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Effect of seawater on photosynthesis and dry matter accumulation in developing rice grains

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Abstract

To understand the physiology of rice under seawater salinity, potted rice plants were irrigated with different concentrations of Japan seawater (electrical conductivity 0.9, 5.7, 11.5, or 21.5 mS cm^{-1}) from 10 d after transplanting (DAT) to 35 DAT, and from 75 to 100 DAT. Seawater salinity decreased the net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, transpiration rate, leaf water and osmotic potentials, and relative water content, and increased leaf temperature. The contents of chlorophylls, carotenoids, and total sugars significantly decreased in the leaves but content of non-reducing sugars decreased only slightly. With increasing salinity the Na^+ concentration increased, while Ca^{2+}, Mn^{2+}, and K^+ concentrations decreased. Salinity decreased the contents of sugars and proteins, dry mass, and rate of dry mater accumulation in developing grains.

Additional key words: Ca; carotenoids; chlorophylls; growth stage; K; Mn; Na; *Oryza sativa* L.; proteins; salinity; sugars.

Introduction

The net photosynthetic rate (P_N) depends on over 50 individual reactions, each of which potentially has a unique response to an environmental variable. Salt-stress is associated with mild reduction in leaf water content and cell volume (Bethke and Drew 1992). This leads to increase in ion concentration, which could inhibit activities of ribulose-1,5-bisphosphate carboxylase, NADP-dependent malate dehydrogenase, and phospho*enol*pyruvate carboxylase (Stiborová *et al.* 1987, Soussi *et al.* 1998). Salinity affects membrane permeability, enzyme kinetics, pH, phytohormonal status, concentrations of the ions and metabolites, and these facts may indirectly affect photosynthesis (Sage and Reid 1994). Previously, we have reported that salinity changes P_N and its related parameters, including osmotic and leaf water potential, transpiration rate (E), internal CO_2 partial pressure (c_i), and contents of biochemical constituents such as chlorophyll (Chl), carotenoids (Car), proteins, and sugars in the rice leaves (Sultana *et al.* 1999). Effects of salinity on various photosynthetic parameters are often studied (*e.g.* Agastian *et al.* 2000).

High salinity induces deficiencies of essential nutrient elements such as Ca, Mn, K, and Mg which in turn reduce the growth of plant (Cramer and Nowak 1992, Khan *et al.* 1997, Lutts *et al.* 1999). The high concentration of external Na^+ inhibits K^+, Ca^{2+}, and Mn^{2+} absorption by plant roots, particularly through the low-affinity systems. The function of Mn^{2+} at the cellular level of plant is to bind firmly to lamellae of chloroplasts, possibly to the outer surface of thylakoid membranes, affecting the chloroplast structure and photosynthesis (Lidon and Teixeira 2000). Cytosolic Ca^{2+} is not only involved in biosynthesis and intracellular transport of proteins but also in signalling components of rice plant (Kashem *et al.* 2000a). The role of K^+ is vital for osmoregulation and protein synthesis, maintaining cell turgor and stimulating photosynthesis (Peoples and Koch 1979).

Although high salinity affects all stages in the growth and development of rice, dry matter accumulation and grain yield are much more depressed by salt than the vegetative growth (Sultana *et al.* 1999). A reduction of grain yield under high salinity might result from loss of

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Abbreviations: c_i – internal CO_2 concentration; Car – carotenoids; Chl – chlorophyll; DAT – days after transplanting; E – transpiration rate; EC – electrical conductivity; g_s – stomatal conductance; NRS – non-reducing sugars; P_N – net photosynthetic rate; RS – reducing sugars; RWC – relative leaf water content; ψ_s – osmotic potential; ψ_w – leaf water potential.

photosynthetic capacity due to the effects of salinity on leaf development or longevity, effects on panicle development, reduced production of assimilates, ability to utilise photosynthates for growth, and/or an increased utilisation of photosynthates in respiration (Wignarajah 1990).

Rice is a salt sensitive crop with peak sensitivity in the early seedling and the early reproductive stages (Yeo *et al.* 1990, Lutts *et al.* 1995). To understand the effect of seawater on photosynthesis, changes in biochemical constituents and dry matter accumulation in developing grains were the main objectives of this study.

Materials and methods

Oryza sativa L. cv. Koshihikari (moderately salt tolerant), was germinated in a tray filled with silty clay-loam soil in a glasshouse at Niigata University, Japan. The experiment was conducted from April to September 1999 using a randomised block design with 3 replications. Each replicate contained 10 pots and each pot had 3 seedlings.

Twenty-five-day-old seedlings were transplanted (May 15) to 3 000 cm^3 plastic pots filled with fertilised sandy loam soil with pH 7.4. The pots were kept in a well-ventilated glasshouse during the whole growing season. The average air temperature and average relative humidity in the glasshouse were in the ranges of 22-32 °C and 60-80 %, respectively. Fertilisers were applied at 12 g(N) m^{-2} (NH_4Cl), 8 g(P) m^{-2} (superphosphate), and 10 g(K) m^{-2} (KCl). Half of the N was applied as a basal dose and a quarter was applied at tillering stage. The rest nitrogen fertiliser was applied at panicle initiation stage. Seawater was collected from Japan Sea (near Niigata University, Japan) and its electrical conductivity (EC) was 61 mS cm^{-1}. From May 25 (10 d after transplanting, DAT) to June 20 (35 DAT) and flag leaf stage (30 July, 75 DAT) to milking stage (25 August, 100 DAT), each pot were watered with seawater (EC 0.9, 5.7, 11.5, or 21.5 mS cm^{-1}). These solutions were prepared by diluting seawater with de-ionised water and were applied far in excess of the saturation capacity of the soil. In order to maintain the required soil medium salt contents, the EC of the soil medium was measured periodically by portable EC meter and required amount of treated water was added.

P_N, stomatal conductance (g_s), intracellular CO_2 concentration (c_i), transpiration rate (E), and leaf temperature (T_l) of the flag leaves were measured at tillering (35 DAT, June 20) and milking (100 DAT, August, 25) stages with a portable photosynthesis system (*SPB-H3, ADC Corporation*, England). Leaf water potential (ψ_w) was then measured with the same flag leaf in a pressure chamber (*Soil Moisture Equipment Corporation*, Santa Barbara, CA, USA). All measurements were taken on the same day between 09.00 and 11.00. For measurement of osmotic potential (ψ_s), flag leaf blades were removed, sealed in a polyvinyl bag, and immediately frozen in dry ice. The leaf samples were then pressed with a garlic squeezer to extract the cell sap. The osmotic potential was determined with a psychrometer (*Wescor RH52*, Logan, Utah, USA). Relative water content (RWC) was measured according to Sultana *et al.* (1999).

Oven-dried samples collected 35 DAT were digested with HNO_3-$HClO_4$, and Na^+, Ca^{2+}, Mn^{2+}, and K^+ were determined by the atomic absorption spectrophotometer. At 35 and 100 DAT pigments were extracted from fresh top leaves with acetone. Sugars were extracted from oven dried leaves/grains (ovaries) with ethanol. The pigments were estimated from the acetone extract by the method of Mahadevan and Sridhar (1982) and sugars were estimated by the method of Dey and Harborne (1990). Protein was estimated by the dye-binding procedure of Bradford (1976) with γ-globulin as a standard.

For the dry mass of the developing grain, 20 grains (de-hulled grain) were collected at 95, 102, 109, and 116 DAT for each treatment and dried them at 60 °C for 48 h. For statistical analysis, MSTAT-C (1989) software program was used and the significance of differences was evaluated by the LSD test.

Results

P_N was decreased when the concentration of seawater increased from 5.7 to 21.5 mS cm^{-1}. g_s was reduced by 80 % while c_i was decreased only by 29 % at 21.5 mS cm^{-1}, respectively. T_l increased by 15.7 % in 21.5 mS cm^{-1} seawater treated plants, and also E decreased with the increased of salinity (Table 1). Leaf water potential, osmotic potential, and RWC significantly decreased with increasing salinity at both growth stages (Table 1).

Total chlorophyll (Chl) content was decreased with the increase in seawater concentration and the Chl *b* was more sensitive to salinity than Chl *a* (Table 2). The content of carotenoids (Car) was decreased by 60 % in 21.5 mS cm^{-1} seawater-treated plants. Salinity inhibited the synthesis of photosynthates. Reducing sugars (RS) were more sensitive than non-reducing sugars (NRS). The NRS content was 50 % of that of RS in the control plants at both stages, but this ratio increased with the increase of salinity. Seawater salinity decreased the synthesis of proteins in the leaves (Table 2).

Salinity had a slight effect on grain dry matter accumulation at lower concentration of salinity and initial stages of growth, but the effect was aggravated by the high concentration and long duration of salinity (Table 3). The average accumulation rate of dry matter in the devel-

Table 1. Effects of Japan seawater on net photosynthetic rate (P_N), stomatal conductance (g_s), internal CO_2 partial pressure (c_i), transpiration rate (E), leaf water potential (ψ_w), osmotic potential (ψ_s), relative leaf water content (RWC), leaf temperature (T_l), and mineral elements [g kg^{-1}] of rice at 35 DAT and 100 DAT. Means of three replications. Different letters indicate significant differences among the treatments at 1 % level of significance based on the LSD test.

Parameter	Growth stage (DAT)	Seawater concentration [mS cm^{-1}] 0.9	5.7	11.5	21.5	LSD (1 %)
P_N [µmol(CO_2) m^{-2} s^{-1}]	35	21.77b	18.3c	13.03d	8.13e	2.91
	100	25.3a	21.5b	15.9 cd	9.03e	
g_s [mol(CO_2) m^{-2} s^{-1}]	35	0.89b	0.50c	0.35d	0.19	0.14
	100	1.57a	0.82b	0.55c	0.29 de	
c_i [cm^3 m^{-3}]	35	222d	192e	178e	165 ef	19.8
	100	307a	293a	275c	219d	
E [mol m^{-2} s^{-1}]	35	7.58b	4.88d	3.83e	2.33f	0.63
	100	8.83a	6.37c	4.27d	2.93f	
ψ_w [-MPa]	35	0.65f	0.97e	1.14 cd	1.89b	0.15
	100	0.91e	1.03 de	1.27c	2.06a	
ψ_s [-MPa]	35	0.86g	1.03f	1.63d	2.55b	0.14
	100	1.28e	1.65d	2.05c	2.8a	
RWC [%]	35	68.7a	65.0b	61.0c	54.3d	2.9
	100	67.0a	61.0c	53.7d	49.0e	
Leaf temperature (T_l) [°C]	35	29.2f	30.9e	33.2cd	33.8bc	1.0
	100	32.4d	33.6c	34.6ab	34.9a	
Na	35	1.2d	2.2c	3.0b	4.0a	0.6
Ca	35	1.2a	1.03b	0.85c	0.65d	0.09
Mn	35	0.087a	0.075b	0.066c	0.056d	0.007
K	35	15.1a	12.6b	11.3b	9.5c	1.3

oping grain (ovary) during 95 to 116 DAT was 0.35 mg d^{-1} in the control but this rate was decreased to 0.08 mg d^{-1} in the 21.5 mS cm^{-1} seawater-treated plants (Table 3). The sugar and protein contents in the grain were significantly inhibited by salinity. The ratio RS/NRS had a similar trend as in leaves (values not shown).

At tillering stage, the concentrations of Na^+ increased by 57, 114, and 186 % in 5.7, 11.5, and 21.5 mS cm^{-1} seawater-treated plants compared to control, respectively. Seawater salinity decreased the contents of Ca^{2+}, Mn^{2+}, and K^+ in the plants (Table 1).

Discussion

Seawater reduced the rate of photosynthesis and related parameters (Tables 1 and 2). The rate of photosynthesis depends on stomatal and non-stomatal components (Bethke and Drew 1992), and each of the components has a unique response to an environmental variable. g_s is related to turgor pressure of cells. The turgor pressure is controlled by solute regulation within the guard cell protoplast and the RWC of epidermal tissues. External high salt solution enhanced the accumulation of Na^+ and Cl^- that altered the osmotic activity, causing a reduction in water potential and an influx of water from the surrounding cells. Excessive Na^+ inhibits K^+, Ca^{2+}, and Mn^{2+} absorption by plant roots and decrease in translocation of nutrients from root to leaves causes nutrient deficiency in the plant (Hasegawa *et al.* 2000). Seawater salinity induced accumulation of toxic ion (Na^+ and Cl^-) and that activated water deficit, important mineral nutrient deficiency, and ionic imbalance in the cytosol, leading to creating an unfavourable environment and thus reducing P_N (Hasegawa *et al.* 2000, Lidon and Teixeira 2000).

Seawater salinity diminished the contents of Chl, Car, and proteins in the leaves (Table 2). A decrease in Chl concentration in salinised plants could be attributed to increased activity of the Chl-degrading enzyme chlorophyllase (Reddy and Vora 1986). Ion imbalance in leaves also adversely affects Chl concentration (Yeo and Flowers 1983). The decrease in Car content under salt stress leads to degradation of β-carotene and formation of zeaxanthin, which are apparently involved in protection against photoinhibition (Sharma and Hall 1991). As salinity adversely influenced the photosynthetic process, photosynthetic production (*e.g.* of sugar) was inhibited (Sultana *et al.* 1999). Kerepesi *et al.* (1998) also found that sugar contents of leaves decreased in tolerant genotypes of wheat under NaCl stress but drought stress increased the content of water soluble saccharides which are linked to stress tolerance. The increase in the ratio of sugars was presumably due to conversion of RS to NRS because salinity induced the sucrose synthesising enzyme sucrose phosphate synthase (Kashem *et al.* 2000b). Plant

Table 2. Effect of Japan seawater on biochemical constituents of fresh rice leaves at 35 and 100 DAT except sugars (from oven-dried samples). Means of three replications. Different letters indicate significant differences among the treatments at 1 % level of significance based on the LSD test.

Parameter	Growth stage (DAT)	Seawater concentration [mS cm^{-1}] 0.9	5.7	11.5	21.5	LSD (1 %)
Chlorophyll (*a*+*b*) [g kg^{-1}]	35	5.65a	2.38c	1.84cd	1.00e	0.69
	100	3.89b	3.40b	2.41c	1.38de	
Chlorophyll *a* [g kg^{-1}]	35	4.10a	2.03d	1.59e	0.77g	0.15
	100	2.61b	2.27c	1.73e	1.05f	
Chlorophyll *b* [g kg^{-1}]	35	1.55a	0.35e	0.25fg	0.22g	0.09
	100	1.31b	1.18c	0.73d	0.32ef	
Carotenoids [mg kg^{-1}]	35	139a	108c	90d	56g	7.8
	100	125b	95d	82f	56g	
Total sugars [g kg^{-1}]	35	183a	150b	132c	105d	12.1
	100	155b	125c	91e	74f	
Reducing sugars (RS) [g kg^{-1}]	35	122a	95c	80d	57e	8.4
	100	104b	75d	47f	38g	
Non-reducing sugars (NRS) [g kg^{-1}]	35	61a	55ab	52b	48bc	7.2
	100	51bc	50bc	44cd	36d	
RS : NRS	35	0.50e	0.58d	0.65c	0.84b	0.03
	100	0.49e	0.67c	0.94a	0.95a	
Proteins [g kg^{-1}]	35	8.50b	7.57bc	6.20de	5.20f	0.95
	100	9.50a	8.20bc	7.10cd	6.10ef	

synthesises sucrose to prevent the important organelles of the cells from stresses (Crowe and Crowe 1992, Kashem *et al.* 2000b).

Seawater reduced the growth of grain and decreased the rate of dry matter accumulation by about 77 % (Table 3). The reduction in dry matter at the grain filling stage might be through inhibition of photoassimilation at an earlier stage, because high salinity reduces the contents of photosynthetic pigments, proteins, and soluble sugars in the leaves. This change might cause the decline in leaf photosynthesis leading to the poor sugar accumulation in the grains (Sultana *et al.* 1999). The translocation of photosynthates depends on pre-anthesis reserves in the plant, current photoassimilate production, and phloem loading. The phloem loading depends on potential, which is affected by salinity. The application of seawater decreased the rate of dry matter accumulation from 0.35 to 0.08 mg d^{-1} in 21.5 mS cm^{-1}-seawater treated plants (Table 3). The toxic ions of Na^+ might interfere with phloem loading and thus translocation of assimilate reserves was inhibited in the salinised plants. Kobata *et al.* (1992) suggested that carbon assimilation and hence dry matter production were more affected than was remobilization of assimilates during the post-anthesis water deficit. They also concluded that under salinity, both photosynthesis and translocation of photosynthates to the grain are important for grain growth.

Finally, these results suggest that the deleterious effect of seawater on grain growth is partly due to the inhibition of photosynthesis that causes pre- and post-anthesis reserve in the plant and reducing the supply of assimilates to the grain.

Table 3. Effect of Japan seawater on dry matter accumulation and biochemical constituents [g kg^{-1}] of developing rice grains. The values are the mean of three replications. Different letters indicate significant differences among the treatments at 1 % level of significance based on the LSD test.

Parameter	Growth period (DAT)	Seawater concentration [mS cm^{-1}] 0.9	5.7	11.5	21.5	LSD
Grain dry mass [mg $grain^{-1}$]	95	6.57gh	5.77i	4.93jk	4.37k	0.77
	102	8.8ef	8.4f	7.23g	4.99jk	
	109	11.0c	10.1 d	8.5f	5.7 ij	
	116	14.0a	11.8b	9.3e	6.0 hi	
Dry matter accumulation [mg d^{-1}]	104	0.35a	0.28b	0.21c	0.08d	0.08
Total sugars	104	123a	100b	71c	45d	9.2
Reducing sugars	104	78a	60b	49c	30d	8.4
Non-reducing sugars	104	45a	40b	22c	15d	6.6
Proteins	104	12.1a	10.1ab	8.5bc	6.5bc	2.1

External excess of salt interferes with the uptake of other essential mineral nutrients. Salt-induced nutrient element deficiency and alteration of physical and biochemical parameters greatly influence the whole metabolism of plant resulting in reduced grain growth.

References

Agastian, P., Kingsley, S.J., Vivekanandan, M.: Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. – Photosynthetica **38**: 287-290, 2000.

Bethke, P.C., Drew, M.C.: Stomatal and nonstomatal components to inhibition of photosynthesis in leaves of *Capsicum annuum* during progressive exposure to NaCl salinity. – Plant Physiol. **99**: 219-226, 1992.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. – Anal. Biochem. **72**: 248-253, 1976.

Cramer, G.R., Nowak, R.S.: Supplemental manganese improves the relative growth, net assimilation and photosynthetic rates of salt-stressed barley. – Physiol. Plant. **84**: 600-605, 1992.

Crowe, J.H., Crowe, L.M.: Membrane integrity in anhydrobiotic organisms: Toward a mechanism for stabilizing dry cell. – In: Somero, G.N, Osmond, C.B.. Bolis, C.L. (ed.): Water and Life. Pp. 87-103. Springer-Verlag, Berlin 1992.

Dey, P.M., Harborne, J.B.: Methods in Plant Biochemistry. Vol. 2. – Academic Press, San Diego 1990.

Hasegawa, P.M., Bressan, R.A., Zhu, J.-K., Bohnert, H.J.: Plant cellular and molecular responses to high salinity. – Annu. Rev. Plant Physiol. Plant mol. Biol. **51**: 463-499, 2000.

Kashem, M.A., Itoh, K., Iwabuchi, S., Hori, H., Mitsui, T.: Possible involvement of phosphoinositide-Ca^{2+} signaling in the regulation of α-amylase expression and germination of rice seed (*Oryza sativa* L.). – Plant Cell Physiol. **41**: 399-407, 2000a.

Kashem, M.A., Sultana, N., Ikeda, T., Hori, H., Loboda, T., Mitsui, T.: Alteration of starch-sucrose transition in germinating wheat seed under sodium chloride salinity. – J. Plant Biol. **43**: 121-127, 2000b.

Kerepesi, I., Galiba, G., Bányai, E.: Osmotic and salt stresses induced differential alteration in water-soluble carbohydrate content in wheat seedlings. – J. agr. Food Chem. **46**: 5347-5354, 1998.

Khan, M.S.A., Hamid, A., Salahuddin, A.B.M., Quasem, A., Karim, M.A.: Effect of sodium chloride on growth, photosynthesis and mineral ions accumulation of different types of rice (*Oryza sativa* L.). – J. Agron. Crop Sci. **179**: 149-161, 1997.

Kobata, T., Palta, J.A., Turner, N.C.: Rate of development of postanthesis water deficits and grain filling of spring wheat. – Crop Sci. **32**: 1238-1242, 1992.

Lidon, F.C., Teixeira, M.G.: Rice tolerance to excess Mn: Implication in the chloroplast lamellae and synthesis of a novel Mn protein. – Plant Physiol. Biochem. **38**: 969-978, 2000.

Lutts, S., Bouharmont, J., Kinet, J.-M.: Physiological characterisation of salt-resistant rice (*Oryza sativa* L.) somaclones. – Aust. J. Bot. **47**: 835-849, 1999.

Lutts, S., Kinet, J.-M., Bouharmont, J.: Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. – J. exp. Bot. **46**: 1843-1852, 1995.

Mahadevan, A., Sridhar, R.: Methods in Physiological Plant Pathology. 2nd Ed. – Sivakami Publ., Madras 1982.

MSTAT Development Team: MSTAT User's Guide: A Microcomputer Program for the Design, Management, Analysis of Agronomic Research Experiments. – Michigan State University, East Lansing 1989.

Peoples, T.R., Koch, D.W.: Role of potassium in carbon dioxide assimilation in *Medicago sativa* L. – Plant Physiol. **63**: 878-881, 1979.

Reddy, M.P., Vora, A.B.: Changes in pigment composition, Hill reaction activity and saccharides metabolism in bajra (*Pennisetum typhoides* S & H) leaves under NaCl salinity. – Photosynthetica **20**: 50-55, 1986.

Sage, R.F., Reid, C.D.: Photosynthetic response mechanisms to environmental change in C3 plants. - In: Wilkinson, R.E. (ed.): Plant–Environment Interactions. Pp. 413-499. Marcel Dekker, New York – Basel – Hong Kong 1994.

Sharma, P.K., Hall, D.O.: Interaction of salt stress and photoinhibition on photosynthesis in barley and sorghum. – J. Plant Physiol. **138**: 614-619, 1991.

Soussi, M., Ocaña, A., Lluch, C.: Effect of salt stress on growth, photosynthesis and nitrogen fixation in chick-pea (*Cicer arietinum* L.). – J. exp. Bot. **49**: 1329-1337, 1998.

Stiborová, M., Kšinská, S., Březinová, A.: Effect of NaCl on the growth and biochemical characteristics of photosynthesis of barley and maize. – Photosynthetica **21**: 320-328, 1987.

Sultana, N., Ikeda, T., Itoh, R.: Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. – Environ. exp. Bot. **42**: 211-220, 1999.

Wignarajah, K.: Growth responses of *Phaseolus vulgaries* to varying salinity regimes. – Environ. exp. Bot. **30**: 141-147, 1990.

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

Yeo, A.R., Yeo, M.E., Flowers, T.J., Flowers, S.A.: Screening of rice (*Oryza sativa* L.) genotypes for physiological characters contributing to salinity resistance, and their relationship to overall performance. – Theor. appl. Genet. **79**: 377-384, 1990.