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Counteractive Effects of ABA and GA_3 on Extracellular and Intracellular pH and Malate in Barley Aleurone[1]

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Barley (*Hordeum vulgare* L.) aleurone layers are known to constitutively acidify their surroundings, primarily by L-malic acid release (J. Mikola, M. Virtanen [1980] Plant Physiol 66: S-142). Here we demonstrate the antagonistic effects of the plant hormones gibberellic acid (GA_3) and abscisic acid (ABA) on the regulation of extracellular pH (pH_e) of barley aleurone layers. We observed a strong correlation between ABA-induced enhancement of extracellular acidification and an ABA-induced increase in L-malic acid release. In addition, ABA caused an increase in intracellular L-malate level. GA_3 caused a slight decrease in intracellular L-malate level and was able to inhibit the ABA-induced increase in L-malate intracellular concentration and release. In addition, this ABA-induced L-malate release could be completely inhibited by GA_3. The ABA-induced release of L-malic acid could not account for the total ABA-induced pH_e decrease, suggesting the existence of an additional mechanism involved in the regulation of pH_e. It has been reported that ABA induces an intracellular pH (pH_i) increase, possibly due to the activation of plasma membrane proton pumps (R. Van der Veen, S. Heimovaara-Dijkstra, M. Wang [1992] Plant Physiol 100: 699–705). A pH_i increase, such as that caused by ABA, might be correlated with the intracellular L-malate increase as suggested by the pH stat model of D.D. Davies ([1986] Physiol Plant 67: 702–706). We studied if the effects of GA_3 on L-malate concentration were correlated with changes in pH_i and found that GA_3 caused a pH_i decrease and that GA_3 and ABA could interfere in the regulation of pH_i. In addition, we were able to mimic the effect of both hormones on L-malate release by bringing about artificial pH_i changes with the weak acid 5,5-dimethyl-2,4-oxazolidinedione and the weak base methylamine. The physiological meaning of the effects of GA_3 and ABA on the regulation of both pH_e and pH_i during grain germination are discussed.

Several processes during barley (*Hordeum vulgare* L.) grain germination are influenced by pH: α-amylase and several proteases have acidic pH optima and Ca^{2+} liberation and metabolite uptake by the scutellar epithelium are facilitated by low pH (Hamabata et al., 1988). In addition, the response of barley aleurone layers to GA, a phytohormone known to play an important role in stimulation of grain germination (Akazawa, 1972), is enhanced at low external pH (Sinjorgo et al., 1993). Therefore, changes in pH could be a mechanism by which processes during germination are controlled.

Barley aleurone layers are generally known to acidify their surroundings, mainly due to a constitutive release of L-malic acid (Mikola and Virtanen, 1980). Macnicol and Jacobsen (1992) reported that during grain maturation the pH of the endosperm decreases. This acidification seems to be brought about by the aleurone and involves malic acid secretion. Both ABA and GA_3 have been reported to increase the extracellular acidification of mature barley aleurone layers (Drozdowicz and Jones, 1992). These authors suggested that GA_3 stimulates phosphate and organic acid release by the aleurone layers. No stimulation of extracellular acidification was observed when aleurone layers of wheat were treated with GA_3 (Hamabata et al., 1988).

Since we are interested in the mechanism of GA and ABA action in barley aleurone cells, we studied the effect of these hormones on the regulation of extracellular pH and L-malate release by barley aleurone. In addition, we investigated the possibility that some of the antagonistic actions of ABA and GA are achieved by counteractive effects on the regulation of pH_i.

MATERIALS AND METHODS

Materials

L-Malic acid was from Sigma. Cellulase R-10 was from Yakult Honsha (Tokyo, Japan). Gamborg B5 was from Flow Laboratories (Irvine, UK). PVP K25 was from Fluka Chemie (Tilburg, The Netherlands) and Pipes was from Janssen Chemicals (Tilburg, The Netherlands). Malate dehydrogenase, citrate synthase, acetyl-CoA, and NAD were from Boehringer Mannheim (Mannheim, Germany). All other chemicals were from Merck (Darmstadt, Germany).

Isolation of Aleurone Layers and Aleurone Protoplasts

Barley (*Hordeum vulgare* L. cv Himalaya, harvest 1985; Department of Agronomy, Washington State University, Pullman, WA) grains were de-embryonated and cut into halves longitudinally. To sterilize the half-grains, they were briefly rinsed with 70% ethanol and incubated for 30 min in 0.1% hypochlorite while shaking. After washing in H_2O they were rinsed for 10 min in 10 mM HCl, washed again, and

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Abbreviations: DMO, 5,5-dimethyl-2,4-oxazolidinedione; pH_e, external pH; pH_i, intracellular pH; MA, methylamine; MDH, malate dehydrogenase.

then incubated for 3 d in water at 25°C in the dark. After this, the aleurone layer and starchy endosperm could be easily separated. Aleurone protoplasts (containing only small vacuoles) were prepared as described by Wang et al. (1991). The buffer used for washing and incubation of protoplasts was a 10 mM Na/K phosphate buffer (0.5 M mannitol, 10 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM KH_2PO_2/Na_2HPO_4, pH 7.0, 830 mOsm).

Measurement of pH_e

Ten aleurone layers were incubated in 1 mL of H_2O for 1 d at 25°C in the dark. The pH_e was measured with a standard Pharmacia glass electrode. For measurement of the effect of L-malic acid on the pH_e, a sample of 500 μL was taken from the medium after the incubation period and supplemented with a certain amount of L-malic acid, after which the pH was again registered.

Measurement of pH_i, Null-Point Method

The null-point method is based on the principle that when the plasma membrane of cells in a weakly buffered solution is permeabilized, the pH_e will change unless it is equal to the pH_i of the cells. After subjecting the protoplasts to the different treatments described in the text, they were washed and resuspended in weak phosphate buffer at a concentration of 2×10^6 protoplasts/mL. This buffer was similar to that described above but with only 2 mM Na/K phosphate. The pH_e was adjusted to the desired value with NaOH or HCl, and subsequently digitonin (0.005%, w/v) was added to permeabilize the plasma membrane. The resulting pH changes were recorded with a combined pH monitor (Pharmacia, Uppsala, Sweden) and a pen recorder. The values of pH_e, at which no apparent shift of pH was recorded after permeabilization, were taken as a close estimate of pH_i. In all determinations correction for the background acidification rate (mainly due to stirring CO_2 into the suspension) was made. Experiments were performed at room temperature (about 22°C). A more detailed study of this method was presented by Van der Veen et al. (1992).

Measurement of L-Malate

The determination of malate and MDH (EC 1.1.1.37) activity were both based on the measurement of ΔA_{340}, which resulted from NAD reduction in the coupled reaction with citrate synthase (EC 4.1.3.7). For determination of the extracellular malate, the incubation medium (H_2O, see "Measurement of pH_e") was assayed. For intracellular malate measurements, the aleurone layers were ground under liquid nitrogen and the powder was dissolved in 400 μL of distilled water (0°C) and sonicated for 5 s. Intracellular MDH was inactivated by incubation for 5 min at 100°C and then the slurry was centrifuged (5 min, 4°C). The final reaction mixture consisted of 100 mM KH_2PO_4 (pH 7.4), 300 μM acetyl-CoA, 750 μM NAD, 0.2 unit of citrate synthase, and 0.3 unit of MDH in a total volume of 200 μL. A_{340} was measured at t = 0 and after an incubation of 45 min at 25°C. ΔA_{340} was related to a standard curve of L-malate.

Statistics

Data are presented as means ± SE, with n = the number of measurements. Differences between values were tested with Student's t test with a confidence boundary >0.95.

RESULTS

The Effects of GA_3 and ABA on Extracellular pH_e

We measured the effect of GA_3 and ABA on pH_e of barley aleurone layers and found that ABA (10 μM) stimulated extracellular acidification, as was reported earlier by Drozdowicz and Jones (1992). The presence of GA_3 (10 μM) alone did not bring about any significant change in extracellular acidification, but it did partly inhibit the extracellular acidification caused by ABA (Fig. 1). Addition of $CaCl_2$ (20 mM) to the aleurone layers, as is often done to increase the effect of GA on α-amylase induction (e.g. Drozdowicz and Jones, 1992), did not influence these pH_e changes (data not shown).

A Correlation between pH_e Decrease and L-Malate Release

A possible mechanism to achieve extracellular acidification could be the stimulation of organic acid release. Since the organic acid L-malic acid is reported to be the main acidifying component secreted by aleurone layers (Mikola and Virtanen, 1980), we studied the effect of ABA and GA_3 on the level of extracellular L-malate. ABA (10 μM) increased the amount of released malate to 400% compared to nontreated aleurone layers, whereas GA_3 (10 μM) inhibited L-malate release by the aleurone layer by about 50%. The stimulation of L-malate

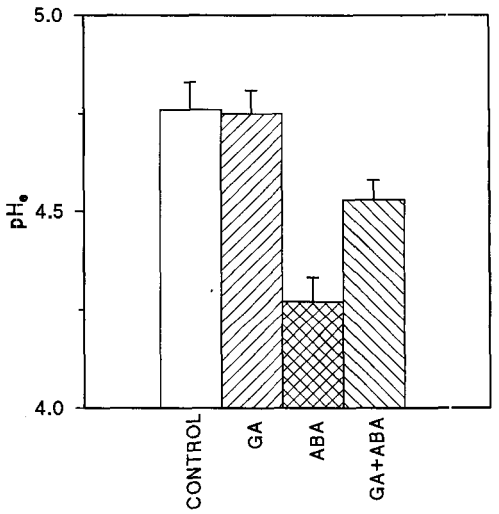

Figure 1. The effect of GA_3 and ABA on pH_e. Ten barley aleurone layers were incubated in 1 mL of H_2O for 16 h in the absence or presence of 10 μM GA_3 or 10 μM ABA. The pH_e of the medium was measured using a glass pH electrode. Control, Nontreated layers; GA, incubated with GA_3; ABA, incubated with ABA; GA + ABA, incubated with GA_3 and ABA. The mean values ± SE of eight independent experiments are presented.

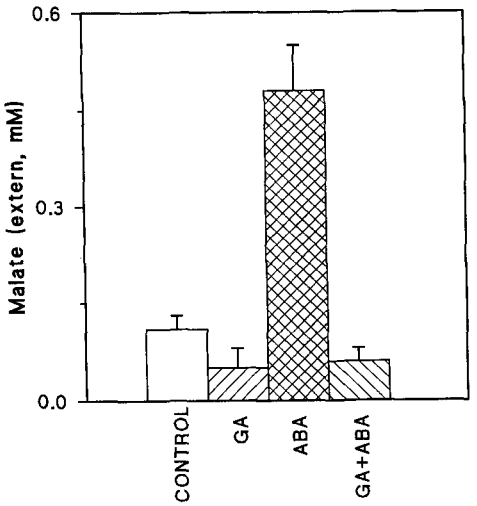

Figure 2. The effect of GA_3 and ABA on extracellular malate concentration. Ten barley aleurone layers were incubated in 1 mL of H_2O for 16 h in the absence or presence of 10 μM GA_3 or 10 μM ABA. The L-malate content in the incubation medium (H_2O) was measured as described in "Materials and Methods." The mean values ± SE of eight independent experiments are presented.

release, brought about by ABA, could be completely overridden by GA_3 (Fig. 2).

The time course of the pH_e decrease and extracellular L-malate increase shows that ABA causes a detectable (and significant) pH_e decrease when incubated for 4 h with ABA. The L-malate increase shows a detectable (and significant) difference between ABA-treated and nontreated layers when incubated for more than 4 h (Fig. 3).

To investigate the quantitative role of malic acid in the ABA-induced extracellular acidification, we treated aleurone layers either with or without GA_3, ABA, or both, and measured the pH_e and L-malic acid in the incubation medium (water). We then removed the aleurone layers and added malic acid to the medium to reach the same concentration of L-malate as was present in the medium of ABA-treated layers (0.48 mM). If L-malic acid was added to the medium of untreated aleurone layers, the pH_e dropped approximately 0.3 unit. We found a similar drop of pH if L-malic acid was added to the medium of GA_3-treated layers (Fig. 4A). This indicates that an increase of L-malic acid in the medium, up to the concentration of ABA-treated layers, was not sufficient to completely mimic ABA's acidifying effect.

Although GA_3 itself had no effect on extracellular acidification (Fig. 4A), it partly counteracted the effect of ABA on pH_e (Fig. 4A) and it completely inhibited the L-malate release caused by ABA (Fig. 2). If we added L-malic acid (to a final concentration of 0.48 mM) to the medium of ABA + GA_3-treated aleurone layers, the pH dropped to the same value as was observed in the medium of ABA-treated layers (Fig. 4, A and B). These observations strongly suggest that extracellular acidification induced by ABA was brought about by L-malic acid release, which could be inhibited by GA_3, and by an additional mechanism that was unaffected by GA_3.

The Effect of GA_3 and ABA on pH_i and L-Malate Concentration

Intracellular L-malate measurements showed that ABA treatment increased the intracellular malate content, whereas GA_3 brought about a decrease (Fig. 5). Again, GA_3 was able to completely inhibit the ABA-induced increase. Hence, the ABA-induced release of L-malate as described in the preceding section, as well as the inhibition of the L-malate release by GA, are correlated with the effects of these hormones on intracellular L-malate concentrations. Davies (1986) suggested that modulation of cytosolic malic acid concentrations plays a crucial role in the biophysical pH stat of the cell. pH_i increase would bring about malic acid increase, thus stabilizing pH_i. Since ABA is reported to increase pH_i (e.g. Gehring et al., 1990; Van der Veen et al., 1992), the L-malate increase caused by ABA might be brought about via an ABA-induced pH_i increase. GA_3 has been reported to cause a short, transient acidification of the cytosol of maize hypocotyls (Irving et al., 1992). If GA has an acidifying effect in barley aleurone cells, then the inhibitory effect of GA on the ABA-induced

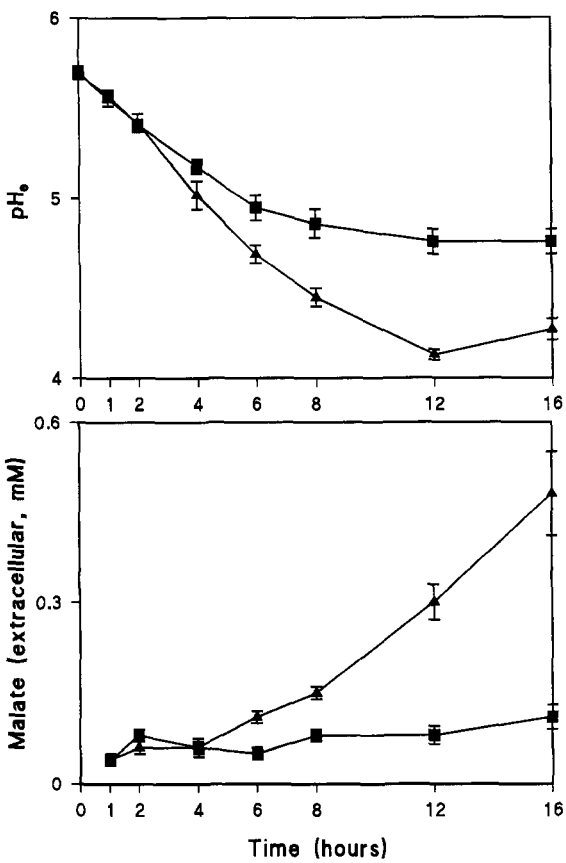

Figure 3. Time course of ABA-induced pH_e decrease and extracellular L-malate increase. Ten barley aleurone layers were incubated in 1 mL of H_2O for 16 h in the absence (■) or presence (▲) of 10 μM ABA. The pH_e of the medium was measured using a glass pH electrode, the L-malate in the incubation medium was measured as described in "Materials and Methods." The mean values ± SE of three independent experiments are presented.

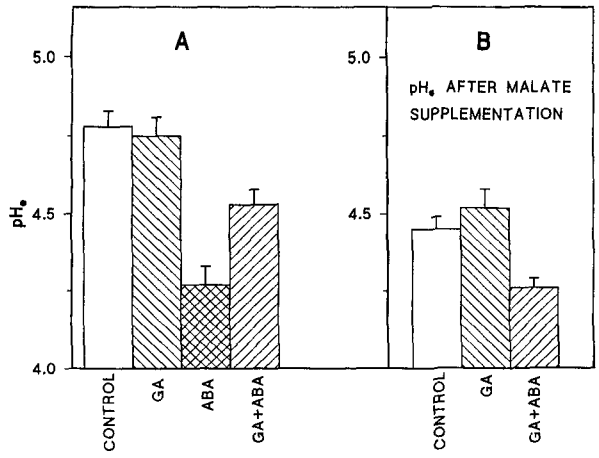

Figure 4. The contribution of the ABA-induced malate release to the ABA-induced pH_e decrease. Ten barley aleurone layers were incubated in 1 mL of H_2O for 16 h in the absence or presence of 10 μM GA_3 or 10 μM ABA. The aleurone layers were then removed from the medium and L-malate concentration and pH_e were measured as described in "Materials and Methods." A shows the pH_e of the medium just after incubation; control, nontreated control; ABA, incubated with ABA; GA, incubated with GA_3; GA + ABA, incubated with GA_3 and ABA. B shows the pH_e of the same media obtained after supplementation of these media with L-malic acid. An amount of L-malic acid was added to achieve a final concentration that was equivalent to that of the medium of ABA-treated layers (0.48 mM). The mean values ± SE of three independent experiments are presented.

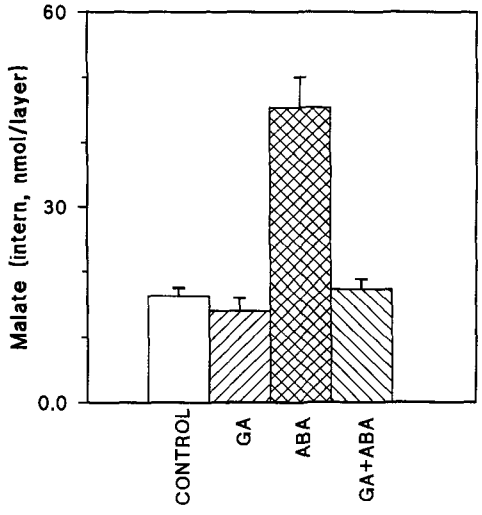

Figure 5. The effect of GA_3 and ABA on the intracellular malate concentration in barley aleurone layers. Ten barley aleurone layers were incubated in 1 mL of H_2O for 16 h in the absence or presence of 10 μM GA_3 or ABA. The aleurone layers were extracted and L-malate content was measured as described in "Materials and Methods." Control, Nontreated layers; ABA, incubated with ABA; GA, incubated with GA_3; GA + ABA, incubated with GA and ABA. The values ± SE of six independent experiments are presented.

L-malate increase might be mediated by a counteractive effect on the ABA-induced pH_i increase. Therefore, we investigated the effect of GA_3 on the ABA-induced pH_i increase.

The effect of GA_3 on the intracellular pH in barley aleurone protoplasts was studied using the null-point method. The same method was used successfully to study the ABA-induced pH_i changes in barley aleurone protoplasts (Van der Veen et al., 1992) and is discussed in the report of that study in more detail. The average pH_i of untreated protoplasts was 6.99 ± 0.01 (n = 24). Different batches of protoplasts had a slightly different basal pH_i, resulting in small variations in the mean pH_i between the different experiments. Addition of GA_3 (10 μM) induced a decrease in pH_i of 0.18 pH units (±0.02, n = 22) on average, reaching a new steady-state level at 45 min after addition of the hormone (Fig. 6). The acidification was GA_3 dose dependent (Fig. 7), with a half-maximal induction at 4×10^{-9} M.

The effect of GA_3 on pH_i was opposite to that of ABA and was achieved in about the same time span (see also Van der Veen et al., 1992). The counteractive effects of GA_3 and ABA were studied by adding these two hormones simultaneously to the protoplasts. The results, presented in Figure 8, show that the combination of both hormones brought about intermediate shifts in pH_i. Looking at long-term effects of ABA and GA_3 on pH_i, we found that approximately 6 h after the addition of the hormones the pH_i of the protoplasts had returned to the level of untreated protoplasts (Table I).

We have shown that L-malate release induced by ABA was detectable 6 h after addition of the hormone. The increase of intracellular L-malate induced by ABA was not yet detectable 4 h after addition of the hormone (data not shown). Thus, it appears that modulation of the pH_i induced by either GA_3 or ABA occurs before we can detect changes in intracellular (and extracellular) L-malate concentration. This points to an

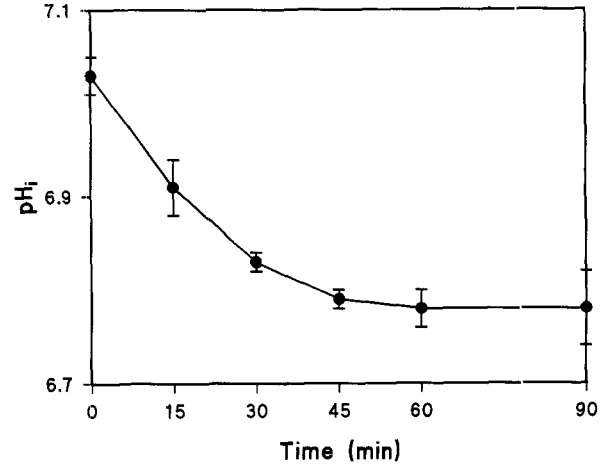

Figure 6. Time course of GA_3-induced pH_i decrease. Barley aleurone protoplasts (8×10^5/mL) were incubated in 10 mM phosphate buffer (pH 7.0) with or without 10 μM GA_3. At different times samples were collected for pH_i measurements. The samples were washed twice with 2 mM phosphate buffer (pH 7.0) and pH_i was determined with the null-point method (see "Materials and Methods"). The mean values ± SE of three independent experiments are presented.

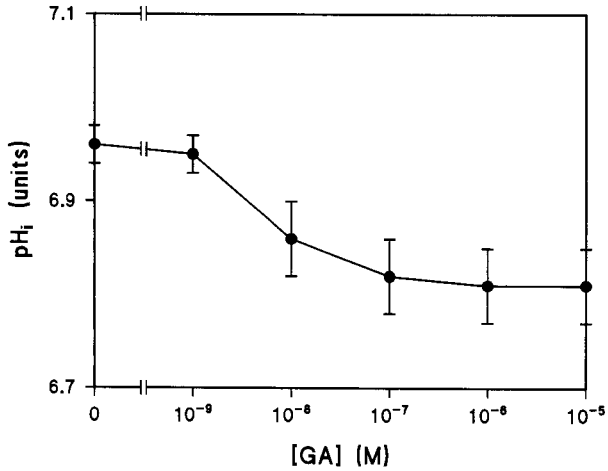

Figure 7. Dose-response curve of the GA_3-induced pH_i decrease. Barley aleurone protoplasts (8×10^5/mL) were incubated in 10 mM phosphate buffer (pH 7.0) with indicated concentrations of GA_3. After 1 h, the protoplasts were washed twice in 2 mM phosphate buffer (pH 7.0) and pH_i was measured with the null-point method (see "Materials and Methods"). The mean values ± SE of four independent experiments are presented.

underlying mechanism such as that described by Davies' pH stat model (Davies, 1986), in which modulation of L-malate concentration is triggered by changes in pH_i. To determine if changes in pH_i, such as those brought about by ABA and GA, can be responsible for the respective increase or decrease in L-malate concentration, we artificially modified pH_i using

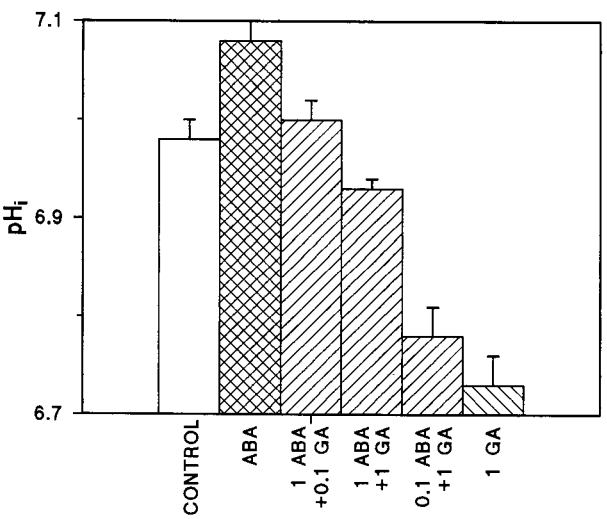

Figure 8. Opposing effects of GA_3 and ABA on pH_i. Barley aleurone protoplasts (8×10^5/mL) were incubated in 10 mM phosphate buffer (pH 7.0) with different concentrations of GA_3 and ABA. After 1 h, the protoplasts were washed twice with 2 mM phosphate buffer (pH 7.0) and pH_i was measured with the null-point method (see "Materials and Methods"). Control, Nontreated layers; ABA, incubated with 1 μM ABA; 1 ABA + 0.1 GA, incubated with 1 μM ABA and 0.1 μM GA_3; 1 ABA + 1 GA, incubated with 1 μM ABA and 1 μM GA_3; 0.1 ABA + 1 GA, incubated with 0.1 μM ABA and 1 μM GA_3; 1 GA, incubated with 1 μM GA_3. The mean values ± SE of three independent experiments are presented.

Table I. *The long-term effects of GA_3 and ABA on pH_i of barley aleurone protoplasts*

Aleurone protoplasts (8×10^5/mL) were incubated in 10 mM phosphate buffer (pH 7) at 25°C, with or without 1 μM ABA or GA_3, and harvested after 1 or 6 h. pH_i was measured as described in "Materials and Methods." The mean values ± SE of three independent experiments are presented.

Experiment	1 h	6 h
Control	6.97 ± 0.01	7.00 ± 0.01
ABA (1 μM)	7.10 ± 0.01	6.99 ± 0.03
GA_3 (1 μM)	6.76 ± 0.02	7.06 ± 0.01

the weak base MA and the weak acid DMO. The effects of MA and DMO (7.5 mM) on intracellular pH in barley aleurone protoplasts have been described by Van der Veen et al. (1992). We studied the effect on intact aleurone layers. Although we were not able to measure the effect on pH_i in walled cells, we expect the effect to be comparable to that in protoplasts, since these chemicals should be able to pass the cell wall. MA is able to cause an increase in pH_i comparable to that of ABA (Van der Veen et al., 1992). When applied to aleurone layers, 5 mM MA caused an increase in the amount of L-malate released (Table II). This increase was seen consistently in separate experiments, although the extent of the increase varied. When applied together with ABA it did not significantly affect L-malate release. This might be explained by the finding of Van der Veen et al. (1992) that 7.5 mM MA does not further increase the ABA-induced alkalinization of pH_i. In addition, we found that 5 mM DMO, which is able to decrease pH_i by approximately 0.2 pH unit (similar to GA's effect) and is also able to do so in the presence of ABA (Van der Veen et al., 1992), could inhibit L-malate release both in the absence and in the presence of ABA (Table II). These data support Davies' hypothesis that increase in pH_i inhibits MDH, thus causing an increase in L-malate. Acidification of the intracellular pH, on the other hand, would be favorable for MDH activity, thereby causing a decrease in L-malate concentration.

Table II. *The effect of artificially induced pH_i changes on the amount of L-malate release by barley aleurone layers*

Ten aleurone layers were incubated in 1 mL of 10 mM phosphate buffer (pH 6.6 or 7.4) at 25°C, with or without 10 μM ABA, 5 mM DMO, or 5 mM MA. After 16 h samples were taken to determine L-malate concentration as described in "Materials and Methods." Results ± SE of four independent experiments are presented.

Experiment	L-Malate
	mM
Control (pH_e 6.6)	0.19 ± 0.04
DMO (pH_e 6.6)	0.09 ± 0.01
ABA (pH_e 6.6)	0.54 ± 0.17
ABA + DMO (pH_e 6.6)	0.13 ± 0.05
Control (pH_e 7.4)	0.27 ± 0.05
MA (pH_e 7.4)	0.41 ± 0.08
ABA (pH_e 7.4)	0.61 ± 0.15
ABA + MA (pH_e 7.4)	0.55 ± 0.11

DISCUSSION

GA and ABA are widely studied antagonists (e.g. Jacobsen and Beach, 1985; Skriver and Mundy, 1990). The interaction of GA_3 and ABA is complex, as shown by their effect on gene expression; ABA is able to completely inhibit GA-induced gene expression (α-amylase), whereas GA does not have any effect on ABA-induced gene expression (Rab) (e.g. Skriver and Mundy, 1990; Van Beckum et al., 1993).

The opposite effects of ABA and GA on pH_e and L-malate concentration described here illustrate the complex interaction between both hormones; GA_3 appears to completely inhibit ABA-induced L-malate accumulation (Figs. 2 and 5). To our knowledge this is the first report of an action of ABA that can be "overruled" by GA_3. GA_3 itself did not induce a significant decrease of pH_e. Drozdowicz and Jones (1992) claimed that GA_3 caused an acidification of the pH_e, but whether this effect was significant was not reported. Although GA_3 is able to inhibit the ABA-induced L-malate release completely, it inhibited the ABA-induced extracellular acidification only partly. This suggests that L-malic acid release can account for part of the ABA-induced pH_e drop and that ABA induces an additional acidifying mechanism. Other organic acids have been reported to take part in the acidification of the endosperm by the barley aleurone layer (Macnicol and Jacobsen, 1992) but seemed to play a minor role compared to malic acid. Mikola and Virtanen (1980) reported that phosphate and amino acids contribute to the constitutive acidification caused by mature aleurone layers, albeit to a minor extent. It was later suggested that the release of these components was inhibited by ABA (Drozdowicz and Jones, 1992). Van der Veen et al. (1992) demonstrated that ABA induced an increase of pH_i in barley aleurone. This intracellular alkalinization could be inhibited by diethylstilbestrol and zearalenone, two plasma membrane H^+-ATPase inhibitors. Therefore, it is tempting to propose that ABA stimulates the plasma membrane proton pump, possibly causing the pH_i increase. The ABA-induced extracellular acidification could then consist of a dual mechanism: induction of L-malic acid release, which can be completely inhibited by GA_3, and stimulation of plasma membrane H^+-ATPases, which seems unaffected by GA_3.

The mechanism by which malic acid is released is not clear. L-malate could be released as an organic acid or could be transported as a divalent anion, balancing (separate) H^+ release. The latter option seems to be more likely, since malate is present mainly as the divalent anion at a pH_i around 7. One transport mechanism by which malate can be transported out of the cell is a channel like the guard cell anion channel 1, which is permeable for malate (Hedrich and Marten, 1993). In either case the mechanism apparently would be completely inhibited by GA.

The pH_i increase caused by ABA could be the trigger for L-malate increase, as suggested by Davies' pH stat model (Davies, 1986). We have shown that GA_3 induces a decrease of pH_i and that it counteracts the effect of ABA on pH_i (Fig. 8). If the (transient) pH_i increase caused by ABA is the trigger for L-malate increase, then the opposing effect of GA_3 on pH_i might well explain its inhibitory effect on L-malate production and release. This hypothesis is supported by the data obtained with the metabolically inert weak acid DMO and the weak base MA. By artificially mimicking GA's effect on pH_i, we observed an effect on L-malate release similar to that of GA: L-malate release was inhibited in the absence of ABA and DMO completely inhibited the ABA induction of L-malate release. Moreover, we could mimic the ABA-induced release of L-malate by increasing pH_i with the aid of MA (Table II). Van der Veen et al. (1992) reported that 7.5 mM MA was not able to increase pH_i to a level higher than that achieved by ABA alone. In our system it did indeed not significantly affect the ABA-induced L-malate release. To obtain favorable conditions for uptake of the weak acid or base, the extracellular pH was adjusted to values more close to their respective pK_a's. In this way the amount of acid or base that can enter the protoplast is increased, since these compounds are able to pass the membrane in their uncharged form. The increase of extracellular pH, used to facilitate MA uptake, also seemed to cause a slight increase in L-malate release itself. This might be caused by a small effect of this extracellular pH (which is higher than pH_i) on the intracellular pH. The pH stat model of Davies (1986) based on the effect of pH_i on malate dehydrogenase can explain the effects on the L-malate concentration but does not account for the increased release of L-malate, unless this can be considered as an "overshoot" of the mechanism.

Another question that arises from this study concerns the mechanism behind the GA-induced intracellular acidification. If activation of plasma membrane proton pumps is indeed responsible for the part of the ABA-induced extracellular acidification that is not affected by GA_3, then the GA_3-induced pH_i decrease cannot be explained by inhibition of the same H^+-ATPases. Possibly there are more or less independent, opposing mechanisms by which ABA and GA_3 modulate pH_i. The precise mechanism by which GA_3 and ABA modulate pH_i is open for further investigation.

The effect of plant hormones on pH_e is believed to play a role in the regulation of germination (e.g. Hamabata et al., 1988). In principle, acid surroundings are favorable for several processes during germination, such as hydrolase activity, Ca^{2+} release, and metabolite uptake by the scutellar epithelium (Hamabata et al., 1988). On the other hand, without addition of hormones to aleurone layers, extracellular acidification is achieved to approximately pH 4.8, quite sufficient to create such favorable surroundings. There may be something significant about the ABA-induced L-malate increase apart from its suggested function in a biophysical pH stat. Hedrich and Marten (1993) showed that extracellular malate increased the anion permitivity of the anion channel guard cell anion channel 1 and induced closure of the stomata, thereby mimicking ABA's effect. The fact that the change in L-malate concentration described here is not restricted to the intracellular concentration suggests that L-malate has other functions as well. It would be interesting to determine if malate has a role as signal molecule in barley aleurone.

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