The effect of fluctuating substrate salinity on the yield and flag leaf photosynthesis of wheat

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The effects of constant and fluctuating substrate salinity on flag leaf photosynthesis and grain yield of wheat were investigated. Plants exposed to salinity fluctuating around a mean integrated value of 25 mol m^{-3} NaCl (0–50 mol m^{-3}) and around 50 mol m^{-3} NaCl (25–75 mol m^{-3}) had yields 36 and 38% higher, respectively, than those receiving the corresponding constant salinity. Plants treated with salinity fluctuating from 50 to 100 mol m^{-3} NaCl had a 14% lower yield than those receiving 75 mol m^{-3} NaCl. Flag leaf photosynthesis did not vary between treatments and during salinity fluctuations, suggesting that differences in flag leaf longevity caused the observed differences in yield. Results suggest plants grown under constant salinity are unlikely to behave in the same way as those in field situations, where fluctuating rather than constant salinity occurs.

Die invloed van konstante en wisselende substraatsaliniteit op fotosintese en graanopbrengs van koring is ondersoek. Plante wat blootgestel is aan saliniteit wat gevarieer het rondom gemiddelde geïntegreerde waardes van 25 mol m^{-3} (0–50 mol m^{-3}) en 50 mol m^{-3} (25–75 mol m^{-3}), het opbrengste van respektiewelik 36 en 38% hoër gehad as plante wat aan konstante saliniteit blootgestel was. Plante blootgestel aan saliniteit wat gevarieer het van 50 tot 100 mol m^{-3} NaCl het egter 'n 14% laer opbrengs gehad as plante blootgestel aan 75 mol m^{-3} NaCl. Aangesien vlagblaarfotosintese nie tussen behandelings gevarieer het nie, kan die verskille in opbrengs moontlik toegeskryf word aan verskille in die lewensduur van die vlagblare. Die resultate dui aan dat plante wat onder konstante saliniteit groei, nie op dieselfde wyse sal reageer nie as plante in die natuur waar die saliniteit wissel.

Keywords: *Triticum aestivum*, salinity stress, fluctuations.

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Introduction

In areas prone to excess substrate salinity stress, the concentration of salts in the soil is often not constant but can fluctuate considerably with time (e.g. Moolman *et al.* 1983; Singh & Narain 1980). Tomar and Gupta (1985) showed that within one year the conductivity of the water table of an Indian soil varied between equivalent salinities of 10 and 230 mol m^{-3} NaCl. Under semi-arid conditions soil salinity may increase considerably during the irrigation cycle, especially when plants are irrigated with saline water (Meiri 1985). The rate of such an increase will vary with the external climatic conditions and the physical properties of the soil. Despite this, in most investigations into the effects of substrate salinity on plants, workers have grown plants at constant average salinity. Little work has been carried out on the effects of fluctuations in substrate salinity on plants. Ball and Farquhar (1984), Clipson (1987), Ewing *et al.* (1989), Flanagan and Jefferies (1989) and Plaut and Federman (1991) have studied the effects of a single increase in salinity on photosynthesis. However, little is known about the long-term effects of salinity fluctuations on plant growth. The aim of the present study was to test the effects of fluctuations in substrate salinity on the growth and flag leaf photosynthesis of wheat, and to compare results with plants grown at average constant salinity.

Materials and Methods

Growth of plants

Spring wheat (*Triticum aestivum* L. cv. SST 66) was grown in acid-washed sand, six plants per 450-cm^{-3} plastic pot. Two experiments were carried out. In the first, the yield parameters and the nutrient concentrations of the plants were determined. In the second, flag leaf photosynthesis was measured. In both experiments, plants were grown in naturally lit thermostatically controlled glass phytotrons. The first was carried out at the University of Natal, Pietermaritzburg, RSA, and the second at Rhodes University, Grahamstown, RSA. For the first experiment, the mean minimum and maximum daily temperatures were 13.5 and 33.2°C respectively, and for the second, 22.7 and 29.0°C. The transmittance of the phytotrons to solar radiation was greater than 80%, and day length was approximately 12 h for each experiment. Each treatment comprised at least 4 pots arranged in a randomized block design.

Plants were watered daily with 250 ml of 20% Hoagland's nutrient solution (Hewitt 1966) amended with 0, 25, 50, 75 or 100 mol m^{-3} NaCl. The concentration of nutrients in the solution was as follows: N, 3.0 mol m^{-3}; P, 0.2 mol m^{-3}; K, 1.2 mol m^{-3}; Ca, 1.0 mol m^{-3}; Mg, 0.4 mol m^{-3}; S, 0.4 mol m^{-3}; Fe (supplied as FeEDTA), 3.0 mmol m^{-3}; B, 9.4 mmol m^{-3}; Mn, 1.82 mmol m^{-3}; Zn, 160 µmol m^{-3}; Cu, 30 µmol m^{-3}; and Mo, 22 µmol m^{-3}. The void volume of the pots was 240 ml. The volume of nutrient solution used was intentionally much greater than the water requirements of the plants to prevent NaCl accumulation in the pots. In the first experiment, five constant salinity treatments (0, 25, 50, 75 and 100 mol m^{-3} NaCl) and four fluctuating treatments (0–50 mol m^{-3}, 25–75 mol m^{-3}, 50–100 mol m^{-3} and 0–100 mol m^{-3} NaCl) were used. In the second experiment, four constant salinity treatments (0, 25, 50 and 100 mol m^{-3} NaCl) and two fluctuating salinity treatments (0–50 mol m^{-3} and 0–100 mol m^{-3} NaCl) were used. After an initial ten-day period of gradually preconditioning appropriate treatments to salinity, changes in salt concentrations were carried out over an eight-day cycle. For example, in the 0–50 mol m^{-3} fluctuating treatment, plants received

250 ml of 0 mol m^{-3} NaCl daily for the first four days, and then 250 ml of 50 mol m^{-3} NaCl daily for the next four days.

Determination of yield parameters and tissue nutrient concentrations

In the first experiment, plants were grown to maturity (112 days), and the ears and the senescent flag and penultimate leaves excised. All plant parts were dried at 80°C to constant mass and then weighed. Grain from all the plants in one pot were combined, a sample taken and grain N determined using micro-Kjeldahl digestion followed by steam distillation and titration of the ammonium collected. The Na and K concentrations of the flag and penultimate leaves were determined by atomic absorption spectrophotometry using an air/acetylene flame. About 50 mg of material was digested in 2 ml of HNO_3, dried, and the residue dissolved in 10 ml of 1 mol l^{-1} HNO_3. All standards and samples were spiked with 1 g kg^{-1} Cs to prevent ionization of Na and K.

Determination of photosynthetic rates

In the second experiment, flag leaf photosynthesis was determined using an ADC Mark III portable IRGA. The Parkinson leaf chamber was modified by the attachment of a cooling unit. Light was provided by a high-pressure sodium lamp. Preliminary experiments showed that the optimum conditions for photosynthesis were a temperature of 25°C, with photosynthetically active radiation at 1000 μmol m^{-2} s^{-1}. The relative humidity was maintained at 50% throughout all experiments. Plants were pretreated for at least 30 min under these conditions, and then five replicate readings were taken for each leaf. Two eight-day experiments were carried out. In the first, the photosynthetic rates of four randomly selected plants from each treatment of constant salinity of 0, 25 and 50 mol m^{-3} and a fluctuating salinity of 0–50 mol m^{-3} NaCl, were measured daily. In the second, the photosynthetic rates of four randomly selected plants from treatments of constant salinity of 0, 50 and 100 mol m^{-3} and a fluctuating salinity of 0–100 mol m^{-3} were measured daily. The first experiment was started approximately one week after the plants started anthesis (71 days after sowing) and the second immediately after the end of the first. Measurements were thus made when grain growth was maximal (Herzog 1986). Although photosynthetic rates were always measured on green leaf tissue, by the end of the second eight-day experiment, the tips of the leaves of the plants receiving 100 mol m^{-3} NaCl had started to senesce. In both experiments, for plants receiving a fluctuating salinity treatment, the first four days of each experiment corresponded to a low concentration of NaCl in the fluctuating salinity cycle, and the second four to a high concentration of NaCl. The rate of net photosynthesis, *A*, was determined using the equations given by Postl and Bolhàr-Nordenkampf (1993). Data were analysed using the Spline program of Hunt and Parsons (1974). Data points in Figures 1 and 2 are fitted values with the 95% confidence limits for the highest and lowest point at each time.

Results and Discussion

Table 1 gives the effect of salinity on the yield components, grain N and the Na and K concentrations of the flag and penultimate leaves at final harvest of wheat. Under the growth conditions used, little tillering occurred, and most plants produced a single culm. None of the plants ever displayed any obvious nutrient deficiency symptoms. Despite using small pots to facilitate fluctuations in substrate salinity, yields per ear were typical of field-grown plants. Data in Table 1 for grain number and grain mass refer to the main stem, while the data for yields include grain from all tillers that the plants produced.

Salinity of 25 and 50 mol m^{-3} NaCl reduced yield mainly by reducing mean grain mass. Salinity of 75 and 100 mol m^{-3} NaCl reduced yield by reducing both grain number and grain mass. Plants exposed to salinity fluctuating around 25 mol m^{-3} NaCl (0–50 mol m^{-3}) and around 50 mol m^{-3} NaCl (25–75 mol m^{-3}) had yields of, respectively, 36 and 38% higher than those receiving the corresponding constant salinity. Increased grain mass rather than grain number caused the improved performance of plants grown with fluctuating salinity. The yield parameters of plants treated with fluctuations from 0 to 100 mol m^{-3} NaCl were intermediate between those of plants receiving a constant 50 mol m^{-3} and fluctuations from 25 to 75 mol m^{-3}. However, plants treated with salinity fluctuating from 50 to 100 mol m^{-3} NaCl had a 14% lower yield than those receiving 75 mol m^{-3} NaCl.

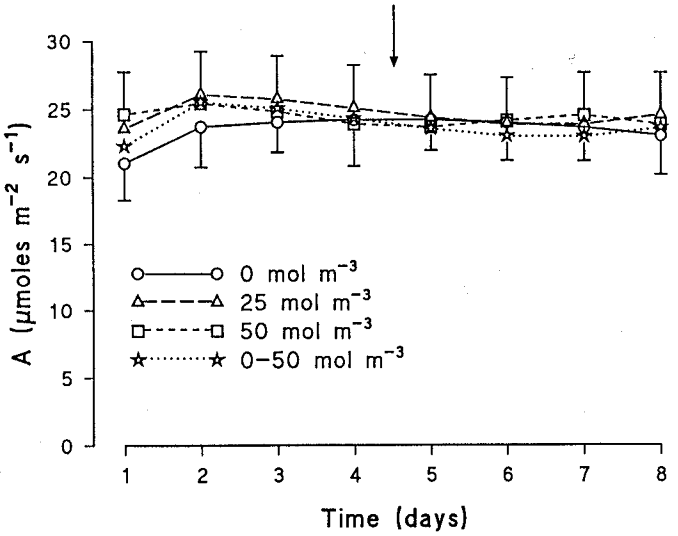

Figure 1 The effect of constant salinity of 0 mol m^{-3}, 25 mol m^{-3}, 50 mol m^{-3} and salinity fluctuating from 0 to 50 mol m^{-3} on net photosynthesis of the flag leaf of wheat. Points plotted are fitted values (32 data points per treatment), with the 95% confidence limits for the highest and lowest point at each time. The arrow represents the time when the salinity was increased for the fluctuating-salinity treatment.

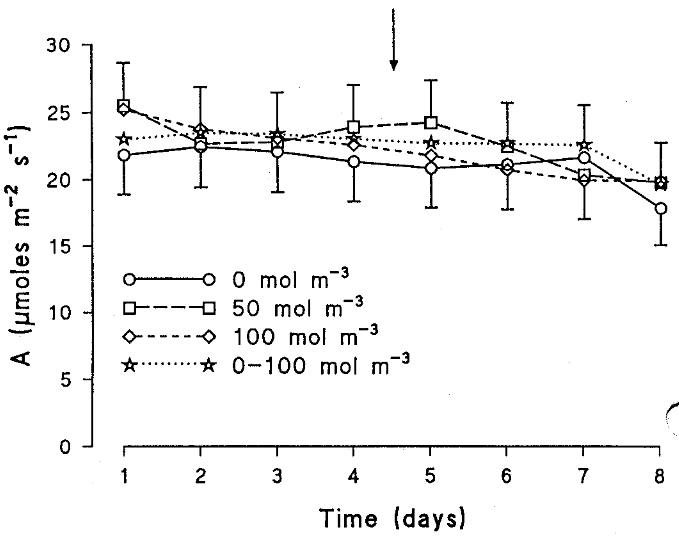

Figure 2 The effect of constant salinity of 0 mol m^{-3}, 50 mol m^{-3}, 100 mol m^{-3} and salinity fluctuating from 0 to 100 mol m^{-3} on net photosynthesis of the flag leaf of wheat. Points plotted are fitted values (32 data points per treatment), with the 95% confidence limits for the highest and lowest point at each time. The arrow represents the time when the salinity was increased for the fluctuating-salinity treatment.

Table 1 The effect of constant and fluctuating salinity on yield, yield components, N concentration and amount in grain, and the Na and K concentrations of the flag and penultimate leaves of wheat at final harvest[a]

	Yield (mg)	Grain number	Mean grain mass (mg)	Straw mass (mg)	Mass of flag and penultimate leaves (mg)	Grain N concentration (%)	Amount of grain N (mg per plant)	[Na] of flag and penultimate leaves (μmol / g dry mass)	[K] of flag and penultimate leaves (μmol / g dry mass)
NaCl (mol m^{-3})									
0	1647	43.8	37.8	1368	138	3.08	50.7	2	60.3
25	533	40.3	12.9	1226	138	4.11	20.7	218	46.3
0–50	726	39.0	18.4	1291	141	3.79	25.4	195	55.4
50	325	31.4	11.0	903	112	4.27	13.7	233	58.3
25–75	448	28.6	15.3	857	99	3.92	16.7	237	63.5
0–100	389	28.2	13.1	852	93	4.28	16.4	245	54.5
75	293	15.7	17.8	400	47	3.51	10.3	259	50.8
50–100	251	18.9	12.3	497	56	3.83	9.3	251	61.5
100	52	7.8	6.0	220	21	4.17	2.3	217	52.3
L.S.D.	127	4.7	3.0	275	17	0.51	11.4	33	8.3

[a] For the yield components and the cation data, $n = 24$ and the residual mean square had 207 degrees of freedom. For the N data, $n = 4$ and the residual mean square had 27 degrees of freedom. L.S.D. = Least significant difference ($P = 0.05$).

The lower yield was caused by a significant reduction in mean grain mass. These results agree with the limited data available, suggesting that varying salinity is better for plants than constant salinity when the mean salinity is low, but that the reverse is true when the mean salinity is high (Bernstein & Pearson 1954; Meiri 1985; Lin & Sternberg 1993). Recently, Naresh *et al.* (1993) compared the effects of irrigating wheat with a mixture of high- and low-quality water with plants receiving alternating applications of high- and then low-quality water. Plants irrigated with water of fluctuating salinity had slightly higher yields than those receiving a constant average salinity, but this difference was not significant. It is difficult to compare the results of Naresh *et al.* with the data presented here, as even plants exposed to water of a constant salinity had yields that were reduced by less than 10% compared with unsalinized controls. However, the effects of fluctuating substrate salinity on plants can clearly be different to those of constant average salinity.

Salinity stress reduced the mass of the straw and the flag and penultimate leaves (Table 1). Plants receiving fluctuating salinity had straw and leaf masses similar to plants grown at corresponding constant salinity. Salinity stress increased the concentration of grain N, but decreased the amount of grain N per plant. Salinity fluctuations changed the N concentration of the grain in the reverse direction to yield. Thus, a fluctuation of 0–50 mol m^{-3} NaCl increased yield and decreased the concentration of N in the grain compared with plants receiving 25 mol m^{-3} NaCl. Salinity stress tended to reduce the K concentrations of the flag and penultimate leaves. Plants treated with fluctuating salinity tended to have higher K concentrations compared with the corresponding constant salinity.

Visual observations and the much greater effect of NaCl on grain mass rather than grain number, suggest that premature senescence of the flag and penultimate leaves caused most of the salinity-induced yield reductions in our experiment. In wheat, the flag and penultimate leaves usually provide about 80% of the carbohydrates used to fill the grain (Austin *et al.* 1977). The Na concentrations of the flag and penultimate leaves at harvest were similar for all treatments (Table 1). This suggests that Na accumulated to a certain critical concentration in the leaves and then senescence occurred.

It is not possible to explain differences in yield between the various treatments based on flag leaf photosynthesis (Figures 1 and 2). Photosynthetic rates were similar in plants grown at 0, 25, 50 and 100 mol m^{-3} NaCl. Unsalinized plants tended to have lower photosynthetic rates than those treated with NaCl, although at any given time this was not significant (Figures 1 and 2). Table 2 gives the mean photosynthetic rates for the four treatments in the two eight-day experiments. Plants from the 0 and 50 mol m^{-3} treatments photosynthesized slightly slower during the second eight-day experiment. In the second experiment, plants exposed to 50 mol m^{-3} NaCl photosynthesized at a significantly faster rate that those exposed to 0 mol m^{-3} NaCl. Visual observations suggested that the lower leaves of the salinized plants senesced prematurely, and a compensatory increase in flag leaf photosynthesis to maintain grain filling may have occurred.

In plants grown at fluctuating salinity, photosynthetic rates were similar to those of plants grown at constant salinity, and did not change during the salinity fluctuation cycle (Figures 1 and 2, Table 2). Other workers (Ball & Farquhar 1984; Clipson 1987; Ewing *et al.* 1989; Flanagan & Jefferies 1989; Plaut & Federman 1991) have shown that a single increase in salinity can reduce photosynthesis in a variety of plant species. In the present experiment, photosynthesis in the wheat had clearly acclimatized to both salinity and to salinity fluctuations. Preliminary results (not shown) suggest that wheat grown at a constant salinity of 0 mol m^{-3} NaCl displayed a large drop in photosynthesis when suddenly salinized. These results agree with the view of Munns (1993) that salinity affects carbon assimilation per plant by reducing leaf surface area rather than reducing the rate of photosynthesis. Rates of photosynthesis during grain filling alone cannot explain the effect of NaCl on the yield parameters.

The most likely explanation for the improved performance of

Table 2 The effect of constant and fluctuating salinity on net flag leaf photosynthesis in wheat[a]

	NaCl (mol m^{-3})	Net photosynthesis (μmol CO_2 m^{-2} s^{-1})
Experiment 1	0	23.5 a
	25	24.7 a
	50	24.2 a
	0–50	23.9 a
L.S.D.		2.0
Experiment 2	0	21.0 a
	50	22.8 b
	100	22.2 ab
	0 - 100	22.8 b
L.S.D.		2.0

[a] Each figure is the mean of four readings taken daily for eight days; thus n = 32 and the residual mean square has 127 degrees of freedom. Within each experiment, figures followed by the same letter are not significantly different using Duncan's multiple-range test (P = 0.05). L.S.D. = Least signficant difference (P = 0.05).

plants grown at fluctuating salinity is that the leaves of these plants took longer to accumulate critical leaf Na concentrations. Certainly, Schachtman and Munns (1992) have shown that salt-tolerant accessions of *Triticum* species had lower rates of leaf Na accumulation than salt-sensitive accessions. It is possible that wheat may remove some leaf Na in the phloem during the low phase of the salinity fluctuation cycle (Hodson *et al.* 1985; Lessant & Marschner 1978), but more work is needed to test this.

In conclusion, the reaction of wheat to fluctuating substrate salinity differs from that of plants exposed to constant average salinity. The response of plants to fluctuating salinity stress deserves further investigation, particularly as plants in the field frequently experience fluctuating rather than constant substrate salinity.

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