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Implementation in Africa of serological diagnostic test for cassava mosaic geminiviruses*

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Abstract/Résumé

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. It s 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/Mots clés: African and East African cassava mosaic geminivirus, ACMD, monoclonal antibody, diagnostic test

Mise en oeuvre de tests sérologiques de diagnostic des geminivirus de la mosaïque du manioc

La nécessité d'un contrôle efficace des geminivirus de la mosaïque en Afrique au sein des programmes nationaux a abouti à la mise au point d'une méthode biotin - streptavidin du test ELISA entièrement basée sur des anticorps monoclonaux. Cette méthode a été évaluée au Kenya et en Ouganda (pays dotés de laboratoires peu sophistiqués), où il a été possible de détecter et de différentier le virus de la mosaïque africaine du manioc (ACMV) et celui de la mosaïque est-africaine du manioc (EACMV). De nouveaux résultats concernant la distribution du dernier virus ont été obtenus. En effet, six échantillons de feuilles de manioc récoltés dans la région du Lac Victoria (à l'Ouest du Kenya) ont donné des réactions qui suggèrent l'existence d'EACMV. Par ailleurs, la présence de ce virus a été uniquement mentionnée en Afrique de l'Est. Les potentialités de la technique de diagnostic sont grandes pout tester le matériel génétique amélioré et les réserves de matériel sain quant à la présence éventuelle de virus de la mosaïque du manioc. La technique est bon marché et durable et peut satisfaire la demande pour des prospections régionales régulières de diagnostic des virus de la mosaïque du manioc en Afrique.

Introduction

African cassava mosaic disease (ACMD) is prevalent in sub-Saharan Africa. Yield losses caused by the disease in susceptible and sensitive cassava varieties is enormous, ranging from 20–95% (Seif 1982, Terry & Hahn 1980). The disease is caused by two distinct geminiviruses namely African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV) (Hong et al. 1993, Swanson & Harrison 1994).

Breeding for resistance and cleaning of infected materials are recommended control measures for ACMD (Hahn et al. 1980, Adejare & Coutts 1981). Serological diagnostic tests for ACMV and EACMV can be useful for both of these interventions. Some regional exchange of improved cassava clones/germplasm between countries is essential for rapid advance in the overall improvement of cassava production in Africa. Restrictions on movements of materials owing to risk of viruses are overcome through serological testing of such materials. The cleanliness of infected materials that are freed of the viruses through meristem tip culture could be ascertained by serological tests. Although a very low concentration of the viruses in such plants may escape detection, a high level of confidence could be achieved.

Serological diagnostic tests could be used also in extensive surveys on ACMV and EACMV to obtain further information about the occurrence and spread of the viruses in Africa. There is therefore the need to have an appropriate serological diagnostic test for National Programmes to enhance their efforts in improving cassava production. Such a technique should be simple, cheap, robust and sustainable. It should also cover all known forms of geminiviruses causing cassava mosaic disease (CMD), namely ACMV, EACMV and Indian cassava mosaic virus (ICMV).

Candidate diagnostic methods

Clark & Adam (1977) reported on a serological technique known as enzyme-linked immunosorbent assay (ELISA). In this technique, polyclonal antibody is used to coat microtitre plate to trap virus particles and the virus is detected by another polyclonal antibody that is conjugated to an enzyme (alkaline phosphatase). This technique cannot distinguish between ACMV and EACMV.

The advent and use of monoclonal antibodies (MAbs) (Köhler & Milstein 1975, Thomas et al. 1986) makes it possible to differentiate between the two viruses in a triple antibody sandwich (TAS) ELISA. Polyclonal antibody is still used to trap the virus particle. In addition to being highly specific,

MAbs have other advantages over polyclonal antibodies. Unlike polyclonal antibodies, MAbs can be produced in large quantities in different batches with little or no variation in quality and affinity of the antibodies (Halk & De Boer 1985). This leads to less variation in research results from different laboratories. The production of MAbs is more sustainable than that of polyclonal antibodies. The hybridoma cell lines which secrete the MAbs can be stored under low temperatures until needed for the production of MAbs. Such is not possible with polyclonal antibody production. Because of these advantages, ACMV MAbs are more readily available than the polyclonal antibodies. Thus, to ensure extensive and regular diagnostic tests of cassava mosaic viruses in Africa, the best choice of ELISA method is one fully based on MAbs.

In using MAbs both for trapping and detection of the viruses, three options of ELISA can be adopted. These are $F(ab^1)_2$ ELISA, double antibody sandwich (DAS) ELISA, and avidin/streptavidin ELISA. The use of pepsin enzyme to cut F_c fragment of the MAbs does not always produce the desired $F(ab^1)_2$. In DAS ELISA, the conjugation of alkaline phosphatase enzyme to the MAb often highly reduces its sensitivity. In biotin-streptavidin ELISA, the biotin is of low molecular weight and when it is conjugated to MAb, it has little or no adverse effect on the sensitivity of the MAb. In addition, biotinylation of MAb is quite simple and reliable. This method of ELISA was thus selected for the development of a serological diagnostic test using ACMV MAbs.

Methods

Development of a diagnostic technique at SCRI UK. Scottish Crop Research Institute (SCRI) is a centre of excellence for CMD studies. From sample materials which were collected in Africa and maintained in SCRI glasshouses, a panel of MAbs against cassava geminiviruses has been produced (Thomas et al. 1986). They are designated as SCR with serial numbers based on the epitopes they detect. MAbs SCR 17, 20, 23, 33, 58 and 60 were selected to develop a biotin-streptavidin system of ELISA. The last two MAbs are specific for Indian cassava mosaic virus (ICMV), the geminivirus of cassava in Indian and Sri Lanka (Swanson & Harrison 1994). This is to ensure testing for the presence of ICMV in cassava materials coming into Africa. SCR 17 and 23 can react with EACMV and ACMV while SCR 33 reacts only with the latter. SCR 20 can detect the three geminiviruses.

These MAbs were in culture fluid supernatant. They were purified using Protein G Column. A series of tests were conducted with them and it was found that a mixture of SCR 17, 20 and 58 was suitable to replace polyclonal antiserum for coating wells of microtitre plates to trap ACMV, EACMV and ICMV. SCR 23, 33 and 60 were labelled with biotin and were used effectively to detect the trapped viruses in ELISA in which streptavidin/alkaline phosphatase conjugate has affinity for the biotin. A yellow colour develops after incubation with a substrate. Typical reactions of the biotinylated MAbs with the geminiviruses in a biotin-streptavidin system of ELISA is shown in Table 1. Thus a biotin-streptavidin system of ELISA wholly based on MAbs was developed (Ogbe & Harrison, unpublished data). This was tested whilst at SCRI on some isolates of ACMV, EACMV and ICMV (Table 2).

Table 1. Reactions of biotinylated monoclonal antibodies (MAbs) SCR 23, SCR 33 and SCR 60 with cassava mosaic geminiviruses.

Biotinylated MAb	Geminivirus ACMV	EACMV	ICMV
SCR 23	+	+	-
SCR 33	+	-	-
SCR 60	-	-	+

+ Reaction; - No reaction

Table 2. Evaluation of a biotin-streptavidin system of ELISA based on monoclonal antibodies (MAbs) to detect and differentiate ACMV, EACMV, and ICMV in cassava*

Origin	Virus isolate	MAb for detection SCR 23	SCR 33	SCR 60
Ghana	ACMV Mc/004	1.83@	2.43	0.00
Tanzania	ACMV Ukerewe 2+	0.17	2.33	0.00
Tanzania	EACMV Dar. 3	1.93	0.00	0.00
Madagascar	EACMV Antisohy H 2	2.03	0.00	0.00
India	ICMV 14	0.00	0.00	2.40
India	ICMV 17	0.00	0.00	1.76
India	ICMV S 1315+	0.00	0.00	0.18
	Uninfected sap	0.00	0.00	0.00

* Wells were coated with a mixture containing IgG of SCR 17,SCR 58 both at 1/500 dilution and SCR 20 (crude ascitic fluid) at 1/5000 dilution. Infected sap of cassava leaves was diluted 1/10. MAbs for detection were biotinylated and were used at 1/1000 dilution. Streptavidin/alkaline phosphatase conjugate was used at 1/5000 dilution to detect biotinylated MAbs;
@ A_{405}nm values after incubation with substrate for 16hr at 4°C
+ Leaves in these samples had only weak symptoms.

It should be noted that detection of EACMV and ICMV is obtained by reaction to only one MAb i.e. SCR 23 and SCR 60, respectively. However, detection of ACMV is by reaction to either SCR 23 and/or SCR 33. Thus EACMV cannot be differentiated if ACMV is also present. This could be regarded as a weak feature of this diagnostic system.

Evaluation of the diagnostic technique in Africa conditions. A diagnostic survey on ACMV and EACMV using the biotin-streptavidin system of ELISA was carried out in Kenya and Uganda in March 1996. This was to test its effectiveness under conditions that would be encountered in less sophisticated laboratories. Young cassava leaf samples were collected from symptomatic plants with symptoms ranging from mild to severe. In all 317 samples were tested in the following proportions: Kenya - 160 and Uganda - 157.

Regarding appropriateness and robustness of the diagnostic technique, it is pertinent to note that some of the tests were carried out under substandard conditions. For example, ELISA plates were incubated at room temperature of between 28°C and 32°C instead of the recommended temperature of 37°C. Also in some of the tests, distilled water for motor batteries sold in petrol filling stations was used to wash the ELISA plates. In addition, the MAbs were transported between Nigeria and Kenya/Uganda under conditions that were not entirely satisfactory (not cool throughout the period of the trip). In spite of these limitations the use of the diagnostic technique was successful.

Table 3. Reactions in ELISA of selected samples tested in Kenya and Uganda*

Country	Sample no	MAb for detection SCR 23	SCR 33
Kenya (East)	61	+++@	-
Kenya (East)	79	++	-
Kenya (West)	91	+++	+++
Kenya (West)	121	++	++
Kenya (West)	141	+++	-
Kenya (West)	159	+++	-
Uganda	21	++	+++
Uganda	43	+++	+++
Uganda	81	+	++
Uganda	142	+	++

* Wells were coated with mixture of IgG SCR 17 and SCR 20 at 1/500 dilution. Sap of cassava leaves was diluted 1/10. MAbs for detection were biotinylated and were used at 1/500 dilution. Streptavidin/alkaline phosphatase conjugate was used at 1/5000. @ - (No reaction), + (Weak reaction), ++ (Strong reaction) and +++ (Very strong reaction). Observation were recorded after incubation with a substrate for 16 hours at 4^0C.

Results

The survey confirmed the report of Swanson and Harrison (1994) about the occurrence of EACMV on the Kenya coast and ACMV to the west in Kenya and in Uganda (Table 3). The survey also produced results that suggested a new finding for EACMV distribution in Kenya. Six samples collected in Lake Victoria region (Kenya west) gave typical reactions of EACMV (Table 3). Hitherto, EACMV was only reported to occur at coast Kenya (Swanson & Harrison 1994).

Four samples collected on the Kenya coast along Mtwapa-Malindi road in Kilifi District did not react with MAbs SCR 23 and 33. Biotinylated MAb SCR 60 was not available at the time of the survey to test the four samples for ICMV but a 'backstop' TAS-ELISA test using bean golden mosaic virus (BGMV) MAb, gave strong reaction indicating a geminivirus was present. Some samples gave weak reactions though such reactions implicated the presence of ACMV. These were collected in the border areas of Kenya and Uganda (Table 4).

Table 4. Weak reactions of some samples suspected to contain ACMV in the border districts of Kenya and Uganda*

Country	Sample no.	MAbs used for detection SCR 23	SCR 33
Kenya	125	-@	+
	126	+	+
	127	+	+
Uganda	6	+	+++
	44	+	+++
	63	-	+
	74	-	+

*, @ as in Table 3.

Discussion

The use of MAbs SCR 17 and 23 in serological tests to detect both ACMV and EACMV and the use of SCR 19, 29 and 33 to detect only ACMV and thereby differentiate it from EACMV has been documented (Swanson and Harrison 1994). This test was proved reliable as it corroborated the nucleic acid analysis (Hong et al. 1993). On this basis, that a "simple" diagnostic technique was developed which involved some couples of MAbs for easy handling by National Programs.

The reasons for weak or non-reaction of MAbs SCR 23 and 33 with some of the samples could mean that selection of the MAbs for detection and differentiation of ACMV and EACMV was carried out with fewer forms of isolates of these viruses than actually existed in Africa. This could be a limitation in the use of MAbs in serological tests as some forms of the virus isolates might escape detection. On the other hand, the weak or non-reaction of these MAbs with some of the samples might actually indicate occurrence of strains of the two viruses. This calls for further investigation.

Nevertheless, there is a good level of confidence for the use of the diagnostic technique by national programs to test for the presence of cassava geminiviruses in improved germplasm prior to their regional exchange. With few exceptions the results obtained were as expected. The six samples with reactions that were typical of EACMV instead of the expected reactions of ACMV should still be observed. Such reactions could be a demonstration of limitations on the part of the MAbs. On the other hand, EACMV might actually occur in western Kenya which has not been recorded before now, probably due to limited survey activities.

The results call for extensive survey of cassava mosaic viruses in Africa. The diagnostic technique can meet the demand, but could be complemented with DNA studies to obtain regional groupings of isolates of ACMV and EACMV. This will enhance quarantine services and breeding for resistance. Using ELISA with DNA studies may also elucidate the etiology of the severe outbreak of ACMD in Uganda.

Recommendations

The following recommendations would help effective serological diagnostic tests of cassava mosaic viruses in Africa:

1. Sustainability is still a problem. A continued link with SCRI is necessary in the area of production and biotinylation of (E)ACMV MAbs. It is hoped that cell lines of the relevant MAbs can be released by SCRI for massive production of the MAbs and for their distribution to national programs. Presently, cassava is mainly used for food in Africa and measures to increase production of the crop will be of great help to most Africans.
2. The MAbs together with the appropriate buffer solutions should be made available to National Programme as a kit.
3. On their parts, National Programmes should strive to have and maintain basic facilities such as generator to ensure regular electricity supply and refrigerator for adequate keeping of antibodies and buffer solutions.
4. The serological diagnostic test areas yet the most appropriate tool for widespread use in Africa for detection of the geminiviruses causing cassava mosaic disease.

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Breeding for resistance to mosaic disease in Uganda

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Abstract/Résumé

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 for ACMD as: limited variability for the triat, unfavourable associations with the trait and lack of fast multiplication techniques to enable released varities reach 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/Mots clés: Africa cassava mosaic disease, cassava,Uganda

Amélioration pour la résistance a la maladie mosaïque africaine du manioc en Ouganda

Le manioc est une importante culture alimentaire en Ouganda cultivée et utilisée largement dans toutes les régions du pays. Cependant, cette culture reste sous la menace de la maladie de la mosaïque africaine du manioc (ACMD) Cet article passe en revue l'amélioration du manioc pour la résistance à l'ACMD en tant que stratégie majeure de lutte adoptée des années durant, en l'absence de méthodes de lutte alternatives écologiquement durables. Cet article identifie aussi les contraintes à l'amélioration pour la résistance à l'ACMD comme étant: la variabilité limitée pour le caractère, les associations non favorables avec le caractère et le manque de techniques de multiplication rapide pour permettre aux variétés résistantes 'diffusées' d'atteindre rapidement les paysans. Les solutions suggérées pour surmonter ces problèmes comprennent: une collecte plus massive de matériel génétique et une évaluation pour la résistance à l'ACMD, la mise à la disposition des paysans d'autant de variétés résistantes que possible et l'emploi de la biotechnologie pour une plus rapide multiplication de matériels résistants pour les paysans.

Introduction

The role played by cassava in the food economy of Uganda cannot be over-emphasized. It is the second most important food crop after bananas and projections into the future show that in the near future it will be the major staple, replacing bananas. Cassava's comparative advantage over other food crops lies in the crop's inherent nature of withstanding marginal conditions. It therefore can do well where other crops are marginalised. Cassava also excels over other food crops by its relative ability to store in the soil long after its maturation, hence its use as a famine reserve crop. In Uganda, the crop is consumed in a number of ways including fresh boiled and processed forms. Besides its role as a food crop, cassava is also becoming an important cash crop with growing markets for its fresh roots, their derived products and stems. The status of cassava in Uganda is, however, threatened by many production constraints. The most important of these are: the African Cassava Mosaic Disease (ACMD), the Cassava Green Mite (CGM), the Cassava Mealybug (CM), Cassava Bacterial Blight (CBB) and the Cassava Anthracnose Disease (CAD).

African cassava mosaic disease (ACMD)

African cassava mosaic disease (ACMD) is caused by the African Cassava Mosaic Virus (ACMV) (Swanson & Harrison