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Cell Proliferation on Potato-Parenchyma induced by the Excitatory Substance of the *Solanaceae*

By

Karl UMRATH † and Zdenka BENEŠ *)

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Summary

UMRATH K. † & BENEŠ Z. 1987. Cell proliferation on potato-parenchyma induced by the excitatory substance of the *Solanaceae.* – Phyton (Austria) 26 (2): 149–155. – English with German summary.

A method is developed permitting to test drops of plant extracts or of solutions of liberating substances on dry disks of potato tubers.

Experiments give evidence that cell proliferation is caused by an aromatic acid with a carboxylic acid group in trans position and with hydroxyl groups on the side chain and on the ring. This acid is by definition the excitatory substance of the *Solanaceae,* as the excitatory substance is defined as the substance inducing cell proliferations and leaf movements in sensitive plants.

Suberinization occurs with many of the liberating substances that induce cell proliferation, but not with plant extracts. A brown superficial layer is caused probably by oxidized phenols.

Zusammenfassung

UMRATH K. † & BENEŠ Z. 1987. Zellproliferation am Kartoffelparenchym, ausgelöst durch die Erregungssubstanz der Solanaceen. – Phyton (Austria) 26 (2): 149–155. – Englisch mit deutscher Zusammenfassung.

Es wird eine Methode entwickelt, die es erlaubt, Tropfen von Pflanzenextrakten oder von Lösungen freisetzender Substanzen an trockenen Scheiben von Kartoffelknollen zu untersuchen.

Nach unseren Versuchen werden Zellproliferationen durch eine aromatische Säure mit der Carboxylgruppe in trans-Stellung und mit Hydoxylgruppen an der

*) Anschrift für Anforderung von Sonderdrucken: Prof. Dr. O. HÄRTEL, Institut für Pflanzenphysiologie, Karl-Franzens-Universität Graz, Schubertstraße 51, A-8010 Graz.

Seitenkette und am Ring bedingt. Diese Säure ist definitonsgemäß die Erregungssubstanz der Solanaceen, da die Erregungssubstanz als die Substanz definiert ist, welche Zellproliferationen und an sensitiven Pflanzen Blattbewegungen auslöst.

Korkbildung tritt bei vielen freisetzenden Substanzen, die Zellproliferationen bedingen, ein, aber nicht bei Pflanzenextrakten. Eine braune oberflächliche Schichte wird wahrscheinlich durch oxidierte Phenole bedingt.

Introduction

Cell divisions in the parenchyma of potato tubers were often investigated on sections of wounded tubers. HABERLANDT 1913 was the first to make such an investigation and in 1921 he ascribed the initiation of cell divisions to wound-hormones. UMRATH 1930 proposed an excitatory substance of different chemical character for every family or subfamily inducing cell divisions and leaf movements of sensitive plants. Later ROSENSTOCK 1963 brought the cell divisions in connexion with the water content of the tissue. ENGLISH, BONNER & HAAGEN-SMIT 1939 ascribed the cell divisions to the traumatic acid. In the paper presented here cell proliferations on the parenchyma of potato tubers were investigated by using plant extracts filtrated through different filters as well as some liberating substances. UMRATH & THALER 1980 pointed out the chance for liberation in connection with the chemical structure on the one hand of the substance to be liberated and on the other hand of the liberating substance.

Methods

Slices of tubers and leaves of *Solanum tuberosum* were extracted with distilled water by short boiling. After half an hour to three hours the plant remainders were removed with as little water as possible. The extracts were tested in this form as well as after filtration either through a column of polyamide (Woelm) to retain phenols and possibly other aromatic compounds, or after filtration through a column of anion exchange resin (Amberlite CG 400 II), to retain acids. The columns were 0,6 cm in diameter and 5 to 7 cm long.

Tubers of potatoes, *Solanum tuberosum* L., bought in a shop, were cut into disks of about 5 mm thickness. The liquid out of the parenchyma was wiped off with filter paper. Then every disk was put in a Petri-dish of 9 cm diameter on dry filter paper. After 12 hours the disks were so far dry that drops of water put on them did not flow away. Now from every solution to be tested and from distilled water, all at pH 6,8 by addition of sodium bicarbonate, two big drops were put on one of the disks. The drops had vanished on the second or on the third day. The results were examined on the third day. The spots where water or an inactive solution had been set up were not recognizable.

Recognizable spots including the surrounding untreated parts of the surface were cut diametrically using a razor blade. The slices were ex-

amined microscopically with 50 to 250 fold magnification. Cell proliferations, elongations, and discolouring of the spots were recorded. Treatment with Sudan III served for detection of suberinization.

The experiments has been performed at room temperature (20–22° C).

In the text indolyl-3-acetic acid is abbreviated as IAA, 5-hydroxyindolyl-3-acetic acid as 5-OH-IAA.

Results and their discussion

1. Extracts of *Solanum*

Slices of potato tubers were extracted in the fivefold amount of water. This extract caused abundant cell proliferations on tomato parenchyma, but no suberinization. The effectiveness of the extract was reduced considerably by filtration through polyamide, retaining phenols, and still more by filtration through anion exchange resin, retaining acids.

Leaves of *Solanum tuberosum* were extracted in the tenfold amount of water (g : g). The extract had a good effect on cell proliferation, but none on suberinization. The effect was greatly diminished by filtration through anion exchange resin, retaining acids, and still more by filtration through polyamide, retaining phenols.

2. Auxins

UMRATH & THALER 1981 showed 5-OH-IAA acetic acid to be an auxin. On etiolated *Lupinus* hypocotyls 5-OH-IAA induces elongation if applied unilaterally in lanoline-water paste in 0,005 to 450 mM concentrations. IAA has this effect only in 0,005 to 1 mM concentration. If the concentration in the paste is 15 mM or higher IAA reduces elongation appearently by liberating the excitatory substance of the *Fabaceae*. On *Mimosa pudica* 1 mM IAA induces leaf-movements, whereas 5-OH-IAA does it scarcely in 10 mM concentration (UMRATH & THALER 1981).

We used often 5-OH-IAA because of its better solubility in aqueous media. One gets easely 6 mM solutions, whereas of IAA one gets only 3 mM solutions.

25 mM phenylvaleric acid caused a marginal elevation with a central depression, both with a brown superficial layer, without suberinization. Addition of 3 mM IAA to the 25 mM phenylvaleric acid (PVA) diminished the marginal elevation and deepened the central depression much under the untreated surrounding (Table 1). There was a brown superficial layer and suberinization. Addition of 3 mM 5-Oh-IAA to the 25 mM PVA minimized the marginal elevation, deepened the central depression and caused a brown superficial layer and suberinization. 6 mM 5-OH-IAA in the combination supressed the marginal elevation completely, deepened the depression and caused a still darker superficial layer.

For an understanding of the depressions mentioned just one must keep in mind that on a cut potato surface cell proliferations occur. Our method of testing does not exclude these cell proliferations, but it facilitates greatly to recognize what a drop of solution causes additional cell proliferation or suppresses cell proliferation with respect to the surrounding.

Table 1

Effects of 25 mM phenylvaleric acid (PVA) and added IAA and 5-OH-IAA respectively on potato discs

Substance	Marginal elevation	Central depression	Brown layer	Suberin-ization
25 mM PVA	++	+	+	–
25 mM PVA + 3 mM IAA	+	++	+	+
25 mM PVA + 3 mM 5-OH-IAA	(+)	+++	+	+
25 mM PVA + 6 mM 5-OH-IAA	–	++++	+++	+

3 mM IAA, 5-OH-IAA or indolyl-3-propionic acid did not cause any reaction. Possibly a poor liberation of the excitatory substance was conteracted by their peculiar depressive action. 3 mM indolyl-3-butyric acid caused a narrow brownish layer, scarcely elevated, but suberinized. This can be interpreted as a prevailing liberation of the excitatory substance.

It is a peculiarity of the excitatory substance of the *Solanaceae* that it is best liberated by indolyl-3-butyric acid. BIELENBERG & al. 1984 showed that in the *Mimosaceae* and in the *Fabaceae* the corresponding excitatory substance is liberated best by IAA, less by indolyl-3-propionic acid and least by indolyl-3-butyric acid. We found this same sequence using tendrils of *Vitis vinifera (Vitaceae)* and of *Cucumis melo (Cucurbitaceae)*.

6 mM 5-OH-IAA caused a dark brown layer, scarcely elevated, with suberinization. With 3 mM the brown layer was not as dark as with 6 mM.

The results with extracts of *Solanum* in the first section indicate that the excitatory substance of the *Solanaceae* is an aromatic acid. The greater ability of indolyl-3-butyric acid to liberate this substance as compared with indolyl-3-propionic- or -acetic acid indicates that the carboxylic acid group of the excitatory substance is situated on a long side chain.

3. Liberating substances

We used 29 different liberating substances. Only a few liberating substances induced suberinization. Trans-cinnamic acid was most effective in this respect and trans-3-hydroxycinnamic acid (meta coumaric acid) was also effective, but substances with hydroxy- or methoxy-groups in position 2, 4 or 3 and 4, as caffeic acid, ferulic acid and hydrocaffeic acid were ineffective. Valeric acid and phenylvaleric acid induced suberinization.

25 mM phenylvaleric acid induced suberinization also when 3 mM IAA acid or 3 mM 5-OH-IAA was added, but suberinization did not occure if 6 mM 5-OH-IAA was added. All these findings suggest that hydroxy-groups in certain positions on ring of 6 C-atoms conteract the appearance of suberinization. Two further substances inducing suberinization were traumatic acid and lauric acid.

A brown superficial layer was caused by the acids from valeric acid to lauric acid and from phenylacetic acid to phenylvaleric acid. In the latter group the efficacy was enhenced by hydroxy-groups in position 2 or 3 and mostly by those in position 3, 4, as in caffeic acid and in hydrocaffeic acid.

Cell proliferations were caused by traumatic acid and lauric acid. The acids from valeric acid (= pentanoic acid) to capric acid (= decanoic acid) caused depressions on the potato parenchyma. The acids from phenylacetic acid to phenylvaleric acid caused all aboundant cell proliferations. A hydroxy-group on the side chain in α-position diminished the effect on cell proliferation. This indicates a hydroxy-group on the side chain of the excitatory substance. Hydroxyl-groups on the phenol ring of liberating substances in position 2, 3 or 4 diminish their effects. Therefore the excitatory substance of the *Solanaceae* has probably one or more hydroxyl groups on its phenol ring.

The following results with liberating substances indicate, that carboxylic acid group of the excitatory substance of the *Solanaceae* is in trans position. Traumatic acid (= trans-2-dodecenedioic) acid is much more effective than dodecanedioic acid. Transcinnamic acid (= trans-phenylpropionic acid) is much more effective than phenylpropionic acid. Crotonic acid (= trans-2-butenoic acid) is better effective than butyric acid.

It remains to explain the greater cell proliferating effect of cis-4-hydroxycinnamic acid as compared with trans-4-hydroxycinnamic acid. Cis-4-hydroxycinnamic acid is best suitable to liberate xanthoxin (UMRATH 1984 a).

BRUINSMA, FRANSSON & KNEGT 1980 found, xanthoxin is responsible for phototropism of *Helianthus annuus* and may according to UMRATH 1984 a be regarded as the excitatory substance of the *Asteraceae*. Therefore we have extracted leaves of *Helianthus annuus* in the tenfold amount of water, to test the extract on potato parenchyma. The extract was green and muddy and a drop of it on a potato disk covered the place with a compact layer. Therefore firstly we have filtrated the extract through filter paper and got a clear yellow solution. A part of it was filtered through polyamide to retain xanthoxin as a phenolic combination. Overnight both solutions got just recognizably muddy and the solution not filtered through polyamide got green. On potato parenchyma the extract not filtered through polyamide induced a slight elevation with new small cells in the treated area. The extract filtered through polyamide induced scarcely an elevation and no new cells. These results indicate that xanthoxin induces cell proliferations

on potato parenchyma and that such an effect by cis-4-hydroxycinnamic acid may be caused by the liberation of xanthoxin.

Conclusions

The excitatory substance is defined as the substance inducing cell proliferations and leaf movements in sensitive plants (UMRATH 1930). Hence one can conclude from the experiments that the excitatory substance of the *Solanaceae* is an aromatic acid with the carboxylic acid group in trans position with hydroxyl groups on the ring and with a relatively long side chain with at least one hydroxyl group.

UMRATH 1984 b characterized the excitatory substance of 7 plant groups to some extent. He found the excitatory substance of 5 families to be aromatic and if we add that of the *Solanaceae* we have 6 families with an aromatic excitatory substance. Only the two investigated subfamilies of the *Leguminosae* have aliphatic excitatory substances, chemically very similar to one another (UMRATH & SOLTYS 1936). In most families the excitatory substance is an acid, only in the *Asteraceae* it is an aldehyde, xanthoxin, with the aldehyde group in cis-trans-position. A carboxylic acid group in trans position was found hitherto only on the excitatory substance of the *Mimosaceae* and of that of the *Fabaceae,* both subfamilies of the *Leguminosae.* Now we can add that of the *Solanaceae.*

The reduction of cell proliferation on potato disks IAA and by 5-OH-IAA shows that the excitatory substance of the *Solanaceae* is an antagonist of the auxins, as are excitatory substances in general.

We can not say much about suberinization. The excitatory substance in extracts of *Solanum* does not release suberinization. Only some of the liberating substances used by us induce suberinization. This is in accordance with the view of ROSENSTOCK 1963, that suberinization is independent of cell proliferation.

Some substances cause a brown superficial layer on the potato parenchyma. Most effective in this respect, are 3,4-dihydroxyphenyl-compound, as caffeic acid, which give off in solution a coloured substance, probably a phenol. So the brown superficial layer may consist of oxidized phenols. 5-OH-IAA acid has a hydroxy-group on the ring with six C-atoms of the indol sceleton, comparable to the 3-position on a phenol ring. It induces an intensive brown layer without suberinization. Also some aliphatic liberating substances with six to ten C-atoms cause a brown layer with a depression below. Aliphatic acids with six to ten C-atoms and with hydroxy-groups only near the carboxylic acid group are substances that would most likely liberate 5-OH-IAA. This allows to guess the possibility that substances causing a brown superficial layer with a depression below do this by liberating 5-OH-IAA.

If the wound hormones of HABERLANDT 1913, 1921, 1923 are identified with the excitatory substances of the different families our results about cell proliferation on potato parenchyma are in accordance with the view of HABERLANDT 1921, 1923, although his experiments are not conclusive.

References

BRUINSMA J., FRANSSEN J. M. & KNEGT E. 1980. Phototropism as a phenomenon of inhibition. – In SKOOG F. (ed.) Plant growth substances 1979. – Spinger Verlag Berlin–Heidelberg–New York.

ENGLISH, J. Jr., BONNER J. & HAAGEN-SMIT A. J. 1939. The wound hormones of plants. IV. Structure and synthesis of a traumatin. – J. Amer. Chem. Soc. 61: 3434–3437.

HABERLANDT G. 1913. Zur Physiologie der Zellteilung. – Sitzungsber. d. Preuß. Akad. d. Wiss. 16: 318–345.

— 1921. Zur Physiologie der Zellteilung. 6. Mitt. Über Auslösung von Zellteilungen durch Wundhormone. – Sitzungsber. d. Preuß. Akad. d. Wiss. 8: 221–234.

— 1923. Wundhormone als Erreger von Zellteilungen. – Beitr. Allg. Bot. 2: 733–750.

ROSENSTOCK G. 1963. Physiologische und anatomische Studien zum Problem der Wundheilung. I. Untersuchungen über Zusammenhänge zwischen dem Wassergehalt verletzten Kartoffelparenchyms und dessen Vernarbungsreaktionen nebst Bemerkungen zur Theorie des Wundreizes. – Beitr z. Biologie d. Pflanzen 38: 275–319.

UMRATH K. 1930. Über Erregungssubstanzen. – Jb. wiss. Bot. 72: 705–719.

— 1984 a. Liberation of xanthoxin. – Phyton (Austria) 24: 79–85.

— 1984 b. Comparative investigations on the excitatory substances of some plant families according to experiments with tendrils. – Phyton (Austria) 24: 305–315.

— & SOLTYS A. 1936. Über die Erregungssubstanz der Papilionaceen und ihre zellteilungsauslösende Wirkung. – Jb. wiss. Bot. 84: 276–289.

— & THALER I, 1980. Auslösung von Blattbewegungen bei *Mimosa* und von Krümmungen von *Lupinus*-Hypokotylen, gedeutet durch Freisetzung von Erregungssubstanz und Auxin. – Phyton (Austria) 20: 333–348.

— & — 1981. 5-Hydroxyindolyl-3-essigsäure als ein Auxin. – Ber. Deutsch. Bot. Ges. 94: 143–149.