Characterization of newly discovered viruses in declining birch – a continutive study in Berlin

Eilisha Bright Opoku1, Maria Landgraf1, Martina Bandte1, Susanne von Bargen1, Martin Schreiner1, Barbara Jäckel1, Carmen Büttnner1

1 Humboldt-Universität zu Berlin, Laborwissenschaftliche Fauleit, Fachgebiet Phytopathologie, Loccumstr. 55-57, D-14195 Berlin; Pflanzenenschutzamt, Mohner Allee 137, D-12147 Berlin. Anرهابه ویرایشگر: Eilisha Bright Opoku, Opoku@hu-berlin.de

Roadside trees have an immense impact on the health of human population and have to be protected and preserved. Virus infections of broad-leaved trees are widespread in urban areas and in forests, which might play a major role in tree decline. Viral infections are mostly recognized by mosaic-like leaf patterns, chlorotic ringspots, lines and mottling of light and dark green color as well as deformation (Fig 1). To gain a more detailed view on epidemiology of a viral complex in birch, the study was continued in 2017 with 107 birch tree samples in Berlin.

Data from next generation sequencing has shown the complexity of the birch virome. Based on molecular biological diagnostics of Cherry leaf roll virus, Apple mosaic virus, Carle- and Badenavirus from birch in 2015 and 2016, data on distribution of the viral complex was collected in the urban landscape of southern Berlin. Characterization of newly discovered viruses is one major goal in the next years to determine pathogenicity and evaluation of impact in urban green and forests. Especially interesting for management and maintenance of urban green is the mode of viral transmission.

Primers were designed for viral detection of infected leaves from different sites in Berlin as shown in map below (Fig 2). Investigation of plant viruses was done by using RT-PCR to test for Cherry leaf roll virus (CLRV) and Apple mosaic virus (ApMV), which are commonly associated with deciduous trees. The diversity of symptoms and the newly discovered viral species (Carle- and Badenavirus) in Betula spp. led to the assumption of a mixed infection with unknown viral origin. Biostest experiment was carried out to check if newly found Carle- and Badenavirus were mechanically transmissible. This was done using Chenopodium quinoa since other plants showed no symptoms (Fig 3). Symptoms like ringspots and intercostal chlorosis were observed three weeks after inoculation and evaluated by RT-PCR (Fig 4).

Transmission experiments for Badenaviruses using biostest plants (Chenopodium quinoa) and mechanical inoculation by infected birch leaves proved to be effective. First positive results were obtained for Chenopodium quinoa despite the narrow host range of Badenaviruses (Fig 5). This gave the impression that Badenaviruses found in birch are mechanically transmissible. Consequences for hygienic practices during tree management and maintenance will be examined once confirmation of Badenavirus pathogenicity is known.

Future studies will reveal whether Carleaviruses are mechanically transmissible using biostest experiments in fulfillment of Koch’s Postulates. Once this is confirmed, birch leaf symptoms can be correlated with newly found plant viruses. Characterization and localization Apple mosaic virus, newly found Carle- and Badenaviruses will also be carried out using tissue printing technologies. Viruses will be localized within the symptomatic plants based on microscopic technologies to determine if they contribute to birch leaf rold diseases.

Methods

Results

<table>
<thead>
<tr>
<th>Virus</th>
<th>Specific primers</th>
<th>Number of tree samples n=107</th>
</tr>
</thead>
<tbody>
<tr>
<td>Badenavirus K</td>
<td>amplified at 300bp</td>
<td>59</td>
</tr>
<tr>
<td>Badenavirus 5G</td>
<td>amplified at 242bp</td>
<td>66</td>
</tr>
<tr>
<td>Carleivirus</td>
<td>amplified at 197bp</td>
<td>13</td>
</tr>
<tr>
<td>CLRV amplified</td>
<td>at 627bp</td>
<td>20</td>
</tr>
<tr>
<td>ApMV amplified</td>
<td>at 204bp</td>
<td>Not yet completed</td>
</tr>
</tbody>
</table>

Fig 5. Gel electrophoresis for detection of Badenavirus using RT-PCR with specific primer Badenavirus from birch samples.

Fig 6. Detection of Badenavirus in C. quinoa using RT-PCR amplified at 242bp.

Outlook

Future studies will reveal whether Carleaviruses are mechanically transmissible using biostest experiments in fulfillment of Koch’s Postulates. Once this is confirmed, birch leaf symptoms can be correlated with newly found plant viruses. Characterization and localization Apple mosaic virus, newly found Carle- and Badenaviruses will also be carried out using tissue printing technologies. Viruses will be localized within the symptomatic plants based on microscopic technologies to determine if they contribute to birch leaf rold diseases.

References

3. Acknowledgement

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