THE EFFECT OF OSMOTIC PRESSURE ON P^{32}-PHOSPHATE ABSORPTION BY EXCISED RICE ROOTS AND THE ADAPTATION OF RICE ROOTS TO VARYING OSMOTIC PRESSURES[1]

TEH-CHIEN SHEN and YUH-JANG SHIEH[2]

(Received January 22, 1966)

The forces involved in the transfer of water from soil to plant roots may be attributed mainly to the hydrostatic pressure and the osmotic potential of soil water (Day, 1942). In general, plant growth is retarded by a high moisture potential of the soil. Magistad and Truog (1943) reported that the osmotic pressure (OP) of a soil solution at the wilting point (15 atm) varies greatly in different soils. Successful crop production was found on soils which had the OP of 4 atm or less. Investigations on the relationship between external OP and plant reaction reviewed by Stocking (1956) were concerned mostly with soil salinity and salt species. Part of the work showed that xerophytic species had cell saps of a high OP.

Stoddard (1953) and Herrick (1933) observed that soil moisture and humidity were the major factors affecting the OP of plant tissues. Increased drought conditions resulted in an increasing osmotic value throughout the aerial parts of the plants. A measure of the osmotic values was a reliable index of the relative potential ability of a plant to compete for water under conditions of stress due to a deficiency of water.

Onodera (1925) found that the OP of the cell sap of upland rice was not sensitive to a slight change in soil moisture at the moist state but showed a remarkable elevation at limiting dryness. Paddy rice, however, was more sensitive to slight changes in soil moisture in the moist state, but did not show any remarkable elevation at limiting dryness. Kishida (1935) reported that the OP of the upland rice was higher than that of the paddy rice; the OP of drought-resistant varieties was higher than that of non-drought-resistant varieties.

Danielson and Russell (1957) measured the amount of rubidium absorbed by corn seedlings from aqueous media of different OPs from 0 to 12 atm and

(1) This study was partially supported by the National Council on Science Development.

(2) Associate Research Fellow of Institute of Botany, Academia Sinica, and concurrently Associate Professor in the Department of Agronomy, National Taiwan University and Research Assistant in Dept. of Agron., respectively.

concluded that OP did not have a direct influence upon the intake of mineral nutrients.

This report presents the data concerning the absorption of P^{32}-phosphate by excised roots of paddy and upland rice plants and the adaptability in nutrient absorption of rice roots to the OP of external solutions.

Materials and Methods

Preparation of root materials: Seeds of a paddy rice variety, Taichung No. 65 and an upland variety, Nunghsuan No. 1 were soaked in tap water at 28–30°C for two days. When the seminal roots started to emerge from the husk, the seeds were spread on a layer of cheesecloth which was supported by an aluminum frame 15 cm in diameter. The frame was set on a large petri dish. The corners of the cheesecloth were dipped into the water in the dish. A second cheesecloth was spread over the seeds. The cover of the petri dish was placed over the seeds to reduce evaporation. The roots were grown at 28–30°C in the humid atmosphere (R_A) or in the water (R_{M0}) underneath the cheesecloth. Roots designated as R_{M2} were grown in water first, then transferred to a mannitol solution of the OP of 4.97 atm for 1 day. R_{M4} roots were further transferred into mannitol solution of the OP of 9.94 atm for another day. The OP of the mannitol solution was calculated using the Morse equation. R_A and R_{M0} roots were excised just below the cheesecloth when they attained the length of 2–3 cm. R_{M2} and R_{M4} roots were cut 2–3 cm from the tips.

Absorption experiments: Two mM KH_2PO_4 solution was prepared for the absorption experiments. The OP of solutions was adjusted with mannitol. Excised roots were rinsed with distilled water, and gently blotted on dry soft lintless paper. One gram samples were weighed out and transferred into a 250 ml Erlenmeyer flask. One hundred ml of the experimental solution was then poured in. Radioactive phosphate[(3)] was added into the flasks at the beginning of the absorption period. The flasks were placed in a water bath maintained at 30°C. The solutions were aerated. After the 3-hour absorption period, roots were separated from the solutions with a copper screen, washed, evenly spread in planchets of the diameter of 5 cm, and then dried at 40°C.

Radioactive assay: The samples were counted with a thin window (1.4mg/cm^2) Geiger-Müller tube connected to a conventional scaler. In most cases, samples were counted to more than 10,000 counts during the 10-minute measuring period. The dry weight of the roots of each sample was determined. Counts per minute per mg dry weight of root was used as the measurement of absorption.

(3) Courtesy of Dr. S. J. Yeh, Institute of Nuclear Science, National Tsing Hua University.

Results

R_A roots of paddy and upland rice were washed on the copper screen with 300 ml of water for less than 5 seconds after the absorption period. The amount of phosphate absorbed by upland rice roots decreased with increasing OP of the experimental solutions. This trend was less evident in paddy rice roots (Fig 1). However, when roots were soaked with a large amount of water for a long period, marked decreases in P^{32} absorption with increasing OP of test solutions was observed in both upland and paddy rice roots (Fig 2). It was evident that a large proportion of P^{32} activity in roots, which absorbed phosphate from solutions of high OP (above 7 atm), was lost into the soaking water. Preliminary work showed that one-hour soaking in 6 changes of 200 ml of water was enough for obtaining equilibrium. During the absorption period, R_A roots plasmolyzed in the solutions of 7.46 atm OP. They plasmolyzed seriously in the solutions of the high tonicities of 9.94 and 12.43 atm.

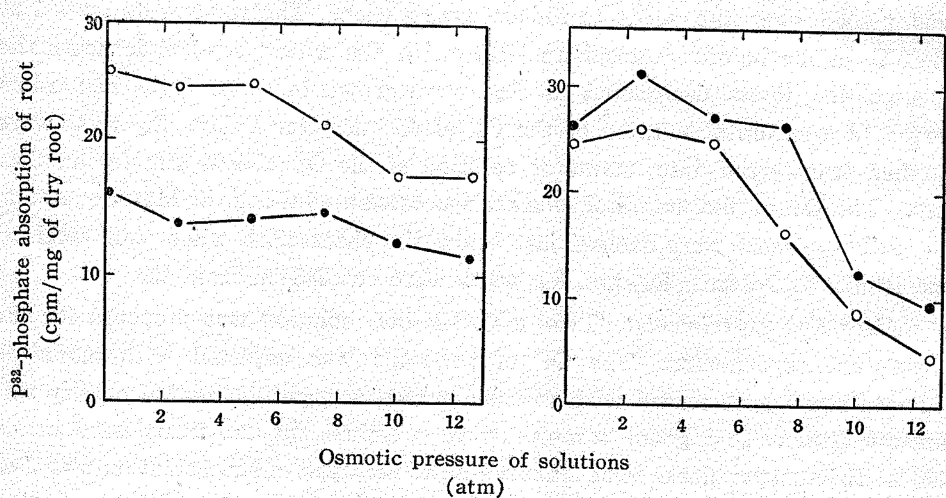

Fig. 1 (left). Absorption of P^{32}-phosphate by R_A roots in solutions of different OPs. 1.60 μc P^{32} was applied in each 100ml test solution. Roots were washed with 300ml of water for about 5 seconds after absorption. •, paddy rice; ○, upland rice.

Fig. 2 (right). Absorption of P^{32}-phosphate by R_A roots in solutions of different OPs. 1.55 μc P^{32} was applied in each 100ml test solution. Roots were soaked in 6 changes of 200 ml of water in an one-hour period. •, paddy rice; ○, upland rice.

Further experiments were conducted with roots grown in solutions of different OPs. R_{M0} roots of both paddy and upland rice varieties acted similarly to R_A roots. R_{M2} and R_{M4} roots absorbed considerable amounts of P^{32}-phosphate from the test solutions of the OPs of 9.94 and 12.43 atm (Fig 3). It seemed that upland rice roots acquired a higher adaptability to high OP than paddy rice roots. Figure 3B shows that maximum absorption of upland rice roots occurred in test solutions whose OP was higher than those of the cultural solutions of these roots. R_{M0} roots started to plasmolyze in the test solutions of the OP of 7.46 atm as R_A roots did. R_{M2} roots plasmolyzed only slightly in the test solutions of 12.43 atm OP. R_{M4} roots kept turgid in all of these test solutions. Roots grown in solutions of high OP had more dry matter in the 1-gm fresh root samples than those grown in water or solutions of low OP. Average dry weight of the samples of R_{M0}, R_{M2} and R_{M4} roots were 64.9, 81.7 and 93.7 mg for paddy rice and 52.8, 79.8 and 90.4 mg for upland rice, respectively.

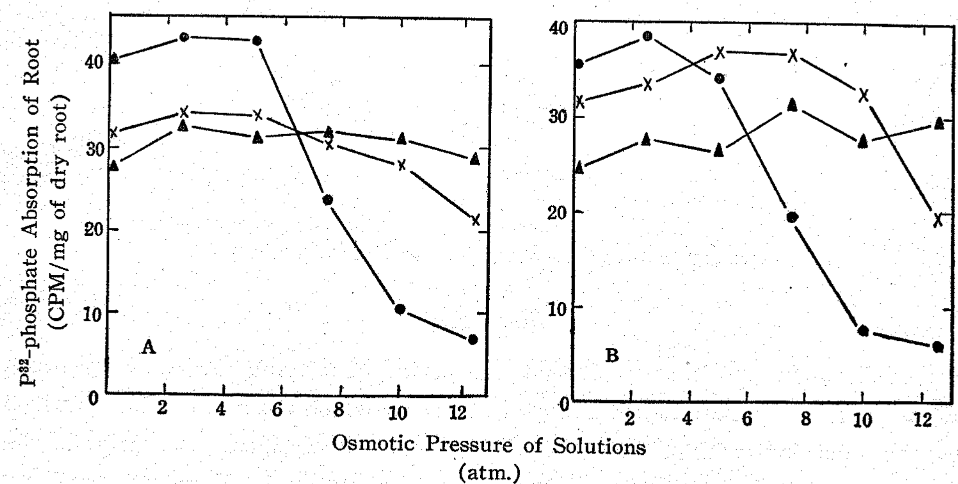

Fig. 3. Absorption of P^{32}-phosphate by R_{M0} (•), R_{M2} (×) and R_{M4} (▲) roots of paddy (A) and upland (B) rice in solutions of different OP. 1.52 μc P^{32} was applied in each 100 ml test solution. Roots were soaked with water for 1 hour after absorption period.

Two sets of R_{M0} and R_{M4} root samples of upland rice were prefed with P^{32}-phosphate in 2mM KH_2PO_4 solution. The samples were washed with 300 ml of water and then immersed in mannitol solutions of different OPs for two hours. The roots were again soaked in water for an hour before drying and counting. It was found that a great amount of prefed P^{32} in the samples treated with mannitol solutions of high concentrations was lost from R_{M0} roots during the soaking period. The amount of P^{32} remained in these roots (Fig 4) was closely similar to the accumulation curve of R_{M0} in Fig 3B. On the other hand, the amount of prefed P^{32} in R_{M4} roots was not affected by the immersion into mannitol solutions.

All data presented in this report are the averages of two replications. The results obtained from experiments conducted at different times showed the same tendency.

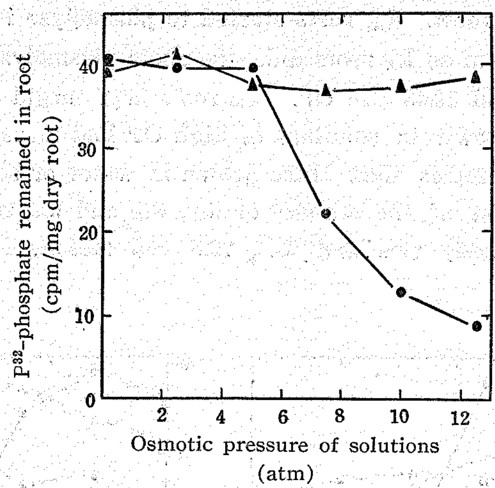

Fig. 4. The loss of prefed P^{32}-phosphate from R_{M0} (•) and R_{M4} (▲) roots of upland rice caused by 2-hour immersion in mannitol solutions of different OP. The samples were soaked in 6 changes of 200 ml of water for one hour before drying.

Discussion

Preliminary work showed that the period of soaking did not have much effect on P^{32}-phosphate accumulation in R_A roots which absorbed in the solutions of the OPs lower than 7.46 atm. The amount of phosphate remained in the R_A roots treated in solution without mannitol was only slightly reduced by the one-hour soaking in water. It was increased a little in the R_A roots that had absorbed in solutions of the OPs of 2.48 to 7.46 atm. Thus, a peak of absorption generally appeared in this region (Fig 2 and 3). It was noted that the roots treated with mannitol solutions tended to absorb atmospheric moisture during the cooling and weighing period if the residual mannitol adhering on the root surface was not thoroughly washed away. Precaution was paid to avoid this to happen by cooling samples in a desiccator before weighing. However, a tendency in increasing dry weight with increased mannitol concentrations still existed when roots were only briefly washed with 300 ml of water after absorption. This may account for the fact that the peak of absorption was not observed in the first experiment (Fig 1) when counts per minute per mg dry weight of root was used as the measurement of absorption.

Ferguson *et al.* (1958) reported that mannitol could be classified as unable to function as a carbon source for tomato root culture. One percent mannitol in a sucrose containing medium reduced the growth value. Thimann *et al.* (1960) reported that washed potato discs floated on a hypertonic solution of mannitol or Carbowax 1500 lost weight at first and then after periods between 3 hours and 3 days, gained weight again. It was found that little externallyapplied mannitol entered into the discs. The osmotic recovery was thus mainly attributed to the temporary liberation in the cells of osmotic material. When rice roots of intact seedlings were transfered from water to 4.79 atm mannitol solution in this experiment, the rate of growth was only slightly slowed down. When they were further transfered into 9.94 atm mannitol solution, growth was halted temporarily. R_{M4} roots used in this experiment were harvested when they remained in this solution for 24 hours and had resumed growth.

It was evident that both paddy and upland rice roots acquired the adaptability of growth and absorption in iso- or even hypertonic media. The adaptation was manifested by the increases in dry weight and P^{32} accumulation from solutions of high tonicity. In the intact rice roots, reserve materials from seeds might serve as a source of osmotic active materials in addition to those released by the root cells. The increased OP of protoplasm enabled the cells to keep turgid in solutions of high tonicity. This is believed to be essential for ion accumulation in rice roots. When roots plasmolyzed in the test solutions, a large portion of the phosphate ions which had entered the roots might be lost to the outside during subsequent soaking in water. Two possible reasons may be considered. A. The free space of roots might be markedly increased when roots were plasmolyzed in hypertonic solutions. The ions which diffused into this space might be washed out during the soaking period. Preliminary work using 16.8μc P^{32} in each 100 ml test solution showed that there was a rising tendency in absorption when the OP of the solution increased to 7.46 atm. The amount of P^{32} applied in the present work was reduced to 1.5–1.6μc in each 100-ml test solution so as to minimize this effect. B. The permeability of the cell membrane to phosphate ions might be increased by plasmolysis and thus increased the rate of the exosmosis of phosphate from root cells. Stiles and Jørgensen (1915, 1917) found that inorganic salts and organic substances increased the exosmosis of plant tissues. The rate of exosmosis was a measure of the toxicity of the solutes in external solutions; the higher the concentration the more rapid the exosmosis. Helder (1956) mentioned that phosphate and other inorganic salts were usually present in the exudate of roots and other plant tissues along with easily diffusible part of the cell constituents as the consequence of increased permeability of cell membrane induced by salts, toxic materials or metabolic inhibitors. Figure 4 shows that prefed P^{32}-phosphate might be lost from R_{M0} roots but not from R_{M4} roots to the mannitol solutions of high OP. The exosmosis happened only on plasmolyzed roots. The amount of P^{32} remaining in R_A roots was closely similar to the absorption curve of the same kind of roots (Fig 3B). Therefore, the decrease in accumulation may be accounted for mainly by the increased exosmosis caused by plasmolysis. Burg *et al.* (1964)

reported recently that a high concentration of mannitol and other suitable osmotic agents prevented the exosmosis of sugar and water loss from apple, other fruits and pea sections. In these cases, the OPs of the experimental material should be considered. The fresh weight of apple tissue sections did not change markedly even in solutions of 20–25 atm OPs. On the other hand, there was a marked tendency to increase sugar loss from the young tissue of etiolated pea seedlings when the concentration of external KCl solution exceeded 0.25 M (Burg et al. 1964, Fig 9).

Summary

The absorption of P^{32}-phosphate by excised roots of a paddy and an upland rice variety was studied. The osmotic pressure of the experimental solutions was adjusted with mannitol.

The amount of P^{32}-phosphate absorbed by excised upland rice roots decreased with increasing osmotic pressure of the solutions. This trend was less marked for paddy rice roots. If active absorption was expressed by the amount of ions taken up into the cells and maintained therein against loss, the ability of ion accumulation of both paddy and upland rice roots was greatly reduced by high osmotic pressure of the solution.

Rice roots adapted to high external osmotic pressure by increasing internal osmotic pressure and dry matter content. This adaptation enabled rice roots to accumulate phosphate from solutions of high osmotic pressure as well as from hypotonic solutions.

Plasmolysis accompanied the decrease in phosphate accumulation. It was suggested that plasmolysis increased the permeability of cell membrane and enhanced the exosmosis of phosphate from root cells.

滲透壓對稻根吸收放射性磷酸量之影響及稻根對滲透壓之適應力

申德建　　謝昱暐

根之吸水受土壤水分含量之影響。本試驗係以甘露醇調節溶液之滲透壓，而觀察切離之水稻及陸稻根在不同水分供應情況下，養分吸收量所受之影響。

稻根吸收放射性磷酸量，因溶液滲透壓之增高而減少，此種趨勢在陸稻較為明顯。

在滲透壓高於 7.46 大氣壓力之溶液中，稻根發生脫水現象。根所吸收之磷酸，因脫水而流失於根外。脫水之程度愈大，根內可蓄積之磷酸量愈低。此種現象，可能由於細胞質膜之可透性因脫水而增大。

稻根對外界之滲透壓有適應能力，在滲透壓較高之溶液中培育之稻根，其細胞液之滲透壓及乾重均較高。因此，在高濃度之甘露醇溶液中，可保持其膨壓，並蓄積多量之磷酸。以此觀之，陸稻根對滲透壓之適應力較高，在比其培養溶液滲透壓較高之溶液中，可表現最高之養分吸收能力。

Literature Cited

BURG, S. P., E. A. BURG and R. MARKS. Relationship of solute leakage to solution tonicity in fruits and other plant tissues. Plant Physiol. **39**: 185-195. 1964.

DANIELSON, R. E. and M. B. RUSSELL. Ion absorption by corn roots as influenced by moisture and areation. Soil Sci. Soc. Amer. Proc. **21**: 3-6. 1957.

DAY, P. R. The moisture potential of soil. Soil Sci. **54**: 391-400. 1942.

FERGUSON, J. D., H. E. STREET and S. B. DAVID. The carbohydrate nutrition of tomato roots. V. The promotion and inhibition of excised root growth by various sugar and sugar alcohols. Ann. Bot. **22**: 513-524. 1958.

HELDER, R. J. The loss of substances by cells and tissues (salt glands). in: Ruhland, W. (ed) Encyclopedia of Plant Physiology. Vol. II: 468-488. 1956.

HERRICK, E. M. Seasonal and diurnal variations in the osmotic values and suction tension values in the aerial portions of *Ambrosia trifida*. Amer. J. Bot. **20**: 18-34. 1933.

KISHIDA, K. On the adaptation of the osmotic pressure in the root of rice plants. J. Sapporo Soc. Agr. Fores. **26**: 401-434. 1935.

MAGISTAD, O. C. and E. TRUOG. Soil solution concentration at the wilting point and their correlation with plant growth. Soil Sci. **55**: 351-360. 1943.

ONODERA, J. On the studies of drought resistance, morphological and physiological modifications and variations of yields for various soil-moisture contents in rice plants. Proc. Crop Sci. Soc. Japan **3**: 91-116. 1925.

STILES, W. and I. JØRGENSEN. Studies in permeability. I. The exosmosis of electrolytes as a criterion of antagonistic ion action. Ann. Bot. **29**: 349-367. 1915.

STILES, W. and I. JØRGENSEN. Studies in permeability. IV. The action of various organic substances on the permeability of the plant cell and its bearing on Czapek's theory of the plasma membrane. Ann. Bot. **31**: 47-76. 1917.

STOCKING, C. R. Osmotic pressure or osmotic value. in: Rubland, W. (ed) Encyclopedia of Plant Physiology. Vol. II: 57-70. 1956.

STODDARD, L. A. Osmotic pressure and water content of prairie plants. Plant Physiol. **10**: 661-680. 1953.

THIMANN, K. V., G. M. LOOS and E. W. SAMUEL. Penetration of mannitol into potato disks. Plant Physiol. **35**: 848-853. 1960.