BIOLOGIA PLANTARUM 37 (2): 265-271, 1995

Sodium and chloride ions contribute synergistically to salt toxicity in wheat

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Abstract

The effects of supplying excess mineral salts, involving sodium as a cation and a range of counteranions, including chloride, on the growth and photosynthetic capacity of a salt susceptible bread wheat were studied. Plant performance was much more affected by the NaCl treatment than by the same concentration of either of the two component ions. With the exception of K^+, other alkali metal chlorides also greatly inhibit plant growth and the electron flow through photosystem 2. The ranking of toxicity of these cations is $Li^+ > Na^+ > K^+$. The synergistic effect of sodium (and other alkali and alkaline earth metals) and chloride shows that neither of these ions alone is responsible for salt stress induced damage.

Key words: alkali metals, chlorophyll fluorescence, growth, photosystem 2, *Triticum*.

Introduction

The productivity of significant areas of arable land, particularly those under irrigation, has been damaged by salinity. In saline soils, the dominant cation is usually, although not always, sodium; while the balancing anion is mainly chloride, along with a significant presence of sulphate and bicarbonate. The depressive effect of NaCl on plant growth is not merely due to osmotic stress, as shown in wheat, for example, by Termaat and Munns (1986). Excess sodium is frequently assumed to be largely responsible for the stress, although high levels of chloride are often found in salinized plant tissue (Kingsbury and Epstein 1986, Gorham *et al.* 1990, Cruz *et al.* 1990). Many studies have therefore treated tissue sodium concentration in NaCl-stressed plants as a metric of tolerance, despite numerous and diverse examples of the lack of correlation between salt sensitivity and leaf ionic concentrations

Received 21 October 1994, *accepted* 29 November 1994.

Abbreviations: DWT - dry mass at flowering; F_0 - initial fluorescence; F_m - maximum fluorescence; F_v - variable fluorescence ($F_v = F_m - F_0$), PS - photosystem; SPNO - spikelet number of the main stem; TNO - tiller number

Acknowledgement: We wish to thank Dr. A. Yeo and Prof. T. Flowers (University of Sussex) for their constructive reading of this manuscript.

(Greenway and Munns 1980). The effects of Na^+ on plant growth, cell elongation and Ca^{2+} homoeostasis have been reviewed by Rengel (1992). However, the sodium ion is not universally toxic, most demonstrably so in halophytes. For example, extreme differences in sensitivity to Na^+ exist between *Lycopersicon cheesmanii* and *L. esculentum*, such that the former can tolerate 200 mM Na^+ but not 200 mM K^+, and the latter exactly the reverse (Rush and Epstein 1981). In cereals too, nutritional studies have shown that Na^+ can, at least partially, substitute for K^+, and that Na^+ was actually beneficial to wheat (reviewed in Hewitt 1952). Rather less attention has been given to the possible toxicity of excess levels of Cl^-, or of any of the other common soil anions. *In vitro* experiments show that Cl^- interferes with protein synthesis (discussed by Flowers and Dalmond 1992), while *in vivo* studies have implicated Cl^- as phytotoxic in soybean and various woody species (reviewed by Greenway and Munns 1980). There is also a suggestion that high levels of soil Cl^- can interfere with uptake of nitrate, leading to N starvation (reviewed by Grattan and Grieve 1992).

High levels of ions, other than sodium, are universally present both in saline soil and in hydroponic solutions. Even very salt sensitive plant species, such as rice, exclude the majority of external ions from their xylem sap. Nevertheless, without specific mechanisms for exclusion, some of these ions will inevitably be absorbed by the plant. It seems unlikely that the plant response is independent of the presence of excess concentrations of these ions. In this study, we have sought to dissect the role of both the balancing anions and of the competing alkali cations which occur in saline soils, as measured by the performance of a salt sensitive wheat variety.

Materials and methods

The Mexican bread wheat (*Triticum aestivum* L.) cultivar Glennson was used in all experiments, as this genotype is highly sensitive to damage by NaCl stress. Two separate experiments were conducted in a lit (*Osram HQI*, 16 h photoperiod, giving an irradiance of 458 $\mu mol\ m^{-2}\ s^{-1}$) and heated (around 20 °C) greenhouse over a five month period between December and April. Plants were grown in vermiculite-filled pots, as suggested by Timm *et al.* (1991), six plants to a 25 cm diameter, 25 cm high pot. The nutrient solution was watered from the top and the excess collected in a bowl under the pot. The level of liquid in the bowl was thereafter maintained by daily replenishment with water, and the solutions were changed every two weeks. The nutrient solution was as described in Martin *et al.* (1994). Salinization began once the first seedling leaf was fully emerged. The various salt treatments are detailed in the legends to Tables 1 and 2. The solutions were made up to give equimolar concentrations of the ions under test within each experiment. The cations involved were Na^+, K^+, Li^+, Mg^{2+}, Ca^{2+}; and the anions were Cl^-, HPO_4^{2-}, SO_4^{2-} and NO_3^-.

The *PEA* (*Plant Efficiency Analyzer, Hansatech Instruments Ltd*, King's Lynn, U.K.) allows non-destructive measurement of photosynthetic parameters, and has been used for this purpose on various crop plants (Smillie and Knott 1982). *PEA*

readings were taken on the fully emerged fourth leaf of each plant, as the ionic concentrations of this leaf has been shown to be representative of the whole shoot (Taeb 1991). The recordings were taken 2 cm from the leaf tip and 0.5 cm from the mid-rib. The parameters F_0 (corresponding mostly to the PS 2 pigment level - Krause and Weis 1984), F_v and F_v/F_m (measuring the electron flow through the PS 2 during photosynthesis, and changes in the thylakoid membrane ultrastructure - Öquist *et al.* 1982, Papageorgiou 1975), and the area under the fluorescence curve (size of electron donor pool - Bennoun and Li 1973) were recorded. At maturity, shoot dry mass, tiller number and spikelet number of the main tiller were measured.

Results were statistically analysed using the computer package *Genstat 5* (Payne *et al.* 1987).

Results

The effects of excess Na^+ and Cl^-, both separately and together: As expected from the known sensitive response of cv. Glennson to medium-high NaCl, there was a drastic reduction in vegetative and reproductive growth when challenged with 180 mM NaCl. However, the relatively mild response to Na^+ and Cl^- separately was less expected. Although both these ions at 180 mM (or, although less probably, the presence of moderately higher than normal levels of the counterions) each reduced growth, Cl^- appeared to be more injurious than Na^+. However the major observation is that these effects were non-additive; there was a pronounced synergistic effect of the two ions, such that both had to be present to exert the maximum damage to growth. The *PEA* data generated similar patterns to the whole plant data. Where the parameters differed between the treatments (F_v, area), the NaCl treatment always gave the lowest reading (Table 1).

Table 1. The effect of 180 mM solutions of Na^+, Cl^- and NaCl on mean shoot dry mass at flowering (DWT) in g, tiller number (TNO), spikelet number of the main tiller (SPNO) and the chlorophyll fluorescence parameters F_0, F_v, F_v/F_m and area under the fluorescence curve. SE - standard error of differences between treatment means. In addition to optimized nutrient solution, the four treatments contained: control - no additive; Na^+ - 45 mM NaCl, 22.5 mM Na_2SO_4, 22.5 mM Na_2HPO_4, 45 mM $NaNO_3$; Cl^- - 45 mM NaCl, 22.5 mM $CaCl_2$, 22.5 mM $MgCl_2$, 45 mM KCl; NaCl - 180mM NaCl.

	Control	Na^+	Cl^-	NaCl	SE
DWT	4.3	3.1	2.1	0.8	0.80
TNO	4.2	3.6	2.4	1.4	0.81
SPNO	15.8	15.8	11.2	0.0	2.24
F_0	489	465	512	445	49.8
F_v	2450	2389	2493	2102	245.0
F_v/F_m	0.98	0.99	0.98	0.97	0.021
Area	56960	55120	60080	42260	10084.8

The differential effect of the alkali and alkaline earth metal chlorides on plant growth and photosynthesis: Equimolar concentrations of the five alkali/alkaline earth metals (Na^+, K^+, Li^+, Ca^{2+} and Mg^{2+}) with chloride as the balancing anion, also resulted in differences in plant response. Once again the NaCl treatment severely inhibited plant growth, although less drastically at this lower concentration of 140 mM than at 180 mM as in the previous experiment. K^+ at 140 mM did not have much effect on plant performance, but the other cations affected plant growth as much as, if not more than Na^+ (Table 2). Li^+ had the most pronounced toxic effect. In this treatment, the plants hardly grew, and died before the appearance of visible floral structures. The PEA measurements again reflected the same trend as in the previous experiment. Neither F_0 nor the ratio F_v/F_m differentiated consistently between the treatments, whereas the other two parameters varied largely in line with the whole plant response.

Table 2. The effect of 140 mM chloride salts of the alkali/alkaline earth ions Ca^{2+}, Mg^{2+}, K^+, Na^+, and Li^+ in optimized nutrient solution, on the growth and photosynthetic characteristics detailed in Table 1. Control - no salt added.

	Control	Ca^{2+}	Mg^{2+}	K^+	Na^+	Li^+	SE
DWT	3.8	0.8	0.8	3.3	1.2	0.0	0.50
TNO	4.7	2.3	1.0	3.0	2.3	1.0	0.60
SPNO	14.0	10.0	9.3	16.0	12.7	0.0	1.60
F_0	453	473	413	473	435	449	58.0
F_v	2395	2194	2059	2210	2179	1224	159.6
F_v/F_m	1.00	0.97	0.98	0.97	0.98	0.82	0.061
Area	50967	47600	38703	47367	43967	20763	7509.2

Discussion

Kingsbury and Epstein (1986) attempted to separate the toxic effects of sodium and chloride by comparing growth rates of a salt sensitive and a salt tolerant wheat in a series of isosmotic solutions. They concluded that the toxicity of NaCl solutions to the sensitive wheat was a function of the Na^+, rather than the Cl^- ion. This conclusion could be questioned on the basis that the sole counteranion in the sodium treatment was nitrate (which was at 120 mM in the culture solution), since high concentrations of nitrate are themselves phytotoxic. In perennial ryegrass, for example, shoot growth inhibition occurred at concentrations above 14.3 mM NO_3^-, reaching a 50 % reduction at 143 mM (Clement *et al.* 1978). Furthermore, the chloride treatment in the Kingsbury and Epstein (1986) study was also depressive of yield, although not to the same degree as the NaCl treatment. In contrast to the conclusions of Kingsbury and Epstein (1986), the present data indicate that, with respect to the response of wheat to NaCl stress, the sodium ion *per se* is not particularly phytotoxic. If anything, of the two ions Na^+ and Cl^-, the latter is the more damaging, although the full toxic effect only comes about when both are

simultaneously present in excess. The toxicity is expressed as a reduction in both plant growth and in certain aspects of the effectiveness of the photosynthetic apparatus. When other monovalent alkali metals substitute for sodium, their toxicities can be ranked $Li^+ > Na^+ > K^+$, both on the basis of shoot dry matter production and PS 2 efficiency. The fact that such a ranking can be obtained confirms that the toxicity of these ions cannot merely be an osmotic effect, as otherwise the KCl treatment would be expected to be as injurious as the NaCl or LiCl ones. The argument that low plant water potential, resulting from the high salt concentration of the medium, itself is the prime agent of reduced plant growth has been convincingly rebutted by Termaat and Munns (1986) and Munns (1993). A similar detrimental effect on plant growth was seen with the divalent cations Mg^{2+} and Ca^{2+}. On this basis, a cocktail of countercations involving Na^+, Ca^{2+}, Mg^{2+} and K^+ in a given chloride concentration would be as, if not more toxic as the same concentration of NaCl. The fact that this did not occur may be explicable on the grounds that one constituent of the cocktail was K^+, and this ion is scarcely toxic; thus the cocktail involved a lower effective cation concentration than did the NaCl treatment.

The chlorophyll fluorescence data show that the PS 2 was impaired only by treatments which generated plant death before physiological maturity. Presumably this reflects the measurement of tissue which is already physiologically (although not visibly) senescent. Thus the effect of salinity on photosynthesis is not a direct one, supporting the position of Munns (1993).

Ca^{2+} homoeostasis is a critical factor in maintaining membrane integrity, and the displacement by Na^+ of these ions from both the plasmalemma of root cells (Cramer *et al.* 1985) and also from the cell wall (Gillet *et al.* 1994) has been shown. This displacement activity is not unique to sodium, and is effected by other alkali metal ions to varying degrees, such that the ions can be ranked in their effectiveness ($Li^+ > Na^+ > K^+$) (Lynch *et al.* 1987). The order also agrees very well with the inhibitory effects of the chloride salts of these ions on plant growth and PS 2 activity seen in the present study. The similar negative effects of excess Mg^{2+} may also be the result of this displacement activity. Surprisingly, excess Ca^{2+} too induced a marked negative response in our experiments. The addition of Ca^{2+} is generally found to be ameliorative of NaCl-induced stress (Lynch *et al.* 1987, Yamanouchi *et al.* 1983), probably as it lessens the displacement of Ca^{2+} by Na^+ through competition between free ions at the plasmalemma. However, when Ca^{2+} is present at the plasmalemma in excess, having been translocated up the xylem, one can no longer invoke a displacement model. Instead we would suggest that these excess external concentrations of Ca^{2+} cause a leakage of Ca^{2+} into the cytoplast, and that this provides the signal to the plant that triggers a whole chain of events with far-ranging effects on the plant's biochemical and physiological status. Among these may be included the impetus to flower, since it has been shown that both vernalisation and appropriate photoperiodic treatments produce a flux of xylem Ca^{2+} (Minorsky 1985, Friedman *et al.* 1989); after all, one of the salient features of the plant's response to salt stress is the bringing forward of flowering time (Francois *et al.* 1986, Maas and Grieve 1990), and it has even been suggested that the extent of

this advance could be used as a measure of the salt tolerance of wheat (Taeb 1991).

What might be the role of Cl^- in the salt toxicity response? Clearly its effect is substantial, given the difference between the NaCl and the Na^+ treatment effects (Table 1). We have also noticed that this pattern is repeated with other metal cations, with the exception of K^+, where KCl toxicity is mild (unpublished data). Since it is well-established that plants stressed with NaCl take up significant quantities of Cl^-, this suggests that the toxicity of Cl^- is only manifested when the activity of the cations allows influx of Cl^- into the cytoplast. A possible *in vivo* effect of Cl^- on plant cell function has been suggested by the sensitivity of leaf RuBP carboxylase levels, and therefore rate of assimilation of CO_2, to leaf Cl^- level in *Prunus* sp. (Ziska *et al.* 1990), which correlates with the known inhibitory effect of Cl^- on protein synthesis *in vitro*. Thus it is certainly conceivable that excess Cl^- ions in the cytoplasm could act to reduce growth and photosynthesis of the plant. If these effects were combined with those generated by the loss of Ca^{2+} homoeostasis, a drastic loss of plant viability could readily ensue.

We interpret these results to mean that the toxicity of NaCl is not merely the result of uptake of excess Na^+, a belief that lies behind many attempts to select for salt tolerance on the basis of tissue Na^+ levels. Rather we would propose that damage is the combined effect of perturbation of Ca^{2+} homoeostasis at the plasmalemma and influx of chloride or efflux of potassium through a membrane whose physiological properties have been altered. There is circumstantial evidence from *in vitro* studies for the toxicity of the Cl^- ion, but these still need to be tied into a general response of the whole plant to NaCl stress.

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