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A mild strain of broad bean mottle virus from faba bean (Vicia faba L.) in the Sudan

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Abstract

A new strain of broad bean mottle virus, isolated from faba bean (*Vicia faba* L.) in the Sudan, is described. It differs considerably from known isolates by its nearly symptomless infection of faba bean in spite of high concentrations of the virus in infected plants. It does not differ from regular isolates in gel-diffusion serology, light and electron microscopy, host range and symptoms in major hosts other than faba bean. It may constitute a potential threat to other food legumes in the region.

Additional keywords: cross-protection, virus variation.

Field surveying of faba bean (*Vicia faba* L.) for viruses in West Asia and North Africa, the region served by the International Center for Agricultural Research in the Dry Areas (ICARDA), by means of sample testing by ELISA demonstrated a high incidence of broad bean mottle virus (BBMV) in that region (Makkouk et al., 1988a, b; Fortass and Bos, 1991). Only slight differences were observed between four isolates from different countries in the region when they were compared biologically and serologically, but all were highly pathogenic on faba bean. When some new plant samples from the Sudan were recently tested biologically, a deviant isolate was obtained that is practically symptomless on faba bean.

The isolate SuV256 was recovered from a $CaCl_2$-desiccated faba-bean leaf sample collected in the Sudan in 1986. The mechanically transmissible isolate was propagated and maintained in faba bean 'Compacta', and during the investigations also stored in leaf material at −20 °C. It was compared with two BBMV isolates from the IPO collection, viz. a Tunisian isolate (TV75-85), earlier described by Makkouk et al. (1988b), and a relatively mild Moroccan isolate of the virus (FN1) still under investigation (Fortass and Bos, in preparation). For serology, an antiserum to the Moroccan isolate MV90-85 as well as the homologous Moroccan virus isolate (Makkouk et al., 1988b) were used.

When first isolated on a few plant species including faba bean, the virus escaped recognition because of the very mild and transient reaction of faba-bean plants to infection. After further mechanical transfer of the virus to several other plant species, however, many of them reacted with symptoms highly characteristic of BBMV. For example, *Chenopodium amaranticolor* and *C. quinoa* rapidly produced numerous pin-point local lesions, and *Pisum sativum* 'Castro' reacted as early as 2–3 days after inoculation with many small etch lesions, rapidly enlarging and coalescing into large areas of desiccating tissue which led to withering of inoculated leaves and was followed by systemic stem and apical necrosis. In 'Rondo' there were fewer local lesions and they remained smaller than in 'Castro', while systemic necrosis indicated a tendency towards hypersensitivity. Faba bean 'Compacta' always reacted severely to common isolates of the virus including TV75-85 (Makkouk et al., 1988b) and to FN1 (Fig. 1B, C; Fig. 2B, C). SuV256 only occasionally caused some diffuse chlorotic or necrotic local lesions, appearing 2–6 days after inoculation, and the systemic vein chlorosis and chlorotic vein banding was very diffuse and mild and sometimes developed into general leaf chlorosis (Fig. 1A) some two days later. Plants inoculated with SuV256 completely and permanently recovered from the disease in another six days (Fig. 2A), in contrast to the common isolates with which recovery usually is temporary (Fig. 2B, C) and symptom development is cyclic (Makkouk et al., 1988b). Symptoms of SuV256

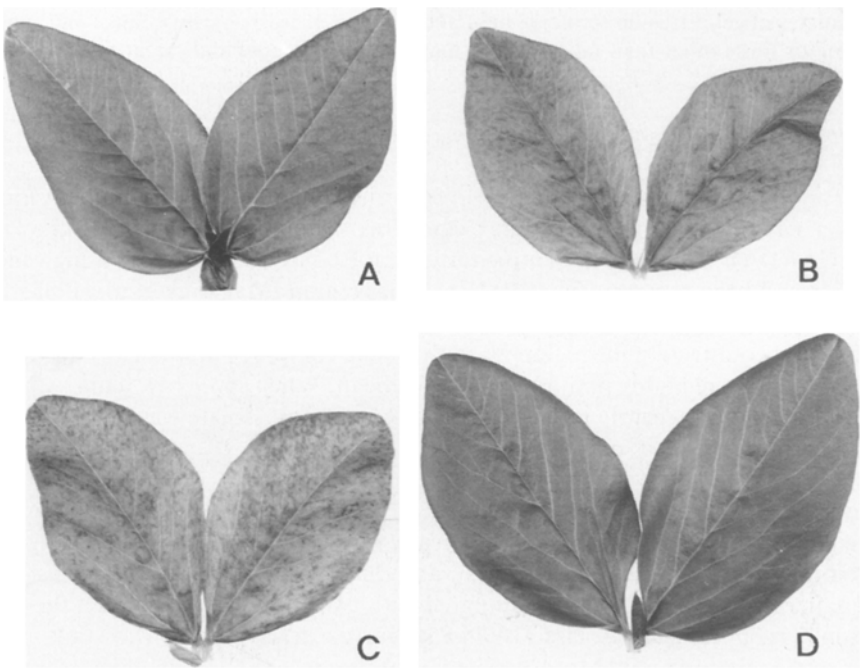

Fig. 1. First systemic symptoms of broad bean mottle virus in faba bean 'Compacta', 10 days after inoculation: isolate SuV256 (A) compared with FN1 (B) and TV75-85 (C); D, uninoculated control.

were also very mild on other faba-bean genotypes vulnerable to common isolates of the virus (Fortass and Bos, in preparation).

In epidermal strips from faba-bean leaves inoculated with SuV256 and stained with phloxine and methylene blue in Christie's solution, enlarged nucleoli, granulated and vesiculated material scattered throughout the cells or accumulating in more or less dense inclusion bodies were observed with the light microscope as described earlier for BBMV (Makkouk et al., 1988b).

Electron microscopy of crude plant sap revealed large quantities of isometric particles with a diameter of about 25 nm. Stained with uranyl acetate, they showed a dense particle centre characteristic of BBMV (Fortass and Bos, 1991).

Serological tests with the antiserum to the Moroccan isolate MV90-85 (Makkouk et al., 1988b) yielded very high ELISA readings in faba bean throughout plant life, and in pea, chickpea, and lentil, when tested early, similar to those with the homologous virus isolate. ELISA also revealed a very high virus titre in *Nicotiana clevelandii*. Quantitatively there was no appreciable difference in reaction of the MV90-85 antiserum to the homologous virus isolate, the Tunisian isolate TV75-85, and SuV256. In agar-gel diffusion tests, when these three isolates and another Moroccan isolate FN1 were applied to adjacent wells, the respective precipitin lines perfectly fused with each other without spur formation.

Fig. 2. Advanced systemic symptoms of broad bean mottle virus in faba bean 'Compacta', 17 days after inoculation: isolate SuV256 (A) compared with FN1 (B) and TV75-85 (C); D, uninoculated control. Note the lack of difference between A and D.

In cross-protection tests with faba-bean plants 'Compacta', prior inoculation with SuV256 did show some degree of protection against challenge inoculation with the symptom-producing isolates FN1 and TV75-85. All plants inoculated with SuV256 became infected, and symptoms were produced. Out of 16 plants challenge-inoculated with FN1 10 days after protective inoculation, 9 plants showed much weaker symptoms and 7 plants slightly weaker symptoms than the controls singly inoculated with FN1, and symptom development was retarded. Out of the 16 plants challenge-inoculated with TV75-85, 14 plants showed weak symptoms only, and only 2 plants showed severe symptoms 8 days after challenge-inoculation. However, during the second cycle of symptom development, the symptoms of this isolate were nearly as severe as in the control plants singly inoculated with TV75-85.

Thus, the deviant virus isolate was unequivocally identified as a newly detected strain, unusually mild on faba bean but of normal pathogenicity to other legumes. It was indistinguishable from regular isolates of BBMV in serology (gel-diffusion serolgy and ELISA), light and electron microscopy, and in symptoms on major hosts and test plant species, but it could be easily distinguished by its initial mild reaction on faba bean and the subsequent complete recovery of infected plants.

This report provides the first evidence of substantial biological variation of BBMV. The strain may occur undetected in faba bean, and possibly come along in its seed (Makkouk et al., 1988b). When occurring in faba-bean crops, it may impose a threat to other sensitive legume species grown nearby, if efficient vectors are around. It may already occur widely in other crops, but may do so unnoticed, e.g. in field peas, where necrotic symptoms may not be recognized by patholgists as caused by virus (Makkouk et al., 1988b). Our results demonstrate the absolute necessity of biological sample testing to supplement serological routine testing for the detection of biologically and pathologically deviant strains of known viruses. The new strain is included in further detailed studies with more common isolates of the virus, especially for interaction with genotypes of the major food legumes grown in the region (Fortass and Bos, in preparation).

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