MOLECULAR CHARACTERIZATION OF RASPBERRY BUSHY DWARF VIRUS ISOLATES FROM POLAND

M. Cieślińska, J. Wójcik-Seliga, Research Institute of Horticulture, Skierniewice, Poland

INTRODUCTION

Raspberry bushy dwarf virus (RBDV), the genus Idaeovirus is a pollen-transmitted agent, infecting wild and cultivated *Rubus* sp. plants. Many infected raspberry and blackberry cultivars do not show any symptoms but in the others RBDV induces yellows disease and fruit deformation (Fig. 1). The virus genome consists of two molecules of single-stranded (ss) RNA. RNA-2 contains two genes, encoding the movement protein (MP) and the coat protein (CP). The aim of the study was to detect and characterize the virus isolates infecting raspberry, blackberry and hybrids of these species in Poland.

METHODS

Leaf samples from 110 *Rubus* sp. plants were collected in several regions of Poland and tested by DAS-ELISA for RBDV. RT-PCR with primers specific to coat protein (CP) and movement protein (MP) genes of RBDV were conducted to confirm the ELISA positive results. The complete sequences of RNA-2, including CP and MP genes, (~2.2 kb) of five isolates were amplified with primers FM-2/RC-2 (Ellis et al., 2005. Plant Management Network). Molecular characterization of the virus isolates was determined by restriction fragment length polymorphism (RFLP) analysis using *Alu*I, *Bfa*I, *Rsa*I, and *Hha*I enzymes, as well as by sequencing and phylogenetic analyses of RNA-2. The sequences were analysed using Lasergene 7.1 software (DNASTAR) and phylogenetic analysis was conducted by the neighbor-joining method of MEGA6.

Reaction of *Chenopodium quinoa, C. amaranticolor* and *Cucumis sativus* sap inoculated with RBDV isolates were also investigated.

RESULTS

Based on DAS-ELISA and RT-PCR results the presence of RBDV was detected in eight samples: six from red raspberry (Pol, Nor, JSqu, Vet, Karm, Marc), one from wild blackberry (WB) and from loganberry (Log). The reaction of herbaceous hosts differed in intensity of the symptoms depending on RBDV isolate used for mechanical inoculation (Fig. 2 A, B). The RFLP patterns of the amplicons digested singly with *Alu*I, *Bfa*I, *Rsa*I, and *Hha*I enzymes and analysis of RNA-2 sequence fragments resulted in significant variability of the five RBDV isolates (Fig. 3, 4). The nucleotide sequences of RNA-2 of these isolates were 97.2-99.5% similar to each other and shared 95.5-98.7% and 95.4-96.1% identity with the sequence of raspberry and grapevine reference strains, respectively. Phylogenetic analysis showed that Polish isolates of RBDV formed well supported group with raspberry strains (GeneBank acc. nos. EU796088-89).

Fig. 1. Symptoms induced by RBDV on leaves and fruits of infected red raspberry

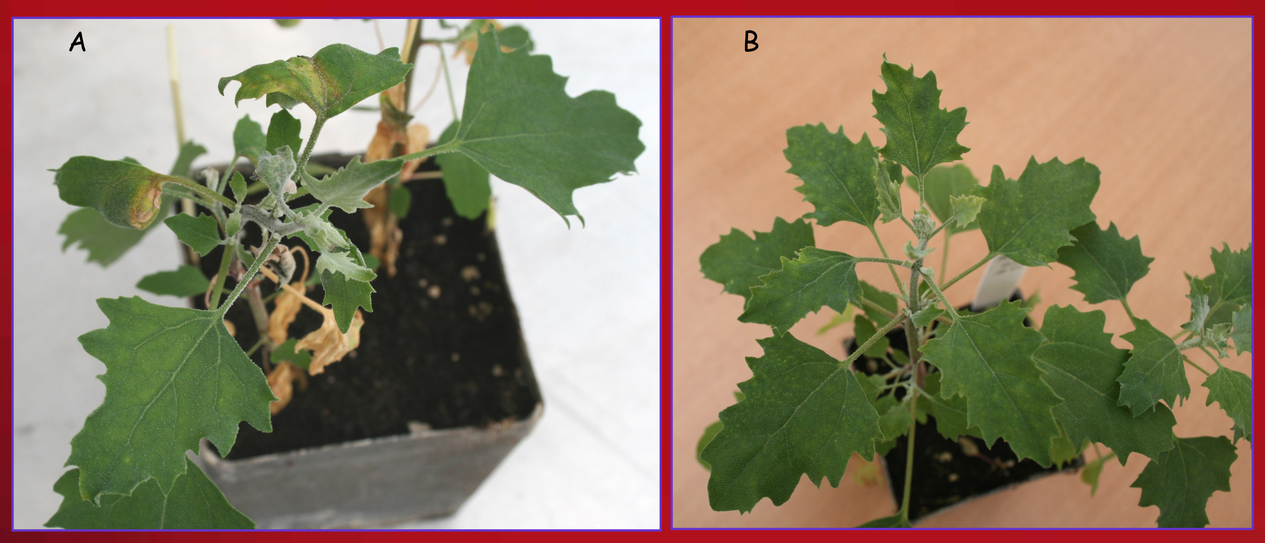

Fig. 2. Symptoms induced by Log (A) and Pol (B) isolates of RBDV on *Chenopodium quinoa*

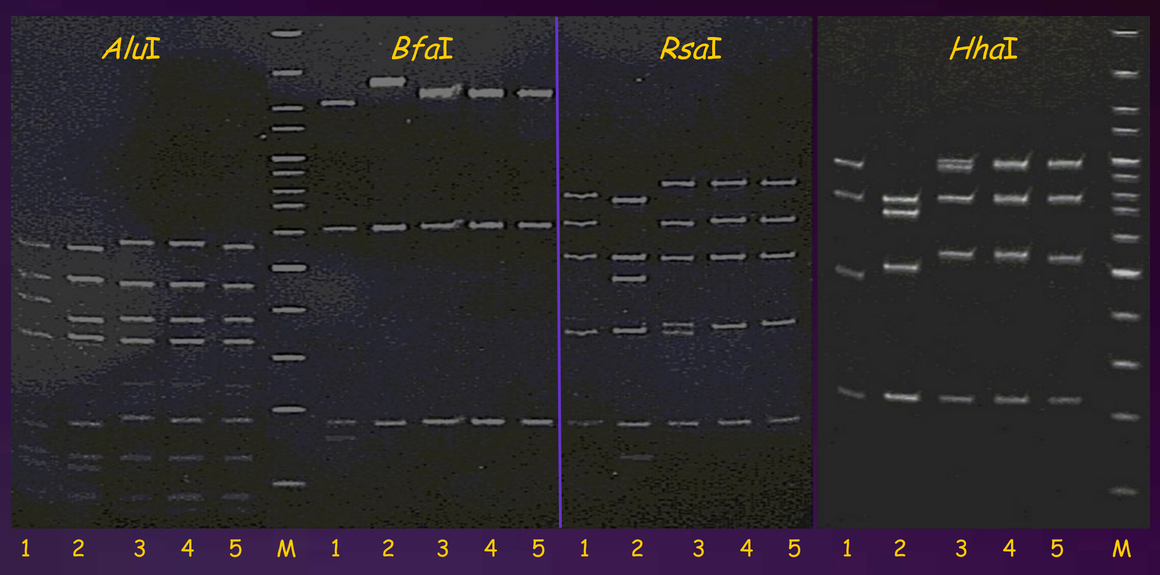

Fig. 3. RFLP patterns after digestion of RT-PCR products amplified with primer pair FM-2/RC-2. M - DNA ladder 100 bp. 1. Pol; 2. Karm; 3. Marc; 4. JSqu; 5. Log

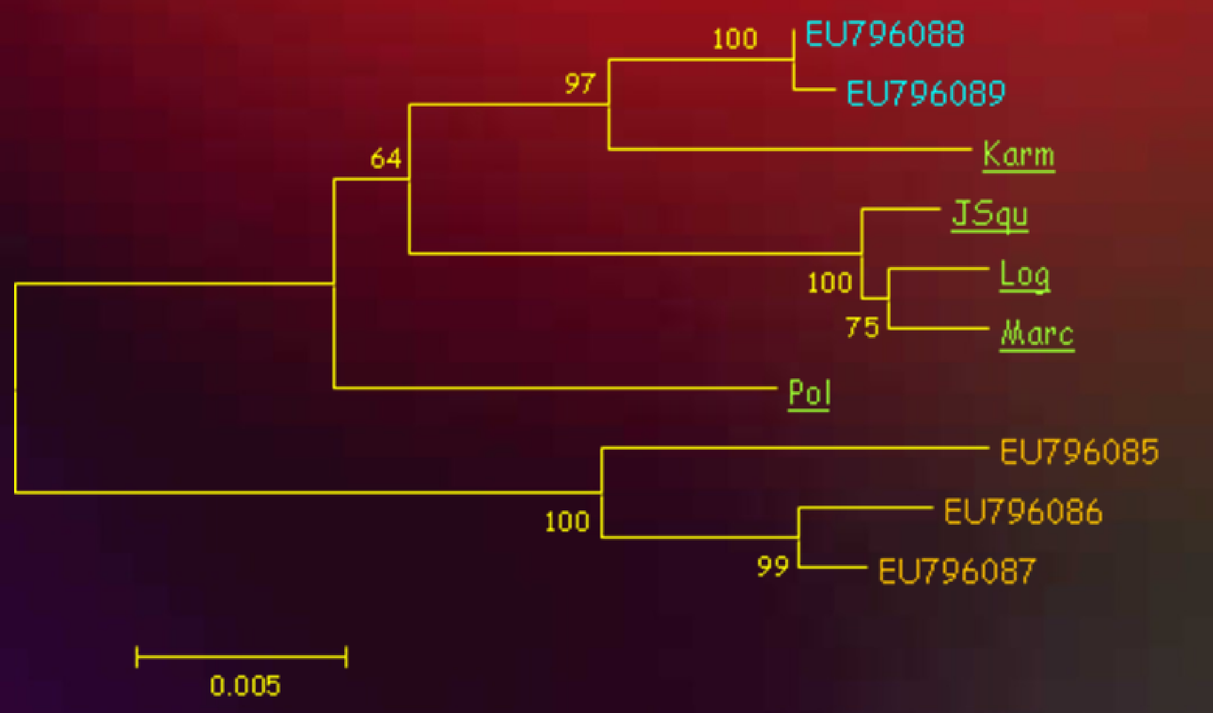

Fig. 4. Phylogenetic tree of the partial RNA-2 of the RBDV isolates (approx. 2.2 Kbp) from Poland (underlined) and the reference strains (GeneBank accession numbers) from red raspberry and grapevine. The tree was constructed using the neighbour-joining method (MEGA6). Bootstrap values (% replication) are showed by each branch node.

CONCLUSION

Based on sequence analysis of RNA-2 it has been showed that RBDV isolates found in Poland varied in their molecular properties. They also induced different symptoms on mechanical inoculated herbaceous indicator plants.

ACKNOWLEDGEMENT

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