Plant and Soil 103, 245–250 (1987)

PLSO 7021

The response of two rice cultivars to external Na/Ca ratio

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Received 15 September 1986. Revised April 1987

Key words: calcium deficiency, *Oryza sativa*, salinity, sodium:calcium ratio

Abstract

The response of the rice cultivars 'M9' and 'M-201' to nutrient cultures salinated at −0.4 MPa with varying ratios of Na and Ca was studied. Although the dry matter production of both cultivars was sensitive to the Na/Ca ratio, this correlation was significant only for M-201. Calcium nutrition was severely affected by the composition of the external solution, and the laminae exhibited Ca-deficiency symptoms at Na/Ca molar ratios of 78 and 198. Sodium concentration in the shoot decreased as the Na/Ca ratio in the external solution decreased. Patterns of Na and Cl distribution in the shoot tissues were similar; both ions were accumulated preferentially in the tillers and older leaves. The Na-induced inhibition of Ca uptake and transport appears to be more limiting to shoot growth of M9 and M-201 than Na toxicity per se.

Introduction

Rice (*Oryza sativa* L.) is often grown successfully during the reclamation of soils that not only have a substantial exchangeable sodium percentage (ESP), but also are low in available calcium (Pearson and Ayres, 1960). Several procedures for evaluating salt tolerance of rice cultivars are based on this relative resistance to high Na/Ca ratios in the root media. In the Ponnamperuma screening technique (Ponnamperuma, 1976), rice seedlings are grown in clay soils treated with 0.5% NaCl. The salinity level of this medium, as measured by the electrical conductivity of the saturated-soil extract (EC_e) is 8–10 dS · m^{-1}. A R Yeo, T J Flowers and their co-workers at the University of Sussex generally use nutrient solution cultures salinated with NaCl (50 mol m^{-3}) for their physiological and anatomical studies of salt-stressed rice (Flowers *et al.*, 1985; Flowers and Yeo, 1981; Yeo *et al.*, 1981; Yeo and Flowers, 1982, 1983, 1984). Although seedlings of the elite breeding line, 'IR 2153-26-3-5-2,' were moderately tolerant of NaCl at this concentration (Flowers and Yeo, 1981), the Na/Ca ratios in the cultures had no appreciable influence on either leaf -Na or shoot growth over a wide range of Na/Ca ratios (5 to 500) (Yeo and Flowers, 1985). Likewise, both survival rate and Na accumulation in the shoots of the cultivars 'Yamabiko' (Yamanouchi *et al.*, 1983) and 'Akebono' (Kawasaki and Moritsugu, 1973) were virtually unaffected by the level of Ca in solution if the root media contained less than 0.25% Na. However, at higher Na (0.50 to 1.25%), the performance of Yamabiko was significantly better at 40 ppm Ca than at 4 ppm Ca (Yamanouchi *et al.*, 1983).

The objective of this study was to assess the response of rice grown in iso-osmotic nutrient cultures (osmotic potential = −0.4 MPa) of varying Na/Ca ratios. Seedling performance was evaluated with respect to dry matter production, visual symptoms of ion toxicity/deficiency injury, shoot element content, and distribution of elements in shoot organs.

Materials and methods

Two rice cultivars, 'M9' and 'M-201', were chosen as the experimental plants. Both cultivars are short-statured, early maturing, medium grain types. M-201 has the distinct yield advantage and has generally replaced M9 in most of the rice growing areas of California (Carnahan *et al.*, 1978; 1982). Seeds were sterilized in a mercuric chloride solution (0.1%) for one minute and germinated in distilled water. Four-day-old seedlings were placed on cheesecloth supported between 2 plastic grids with 1.7 cm^2 openings. The seedlings, separated by the grid partitions, were covered with moist vermiculite. The grid assemblies were transferred to the glasshouse and supported over plastic pots containing 28 l of tap water. Three days later, the tap water was replaced by Yoshida solution (Yoshida *et al.*, 1972). The composition of this nutrient solution was modified by substituting KH_2PO_4 for NaH_2PO_4; iron (0.036 mol m^{-3}) was supplied as sodium ferric diethylenetriamine penta-acetate. The plants were thinned to 24 per pot.

Fifteen days after germination when the seedlings had reached the third-leaf stage, salination was initiated using mixtures of $NaCl—CaCl_2$. Salts were added in 3 equal increments over a 3-day period until an osmotic potential of −0.4 MPa was reached. Salt-treatments were chosen to study ion imbalances that might occur over a wide range of Na/Ca ratios (Table 1). All solutions were changed weekly and the pH was maintained at 4.0. Each treatment was replicated three times.

Shoot material was harvested 38 days after germination (23 days after initiation of salination). The unstressed plants had 7 fully expanded leaves on the main culm and at least 2 well-developed tillers, while the salinated seedlings had reached the 6th leaf stage with 1, rarely 2 tillers. From each pot, 10 complete shoots were removed and weighed. The remaining shoots (10 to 14 per pot) were subdivided as follows: leaves 1, 2, and 3 which had emerged prior to salination; all younger blades; sheaths; and tillers. The plant material was carefully rinsed to remove surface contamination without extracting shoot constituents (Grattan *et al.*, 1981). The tissues were dried at 65°C, weighed and ground in a blender.

Sodium, calcium, magnesium, potassium, iron, manganese, and zinc were determined on nitric-perchloric acid digests of the plant tissues by atomic absorption spectrophotometry, and phosphorus content by the molybdate-vanadate colorimetric method (Kitson and Mellon, 1944). Chloride was determined on nitric-acetic acid extracts by the coulometric-amperometric titration procedure (Cotlove, 1963).

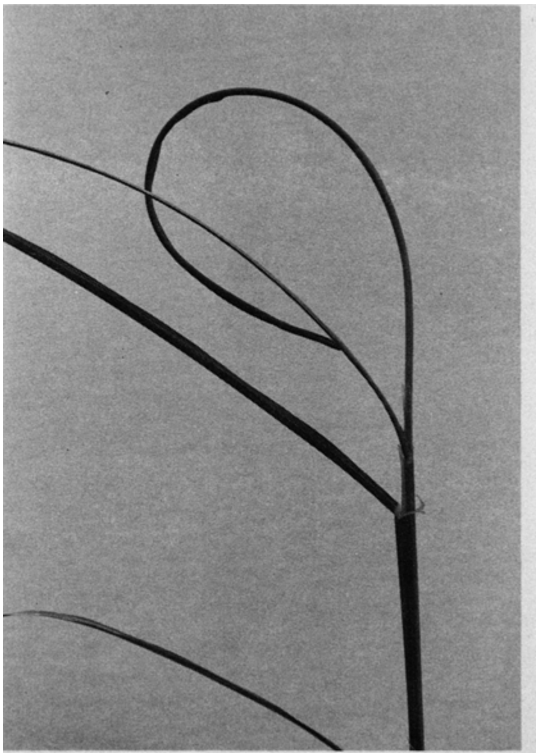

Fig. 1. Calcium deficiency symptom in rice seedling cv. M-201. Na/Ca ratio = 78; OP = −0.4 MPa.

Results

Shoot injury

Twenty-one days after germination, the non-salinated control shoots had grown to the 4th-leaf stage. At this time (6 days after initiation of salination), shoot growth of both cultivars was inhibited by the salt treatments. Development of the 4th leaf was delayed by 3 or 4 days. At the highest Na/Ca ratio (198 on a molar basis), many of the shoots exhibited acute Ca deficiency symptoms. The emerging blades were tightly rolled; the tips were severely withered, often necrotic (Yoshida, 1981). Occasionally, adjacent blades adhered to one another in the "bull-whip" configuration that is typical of severe Ca deficiency in cereals (Kawasaki and Moritsugu, 1973). An increment of Ca that

Fig. 2. Effect of Na/Ca ratio (198) on the growth of M-201 showing collapse of lamina and rolling of youngest leaf.

decreased Na/Ca to 78 did not correct this disorder (Fig. 1); Ca levels of 4.4 mol m^{-3} or higher were required to alleviate this type of injury.

Both M9 and M-201 exhibited chlorotic or white bands on the laminae. While this disorder has been reported to be characteristic of salinity damage, it has not been specifically associated with Ca deficiency (Ponnamperuma, 1984; Yoshida, 1981). Again, the degree of injury was greater at the highest Na/Ca ratios (198 and 78). The white areas became structurally weak and eventually necrotic (Fig. 2). As the Ca level in the cultures increased, fewer shoots were affected, the bleaching was not so intense, and blade collapse rarely occurred.

Shoot growth

Growth of rice seedlings was markedly inhibited after 23 days exposure to nutrient cultures salinated at an osmotic potential of −0.4 MPa (Table 1, Fig. 3). Both cultivars responded similarly to the composition of the root media. Fresh and dry matter production was lowest at high external Na/Ca; maximum shoot growth of salinized plants occurred when Na/Ca was 18, and then declined with increasing Ca. The correlation between Na/Ca and seedling growth was highly significant for M-201,

Table 1. The effect of external Na/Ca ratio on the dry weight and ion concentration in the shoots of *Oryza sativa* cvs M9 and M-201

Cultivar	Salt treatments				Plant shoot							
	Na	Ca (mol m^{-3})	Na/Ca		Dry weight (mg shoot^{-1})	Na (mmol kg^{-1} dry weight)	Ca	K	Mg	Cl	P	Fe (mg kg^{-1} d.w.)
M9	0	1.00	(Control)		769	4	81.2	1272	211	180	238	189
	85.2	0.43	198		278	1264	38.	534	140	1339	201	213
	84.3	1.08	78.1		271	1389	55.6	529	154	1475	190	196
	79.5	4.43	17.8		395	790	90.3	624	120	949	203	178
	73.2	9.15	8.0		391	730	147	667	119	1006	201	156
	65.7	14.08	4.7		341	811	245	670	120	1300	196	161
				$LSD_{0.05a}$	NS	282	29.9	NS	NS	NS	NS	24
				$LSD_{0.01}$	NS	385	40.8	NS	NS	NS	NS	32
M-201	0	1.00	(Control)		660	6	72.9	1132	171	160	215	168
	85.2	0.43	198		214	1294	36.1	474	124	1382	181	182
	84.3	1.08	78.1		274	976	47.9	505	123	1043	189	173
	79.5	4.43	17.8		396	676	77.8	560	101	793	191	163
	73.2	9.15	8.0		341	710	153	602	102	1164	187	140
	65.7	14.08	4.7		333	852	244	630	115	1049	180	151
				$LSD_{0.05a}$	35	250	55.3	40	NS	NS	NS	16
				$LSD_{0.01}$	48	341	75.4	55	NS	NS	NS	22

[a] Control values are not included in analysis of variance.

ig. 3. Response of M9 (a) and M-201 (b) to the external Na/Ca ratio. Each pot contains 20 36-day-old seedlings.

but only marginally so for M9. The decrease in dry weight at Na/Ca ratios of 8 or less may be a consequence of Ca toxicity (Yamanouchi *et al.*, 1983).

Despite the extensive shoot injury at the Na/Ca ratio of 198, all seedlings of both cultivars survived for 23 days under this treatment. A small varietal difference, however, was apparent in seedling survival at other Na/Ca ratios. For M9, survival was 97% at ratios of 78 and 18, then decreased to 94 and 88% as the Ca in the cultures increased to 9 and 14 mol m^{-3} respectively. The survival of all salinized M-201 seedlings was high (98.9%); fatalities were not related to external Na/Ca.

Shoot element composition

The diagnosis of the visual symptoms of Ca deficiency in rice seedlings grown at high Na/Ca was confirmed by analysis of the complete shoots as well as of the separate shoot tissues (Table 1, Fig. 4). At Na/Ca = 198, shoot-Ca was less than half of that present in the unsalinated shoots. Shoot-Ca was also sub-optimal when Na/Ca = 78, although the Ca concentration in the external solution (1 mol m^{-3}) is generally considered adequate for hydroponically-grown rice (Yoshida *et al.*, 1972). At Na/Ca = 18, Ca concentration in the saline cultures was four-fold higher than in the control nutrient solution. Despite this increase in external Ca level, Ca accumulation in the shoots of the salt-treated plants rose only 5–10% above the level in the control shoots. In all shoot parts, Ca levels increased as the external Na/Ca decreased. Ca was preferentially accumulated by the older leaves.

Sodium accumulation in salt-stressed rice shoots is highly variable (Flowers and Yeo, 1981). In the case of M9 and M-201, the standard deviation was often 35 to 40% of the mean Na concentration. Nevertheless, the decrease in Na/Ca ratio caused a significant decrease in shoot-Na (Table 1). This decline in Na concentration, however, was a consequence of dilution by shoot growth rather than a reduction of net Na uptake. In spite of the large variances in shoot-Na, total Na content in μmol per shoot was 315 $\pm$ 55 and 264 $\pm$ 56 for M9 and M-201, respectively (n = 15). Sodium was most strongly accumulated in the tillers and older leaves (Fig. 4). Chloride accumulation was also highly

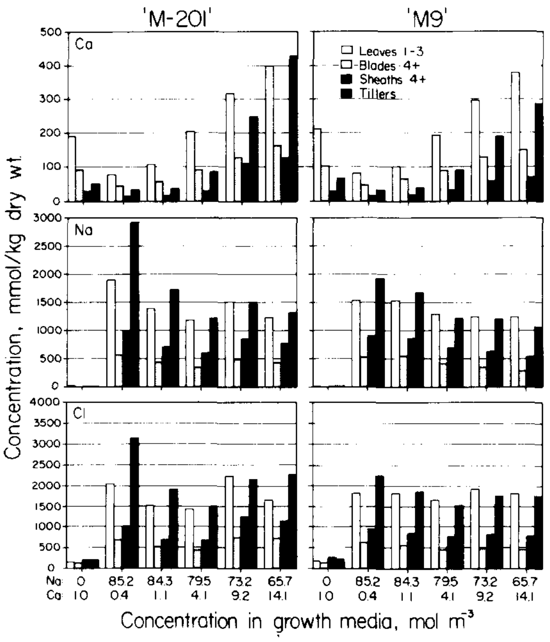

Fig. 4. Distribution of calcium, sodium, and chloride in plant tissues of rice cultivars M-201 and M9.

variable, but was not correlated with the external Na/Ca ratio. Patterns of Cl and Na distribution in the shoot tissues were similar.

The K content of the stressed shoots was reduced to about half of the concentration present in the non-salinated shoots. The gradual increase in shoot-K with large increases in external Ca was significant only for M-201. Shoot-Mg and -P decreased with salinity, but the Na/Ca ratio had no further effect.

Root-media composition also affected the iron nutrition of both cultivars. Increases in substrate Ca caused a significant decrease in shoot-Fe. No distinct relationship was evident between shoot-Mn or -Zn and the Na/Ca ratio in solution. (Data not presented).

Discussion

Solution culture experiments conducted in this Laboratory have shown that cereals, *e.g.* barley, corn, and sorghum, often exhibit symptoms of severe Ca deficiency in response to high external Na/Ca ratio. Although we have observed substantial differences between cultivars in the sensitivity to Na/Ca, this injury frequently occurred even when the Ca concentration in solution was well above that required for normal plant growth under non-saline conditions. The extent of injury was correlated with the total osmotic potential of the cultures. Corn seedlings (cv. DeKalb XL-75), for example, were extensively damaged at -0.4 MPa (Na/Ca = 34), but only slight injury was apparent at -0.3 MPa (Na/Ca = 26) (Maas and Grieve, 1987).

The results of the present study demonstrate that the rice cultivars, M9 and M-201, in common with certain other cereal genotypes, were sensitive to the Na/Ca composition in iso-osomotic cultures (OP = -0.4 MPa). At high Na/Ca (198 and 78), this response was characterized by severe Ca deficiency in the shoot. The disorder was particularly obvious in the blades that, at the time of harvest, comprised the physiologically active centers of the plant (Tanaka, 1961). When the Na/Ca ratio was reduced to 18, this nutritional problem was largely corrected and shoot growth was stimulated. The resulting decrease in shoot-Na concentration, even at high external Na (80 mol m^{-3}), was due to the diluting effect of increased growth, not to a reduction in total Na uptake.

Based on seedling survival, the tolerance of M9 and M-201 to high substrate Na was considerably greater than has been reported for several dwarfed lines developed by the International Rice Research Institute. The IRRI lines were grown in Yoshida nutrient cultures salinated with NaCl (50 mol m^{-3}). The period in which 50% of the individuals died, designated as D_{50}, for cultivars 'IR 26', 'IR 28', and 'IR 2153' was 9, 11, and 23 days respectively (Flowers and Yeo, 1981). In contrast, exposure for 23 days to NaCl concentrations that ranged from 66 to 85 mol m^{-3} resulted in the death of 1.1 and 4.4% of the total population of M-201 and M9, respectively.

At all Na/Ca ratios, Na accumulation increased with the age of the shoot tissue. This distribution pattern in rice has been described in detail by Yeo and Flowers (1982). Na concentrations in the physiologically active leaf blades of M9 and M-201 did not greatly exceed 500 m mol kg^{-1} dry weight. The older leaves accumulated high concentrations of either Na or Ca at opposite extremes of the Na/Ca range. The premature senescence of these older blades was most probably the result of Na toxicity at high Na/Ca and Ca toxicity at low Na/Ca. Ca toxicity may also be a factor in the reduction of shoot growth at high external Ca (Yamanouchi *et al.*, 1983).

Physiological studies have shown the importance of Ca in cellular systems that stabilize cell wall structure, maintain membrane integrity and selectivity, and that regulate ion-transport processes (Hanson, 1983). Membrane permeability may be increased by the Na-displacement of Ca ions that are associated with the external surface of the plasmalemma (Cramer *et al.*, 1985). Under conditions of high Na/Ca, this response may lead to the influx of intercellular Na and to the displacement or screening of Ca from the apoplastic cation exchange sites of the root xylem (Lynch and Läuchli, 1985). The Ca deficiency symptoms that we observed in rice grown at high external Na/Ca ratios may have resulted from a similar Na-inhibition of Ca movement whereby both Ca uptake and transport to the shoot were reduced.

The sensitivity of M9 and M-201 to the Na/Ca composition of the saline cultures contrasts sharply with the performance of the rice cultivar IR2153 studied by Yeo and Flowers (1985). Even at extreme Na/Ca ratios (5 to 500), dry matter production of this elite breeding line was not greatly affected, and no disorders attributable to Ca deficiency were reported. Although the disparate experimental conditions that were used in the studies of IR2153 and M9/M-201 may have accentuated this differential response, the results emphasize the wide variability among rice genotypes to the injurious effects of salinity stress.

Acknowledgements

The authors thank Donald A Layfield for analytical services and Ronald Pilkington for technical assistance. Seeds of M9 and M-201 were provided by the California Rice Experiment Station, Biggs, California.

References

Carnahan H L, Johnson C W, Tseng S T and Mastenbroek J J 1978 Registration of 'M9' rice. Crop Sci. 18, 357–8.

Carnahan H L, Johnson C W, Tseng S T, Oster J J and Hill J E 1982 Registration of M-201 rice. Crop Sci. 22, 1087–8.

Cotlove E 1963 Determination of true chloride content of biological fluids and tissues. II. Analysis by simple, nonisotopic methods. Anal. Chem. 35, 101–105.

Cramer G R, Läuchli A and Polito V S 1985 Displacement of Ca^{2+} by Na^{+} from the plasmalemma of root cells. Plant Physiol. 79, 207–211.

Flowers T J, Duque E, Hajibagheri M A, McGonigle T P and Yeo A R 1985 The effect of salinity on leaf ultrastructure and net photosynthesis of two varieties of rice: further evidence for a cellular component of salt-resistance. New Phytol. 100, 37–43.

Flowers T J and Yeo A R 1981 Variability in the resistance of sodium chloridesalinity within rice (*Oryza sativa* L.) varieties. New Phytol. 88, 363–373.

Grattan S R, Maas E V and Ogata G 1981 Foliar uptake and injury from saline aerosol. J. Environ. Qual. 10, 406–409.

Hanson J B 1983 The roles of calcium in plant growth. In Current Topics in Plant Biochemistry and Physiology. Eds. D D Randall, D G Blevins and R Larson. pp 1–24. University of Missouri, Columbia.

Kawasaki T and Moritsugu M 1973 Calcium effect on the absorption of monovalent cations by plant roots. VII. The effect of calcium on the absorption of monovalent cations by plant—especially in relation to salt injury. (2. Barley and paddy rice plants). J. Sci. Soil Manure, Japan 44, 133–137.

Kawasaki T and Moritsugu M 1979 A characteristic symptom of calcium deficiency in maize and sorghum. Comm. Soil Sci. Plant Anal. 10, 41–56.

Kitson R E and Mellon M G 1944 Colorimetric determination of phosphorus as molybdovanado phosphoric acid. Ind. Eng. Chem. Anal. Ed. 16, 379–383.

Lynch J and Läuchli A 1985 Salt stress disturbs the calcium nutrition of barley (*Hordeum vulgare* L.) New Phytol. 99, 345–354.

Maas E V and Grieve C M 1987 Sodium-induced calcium deficiency in salt stressed corn. Plant, Cell Environ. (*In press*)

Pearson G A and Ayres A D 1960 Rice as a crop for salt-affected soil in process of reclamation. Production Research Report No. 43.Agric. Res. Ser. USDA, 13 pp.

Ponnamperuma F N 1976 Screening rice for tolerance to mineral stresses. *In* Plant Adaption to Mineral Stress in Problem Soils. Eds. M J Wright and S A Ferrari. pp 341–353. Cornell University, Ithaca, New York.

Ponnamperuma F N 1984 Role of cultivar tolerance in increasing rice production on saline lands. *In* Salinity Tolerance in Plants: Strategies for Crop Improvement. Eds. R C Staples and G A Toenniessen pp 255–271. Wiley International, New York.

Tanaka A 1961 Studies on the nutrio-physiology of leaves of rice plant. J. Fac. Agric. Hokkaido Univ. 51, 449–550.

Yamanouchi M, Shimada Y and Yoshida S 1983 The effects of calcium ion to reduce the toxicity of sodium chloride for rice plant. Jap. J. Soil Sci. Plant Nutr. 54, 499–504.

Yeo A R, Caporn S J M and Flowers T J 1985 The effect of salinity upon photosynthesis in rice (*Oryza sativa* L.): Gas exchange by individual leaves in relation to their salt content J. Exp. Bot. 36, 1240–1248.

Yeo A R and Flowers T J 1982 Accumulation and localisation of sodium ions within the shoots of rice (*Oryza sativa*) varieties differing in salinity resistance. Physiol. Plant. 56, 343–348.

Yeo A R and Flowers T J 1983 Varietal differences in the toxicity of sodium ions in rice leaves. Physiol. Plant. 59, 189–195.

Yeo A R and Flowers T J 1984 Mechanisms of salinity resistance in rice and their role as physiological criteria in plant breeding. *In* Salinity Tolerance in Plants: Strategies for Crop Improvement. Eds. R C Staples and G A Toenniessen. pp 151–170. Wiley International, New York.

Yeo A R and Flowers T J 1985 The absence of an effect of the Na/Ca ratio on sodium chloride uptake by rice (*Oryza sativa* L.). New Phytol. 99, 81–90.

Yoshida S, Forna D A, Cock J H and Gomez K A 1972 Laboratory Manual for Physiological Studies of Rice. International Rice Research Institute, Manila.

Yoshida S 1981 Fundamentals of Rice Crop Science. International Rice Research Institute. Manila.