Evaluation of the promoter activity of caulimoviruses associated with Dahlia spp by transient expression of the beta-glucoronidase gene (GUS)

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Abstract

One of the most common promoters used for constitutive gene expression was derived from a plant virus. The 3SS promoter from Cauliflower mosaic virus (CaMV) drives high levels of transgene expression; directs constitutive expression and displays no tissue-specificity. Due to these properties, the CaMV 3SS promoter is widely applied in expressing foreign genes in plants. However, some limitations including insufficient promoter activity or strong down-regulation necessitated the exploration of more efficient promoters from other caulimoviruses. Dahlia mosaic virus (DMV) and two new caulimoviruses tentatively designated as Dahlia common mosaic virus (DCMV) and an endogenous sequence (DMV-D10) have been reported from dahlia (Dahlia variabilis) plants. Not much information is known about the promoters from these caulimoviruses and they represent a potential source for new promoters. Based on sequence comparisons and promoter prediction programs, we identified the putative 3SS promoter in DMV, DCMV and DMV-D10 from cultivated and wild dahlia species. The intergenic regions containing the putative 3SS promoter were separately cloned into pCAMBIA1281Z, a binary vector. Constructs were delivered into Agrobacterium tumefaciens by electroporation and agroinfiltrations were done into Nicotiana tabacum, N. benthamiana and Verbascum enceoloides. The activity and strength of the putative promoters from DMV, DCMV and DMV-D10 was determined by GUS assays. Preliminary results from qualitative GUS assays demonstrated that DMV, DCMV and DMV-D10 promoter activity is similar to the one showed by 3SS promoter of CaMV. Transient expression showed stronger activity in N. benthamiana leaf tissue in comparison to N. tabacum leaf tissue for all constructs. Deletion analysis of the 3' and 5' end of the promoter region in order to establish the optimal boundaries for maximal promoter activity is underway. Quantitative GUS assays are also in progress. Since promoter selection has become increasingly important for successful gene transfer and expression of transgenes in plants, the findings could provide important information on new promoters for gene expression in plants.

Materials and Methods

The putative 3SS promoter in DMV, DCMV and DMV-D10 was identified analyzing the sequences of the intergenic region (IGR) in the program Promoter 2.0 prediction server (www.cbs.dtu.dk/services/promoter) from cultivated and wild dahlia species.

Putative 3SS promoter regions were amplified by PCR using DNA extractions (QIAGEN kit) from affected Dahlia plants and specific primers. Amplicons were cloned into pGEMT-easy vector (Promega) and sub-divided separately into pCAMBIA1281Z (http://www.cambio.co.uk), a vector specially designed for promoter examination.

Confirmation of successful cloning and promoter orientation was done by sequencing.

pCAMBIA1304 was used as positive control since this vector contains the 3SS promoter from CaMV whereas pCAMBIA 1281Z promoter-less was used as negative control.

All constructs made were delivered to Agrobacterium tumefaciens by electroporation.

Bacteria were prepared for agroinfiltration into Nicotiana tabacum, N. benthamiana and Verbascum enceoloides (three replicates each plant) following the procedure described by Vaghhochipwala and Mysore from the Plant Virology Protocols.

The activity and strength of the putative promoters from DMV, DCMV and DMV-D10 was determined by GUS assays. Qualitative GUS assay was performed according to the protocol described originally by Jefferson and adjusted at the WISU Plant Transformation Core. By electroporation.

Results

Results from qualitative GUS assays are summarized in Table 1.

The promoter activity of DMV, DCMV and DMV-D10 from cultivated (Dahlia variabilis) and one wild species (Dahlia Sherffii) was similar to that of CaMV.

Transient expression showed stronger activity in N. benthamiana leaf tissues (Figure 2) in comparison to N. tabacum leaf tissue (Figure 3).

Agroinfiltrations in V. enceoloides, host for DMV (Brunt, 1971) was difficult to perform and this attempt was not successful.

Table 1. Results from qualitative GUS assays

<table>
<thead>
<tr>
<th>Construct</th>
<th>Promoter Activity</th>
</tr>
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<tbody>
<tr>
<td>DMV-D10 1281Z (R1)</td>
<td>+</td>
</tr>
<tr>
<td>DMV-D10 1281Z (R2)</td>
<td>+</td>
</tr>
<tr>
<td>DMV-D10 1281Z (R3)</td>
<td>+</td>
</tr>
<tr>
<td>DCMV 1281Z (R1)</td>
<td>-</td>
</tr>
<tr>
<td>DCMV 1281Z (R2)</td>
<td>-</td>
</tr>
<tr>
<td>DCMV 1281Z (R3)</td>
<td>-</td>
</tr>
<tr>
<td>DMV-D10 (R1)</td>
<td>+</td>
</tr>
<tr>
<td>DCMV-D10 (R1)</td>
<td>-</td>
</tr>
<tr>
<td>DCMV-D10 (R2)</td>
<td>-</td>
</tr>
</tbody>
</table>

Deletion analysis (5' and 3' end) of the promoter regions are underway and should be performed to determine transient activity of all evaluated promoters. From the analogy with other promoters of caulimoviruses, we expect that the activities of promoters tested are similar or maybe higher than the activity of the CaMV 3SS promoter.

Conclusions and Future Research

DMV-, DCMV- and DMV-D10- putative 3SS promoter drives high levels of gene expression as CaMV 3SS promoter in N. benthamiana leaf tissue. N. tabacum leaf tissue displayed less activity of promoters. Agroinfiltrations in V. enceoloides were not successful and therefore have to be repeated.

Quantitative GUS assays are underway and should be performed to determine transient activity of all evaluated promoters.

To evaluate stable expression of the GUS gene, transgenic plants should be obtained using agrobacterial transformation procedures.

Deletion analysis (5' and 3' end) of the promoter regions are also in progress and should be performed to determine the optimal boundaries for maximal promoter activity.

From the analogy with other promoters of caulimoviruses, we expect that the activities of promoters tested are similar or maybe higher than the activity of the CaMV 3SS promoter.

References