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Biological and serological diversity of a potyvirus infecting maize in the Mediterranean area

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Summary — Several years ago we reported the occurrence of potyvirus infecting maize in southern France and in Spain and Italy, the most prevalent virus being maize dwarf mosaic virus (MDMV) strain A. MDMV is present on dent and sweet corn mainly in areas where Johnson grass (*Sorghum halepense* L), the reservoir host, is abundant. The aim of our work was to obtain a good and reliable diagnostic method and to start to study the biodiversity of MDMV. We have obtained a good specific antiserum directed against the capsid protein of the virus. In 1991, we started a collection now made up of more than 100 isolates from southern France, the northern part of Italy and some areas of northern Spain. The molecular weights of the capsid protein of all the isolates were determined by the immunoblot method. We have been able to classify the isolates in different groups. The behaviour of the isolates belonging to each group defined by Western blot was analysed by mechanical inoculation on several maize inbred lines. We have thus demonstrated a biodiversity in MDMV. This situation is important for the breeder and allows a better understanding of the behaviour of inbred lines of maize in different areas.

maize dwarf mosaic virus A (MDMV-A) / *Zea mays* L = maize / biodiversity

Résumé — Diversité biologique et sérologique des potyvirus infectant le maïs en zone méditerranéenne. *Le potyvirus dominant sur maïs dans la moitié sud de la France et la partie nord de l'Espagne et de l'Italie est le virus de la mosaïque nanisante du maïs souche A (MDMV-A). Ce virus est présent principalement en production de maïs doux et de maïs semence dans les zones ou le sorgho d'Alep (*Sorghum halepense *L) est abondant. Notre travail avait pour but de mettre au point une méthode de diagnostic et d'amorcer l'étude de la biodiversité de ce pathogène. Une collection de plus de 100 isolats a été constituée. Nous avons pu classer les isolats en 2 groupes principaux d'après le poids moléculaire de leur protéine capsidique et de leur comportement sur différentes lignées de maïs. Ces observations nous ont permis de démontrer l'existence d'une biodiversité chez le MDMV-A. Ces résultats importants pour la sélection de lignées permettent de mieux comprendre les réactions différentes des lignées suivant les localités.*

virus de la mosaïque nanisante du maïs-souche A (MDMV-A) /* Zea mays *L = maïs / biodiversité

* Correspondence and reprints

INTRODUCTION

Maize in the Mediterranean area can be infected by several viruses; the most prevalent of which is maize dwarf mosaic virus (MDMV) strain A. This virus can be identified by serology. Having observed differences in reactions of several inbreds and hybrids in different areas of southern France and northern Spain and Italy, we decided to study this biodiversity. The identification of strains is important in breeding for host plant resistance and epidemiology.

Potyvirus produce proteinaceous inclusions (Purcifull *et al,* 1973). Alper *et al* (1984) have shown that these proteins can be diagnostic for different viruses belonging to the potyvirus group. Jensen *et al* (1986) have shown variations in inclusion protein size, capsid protein size, host range and symptom expression induced by sugarcane mosaic viruses. We wanted to know if such proteins can be used to distinguish MDMV isolates characterized by host reaction.

MATERIALS AND METHODS

Virus

MDMV isolated from Johnson grass (*Sorghum halepense* L) in the Montpellier area was maintained and multiplied in 'Golden Giant' sweet corn held in the greenhouse. Virions were purified according to the protocol of Gough and Shukla (1981) with slight modifications. Virus purity was estimated by acrylamide gel electrophoresis (SDS-PAGE), and virus yield determined spectrophotometrically. To purify the cytoplasmic inclusion protein (CIP), we followed the method proposed by Hiebert *et al* (1984).

Antisera production

Antisera for virus and cytoplasmic inclusion protein were raised by immunizing New Zealand rabbits with purified intact virions or protein.

Sources of isolates

From 1989 onwards, more than 100 isolates were collected in the south part of France and in the north of Spain and Italy. Leaf material obtained from inbred lines or from Johnson grass were tested directly or after multiplication on 'Golden Giant' corn sweet. All the isolates were kept at –40°C.

Electroblot immunoassay (EBIA)

Capsid and cytoplasmic inclusion proteins were separated by SDS PAGE, electrotransferred onto nitrocellulose membrane and detected immunologically.

Inoculation of inbred lines

Several inbred lines were grown in pots in a greenhouse. About 8–9 d after sowing, the plants were mechanically inoculated with some isolates and moved to a controlled environment chamber at 24°C and 16 h of illumination per day for the remainder of the experiment.

RESULTS

Antisera

The antisera against virions collected 3 weeks after the first immunization had a good titer in ACP-ELISA (1 to 1 048 576) rising later up to 1 to 8 097 152. In DAS-ELISA the limit of detection of purified MDMV was 1 ng/ml. No antisera were obtained against the CIP.

Molecular masses of capsid protein

The isolates could be resolved into 2 groups by the size of their coat protein. One was called PER with a capsid protein of 36 kDa, the other was named LAV with a capsid protein of 35 kDa. For an isolate obtained from USA-Illinois (Ford) we found 35.5 kDa. Among our isolates collected in 1994, we found approximately the same number belonging to each group. The distribution of the type isolate in the prospected area is random. The sizes obtained for the coat protein are similar to those published by Jensen *et al* (1986). These authors also found differences in protein properties not related to geographic origin.

Reaction of inbred lines

Using the disease index method (Kuhn and Smith, 1977; Alliot *et al,* 1985), we have also been able to separate the 3 groups of isolates (LAV-PER-USA) following the reactions obtained on several inbreds of maize.

DISCUSSION

The results above show serological differences between MDMV isolates which correspond to biological differences. Jensen *et al* (1986) also showed among 16 isolates of sugarcane mosaic viruses and MDMV many variations in capsid protein size, host range and symptom expression. Variations in MDMV may be greater than we generally suppose. Knowledge of this variability may be useful in characterizing isolates to improve identification, in epidemiology studies, in developing inbred lines. We are trying to obtain an antiserum against CIP to see whether we can find a variation for this protein.

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