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Wounding-induced cell wall pH shifts in coleoptile segments of various *Poaceae*

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Abstract

Wounding-induced extracellular pH shifts were characterized previously in excised segments of maize (*Zea mays* L.) coleoptiles. In the present study it is demonstrated that similar pH shifts also occur in *Triticum aestivum* L., *Secale cereale* L., *Hordeum vulgare* L., *Avena sativa* L., *Sorghum durra* (Forsk.) Stapf, and *Setaria italica* (L.) Beauv., with characteristic quantitative differences between the species. Indole-acetic acid induces pronounced drops of the medium pH in all species except *Setaria italica*.

Additional key words: auxin, *Avena sativa, Hordeum vulgare, Secale cereale, Setaria italica, Sorghum durra, Triticum aestivum.*

Introduction

In studies on the regulation of growth on the cellular level it is often inevitable to perform experiments on excised organ segments instead of whole plants. In such cases care must be taken to identify effects caused by the excision, and to distinguish them from physiologically relevant phenomena. Without this distinction, the significance of the behaviour observed for growth regulation *in situ* can hardly be soundly evaluated.

In plant cells, the driving force for most plasma membrane transport processes is an electrochemical proton gradient, which is built up by the plasma membrane H^+-ATPase. Thus it is hardly surprising to find transient membrane potential drops, together with transiently increasing extracellular pH values in plant organ segments following wounding by excision. Recently such membrane effects have been reported from wheat root segments (Pakhomova 1996). Transient extracellular pH maxima have been described in segments of dicot stems (Marrè *et al.* 1974, Parrish and Davies 1977, Brummell and Hall 1981) and maize coleoptiles (Jacobs *et al.* 1984, Karcz *et al.* 1995).

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Abbreviations: AE - acid equilibrium; FM - fresh mass; IAA - indole-3-acetic acid; NP - neutral peak.

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In the latter object, we have utilized the transient pH peak to study the conditions necessary for a reliable estimation of cell wall pH changes (Peters and Felle 1991a, 1991b). We had concluded that the transient rise in extracellular pH is an expression of a wounding-induced breakdown of cellular energetics, followed by a slow recovery.

While the phenomonon is fairly well characterized in maize, data from other *Poaceae*, which also are used frequently in physiological studies, are less complete. In the present study comparative data on the spontaneous development of extracellular pH in excised coleoptile segments from seven species are reported and discussed.

Materials and methods

Seeds of *Zea mays* L. cv. Consul or cv. Santos (KWS, Einbeck, Germany) were soaked in tap water for 6 h, sown on moist vermiculite in plastic boxes, and were kept in the dark at 25 °C for 94 to 108 h. Seedlings with coleoptiles 15 to 20 mm long were selected, and coleoptiles were abraded with emery cloth (*Vitex KK14F Nr.392 "rouge", VSM* AG, Hannover, Germany; *cf.* Peters and Felle 1991a). Segments (3 to 13 mm below the apex) were excised, and the primary leaf removed. 24 segments were threaded upright on nylon strings in pairs, and were incubated in 2 cm^3 of medium containing 1 mM KCl, 0.1 mM NaCl, and 0.1 mM $CaCl_2$, adjusted to pH 5.6 with HCl/KOH, which was vigorously aerated [16 to 18 cm^3(air) cm^{-3}(medium) min^{-1}]. A pH electrode (*Ingold U 402 M6-S7*) was inserted, and pH was monitored continuously on a chart recorder. Up to five tests were performed in parallel.

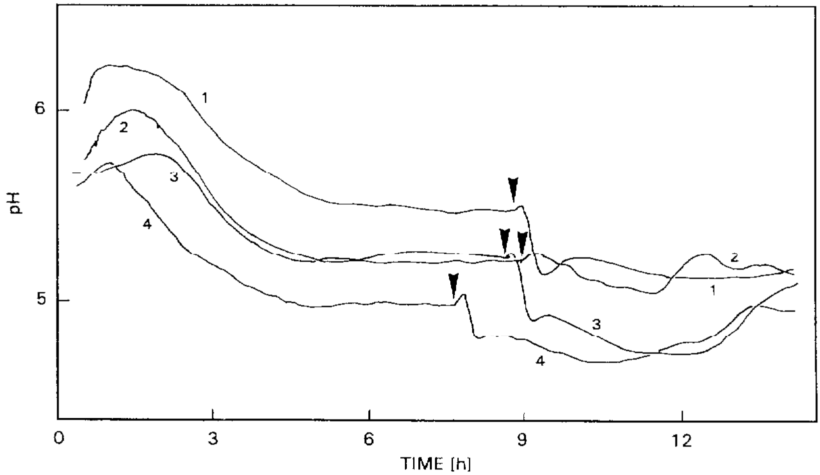

Fig. 1. Changes of the medium pH evoked by abraded coleoptile segments of *Secale cereale* (*track 1*), *Setaria italica* (*track 2*), *Avena sativa* (*track 3*), and *Sorghum durra* (*track 4*). 520 - 570 mg FM of coleoptile segments were incubated in 2 cm^3 of medium (containing 1 mM KCl, 0.1 mM NaCl, and 0.1 mM $CaCl_2$, initial pH 5.6), which was vigorously aerated. Excision took place at time 0. Addition of 10^{-5} M IAA is indicated by *arrows*. The tracks are representative examples of four experiments performed in parallel. Note the transient pH maximum following excision (NP), and the steady state value established after 5 - 6 h (AE).

Similar protocols were followed with *Avena sativa* L. cv. Flämingsvita (*KWS*, Einbeck, Germany), *Triticum aestivum* L., *Secale cereale* L., *Hordeum vulgare* L., *Sorghum durra* (Forsk.) Stapf, and *Setaria italica* (L.) Beauv. Seeds of the latter species were collected in the Botanical Garden of Gießen University. Experimental details were accommodated to seedling size; in all cases fresh mass of segments per volume of bathing medium was about 270 mg cm^{-3}, equaling that one in experiments with maize. Coleoptile segments of *Sorghum* and *Setaria* were only 5 mm long; with these species the preparation was performed under a stereomicroscope.

Results

Following excision maize coleoptile segments induce a characteristic development of the pH in aerated incubation media. This development, which reflects changes in the apoplast (= extracellular) pH, is characterized by a transient maximum at about pH 6.3 ("Neutral Peak", NP), followed by the establishment of a stable state ("Acid Equilibrium", AE) at about pH 4.8, some 6 - 7 h after excision (Peters and Felle 1991a). Qualitatively similar NP-AE-type pH development, distinct in quantitative terms only, was observed in all *Poaceae* species tested (Fig. 1). The range of the endogenously controlled pH shift is largest in maize (Fig. 2); cv. Goldprinz closely resembles the maize cultivars tested here (H. Lüthen, personal communication). All species acidify the medium in response to 10^{-5} M indole-acetic acid (IAA). This effect is strongest in *Avena sativa*, but is particularly weak in *Setaria italica* (Fig. 2).

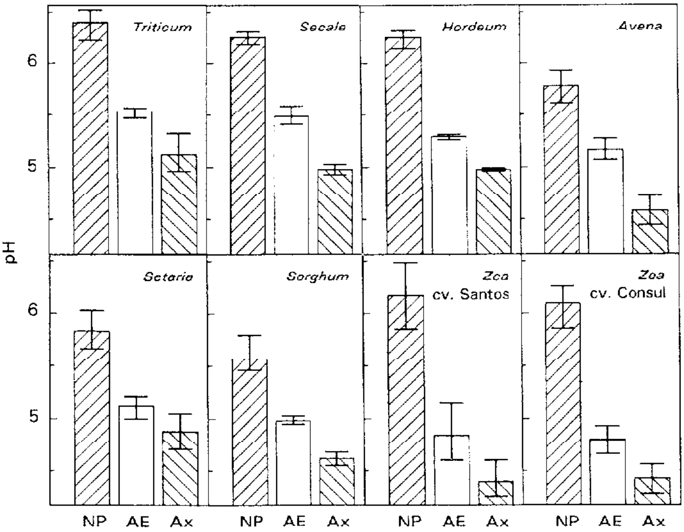

Fig. 2. Mean pH values at NP and AE, and the minimum pH value induced by 10^{-5} M IAA (Ax) in pH tests with 7 *Poaceae* species (there are two cultivars of *Zea mays*); experimental details as described for Fig. 1. Mean values of 5 experiments (14 in *Zea* cv. Consul, 64 in *Zea* cv. Santos) are given; *bars* represent the total range of values in the particular experiment. Note that species without mesocotyl (*Triticum*, *Secale*, *Hordeum*) tend to have higher pH values in the AE; note also the weakness of the auxin response in *Setaria*.

Discussion

pH shifts of the NP-AE-type do not only take place in *Zea mays* as reported before (Jacobs *et al.* 1984, Peters and Felle 1991a, Karcz *et al.* 1995), but are generally occurring in grass seedling coleoptile segments. The reason why the effect has been overlooked before is probably the one previously suggested in the case of *Zea mays* (Peters and Felle 1991a), namely insufficient densities of living tissue in the incubation media.

NP-AE-type pH shifts correlate with changes in other physiological parameters: *1*) the growth rate of coleoptile segments decreases sharply after excision, and partly recovers within a few hours. The effect, known as the "spontaneous growth response" (Evans and Schmitt 1975), has been scrutinized in *Zea* (Evans *et al.* 1977, Hatfield and LaMotte 1984, Parsons *et al.* 1988), and was also demonstrated in *Phalaris* (Rothert 1894), *Hordeum* (Stark 1917), *Triticum* (MacDowell and Sirois 1976, Bleiss and Ehwald 1993), and *Avena* (Rothert 1894, Söding 1929, Köhler 1956, Cline *et al.* 1974, Parsons *et al.* 1988). *2*) The transient growth decline appears paralleled by endogenous levels of IAA in *Zea* and *Avena* (Weiler *et al.* 1981, Iino and Carr 1982). *3*) Auxin sensitivity (in terms of maximum inducible growth rates) rises during the "spontaneous growth response" in *Zea* (Vesper and Evans 1978), *Avena* (Rietsema 1950), and *Triticum* (MacDowell and Sirois 1977). *4*) Decapitated coleoptiles loose their sensitivity to light and gravity; the recovery of sensitivity after some hours has led to the notion of the "regeneration of a physiological tip" (Rothert 1894). Taken together, upon excision or decapitation coleoptile segments of all species tested pass through a state of physiological disorder, which is well defined by the cessation of growth and tropic reactivity. No mechanistic explanation of the underlying processes has been established yet. In coleoptiles auxin induces cell wall acidification, and growth rates partly depend on this factor (Rayle and Cleland 1992). The general occurrence of NP-AE-type pH shifts following coleoptile segment excision among the *Poaceae* opens the possibility that cell wall pH might be involved in the "regeneration of a physiological tip", and that auxin exerts its long-suspected role in this process via the control of extracellular pH.

All species tested exhibit a significant acidification response to auxin (Fig. 2). However, the effect is weak and slow in *Setaria*. Noteworthily, seedlings of the subfamily *Panicoideae s.l.* (including *Setaria*) differ physiologically from other *Poaceae*, as noted already by Rothert (1894). In these grasses the coleoptile ceases to grow at an early stage, and seedling shoot elongation growth is brought about mainly by the mesocotyl. Coleoptile decapitation causes growth rate reduction in the mesocotyl; similarly, light and gravity perception takes place in the coleoptile, whereas tropic curvatures occur in the mesocotyl. Thus, coleoptiles of *Setaria* differ from those of other species tested, in that they lack capacitance to elongate or react themselves to tropic stimuli (*Sorghum* might be intermediate, Rothert 1894). The reduced pH response to auxin in *Setaria* would appear to accord with the acid-growth theory of auxin action in this case; this result calls for further investigation.

Coleoptile segments from species lacking a mesocotyl (*Triticum*, *Secale*, *Hordeum*) establish a higher steady state pH in the AE than those species, which possess one (Fig. 2). At this stage I am unable to offer an explanation for this fact.

Previously we had suggested that inquiries into the interrelation of cell wall pH and growth, and the mode by which auxin acts on both in *Zea* coleoptiles, should be performed not before the establishment of the AE (Peters and Felle 1991a,b). This, we argued, was necessary to avoid having to interprete the auxin response against a dynamic background (see also Rietsema 1950, Prat 1978, Bleiss and Ehwald 1993). The present study shows that the argument can be extended to coleoptile segments in general.

References

Bleiss, W., Ehwald, R.: Transient changes in length and growth of wheat coleoptile segments following treatments with osmotica and auxin. - Physiol. Plant. **88**: 541-548, 1993.

Brummell, D.A., Hall, J.L.: Medium acidification by auxin- and fusicoccin-treated peeled stem segments from etiolated seedlings of *Pisum sativum*. - J. exp. Bot. **32**: 635-642, 1981.

Cline, M.G., Edgerton, M., Rehm, M.M.: Accelerated endogenous growth in *Avena* coleoptile segments. - Planta **120**: 213-214, 1974.

Evans, M.L., Schmitt, M.R.: The nature of spontaneous changes in growth rate in isolated coleoptile segments. - Plant Physiol. **55**: 757-762, 1975.

Evans, M.L., Simon, M., Vesper, M.J.: Further characterization of the spontaneous growth response in *Zea* coleoptile segments. - Plant Cell Physiol. **18**: 441-452, 1977.

Hatfield, R.D., LaMotte, C.E.: IAA-induced growth responses of decapitated corn seedlings. - Plant Physiol. **74**: 302-306, 1984.

Iino, M., Carr, D.J.: Sources of free IAA in the mesocotyl of etiolated maize seedlings. - Plant Physiol. **69**: 1109-1112, 1982.

Jacobs, M., Lomax, T., Hertel, R.: A comparison of the auxin specifity of medium acidification and elongation in maize coleoptiles. - Plant Sci. Lett. **34**: 35-41, 1984.

Karcz, W., Stolarek, J., Lekacz, H., Kurtyka, R., Burdach, Z.: Comparative investigation of auxin- and fusicoccin-induced growth and H^+-extrusion in coleoptile segments of *Zea mays* L. - Acta Physiol. Plant. **17**: 3-8, 1995.

Köhler, D.: Über die Beziehungen zwischen der Länge von Haferkoleoptilen und der Wachstumsgeschwindigkeit ihrer isolierten Ausschnitte. - Planta **47**: 159-164, 1956.

MacDowell, F.D.H., Sirois, J.C.: Simple photometric auxanometers of high sensitivity. - Plant Physiol. **58**: 253-256, 1976.

MacDowell, F.D.H., Sirois, J.C.: Importance of time after excision and of pH on the kinetics of response of wheat coleoptile segments to added indoleacetic acid. - Plant Physiol. **59**: 405-410, 1977.

Marrè, E., Lado, P., Rasi-Caldogno, F., Colombo, R., de Michelis, M.I.: Evidence for the coupling of proton extrusion to K^+ uptake in pea internode segments treated with fusicoccin. - Plant Sci. Lett. **3**: 365-379, 1974.

Pakhomova, V.: Electrogenic K^+/H^- exchange in excised wheat roots. - Biol. Plant. **38**: 495-499, 1996.

Parrish, D.J., Davies, P.J.: On the relationship of extracellular pH and growth of excised pea stems. - Plant Physiol. **59**: 574-578, 1977.

Parsons, A., Firn, R.D., Digby, J.: The role of the coleoptile apex in controlling organ elongation (Parts I and II). - J. exp. Bot. **39**: 1331-1354, 1988.

Peters, W.S., Felle, H.: Control of apoplast pH in corn coleoptile segments. I. The endogenous regulation of cell wall pH. - J. Plant Physiol. **137**: 655-661, 1991a.

Peters, W.S., Felle, H.: Control of apoplast pH in corn coleoptile segments. II. The effects of various auxins and auxin analogues. J. Plant Physiol. **137**: 691-696, 1991b.

Prat, R.: Gradient of growth, spontaneous changes in growth rate and response to auxin of excised hypocotyl sections of *Phaseolus aureus*. - Plant Physiol. **62**: 75-79, 1978.

Rayle, D.L., Cleland, R.E.: The acid growth theory of auxin-induced cell elongation is alive and well. - Plant Physiol. **99**: 1271-1274, 1992.

Rietsema, J.: A modified cylinder test of high sensitivity for growth substances. - Proc. konink. Akad. Wetensch. (Amsterdam) **52**: 1194 1204, 1950.

Rothert, W.: Über Heliotropismus. - Beitr. Biol. Pflanz. **7**: 1-212, 1894.

Söding, H.: Weitere Untersuchungen über die Wuchshormone der Haferkoleoptile. - Jahrb. wiss. Bot. **71**: 184-213, 1929.

Stark, P.: Beiträge zur Kenntnis des Traumatropismus. - Jahrb. wiss. Bot. **57**: 461-552, 1917.

Vesper, M.J., Evans, M.L.: Time-dependent changes in the auxin sensitivity of coleoptile segments. - Plant Physiol. **61**: 204-208, 1978.

Weiler, E.W., Jourdan, P.S., Conrad, W.: Levels of indole-3-acetic acid in intact and decapitated coleoptiles as determined by a specific and highly sensitive solid-phase enzyme immuno assay. - Planta **153**: 561-571, 1981.