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THE USE OF THE ENZYME-LINKED IMMUNOSORBENT ASSAY AND IMMUNOSORBENT ELECTRON MICROSCOPY FOR THE DETECTION AND CHARACTERIZATION OF CASSAVA COMMON MOSAIC VIRUS

(Usage du Test ELISA et de l'immunoélectromicroscopie pour détecter et caractériser une souche de la mosaïque commune du manioc)

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SUMMARY

The double-antibody sandwich form of the enzyme-linked immunosorbent assay (ELISA) provided a rapid and sensitive means for the detection of cassava common mosaic virus (CCMV) in cassava (*Manihot esculenta* Crantz). The ELISA procedure is particularly useful in a large-scale CCMV indexing program because many more plants or plantlets can be assayed per day than by graft indexing or bioassays. The horseradish peroxidase : O = phenylenediamine enzyme-substrate combination is a sensitive signal for detecting CCMV. The immunosorbent electron microscopy (ISEM) technique was used to serologically compare a Colombian isolate with CCMV and other members of the potexvirus group by observing the number of virus particles selectively absorbed by different potexvirus antisera. The ISEM method also increased the number of virus particles adsorbed on the surface of the electron microscope grid available for observation and measurement.

RESUME

Le test immunoenzymatique à double anticorps "ELISA" fournit une méthode rapide et sensible pour détecter la mosaïque commune du manioc (CCMV) dans les plants de Manioc.

Le test ELISA est particulièrement utile pour des tests d'indexation à grande échelle, car il permet d'examiner beaucoup plus de plantes par jour que par les tests utilisant le greffage ou les hôtes différentiels. La combinaison enzyme-substrat peroxydase du radis noir - orthophénylène-diamine peut servir de signal particulièrement sensible pour détecter CCMV. L'immunoelectromicroscopie (ISEM) a été utilisée pour comparer sérologiquement une souche colombienne de CCMV et

d'autres membres du groupe "potexvirus" en observant le nombre de particules virales sélectivement adsorbées par les antisérums correspondant aux divers potexvirus. Cette méthode permet aussi d'augmenter le nombre de particules virales fixées sur les grilles pour observation et mensuration au microscope électronique.

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Cassava common mosaic virus (CCMV), a plant potexvirus with elongated rod-shaped particles, is present in cassava from many Latin American countries. The field incidence of CCMV is usually low however the yield of infected plants can be reduced by as much as 60 per cent. Although there are no reported vectors of CCMV this virus is readily mechanically transmitted and efficiently disseminated in infected planting material. The risk of introducing CCMV into new areas via infected vegetative material is high therefore sensitive virus detection methods are required for indexing cassava germplasm for the presence of CCMV. The double antibody sandwich form of the enzyme-linked immunosorbent assay (ELISA) and immunosorbent electron microscopy (ISEM) are available for detecting CCMV in cassava.

The ELISA test is conducted in small wells in a polystyrene or polyvinyl cloride microtiter plate containing 96 sample wells. Sample preparation consists of grinding the plant tissue sample in 20 to 100 volumes of extraction buffer with a mortar and pestle. We found that buffer dilutions greater than 1/10 worked better for cassava tissue. Depending on the available labor up to several hundred plants can be indexed/day with results available within 36 hours. If required many more samples can be processed using commercially available tissue extractors and automated ELISA systems. We routinely use CCMV gamma globulins conjugated to horseradish peroxidase as the detecting antibodies but alkaline phosphatase can also be used. Using ELISA CCMV can be detected in small leaf disks from infected cassava plants or in small leaves from plantlets.