Jour. Agric. Physics, Vol. 1, No. 1, pp. 156-160 (2001)

Mechanism of Tolerance to Osmotic Stress : Comparative Study by ^{31}P Nuclear magnetic Resonance Spectroscopy and Proton NMR Imaging

SHANTHA NAGARAJAN
Indian Agricultural Research Institute Regional Station, Karnal-132 001

ABSTRACT

The adaptive mechanism of pearl millet to osmotic stress as compared to maize was studied at cellular level using ^{31}P NMR spectroscopy and ^{1}H NMR imaging. *In vivo* spectroscopy of root segments challenged with hyper osmotic shock showed a large build up of phosphocholine and reduction in glucose 6P in both crops. But energy level as given by UDPG did not disappear in pearl millet even after 17h of stress as it did in maize. The osmotic shock produced large vacuolar alkalinization and a decrease in pH across tonoplast membrane in maize roots. But pearl millet was able to adapt to stress with only marginal alkalinization of the vacuole and maintained the pH across tonoplast membrane. The distribution and binding of water in intact young stems of pearl millet and maize were obtained non-invasively by ^{1}H NMR imaging when the roots were exposed to osmotic shock. In maize, the vascular tissue dried and the movement of water to growing shoot regions was stopped and the plant wilted irreversibly. Pearl millet on the contrary, adapted to stress by shrinkage of the stem region and maintained movement of water through diffusion which helped in the recovery of the plant once the stress was relieved.

Introduction

Many physiological and biochemical responses to osmotic shock in higher plants and algae have been well studied (Aspinall and Paleg 1981; Morgan 1982; Rhodes 1987). But not much work has been done on *in vivo* mechanism by which plants perceive water stress and transduce it into biochemical response. High resolution ^{31}P NMR technique permits the study of intracellular pH regulation, phosphate compartmentation and energy metabolism non-invasively. NMR imaging is largely based on the deduction of the water signal and can reveal information about water distribution and binding in a tissue, spatial dependence of the relaxation times and some time dependent property of the image. Both these techniques were used to compare the response of relatively tolerant species pearl millet (*Pennisetum americanum*(L) Leeke) with that of susceptible plant maize (*Zea mays* L.) to osmotic shock to understand the their adaptive mechanism.

Materials and Methods

Seeds of maize (cv. LG 11) and pearlmillet (cv. MH 179) were germinated in sand bed and transferred to a tank containing nutrient solution(50% Hoagland) in a green house under natural light condition. Roots excised from 6 days old seedlings in case of maize and secondary roots excised from two weeks old plants in case of pearl millet were used for ^{31}P NMR experiments. One month old plants with 6 fully emerged leaves were used for the NMR imaging experiments.

Experiment I : Root segments of 6-7 cm length at an approximate density of 0.35g FW cm^{-3} were placed in a 20mm diameter NMR tube and oxygenated buffer(1mM KCl, 0.5mM CaSO44, 10mM Mes[pH 6.0]) was circulated through the sample at a rate of 15ml.min^{-1} to keep the tissue metabolically active. ^{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. The pH values of the intracellular compartments were calculated from the chemical shifts of Pi resonance after construction of calibration curves as described by Roberts *et al.*(1981). After recording spectra for 2 h in standard buffer, the circulating solution was changed to the osmoticum (PEG-6000 solution in buffer to give an osmotic potential of -1.35 Mpa) and hourly spectra were recorded for 17h. For control spectra, the standard buffer was circulated for 16h and spectra were recorded.

Experiment II: One month old plants with 6 fully emerged leaves were used for the imaging experiments. The roots were kept in nutrient medium with aeration below the probe and images were obtained of the stem portion 15 cm below the apex. The spectrometer part of the MRI instrument

consists of Surrey Medical Instruments Systems operating at 20.35 MHz. The probe (10 X10 X20cm) is custom engineered (Doty Scientific Inc.,USA) containing a solenoid RF coil of 3cm length and diameter, surrounded by a set of active shielded gradients. The magnetic field of 0.47 Tesla was generated with an electromagnet system and the spectrometer was operated at 20.35 MHz. Images were calculated in a 128X128 matrix after Fourier transformation. The control images were taken with roots in the aerated nutrient medium after equilibrating the plants in the laboratory condition. The nutrient medium was replaced by a solution of aerated PEG-6000 in nutrient medium giving an osmotic potential of -0.9 MPa by a capillary and pump system without disturbing the position of the stem in the probe. The images were taken at different intervals of time till the plants showed visible symptoms of wilting. Then the rooting medium was reversed to nutrient solution and recovery was observed.

Results and Discussion

Experiment I : The changes in the phosphate resonance when the root segments were exposed to osmotic shock are given in Fig.1A&B for maize and pearl millet, respectively. In maize, immediately after the shock, there was vacuolar peak broadening (Pi accumulation) and after long hours of exposure to hyper osmoticum (>8h), apparently there was build up of phosphocholine and a decrease of glucose 6P and UDPG in both crops. In case of pearl millet, there was build up of phosphocholine and vacuolar broadening, but the level of UDPG was maintained even up to 17h of stress. Similar increases in phosphocholine and vacuolar phosphate contents were reported by Spickett *et al.*(1992) in maize root tips in a 4h experiment. This progressive decrease in glucose-6P and UDPG levels accompanied by Pi accumulation in the vacuole and phosphocholine in the cytoplasm have been attributed to membrane breakdown during stress due to utilization of lipids as an energy source (Rhodes, 1987). The changes in cytoplasmic and vacuolar pH values and the pH gradient across tonoplast (calculated as the difference between vacuolar and cytoplasmic pH) obtained during control run with only perfusion buffer and with hyper osmotic shock of -1.35 MPa for maize and pearl millet root segments are shown in Fig.2&3, respectively. In maize root segments, the osmotic shock resulted in large vacuolar alkalinization and a sharp reduction in pH gradient across tonoplast. But in pearl millet, the osmotic

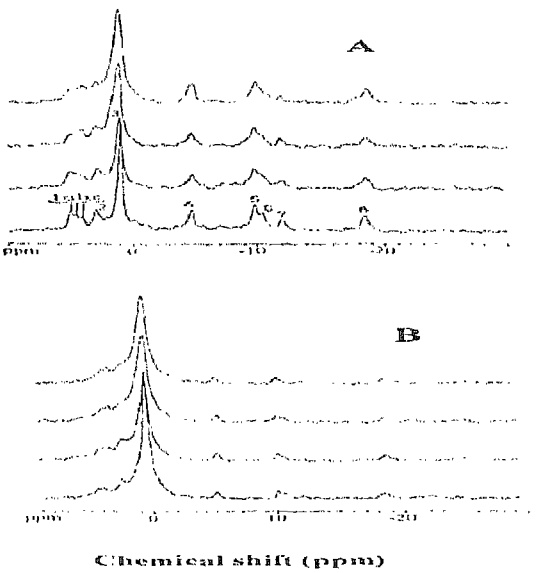

Fig. 1. ^{31}P NMR spectra of root segments treated with hyper osmotic shock of -1.35 MPa using PEG-6000 in the perfusion buffer: (A) maize (B) pearlmillet. The resonance assignments are as follows: 1a, Glucose 6P; 1b, Fructose 6P and other phosphomonoesters; 1c, phosphocholine; 2, cytoplasmic phosphate; 3, vacuolar phosphate; 4, γ-phosphate of NTP and β-phosphate of nucleoside diphosphate; 5, α-phosphates of NTP and nucleoside diphosphate; 6, UDPG and NAD(P)(H); 7, UDPG and 8, β-phosphate of NTP. Base spectra is that of control with only buffer and the subsequent ones are those taken every 4h after changing to PEG solution in buffer.

shock produced small alkalinization vacuole and almost no change in the pH gradient across tonoplast. In both cases there was some transient alkalinization of cytoplasm. The large reduction in δpH due to osmotic shock in maize roots may be due to the dissipation of the primary tonoplast pumps (H+-ATPase and H+-PPase) that normally maintain pH gradient across tonoplast (Reuveni *etal.* 1987). But root segments of pearl millet were able to match the external osmoticum for a longer period with minimum change in δpH which may be described as its adaptive mechanism to osmotic stress.

Experiment II : The images constructed by ir-cpmg and pfg-cpmg sequences for control and stress conditions are shown in Fig.4&5 for maize and pearl millet stems, respectively. In maize, the plant showed wilting symptoms after 20h of stress and failed to recover when the root medium was

changed to nutrient solution. The amplitude images (Fig.4A a&b) of the stem taken when the roots were in nutrient solution and when they were in PEG-6000 solution respectively indicated a decrease in the amplitude due to osmotic stress. This showed that the stem did not act as a reservoir of water for supply to the growing regions. In case of flax stems during dehydration the older stem may loose water to the young stem part (Boyer, 1972). Such adaptations were missing in maize plant. The R1 and R2 weighted images showed regions near the periphery and at the centre with higher R1 s and R2s (lower T1 & T2 s). In squash stem, the proton NMR imaging showed relatively shorter T1s for the xylem tissue which has been explained by the authors as due to the strong binding of a portion of water to cellulose cell walls in the conducting cells (Veres *et al.*, 1991). By analogy, the regions near the periphery and at the centre with higher R2s (lower T2s) may be identified with vascular bundles. The decrease in the diffusion constant (Fig.4Db) after the imposition of stress also showed that movement of water to the apical region through diffusion was reduced. This perhaps was the reason for the complete wilting of the plant in 20h of osmotic stress and no recovery when reversed back to nutrient medium. In pearl millet, the plant showed wilting symptoms only after 70h of stress and was able to recover nearly to the unstressed status when transferred to the nutrient medium. The amplitude, R1 and R2 weighted images obtained under normal and stress

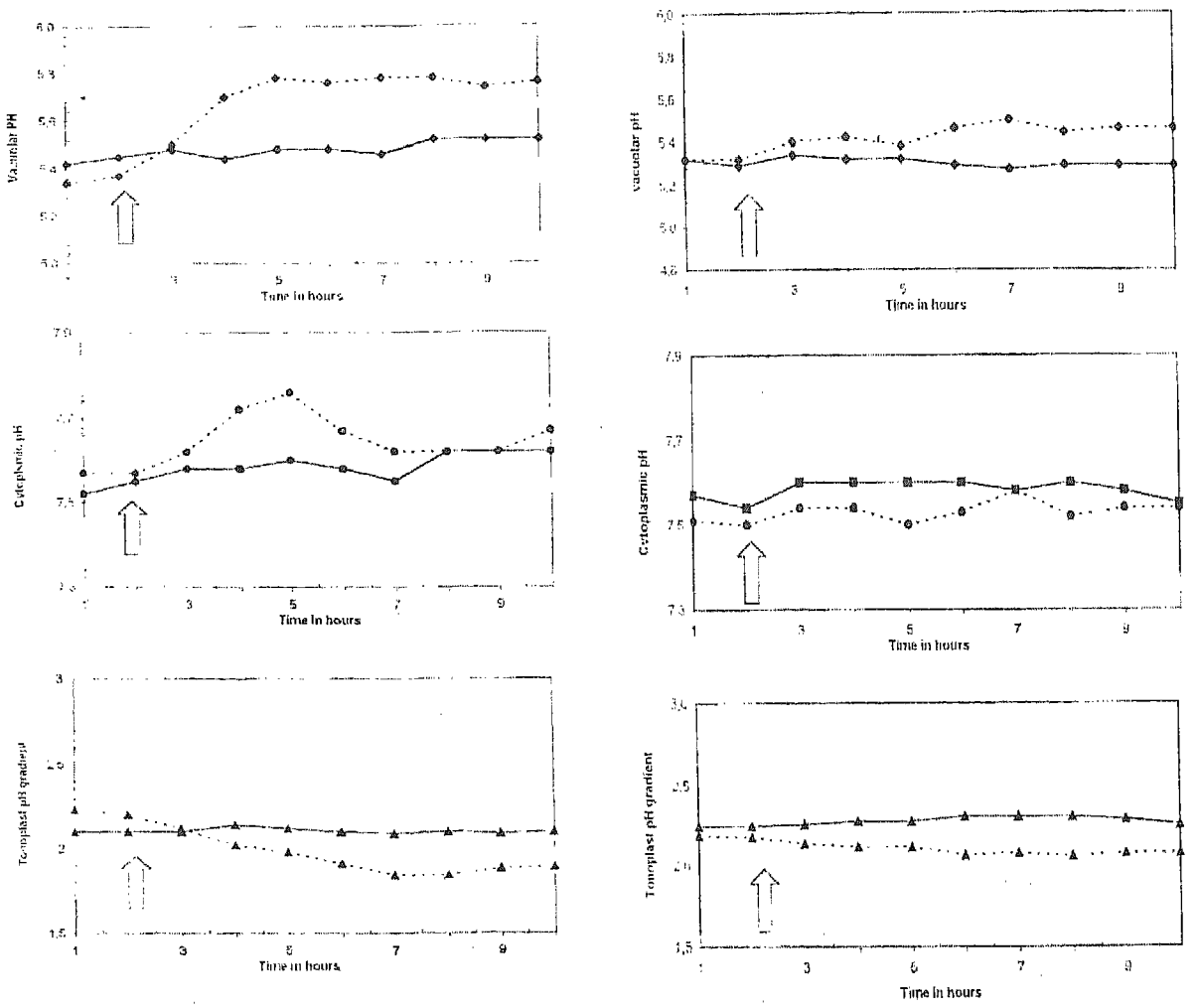

Fig. 2&3. Time course changes in vacuolar pH, cytoplasmic pH, pH gradient across the tonoplast of excised root segments of (2) maize and (3) pearlmillet as affected by -1.35 MPa PEG-6000. Each point was an average of two files. Solid lines are for control and dotted lines are for stress. Arrow indicates the imposition of osmotic shock.

conditions are given in Fig. 5. The most notable change was 12 % shrinkage in the tissue in 70 h of osmotic stress (Fig.5 Ab,R1b & R2b). During a period of soil drying, cell turgour decreases and plant tissues may shrink more or less progressively during the day if atmospheric conditions are conducive to high transpiration throughout the drought (Kozlowski, 1972). Therefore, the shrinkage in pearl millet stem suggests that transpiration continued even under stress. The increase in the intensity of amplitude weighted images during stress can be explained as an increase in number of protons per pixel as a result of shrinkage. The diffusion weighted images taken after the imposition of the stress (Fig. 5 Db) showed that there was an increase in the diffusion constant in the pith parenchyma region which indicated that there was movement of water to the growing apical regions of the stem through diffusion. This continued transport of water to the growing part of the plant even under osmotic stress could have helped the plant to recover once the stress was relieved.

Conclusion

Both high resolution Phosphorus NMR spectroscopy and proton NMR imaging have non-invasively shown the better adaptation mechanism of pearl millet to osmotic stress as compared to maize. The pearl millet root segments were able to withstand the hyper osmotic shock with minimum changes in intracellular pH values and energy levels

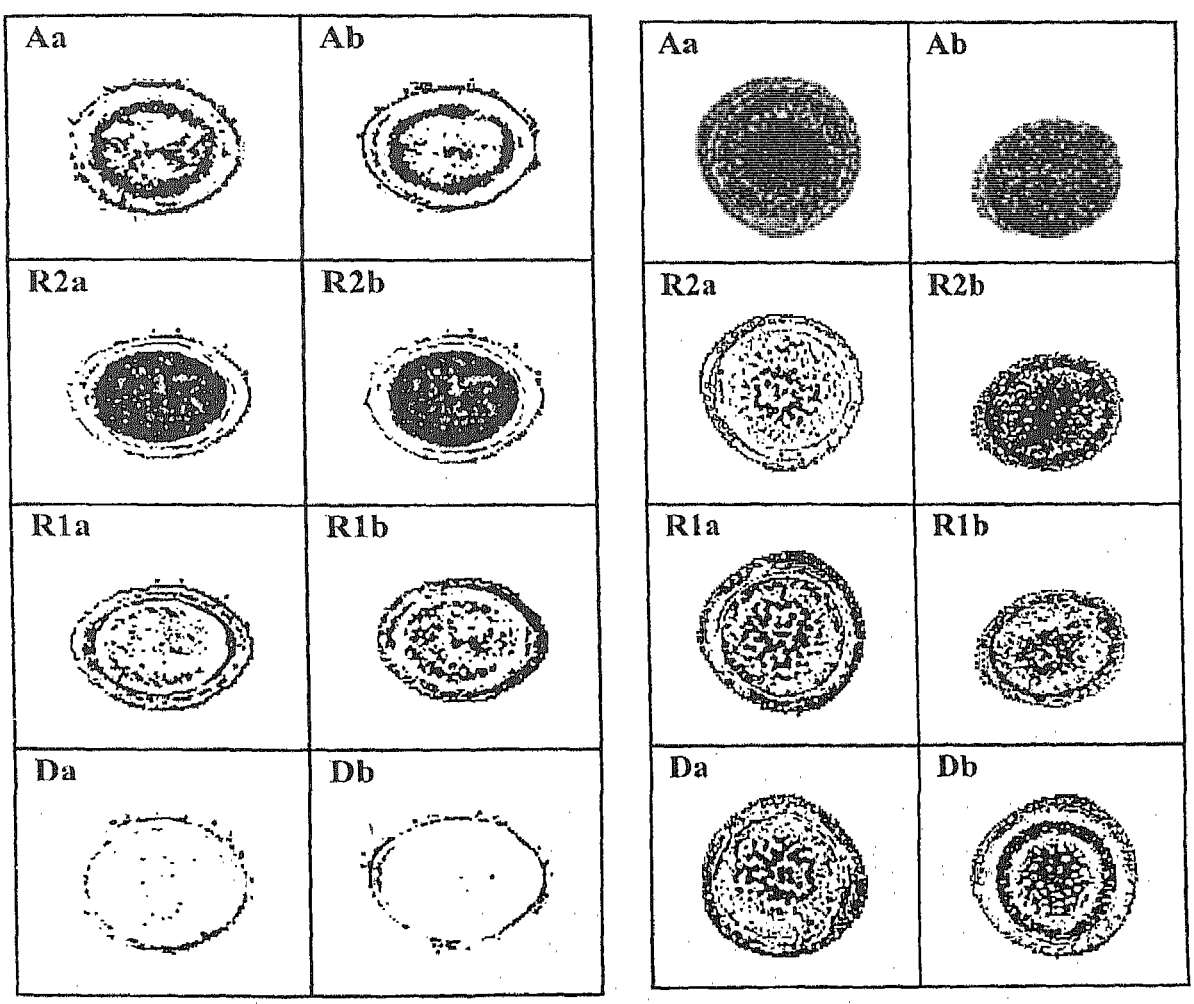

Fig. 4&5. ^1H NMR images of one month old (4) maize and (5) pearl millet stem with roots in nutrient medium or in PEG-6000 solution of -0.9 MPa potential for 20h by inversion recovery spin echo method and pulsed gradient spin-echo method. A-Amplitude weighted images; R_2-R_2 weighted images; R1-R_1 weighted images and D-Diffusion weighted images. a-control with roots in nutrient solution; b-stress with roots in PEG-6000 solution.

as compared to maize root segments. In the intact plant when the roots were kept in osmoticum, pearl millet adjusted to the stress by shrinkage of the stem tissue and continued movement of water to the apical regions through diffusion. But these adaptations were missing in maize stem which resulted in the permanent wilting of the plant.

Acknowledgements

The author wants to acknowledge the Commission on European Communities for the award of 'Marie Curie' post-Doctoral fellowship.

References

Aspinall, D. and Paleg, L.G. 1981. Proline accumulation: Physiological aspects. In: The physiology and Biochemistry of drought Resistance in Plants (Eds. Paleg, L.G and Aspinall, D.), Pp. 205-240, Academic Press, New york.

Boyer J.S. 1972. Resistance to water transport in soybean,bean and sunflower. *Crop Science*, **11** : 403-407.

Kozlowski T.T. 1972. Shrinking and swelling of plant tissues. In: Water deficits and plant growth (Ed. T.T. Kozlowski), Vol.III. pp. 1-57, Academic Press, New York.

Morgan, J.M. 1982. Osmoregulation and water stress in higher plants. *Annul Review of Plant Physiology*, **35** : 299-319.

Reuveni M., Colombo R., Lerner H., Pradet A and Poljakoff-Mayber A. 1987. Osmotically induced proton extrusion from carrot cells in suspension culture. *Plant Physiology*, **85** : 383-388

Rhodes, D. 1987. Metabolic responses to stress, In: The Biochemistry of Plants, Vol.12, (Ed. D.D. Davies), Pp. 201-204, Academic Press, San Dieago.

Roberts J.K.M., Wade-Jardetzky N. and Jardetzky O.1981. *Intracellular pH measurements by 31P nuclear magnetic* resonance. Influence of factors other than pH on 31P chemical shifts. *Biochemistry*, **20** : 5389-5394

Spickett C.M., Smirnoff N. and Ratcliffe R.G. 1992. Metabolic response of maize roots to hyperosmotic shock. An *in vivo* 31P nuclear magnetic resonance study. *Plant Physiology*, **99** : 856-863.

Veres J.S., Cofer G.P. and Johnson A.G. (1991) Distinguishing plant tissues with magnetic resonance microscopy. American *Journal of Botany*, **78** : 1704-1711.