Effect of osmotic stress on abscisic acid efflux and compartmentation in the roots of two maize lines differing in drought susceptibility

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Abstract

Roots of two *Zea mays* L. lines (drought-resistant Polj 17, and drought-susceptible F-2) were exposed to osmotic stress induced by sorbitol (osmotic potential -1.0 MPa). The following parameters were determined in cortex cells: membrane permeability for abscisic acid (ABA), ABA fluxes across membranes, pH values and ABA content in cytoplasm and vacuole. Osmotic stress induced different distribution of ABA within cell compartments in the investigated lines. ABA transport in the F-2 line occurred according to the intracellular pH gradient and the anion trap concept. In Polj 17, however, osmotic stress did not cause any significant effect on pH gradient and compartmental ABA content, but had a stimulating effect on ABA efflux from cytoplasm to apoplast and than via xylem to the leaf. These findings indicate different mechanisms of ABA transport between the investigated lines in response to osmotic stress.

Additional key words: compartmental analysis, osmotic stress, root cortex cells, *Zea mays* L.

Introduction

Drought is one of the most limiting environmental stresses for plant production. Plants respond to drought and other environmental stresses by producing abscisic acid (ABA), which causes a variety of plant responses including stomatal closure and growth reduction. Much of the present knowledge of ABA biosynthesis and distribution has been derived from leaf studies. Pierce and Raschke (1981) showed that the reduction of leaf pressure potential induces ABA biosynthesis. Regarding the rapid changes of ABA content in leaf mesophyll, Heilman *et al.* (1980) pointed out the importance of redistribution of the existing ABA in the leaf. Hartung *et al.* (1982, 1988) found ABA to be distributed among leaf cell compartments depending on transmembrane pH gradient. This mechanism was described in detail in mathematical models for the leaf, proposed by Slovik *et al.* (1992), and for the root, by Daeter *et al.* (1993). Both models showed that ABA is distributed among cell compartments according to the Henderson-Hasselbalch equation. It means that the existing ABA is trapped in more alkaline cell compartments (cytoplasm).

Besides leaves, roots are also capable of producing ABA in response to drought or osmotic stress, and Behl and Hartung (1984) showed similarity in ABA distribution among leaf and root cell compartments. ABA may act as a "signal molecule", informing the leaves about reduced water availability in the soil (Dodd *et al.* 1996). Such control has been termed non-hydraulic or chemical signaling. It is thereby distinguished from hydraulic signaling provoked by xylem sap pressure change caused by reduced soil water availability. However, Slovik *et al.* (1995) showed that ABA in the leaf may also generate chemical signals in the phloem, since changing water relations in the leaves release ABA into the apoplast and it is then taken up by alkaline phloem.

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Abbreviations: ABA - abscisic acid, $\Phi_{oc,co}$ and $\Phi_{cv,vc}$ - flux across plasma membrane and tonoplast; ψ_o- osmotic potential; Ps_{pla} and Ps_{ton} - plasma membrane and tonoplast permeability coefficients for ABAH; k_{ca}, k_{cv} , k_{vc} - rate constants of one way fluxes from cytoplasm to apoplast, from cytoplasm to vacuole and from vacuole to cytoplasm, respectively.
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Substantial evidence indicated genetic variation in drought-induced ABA accumulation capacity. Behl and Hartung (1984) showed that higher cytoplasmatic and vacuolar ABA contents in a stress-tolerant barley cultivar correlated well with its higher ABA accumulation capacity. Similar investigation has not been done on maize although natural genetic variation in maize leaf ABA content has been found (Quarrie 1991). This experiment therefore aimed to investigate the mechanism of ABA distribution in root cortex cells of two maize lines differing in both drought resistance and leaf ABA accumulation capacity. To compare the mechanism of ABA transport and distribution in the roots of these lines, we used an efflux compartmental ABA analysis.

Materials and methods

Plants: The genotypes used for the assays were two inbred maize (*Zea mays* L.) lines provided by the Maize Research Institute (Belgrade, Yugoslavia). The lines had previously been classified as drought-resistant or "high-ABA" (Polj 17) and drought-susceptible or "low-ABA" (F-2), according the germination tests, visual estimation in field conditions and the leaf detached tests (Pekić and Quarrie 1987). Seeds of these two lines were germinated for 4 d in a growth chamber at 25 (C in the dark until primary roots were about 5 - 7 cm long. Root segments (approximately 2 cm long, cut 5 mm above the root tip, with the central cylinder removed) were used in all experiments.

Efflux compartmental analysis: Efflux experiments were done according to procedures proposed by Behl *et al.* (1981), Behl and Hartung (1984) and Daeter and Hartung (1990). At the first part of efflux experiments, root cortex segments were preincubated for 60 min in 4 cm^{-3} of assay medium containing: 2 mol m^{-3} $CaCl_2$, 6 mol m^{-3} KCl, 2 mol m^{-3} K_2HPO_4, 25 mol m^{-3} MES, 1.5 mol m^{-3} KNO_3, 1 mol m^{-3} $MgSO_4$, 1 mol m^{-3} glucose, 10^{-2} mol m^{-3} tetcyclacis (*BASF*, Limburgerhof, Germany, Germany) and 10^{-4} mol m^{-3} ABA (*Sigma-Aldrich*, Dorset, UK), pH 6.0. Root cortex segments were then loaded for 18 h (loading period) in 4 cm^{-3} of fresh assay medium in which 7 400 Bq of ^{3}H-ABA (*Amersham*, Radioactive Centre, Bucking, UK) and 50 U per cm^{-3} penicillin G (to prevent bacterial growth) were added. Over the period, equilibrium between external radioactive ABA and internal radioactive ABA in tissue was achieved. After loading period root cortex segments were repeatedly eluted (efflux period) with fresh non-radioactive assay medium (assay exchange medium) in proposed time intervals (Daeter and Hartung 1990). Tetcyclacis, an inhibitor of cytochrome P_{450} dependent monoxygenases (Rademacher *et al.* 1987; Daeter and Hartung, 1990), was added in assay and exchange assay medium to prevent possible ABA hydroxylation into phaseic (PA) and dihydrophaseic acid (DPA) in root tissues during loading and efflux periods.

In stress experiments, after the loading period (control assay medium), root cortex segments were eluated with assay exchange medium, which additionally contained 10^3 mol m^{-3} sorbitol (*Sigma-Aldrich,* Diesenhofen, Germany) as an osmoticum (adjusted at ψ_o = -1.0 MPa). Finally, all elutes were dried at 60 °C overnight. ABA was extracted from root segments overnight in 0.5 cm^3 of 80 % methanol. Sample radioactivity was determined by using liquid scintillation counter (*LKB mod 1210*, Turku, Finland).

Calculation: Graphical and mathematical analyses of the time-dependent ^{3}H-ABA efflux curves were performed according to MacRobbie (1981) and Behl and Hartung (1984, 1987), using a generally accepted biphasic model. ABA concentration in cell compartments, permeability coefficients (Ps) and ABA fluxes ($\phi_{oc,cv}$) across both membranes were calculated as described by Daeter and Hartung (1990) and Baier and Hartung (1991), assuming that the ratio between the length and diameter of the cortex cells is the same in control and stress conditions (6:1) and cytoplasm/vacuole volume ratio 1.5/98.5 and 2/98, respectively. The required cell dimensions for all calculations were determined using conventional microscopy and electron scanning microscopy and data (not shown) were statistically processed by computer programme (Jovanović 1998). The cytoplasmatic and vacuolar pH values were calculated according to the equation of Wadell and Butler (1959) assuming that ABA is distributed according to Henderson-Hasselbalch-equation.

Results

Loss of ^{3}H-ABA from the previously labelled root cortex tissues and residual ^{3}H-ABA content showed normal biphasic patterns when plotted semilogarithmically *versus* time (not shown). When steady-state conditions was reached over the loading period (as prerequiste for compartmental analysis) influx and efflux at the plasmalemma and tonoplast are numerically equal and the individual one-way fluxes and contents of ABA could be determined (Behl and Hartung 1984, 1986, MacRobbie 1981).

One-way ABA fluxes across both membranes in Polj 17 showed that osmotic stress significantly increased ABA flux across plasma membrane (nearly twice compared with control) but ABA flux across tonoplast decreased (Table 1). In F-2, however, both plasma membrane and tonoplast ABA fluxes were higher under stress conditions compared to control. The comparison of lines revealed that ABA fluxes across both membranes in F-2 were significantly lower then in Polj 17.

The results of compartmental ABA analysis for vacuole and cytoplasmatic concentrations calculated on the volume basis (see Materials and methods) showed

Table 1. ABA concentrations [mmol m^{-3}] in vacuole and cytoplasm, one-way ABA fluxes [10^{-21} mol $cell^{-1}$ s^{-1}], internal pH values, and permeability coefficients [10^{-9} m s^{-1}] of the membranes in maize cortex cells under control and stress conditions. The external ABA concentration was 10^{-4} mol m^{-3} at pH 6.0. The osmotic potential of the sorbitol medium was adjusted to 1.0 MPa. Means of four replicates ± SE.

		Polj 17 control	stress	F-2 control	stress
ABA	vacuole	0.21 ± 0.02	0.18 ± 0.01	0.22 ± 0.02	0.21 ± 0.02
	cytoplasm	1.99 ± 0.11	1.97 ± 0.30	1.18 ± 0.17	1.99 ± 0.22
One-way ABA fluxes	$\phi_{oc,co}$ - plasmalemma	0.73 ± 0.11	1.36 ± 0.05	0.30 ± 0.01	0.45 ± 0.15
	$\phi_{vc,cv}$ - tonoplast	0.54 ± 0.07	0.33 ± 0.01	0.17 ± 0.00	0.22 ± 0.01
pH	vacuole	6.37 ± 0.04	6.28 ± 0.01	6.34 ± 0.04	6.32 ± 0.05
	cytoplasm	7.35 ± 0.02	7.31 ± 0.06	7.06 ± 0.06	7.32 ± 0.05
Ps	Ps_{pla} - plasmalemma	13.25 ± 3.10	24.65 ± 6.12	8.54 ± 2.31	12.50 ± 2.41
	Ps_{ton} - tonoplast	9.68 ± 1.73	6.66 ± 0.23	3.25 ± 0.36	6.09 ± 0.24

that in Polj 17 osmotic stress reduced ABA concentration in the vacuole, whereas cytoplasmatic ABA concentration remained unchanged (Table 1). In F-2, however, ABA concentration in the vacuole did not change during the osmotic stress treatment, while cytoplasmatic ABA increased. Therefore, the ratio between vacuolar and cytoplasmatic ABA content in that line increased during stress, which indicates that some of the ABA from vacuole is transported into cytoplasm. Comparison between lines, however, did not reveal significant difference in stress-induced ABA content in the investigated cell compartments.

Calculated vacuolar and cytoplasmatic pH values (Table 1) showed that stress had little effect on Polj 17. In F-2, however, cytoplasmatic pH increased. A comparison of pH values between lines revealed significant genotypic difference only for the control treatment, in which cytoplasmatic pH was approximately 0.3 pH units higher in Polj 17 than in F-2.

To understand the mechanism of ABA transport between different cell compartments we calculated plasma membrane (Ps_{pla}) and tonoplast (Ps_{ton}) permeability coefficients for ABAH in Polj 17. During stress treatment, Ps_{pla} significantly increased, although Ps_{ton} significantly decreased (Table 1). In F-2, sorbitol stress increased both Ps_{pla} and Ps_{ton}. A comparison between lines showed that Ps_{pla} values were lower under both control and stress conditions in F-2, compared with Polj 17, although Ps_{ton} values were different only in controls (higher in Polj 17 than in F-2).

Table 2. Effect of osmotic stress on rate constant of one-way ABA fluxes across root cortex cell membranes: k_{co} (cytoplasm ⇒ apoplast), k_{cv} (cytoplasm ⇒ vacuole), and k_{vc} (vacuole ⇒ cytoplasm). Experimental conditions were the same as at Table.1.

	Polj 17 control	stress	F-2 control	stress
k_{co} [10^{-6} s^{-1}]	93.00 ± 9.03	160.00 ± 24.15	133.00 ± 20.12	102.00 ± 9.02
k_{cv} [10^{-6} s^{-1}]	67.00 ± 14.01	42.00 ± 10.20	48.02 ± 3.03	50.00 ± 4.00
k_{vc} [10^{-6} s^{-1}]	9.72 ± 1.61	8.88 ± 0.75	4.44 ± 2.20	10.83 ± 0.75

One-way ABA fluxes are known to be influenced by ABA contents of the originating compartment (Behl and Hartung 1984) and therefore the ratios between one-way fluxes and ABA contents were calculated (Table 2). Those ratios represent the rate constants (k) of the individual one-way ABA fluxes and describe more

precisely the effect of osmotic stress on ABA transport in cortex cells. The acquired results for Polj 17 showed that ABA transport from cytoplasm to apoplast (k_{ca}) increased under stress condition, while transport from cytoplasm to vacuole (k_{cv}) decreased, and ABA transport from vacuole to cytoplasm (k_{vc}) remains unchanged (Table 2). This indicates that osmotic stress had an enhancing effect on ABA transport from cytoplasm to apoplast.

These results also showed that a reverse process occurs in F-2 (Table 2). ABA transport from cytoplasm to vacuole (k_{cv}) did not significantly change under stress treatment, transport from vacuole to cytoplasm (k_{vc}) increased, while ABA transport from cytoplasm to apoplast (k_{ca}) decreased.

Discussion

Behl and Hartung (1984) showed that, under stress condition, ABA was stored mainly in the vacuole compartment of root cells. In our experiments, *ca.* 90 % of total ABA was accumulated in cytoplasm in both lines. Under osmotic stress, the drought-resistant barley cultivar had 3 times higher cytoplasmatic and 5 times higher vacuolar ABA concentration than the stress-susceptible cultivar (Behl and Hartung 1984). However, the comparison of our lines, which also differed in stress tolerance and ABA accumulation capacity in the leaves (Pekić and Quarrie 1987), did not support such conclusion. The investigated lines showed differences only in cytoplasmatic ABA content in control conditions, which was significantly higher in the drought-resistant line Polj 17 than in the drought-susceptible line F-2. The ratio of ABA concentration in vacuole and cytoplasm in Polj 17 did not change under stress. However, this ratio increased (from 1:5 to 1: 10) in F-2, indicating that ABA from vacuole was transported into cytoplasm. The analysis of rate constants of one-way ABA fluxes (Table 2) showed that osmotic stress caused a significant increase of ABA transport to apoplast in Polj 17. However, the results acquired for F-2 indicated that an increased transport from vacuole to cytoplasm could lead to accumulation of ABA in cytoplasm.

There are two possible mechanisms of transmembrane ABA transport: *1*) transport can be driven by an active transport system, and *2*) by diffusion as an undissociated, lipophilic ABAH molecule (Hartung *et al.* 1990). They demonstrated that the movement of ABA across guard cell plasma membrane occurred exclusively by diffusion. Similar results have also been reported for barley (Behl and Hartung 1984), bean (Hartung and Dierich 1983) and alfalfa roots (Wolf *et al.* 1991). In our experiment we assumed the same mechanism of transmembrane ABA transport. This assumption is based on our previous results for Polj 17, which showed that an increase in ABA concentration (from 10^{-4} to 10^{-1} mol m^{-3}) in efflux medium caused highly significant (r = 0.986) linear increase of ABA flux across plasma membrane (Jovanović *et al.* 1992, Jovanović 1998). The highly significant correlation (r = 0.998) between ABA concentrations in cytoplasm and ABA flux across tonoplast also indicated that ABA was transported through tonoplast by diffusion.

Hartung and Slovik (1991) reported that cell pH changes *in vivo* could trigger redistribution of the existing ABA in guard cell compartments and thus increase ABA content at the site of action of guard cells. This could take place long before the beginning of *de novo* ABA biosynthesis. According to Wilkinson and Davies (1997), if ABA accumulates in compartments with higher pH, such pH increase could be a mechanism of ABA accumulation in the vicinity of stomata. In our experiments, medium pH was maintained constant (6.0) and ABA flux across membranes was therefore dependent on cytoplasmatic and vacuolar pH changes. Reduced osmotic potential in the medium resulted in slight pH changes in both compartments (Table 1) and vacuole-cytoplasm-apoplast pH gradients therefore remained nearly constant in Polj 17. However, Heilmann *et al.* (1980) and Daeter *et al.* (1993) reported that drought had an alkalization effect on cytoplasm, resulting in increased ABA transport across root cortex symplast. Our results for Polj 17, however, showed that osmotic stress induced a mild acidification of cytoplasm and vacuole (the inter-cellular pH gradient did not significantly change), which indicates that ABA transport across cortex cells could not be explained by the intracellular pH gradient. Sorbitol, however, had the reverse effect on cytoplasmatic pH in F-2. The cytoplasmatic and vacuolar pH of F-2 corresponds with an effective anion trap (Hartung *et al.* 1982) and could explain the observed increase in cytoplasmatic ABA.

Although our investigations focused on cortex cells alone and excluded the central cylinder, the findings confirmed significant genotypic differences between the investigated lines regarding stress-induced efflux and compartmentation of ABA. In the drought-resistant line Polj 17, osmotic stress activated a mechanism of enhanced ABA transport from cytoplasm to apoplast, although in the drought-susceptible line F-2 it increased the permeability and ABAH flux of tonoplast, which resulted in ABA accumulation in cytoplasm. Based on these results we assumed that efficient transport and redistribution of existing ABA in the investigated lines exposed to osmotic stress is more important than the amounts of stress-induced accumulation of root-sourced ABA. Further investigation based on this data and focused on problems of adaptation and resistance of maize to drought would be highly worthwhile.

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