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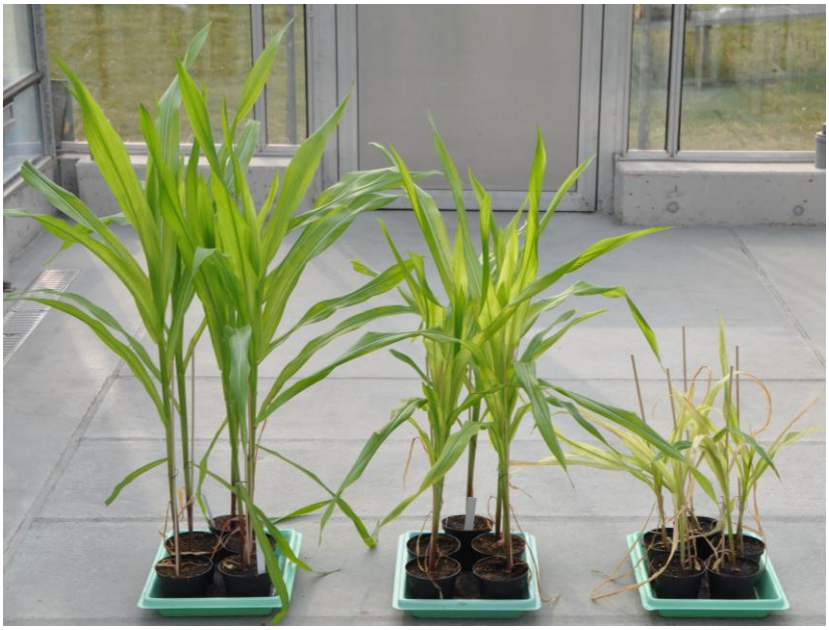
Data Sheet on
Maize Lethal Necrosis (MLN) Disease

Maize lethal necrosis is a serious disease of maize which from its first appearance in Kenya in 2011 (Wangai et al., 2012) is now found in many countries of East Africa where maize is grown. The maize lethal necrosis disease (syn. corn lethal necrosis, CLN) is known to naturally affect varieties of maize (*Zea mays*) resulting in chlorotic mottling of the leaves, severe stunting and necrosis, often leading to plant death. MLN is caused by a mixed infection between *Maize chlorotic mottle virus* (MCMV, genus *Machlomovirus*) and potyviruses infecting maize. In Kenya and other countries, most frequently it is *Sugarcane mosaic virus* (SCMV) in synergism with MCMV causing MLND. Single infections of MCMV or SCMV cause only mild mosaic or mottling symptoms and a moderate reduction of growth (Fig. 1). In mixed infections, early infected plants appear stunted and show a general chlorosis, leaf bleaching and necrosis.



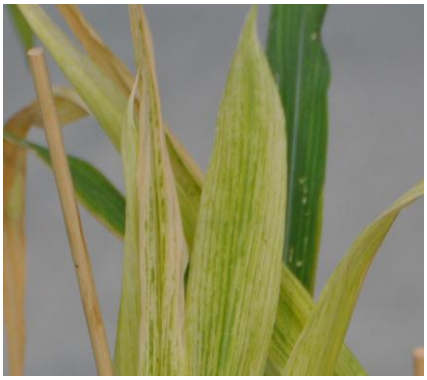


Fig.1: Symptoms of MCMV and SCMV on maize. Maize infected with SCMV (left), MCMV (middle) and synergistic effect of mixed infection of MCMV and SCMV (right and cutout detail).

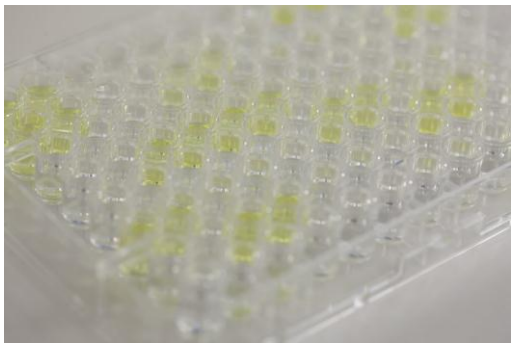
Both, MCMV and SCMV are readily transmitted by mechanical means and are known to be seed transmitted. In addition, MCMV can be experimentally transmitted by thrips (Cabanas et al., 2013) while SCMV is vectored by aphids.

Infectious isolates available at DSMZ	Collection no.:	Original host:	Geographic origin:
	MCMV PV-1087	*Zea mays*	Kakamega, Kenya
	SCMV PV-0731	*Zea mays*	Borsdorf, Germany
	SCMV PV-0368	*Zea mays*	Freiburg, Germany

Virus diagnosis: Identification of MLND and the viruses involved in the disease complex is generally by observation of symptoms in the field. However, because single infections of the viruses and early stages of the disease are often inconspicuous and resemble physiological disorders, specific diagnostic tests are to be applied to confirm virus presence and to adequately detect/identify the viruses in the mixed infection.
ELISA tests are the most reliable assays for detection of these viruses in maize as both viruses reach concentrations in maize easily detected by ELISA. Specific polyclonal antibodies developed at the DSMZ Plant Virus Department allow a sensitive and reliable detection and identification of MCMV and SCMV in a standard double antibody sandwich enzyme linked immuno-sorbent assay (DAS-ELISA).

References:

Rochon et al. in Virus Taxonomy, ed. by King, Adams, Carstens & Lefkowitz (Elsevier/Academic Press, London, 2012), pp. 1111–1138
http://www.cabi.org/isc/datasheet/119663
Cabanas et al. (2013) Dissecting the Mode of Maize Chlorotic Mottle Virus Transmission (Tombusviridae: Machlomovirus) by Frankliniella williamsi (Thysanoptera: Thripidae). J Econ Entomol 106, 16-24.
Wangai et al. (2012) First Report of Maize chlorotic mottle virus and Maize Lethal Necrosis in Kenya. Plant Dis 96, 1582-1583.



DAS-ELISA: ELISA procedures are *in-vitro* laboratory assays. The double antibody sandwich ELISA semi-quantifies the virus between two layers of virus specific antibodies (a capture and a detection antibody). Following the immobilization of the capture antibody to the surface of the i.e. 96 well ELISA plate, nonspecific binding sites are blocked prior adding the homogenized samples to the wells. Unbound material is removed by washing before the enzyme-conjugated detection antibody is added. In case of a virus infected sample, this antibody binds to the captured virions. Following repeated washing a substrate is applied to the wells, which is converted by the enzyme to a yellow color in those wells belonging to virus infected samples. This color development can be evaluated by visual inspection or measured by a microplate reader. The test provides results in few hours, is upscalable and requires little instrumentation.

In addition to DAS-ELISA, lateral flow assays (LFA) have been developed at DSMZ to allow rapid on-site diagnosis and confirmation of the viruses in MLN.

positive negative

LFA: Lateral flow assays in specific devices offer a very fast and convenient on-site testing without the need for specialized and costly equipment. They are based on immune-chromatography of virus-specific antibodies and can be performed directly in the field. The homogenized sample is dispensed on the sample spot (S) where the virus reacts with a virus specific antibody (IgG) conjugated to a colored dye. Both migrate by capillarity on the chromatographic membrane. The test line (T) contains antibodies specific for the pathogen to be tested. The control line (C) contains IgGs specific for the antibodies used. If the virus is present in a sample, once reaching the test line, it reacts with the specific polyclonal antibody and the colored conjugate adhering to the virus generates a red line, indicating a positive result. Unbound antibodies continue to migrate to the control line and a red colored line then indicates the correct performance of the test. The assay requires a minimum of preparation and reaches results within a few minutes.

The diagnostic tests have been optimized for detection of SCMV and MCMV in maize and thoroughly validated pursuant to the quality assurance procedures at the DSMZ Plant Virus Department. Virus isolates and ELISA tests are offered as certified reference materials according to ISO Guide 34:2009 and DIN EN ISO 17025:2005 accreditation.

DAkkS
Deutsche
Akkreditierungsstelle
D-RM-18324-01-00

Diagnostic products available:

- MCMV DAS-ELISA set AS-1087 and MCMV ELISA positive control PC-1087
- MCMV lateral flow assay LFA-1087
- SCMV DAS-ELISA set AS-0166 and SCMV ELISA positive control PC-0731
- SCMV lateral flow assay LFA-0731
- combined MCMV/SCMV lateral flow assay LFA-1087/0731

(LFAs are not covered by the accreditation scope.)

ELISA tests and LFA are accompanied with detailed protocols prescribing the testing procedures.
Virus isolates and serological tests are available at the DSMZ Plant Virus Department and can be ordered via web shop (www.dsmz.de), email (plantvirus@dsmz.de) or by fax (+49-511-2616455).