Potato virus strain influence in vitro plants regeneration

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Abstract Virus diseases determine great losses in potato yield and obtaining potato virus free regenerates becomes very important whilst there are no chemical methods for direct viruses eradication. It was observed that the regeneration percentage from meristem culture depends on the viral strain that infects the donor plant and also in a great measure of the explants size. The lowest regeneration percentage was obtained when the inoculums was constituted of the apical meristem without leaves primordia excised from plants infected with both Potato virus X and Potato virus Y, being of about 15.93%. The highest regeneration percentage of about 92.18% was obtained from plants infected with Potato virus S and the explants were constituted of the apical meristem plus four leaves primordia. The lowest regeneration percentages were obtained from explants excised of plants infected with more than one virus strain.

Potatoes are a vegetative propagated crop, and many disease organisms including several viruses and a viroid are disseminated in tubers [17]. The important role that tubers play in virus spread is recognized by the strict requirements for foundation or certified seed production [15].

Seven viruses and spindle tuber viroid are recognized as important from either a production or a seed certification standpoint. The viruses include potato leafroll virus (L), potato viruses Y, X, A, S, M, and alfalfa mosaic virus, with the first three being the most important [13, 14, 17]. Potato leafroll virus (PLRV) causes an important disease of potatoes affecting the quantity and quality of production and may cause a crop to be ineligible for certification. PLRV can be difficult to detect because foliar symptoms are not always obvious. Thus infected tubers or tubers with net necrosis may result from plants without visual symptoms [14]. Potato virus Y (PVY) is one of the most important viruses infecting potatoes. It is readily spread by aphids in a nonpersistent manner as well as mechanically by human activity and may result in severely depressed yields. PVY is tuberborne and can interact with other viruses such as PVX and PVA to result in heavier losses [13]. Potato Virus X (PVX) is PVX is tuberborne and one of the most widely distributed viruses of potatoes because no symptoms develop in some varieties (latent mosaic), the full extent of damage with PVX is not recognized. Mixed infections of PVX with other viruses like PVA and PVA cause more damage than PVX alone [13, 14]. Potato viruses, virus infection, in vitro regeneration

Key words

Potato viruses, virus infection, in vitro regeneration

To establish a successful infection, plant viruses move from cell to cell into the vascular system, where they are systemically translocated throughout the plant [10]. Cell-to-cell movement involves passage through plasmodesmata (PD), the small pores that interconnect cells (reviewed in Roberts and Oparka, 2003). As established by dye-coupling studies, plasmodesmata normally have a size exclusion limit (SEL) of 800 to 1000 D [1]. Thus, it is generally accepted that transport through plasmodesmata is restricted to only small molecules, which would include metabolites, ions, and hormones. It has been shown that some viruses appear to require capsid protein for cell-to-cell movement, whereas others do not [5]. Several studies regarded virus free material getting by in vitro culture of potato tissues [11, 14, 15] have been done but none of them was concerned about the virus strain influence on tissues regeneration from in vitro culture. In this study, direct evidence is presented showing that viral strain which infects the donor material affects regeneration from tissues cultured in vitro, and meristems size is very important in this way. As not all the viruses can traffic cell to cell through plasmodesmata to the apical meristem of the plant this part of the plant should be
free of viruses and can constitute the donor material for healthy plants regeneration.

Material and Methods

Biological material was constituted of four potato cultivars, which results presented no statistically accepted differences. Data taken in consideration were represented by the average values obtained of all four cultivars studied. The average value and the standard deviation of 3-5 samples were calculated using the Microsoft Office Excel 2003 program.

Equal numbers of potato plants were mechanically inoculated with Potato virus A (PVA), Potato virus M (PVM), Potato virus S (PVS), Potato virus X (PVX), Potato virus Y (PVY) and Potato leafroll virus (PLRV) and an equal number of potato plants were inoculated with a mixture of two viruses: Potato virus X and Potato virus Y, Potato virus A and Potato leafroll virus, Potato virus X and Potato virus S, Potato virus Y and Potato virus X, and constituted the samples as shown in table 1. Healthy non-infected plants constituted the control.

Virus material was provided from infected plants (kindly provided by Dr. Nicolae Cojocaru, Potato Research Institute, Brasov, Romania), tested with ELISA test kits PathoScreen PVY, PVA, PVS, PVS, PLRV and PVX from Agdia Inc., IN, USA. Virus infected plant sap was diluted in a ratio of 1:1 with PBS 1x buffer (Na₂HPO₄ -1.09g/l, NaH₂PO₄ – 0.32g/l, NaCl – 9g/l, dissolved in distilled water and add to 1000 mL, pH – 7.2) and used for mechanical inoculation of potato leaves.

Explants were constituted of apical meristems of different sizes: apical meristem without leaf primordia, apical meristem plus one, two or four leaves primordia. Culture medium used for experimentation was constituted of PM [1] added with 5,7x10⁻⁶M indole-3-acetic acid, 4,9x10⁻⁷M indole-3-butyric acid and 8,6x10⁻⁷M gibberellic acid. Regeneration percentage was measured 10 weeks after inoculation.

Results and Discussions

Virus free material from virus infected donors can be easily obtained by in vitro cultures [2]. One of the most used method is meristem culture due to the fact that meristems, especially those constituted of the apical meristem without leaves primordia, are free of viruses [11, 13, 15]. Starting from this type of explants healthy and vigorous plants can be regenerated, that can constitute the either the seeding material itself or donors for seeding material getting [14]. Literature reports showed that root tips of plants infected with one of several viruses have been found to be free of detectable virus [9]. Smith and Schlegel (1964) studied the distribution of Clover yellow mosaic virus in root tip of Vicia faba and found that within the limit of the assay method, the first 0.4 mm of the root tip, which included the root cap and the meristem, were virus free.

Data obtained from regeneration of shoots by meristems culture, sampled from virus infected plants; show that significantly differences were registered between the different viruses’ infections (table 1). Statistically negative results were registered for almost all the samples comparing with the healthy control, independent of the meristem size that the shoots were regenerated of. This fact shows that regeneration capacity is influenced by the virus presence in the plant tissues. More over, significantly differences were registered between the different virus infection samples. The lowest regeneration capacity was registered when plants were infected with both PVY and PVX, being of about 15.93%. This results might be due to the reason that virus Y, that possess the protein HC-Pro involved in cell-to-cell traffic of viral particles through branched plasmodesmata is gating the plasmodesmata allowing virus X pass [5, 7]. Also, virus X that is low sizes [8] and can easily traffic through simple not branched plasmodesmata. Other studies showed that virus X was found even in the apical meristem of shoots [14].

Our studies showed that regeneration percentage was in almost all the cases not significantly different when healthy tissues were generating by in vitro cultures new plants, comparing with the meristems sampled from plants infected with one virus (table 1). But, in the mixed infections, as in our case, some of the viruses are trafficking to the tip of the root infecting also the meristematic cells [13, 14]. There is evidence that some viruses and also in mixed infections may invade the primary meristematic tissues [13].
Regeneration percentage of potato meristems depending on their size and virus strains that infect the donor plants

<table>
<thead>
<tr>
<th>Nr. crt.</th>
<th>Virus</th>
<th>$x \pm s_x$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AM</td>
<td>AM+1</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>24.94 ± 4.15 a</td>
</tr>
<tr>
<td>2</td>
<td>PVA</td>
<td>23.44 ± 3.59 ab</td>
</tr>
<tr>
<td>3</td>
<td>PVM</td>
<td>23.91 ± 3.81 ab</td>
</tr>
<tr>
<td>4</td>
<td>PVX</td>
<td>22.57 ± 3.58 b</td>
</tr>
<tr>
<td>5</td>
<td>PVY</td>
<td>18.75 ± 3.83 cd</td>
</tr>
<tr>
<td>6</td>
<td>PLRV</td>
<td>17.13 ± 3.83 c</td>
</tr>
<tr>
<td>8</td>
<td>PVA+PLRV</td>
<td>18.12 ± 3.62 cde</td>
</tr>
<tr>
<td>9</td>
<td>PVX+PVY</td>
<td>17.57 ± 3.87 de</td>
</tr>
<tr>
<td>10</td>
<td>PVX+PVY</td>
<td>16.81 ± 3.83 e</td>
</tr>
<tr>
<td>11</td>
<td>PVY+PVY</td>
<td>18.96 ± 4.00 d</td>
</tr>
</tbody>
</table>

LSD$_{0.05}=1.503$
LSD$_{0.05}=2.026$
LSD$_{0.15}=2.689$

AM= apical meristem without leaf primordia
AM+1= apical meristem with one leaf primordia
AM+2= apical meristem with two leaves primordia
AM+4= apical meristem with four leaves primordia

The two virus infections were done with the reason that some of the viruses interact with another to traffic cell-to-cell and on the reason that some of the viruses, as PVS and PVX are small size viruses that can easily pass through the unbranched plasmodesmata to the next level of cells [14]. No report specifies if presence of a virus strain in the apical meristem and in the regenerated shoots respectively would influence the regeneration capacity, but our study proved that, depending on the type of the virus strain the results were different. Our study shows that significantly higher regeneration percentage was registered for the samples infected with PVA (91.84%) and PVS (92.18%), viruses that naturally induce mild symptoms [4, 12] than for samples infected with PVX or PVY [3]. These two last mentioned viruses determine high losses in potato yields [8, 17]. We suppose that low regeneration rate might be due to the synthesis of nitric oxide and other reactive oxidants determined by the virus presence in infected cells as a defense mechanism [9], that can inhibit cells activity and also can generate apomixes [18]. Also, viral proteins presence in plant cells determine the decreasing of cells multiplication or even their death by inhibition of the principal plants protein ribulose biphosphate carboxylic oxygenase (rubisco), the main cause of leaves mosaic appearance [17]. Fraser (1997) estimated that TMV infection reduced plant proteins synthesis with 75% during the viral replication period. Infection did not disturb the host polyadenilated RNA and its distribution site [12]. These results show that viral infection influence host proteins synthesis in the translation phase but not in the transcription. Detailed studies identified that the infection front determines in plant cells the following situations: inhibition of at least eleven host proteins by the virus presence in the cells [19], expression of some host genes was induced in association with viral replication [13] and inhibition of some host genes promoters by virus action [12]. Beside host proteins synthesis inhibition viral infection can determine temporary genomic DNA synthesis in the apical meristem of beans plants [13], followed by a temporary inhibition of the mitotic index. Viral infection determines inhibition of ribosomal RNA synthesis in the host cells, both in the chloroplasts, determining mosaics appearance and in the cells cytoplasm determining cells death and leaves tissues necrosis [14]. Virus presence can disturb the expression of membrane lipids transfer [13] and the level of cellular carbohydrates, by the use of plasmodesmata in viral proteins, viral RNA or viral particles transport enabling the nutritive exchange between neighbouring cells [10].

Regeneration from meristems’ capacity depends in a great manner of the explants size, the cultivar, the nutrients in the culture media and the hormonal balance [2, 6, and 12]. Thus for, significant differences existed between the results obtained from meristems...
constituted only of meristematic dome without leaf primordia, in average and the explants constituted of meristematic dome plus four leaves primordia (table 1). The lower regeneration percentage was obtained, when the explants had the smallest size (in average 20.31%). The best results were noticed when the meristem had four leaves primordia (in average 78.92%), the regeneration capacity increasing direct proportionally with the meristem size. These results are normal because meristem viability increases with the explants size [1, 16]. Considering the fact that these researches were a preamble for establishing virus free plants acquiring protocols it has to be taken in consideration especially the results obtained for the meristems without or with one leaf primordia, due to the reason that as the explants size are smaller the virus free plants percentage is higher [6, 15, 16].

References