

Sequence Variability of *Cucumber mosaic virus* (CMV) and its Effects on CMV-Resistance of *Capsicum* sp.

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5 Summary

Cucumber mosaic virus (CMV) is an economically important pathogen on chili in Asia. Due to the lack of commercially available resistant lines and a lack of epidemiological knowledge about the CMV population in Asia, the disease is difficult to manage. To improve this situation 38 CMV isolates originated from Asia, Europe and USA have been characterized and plant lines, originated from a worldwide germplasm collection of the AVRDC (Asian Vegetable Research and Development Centre, Taiwan), have been screened for resistance with those isolates.

The characterization of those isolates were carried out by symptomatology, serology, RT-PCR-RFLP, microarray and phylogenetic analysis using coat protein (CP), movement protein (MP) and the gene silencing suppressor genes (2b). The phylogenetic analyses with a large number of CP, MP or 2b sequences confirmed the previous classification of CMV isolates into serotypes I and II as well as subgroups Ia, Ib. However, the Asian isolates distributed into several subgroups within subgroup Ib with high bootstrap support. Serology and RT-PCR-RFLP could differentiate the serotypes I and II, a further differentiation into the subgroups Ia and Ib was best possible with the microarray technique. This technique was based on probes derived from variable parts of the CP gene. The results of the microarrays showed a clear differentiation of subgroups Ia, Ib and serotype II with oligonucleotide probes for the first time.

Symptomatology studies revealed four phenotypes on *N. glutinosa* and three phenotypes on four resistant chili lines PBC370, PBC549, PBC459 and VC246. However, it was almost not possible to establish a relationship of isolates between sequences and symptoms. The elementary resistance mechanism of two of those chili lines was investigated by grafting and reciprocal crosses and due to these results possible mechanisms of two resistant lines could be proposed. Line PBC370 may have recessive, partially dominant, or polygenic recessive inheritance, whereas the resistance gene involved in line VC246 is probably dominant. Therefore, VC246 may be a new, hopefully, true resistant line that can be used for breeding programs. Although the chili lines were resistant to most of the isolates, some of the isolates were able to overcome this resistance and caused severe symptoms. A reassortment system for mapping of the genetic traits of the CMV genome was established and used to clarify the determinants of chili resistance breaking by these isolates. The result revealed that RNA 2 of isolate AN was responsible for resistance breaking on line VC246. Further studies with reverse genetics confirmed this result and showed that a 1100 bp cassette of the 2a/2b overlapping region was responsible for resistance breaking. On the

basis of a comparison of the 2b gene of resistance breaking and non resistance breaking isolates, two single amino acids changes were selected to examine which might be responsible for this process. However, the results indicated that none of either amino acid is solely involved in resistance breaking of chili, but interestingly changed the symptoms on tobacco.

Moreover, the influence of the interaction and/or compatibility among CMV genome segments to the biological behaviour on tobacco and chili was investigated with a set of six biologically distinct isolates. These results indicated that interaction and/or compatibility among genome segments play an important role in the process of plant-virus interaction so that it was concluded that results of genetic mapping of determinants of CMV should be cautiously explained.

Taken together, the characterization of the population structure of CMV in Asia, the development of a detection method for the differentiation of serotypes and subgroups and the knowledge of the genetic background of some resistant chili lines as well as the biological properties of some resistance breaking isolates may give a basis to improve the Asian chili production in the future.