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Root System of PVY^{NTN}-Infected Potato Cultivar 'Igor' Grown in vitro

By

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Summary

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Morphological and histological characteristics of a root system were analysed in healthy and PVY^{NTN}-infected potato plants (cv. 'Igor') grown in stem node tissue culture. After 35 days of growth the number and width of secondary roots were reduced, as well as the width of the primary roots. Histological analysis of root apical meristems showed more pronounced differences between healthy and infected plants in the structure of the central stele rather than the cortex.

Introduction

Plant growth and development are mediated by apical meristems that are the primary sites of plant morphogenesis. The apical root meristem is composed of founder cells with very rare mitotic activity and initial cells which divide frequently and contribute cells to all root tissues - epidermis, cortex, pericycle, endodermis, vascular elements, and root cap (SCHIEFELBEIN & al. 1997).

The infection of potato cultivar 'Igor' with the potato virus Y^{NTN} (PVY^{NTN}) results in the potato tuber necrotic ringspot disease (PTNRD), (LE ROMANCER & al. 1994). Diseased plants are suppressed in the growth and development of their shoots and roots and have characteristic symptoms (LE ROMANCER & al. 1994).

The aim of our work was to investigate how the observed morphological changes in roots of infected potato plants are related to the apical root meristem structure.

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Material and Methods

Plant material

Single stem node cuttings from 6 weeks old healthy and PVY^{NTN}-systemically infected potato plants (*Solanum tuberosum* cv. 'Igor') were propagated in vitro on MS medium and grown in growth chambers as described (DOLENC & al. 1997). Morphological and histological analysis of roots and apical root meristems was done on the 18th and 35th day of growth. On the same days fresh and dry weights were measured.

Morphological analysis

Root systems of 18 and 35 days old healthy and infected plants were fixed in 3:1 (v/v) absolute ethanol:acetic acid for at least 24 hours at 4°C, hydrolized in 5M HCl, Feulgen stained overnight and stored in 45% (v/v) acetic acid. For this research, common root terminology was adapted to the object of experiment. Primary roots grow directly on the stem of newly formed plants. Secondary roots are lateral roots, developed on the primary roots. The number, length and width of primary and secondary roots and the size of root tips were measured with a stereomicroscope.

Histolgical analysis of apical root meristems

10 µm thick longitudinal tissue sections of root tips of primary roots of 18 and 35 days old healthy and infected plants were prepared for the light microscopy as described for apical shoot meristems (DOLENC & al. 1997). The width and cell file number of the root apical meristem, cortex and stele were measured.

Statistical analysis

The Mann-Whitney U-test was used for statistical analysis between healthy and infected plants on the same day of growth.

Results and Discussion

The uniform circular arrangement of root tissue layers (SCHIEFELBEIN & al. 1997) was observed in mature roots and also in the root apical meristems irrespective of the virus infection.

PVY^{NTN}-infection of potato cv. 'Igor' grown in vitro caused a reduction of root growth and development. The effect was even more pronounced at the end of cultivation after 35 days.

The fresh and dry weights of roots of the infected plants were lower a maximum of 65% compared to healthy ones (Table 1).

Table 1. Root fresh and dry weight (mg) of healthy and PVY^{NTN} -infected potato cv. 'Igor' after 18 and 35 days of growth.

	18 days		35 days	
	Healthy	Infected	Healthy	Infected
Fresh weight	29.72 ± 7.96	14.42 ± 3.01	125.00 ± 14.00	42.93 ± 4.29 ***
Dry weight	1.72 ± 0.41	0.79 ± 0.10	7.42 ± 0.88	2.89 ± 0.27 ***

Values are the means of replicates ± SEM (N=10); (***) P≤ 0.001.

After 35 days of cultivation the root system of infected plants was lessened mainly due to the 35% lower number of secondary roots, while the number of primary roots did not alter. Primary and secondary roots of the infected plants were significantly, for 15% narrower then the roots of healthy controls (Table 2).

Table 2. Primary and secondary root morphology of healthy and PVY^{NTN} -infected potato cv. 'Igor' after 18 and 35 days of growth.

	18 days		35 days	
	Healthy	Infected	Healthy	Infected
Primary roots				
Number	5 ± 0	5 ± 1 ns	4 ± 0	4 ± 0 ns
Length (mm)	25.65 ± 1.85	23.14 ± 1.81 ns	42.20 ± 3.89	42.26 ± 4.20 ns
Width (mm)	0.27 ± 0.01	0.25 ± 0.01 ns	0.32 ± 0.02	0.27 ± 0.01 *
Secondary roots				
Number	24 ± 1	15 ± 3 *	37 ± 6	23 ± 3 *
Length (mm)	5.2 ± 0.64	5.24 ± 0.50 ns	13.86 ± 1.09	17.74 ± 2.85 ns
Width (mm)	0.21 ± 0.01	0.22 ± 0.01 ns	0.27 ± 0.01	0.23 ± 0.01 *

Values are the means of replicates ± SEM (N=10); (ns) – non significant, (*) P≤ 0.05.

Root branching was estimated by different characteristics. The number of secondary roots (developed roots and primordia) on a single primary root was 6 after 18 days and 9 after 35 days in healthy controls. In infected plants there was a reduction of the secondary roots (3 and 7 after 18 and 35 days, respectively) mainly due to the significantly lower number of root primordia compared to the control (3 and 2**, P<0.01 after 18 days; 4 and 2*, P<0.05 after 35 days). The area of secondary roots expressed as a length of the primary root where the secondary roots grow was smaller by 20% in infected plants than in healthy ones (17.7 and 14.4 after 18 days; 33.7 and 26.7 after 35 days). Although the differences were not statistically significant, the distance between successive secondary roots was longer by 40% in infected potato plants after 35 days of cultivation.

The smaller size of mature root system in plants infected with the virus reflected in the root apical meristems. Root tips of infected plants were narrower and shorter up to 10% in comparison with healthy potato plants. Total meristem width was smaller because of a lower cell file number (Table 3). The central stele

of the meristem was more affected than the cortex. Cells in the stele had an 8% smaller cell volume compared to the stele of healthy plants. This, together with the 20% reduced file number in the stele, resulted in a total 20% reduction of the stele width. The cortex width and cell file number did not diminish so drastically and a tendency to a larger cell volume was detected (data not shown). As cells in the stele differentiate into vascular elements (SCHIEFELBEIN & al. 1997), their smaller number could result in less developed conducting part of narrower mature roots. Because of the reduced capacity to conduct water and nutrients, the overall plant growth, development and other physiological processes can be affected.

Table 3. Width (μm) and cell file number in root apical meristem of healthy and PVY^{NTN} -infected potato cv. 'Igor' after 18 and 35 days of growth.

	18 days		35 days	
	Healthy	Infected	Healthy	Infected
Width (μm)				
Stele	82.5 ± 2.5	71.6 ± 3.8 **	86.3 ± 3.1	68.0 ± 2.8 ***
Cortex	41.2 ± 2.0	41.8 ± 1.4 ns	56.2 ± 2.0	48.7 ± 2.8 *
Total[a]	164.3 ± 5.1	154.7 ± 5.1 ns	198.7 ± 4.8	164.5 ± 7.9 **
Cell file number				
Stele	12 ± 0	10 ± 1 *	12 ± 0	10 ± 0 ***
Cortex	4 ± 0	4 ± 0 ns	5 ± 0	4 ± 0 ***
Total	20 ± 0	18 ± 1 *	22 ± 0	18 ± 1 ***

Values are the means of replicates ± SEM (N=10); (ns) – non significant, (*) P≤ 0.05, (**) P≤ 0.01, (***) P≤ 0.001.

[a]Total represents stele (width or cell file number) together with two times cortex (width or cell file number).

The reduced root systems and apical root meristems of infected plants can be due to more than one parameter that changed during the virus infection (FRASER 1992). A very important part of development regulation are plant growth regulators. Among others, cytokinins regulate root formation and further development (HINCHEE & ROST 1986). In infected potato plants, the cytokinin concentration changes and therefore also its ratio to other regulators, resulting in altered plant metabolism (DERMASTIA & al. 1995, DERMASTIA & RAVNIKAR 1996). Plants with an increased cytokinin production are smaller and their root growth is also reduced (BRZOBOHATY & al. 1994). The cytokinin concentration in PVY^{NTN}-infected potato cv. 'Igor' is higher than in healthy plants (DERMASTIA & RAVNIKAR 1996) and could be one of the reasons for the observed root reduction. Another plant growth regulator, jasmonic acid that acts as a signal molecule in plant defence, is also affected by PVY^{NTN}. The infection causes its accumulation in roots (PETROVIČ & al. 1997). Its high concentration in roots could be supraoptimal and thus may cause root growth inhibition (RAVNIKAR & al. 1992).

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