Metabolic response of roots to osmotic stress in sensitive and tolerant cereals — Qualitative in vivo $^{31}$P nuclear magnetic resonance study

Shanthaa Nagarajan*, Cor Dijkema and Henk Van As

Department of Molecular Physics, Agricultural University, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands

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High resolution $^{31}$P nuclear magnetic resonance (NMR) spectroscopy was used to investigate the changes in phosphate metabolism and intracellular pH in intact root segments of relatively osmotic stress sensitive species maize (Zea mays L) and insensitive species pearl millet (Pennisetum americanum (L) Lecke) exposed to hyper osmotic shock. The results were used to understand the adaptive mechanism of the two species. The hyper osmotic shock resulted in large build-up of phosphocholine and decrease in glucose 6-phosphate (G-6P) and UDPG levels in both the crops. The osmotic shock produced a large vacuolar alkalization and decrease in pH across tonoplast membrane in maize roots. However, the roots of pearl millet were able to adapt to the stress and maintained pH gradient across tonoplast with marginal vacuolar alkalization. This may be attributed to the sustained activity of primary tonoplast pumps and increased activity of H$^+$-ATPase that normally maintain pH gradient across tonoplast.

Water deficit is a major factor limiting crop production in many areas, and consequently, it is important to gain an understanding of how plants respond and acclimatize to water stress. NMR offers a technique which allows the metabolic responses of plant tissues to be investigated non-invasively.

This approach has been used by Spickett et al. to study the response of maize root tips to hyper osmotic shock. They have proposed a model for some of the biochemical events that occur during early stages of adaptation to osmotic stress. It is ideal to use an active tissue like root tips. However, in field conditions, root tips form only a minor portion of the active root mass in the soil. Therefore, in our study, we have used root segments of maize and compared its response to hyper osmotic shock with that of a tolerant species pearl millet to understand the adaptive mechanism to osmotic stress.

Materials and Methods

Plant material for NMR experiments

Seeds of maize (Zea mays L cv. LG 11) and pearl millet (Pennisetum americanum (L) Lecke cv. MH 179) were germinated in sand beds (moistened at the rate of 120 ml/kg of sand. Five days later, they were transplanted carefully in the nutrient tank with 10% Hoagland solution. Roots excised from six days old seedlings in case of maize and secondary roots excised from two weeks old plants in case of pearl millet were used as experimental material after washing five times in deionized water for removing adsorbed nutrient solution.

NMR experiment

Root segments of 6-7 cm length (approximate density of 0.35 g fresh wt.cm$^{-3}$) were placed in a NMR tube (20 mm dia.). Oxygenated buffer (1 mM KCl, 0.5 mM CaSO$_4$, 10 mM Mes[pH 6.0]) was circulated through the sample at a rate of 15 ml/min using a modification of a system described for compressed plant cells. $^{31}$P NMR spectra were recorded on a Bruker AMX 300 spectrometer, operating at a phosphorus frequency of 121.49 MHz, using a double tuned $^{13}$C/$^{31}$P probe head. All the spectra were acquired with a spectral window of 10,000 Hz. Spectra were recorded from tissue using 60°C pulse angle and 0.5 s re-cycle time. Each spectrum was the average of 7200 transients for 1 hr. Each free induction decay (FID) contained 8 k data points and spectra were obtained by Fourier transformation of the FID's that were zero filled to 16 k (applying a Lorentzian line broadening of 25 Hz). Chemical shifts were obtained relative to the signal from a capillary containing 250 mM MDP (resonating at 17.00 ppm relative to the signal from 85% H$_3$PO$_4$). The...
intracellular pH was estimated from a standard curve of chemical shift established as a function of pH. For this, a solution containing 1 mM KH$_2$PO$_4$, 1 mM G-6P, 100 mM KCl and 2 mM MgSO$_4$ was used according to Roberts et al.$^3$.

The tissue was allowed to equilibrate in oxygenated standard buffer for 30 min., before the NMR experiment. It was transferred to the NMR probe and after recording spectra for 2 hr in standard buffer, the circulating solution was changed to the osmotic potential of -1.35 MPa (PEG-6000 solution in buffer gives an osmotic potential of -1.35 MPa, which was calculated using equations described by Money$^6$ and pH was adjusted to 6.0). Hyper osmotic shock of -1.35 MPa was chosen following Spickett et al.$^3$, who have used this potential to produce a hyper osmotic stress for the same maize cultivar LG 11. As the PEG solution was highly viscous, the tuning and matching was re-done and the anaerobic situation was avoided by vigorously stirring the circulating medium with a magnetic stirrer in the reservoir outside the probe. A similar experiment was conducted with pearl millet root segments. For control spectra, the standard buffer was circulated and data recorded for both crops.

Results and Discussion

NMR spectra

The changes in the phosphate resonance when the root segments were exposed to osmotic shock are given in Fig.1 A and B for maize and pearl millet respectively. Levels of ATP represented by β-ATP peak showed that the tissue was healthy till the end of the experiment. Therefore, anaerobiosis was not a problem even with PEG-6000 in the perfusion medium as judged from the stability of ATP levels. Immediately after the shock, there was vacuolar peak broadening and after long hours of exposure to hyper osmoticum (>8 hr), apparently there was a build-up of phosphocholine and a decrease of G-6P and UDPG in both kinds of roots. The results of hyperosmotic shock broadly tallied with those reported by Spickett et al.$^3$ in a similar experimental set-up using maize root tips. The most notable change reported in their four hourly experiment was an increase in phosphocholine and vacucular phosphate contents with a transient increase in cytoplasmic phosphate. In the root segments of both the crops, we noticed a build-up of phosphocholine after 8 hr of hyperosmotic shock. There was an evidence of Pi accumulation in the vacuole since an increase in the width of the vacuolar peak at half height was observed. Roby et al.$^4$ have observed that perfusion of compressed sycamore cells (higher plant cells) with sucrose free culture medium triggered a progressive decrease in G-6P and UDPG
over 30 hr. These changes were accompanied by a \( \text{Pi} \) accumulation in the vacuole and a phosphocholine accumulation in the cytoplasm. These changes have been attributed to the membrane breakdown during stress due to utilization of lipids as an energy source. Such effects were reversed when an external carbon source was added. Therefore, both substrate starvation and osmotic shock led to membrane breakdown and phosphocholine build up in these root segments.

**Intracellular pH**

The changes in cytoplasmic \( pH \) values obtained during control run (only perfusion buffer) and with hyper osmotic shock of -1.35 MPa are shown in Fig. 2 A and B for maize and pearl millet root segments are shown respectively. In maize root segments, the osmotic shock resulted in alkalinization of cytoplasmic \( pH \) immediately after the imposition of the stress, which reduced to control levels with the passage of time. In pearl millet, the osmotic shock produced marginal alkalinization after 5 hr of exposure to osmotic shock which also reduced to control levels with time. The vacuolar \( pH \) changes are given in Fig. 3 A and B for the root segments of both the crops. The \( pH \) gradient across tonoplast (calculated as difference between vacuolar and cytoplasmic \( pH \)) are depicted in Fig. 4 A and B. There was a large vacuolar alkalinization and a sharp reduction in \( pH \) gradient across tonoplast in maize roots. In case of pearl millet, there was marginal vacuolar alkalinization after 5 hr of exposure to the osmotic shock. The gradient in \( pH \) across tonoplast membrane was maintained throughout the stress period with no significant reduction. The large reduction in \( pH \) due to osmotic shock in maize roots may be due to the deceleration of the primary tonoplast pumps (H\(^+\)-ATPase and H\(^+\)-PPase), which normally maintain \( pH \) gradient across tonoplast. But they were able to match the external osmoticum in pearl millet roots, which may be described as its adaptive mechanism to osmotic stress. Similar results were reported by Spickett et al.\(^7\) in the root tips of *Spartina anglica* when challenged with high salt concentrations as compared to maize root tips. *S. Anglica* root tips accumulated sodium more slowly than did maize, with no change in cytoplasmic \( pH \) and relatively small change in vacuolar \( pH \). Association of H\(^+\) extrusion
and cytoplasmic pH regulation in root tips acclimated to low oxygen stress has been reported. Increase in plasma membrane H+ ATPase activity in response to saline stress and nutrition stress have been demonstrated. A plant under stress may require synthesis of compatible solutes as well as increased rates of ion extrusion. Therefore, ability of a plant to tolerate stress and its subsequent adaptation to stress may be determined by its intrinsic capacity to sustain the activity of H+ pumps. However, in higher plants it is incorrect to assume a direct relationship between vacuolar pH maintenance and tolerance to osmotic stress and, it partly explains the tolerance mechanism. There are numerous other physiological adaptations, such as stomatal closure, stimulation of compatible solute production, changes in gene expression and developmental pattern and continued photosynthesis, which play a role in the tolerance of the crop to osmotic stress.

In the root segments of the relatively susceptible crop maize, hyper osmotic stress reduced pH gradient across tonoplast with a large vacuolar alkalization. However, the root of tolerant crop pearl millet was able to adapt to the stress and maintained the pH gradient across tonoplast with marginal cytoplasmic and vacuolar alkalization. This may be attributed to its sustained H+-ATPase activity of plasma membrane.

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References