BIOLOGIA PLANTARUM 40 (2): 317-320, 1997/98

BRIEF COMMUNICATION

Tobacco polyamines as affected by stresses induced by different pathogens

A. EDREVA

D. Kostov Institute of Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

Abstract

A decrease of the polyamine content of tobacco leaves inoculated with fungal, bacterial and viral pathogens differing in symptom severity (mosaic, chlorosis, necrosis) was observed. The extent of the decrease was not related to the type of pathogens but to the severity of damage symptoms; hence, the polyamine decline may be regarded as a common response of tobacco to damage produced by pathogens.

Additional key words: Alternaria tenuis, Erysiphe cichoracearum, Nicotiana tabacum, Peronospora tabacina, Pseudomonas tabaci, thin layer chromatography, tobacco mosaic virus.

Regulatory functions of polyamines (PA) in prokaryotic and eukaryotic cells have been recognized; specific properties of PA molecules (short chained polyvalent organic cations carrying two terminal positive charges) are suggested to underlie these functions (Galston and Kaur-Sawhney 1990). In plants, research on the role of PA in the regulation of growth and development and in stress phenomena is rapidly increasing during the last decade (Bagni 1989). Abundant information is available on the involvement of PA in plant responses to various abiotic stresses (Flores 1991), whereas PA in pathogenic stresses is a neglected aspect, scanty data are reported concerning mainly the effect of some biotrophic fungi on PA in cereals (Greenland and Lewis 1984, Walters and Wylie 1986, Machatschke *et al.* 1990).

The aim of the present work was to follow the changes of PA in tobacco subjected to stress caused by fungi, bacteria and viruses, producing varying damage symptoms on leaves.

Received 21 April 1997, *accepted* 29 July 1997.

Abbreviations: f. m. - fresh mass; PA - polyamines; TMV - tobacco mosaic virus.

Acknowledgments. The financial support of the National Science Fund (Contract No K - 409/ 1994) is gratefully acknowledged.

Fax: (+359) 2 757087; e-mail: ttsonev@obzor.bio21.acad.bg

The experimental design involved tobacco plants, *Nicotiana tabacum* L. cv. Nevrokop 5 inoculated with the following pathogens: *a*) fungi - *Peronospora tabacina* A. (blue mold), an obligative endoparasite, *Erysiphe cichoracearum* D.C. (powdery mildew), an obligative ectoparasite, *Alternaria tenuis* N., a facultative saprophyte; *b*) bacteria - *Pseudomonas tabaci* W. *et* F. (wild fire); and *c*) virus - tobacco mosaic virus (TMV). Some of these pathogens brought about severe damage symptoms on leaf tissue which later become necrotic (*P. tabacina*, *A. tenuis*, *P. tabaci*); other caused milder damage symptoms - mosaic, chlorotic spots (TMV, *E. cichoracearum*).

Tobacco plants were grown in sterilized soil in greenhouse (20 - 25 °C average day temperature, 60 - 80 % relative humidity, and 360 μmol m^{-2} s^{-1} photon flux density). Uniformly developed plants (about 3-months-old) were transferred to a growth chamber (25 ± 1 °C, 60 - 80 % relative humidity and 60 μmol m^{-2} s^{-1} photon flux density, 12-h photoperiod). A week later plants were used for inoculation.

Two post-inoculation stages were followed up: initial (first visible symptoms appearance) and late (4 d after the appearance of symptoms); stages corresponded to aggravation of tissue damage. Inoculated leaves from middle stalk position were analyzed. Leaves of healthy not inoculated plants were used as controls.

Commonly reported methods for inoculation were applied (Király *et al.* 1970). Conidial suspensions of fungal pathogens were sprayed on leaves: conidial concentrations were 5×10^4, 5×10^5 and 4×10^5 conidia per cm^3 for *P. tabacina*, *E. cichoracearum* and *A. tenuis*, respectively. The cell suspension of the bacterium *P. tabaci* contained 10^8 cells per cm^3. Virus inoculum was prepared from virus-infected leaves with 0.01M sodium phosphate buffer, pH 7.0. The ratio of leaves to buffer was 1/5 (m/v).

The method of Delétang (1977) for determination of PA was applied; it is based on a combination of ion exchange column chromatography, thin layer chromatography and colorimetry. Fresh leaf material was extracted with 80 % methanol. Extracts were depigmented with petroleum ether and loaded on a column packed with cation exchange resin, *Amberlite CG50* (H^+) (100 - 200 mesh). The PA retained on the resin were eluted with 3 M acetic acid, and eluates evaporated under reduced pressure to eliminate acetic acid. The resulting water residue contained free PA. To separate individual PA components, an ascending one-dimensional thin layer chromatography on cellulose plates, 0.1 mm (*Merck*) was applied. Chromatograms were developed in the system ethylene glycol monomethyl ether : propionic acid : water [70:15:15 (v/v)] and sprayed with a ninhydrine solution to detect PA (violet colored products). Spots corresponding to individual PA were identified using reference substances, eluted from chromatograms and quantified by colorimetry at 575 nm. Experiments were repeated three times, with three replicates per experiment, using leaves of 10 plants per replicate. Significance was given by the Student's *t*-test at $P < 5$ %.

In leaves of healthy plants (controls) three PA compounds were detected: putrescine, spermidine and cadaverine (traces). Presence of spermine was not established. Putrescine was the predominant PA. This confirms data of Madsen *et al.*

(1985), pointing that putrescine and spermidine are the major PA in leaves of different tobacco genotypes. It is noteworthy that in leaves inoculated with the pathogens under study the same three PA compounds were found, *i.e.* no qualitative shifts in the PA pattern occurred.

Table 1. Content of free putrescine and spermidine [nmol g^{-1}(f. m.) and % of the corresponding controls] in tobacco leaves inoculated with fungal, bacterial and viral pathogens. C - controls - leaves of healthy plants; I - inoculated leaves. Initial stage - appearance of the first visible symptoms; late stage - 4 d after the appearance of symptoms. Data are means from three experiments each with three replicates. Standard deviations are less than 10 % of the means. Values in the same column followed by different letters are significantly different at $P < 5$ %. Values for inoculated plants are significantly different from that for corresponding controls.

Polyamines	Stage	Fungi								
		P. tabacina			*E. cichoracearum*			*A. tenuis*		
		C	I	[%]	C	I	[%]	C	I	[%]
Putrescine	initial	205 a	146 b	71	195 a	166 b	85	204 a	150 b	74
	late	201 a	105 c	52	192 a	152 c	79	208 a	121 c	58
Spermidine	initial	69 a	51 b	74	72 a	63 b	88	70 a	53 b	76
	late	71 a	40 c	56	69 a	58 b	84	68 a	43 c	63

Polyamines	Stage	Bacteria			Virus		
		P. tabaci			TMV		
		C	I	[%]	C	I	[%]
Putrescine	initial	202 a	137 b	68	203 a	180 b	89
	late	198 a	83 c	42	210 a	170 c	81
Spermidine	initial	74 a	51 b	69	81 a	69 b	85
	late	76 a	39 c	51	79 a	63 b	80

All pathogenic agents brought about a decrease of both putrescine and spermidine (Table 1). The effect of each pathogen was evident at the initial stage and became more prominent at the late stage, *i.e.* when damage was more pronounced. The extent of PA decrease was not dependent on the nature of pathogens - fungal, bacterial or viral - but on the degree of tissue damage: pathogens producing severe damage (*P. tabacina, P. tabaci, A. tenuis*) induced more important PA decline that pathogens producing mild damage symptoms, such as chlorosis and mosaic (*E. cichoracearum*, TMV).

These data suggested that reduction of PA is a non-specific response of plants to tissue damage; it may be caused by different pathogenic agents. The finding is compatible with the view that a high PA titer is characteristic for young, actively metabolizing tissues, whereas low PA levels are typical for tissues of slowed metabolic functions, such as senescent leaves (Galston and Kaur-Sawhney 1990).

Our results are in accordance with reports about other plants, inoculated with damage-producing fungi, such as *Rhizopus stolonifer* infected tomato (Bakanashvili *et al.* 1987) and *Fusarium solani* infected peas (Teasdale and Hadwiger 1977), where

a decline of PA was also established. A similar response was observed in leaves of tomato and *Gynura aurantiaca* plants following damage induced by citrus exocortis viroid inoculation (Bellés *et al.* 1991). The expression of damage relates to a decrease of PA. The latter may be accounted for by shortage of key metabolites for PA biosynthesis at the expenses of enhanced ethylene biosynthesis; it is known that PA and ethylene share common biosynthetic pathway and compete for common precursor, S-adenosyl methionine (Kushad and Dumbroff 1991). This assumption is justified by the fact that increased ethylene evolution is a characteristic feature of stress and senescence in plants (Yang and Hoffman 1984). Moreover, a view has long been developed (Udvardy and Farcas 1966) that natural senescence is accelerated following pathogen invasion.

References

Bagni, N.: Polyamines in plant growth and development. - In: Bachrach, U., Heimer, Y.M. (ed.): The Physiology of Polyamines. Vol. II. Pp. 108-120. CRC Press, Boca Raton 1989.

Bakanashvili, M., Barkai-Golan, R., Kopeliovitch, E., Apelbaum, A.: Polyamine biosynthesis in *Rhizopus*-infected tomato fruits: possible interaction with ethylene. - Physiol. mol. Plant Pathol. **31**: 41-50, 1987.

Bellés, J.M., Carbonell, J., Conejero, V.: Polyamines in plants infected by citrus exocortis viroid or treated with silver ions and etephon. - Plant Physiol. **96**: 1053-1059, 1991.

Delétang, J.: La production des alcaloïdes et ses relations avec la rhizogénèse chez *Nicotiana tabacum*. - Ann. Tabac SEITA (Section 2) **14**: 5-105, 1977.

Flores, H.E.: Changes in polyamine metabolism in response to abiotic stress. - In: Slocum, R.D., Flores, H.E. (ed.): Biochemistry and Physiology of Polyamines in Plants. Pp. 213-228. CRC Press, Boca Raton 1991.

Galston, A.W., Kaur-Sawhney, R.: Polyamines in plant physiology. - Plant Physiol. **94**: 406-410, 1990.

Greenland, A.J., Lewis, D.H.: Amines in barley leaves infected by brown rust and their possible relevance to formation of "green islands". - New Phytol. **96**: 283-291, 1984.

Király, Z., Klement, Z., Solymosy, F., Vörös, J.: Methods in Plant Pathology. - Akadémiai Kiadó, Budapest 1970.

Kushad, M.M., Dumbroff, E.B.: Metabolic and physiological relationships between the polyamine and ethylene biosynthetic pathways. - In: Slocum, R.D., Flores, H.E. (ed.): Biochemistry and Physiology of Polyamines in Plants. Pp. 77-92. CRC Press, Boca Raton 1991.

Machatschke, S., Kamrowski, C., Moerschbacher, B.M., Reisener, H.J.: Polyamines levels in stem rust infected wheat leaves and effects of alpha-difluoromethylornithine on fungal infection. - Physiol. mol. Plant Pathol. **36**: 451-459, 1990.

Madsen, J.P., Bush, L.P., Gay, S.L.: Effect of curing on polyamine content of leaves of *Nicotiana tabacum* L. genotypes with different alkaloid levels. - J. agr. Food Chem. **33**: 1182-1185, 1985.

Teasdale, J.R., Hadwiger, L.A.: Effect of pisatin inducing fungi in pea polyamines. - Phytochemistry **16**: 681-682, 1977.

Udvardy, J., Farcas, G.: Drifts in enzyme levels in detached senescing barley leaves and their relation to enzyme changes. - Acta phytopathol. **1**: 19-27, 1966.

Walters, D.R., Wylie, M.A.: Polyamines in discrete regions of barley leaves infected with the powdery mildew fungus, *Erysiphe graminis*. - Physiol. Plant. **67**: 630-633, 1986.

Yang, S.F., Hoffman, N.E.: Ethylene biosynthesis and its regulation in higher plants. - Annu. Rev. Plant Physiol. **35**: 155-189, 1984.