Pages 10-13

Cassava in Africa

F.I. Nweke & J.K. Lynam

ABSTRACT

This paper based on farm level information collected from 10 major cassava producing countries show that cassava production is a commercial activity. Expanded production is however hampered by reliance on traditional production and processing technologies which result in high production costs and low product qualities. The high production costs and the low qualities in turn result in restricted product markets.

Key words: cassava, manioc, Africa

Pages 13-19

African cassava mosaic virus disease: the magnitude of the problem

J.M. Thresh, G.W. Otim-Nape, J.P. Legg & D. Fargette

ABSTRACT

This paper considers the various estimates that have been made since 1956 of the losses caused by African cassava mosaic disease (ACMD). It is suggested from the limited evidence available that the overall incidence of ACMD is currently 50-60% and that diseased plants sustain losses of 30-40%. On these plausible assumptions losses in Africa are 15-24%, equivalent to 12-23 million tonnes compared with actual production estimates of 73 million tones. Such losses are equivalent to $1200-2300 million at a conservative value of $100 per tonne. However, it is concluded that these figures should be treated with caution and that definitive estimates of the losses caused by ACMD are not possible because of the limited information available on the incidence and severity of the disease and on the relationship between disease severity and crop loss. Proposal are made for a new approach to estimating yield loss utilizing the experience gained from the previous limited surveys and field trials

Meanwhile, in considering the importance of ACMD and the need for control measures it is appropriate to distinguish three contrasting situations. In the epidemic areas, as currently experienced in much of Uganda, ACMD is prevalent and causes such severe losses that control measures are essential and emergency relief is justified. In the large tracts of Africa where ACMD is epidemic the disease is prevalent but the situation is relatively stable, losses are acceptable and control measures are optional. Losses are unimportant and control measures are unnecessary where ACMD is benign, as in the upland areas of Burundi, Tanzania and Malawi.

Key words: African cassava mosaic diseases, prevalence, yield loss, severity.

Pages 19-22

Properties, differentiation and geographical distribution of geminiviruses that cause cassava mosaic disease

B.D. Harrison, Y.L. Liu, X. Zhou, D.J. Robinson, L. Calvert, C. Munoz & G.W. Otim-Nape

ABSTRACT

Previous work has detected three different whitefly-transmitted geminiviruses in mosaic-affected cassava: African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV) and Indian cassava mosaic virus (ICMV). ACMV is now recorded from 17 countries in Africa west of the Rift Valley, EACMV from 5 East African countries and Madagascar, and ICMV from India and Sri Lanka. The three viruses are readily detected and distinguished by TAS-ELISA with a small set of monoclonal antibodies raised against particles of ACMV or ICMV. The nucleotide sequences of their genomic DNA molecules differ as much from one another as from those of whitefly-transmitted geminiviruses that infect species other than cassava. Thus, the three viruses can also be identified by the polymerase chain reaction (PCR) with virus-specific oligonucleotide primers, or by the patterns of fragments obtained by restriction endonuclease treatments of the products of PCR with degenerate primers. Recently an epidemic of an extremely severe form of cassava mosaic disease has spread through much of Uganda into areas where ACMV already occurred. The epidemic is linked to the occurrence of a new form of cassava geminivirus, the Uganda variant (UV), which is now widely distributed in Uganda. UV is serologically indistinguishable from ACMV but unlike EACMV. However, it has some nucleotide sequences typical of EACMV and unlike those of ACMV, suggesting that recombination between ACMV and EACMV may have played a part in its origin. Many of the most severely affected cassava plants contain both UV and ACMV.

Key words: cassava, cassava mosaic disease, geminiviruses, Uganda variant geminiviruses

Pages 23-28

Presence of a new virus closely related to East African cassava mosaic geminivirus, associated with cassava mosaic outbreak in Uganda

D. Deng, W.G. Otim-Nape, A. Sangare, S. Ogwal, R.N. Beachy & C.M. Fauquet

ABSTRACT

N-terminus nucleotide fragments of germinivirus coat protein coding sequences have been amplified by PCR from cassava in Uganda, expressing mild and severe symptoms of mosaic disease and collected in different locations relative to the expansion of the current epidemic of the disease in the country. Similar amplifications have been done with single whitefly samples collected in the same locations. Sequences obtained from the amplified fragments and RFLP mapping show the presence of two germinivirus species, one similar to African cassava mosaic virus (ACMV) and one typical of East African cassava mosaic virus (EACMV-Ma). The presence of the former one is correlated to mild symptoms on cassava and to whiteflies collected Ahead of the front or at the front of the epidemic, while presence of the latter is correlated with severe symptoms on cassava and with the samples of whiteflies from Behind the front. The complete sequence of the coat protein (CP) of a clone amplified from a plant expressing severe symptoms show an apparent recombination between the CP of the ACMV and the CP of the EACMV. While the sequence of the pre coat protein of the same clone is not related to ACMV. By showing the presence of two viral entities, this work clarifies the epidemiological situation of the cassava mosaic epidemic in Uganda but poses a number of questions relating to the possible recombination between viral species, to the emergence of new geminiviruses and to the biodiversity of germiniviruses of cassava in Africa.

Key words: germinivirus, mosaic virus, cassava, Uganda, East Africa

Pages 28-32

Genetic relationships among cassava clones with varying levels of resistance to African mosaic disease using RAPD markers

Hodeba Douwehan Mignouna and Alfred Gilbert Olonju Dixon

ABSTRACT

Thirty-five cassava landraces were collected in various countries of West Africa. Thirty-four of them were found resistant to the African cassava mosaic disease (ACMD) and suspected of carrying different sources of genes for resistance. The 35 landraces together with five improved varieties and one resistant genetic stock (58308) of cassava were subjected to RAPD (random amplified polymorphism DNA so as to discriminate the cassava varieties and determine the genetic diversity among them. DNA extracted from cassava leaves was purified and used with nine selected random primers (10-mer) in the polymerase chain reaction (PCR), this generated 74 amplified fragments. Only the 54 polymorphic fragments were analyzed using principal component analysis and 31 were finally selected as the most informative to effectively characterize the cassava genotypes. The dendrogram constructed from the similarity matrix generated identified six clusters at the 60% similarity level among the 41 cassava genotypes. Clone 58308 (until recently, the only and extensively used source of resistance genes for ACMD) and 30572, an improved cultivar and a derivative of clone 58308, were found in the same cluster group. All the resistant landraces were genetically distant from the resistant genetic stock (58308) and 30572.

Key words: Cassava landraces, DNA fingerprinting, African cassava mosaic disease, genetic diversity

Pages 33-36

Implementation in Africa of serological diagnostic test for cassava mosaic geminiviruses

F.O. Ogbe, G. Thottappilly & F.M. Quin

ABSTRACT

The need for effective control of cassava mosaic geminiviruses in Africa by National Programmes led to the development of a biotin-streptavidin method of enzyme-linked immunosorbent assay (ELISA) which is wholly based on monoclonal antibodies. Its evaluation in Kenya and Uganda (in less sophisticated laboratories) successfully detected and differentiated African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV). A new finding about the distribution of the latter was observed as six cassava leaf samples collected in Lake Victoria region (Kenya west) gave reactions that suggested the occurrence of EACMV. Hitherto in East Africa, the virus was reported to occur only at the coast. The diagnostic technique has great potential for testing improved germplasm and stocks of healthy materials for the presence of cassava mosaic viruses. The technique is cheap and sustainable and suitable for regular regional diagnostic surveys of cassava mosaic viruses in Africa.

Key words: African and East African cassava mosaic germinivirus, ACMD, monoclonal antibody, diagnostic test

Pages 36-42

Breeding for resistance to mosaic disease in Uganda

G.N. Ssemakula, Y.K. Baguma, G.W. Otim-Nape, A. Bua & S. Ogwal

ABSTRACT

Cassava is an important food crop in Uganda grown and utilized widely in all parts of the country. The crop, however, continues to be threatened by African Cassava Mosaic Disease (ACMD). This paper reviews breeding of cassava for ACMD resistance as the major control strategy that has been adopted over the years, in the absence of alternative, ecologically sustainable methods of control. The paper also identifies constraints to breeding for ACMD as: limited variability for the trait, unfavorable association with the trait and lack of fast multiplication techniques to enable released varieties reaches farmers at a fast rate. Suggested solutions to overcome these problems include: a more massive germplasm collection and evaluation for ACMD resistance, release of as many resistant varieties to farmers as possible and use of micropropagation for faster multiplication of resistant materials for farmers.

Key words: Africa cassava mosaic disease, cassava, Uganda

Pages 42-43

Epidemic of severe cassava mosaic disease in Uganda and efforts to control it

Otim-Nape G.W., A. Bua, Y. Baguma & J.M. Thresh

ABSTRACT

Cassava mosaic disease, caused by a whitefly-transmitted geminivirus has been known in Uganda since 1928. Severe epidemics were reported from 1933 to 1944 and were controlled by use of resistant cassava varieties and sanitation. However, since 1988 an epidemic of a more severe mosaic disease, first reported in Luwero district, has spread through the country from north to south devastating crops. Since 1988, the epidemic has spread c. 140 km southwards towards Kampala. By May 1995, it was only c. 20 km from Kampala and was continuing to spread southwards along a broad front at a rate of c. 15-20 km per annum. The front is characterized by large numbers of whiteflies (*Bemisia tabaci*) and by a high incidence of recent infection cause by veaorborne virus. The lower leaves of plants infected in this way seem healthy while the youngest leaves show severe symptoms. They are reduced in size and show marlced distortions at~d malformation which give the infected plants a paint-brush-like appearance. The plants harbor numerous adult whiteflies on the young shoots and large nymphal populations on the undersides of lower leaves. Fifteen to twenty kilometers behind the front, all plants show severe mosaic symptoms due to the use of cuttings from plants infected by whiteflies the previous year. The plants are severely stunted and produce little or no yield. Consequently farmers become discouraged and give up cassava cultivation. Over 150,000 ha of cassava that formally produced over 2.2 million tonnes (U.S. $ 440 million) of fresh cassava roots per annum have been lost in this way. The causes of the epidemic are being investigated. A new biotype of *B. tabaci* a more aggressive strain of the virus, or both are suspected to be involved. To control the disease and restore food security in the country, technologies appropriate to farmers had to be developed and transferred quickly to them. A survey was undertaken in areas severely affected by cassava mosaic to ascertain the extent of farmers' knowledge and their practices for controlling the disease. Results showed that farmers were aware of the disease and used what they thought were resistant varieties, selected healthy planting materials, rouged infected plants and changed varieties in attempts to control the disease. This information was used as a basis for improved control methods. When they were compared with local varieties in multilocational and on-farm trials, the improved genotypes TMS 30572, TMS 60142 and TMS 30337 proved superior and acceptable according to farmers' selection criteria and were released as varieties Migyera, Nase 1 and Nase 2, respectively. A national network of cassava workers (NANEC) was created to tackle the problem of technology transfer. Similarly a scheme for multiplication and distribution of 'clean' stocks of the improved varieties was worked out and used by NUANCE. Stocks were multiplied

by institutional farms, by village farming groups especially women, and by individual farmers. By 1966, c. 10,000 ha of the improved varieties were being grown and had been distributed to farmers in 26 districts seriously affected by the disease. All extension agents and most farmers in most districts were trained in mosaic control, and in improved cassava multiplication and production. The causes and spread of the epidemic, approaches to its control and cassava multiplication strategies are discussed.

Pages 44-48

Effect of African cassava mosaic disease on growth and yield components of virus-tested cassava genotypes derived from meristem culture in early and late planting periods in three agroecologies of Nigeria

A.O. Akano, G.I. Atiri, S.Y.C. Ng & R. Asiedu

ABSTRACT

A delivery system for cassava planting material with good health status (particularly indexed free from African cassava mosaic virus (ACMV) has been proposed as a way to improve and sustain higher root yield. To evaluate the possible benefits derived from use of ACMV-indexed plants, seven cassava genotypes with different levels of susceptibility to African cassava mosaic disease (ACMD) were evaluated during early and late growing periods in three agroecologies in Nigeria – the humid rain forest (representative location: Onne), forest-savanna transition (Ibadan) and northern Guinea savanna (Zaria). Cuttings for the experiments were from two sources – farmers' field and materials originally from in vitro culture (indexed free from ACMV) which had undergone one year of field planting at each respective location. The growth, yield performance as well as incidence and severity of the disease in the two sets of planting materials were compared. For both growth periods at Ibadan and Onne, disease incidence and severity of most of the genotypes derived from cuttings regenerated through tissue culture were not significantly different ($p < 0.05$) from those from farmers' field. However, one susceptible genotype TME 1 (a local landrace), derived from tissue culture had significantly lower ($p < 0.05$) incidence and severity than those from farmers' fields. In contrast, at Zaria disease was completely absent from plants of all the genotypes from tissue culture materials, and from some improved cultivars from farmers' fields (presumably clean from cuttings) in both growth periods. This confirms absence of spread of the disease in the field in the Northern Guinea savanna and suitability of the zone for multiplication of cassava planting materials. At Ibadan and Onne, there were no significant differences ($p < 0.05$) in storage root yield and shoot weight, between plants derived from the two different sources. At Zaria, the late planting of the moderately resistant cultivars (TMS 4(2) 1425 and TMS 30555), the late and early planting of the susceptible cultivar (TMS 91934), derived from tissue culture had significantly higher ($p < 0.05$) storage root yield than those from farmers' fields. Root yields generally were poor at Zaria and for those plants that showed ACMD symptoms, fresh root yield was not significantly correlated with disease ($p < 0.05$). These findings support the view that use of virus-tested propagation materials as a control measure for ACMD, particularly in zones of high disease pressure is not very successful. Growth and yield reductions attributable to

ACMD among the improved cultivars were low. Further in-depth studies to quantify yield losses in the three agrorcologies are still required.

Key words: cassava, African cassava mosaic disease, meristem culture, Nigeria

Effect of African cassava mosaic disease on the yield of cassava derived from cuttings from tissue culture plants in two agroecological zones in Nigeria

A.O. Akano, R. Asiedu & S.Y.C. Ng

ABSTRACT

Cuttings from five cassava genotypes selected based on different levels of susceptibility to African cassava mosaic disease (ACMD) were made from symptom less plants at the humid rain forest (representative location: Onne) and northern Guinea savanna (representative location: Zaria). These plants were derived from tissue culture plants that had been through two growth cycles on the field. Also, the cuttings were planted alongside those from infected cassava plants and were separated in space by planting maize crops around the disease free plantings. A wider spacing of 1.5m x 1.5m was used in the trial to reduce the effect of plant density compensation. At three months after planting (MAP), secondary infection was observed within the ACMV-free planting only at Onne. This also coincided with removal of the maize crop. The incidence and severity of the disease as well as plant height were recorded for individual plants of the two classes of plantings at 3 and 6 MAP. Relationships between the fresh root and dry matter yield at 12 MAP and the differential reactions of the genotypes and plants to disease infection pressure, will be reported. A guide to deployment of disease-free cuttings as a phytosanitary measure in controlling ACMD will also be discussed.

Pages 49-52

A new isolate of African cassava mosaic virus in South Africa

L.C. Berrie, K. Palmer, E.P Rybicki, S.H. Hiyadat, D.P. Maxwell & M.E.C Rey

ABSTRACT

African Cassava Mosaic Virus (ACMV) is widespread in cassava in Africa and causes serious loss in yields. Several isolates of ACMV have been reported, namely the West African, Kenyan "type" and East African (Malawi) isolates. ACMD has been reported in South African and the purpose of this investigation was to compare the South African isolate of ACMV with other isolates. Mechanical and whitefly transmissions and TAS-ELISAs with monoclonal antibodies were performed. PCR amplification and cloning of a partial DNA A fragment (1400nt spanning the common region (CR), AV2, coat protein (CP) and partial AC1 ORF) was performed. Sequencing of the core and entire coat protein regions was accomplished using "perimeter walking" and subcloning strategies. Amino acid (aa) similarity comparisons of the coat protein were made between SACMV and other whitefly-transmitted geminiviruses. Sequences were obtained from Genebank or the literature and analyzed using Geneproversion 4.0 software (Riverside Scientific) or CLUSTAL W (1.6) multiple sequence alignment. Mechanical and serological results placed SACMV in group B, the east African isolates. Furthermore, SACMV shared the highest aa similarities in pair wise comparisons with EACMV from Malawi (89%) and Tomato Yellow Leaf Curl Virus (TYLCV) from Israel (87%) in the coat protein ORF, compared to ACMV-Nigeria (80%) and ACMV-Kenya (79%). Similar high aa homologies were obtained with the other isolates of TYLCV. However SACMV clustered most closely with TYLCV- Israel in multiple sequence alignments and phylogenetic tree generation. Amino acid percentage differences were considered great enough (less than 90% aa similarity) between SAMCV and other SACMV in South Africa as a distinct isolate from ACMV, EACMV and TYLCV.

Key words: ACMV, dendogram, cassava, isolate

Pages 52-54

Développement des cultivars assainis de manioc

J. Mabanza, G. Tonnang et J. Mahouka

Résumé

Au niveau de l'évaluation des cultivars africains de manioc, certains aspects, notamment l'assainissement variétal, ont pu être suivis dans le cadre de l'amélioration des cultivars africains de manioc du projet STD2 de l'Union Européenne débuté en 1989. Pour ce faire, le comportment sur le terrain des produits issus des vitrocultures est apparu comme une donnee essentielle au niveau de l'évaluation variétale. L'étude a permis de connaître les potentialites des produits obtenus de vitroculture par rapport aux plantes traditionnelles. II resort que: la culture *in vitro* confère aux plantes une vigueur notable; cette vigueur se manifeste par le caractère développé de l'ensemble des organes de la plante (tige, feuille, racine amylacée) et par une production élevée en raciness amylacées. Elle se manifeste d'autre part par un meillure comportement des plantes vis-à-vis de stress divers, notamment les stress biologiques comme les parasites et l'enherbement dont I'impact est nettement inférieur à celui observé sur les plantes ordinaries. Cette vaguer restorée peut s'exprimer dans les conditions ordinaires de la culture manioc. même vis-à-vis des differents stress biologiques; par ce fait le vitroplant de manioc, peut avantageusement être exploité pour la culture commerciale du manioc.

Mots clés: manioc, cultivars assainis, culture de in vitro

Use of monoclonal and recombinant antibodies to detect cell-wall polysaccharides in cassava

M.N.V. Williams, M.G. Hahn, A.G. Darvill, & P. Albersheim

ABSTRACT

Antibodies provide an inexpensive, simple and sensitive method of analyzing specific molecules in complex mixtures. We have isolated a number of monoclonal and recombinant monospecific antibodies which recognize polysaccharides of the plant cell wall. The specific molecular structures, or epitopes, to which some of these antibodies bind, have been well characterized. Epitopes from hemicellulolistic (xyloglucan) and pectic (polygalacturonic acid, rhamnogalacturanans l and ll) polysaccharides. The integrity of these polysaccharides in the cell wall is important in resistance to plant pathogens and post harvest preservation of vegetables. In addition, pectic polysaccharides are the primary source of dietary fiber. We will use monoclonal antibodies to examine the cell wall polysaccharides of cassava plants and tubers.

Pages 54-55

Screening for and basis of resistance to cassava bacterial blight (*Xanthomonas campestris* pv. *Manihotis*)

R.M. Copper, R. Day, R. Burgin, B. Rodgers-Gray, J. Flood, R. Mepsted, G.G. Henshaw, N. Taylor, R. Gomez-Vasquez & J.R. Beeching

ABSTRACT

Because of the systematic nature of infection by *X.campestris* pv. *manihotis* (XCM), the long growth cycle and the vegetative propagation of cassava, resistant genotypes offer the only term strategy against cassava bacterial blight (CBB). Cultivars with resistance under field conditions continue to be selected for. Resistance is apparently polygenic, and this type of genetic control in many other host-pathogen interactions gives durable resistance, but may be only partial, subject to environmental control, plant age and inoculum pressure. CBB resistance often follows this pattern. The mechanisms of resistance to CBB {and to any disease of cassava} are unknown; the euphorbiaceae have been largely ignored in this respect. We aim to dissect components of resistance in order to: 1) improve current screening methods for CBB resistance; 2) provide new screening methods; 3) provide target genes for future transgenic lines; 4) reveal traits for molecular marker assisted mapping of CBB resistance. In order to investigate resistance, a facile, reproducible, realistic test which concurs with field resistance is required. Few if any tests achieve the latter criterion; but it is likely that each method may reveal different components of complex, multifaceted "field resistance". Thus, petiole stab inoculation clearly distinguished resistant and susceptible reactions but ranked cultivars differently from abaxial leaf infiltration with XCM. Some putative field resistant lines proved to be susceptible. However, field testing relies on uncontrollable incidence and levels of inoculum; also reaction may depend on the XCM isolates prevalent. Recent evidence for "races" based on differential isolate-cultivar interactions is reported elsewhere at this meeting. Leaf infiltration only differentiated cultivars at low inoculum levels, e.g., 10.2 cells/mL; there were no significant differences at 10.8 cells/mL. Most inoculation methods bypass the usual route of entry for XCM which is via stomates. Thus we investigated stomatal density, distribution and characteristics by leaf impressions and by cryo-scanning electron microscopy. On abaxial surfaces stomates were numerous but on most cultivars were covered in extensive wax deposition and in many were sunk into pits as a result of surrounding papillate cells. Consequently, the surface was non-wettable and therefore this route of entry for XCM seems unlikely. Adaxial stomates were found on all cultivars

examined (4 susceptible and 9 resistant). These were superficial, not occluded by wax and were concentrated along the mid-rib and in some cases major veins. The surface was wettable and a suspension of fluorescent particles was deposited along the mid-rib and veins following evaporation. These stomates are therefore more likely portals for entry of XCM by rain splash dispersal. There was a relationship between field susceptibility and high stomatal number (less than 10 microns, and to a lesser extent within 20 microns) adjacent to the mid-rib. Field resistant cultivars had none or very few in this location. Two field resistant cultivars (CG40211 and MCOL2261) which were the most susceptible to stab inoculation had no or very few stomates within 10 microns. It is concluded that the leaf surface characteristics can contribute to field resistance of some genotypes and may provide useful selection criteria. Opening and closing characteristics of stomates as determined by stomatal resistance with a porometer was not related to CBB reaction of two susceptible and two resistant lines. Nevertheless, the inoculation methods as described earlier which introduce XCM into cassava tissues directly, revealed resistance which must be based on cellular responses of the host. Defence related genes and their products are well known from many other host-pathogen systems. Before investigating lines with field resistance to CBB, we are testing the potential responses of cassava by first inducing a rapid, localized hypertensive response with the incompatible pathogen from *Phaseolus* bean, *Pseudomonas syringae* pv. *Phaseolicola*. Thus far, rapid induction of peroxidase, phenylalanine ammonia Iyase (PAL) and an antibacterial compound(s) has been detected; glucanase is constitutive. Also ongoing research here involves challenging suspension cultured cells of cassava with endogenous and microbial “elicitors”. Salicylic acid triggers a rapid, characteristic alkalinization of the medium (K+H+ exchange) and subsequent induction of PAL. This facile system enables biochemical and molecular probing for rapid induction of defence-related genes as revealed in many other host-parasite systems. Another application of suspension cells is in attempts to regenerate (possibly novel) disease resistant or tolerant lines from embryogenic units surviving exposure (co-culture) to XCM or to its pathogenicity factors. We have established conditions for co-culture, regeneration and rescue of cassava cells following removal of XCM cells with certain antibiotics.

Pages 56-58

Heritability estimates for a ACMD resistance for some newly developed cassava clones in Nigeria

R.E.C. Mba & A.G.O. Dixon

ABSTRACT

Data used in this study were generated from three multilocational yield and biotic stress resistance trials of newly developed cassava clones from IITA – UYT 14 (14 clones evaluated at 4 test sites for 3 years); UYT 15 (15 clones evaluated at 12 test sites for 5 years); and UYT 25 (25 clones evaluated at 10 test sites for 5 years) across the major cassava growing areas in the humid forest, savanna transition and savanna agroecologies of Nigeria. Broad-sense heritability (h^2) estimates as well as genetic advance under selection (G_s) for African cassava mosaic disease (ACMD) resistance were computed for these 3 populations. The h^2 estimates from combined analyses over locations and years, were 86%, 82%, and 70%, for UYT's 14, 15 and 25, respectively. At a selection pressure of 2%, genetic gain of ACMD severity scores of 0.58, 0.98 and 0.45 (on a scale of 1 to 5) were got for UYT's 14, 15 and 25, respectively. The values of broad-sense h^2 obtained were high enough to warrant the use of simple selection procedures. Also, the values of G_5 obtained indicate that the progenies from the populations would have very low ACMD severity scores (1.45, 1.43 and 1.45, respectively). These results indicate that resistance to ACMD due to hereditary factors is available amongst the clones tested are that significant gains can be made in resistance to ACMD by selecting from these materials.

Key words: cassava, heritability, ACMD, genetic advance, selection, phenotypic and genotypic coefficients of variation

Pages 59-61

A system to screen and select for resistance to *Fusarium solani*

W. Msikita, J.S. Yaninek, M. Fioklou, H. Baimey, M. Ahounou & R. Fagbemissi

ABSTRACT

Field selection for resistance to *Fusarium* spp is often cumbersome and inaccurate because *Fusarium* spp have many hosts, and can live saprophytically in the soil for a very long time. An experiment was undertaken to develop a system to accurately assess resistance/susceptibility for cassava genotypes in a small space, and in the absence of other pathogens. Three genotypes of cassava (TMS 30572, Agriculture, and Tchukunochi) were used for the experiment. Disease free plants were obtained by culturing nodal cuttings on Murashige and Skoog (MS) media supplemented with sucrose (30 gm/l), myo-inositol (100 mg/l), glycine (100 mg/l), adenine sulfate (80 mg/l), benzylaminopurine (0.05 mg/l), and α-napthaleneacetic acid (0.01 mg/l. After two weeks of culture, shoots were obtained, and rooted on the same medium. Tissue culture-derived plants were transplanted in pots, inoculated with *F. solani* one week later, and assessed for root mortality. Susceptibility indices for tissue culture plants (expressed as root mortality- number of dead roots per total number of roots) and stem cutting-derived plants (expressed as percentage of wilted leaves) for each plant was calculated. Genotypes varied significantly in their reaction to disease both as tissue culture and as stem cutting-derived plants. A reverse trend was observed in susceptibility index among tissue culture and stem cutting-derived plants. TMS 30572 which was the most susceptible among tissue culture-derived was the most resistant among stem cutting-derived plants. Results are discussed with a view to develop an *in vitro* based system to rapidly select for resistance to cassava root rot pathogens.

Key words: in vitro, in vivo, root rots, cassava

Pages 61-63

Cassava bacterial blight in South America: pathogenic and geneticharacterization of the causal agent and its application to screening methods

S. Restrepo, V. Verdier, G. Mosquera, A. Gerstl, R. Laberry, T. Valle & E. Alvarez

ABSTRACT

Cassava bacterial blight (CBB) is a worldwide disease particularly destructive in South America. It is caused by *Xanthomonas campestris* pv. Manihotis (*Xcm*) which can induce a great diversity of symptoms (angular leaf spots and blight symptom, gum exudation and shoot wilting). This pathogen is characterized by a systematic and an epiphytic stage of infection. CBB is managed primarily through the use of resistant cultivars. In order to guide the selection of resistant materials, information on the pathogen variation is needed. The objectives of our study were to describe the pathogen population structure in Colombia, Brazil and Venezuela and to develop an appropriate strategy to screen for varietal resistance. Molecular techniques based on RFLP studies and an analysis of the virulence variation was used to characterize *Xcm* strains. The sampling strategy permitted to assess diversity at different geographic levels (country, region and site). Analysis of 321 strains (165 from Colombia, 40 from Brazil and 96 from Venezuela) were made using molecular markers developed specifically for *Xcm* characterization. Results showed that the pathogen is highly diverse and the multiple correspondence analyses of the molecular data allowed to separate the *Xcm* strains in different genetic clusters. A general correlation was observed between the genetic clusters and the geographic origin of the isolates. A first attempt was made to demonstrate the possible existence of races among *Xcm* strains. Strains representing different genetic and pathogenic clusters detected by the RFLP analysis and the virulence analysis have been selected for inoculation of cassava germplasm collections indigenous to each country. The information obtained provides a preliminary basis to design strategies to identify different sources to the pathogen.

Key words: cassava, cassava blight, genetic characterization, *Xanthomonas campetris*

Pages 64-68

Cassava bacterial blight: recent achievements in understanding the disease

V. Verdier, S. Restrepo, B. Boher, M. Nicole, J.P. Geiger, E. Alvarez & M. Bonierbale

ABSTRACT

Cassava bacterial blight (CBB) is a major disease in Latin America, Africa and some Asian countries, causing a serious crop loss which affects both yield and planting material. The causal agent is *Xanthomonas campestris* pv. Manihotis (*Xcm*). The deployment of resistant varieties is one of the major approaches aimed at controlling this bacterial disease. Understanding the epidemiology of the disease, including knowledge of the pathogen population structure and host-pathogen interactions, is important in order to determine the best strategy for deployment of resistance. Molecular techniques have provided genetic markers that have been used to study *Xcm*/cassava interactions. The population structures of Xcm in Africa and in South America have been documented based on virulence typing and DNA analysis. African bacterial populations are homogeneous while in South America, where the crop originated, the pathogen is highly diverse. Recent studies of Xcm strains in West-Africa showed diversity that suggests host contribution to Xcm differentiation. Biochemical and histochemical studies of Xcm-cassava interactions have evidenced defense mechanisms that limit Xcm development in resistant plants. Progress has been made recently on the genetics of the cassava-Xcm
Interaction; a gene designated *pth*B, was characterized as a member of the *avr/pth* gene family) avr for avirulence, *pth* for pathogenicity and hypersensitive response). An F1-hybrid population developed for genetic mapping in cassava (Fregene et al. this volume) shows a broad range of resistance levels and search for molecular markers linked to resistance genes is in progress. The success of breeding and seed certification programs will depend on the availability of reliable methods for detecting the pathogen in cassava seeds and stakes. Recently, detection methods for Xcm based on dot-blot hybridization and PCR assays were developed and proved to be relatively sensitive. These techniques will be especially useful for detecting the CBB pathogen in sexual seed.

Key words: Xanthomonas, detection, genetic, pathogenic diversity, host parasite interactions, resistance

Pages 69-73

Integrated technology for the treatment and management of cyanoglucosides in cassava starch and sago factory waste waters

C. Balagopalan & P. S

ABSTRACT

Starch and Sago production from cassava (*Manihot esculenta* Crantz) roots is an increasingly important agro-industry. Approximately 4000-6000 liters of waste waters are discharged to the environment per tonne of starch/sago produced. High amounts of cyanogens have been detected in the waste waters generated and discharged from the cassava processing factories. Concentration of total cyanide varied from 10.4 to 27.4 mg/l in the waste waters samples collected from seven starch/sago factories. Concentration of total cyanide in the ground water sources collected near the factories ranged between 1.2 to 1.6 mg. As per the Environmental Protection Act of India (1986) the tolerable limit of cyanide in drinking water and waste waters are 0.05 mg/l and 9.2 mg/l, respectively. An integrated approach was therefore made and a low cost technique was developed to eliminate cyanogens from the waste waters by exposure to sunlight and anaerobic treatment coupled with filtration through sand, gravel and charcoal columns. Three cyanide degrading organisms viz, *Aspergillus* sp, *Saccharomyces* sp. and a *Bacillus* sp. isolated from cassava starch/sago factory waste waters were found to degrade cyanide under static conditions. Among the three organisms *Bacillus* sp. possessed maximum ability to detoxify cyanide. At higher concentrations of 12,000 and 14,000 mg/KCN/100 ml medium the percentage of reduction was to extent of 71.06 and 68.97, respectively. This showed the possibility of integrating microbial treatment with other chemical and physical treatment of cassava starch/sago factory waste waters for cynogen removal.

Key words: Integrated technology, waste waters, starch, sago, cyanoglucosides, CN, KCN, biological oxidation demand, chemical oxidation demand, anaerobic treatment-filtration, charcoal, biodegradation, linamarase, rhodanese, adsorption

Pages 73-76

Genotypic and tissue differences in biosynthetic activity for linamarin production in cassava

Y. Chukwumah, S.Y.C. Ng and M. Bokanga

ABSTRACT

The biosynthesis of cyanogenic glucosides in cassava genotypes with low and high cyanogenic potential has been investigated using a microsomal7enzyme system extracted from seedlings, germinating stem cuttings, tissue culture plantlets and root parenchyma. The results show that there is a wide variation in biosynthetic activity among cassava genotypes and that the biosynthetic activity is not correlated with the accumulation of cyanogenic glucosides in nearly all cassava tissues tested. In stem cuttings, the genotype TMS 91/01682 with the highest root cyanogenic potential (421.60 mg HCNeq.kg – 1) had about the lowest biosynthetic activity (0.13 nmol HCNeq.mg1. h-1) in etiolated leaves; the genotype TMS 82/00058 with root cyanogenic potential of 155.15 mg HCNeq.kg-1 had a biosynthetic activity of 1.92 nmol HCNeq.mg-1.h-1 and 1.71 nmol HCNeq.mg-1. h-1 in etiolated and light-grown leaves respectively, while TMS 30555 with root cyanogenic potential of 81.30 mg HCNeq.kg-1 had a biosynthetic activity of 1.09 nmol HCNeq.mg-1. h-1 and 1.69 nmol HCNeq.mg-1. h-1 in etiolated and light-grown leaves respectively. These findings suggest that the accumulation of cyanogenic glucosides in cassava tissues is not solely controlled by the rate of biosynthesis. The rate breakdown of cyanogenic glucosides or the rate of translocation may have a stronger influence on the levels of cyanogenic glucosides in cassava tissues. Biosynthetic activity has been demonstrated in light-grown materials. In both the low and high cyanogenic potential genotypes, the range of biosynthetic activity determined in light-grown leaves (0.12-2.41 nmol HCNeq.mg-1. h-1) was comparable to that found in etiolated leaves (0.13-1.92 nmol HCNeq.mg-1. h-1). In root parenchyma biosynthetic activity was detected in eight out of the eleven genotypes tested and the level of activity was found to be comparable to the levels found in other tissues. The cyanogenic potential of leaves from light-grown plant materials tended to be higher than that of leaves from etiolated plant materials. This suggests that light reactions in the plant may play an important role in the accumulation of cyanogenic glucosides in cassava tissues.

Key words: Biosynthetic activity, linamarin, cassava, cyanogenic glucosides, microsmal enzyme system

Pages 77-81

Recent advances in molecular and biochemical studies of cyanogenesis in cassava: complexity of the cassava genome

M. Hughes, J. Hughes, K. Brown & S. Liddle

ABSTRACT

Analysis of genomic clones for the cyanogenic α-hydroxynitrile Iyase and (ß-glucosidase (linamarase) genes has indicated that a single genotype of cassava (MBRA534) contains more than one copy of each. Thus the MBRA534 genome contains at least 10 different ß-glucosidase genes (some of which may be allelic) and 7 α-hydroxynitrile Iyase genes (some of which may be allelic). Different ß-glucosidase genes have the same basic intron-exon structure as do different α-hydroxynitrile Iyase genes, although intron sizes as well as sequences can vary. The promoters of a number of these genes have been sequenced and putative control motifs identified. The expression of individual genes has also been investigated in vivo using RTPCR and reporter gene transient expression, following biolistic introduction of the DNA into plant cells. Studies of the α-hydroxynitrile Iyase and (ß-glucosidase proteins has focused on the use of recombinant proteins in yeast and bacteria as a preliminary to their manipulation for optimal production and activity in cassava fermentation processing.

Key words: cyanogenesis, genome organization, α-hydroxynitrile Iyase, ß-glucosidas

Pages 81-84

Reason for use of bitter cassava in southern Tanzania

R. Kapinga, N. Mlingi & H. Rosling

ABSTRACT

Reports indicate that cassava farmers in several areas with food insecurity preferentially grow bitter and toxic cultivars. This seems paradoxical since it oblige adherence to laborious processing of roots to avoid toxic effects. To explore farmers rational for use of bitter and toxic cassava cultivars a rapid assessment procedure was done in Mtwara region. Following key-informant interviews we selected seven villages representing agro-ecological variation in the region. The cultivar preferences were elucidated through focused group discussions and unstructured interviews during observation of processing and visits to fields. Classification of cultivars regarding taste was done on a continuum from very bitter to bitter, cool and very cool. Farmers stated that poisoning may result from consumption of unprocessed bitter roots but not from non-bitter roots. The four reasons for use of bitter cultivars were protection against theft, protection against attacks by vermin, high yields on poor soil and tolerance to pests and diseases. Selection of seedlings from spontaneously planted cassava seeds was reported by women in one village. In another village we learnt from a man that the established processing by sun-drying could be shortened and still yield safe flour from very bitter roots if they were first pounded to achieve complete root tissue disintegration and then completely dried before pounding into flour. This is consistent with chemical studies. Farmers have rational reasons to grow bitter cultivars and can estimate the risk of acute poisoning based on taste of roots and processing applied.

Key words: cassava, bitter, southern Tanzania, farmers

Pages 84-85

The biosynthesis of cyanogenic glucosides is catalyzed by two membrane-bound multifunctional cytochrome P450's and a soluble UDPG- glucosyltransferase

Moller B.L., Soren Bak, Rachel A. Kahn, Fahrendorf T. & Barbara Ann Halkier

ABSTRACT

Cyanogenic glucosides are found in more than 2500 different plant species some of which like cassava are impor~tant crop plants. Upon damage of plant tissues containing cyanogenic glucosides, these will be partly or fully hydrolyzed into cyanohydrins and cyanide. Plant materials containing high arnounts of cyanogenic glucosides like cassava thus needs careful processing before being used as food. When careful processing is not carried out, cassava consumers may be exposed to high levels of cyanide. The reason for doing the work has been to: (1) identify the intermediates involved in the biosynthesis of cyanogenic glucosides; (2) isolate and characterize the enzymes catalyzing the biosynthetic steps; (3) isolate and characterize the structural genes involved; (4) transform cassava with anti-sense constructs to knock-out the first specific step in the pathway tissue- specifically or in total; (5) characterization of the cassava plants obtained especially with respect to their resistance towards pests and predators. Much progress has been obtained within objectives (1) to (3). Other groups have recently been successful in obtaining stable transformation of cassava. In collaboration we hope soon to be able to embark into objectives; (4) and (5). From a nutritional point of view, it would be a great advantage to be able to remove or dramatically lower the content of cyanogenic glucosides in cassava or at least in the tubers. It has been speculated that cassava plants devoid of cyanogenic glucosides would be vulnerable to attack pests. This may be true but not necessarily. Each plant contains hundreds of secondary products and the removal of one is not expected to break down all the different systems of the plant. Those secondary products produced in high amounts are not necessarily the most important ones. These aspects and our knowledge of the intermediates, enzymes and genes involved in the synthesis of cyanogenic will be discussed.

Pages 85-87

Partial characterization of the linamarin-synthesizing enzyme complex from cassava

J.M. Mcmahon & R.T. Sayre

ABSTRACT

Many plants produce cyanogenic glycosides which are hydrolyzed following tissue disruption to liberate hydrogen cyanide. Cassava (*Manihot esculenta*) accumulates the cyanogenic glycosides linamarin in all tissues, with the exception of seeds. Recently, we have demonstrated that the vacuole is the sub-cellular site of linamarin storage, and that linamarin can be synthesized in cell-free vacuole preparations. In this study, we present results on: 1) the partial purification of the enzyme(s) catalyzing the first dedicated step in linamarin synthesis, and 2) tissue and cultivar dependent patterns of linamarin synthesis activity. The active cyanogen synthesizing complex has been solubilized from salt-washed tonoplast membranes using reduced Triton X-100 and purified approximately 200-fold. Based on carbon monoxide difference spectra and substrate-binding studies, it is apparent that a cytochrome P450 catalyzes the initial hydroxylation of valine to form N-hydroxyvaline. We demonstrate that antibodies against an avocado cytochrome P450 as well as chlorpromazine inhibit cyanogen synthesis in RTX-100 solubilized tonoplast membrane fractions. Until recently, it was believed that linamarin was synthesized only in the leaves and subsequently transported to the roots. However, we have demonstrated that cassava roots are capable of synthesizing linamarin at rates comparable to leaves. We have measured the rates of cyanogen synthesis in microsomal fractions from three cassava cultivars of differing cyanogenic potential (CNp). Microsomes isolated from root peel (intermediate CNp) had a linamarin synthesis rate of 4.95×10^{-4} μmol/mg protein/hr, while leaf microsomes synthesized linamarin at a rate 2.46×10^{-4} μmol/mg protein/hr. Root microsomal preparation from the high CNp variety had a higher rate of cyanogen synthesis than either the intermediate or low CNp varieties. These results demonstrate that there is no apparent correlation between linamarin content and synthetic capability.

Key words: cassava, cytochrome P450, cyanide, microsomes

Pages 88-90

Variation of cassava cyanogenic potential in different agro-ecologies

J. Mkumbira, N.M. Mahungu & S.K. Salipira

ABSTRACT

Cassava cyanogenic potential varies among different agro-ecologies, clones, plants of the same clone and tissues of the same plant. Variation of cyanogenic potential in different agro-ecologies is of importance in areas where cassava is consumed raw or with minimum processing. A comparison of cyanogenic potential in seven environments was made in Malawi using eight different cassava clones. Results showed differences in magnitude of cyanogenic potential variation between agro-ecologies than those with relatively high levels. At one environment (Makoka) cyanogenic potential of the clones was consistently low.

Key words: cassava, cyanide, agro-ecologies, environment

Pages 90-94

Comparison of three cyanogen assays for total cyanogens in cassava (*Manihot esculenta* Crantz)

J.D.K. Saka, A.R.K. Mhone & L. Brimer

ABSTRACT

The sensitivity and reproducibility of three methods for determining the total cyanogenic potential (CNp) of fresh and processed cassava were determined and compared. The total cyanogen content of fresh cassava roots and three cassava products (kondowole, makaka, and starch) were analyzed by the acid hydrolysis, microdiffusion with solid state detection and Cooke's enzymatic assays. The total cyanogen contents of the cassava, obtained by the three methods were not significantly different ($p < 0.05$). For example, the CNp of a low cyanogen containing variety, Mwaya were 18.9 ± 1.6, 20.3 ± 0.4 and 20.4 ± 1.4 mg HCN eq. kg^{-1} fresh weight by Cookie's, acid hydrolysis and solid state methods, respectively. However, at very low cyanogen levels, less than 5 mg HCN eq. kg^{-1} fresh weight, the acid hydrolysis method overestimates by 3-5 times. Otherwise, their coefficients of variations are very similar, <10%. The acid hydrolysis and solid state methods are cheap and the latter can be used under field conditions to screen for low or high cyanogenesis. They are strongly recommended for use in laboratories in the developing countries.

Key words: cassava, cyanogenic potential, assay, enzymic assay, acid hydrolysis, picrate, solid state

Pages 94-95

Use of highly cyanogenic cassava cultivars (*Manihot esculenta*) in the production of biodegradable pesticides

F.L.C. Jackson, J. Dubow, D. Foster, and J. Denkevitz

ABSTRACT

Humans and animals of the tropical regions continue to be plagued by hyperendemic vector-borne diseases. The increasing economic and ecological cost of using petroleum-based pesticides has created a great need for biodegradable plant-based pesticides as a more cost-effective and ecologically sound replacement. Cell-sap solutions of the cyanogenic glucosides from cassava (*Manihot esculenta*) are highly effective against the main vectors of schistosomiasis, malaria, and filariasis, three important diseases targeted by the World Health Organization for control. Additionally, the toxic constituents of these cassava-based pesticides degrade within 24 hours, preventing bioaccumulation and limiting ecological damage. Low concentrations of linamarin and lotustralin kill lst, 2nd, and 3rd instar larvae of *Aedes* spp., *Anopheles* spp., and *Culex* spp. Mosquitoes and inhibit pupal development and adult emergence. For these species, the LC50 was approximately 0.48 ppm while the LC90 was approximately 0.97 ppm. Cassava cell-sap solutions also kill young *Bulinus* spp. And *Biomphalana* spp. Snails at concentrations under 1.35 ppm. Even lower concentrations of the cyanogenic glucosides kill the infective forms of *Schistosoma* spp. Given these positive laboratory and field results, under different ecological settings, it is important to retain highly cyanogenic cultivars of Manihot esculenta explicitly for pesticide production.

Isolation of a cDNA clone of the hydroxynitrile Iyase gene from cassava and its expression in different organs

White W.L.B. & R.T. Sayre

ABSTRACT

The presence of cyanogens, mainly acetone cyanohydrin, in cassava root products can pose a serious health hazard to consumers. Hydroxynitrile Iyase (HNL) converts acetone cyanohydrin, produced from the deglycosylation of linamarin, to acetone and hydrogen cyanide. The gene for HNL was cloned from a cassava leaf cDNA library. The 1.1 kb cDNA had a derived protein sequence which differed from the previously published cDNA (Hughes et al. 1994) by 12 amino acids. The derived amino acid sequence was approximately 30% identical and 50% similar to the rice pathogen induced resistance genes pir a and pir b. The area with the highest homology occurs in the first 62 amino acids. These regions have 55% identity and 70% similarity. HNL activity assays and immunoblot indicate that HNL is present in leaves but not stems or roots. Northern, Southern, and immunoblot analyses will be presented. In order to facilitate detoxification of cassava root products, we plan to genetically engineer cassava to overexpress HNL in cassava roots.

Pages 95-96

Reasons for use of "bitter" and toxic cassava in Malawi

Linley Chiwona-Karltun, Ngoma J., Mahungu N.M., Saka J., Tylleskar T. & Rosling H.

ABSTRACT

African farmers subsisting on marginal land reportedly prefer toxic and "bitter" varieties with high levels of cyanogen glucosides. Cassava processing, a woman's task, renders roots storable, non-toxic and improves palatability. In Nkhata-Bay distinct roots are soaked and fermented for 2-7 days, sun dried and processed into flour. In-depth interviews with farmers conducted in 13 communities, linguistically revealed that cassava is grouped as harmful (*Chibaba, Chingakupweteka* words that seem to refer to both toxicity and bitterness) and cool (*Chizzima. Chizika* words that refer to absence of toxicity and bland taste). Farmers identified three groups of reasons for the use of "bitter" cassava: 1) Food security. The necessity to process bitter roots ironically empowered women to decide when and how much to harvest. Farmers consistently stated that "bitterness" guards the crop from attacks by monkeys and other vermin as well as theft by humans. Furthermore, there is less of social obligation to share the fresh toxic roots with neighbors and relatives since the bitter cassava can not be readily eaten as a snack. 2) Yield. The farmers also stated that bitter varieties yielded more than the non-bitter varieties, especially in low-fertility soils and in areas with low rainfall. 3) Quality. Bitter varieties produce whiter flour, a less elastic porridge (*kondowole)* and a more palatable leaf relish, according to the majority of the informants. Furthermore, mechanized milling or improvements in cassava processing that reduce time and energy exerted by women were regarded as high priority in cassava post-harvest technology.

High cassava consumption without cyanide exposure in Kinshasa, Zaire

Rosling H., Banea M., Bikangi N. & Tylleskar T.

ABSTRACT

Background: Bandundu region in Zaire is a major supplier of cassava to the capital Kinshasa. But in this region short-cuts in soaking of roots from bitter and toxic varieties have been shown to cause high dietary cyanide exposure that has been linked to acute poisoning and epidemics of the paralytic disease konzo. Aim: To study if inhabitants in Kinshasa that consume cassava from Bandundu are exposed to cyanide. Methods: Food frequency interviews and collection of urine was made from 66 women in a Konzo affected village in Bandundu and from 205 women in two zones in the outskirts of Kinshasa. Results: The frequency of daily cassava consumption was high in both groups, 100% in Bandundu and 85% in Kinshasa. Soaking and drying was often shortened in Bandundu, whereas all women's in Kinshasa only consumed cassava flour obtained by mechanical milling of dry roots. Mean urinary linamarin was the highest ever recorded in women in Bandundu, 614 compared to 11 mmol/l in those from Kinshasa. The mean urinary thiocyanate, the main cyanide metabolite was 558 in Bandundu and 12 mmolA in Kinshasa. The value found in Kinshasa is lower than that of European reference populations, 27-31 mmol/l, and urinary thiocyanate did not differ between women in Kinshasa consuming cassava from Bandundu and from other regions respectively. Conclusion: High cassava consumption in Kinshasa was not linked to any detectable cyanide exposure although roots originate from a region where such exposure is common. The reasons are probabl- prolonged storing of roots during transport followed by complete drying before mechanical milling. This seems to result in effective removal of cyanogens and the findings suggest that mechanization of milling will secure consumption of cassava with regard to cyanogenesis.

Pages 97-99

Amélioration qualitative des produits du manioc: conservation de la pâte égouttée, pétrie et précuite

E. Avouampo

ABSTRACT

Le manioc, aliment de base au Congo est généralement transformé de manière traditionnelle. Au cours la transformation, les produits semi-finis (raciness rouies, pâtes défibrées et pétrie), sont souvent conserves pendant une durée n'excédant pas 5 jours. Cependant, la pâtes précuite est transformée aussitôt et n'a jamais été conserve. Agricongo, qui étudie le procédé traditionnel en mettant au point le procédé méchanique de fabrication de chikwangue a constaté qu'il est possible de conserver cette pâte pendant plus de 7 jours. Les operations de rouissage, pétrissage, précuisson et emballage/caisson ont été executes de telle sorte que les chikwangues obtenues proviennent des pâtes conserves pendant 0, 2, 7 et 14 jours, et que la cuisson de ces chikwangues se fasse simultanément et ce, de façon standard. Les observations faites du premier au dernier jour de la conservation montrent la presence d'une microflore sur les pâtes conserves ont été compares à celles des pâtes non conserves, les résultats des tests de dégustation ont montré que les chikwangues des pâtes conserves ont été mieux appréciées. La conservation de la pâtes a non seulement un effet bénéfique organoleptiquement, mais qu'elle permet aussi d'obteir un produit capable deter vendu dans la marches comme les autres produits et derives du manioc.

Mots clés: Manioc, pâtes précuite, conservation, qualities organoleptiques, chikwangues

Pages 99-105

Physiological deterioration: towards a molecular understanding

J.R. Beeching, Y. Han & R.M. Cooper

ABSTRACT

Postharvest physiological deterioration severely limits the marketability of cassava, necessitating its prompt consumption or processing upon harvesting. The phenomenon has been described physiologically and to a certain extent biochemically, and is found to bear strong parallels to wound and stress-induced responses in better characterized plant model systems. Exploiting this theoretical background and existing tools rapid progress can be made in understanding physiological deterioration in cassava. A cDNA library from mRNA from wounded cassava tuber tissue was constructed in λ gt 10. From this library a cassava cDNA clone for phenylalanine ammonia-Iyase was identified and isolated using a bean genomic clone as a heterologous probe. Preliminary characterization of this clone is presented here.

Key words: cassava, physiological deterioration

Pages 105-110

Applications de la biotechnologie dans l"industrie de transformation du manioc: Panorama actuel et perspectives

Gerard Chuzel

Résumé

Les trois grandes domains possibles d'applications de la biotechnologie pour la transformation du manioc et sa valorization sont présentés sur la base de resultants de recherché conduits par diverses equips dans le contexte socio-économique latino-américain de transformation du manioc: (1) l'amélioration de la qualité des produits traditionnels fermentés par la maîtrise des processus fermentaires, que ce soit au niveau de procédé, des propriétés fonctionnelles et nutritionnelles des produits ou des mechanisms de détoxication; (2) la transformation bioloque des déchets et sous-produits des industries de première transformation du manioc, que ce soit pour le traitement des eaux résiduaries (eaux de pressage ou eaux d'extraction d'amidon) ou les déchets solides (tourteaux d'extraction, issues) (3) le développement de nouveaux produits, en valorisant les potentialities des souches isolées dans les fermentations traditionnelles, ou en utilisant des enxymes commerciales pour la bioconversions d'amidons en divers hydrolysats

Mots cles: manioc, fermentations, produits traditionnels, déchats

Pages 110-113

Application of microbial starter cultures for new and traditional cassava products

A.J.A. Essers & M.J.R. Nout

ABSTRACT

Traditional fermented cassava products are inventorized; only few novel products exist. Some research on traditional fermentation of cassava has been done, but this has rarely led to application of (improved) starter cultures in commercial practice. Possible reasons for this lack of implementation, such as shortage of insight into consumer demand and economic feasibility, are discussed. Basic questions on objectives, demand, and technological and organizational issues, need for viable implementation of new fermentation technology are formulated; and ways for applying biotechnology to cassava processing are suggested.

Key words: cassava processing, fermentation, starter culture

Pages 113-116

Enhancement of cassava fermentation: Tanzanian village experience

E.M. Urio & N.L.V. Mlingi

ABSTRACT

Cassava is an important food security crop in Tanzania. It forms a secondary staple in ten out of the twenty five regions in the country. Traditional cassava processing methods and ways of consumption are reviewed with respect to four agro-ecological zones. Submerged and heap fermentation practices which involve microbial activity are discussed. Drawbacks and limitations of the methods in relation to safety and quality of the end products are pin-pointed. Future outlook and possible areas of research for the purpose of improvement and promotion of indigenous processing methods are highlighted.

Key words: cassava processing, fermentation, safety and quality

Pages 116-120

Developmental regulation of cassava granule bound starch synthase ll

T.R.I. Munyikwa, E. Jacobsen & R.G.F. Visser

ABSTRACT

We have isolated a 2708 bp cDNA encoding a 751 amino acid polypeptide that shows homology to potato (70%) and pea (72%) granule bound starch synthase II (GBSSII). The derived amino acid sequence of this cassava GBSSII exhibits low sequence homology to cassava GBSSI (35% identity). However within the C-terminus there are regions (with up to 90% homology) which are conserved amongst the starch and glycogen synthase genes. Cassava GBSSII is highly and differentially expressed in leaves, whereas GBSSI shows high constant expression in tubers and little expression in leaves. Expression of these two genes is trancriptionally controlled in relation to the type of tissue where starch synthesis is occurring.

Key words: cassava, granule, starch, development regulation

Pages 120-123

The effects of pre-harvest pruning of cassava upon post-harvest deterioration potential, scopoletin and dry matter contents

G.M. O'Brien, Q. van Oirschot, O. Orozco, A.L. Chaves & J. Mayer

ABSTRACT

The agronomic practice of pre-harvest pruning (removal of most of the aerial part of the plant) is known to significantly reduce the post-harvest physiological deterioration (PPD) susceptibility of cassava roots. The results of a recent experiment involving the pre-harvest pruning of cassava plants and their subsequent harvest at set intervals, point away from any simple causal link between either parenchymal scopoletin or parenchymal DMC and visible PPD. Plants of two cassava cultivars, one susceptible to PPD, and the other highly resistant, were harvested (i) without pruning, and (ii) at various intervals after pruning. Sample roots were taken for the evaluation of scopoletin content, dry matter content and PPD. The three parameters were observed in relation to time after pruning. In the PPD-susceptible cultivar, a sharp downward trend in visible PPD during the post-pruning period was accompanied by a similar trend in the scopoletin contents in samples taken. A significant correlation between the two parameters was observed (R= 0.81), implying that pruning, as well as leading PPD-susceptibility, also led to reduced parenchymal scopoletin contents. In the PPD-resistant cultivar, a notable overall reduction in PPD was observed, and scopoletin content also decreased generally. However, some individual results contradicted the general trend: around 3% of results matched relatively high scopoletin levels (>4 mg/kg, fresh weight basis) with zero visible PPD. With both cultivars it was noted that DMC, which underwent some minor fluctuations after pruning, did not correlate significantly with visible PPD (R < 0.1). The evidence suggests that there is no simple, straightforward link between DMC or scopoletin and PPD.

Key words: cassava, pruning, postharvest physiological deterioration, scopoletin, dry matter

Pages 123-127

PCR amplification, cloning and expression of a phenylalanine ammonia-Iyase gene in cassava *(Manihot esculenta* Crantz)

L.F. Pereira, A. Agyare-Tabbi & L. Erickson

ABSTRACT

With the objective of acquiring some understanding of the molecular mechanisms of both physiological deterioration of cassava roots and plant-pathogen interaction between cassava and *Xanthomonas campestris*, we have amplified and cloned a phenylalanine ammonia-Iyse gene (PAL) from cassava genomic DNA. Sequence analysis of the 520 bp amplified product (PCRPAL) demonstrated high homology with previously isolated PAL genes in other species. PCRPAL is more highly expressed in leaves than in stems, and more highly in young leaves than in mature leaves. Expression of PCRPAL also increases following leaf injury. The results obtained regarding PCRPAL expression are in agreement with the literature, and currently we are measuring expression in roots at different stages of deterioration and in leaves challenged with Xanthomonas *campestris*

K**ey words**: cassava, cloning, PCR amplification, Iyase gene, phenylalanine, ammonia

Enhanced traditional biotechnology for cassava development

M. Bokanga

ABSTRACT

For several millennia, biotechnology has been at the service of mankind, particularly for the production of foods and beverages. Cassava has been used as a substrate for microbial biotechnology in Brazil, one of the probable centre of domestication of the crop, but also in Asia and Africa where the crop was recently introduced. Examples of indigenous biotechnological applications on cassava include the production of sour starch in Latin America, beiju in Brazil, tapai ubi and tape ketela in southeast Asia, gari, attiéké and chikwangue in Africa. Potent alcoholic drinks are also traditionally made from cassava in several countries. The soybean bioprocessing industry in Asia has demonstrated the enormous benefit that can be derived from modernization of traditional bioprocesses. Although the creation of wealth through biotechnology depends upon the involvement of the industrial sector and the business community, farmers who supply the raw material and their knowledge of traditional bioprocesses must participate in the creation and sharing wealth, if a sustainable development is to be achieved.

Pages 128-129

Behavior of starch suspensions from different cassava varieties subjected to thermal treatment and lactic fermentation

V. Pedroarias, A. Kafka, C. Rovedo, S.V. de Fabrizio, C. Suarez, G. Chuzel

ABSTRACT

Cassava starch uses are increasingly wide, particularly as food ingredient or additive. Fermented vegetal milks elaborated with cassava starch suspensions has been recently proposed focusing a raising interest. Concomitantly, microbiological and rheological aspects of such suspensions have become more significant. We have already found that amylolytic lactic acid bacteria (ALAB) are able to accomplish the biotransformation of starch. Also gelified suspensions obtained by the thermal treatment showed dramatic changes in their viscosity, when subjected to the lactic starters action. Although cassava starch is considered free of cyanide (CN), it was observed that the CN content in roots from different varieties correlated with different varieties correlated with differences in the viscoamylograms of the corresponding starch suspensions. It was interesting then to investigate which factors could affect the consistency or texture of fermented starch suspensions. 6% starch suspensions from different sources previously pasteurized (90°C, 20 min with shaking) and fermented by ALAB starter (12°C. 10 h) were used as prototypes of fermented cassava beverages. Starch was obtained at laboratory scale from three varieties MCoLCMC4O, Mcol 1684 and Mcol 1505 (CIAT germplasm collection) Colombia and compared with commercial starch from Argentine (ARG) and Brazil (BRA). The fermentation was followed by several parameters: pH, lactic acid production, apparent viscosity, microbial enumeration, as well as sensorial and rapid tests of fluidness and creaminess used by the industry. In all cases, the pH decreased during the fermentation together with a continuous drop in the viscony associated with an evident increase in the 9uidness and in the lactic acid percentage. The lactic bacterial population remained slightly higher at the end of the fermentation whereas the tenure was not significantly affected. The apparent viscosity measured immediately after pasteurization was similar for the three MCol starches, suggesting that the proposed heating conditions would minimize the differences observed in regards with the CN content in the roots. However, the Mcol 1505 samples showed unusually lower viscosity during the fermentation even though the microbial counts indicated an efficient pasteurization process. To elucidate these observations, starch suspensions of all sources were pasteurized and incubated at 32°C for 24 h without any inoculunL. The viscosities of samples prepared with the commercial starch ARG and BRA were not affected, almost the same occurred with the viscosities of MCoL 1684 and CMC40. But in the case of MCol 1505 the viscosity dropped 53% at 6h

and more than 90°/0 at 24h of incubanon. Commercial starches are usually treated with SO_2 or hypochloride to control undesirable microorganisms. Then, starch MCol 1505 was treated with sodium hypoclhoride at a final concentration of 0.6 mg of acive CI_2 per g of dried starch. This time the incubator without inoculum of the gelified suspension prepared with the treated starch Mcol 1505 did not affect the consistency. Furthermore, the thermoresistant rod responsible for this unexpected amylolyic effect was isolated. Our results indicated that microbiological and processing factors in starch obtention may better determine the rheological properties of fermented cassava milks than the CN content in the root of different cassava varieties. As these two factors and fermentation parameters can be better controlled. Diverse cassava varieties can be used to obtain this kind of health products creating opportunities for processors and new markets for farmers from different regions.

Pages 129-131

Enhancing the nutritive value of cassava crop residues and by-products for livestock feeding through microbial degradation

O.O.Tewe

ABSTRACT

Cassava peels, tender stems, leaves and starch residues which constitute about 25% of the plant are largely discarded as waste after harvest and processing in most African countries. These can serve as valuable sources of energy, protein, minerals and vitamins in livestock feed industry when biodegraded with microbial organisms. Fungi that produce cellulases include *Trichoderma viride, T. roningii* and *Penicillum funiculosum*. *Neurospora sitophila* degrades cell wall of cassava root and reduces its cyanogen content through its pectolytic and cellulolytic enzyme system. *Aspergillus niger* enhances breakdown of non-starch polysaccharides, reduction of cyanide and enrichment of protein in cassava substrates. The efficacy of these micro-organisms in enhancing the nutritive value of cassava crop residues and by products for feeding of poultry and pigs need to be further investigated.

Key words: cassava, nutritive value, crop residues, microbial degradation, livestock feeding

Pages 132-134

Application of molecular markers to describe South African elite cassava cultivars

S. Laminski, E.R. Robinson & V.M. Gray

ABSTRACT

In the past cassava has not been viewed as a major crop in South Africa. Its importance however was realized, and a breeding programme established from which a number of elite cassava cultivars were selected. This selection was largely based on conventional breeding criteria. These methods are not reliable as estimates of genetic diversity due to the heterozygosity of cassava and the varying conditions under which trials were done. Determining genetic diversity among cultivars has increasingly made use of molecular methods. RAPDs and isoenzymes were used in this study to determine the genetic differences and genetic fingerprints of the South African elite cassava cultivars. Results were examined, using the computer programme NTSYS, to determine the reliability of both RAPDs and isoenzymes. A number of known international cassava cultivars were included, to have a reference point in further studies in determining the uniqueness of these elite South African cultivars.

Key words: cassava, molecular markers, South Africa

Pages 135-138

Isolation and characterization of repetitive and microsatelite DNA sequences in cassava

A. Agyare-Tabbi, L.F Pereira & L.R. Erickson

ABSTRACT

Microsatelites or simple sequence repeats (SSRs) are a subclass of tenderly-arranged repetitive DNA sequences which are made up of 2-5 basepair repeat motifs. They have been shown to be an abundant and a highly polymorphic set of molecular markers. In an attempt to develop this marker system for cultivar identification and the estimation of genetic diversity in cassava cultivars and their wild relatives and to understand the roles of repetitive DNA in the genome, we have cloned and characterized repetitive sequences in this species. Southern blots of genomic DNA from two cultivars of cassava were hybridized with ^{32}P-labelled oligonucleotide probes [$(CA)_{10}$, $(GA)_{10}$, $(GATA)_4$] to assess the nature and frequency of SSRs in cassava. Results indicated that (GA) and (GATA) repeats were the most abundant. A genomic library in lambda DASH with inserts between 13-20 kb was screened with the same probes. A combined probe of $(CA)_{10}$ and $(GA)_{10}$ did not give any positive clones, however the $(GATA)_4$ probe yielded 38 positives. Sequence analysis has shown that one of two $(GATA)_4$ –positive clones has a microsatelite locus with $(GATA)_5(GA)_{13}$ repeat region. With primers from the flanking region, this locus was tested for polymorphic variation among cassava cultivars but was found to be monomorphic in the cultivars tested. The other positive clone has homology to genes in LTR-retrotransposons.

Key words: cassava, manioc, microsatelite DNA sequences

Pages 138-139

Application of molecular markers to genetic mapping and germplasm characterization in cassava

M.W. Boniebale, M.M. Maya, E. Barrera, M. Fregene, V. Verdier, J. Bedoya and A.C Roa

ABSTRACT

Molecular maps provide direct measures of genetic distinction at discrete loci throughout a plant genome. The application of such maps to breeding objectives depends on the simultaneous monitoring of molecular (genotypic) and whole plant (phenotypic) variation in a defined set of genetic stocks. In the case of characters that are quantitatively inherited, this is most effectively accomplished by the evaluation of sexual progenies that segregate for a trait of interest. Once markers representing genetic loci that explain a significant proportion of the phenotypic variability are identified, they can be developed as gene tags, and used as correlative screens in selection pro grams. The cassava map described by Fregene et al. (these proceedings) is based on a cross designed to segregate for several important cassava characters. During the development of this map, 150 clones of CM7857 (TMS30572 x CM2177-2) have been propagated for field evaluation, and preliminary information gathered on the range of variability presented for root quality, resistance, and physiological parameters. A wide range of segregation has been observed for resistance to bacteriosis, photosynthetic rate, cyanogenesis, perishability and dry matter content, and experiments are planned to gain increased precision on measurements of the genetic components of this variance. Our goals are to identify the important loci controlling these traits by their consegregation with mapped molecular markers, and apply the resulting genetic information and gene tags to improving selection schemes. Molecular markers also provide neutral characters for quantitative estimates of genetic similarity. An application to the study of diversity within and among *Manihot* species will be discussed.

Assessment of genetic diversity in *Manihot* species with AFLPs

Merideth Bonierbale, Ana C. Roa, Maria M. Maya, Myriam C. Duque and Joe Thome

ABSTRACT

Although cassava is one of world's most important tropical crops, the origin and domestication of the crop, and relationship with other species of the genus *Manihot* have not been clearly explained and are under debate. In this study, 105 genotypes of five wild Manihot species proposed as close relatives or ancestors of cassava, one wild species expected to be more distant, and a selected sample of the crop germplasm were analyzed with AFLP (Amplified Fragment Length Polymorphism) to estimate genetic similarities with the NTSYS program. Results showed individuals grouped according to prior taxonomic classification. *M. aesculifolia, M. brachyloba* and *M. carthaginensis* were the most distant taxa with respect to cassava. *Manihot esculenta* subsp. *flabellifolia* and *M. esculenta* subsp. *peruviana* formed a mixed group closet to the crop. The last group presented major genetic diversity with respect to the crop germplasm. These findings are in agreement with the proposal that the subspecific taxa of *M. esculenta* are most closely related to cassava and support the hypothesis that ancestors of cassava can be found in this group. The crop germplasm presented a narrower range of variation than most wild species. Some of wild taxa showed species specific bands which could be useful for identification and classification of germplasm and introgression studies. The presence of genetic diversity in patches within some of the species suggests the existence of different gene pools within these taxa. In most cases these findings were associated with distinct morphology and ecogeography.

Pages 140-147

Molecular marker (AFLP)-based *Manihot* and cassava numerical taxonomy and genetic structure analysis in progress: implications for their dynamic conservation and genetic mapping

G. Second, A.C. Allem, R.A Mendes, L.J.C.B Carvalho, L. Emperaire, C. Ingram & C. Colombo

ABSTRACT

We are using multilocus molecular markers, with samples representing the geographic and ecological range of distribution of *Manihot* spp; along with classical botany and ecology, to characterize: 1) the genetic structure of cassava in relation to its wild relatives and 2) the domestication process of cassava. Not only a numerical taxonomy of the genus is expected but also the definition of strategies for the conservation and the utilization of these genetic resources. Amplification fragment length polymorphism (AFLP^{TM}) is used on: 1) 276 plants representing a large part of the diversity of the wild species of the genus, and some presumed natural inter-specific hybrids, and 2) 82 plants of cassava representing a) the cultivars in the World collections, and b) the varieties present in one Amazonian field with ethnobotanical record. The two first axis of variation (PCo of the matrix of similarity in pair-wise comparisons) extract, from the total, cassava along with several species that are closely related to it, including hybrids between *M. glazovii* and cassava. Although domestication appears to have involved primarily *M. esculenta* spp. *flabellifolia* and *peruviana*, it seems that some other species have also contributed. The question of the possible role of inter-specific hybridization in the domestication process of cassava is then raised again, particularly since the importance of inter-specific hybridization and also the high ratio of intra/inter-specific variation in the genus are confirmed. The genetic diversity of cassava itself is high, but a diversity nearly equal, although slightly divergent, is found in a single Amazonian field at the level of AFLP. It is shown that a traditional variety can include several related genotypes and the hypothesis of a dynamic management of the diversity of cassava according to the Amazonian tradition is confirmed. The results validate the methodology adopted but it should be desirable to apply it to a sampling even better representative of the genus. They also validate the choice of strategy of dynamic conservation for the wild species. The important genetic recombination suggested to be at the origin of the diversity of cassava gives a favorable perspective for various strategies of genetic mapping and gene tagging, for this crop usually multiplied vegetatively.

Key words: Manihot, cassava, AFLP, molecular markers, genetic resources, dynamic conservations, numerical taxonomy

Pages 147-149

Phylogenetic relationships and genetic diversity in *Manihot* species

B. Schaal, L.J.C.B. Carvalho, T.Prinzie, K. Olsen, M. Hernandez, G. Cabral, & D. Møeller

ABSTRACT

The phylogenetic relationship among species of the genus Manihot has been actively debated. Resolution of species affinities is of critical importance since the domesticated species, *Manihot esculenta*, cassava, provides an important source of carbohydrates for people throughout the tropics. We have investigated the species relationships within the genus *Manihot* by a molecular phylogeny reconstruction and by an analysis of RAPD data. Gene sequences used for phylogeny reconstruction include the ITS-I and ITS-2 regions of nuclear ribosomal DNA, calmodulin, linamarase, and aspartate amino transferase. Genomic DNA is isolated from either fresh or dried leaf material, DNA sequences are amplified by PCR and PCR products are directly sequenced. Sequence data are analyzed using the computer program PAUP while RAPD data are analyzed by NTSYS. DNA analysis indicates a South American origin for cassava with *M. esculenta ssp. Flabellifolia* and *M. esculenta ssp. peruviana* being the nearest wild relatives. Several gene sequences are identical among these two subspecies and cassava, indicating close affinities and most likely, a recent common ancestor. Thus, particular effort should be made to collect and preserve germplasm from *ssp. flabellifolia* and *ssp. peruviana* for future use in cassava breeding programs.

Key words: cassava, phylogeny, *Manihot* species, genetic diversity

Pages 150-151

A molecular genetic map of cassava (*Manihot esculenta* Crantz)

M. Fregene, F. Angel, R. Gomez, F. Rodriguez, P. Chavarriaga, M. Bonierbale, W. Roca, and J. Tohme

ABSTRACT

A genetic linkage map of cassava has been constructed with 132 RFLPs, 30 RAPDs, 3 microsatelites, and 3 isoenzyme markers segregating from the heterozygous female parent of an intraspecific cross. The F_1 cross was made between TMS 30572 and CM2177-2, elite cassava cultivars from Nigeria and Colombia, respectively. The map consists of 20 linkage groups spanning 931.6 cM or an estimated 60% of the cassava genome. Average marker density is one per 7.9 cM. Since the mapping population of an F_1 cross between heterozygous parents, with unique alleles segregating from either parent, a second map was constructed from the segregation of 107 RFLPs, 50 RAPDs, 1 microsatelite, and 1 isoenzyme marker from the male parent. Comparison of intervals in the male and female derived maps, bounded by markers heterozygous in both parents, revealed significantly less meiotic recombination in the gametes of the female than in the male parent. Six pairs of duplicated loci were detected by low-copy genomic and cDNA sequences used as probes. Efforts are underway to saturate the cassava map with additional markers, to join the male and female derived maps, and to elucidate genome organization in cassava.

Key words: manioc, cassava, carte genetique, molecular genetic map, isozyme, RFLP, linkage groups, DNA

Pages 152-154

Screening for embryogenic competence in some cassava (*Manihot esculenta* Crantz) cultivars in Ghana

K.E. Danso & R.K.A Ahiabu

ABSTRACT

Three cassava (*Manihot esculenta* Crantz) genotypes, a local cultivar "*Bosom nsia*", and two IITA improved cultivars TMS 30572 and TMS 4(2)1425 were screened for their embryogenic competence. Young leaf lobes dissected under the microscope were cultured on an induction medium of Murashige and Skoog basal salts supplemented with 16 mg/l 2,4-dichlorophenoxyacetic acid. Cultures were incubated in the dark for 30 days in a growth room. After 15 days all the leaf lobes of the three cultivars developed into embryogenic calli. Calli were transferred to Murashige and Skoog basal salt supplemented with 0.1 mg/l benzylaminopurine for development into mature embryos. The cultivar "*Bosom nsia*" and TMS 4(2)1425 showed higher embryogenic competence than TMS 30572. Embryogenic calli which could not mature into embryos developed either roots without shoots or foliose structures. Investigations into increased embryo production and subsequent conversion into plants are being studied.

Key words: somatic embryogenesis, embryogenic calli, embryogenic competence

Pages 154-158

Friable embryogenic callus formation from thin cell layer explants of cassava

J. Groll, V.M. Gray & D.J. Mycock

ABSTRACT

Thin cells layers isolated from stem internodes or petioles of cassava produced a friable embryogenic callus on media with high cytokinin levels. Any of the following cytokonins: 6-benzylaminopurine (BAP); 6-(γ, γ-dimethylallylamino) purine (2iP); 6-furfurylaminopurine (kinetin); or N-phenyl-N'-1, 2, 3-thiadiazol-5-ylurea (thidiazuron) induced embryogenic callus production at levels of 10 μM when supplemented with 3-indoleacetic acid (IAA) at 0.1 μM. The first signs of embryogenic callus production were observed 12 days after initial plating. Direct root organogenesis from the thin layer tissues occurred even earlier after 5 days, shoot organogenesis was never observed. Embryoids were first observed after three weeks, and after four weeks some embryoids possessed a clear radicle and showed radicle elongation, even at high cytokinin levels. This is not reported by other workers who have used 2, 4-D to induce somatic embryogenesis from leaf lobes. The effect of nodal position on the parent plant on subsequent embryogenic callus production was also investigated. Highest frequency of embryogenic callus formation occurred when the uppermost nodes were used as an explant source. Although at a lower frequency, embryogenic callus also formed from thin cell layers derived from petioles. Previously researchers have shown that somatic embryos of cassava could only be initiated from embryos or leaves, therefore this protocol presents a novel approach to initiating embryogenesis that may compliment the range of existing techniques.

Key words: cassava, Manihot esculenta, somatic embryogenesis, thin cell layers.

Pages 158-132

Analysis of a cassava root specific β-glycosidase promoter

S. Liddle, J. Hughes & M.A Hughes

ABSTRACT

Recent advances in cassava (*Manihot esculenta* Crantz) transformation and regeneration have laid the way open for improvement of cassava by genetic engineering. In order to do this effectively, cassava tissue specific promoters need to be found. In order to achieve this a cassava genomic library was constructed form cultivar MBRA 534 and screened for genes with homology to the cassava cyanogenic β-glycosidase (linamarase) cDNA clone, pCAS5. A number of clones were isolated and one of them, pCBG3, was subcloned and sequenced. The *cbg3* gene codes for a 541 amino acid protein which shows a 75% identity (86% similarity) to the cassava linamarase protein. The gene contains twelve introns of sizes ranging from 79bp to 244bp. Genomic PCR indicates that the linamarase gene also has twelve small introns inserted into the coding region at the same positions as in *cbg3*. Primers specific to the *cbg3* gene have been used in conjunction with RT-PCR to show that this gene is expressed in the roots and not the leaves or cotyledons of three week old seedlings. A 1.3 kb section of the promoter has been sequenced and a number of putative promoter elements identified. The promoter is currently being studied using a transient assay of a reporter construct delivered to cassava tissue using biolistics.

Key words: cassava, glycosidase promoter, root

Pages 163-167

Transformation and gene expression in cassava via tissue electroporation and particle bombardment

H.T. Luong, P.R. Shewry & P.A. Lazzeri

ABSTRACT

Parameters affecting tissue electroporation efficiency of embryogenic cassava tissues were identified and optimized. GUS- expressing regions were observed in proliferating tissues, 5 months post-electroporation, but transformed shoots were not recovered. Using particle bombardment, several constitutive and tuber protein promoter constructs were tested in cassava embryogenic tissues, leaves, stems, roots, and tuberous root discs for transgene expression. Plasmids containing tuber storage protein promoters (patatin, sporamin or ß-amylase) were also found to be transiently expressed at high levels in cassava embryogenic tissues. For bombardment experiments aimed at recovering stably transformed tissues, pre-cultured, immature leaves were co-bombarded with the plasmids pDE4 and pDE110 (CaMV35S-bar-Nos) encoding the gene conferring resistance to PPT. Bombarded explants were induced to undergo somatic embryogenesis and cycles of secondary embryogenesis prior to regeneration under selection pressure. Chimeric, GUS-expressing, matured somatic embryos were obtained, and regenerating tissue, tolerant to PPT selection were shown by PCR analyses to contain the GUS fragment. However, GUS-positive shoots have not been obtained to date, although several PTT-tolerant plants are currently growing under selection. PCR conditions for the *bar* gene have been difficult to optimize, but plants are being analyzed by other methods. Low plant regeneration frequency from somatic embryos was considered to be the limiting factor for transformation experiments. The inclusion of an embryo maturation step on charcoal-supplemented medium and modifications of the regeneration medium significantly increased frequency of plant conversion, while the use of a plant development medium further enhance the vigour and sustainable growth of regenerated plants.

Key words: cassava, gene expression, electroporation, particle bombardment.

Pages 167-169

Identification and characterization of tuber-specific clones in cassava

K.L. Marello, P.R. Shewry & J.R. Beeching

ABSTRACT

The cassava tuber is largely uncharacterized from a molecular perspective, yet future improvements of the crop may rely extensively on a greater understanding of this area. The isolation of tuber-specific genes and their promoters should allow for the selective expression of genes of interest, as a means of biotechnological improvement. As an initial step towards this end a cDNA library was constructed in λgt11 from mRNA isolated from tubers of the cassava cultivar CMC 40. Screening by the polymerase chain reaction confirmed the presence of inserts ranging from 350bp to 2400bp, which were analyzed by cloning into plasmid vectors and partial sequencing to decide upon those of interest. One clone, A41 represents a novel cassava single copy gene with no known homology in the database and is currently under further investigation.

Key words: cassava, polymerase chain reaction, characterization, identification

Pages 169-172

Transformation and culture of cassava protoplasts

S.L. McDonnell & V.M. Gray

ABSTRACT

A rapid single cell *Agrobacterium tumefaciens* based transformation procedure using cassava protoplasts has been developed and tested. The objective of this endeavor was to assess the feasibility of a transformation procedure involving protoplasts. The procedure involved co-cultivation of protoplasts with the *A. tumefaciens* (strain LBA4404) containing the binary vector pBI121 for 24 hours followed by removal of the bacteria via centrifugation and antibiotic treatment (carbenicilin and cenfotaxime). ß-glucuronidase (GUS) activity in the protoplasts was assayed 1 day and 1 week after transformation using a spectrophotometric assay technique base on an assay using p-nitrophenyl-ß-D-glucuronic acid (pNPG) as the substrate. Protoplasts were then cultured in a regeneration medium where cell wall regeneration took place 4 to 5 days after culture, and the first cellular division was observed 14 to 16 days after culture. Viable microcalli, derived from transformed protoplast cultures, formed after one month in the regeneration medium.

Key words: cassava, protoplasts, agrobacterium, transformation, microcalli, regeneration

Pages 172-175

Searching for root specific promoters in cassava

S. Bohl, I. Potrykus & J. Puonti-Kaerlas

ABSTRACT

In genetic engineering of cassava, tissue specific promoters will be of prime importance. The prerequisite for screening for root specific genes is the construction of a cassava genomic library. In order to do this, DNA extraction methods had to be optimized to ensure high yields of good quality DNA from different cassava tissues. Likewise, for extraction of RNA from primary and secondary cassava roots, RNA isolation protocols had to be modified to allow yields of good quality of RNA. A cassava cosmid genomic library was constructed, containing average fragment sizes of 17 kb. The library thus obtained represents 5 equivalents of cassava genome. For heterologous promoter studies, a transient expression was developed for cassava root cambium.

Key words: cassava, genomic library, RNA isolation, transformation

Pages 175-180

Development of meristem gene transfer techniques for cassava

J. Puonti-Kaerlas, P. Frey and I. Potrykus

ABSTRACT

Genetic improvement of cassava via biotechnology is still constrained by lack of efficient, genotype-independent transformation methods. Meristems are the natural growth centres of plants, and thus regeneration from these structures should be less genotype dependent. In order to study a possibility of establishing meristem transformation as a genotype-independent we have developed regeneration methods from cassava meristems via multiple shooting somatic embryogenesis. Different transformation methods are being tested for their compatibility with these regeneration schemes. Shooting conditions have been partly optimized by studying particle penetration into meristems and the effects of DNA concentration and pretreatments on meristem survival and transient transformation frequencies after particle bombardment.

Key words: Cassava, meristem, transformation, regeneration

Pages 181-186

Production of transgenic cassava (*Manihot esculenta* Crantz) via organogenesis and Agrobacterium-mediated transformation

J. Puonti-Kaerlas, H.-Q. Li, C. Sautter & I. Potrykus

ABSTRACT

Biotechnology provides a valuable tool for genetic crop improvement, but until now the lack of reliable transformation systems allowing selection and regeneration of transgenic plants has prevented the use of genetic engineering in cassava. By combining direct shoot organogenesis from somatic cotyledon explants and *Agrobacterium*-mediated gene transfer we have developed the first reproducible method for producing transgenic cassava plants with molecular data to confirm their transgenic nature. After initial screening, two *Agrobacterium* strains were selected for transformation studies, and different aspects of the cocultivation procedure were partially optimized. Using the protocol derived from the optimization studies, transgenic shoots could be regenerated after selection on geneticin or hygromycin. Stable integrative transformation was shown by molecular analyses. The Southern data prove stable integration of the transgenes into the cassava genome, and the transcriptional activity of these genes is demonstrated by the Northern blots. This now opens cassava for genetic engineering, and makes it possible to transfer agronomically important genes into this species, integral for food security in tropical areas.

Key words: cassava, *Agrobacterium*, transformation, regeneration

Pages 187-193

Stable transformation of cassava (*Manihot esculenta* Crantz) by particle bombardment and by Agrobacterium

C. Schöpke, N. Taylor, R. Cárcamo, A.E. González de Schöpke, N.K. Konan, P. Marmey, G.G. Henshaw, R.N. Beachy & C. Fauquent

ABSTRACT

A protocol was established for the introduction of DNA into embryogenic suspension-derived tissues of cassava via microparticle bombardment, for the selection of genetically transformed cells, and for the regeneration of fully transgenic plants from these cells. The plasmid DNA used for bombardment contained a gene encoding neomycin phosphotransferase (*nptll*) and a gene encoding ß-glucuronidase (*uidA*). Selection of bombarded tissue with paromomycin resulted in the establishment of putative transgenic embryogenic calli. In most of these calli ß-glucuronidase (GUS) was detected histochemically. Molecular analysis of paromomycin-resistant embryogenic calli and of plants regenerated thereof confirmed the stable integration of bombarded DNA into cassava genome. After the establishment of a selection and regeneration protocol for microbombarded tissue, we modified this protocol to use it for *Agrobacterium*-mediated transformation of embryogenic suspensions. Embryogenic tissue was infected with two *Agrobacterium* strains containing a plasmid with the *nptll* and the *uidA* genes. Many paromomycin-resistant and GUS positive lines of embryogenic callus were recovered from the infected tissue. Some of these lines are now in the process of shoot regeneration.

Key words: cassava, *Manihot esculenta*, embryogenic suspensions, microbombardment, transformation

Pages 194-195

Plant regeneration from transgenic and non-transgenic embryogenic suspension cultures of cassava *(Manihot esculenta* Crantz)

C. Schöpke, R. Cárcamo, R.N. Beachy & C. Fauquent

ABSTRACT

Although the regeneration of plants from embryogenic suspensions cultures of cassava in principle is possible, the efficiency was very low when we tried to regenerate transgenic embryogenic embryogenic tissue of cassava. A culture system in which plant tissue is placed on a membrane that floats on liquid medium has been reported to overcome possible problems related to the traditionally used semi-solid culture media. As part of our optimization experiments for the regeneration of plants from transgenic embryogenic tissue of cassava, we investigated the use of this system.

Key words: cassava, *Manihot esculenta*, embryonic suspension, lifeRaft, membrane culture

Pages 196-200

Optimization of chemical selection of transgenic friable embryogenic callus of cassava using the luciferase reporter gene system

S.C.H.J. Snepvangers, C.J.J.M. Raemakers, E. Jacobsen & R.G.F. Visser

ABSTRACT

Friable embryogenic callus was bombarded with DNA of the construct pJIT100. This construct contains the firefly luciferase gene and the phosphinoacetyltransferase gene. Luciferase activity was used to evaluate the efficiency of selection of transgenic tissue by phosphinothricin (PPT) during the proliferation, maturation and secondary somatic embryogenesis. Addition of 5 mg/l PPT yielded the highest number of independent transformation events per bombarded dish. Sixteen weeks after bombardment distinct growing clumps of tissue were observed, even on medium without PPT. More than 50% of the distinct growing clumps observed on media supplemented with PPT and none of the clumps observed on a medium without PPT turned out to be luciferase positive. On a medium supplemented with 20 mg/l PPT selection of clumps would be most efficient with respect to proliferation of transgenic tissue. Maturation of friable embryogenic callus was not inhibited by the use of PPT during the proliferation phase. However, not all the mature embryos obtained, were luciferase positive. The fraction of mature embryos was increased if PPT was added to the maturation medium. Mature embryos cultured on a medium supplemented with 2, 4-D formed secondary embryos. Secondary somatic embryogenesis of non-transgenic embryos was completely inhibited if PPT was added to the medium.

Key words: cassava, transformation, luciferase, phosphinothricin

Pages 200-204

Improved procedures for producing embryogenic tissues of African cassava cultivars: Implications for genetic transformation

N.J. Taylor, R.J. Kiernan, G.G. Henshaw & D. Blakesley

ABSTRACT

The inclusion of NAA with picloram in the induction medium and optimization of the state of the mother plants has led to significant improvements in the production of primary embryogenesis across a range of African cassava cultivars. By utilizing the primary embryogenic tissues so produced, friable embryogenic callus and suspension cultures have been established in eight out of ten cultivars attempted to date, demonstrating the applicability of this culture system for the transformation of a significant range of cassava cultivars. Genetic transformation programmes are ready to proceed with these cultivars.

Key words: cassava, embryogenic tissue, genetic transformation, Africa

Pages 204-208

The cassava vein mosaic virus promoter: A new promoter for cassava genetic engineering

B. Vendaguer, K. Konan, A. de Kochko, R.N. Beachy & C. Faquent

ABSTRACT

The cassava vein mosaic (CVMV) is a double stranded DNA virus which infects cassava plants in northern Brazil and has been characterized as a plant pararetrovirus belonging to the caulimovirus subgroup. Two DNA fragments, CVPI of 388 nucleotides from position -368 to +20 and CVP2 of 511 nucleotides from position -443 to +72, were isolated from the viral genome and fused to the *uidA* reporter gene to test promoter expression. Both promoter fragments were able to cause high levels of gene expression in protoplasts isolated from cassava and tobacco cell suspensions. The expression pattern of the CVMV promoters was analyzed in transgenic tobacco and rice plants, and revealed that the GUS staining pattern was similar for each construct and in both plants. The two promoter fragments were active in all plant organs tested and in a variety of cell types, suggesting a near constitutive pattern of expression. Particle bombardments of cassava tissue provide the first evidence that the CVMV promoter is strongly expressed in this important crop. An *nptll*: CVMV fusion gene construct used for selection of cassava transgenic tissue is the first biotechnology application of this new promoter.

Key words: cassava, cassava vein mosaic virus promoter, genetic engineering

Pages 209-212

In vitro cassava research capability at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) - Ghana: potentials and constraints

R.K.A. Ahiabu, K.E. Danso, G.Y. P. Klu, P. Bieler & W. Msikita

ABSTRACT

The Biotechnology and Nuclear Agriculture Research Institute (BNARI) is one of three institutes under the Ghana Atomic Energy Commission. Its main research focus is on the application of nuclear techniques to biological and agricultural research. Cassava *Manihot esculenta* Crantz is one of four vegetatively propagated crops being researched on to improve its genetic constitution using *in vitro* mutagenesis and also to clean planting material using thermotherapy and meristem culture. Genetic improvement is aimed at the selection of mutants resistant to the African cassava mosaic virus (ACMV) disease which is predominant in Ghana. Somatic embryogenesis and shoot tip culture techniques coupled with mutagenic treatments are the main methodologies being employed to select for suitable variants resistant or tolerant to ACMV. This work is sponsored by the International Atomic Energy Agency (IAEA) and the Ghana Government. Results indicate potentials for obtaining desirable mutants but so far, only tolerant variants have been selected and are being multiplied for future replicated trials to evaluate their performance. Thermotherapy and meristem culture for cleaning selected cassava cultivars and the evaluation of these in relation to ACMV infected cuttings is another work being sponsored by the International Institute of Tropical Agriculture (IITA) under a UN project termed Ecologically Sustainable Cassava Plant Protection (ESCaPP) project. Two IITA improved cultivars and a local cultivar are being used in this study. Weaned *in vitro* plantlets of these multiplied in a screen house for trials next year. This poster is to highlight potentials of BNARI and constraints in relation to work at the institute.

Key words: cassava, mutagenesis, mutant, variant

Pages 212-214

In vitro propagation of six Zambian clones of cassava (*Manihot esculenta* Crantz)

W.K. Chishimba* & E.S. Lingumbwanga

ABSTRACT

In vitro propagation of six Zambian cassava clones namely; Luc 55, Luc 78, Luc 133, Luc 289, Luc 304 and Luc 327 was investigated using half-strength Murashige & Skoog and, Linsmaier and Skoog media. Currently, the potential to produce virus-free cassava seed stocks through tissue culture remains completely untapped in Zambia. To apply this system on a massive scale like seed production, a reliable cheap in vitro propagation medium has to be developed. Therefore, the objective of this study was to establish the most suitable medium on which the six Zambian cassava clones can perform very well *in vitro*. Ten stakes per clone were sourced from the Root and Tuber Improvement of the Department of Agriculture in Zambia. The clones were established in a small field plot to serve as source of material for *in vitro* manipulations. After seven months, axillary explants (2 cm long) were excised and disinfected in 50% sodium hypochlorite (3% available chlorine) containing 0.1% Tween 20 as a surfactant. Explants were rinsed 4 times with sterile deionized distilled water under the hood before trimming to about 1cm and culturing on either half-strength Murashige and Skoog medium, or Linsmaier and Skoog medium supplemented with 0, 1, 2, or 3µM benzyladenine. The results consistently showed that superior *in vitro* growth was tenable on half-strength Murashige and Skoog medium without any exogenous application of plant growth regulators. Plants established in the field did not show any abnormalities in growth, form and tuber quality. This study shows that the six clones are numerable to *in vitro* propagation.

Key words: cassava, *in vitro*, propagation

Pages 214-215

Cryoperservation of cassava shoot tips through rapid freezing

R.H. Escobar and W.M. Roca

ABSTRACT

In the last few years we have developed a cassava cryopreservation technique, based on a programmed slow freezing protocol. This technique allows to recover viable cassava plants from shoot tips maintained in L.N (Escobar et al, 1995), and opens the way to the development of a long term conservation system (base gene bank) for this important crop plant. Recently, we have been keen to make this methodology more accessible in terms of cost and time of operation. As an approach to this goal, we have worked with the direct immersion of shoot tips into L.N. The rapid freezing technique resulted in plant recovery rates as high as with the programmed slow freezing protocol. Viability rates 80-90% and plant recoveries of 40-50% have been recorded with CV. MCol 22. Considering the large number of accessions in the world cassava germplasm collection held at CIAT, the rapid freezing technique would not only expedite the process, but make it more operable in terms of cost per accession. The technique will be evaluated with a larger set of cassava genotypes and develop it further for future implementation accession.

Key words: cassava, cryopreservation, shoot tip, rapid freezing

Pages 215

Improvement of plant regeneration from cyclic somatic embryos of cassava (*Manihot esculenta* Crantz)

Zhu, Ji; Huang, Yuwen & Liang, Chengyi

ABSTRACT

Cotyledons from matured somatic embryos of cassava were used as explants. When $AgNo_3$ was added in the induction medium or maturation after culturing for 7 or 15 days, respectively, plant regeneration frequency (PRF) increased from 40.5% (control) to 58.1% in the former treatment and from 36.1% to 61.3% in the later treatment. When ABA was added to the induction or maturation medium, PRF increased from 46.4% to 72.5% during the induction of somatic embryogenesis and from 35.1% to 81.3% in the maturation stage of somatic embryos. Decreasing the concentration of 2, 4-D from 4.0- mg/l to 2.0 mg/l, and combining with 16 mg/l $AgNO_3$ in the induction medium at 7 days after inoculation and 0.25 mg/l ABA in the maturation medium, significantly improved maturation of somatic embryos and their development to form normal shoots. A highest PRF of 95% was obtained, and number of plants produced per explant reached 39.6, higher than any data previously reported.

Pages 216-219

Cassava acclimatization in Mozambique

M.A.B. Jorge

ABSTRACT

A tissue culture laboratory was set up at Umbeluzi Research Station of INIA in 1991, to promote mass production and distribution of healthy selected varieties of cassava (*Manihot esculenta* Crantz). The first transfer of plants to the field the rate of survival was unacceptably low (10-15%). To improve this, four series of trials were conducted in 1995 to determine the best method for acclimatization of tissue culture cassava plantlets. Different combinations of substrate composition, levels of lighting, opening times and opening rates of the humidity chambers were evaluated. Locally adapted humidity chambers versus the recommended IITA and CIAT types were also compared. Parameters used were plant survival and leaves per plant. Light intensity was found to be very critical for plant survival. The best survival rates were 40%, using the local method under 50% of shade as well as the IITA and CIAT methods under 85% of shade. The effect of the substrate composition, opening times and opening rates were not very clear, as there were almost no significative differences among treatments and most of the plants died before the end of the trials. The achieved percentage of survival was yet far from the optimal, thus additional experiments considering other valuables must be carried out to achieve satisfactory survival levels.

Key words: *Manihot esculenta*, tissue culture, humidity chambers, hardening off

Pages 220-221

Rapid multiplication of selected cassava genotypes in Kenya

E.M. Kanguha & A. Maliu

ABSTRACT

Cassava is currently an important crop in Kenya especially in the dry areas. It is rated as the fourth important food crop in the tropics. Tissue culture plays an important role as a means of rapid multiplication of vegetatively propagated crops. Cassava is quiet amenable to this technique. The aim of undertaking this research was to find a media that could be used for the rapid multiplication of our genotypes. Plantlets from the glasshouse were sterilized with alcohol for three minutes and mercuric chloride at 0.01% then washed three times with alcohol for three minutes and mercuric chloride at 0.01% then washed three times with sterile distilled water. A media was adapted from International Atomic Energy Agency (IAEA) laboratories and used for the rapid multiplication of six genotypes in Kenya. The constituents of the media were Murashige and Skoog salts Myo-inositol, thiamine, sugar, the hormones, Benzyl amino purine (BAP), Naphthaleneacetic acid (NAA) and Gibberellic acid (GA_3). The media was hardened by agar. The Murashige and Skoog sailts were prepared. This reverted all the six genotypes into callus. Plantlets were sterilized by the above technique than cultured on the above media but with the hormone NAA excluded. Plants with strong shoots and a good root system were obtained. These are being multiplied for further research in mutation breeding and as a source of clean planting materials for farmers.

Key words: cassava, Kenya, genotypes, multiplication, genotypes

Pages 222-226

An efficient mass propagation system for cassava utilizing nodal explants and auxiliary bud-derived meristems

N.K. Konan, C. Schöpke, R. Cárcamo, R.N. Beachy & C. Fauquet

ABSTRACT

Nodes from 3-5 weeks old in vitro plants of different cassava cultivars were cultured for 2-3 days on semi-solid Murashige and Skoog (MS) basal medium supplemented with 10 mg/l cytokinin to induce the enlargement of auxiliary buds. Subculture of these buds onto medium of the same type resulted in multiple shoot formation within 4-6 weeks. Of the four cytokinins tested (BAP, TDZ, zeatin, kinetin), BAP was the most efficient for inducing shoot development. Optimum results were obtained with the cultivar TMS 30555, in which 63% of the explants produced at least 10 shoots on medium containing 10 mg/l BAP. In cultivars that did not produce shoots, the addition of the surfactant Pluronic F-68 (2% w/v) to the regeneration medium raised the percentage of explants forming at least 5 shoots from 0% to 20-60%, depending on the cultivar. Auxillary buds were also used as a source of meristems. These were dissected in order to test their ability to regenerate into shoots. Shoot formation from meristems of six cultivars was observed after a treatment that included preculture on medium supplemented with 5 mg/l BAP followed by a transfer to medium containing 10 mg/l BAP.

Key words: cassava, *Manihot esculenta*, micropropagation, Pluronic F-68, multiple shoots

Pages 226-227

In vitro culture of *Manihot glaziovii* Muel. Arg. Zygotic embryos

R.A. Mendes, M. de-Goes, J. B. Teixeira & M. Pasqual

ABSTRACT

The most effective factor controlling the *in vitro* germination rates of *M. glaziovii* embryo axes was the temperature. The temperatures of 25 and 30°C were better to stimulate the germination than 20°C. The effect of temperature on the germination rates indicates a tendency of stabilization, nevertheless, it is possible that a slight increase in the temperature will effect positively on the germination. Tests of zygotic embryo culture using WPM nutrient medium supplemented by activated charcoal at high temperatures of 25-30°C are recommended for other *Manihot spp*. In order to check the possibility of using this methodology as an alternative routine for the rescue of wild relatives of cassava.

Key words: Manihot glaziovii, cassava, zygotic embryos, in vitro culture

Pages 228-231

Responses of cassava to polyethylene glycol mediated osmotic stress *in vitro*

S.Y.C. Ng & I.J. Ekanayake

ABSTRACT

Studies were conducted to test the responses of cassava (*Manihot esculenta* Crantz) nodal cuttings to polyethylene glycol (PEG) mediated osmotic stress *in vitro.* Results from seven cassava genotypes indicated that growth of cassava node cuttings was completely inhibited at 20% and 30% PEG in the culture media (representing osmotic potentials of -0.5 MPa and -0.85 MPa, respectively). However, some of the node cuttings of two genotypes, TMS 50395 and TMS 90853, remained green at 20% PEG. The responses of node cuttings to lower PEG concentrations, between 1% to 10% or osmotic potentials of -0.054 and -0.218 MPa, respectively, showed that there were genotypic differences. Varying thresholds were observed for different genotypes in certain growth parameters such as survival, rooting ability, leaf number and plant height. Based on these findings, the potential of PEG as a selective agent for identifying tolerance to waste stress mediated dehydration and related physiological studies in cassava is discussed.

Key words: cassava, polyethylene glycol, stress, in vitro, osmotic

Pages 232-234

Cassava *in vitro* germplasm management at the International Institute of Tropical Agriculture

S.Y.C. Ng & N. Q. Ng

ABSTRACT

In vitro techniques have been applied to the conservation and exchange of cassava germplasm at the international Institute of Tropical Agriculture (IITA). The initial explants, meristems or nodal cuttings, obtained from plants grown in the field/screen house, were cultured on modified Murashige and Skoog (MS) medium. Regenerated plantlets were subcultured to increase the number of cultures before they were transferred to lower incubation temperatures (18 to 24°C) for storage. Embryos from seeds of wild *Manihot* germplasm were cultured on half strength MS medium. Plantlets obtained from these embryos were subsequently cloned and then transferred to the reduced temperature conditions for storage, or transplanted to the field for evaluation. Using meristems/nodal culture technique, some cassava germplasm collections in national programs were successfully transferred to IITA for conservation. Currently, a total of 727 accessions of cassava and related *Manihot* species are maintained in reduced incubation temperature conditions. Cultures can be stored for 8-14 months. For dissemination of selected cassava germplasm to collaborators outside Nigeria, plantlets regenerated from meristems culture were transplanted and indexed for African cassava mosaic virus (ACMV). Plantlets regenerated from nodal cuttings obtained from these virus-tested plants were used for distribution upon request. NARS in more than forty countries in Africa received these certified virus-tested plantlets for evaluation.

Key words: *Manihot esculenta*, germplasm conservation, embryo culture, meristems culture

Pages 234-238

Gestion d'une banque de genes de manioc: experience au Congo

F.R. Otabo, J.C. Moussouami et J. Mabanza

ABSTRACT

Le materiel génétique d'une variété qui disparaît est irreplaçable. Pour séléctionner améliorer ou développer de nouvelles souches adaptable et variées, cette information doit être préservée. La conservation du matériel génétique est donc d'une grande importance dans un programme d'amélioration ou de création variétale. Au Congo des 1975 le programme de selection et d'amelioration du manioc a utilisé des collections vivantes sur le terrain a Loudima, Odziba et Kindamba. Ceci a abouti à la perte d'un grand nombre de matériel végétal. La mise en place d'une banque de génes in vitro de manioc à été réalisée dans le but d'assurer une meilleure conservation des ressources génétiques de manioc disponibles. Des conditions de culture *in vitro* permettant de conserver le germoplasme de manioc en condition de vie ralentie aussi longtemps que possible ont été déterminées. Un milieu de culture a été notamment mis au point. Ce milieu comprend peu de saccharose et un peu de manitol et favorise une conservation des plantes jusq'au delà de six mois sans altérer la faculté de multiplication des plantes. Des observations sur des genotypes d'origines diverses (IITA Zaïre, genotypes locaux) sont réalisées au niveau de la croissance et du développement des plantes. Ce travail a abouti à une meilluere gestion de la vitrothéque par le stockage de plus 100 accessions dans avior recours au nécessaire renouvellement chaque six semaine connu au paravant.

Mots cles: banque de gene, manioc, culture, *in vitro*

Pages 238-242

Regeneration of plants from somatic embryos and friable embryogenic callus of cassava (*Manihot esculenta* Crantz)

C.J.J.M. Raemakers, M.G.M. Rozenboom, K. Danso, E. Jacobsen & R.G.F. Visser

ABSTRACT

In cassava somatic embryogenesis can occur both indirectly (friable embryogenic callus) and directly (secondary somatic embryogenesis). In the direct system, plants were regenerated in a two step procedure. In the first step friable embryogenic callus is cultured for maturation. A Murashige and Skoog based medium gave the best results. Mature embryos were obtained from all tested transgenic friable embryogenic callus lines and from friable embryogenic callus lines derived from protoplasts. The transgenic mature embryos were first multiplied via secondary somatic embryogenesis to increase the number and to obtain synchronized cultures. NAA induced secondary embryos, which had never undergone the process of friable callus induction and maintenance, germinated at high frequency if they were desiccated and cultured on a medium supplemented with BAP. Secondary embryos derived from friable embryogenic callus (transgenic and non-transgenic, or derived from protoplasts) germinated at a much lower frequency. The resulting plants derived from non-transgenic and from protoplast derived friable embryogenic callus were *in-vitro* morphologically similar to the original genotypes. This was in contrast with plants derived from the transgenic friable embryogenic callus lines in which about one quarter yielded plants which had a dwarfed-phenotype.

Key words: cassava, friable embryogenic callus, maturation, germination, transgenic

Pages 243-245

Tissue culture of cassava: a South African perspective

B.R. Woodward, J. Allemann & B.P. O'Regan

ABSTRACT

In South Africa, there has recently been revived interest in cassava as a source of industrial starch as well as a supplement for animal feed. Extensive cultivations and a processing plant have been established in the Northern Province. Our interests in cassava, however, is to be able to provide disease-free planting material through meristems culture and improved cultivars for Southern Africa, as well as provide directed training in cassava tissue culture techniques at our UNESCO/BAC biotechnology training courses. The biotechnology work on cassava will also form an important supplement to the activities of the Developing Agriculture Research Programme at VOPI. About 30 different cassava lines have been established *in vitro* and their performance in tissue culture assessed. Leaf explants of the cultivar MCOL 1505, an ACMD resistant parent line and four other cultivars were induced to form callus and somatic embryos on a medium containing picloram levels. Research has also been initiated on in vitro thermotherapy and meristems cultures to obtain disease-free planting material.

Key words: cassava, tissue culture, South Africa

UNESCO/BAC Training Programme in plant biotechnology at Roodeplaat Vegetable and Ornamental Plant Institute

B.R. Woodward and J.A. Brink

ABSTRACT

The Biotechnology Action Council (BAC) of the United Nations Educational Scientific and Cultural Organization (UNESCO) has established a regional Education and Training Centre (BETCEN) in the field of plant biotechnology at the Roodeplaat Vegetable and Ornamental Plant Institute (VOPI) in Pretoria. As one of five BETCEN's in the world the aim of this programme is to provide technical and scientific training and support in biotechnology in Africa. The various training courses consist of: (1) A basic tissue culture course (2) An advanced tissue culture course (3) A plant molecular marker course and (4) A three-month fellowship at VOPI. All these courses consist of theory and practical work and include, amongst others, crops important in Africa, e.g. sweet potato, potato, cassava and maize.

Studies on induction of somatic embryogenesis and shoot organogenesis in cassava at the South China Institute of Botany

Ma, Guo-Hau; Xu, Quishen; Xian, Yunlan; & Guo, Jun-Yan

ABSTRACT

A culture procedure using NAA as the sole auxin has been developed for the induction of somatic embryogenesis from immature in vitro leaflets. A total of 16 Chinese and South American cultivars were tested by culture on Murashige and Skoog (MS) basal medium supplemented with various concentrations on NAA. 40 mg/l NAA was found to be the most effective and could be used to induce somatic embryogenesis from 12 of the tested cultivars. The frequency of primary embryogenesis varied between the cultivars, with best response occurring from M.Col. 1505 at approximately 25%. In the majority of the cultivars the level of induction frequencies achieved with NAA were comparable to (but not greater then) those records when explants were cultured on MS supplemented with 2, 4-D at 4 mg/l. Somatic embryos of the cultivar Nanzhi 188 were produced by continuous cyclic culture on MS basal medium supplemented with 4 mg/l 2, 4-D. When 4 mm^2 fragments of these embryos were cultured on medium to which had been added various concentrations of cytokinins and auxins, organogenesis or embryogenesis was induced depending the type of growth regulator employed. TDZ and BAP were found to be more effect than kinetin or 2-iP for the induction of organogenesis and could induce shoot formation even when used in combination with NAA (0.2 mg/l) or picloram (0.05 mg/l). The highest frequency of shoots formation took place on medium supplemented with NAA (0.2 mg/l) in addition to either TDZ or BAP at 1.0 mg/l. In this case 45 – 50% of the explants underwent caulogenesis with an average of between 1.5 and 2 shoots produced by each tissue fragment. It is considered that this culture system has potential value for use with *Agrobacterium* transformation protocols.

Pages 246-247

Role of somatic embryogenesis and organogenesis in breeding cold tolerant cassava

Li, HQ; Ma, GH; Zhu, J & Guo, JY

ABSTRACT

Cassava (*Manihot esculenta*) is an important tropical crop in many developing countries. In order to extend its cultivation to the vast marginal land in northern China, we have been studying somatic embryogenesis, organogenesis and plant regeneration. We are aiming to develop systems for genetic transformation and for the induction of somaclonal variation which can be used to improve cold tolerance in cassava. The results obtained to date indicate that the induction of primary somatic embryos via callus could be easily achieved when very young leaf lobes were used as the explant. In addition, by using solid and liquid medium in cyclic culturing, the production of embryos was greatly increased. Over 90% regeneration was achieved by employing maltose as the carbon source and optimizing the use of NAA, ABA, $AgNo_3$ and paclobutrazol in the induction medium. When fragments of cotyledons from the germinated embryos were cultured on medium containing cytokinins with no 2, 4 – D, many shoots were produced from around the cut surface of the fragments. Such tissues are desirable for infection with *Agrobacterium tumefaciens* and by using the strains LBA4404 (pBin9-Gusint) and G418, we have obtained GUS-stained shoots which have been tested by PCR and by Southern and Northern blot hybridization. Somaclonal variation and genetic transformation for cold tolerant cassava are now being further studied.

Pages 248-253

Selection of cassava varieties by farmers in the Lake Zone of Tanzania

R. Kapinga, B. de Steenhuijsen-Piters, S. Kajiru, D. Shwagara, C. Rugutu & N. Mahungu

ABSTRACT

Cassava (*Manihot esculenta* Crantz) is a traditional crop in the food systems of many Tanzanians, more so in the Lake Zone regions (Mwanza, Shinyanga, Mara and Kagera). Studies indicate the need for improved cassava varieties acceptable to farmers. In 1995, Farming Systems and Root/Tubers research programs based in the zone used a participatory research farmer approach to select varieties. Farmers were invited to station research fields to assess varieties on vegetative and root qualities. It was expected that farmers using their selection criteria would be able to identify varieties with desired attributes. Also the approach would help in developing complementary between farmers' evaluations and those of researchers to maximize selection efficiency. Study sites were Ukiriguru Research Station (semi-arid with moderate rainfall of average 800 mm p.a.), and Maruku Agricultural Research Institute (humid area with high rainfall average of 1800-2200 mm p.a.). Eight clones were assessed along with the local varieties Rushura (at Maruku) and Liongo Control (at Ukiriguru). Farmers' selection criteria in order of importance were high root yield, good root characteristics, resistance to pests/diseases; good canopy architecture and high production potential of leaf vegetable; good inground storability; production of planting material and tolerance to drought. Individual farmers assessed clones for vegetative and root characteristics in the field, and when cooked. At Maraku, variety 4(2) 1425 (an IITA variety), was ranked number one by 96% of the total farmers. This was followed by Kiryunukwe/11 (93%), Mulundi/5 (92%) and Kiryunukwe/8 (89%). At Ukiriguru, clone Kiryunukwe/13 was ranked the first by 80% of the total farmers, followed by 4(2) 1425 (70%). Mulundi/5 (60%). Clones from Kiryunukwe family, although selected, were not given for on-farm testing because of their low resistance to cassava mosaic disease. Farmers' perception of varieties varied depending on the food habits and the cassava production objective. Some selection criteria considered to be important by farmers at one site were not necessarily important at another site.

Key words: Cassava, farmers, Tanzania, varieties

Pages 253-257

Transfer and adaptation of Colombian sour starch technology to Uatappy cassava producer processors in Manabi, Ecuador

H. Caballero Vera, J. Villafuerte & S.V. Poats

ABSTRACT

The UATAPPY project (Union of cassava producers, Agricultural workers and processors) is described. It has a primary aim of transferring processing methods to producer-processors in Manabi province of Ecuador. It comprises 17 processor associations (7 for men, 2 for women, 6 mixed) involving a total of 320 members of which 28% are women. UATAPPY is dominated by small scale resource-poor farmers who mainly produce maize and cassava in rainfed intercrop plots on slopes with poor soils. Cassava is processed into sweet starch for human consumption and into sour starch which is sold. With support from various donors the project has been able to build facilities for fermenting cassava roots and processing roots into sour starch as an income generation enterprise. Trials show that the starch could be infected with unwanted fungi such as *Penicilium niger* and *Aspergillus flavus.* However, efforts are being made to fine-tune and improve the system and the processes involved. The family-based operations have generated various multiplier effects that now cover other associated sub-projects. The project spread from farmer to farmer and was widely adopted because equipment and processes were intermediate and appropriate. The sour starch produced through UATAPPY project was sold in local Colombian markets and this has also led to a greater demand for fresh cassava roots and had increased the wage labour opportunities for the poorest segment of the local population.

Key words: cassava, transfer, adoption, processors, Ecuador, producer, Utappy

Pages 257-260

Cassava technology transfer: Lessons from Cameroon and Nigeria

M.O. Akoroda

ABSTRACT

Cassava is an important starchy crop that has become a staple in most of sub-Saharan or Tropical Africa. The many and diverse agricultural environments in which the crop is grown has resulted in wide range of root yields. To raise yields so as to increase production outputs, the use of fertilizer and better culture have been adopted in some cases. However, the more durable intervention has been in the breeding of new clones that are better able to cope with poor or stressful environments and also yield more roots within the same growth duration. The transfer of technology comprises two phases: the assemblage of desirable genes into a few clones and the diffusion of such clones. Dissemination of improved cassava clones to farmers in Cameroon and Nigeria shows that there are numerous separate steps that should be carefully implemented to achieve the effective and efficient delivery of the new genetic technology of improved clones. Besides, the system into which the technology is being delivered would also affect the mode of technology transfer. Lessons from these two countries are discussed.

Key words: cassava, technology transfer, plantable stems, cuttings, biotechnology, biotech-cassava

Closing speech

J. Wagonda-Muguli

It is with great pleasure that I am with you to officiate at the closing of the Third International Scientific Meeting of the Cassava Biotechnology Network (CBN). From the look of things I feel you have had successful deliberations during this week.

I do not need to repeat it to the already converted, that, cassava is one of the most important crops in Africa and that in Uganda, it continues to contribute immensely to household food security and to alleviating poverty through generation of income for our rural farmers. It is also of no doubt that this crop is indeed challenged by numerous constraints–pests and diseases, bitterness or even toxisty and poor utilization base among others. We need innovative approaches to solve these problems. Biotechnology is one such approach. This conference could not have chosen a better theme than "The contribution of biotechnology to cassava development in Africa".

Concerted effort towards application of biotechnology for cassava development in Africa is needed at all levels. I have followed with keen interest the deliberations of this conference. I have particularly been happy to note that you devoted the whole day of Monday on "Biotechnology for genetic improvement of crops" which was particularly targeted to provide interested researchers with a descriptive introduction to biotechnology methods, tools and applications and how it can be usefully integrated into crop research programmes. This is a noble idea because it aims at developing African capacity to utilize biotechnology methods to achieve sustained development for our people. I have also learnt that through collaborative arrangements through our research organization, NARO, and some of you individually or your respective laboratories, you have now managed to unravel the real identity of the causative agent of the devastating cassava mosaic disease in this country. With the cause now known, I'm sure our scientists are now better armed to deal with the problem.

You have also discussed microbial and genetic biotechnology of cassava products and processing, cyanogenesis, genome research, regeneration and genetic transformation of cassava. These are tropical issues that should have tackled the minds of all of you and especially our NARO scientists who must take the lead in our national drive to modernize agriculture. As a challenge, I would wish all of you present here to report at the CBN IV results from your research indicating how you have integrated the biotechnology aspects into your protocols.

It is gratifying to note that the conference participants included distinguished scientists from international, regional and national research centers all over the world. This composition must have led to extensive and effective handling of the topics of discussion given the expertise and extensive experience here assembled.

I wish to emphasize the point that when fora like these are held, there is a need to involve stakeholders and end users of the packages that you develop. I'm happy to note that among

the seminar participants were farmers' representatives and NGOs among others. I must thank you for this vision because technology generated and not transferred represents a wastage of resources and a failure in the development process. It is my sincere hope that the findings and recommendations arising from this important conference will pass beneficially to the clientele. Afterall, the ultimate goal of this conference was to review the available information and progress on cassava biotechnology in Africa and lay strategies for its utilization in eradicating hunger and poverty among rural populace. The strategies developed will go along way towards solving the cassava problems and ensuring household food and income security in the continent.

I wish to thank the Dutch DGIS, German GTZ, CTA, ODA of the UK, USAID, the Rockfeller Foundation and other donors, not only for funding this conference and for providing such effective participation, but also for supporting other development initiatives in Africa. We are indeed very grateful and we look forward to enhanced collaboration in future.

Your input as conference participants is indeed commendable. Without you, this assembly would not have materialized. We have a saying in one of our local languages that "Agali awamu gge galuma enyama". Literally, this means that it is the teeth that are acting together that can bite through a piece of meat. Group action, collaborative and cooperative effort and collective pursuance of common interests are the best available weapons for advancing the African cause. We shall not be able to develop Africa if we are not prepared to work together.

Finally, I wish to believe that you have all had a nice stay in our country. We are looking forward to hosting you another time. With these few remarks, I declare the "Third International Conference on Cassava Biotechnology" closed.

For God and my country.