans were co-added. These analyzes were run in duplicate. Finally, microwave insertion loss was performed by varying the microwave frequency and at the same time, measuring the attenuation of the energy, after passing through the film. The method is well described in Bergo et al. (2006).

Results and Discussion

As expected, the increase in the glycerol concentration on the FFS produced films less stiff and rigid, and more extendible; i.e., it caused a reduction on the tensile strength and increase of the elongation at break of films, respectively (Fig. 1). These trends were probably due to the reduction in interactions between the biopolymers chains. This effect of the plasticizer concentration on the mechanical properties is well known and broadly discussed in the literature (Arvanitoyannis and Biliaderis 1998, Sobral et al. 2001, Mali et al. 2005.

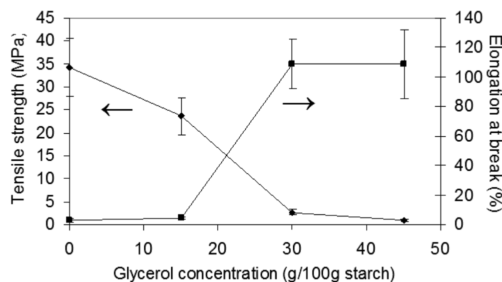

Fig. 1. Effect of the glycerol concentration on tensile strength and elongation at break of cassava starch films

The resulting trends of the mechanical properties of the films could be explained in a general manner, by the effect of the glycerol on the state properties of the films. It can be observed in the DSC curves of the films (Fig. 2), that the glass transition temperature (Tg) decreased from 131.9 to 42.1ºC, when the glycerol content increased from 0 to 45%, respectively; i.e., the films became less glassy with the addition of glycerol. This behavior was also observed by Mali et al. (2002) working with yam starch based films, and by Forssell et al. (1997), dealing with films based on barley starch, in both cases with glycerol. These latter authors also observed an endothermic peak as the one observed in this work (Fig. 2) with samples without glycerol. In this case, this phenomenon, observed at a temperature of about 197ºC in films without glycerol, was due to thermal degradation of starch, which shifted to higher temperatures by the increase of the glycerol content, attaining 218ºC in films with 45% of glycerol.

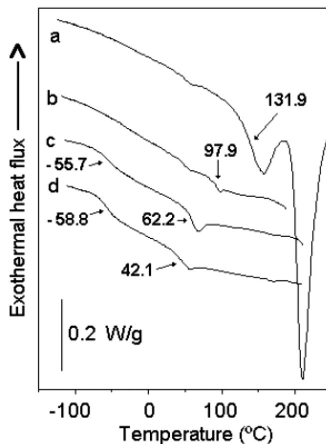

Fig. 2. DSC curves of cassava starch films containing (a) 0%; (b) 15%; (c) 30%; and (d) 45% of glycerol. Numbers indicated in the plots are values of temperature (ºC)

It can be also observed in DSC curves (Fig. 2) of cassava starch films, that another glass transition was visible at very low temperature when glycerol concentration was 30% (Tg = −55.7ºC) and 45% (Tg = −58.8ºC). This behavior was also observed by Forssell et al. (1997), who suggested that a phase separation has occurred between a starch rich phase and glycerol rich phase. This phenomenon may explain the high standard deviation on data of elongation at break of films with 30 and 45% of glycerol. In fact, these

films presented less workability than those with 0 or 15% of glycerol due to a “sticky” character.

Figure 3 shows the X-ray diffractograms (XRD) obtained with the films studied in this work. They can be related to complex structures like B-V type crystal structure, typical of tuber starches (Mali et al. 2002, Famá et al. 2005). According to these XRDs, films with 0 and 15g glycerol per 100 g starch presented an amorphous characteristic, but some tendency to crystalline peaks, at $2\theta \approx 20^{\circ}$, were observed for films with 30 and 45 g glycerol per 100 g starch. Starch films could have amorphous character because the thermal treatment of FFS provoked starch gelatinization, causing disruption of the double helix conformations of the yam starch. However, the increase in glycerol content in the films may have increased the macromolecular mobility, allowing the formation of microcrystalline junctions, i.e. some re-crystallization occurred. Mali et al. (2002) observed that glycerol in FFS did not notoriously influence the X-ray pattern of yam starch films.

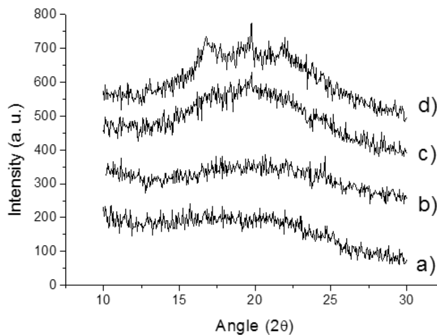

Fig. 3. X-ray diffraction pattern of cassava starch films containing (a) 0%; (b) 15%; (c) 30%; (d) 45% glycerol

Some small differences in terms of band shape and intensity can be observed in the fingerprint of yam starch in the FTIR spectra (Fig. 4), as a result of the glycerol content variation. The peak observed at 1011.8 cm^{-1}, that appeared as a shoulder in films without glycerol, became more prominent and presented a displacement to 1013.4, 1014 and 1014.8cm^{-1}, for glycerol concentrations of 15, 30 and 45%, respectively. These peaks could be associated with COH bond vibrations or solvation, and could also be associated to changes from an amorphous to a semi-crystalline state (van Soest et al.

1995, Vicentini et al. 2005). The displacement of the peak observed from 995 to 997.9 cm^{-1} when the glycerol content increased from 0 to 45%, could also be associated to the amorphous-crystalline transition in these films.

The glycerol also affected the peak normally associated to COC anti-symmetric bridge stretching (van Soest et al. 1995). This peak, initially observed at 1148.6cm^{-1} in films without glycerol, was displaced to 1149.6, 1150.2 and 1150.5 cm^{-1} when the glycerol concentration was 15, 30 and 45%, respectively. It can be therefore suggested that the thin difference between these last two data may be explained by a phase separation, such as that observed in DSC curves (Fig. 2).

Figure 5 shows two examples of microwave insertion loss response as a function of frequency (frequency domain), obtained from that films conditioned in a) silica-gel and b) NaBr. The results show that the main changes are prominent in the region between 11 and 13 GHz. These changes were attributed to the moisture that tends to accumulate in the films, due to the glycerol hygroscopic character.

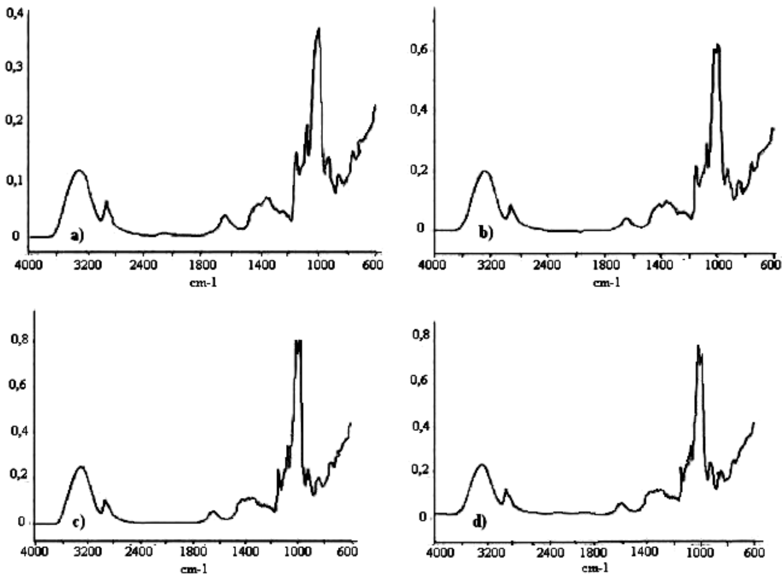

Fig. 4. FTIR spectra of cassava starch films containing (a) 0%; (b) 15%; (c) 30%; (d) 45% glycerol

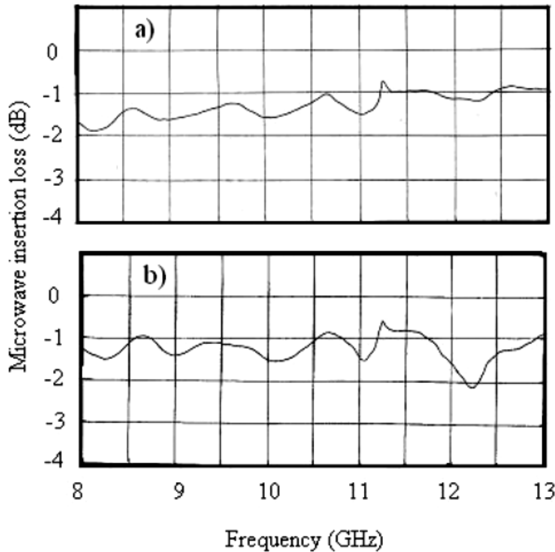

Fig. 5. Example of microwave insertion loss in cassava starch films containing 15% glycerol, conditioned in: a) silica-gel and b) NaBr, for a week

Conclusions

The increase of glycerol content in cassava starch films, increase the macromolecular mobility, probably by solvation of COH bonds of the starch, which allowed some polymer re-crystallization. However, at the macroscopic scale, cassava starch films became less stiff and more flexible. In fact, the glycerol addition had two effects: it caused an increase in the mobility of amylose and amylopectin chains which overcame the opposite effect of the re-crystallinization. Also, the increase of the flexibility of the films with plasticizer could be attributed to a lubrication effect of glycerol, associated to a phase separation observed at the highest concentrations. Microwave insertion loss response as a function of frequency shows that this technique is sensitive to the moisture, due to different conditioning of the films. The moisture affects the region between 11 and 13 GHz.

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