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Relation between the Endogenous Jasmonic Acid and Decreased Potato Virus Y^{NTN} Concentration in Potato Treated with Jasmonic Acid

By

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Summary

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Systemic infection of potato (*Solanum tuberosum* L.) cv. Igor grown in vitro with potato virus Y^{NTN} (PVY^{NTN}) resulted in the accumulation of endogenous jasmonic acid (JA) in plant roots, and in decreased plant fresh and dry weight, especially obvious in plant roots (PETROVIČ & al. 1997). The addition of exogenous 0.1 μM JA to the growth medium reduced virus content (PETROVIČ & RAVNIKAR 1995). In order to find the experimental explanation for lower PVY^{NTN}-concentration in plants treated with 0.1 μM JA, we compared biochemical data on endogenous jasmonic acid with morphological characteristics of JA-treated plants. JA-treatment did not influence the virus-induced changes in distribution endogenous JA between shoots and roots, but it reduced the endogenous JA levels, most visible in infected roots. Simultaneously, JA treatment reduced virus-induced decrease in plant fresh and dry weight, most visible in plant shoots. Present data suggest that the virus may influence the sensitivity of potato plants to exogenous JA treatment.

Introduction

Virulent and aggressive potato virus Y^{NTN} severely affects susceptible potato cultivar Igor. However, the symptoms are partially suppressed when virus infected plants are grown in tissue culture, which is related to increased cytokinin biosynthesis (DERMASTIA & RAVNIKAR 1996) and to the accumulation of endogenous JA in plant roots (PETROVIČ & al. 1997). The PVY^{NTN} content of JA-treated potato plantlets, using a range of JA concentrations added to the growth medium, did not show a clear trend with increasing exogenous JA concentration,

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but was always invariably and significantly lower at 0.1 μM JA compared to the control without JA in the medium (PETROVIČ & RAVNIKAR 1995). We tried to test the hypothesis of a possible relation between the endogenous jasmonic acid and decreased virus concentration in potato plants treated with 0.1 μM JA.

Material and Methods

Plant material

Potato plants (*Solanum tuberosum* L. cv. Igor) grew 6 weeks in stem node tissue culture, on MURASHIGE & SKOOG 1962 - medium, supplemented or not with 0.1μM of JA, (Apex Organics, UK), pH 5.7-5.8. Cultures were kept at 20±2 °C, with a photoperiod of 16 h of light at 50 μM m^{-2} s^{-1} (Osram LI8W 20 lamps).

Isolation and quantification of JA

Plant material and internal standards were prepared as previously described (PETROVIČ & al. 1997). Extracts were separated on nonactivated mini columns Lichlorut RP-18 (500 mg, Merck, Germany) with solvents A (methanol-0.2% acetic acid) and B(water), as follows: 1. application of the sample in 100 μl of pure methanol. 2. Stepwise elution with 2 ml of each: 20, 30, 40, 50 and 60% of A in B. 3. Fractions with 50 and 60% of A in B contained JA; they were combined, evaporated and dissolved in 100 μl of 60% methanol. Samples were further purified with HPLC (Knauer, Germany): 100 ml of a sample was injected, using a reverse phase HPLC C18 Eurochrome column of dimensions 4 times 250 mm (Knauer, Germany), and as a solvent 60% methanol in 0.1% H_3PO_4, with the flow of 1ml min^{-1}. Fractions containing JA were methylated and analysed as previously described (PETROVIČ & al. 1997).

Results and Discussion

To find the explanation for lower PVY^{NTN}-concentration in plants treated with 0.1 μM JA (PETROVIČ & RAVNIKAR 1995), we compared endogenous jasmonic acid levels with morphological characteristics of JA-treated plants.

Fresh weight of virus infected plants treated with JA significantly decreased by 31%, especially in plant roots (57%), compared to healthy plants grown on the same medium (Table 1). Virus infection alone caused similar but stronger decrease in fresh weight (by 44%), of both shoots and roots of infected plants (by 40% and 54%, respectively) (Table 1). However, JA treatment reduced the inhibiting effect of viral infection on fresh weight of shoots. Consequently, the root to shoot ratio of JA-treated plants was reduced from 0.18 in healthy plants to 0.11 in infected ones, as opposed to JA untreated controls, where root to shoot ratio was reduced only from 0.2 in healthy to 0.16 in infected plants (Table 1).

The endogenous concentration of JA in JA-treated infected plants, compared to the healthy JA-treated control, increased significantly in the roots of infected JA-treated plants in both experiments. Increased concentration of endogenous JA was detected also in the roots of infected untreated potato plants (Table 2), as already shown (PETROVIČ & al. 1997). CREELMAN & MULLET 1995 demonstrated a 5-time-increase in JA concentration in soybean leaves stressed by allowing them to lose 15% of their fresh weight. Notably, the exogenous JA did not affect the virus-induced changes in distribution of endogenous JA between shoots and roots. Although there is no significant difference in endogenous absolute JA concentrations between JA-treated plants and control plants, a trend toward their decrease is clearly visible in JA-treated plants, especially in infected roots (Table 2). These can be a consequence of JA transformation to its metabolites and/or JA induced changes in membrane fluidity (VILHAR & al. 1991) which can influence the synthesis of jasmonates.

Table 1. Fresh weight (FW, expressed in g) of healthy and PVY^{NTN}-infected plants of JA-untreated control and plants treated with 0.1 µM JA. decrease : indicates a decrease in fresh weight due to the virus infection, expressed in percentage as a difference toward a 100% of fw of healthy plants in control and JA treatment, respectively. The figures are means of three different experiments, each of 60 plants. * indicates a significant difference against the healthy control and JA treated, respectively, at $p<0.001$.

	CONTROL			0.1 µM JA IN MEDIUM		
MATERIAL	HEALTHY	INFECTED		HEALTHY	INFECTED	
	FW (g)	FW (g)	decrease	FW (g)	FW (g)	decrease
Shoots	0.475	0.275*	40%	0.596	0.436*	27%
Roots	0.096	0.045*	54%	0.105	0.046*	57%
Whole plants	0.571	0.320*	44%	0.701	0.482*	31%
Root to shoot ratio	0.20	0.16		0.18	0.11	

Table 2. Absolute concentrations of endogenous JA, expressed in µg/g of dry weight (DW), in healthy and PVY^{NTN}-infected JA-untreated plants (control) and plants treated with 0.1 µM JA. % of control: indicates the percentage of endogenous JA content compared to the untreated controls. Figures represent 2 different experiments, each with 60 plants.

	CONTROL		0.1 µM JA IN MEDIUM			
MATERIAL	HEALTHY	INFECTED	HEALTHY		INFECTED	
	µg/g DW	µg/g DW	µg/g DW	% of control	µg/g DW	% of control
Experiment 1						
Shoots	4.76	2.01	4.58	96	1.60	80
Roots	3.35	23.83	2.90	86	16.80	70
Whole plants	4.56	4.42	4.23	95	3.38	77
Experiment 2						
Shoots	10.54	4.9	10.70	101	1.93	39
Roots	4.48	49.64	3.55	79	31.90	64
Whole plants	9.22	11.77	9.3	101	5.46	46

The multiplication of the virus was lower in JA-treated plants (70 µg/g of FW) in comparison to control plants (200 µg/g of FW). The reduced rate of PVY^{NTN}-multiplication in potato grown in the presence of 0.1 µM JA correlates with the observed accumulation of endogenous JA in the roots of the infected plants that was lower than that in infected plants grown on the medium without JA (Table 2). At the same time, JA-treatment of infected plants caused a significant increase in fresh weight of shoots, compared to infected plants growing in the absence of JA (Table 1). Consequently, this resulted in a further decrease of root to shoot fresh weight ratio of infected plants, from 0.16 in untreated control to 0.11 in

JA-treated plants. These data suggest that the presence of the virus changes the sensitivity of potato plants to exogenous JA treatment and further support the hypothesis that the induced changes of endogenous JA levels are more important for the induction of different metabolic responses than the absolute concentrations of JA in plants (HARMS & al. 1995, PETROVIČ & al. 1997). The exact reason why only application of 0.1 μM JA and not other JA-concentrations provoked the effect of decreasing virus concentration remains to be established. The responsiveness to JA should be studied in combination with other effects or pathways to further specialise the conditions under which genes respond to this plant growth regulator (CREELMAN & MULLET 1995, PETROVIČ & al. 1997). Although the molecular basis of JA/cytokinin interaction is not known yet, previous results support the idea that JA-induced responses are associated with cytokinin metabolism (DERMASTIA & al. 1994, SANO & al. 1996), which results in changed plant growth and development.

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