Detection of high molecular weight dsRNA persisting in Dianthus species

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ABSTRACT Cryptic plant viruses are seed-borne dsRNA-viruses, which co-exist life-long with the host plant, without inducing any apparent symptoms. Since growth conditions and the host-virus combination (cultivar, strain, isolate, thermotherapy, etc.) are known to influence virus multiplication, we wanted to find out what effect long-term tissue culturing has on the survival of carnation cryptic virus (CarCV). 21 members of Hungarian Dianthus germplasm collection have been aseptically grown for 16 years. Total nucleic acids of these Dianthus species and of Silene vulgaris were separated by non-denaturing gel electrophoresis and the dsRNA-pattern was visualized by immunoblotting using dsRNA-specific monoclonal antibodies. Genomic dsRNAs of CarCV were detected in D. caryophyllus. In four additional species: D. superbus, D. giganteus D. gratianopolitanus and Silene vulgaris several dsRNA-species in the same size range as the genomic dsRNAs of CarCV were detected. We also show that three other cryptoviruses, the beet cryptic viruses BCV1, -2 and –3 can persist under in vitro conditions. Our results indicate that cryptic viruses are so well adapted to their hosts that they can persist after more than a decade of in vitro culturing despite the dramatic change of the environment.


Materials and Methods

Plant material

Eighteen different species of in vitro propagated Dianthus, two members of the D. caryophyllus variety “club” and Silene vulgaris from the Hungarian germplasm collection were used to search for the presence of carnation cryptic virus dsRNAs. The tissue cultures were initiated 16 years ago from sterilized seeds to eliminate single-stranded RNA-viruses and have been grown since then on hormone-free Murashige-Skoog (MS) basal medium in tissue culture tubes.

The in vitro cultured haploid and diploid Beta vulgaris lines were grown at Beta-Research Ltd., Sopronhorpács, Hungary. Diploid shoot cultures were initiated from sterilized seeds and have been aseptically grown for 6-7 years since then. Haploid plants were produced from in vitro cultures of ovules removed before anthesis from closed flowers of male sterile plants and have been grown aseptically for the last 5 years (Potyondi and Heszky 1992).

Total nucleic acid purification and dsRNA-immunoblotting

dsRNAs in crude nucleic acid extracts were detected on immunoblots as described by Lukács (1994). J2, a dsRNA-specific monoclonal antibody was used as primary antibody. This antibody specifically recognizes dsRNA independent
of its nucleotide composition and sequence and does not cross-react with short double strand helices present in single-stranded RNAs (Schönborn et al. 1991). Fifty µg total nucleic acids were loaded onto each lane of the non-denaturing 5% PAA-TBE gel.

Thermotherapy and meristem isolation

Cultures were maintained at 30°C in a growth room for 5 days, followed by 5 weeks at 36°C. After excising the meristem (0.2-2 mm), meristems were transferred to MS medium containing kinetin and α-naphthyl-acetic acid and shoots were regenerated for 6 weeks (Murashige and Skoog 1962).

Results and Discussion

Carnation cryptic virus (CarCV) can be detected in in vitro cultured Dianthus species

In virions purified from CarCV-containing plants three major dsRNA (MW 1.04×10^6, 0.95×10^6 and 0.84×10^6 Da, respectively) and a minor species (0.88×10^6 Da) have been described (Lisa et al. 1981a). The minor dsRNA was not present in all preparations (Lisa et al. 1981b). To find out whether CarCV is able to survive under the selection conditions of in vitro culturing we investigated different carnation species (Table 1) after 16 years of continuous in vitro cultivation. We found that dsRNAs of the same molecular mass as in CarCV are present in D. caryophyllus ‘Chabaud’ and ‘Grenadin’, but not every sample contained all four CarCV bands. The most prominent difference was seen in D. caryophyllus ‘Chabaud’ where only the two smallest (0.95×10^6 and 0.84×10^6 Da) dsRNA fragments were detected (Figure 1). Although the presence of the larger fragments at concentrations below the detection limit cannot be excluded, we conclude that in this case the relative concentration of genomic fragments was altered after prolonged in vitro cultivation. Further experiments should clarify the effect of such alteration on the virus cycle.

In addition to D. caryophyllus, dsRNAs were found in at least six other species. Four of them: D. superbus, D. giganteus D. gratianopolitanus and Silene vulgaris contained dsRNAs in the same size range as CarCV dsRNAs, but whether these dsRNAs are related to CarCV has not yet been proved. Two further species, D. plumarius and D. chinensis also exhibit prominent dsRNA bands but whose molecular mass is totally dissimilar to that of CarCV. The origin of these bands ranging from 1100 to 3600 bp is not known (part of the results are shown in Fig. 1, lanes A).

Figure 1. Detection of dsRNA species in in vitro plantlets regenerated from meristem tip culture after thermotherapy of carnation plants. Symbols: A, untreated control plants, B and C, shoots regenerated from treated and excised single meristems smaller than 0.8 mm (B) or larger than 0.8 mm (C). (1) D. caryophyllus, (2) D. caryophyllus ‘Grenadin’, (3) D. caryophyllus ‘Chabaud’, (4) D. gratianopolitanus, (5) Silene vulgaris.

Influence of thermotherapy and meristem excising in in vitro cultured Dianthus species

Heat treatment or thermotherapy is a frequently used method for efficiently eliminating viruses from plants. However, it does not appear to work when applied to cryptovirus containing plants: After the treatment genomic dsRNAs of CarCV were still detected in D. caryophyllus and the uncharacterized dsRNA-species in D. gratianopolitanus and Silene vulgaris were also not eliminated (Fig. 1).

Beet cryptic virus (BCV1, -2 and -3) dsRNA persist in Beta vulgaris ssp. vulgaris after 6-7 years of in vitro culturing

To find out whether the same behavior is exhibited by other cryptic viruses we investigated tissue cultures of B. vulgaris ssp. vulgaris. In beet plants three kinds of probably unrelated cryptic viruses may occur and even coexist, Beet Cryptic Vi-

Table 1. Occurrence of dsRNA-species in in vitro propagated carnation plants.

<table>
<thead>
<tr>
<th>Species</th>
<th>dsRNA</th>
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<tr>
<td>D. caryophyllus</td>
<td>+</td>
<td>D. sylvaticus</td>
<td>-</td>
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<tr>
<td>D. caryophyllus ‘Grenadin’</td>
<td>+</td>
<td>D. armeria</td>
<td>-</td>
</tr>
<tr>
<td>D. caryophyllus ‘Chabaud’</td>
<td>+</td>
<td>D. giganteus</td>
<td>+</td>
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<tr>
<td>D. chinensis</td>
<td>+</td>
<td>D. deltoides</td>
<td>-</td>
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<tr>
<td>D. giganteiformis</td>
<td>-</td>
<td>D. knapii</td>
<td>-</td>
</tr>
<tr>
<td>D. anatolicus</td>
<td>-</td>
<td>D. carthusianorum</td>
<td>-</td>
</tr>
<tr>
<td>D. superbus</td>
<td>+</td>
<td>D. gratianopolitanus</td>
<td>+</td>
</tr>
<tr>
<td>D. seratinius ssp. regis-stephani</td>
<td>-</td>
<td>D. plumarius ssp. praecox</td>
<td>+</td>
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<tr>
<td>D. fischeri</td>
<td>-</td>
<td>D. gallicus</td>
<td>-</td>
</tr>
<tr>
<td>D. pontederae</td>
<td>-</td>
<td>D. monspessulanus</td>
<td>-</td>
</tr>
<tr>
<td>Silene vulgaris</td>
<td>+</td>
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All three known BCVs could be detected in the cultures after 6-7 years of culturing (results not shown). It was, however, also evident that many individual cultures were free of BCV dsRNA, although we know from many experiments that most individual sugar beet plants harbour at least one BCV.

Taken together our results indicate that cryptic viruses are so well adapted to their hosts that they can persist for 16 years even under the artificial conditions of tissue culturing and that they can even survive thermotherapy. Therefore, when generating virus-free plants by micropropagation it should be taken into account that cryptic viruses may survive and be present even in aseptically grown cultures. In addition, the undetected presence of cryptic viruses may invalidate tests to confirm that plants are virus-free, if these are based upon the isolation of high molecular weight dsRNA or of virus particles.

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References