Qatar Univ.Sci. J. (2005), 25: 98-112

Possible Ionic Effects of NaCl on Two Mexican Wheat Cultivars

Bassam Taha Yasseen
Department of Biological Sciences, College of Arts and sciences,
Qatar University, Doha, Qatar

التأثيرات الأيونية المحتملة لكلوريد الصوديوم على صنفين من القمح المكسيكي

بسام طه ياسين
قسم العلوم البيولوجية – كلية الآداب والعلوم
جامعة قطر – الدوحة – قطر

قورن تأثير كلوريد الصوديوم على النمو وبعض الجوانب الفسيولوجية لصنفين من القمح المكسيكي (كاجيم: متحمل للملوحة، ويوكورا: حساس للملوحة) مع عوامل أزموزية أخرى مثل كبريتات الصوديوم (مركب أيوني) وجلايكول متعدد الأثيلين PEG 1000 (مركب غير أيوني). كانت استجابة عدد الأشطاء والمساحة الكلية للأوراق والعلاقات المائية والتركيب الايوني في الأوراق لمستويات متماثلة من تلك المركبات الأزموزية توحي بأن تأثير الملح كان أساسا أزموزيا لكن لوحظت تأثيرات أيونية خاصة لتلك الأملاح. تمتلك تلك المركبات تأثيرات متشابهة على عدد الأشطاء والمساحة الكلية للأوراق. شهد الجهد المائي والجهد الأزموزي للأوراق هبوطا جوهريا مع هبوط الجهد الأزموزي للمحلول المغذي بيد أن تأثير PEG كان أقل من تأثير الأملاح المستعملة. كذلك فإن تأثير الأملاح كان كبيرا في تقليل مستويات البوتاسيوم والكالسيوم في الأوراق بينما كان تأثير PEG الأقل على تلك العناصر. من جانب آخر فإن تأثير الأملاح في زيادة تركيز الفسفور في الأوراق كان أكثر منه في حالة PEG . كذلك فإن لكافة المركبات الأزموزية تأثيرات متشابهة على تركيز البرولين في أوراق الصنفين قيد الدراسة.

Keywords: *Ionic composition, Mexican wheat (Triticum asestivum L.), Osmotic agents, PEG, Salinity, Specific ion effects, Water relations.*

ABSTRACT

The effect of NaCl on growth and physiology of two Mexican wheat (*Triticum asestivum* L.) cultivars (Cajeme: salt tolerant, and Yecora: salt sensitive) was compared with that of other osmotic agents, Na_2SO_4 (ionic compound) and polyethylene glycol, as PEG 1000 (nonionic compound). The response of the number of tillers, total leaf area, water relations and ionic composition to these osmotic agents revealed that the effect of salt was mainly osmotic, but some specific effects of individual ions were also observed. All these compounds had similar effects on the number of tillers and total leaf area of both cultivars. Water and solute potentials of leaves were reduced as the osmotic potential of the culture solution decreased, but water potential of leaves with PEG did not decrease as much as with NaCl and Na_2SO_4. The ionic agents, NaCl and Na_2SO_4, decreased K^+ and Ca^{2+} in the leaves, while PEG had little effect on leaf content of K^+ and Ca^{2+}. All these agents increased phosphorus in leaves of both cultivars, but PEG had the smallest effect. All these compounds had similar effects on the accumulation of proline in the leaves of both cultivars.

Introduction

When exposed to saline environments, plants must contend with three basic problems: (a) maintaining favorable water relations, (b) coping with potentially toxic ions, and (c) obtaining the required nutrient ions despite the predominance of other ions in the external media [1]. Therefore, there are three major hazards under saline conditions: (a) osmotic stress causing a reduction in the potential of water absorption, (b) specific ion effects usually associated with the excess of Na^+ and Cl^- uptake causing some toxic symptoms such as leaf injury including: marginal necrosis, tip burning, wilting chlorosis or bronzing, and (c) nutrient ion imbalance when the excess of Na^+ and Cl^- leads to considerable disturbances in the uptake and distribution of essential elements such as K^+, Ca^{2+}, Mg^{2+} as well as nitrate and phosphate [2, 3, 4, 5, 6, 7, 8].

In a previous study, it has been reported that the depression in growth of the Mexican wheats due to NaCl could be due to : osmotic effects or toxicity effects from Na^+ or Cl^-, or from both ions [9]. No specific symptoms apparently due to toxicity were observed in that study due to the accumulation of these ions in the leaves of these cultivars at concentrations of NaCl up to 150 $molm^{-3}$ in the culture solution. Thus, the use of isosmotic concentrations of different salts and inert organic compounds can be used in studying the possible osmotic and / or specific ion effects of salts. The present investigation was, therefore, carried out to assess the ionic effects of sodium chloride on growth and some physiological aspects of two Mexican wheat cultivars differing in their salt tolerance; Cajeme: salt tolerant cultivar, and Yecora: salt sensitive cultivar [9, 10, 11].

Materials And Methods

The experiments

Two experiments were made to study the effects of osmotic agents on the growth and physiology of plants:

(a) Experiment (1): effects of NaCl and Polyethylene glycol (PEG 1000).

(b) Experiment (2): effects of NaCl and Na_2SO_4.

Three osmotic concentrations were used in these experiments. Table 1 shows the osmotic potentials (MPa) of NaCl, Na_2SO_4 and PEG and the corresponding concentration ($molm^{-3}$) of these osmotic agents. The experiments were designed as factorial experiments (2 cultivars x 2 osmotic agents x 3 potentials x 4 replicates). All measurements were repeated and standard error was calculated.

Table 1: The osmotic potentials (MPa) of NaCl, Na_2SO_4 and PEG used in experiments (1) and (2) and the corresponding concentrations (molm-3) of these osmotic agents

Osmotic potential (MPa)	NaCl Molm^{-3}	Na_2SO_4 molm^{-3}	PEG 1000 Molm^{-3}
- 0.5 *	0	0	0
- 0.25	55	44	54
- 0.45	110	88	99

* The osmotic potential of the nutrient solution used in the study.

The relationship between the concentration and osmotic potential of PEG was found to have a coefficient of determination (r^2) of 0.976, while those of NaCl and Na_2SO_4 were 0.999. Regression for the osmotic potentials (MPa) against concentrations (molm^{-3}) was developed and was used to determine the isosmotic concentrations of these agents.

Plant material and growth conditions

Wheat (*Triticum aestivum* L.) grains of the cultivars; Cajeme and Yecora were provided by CIMMYT, Mexico. They were germinated in trays of washed perlite and watered with demineralized water. Six seedlings of uniform size were transferred to the nutrient solution when they had two leaves (10 –12 days after sowing). Plastic containers of 27 x 20 x 20 cm in size, were used to hold $10x10^3$ cm^3 nutrient solution. The containers were covered by 3.5 cm-thick polystyrene sheets; with holes of 3 cm in diameter. Each seedling was bandaged with a piece of polyurethane and inserted into a hole in the polystyrene sheet. The root system of the seedlings was submerged in the nutrient solution. Air was continuously bubbled through the nutrient solution using a pump aquarium. The pH of the fresh nutrient solution ranged from 4.8 to 5.3 and after two weeks of use from 5.8 to 6.4. Deionized water was added whenever necessary to replace the water lost by transpiration and evaporation. Modified Long Ashton nutrient solution was used in these experiments; Iron was used as a combination of 0.1 molm^{-3} Fe-EDTA and 0.0144 molm^{-3} Ferrous Sulfate [12]. Nutrient solutions were replaced by fresh solutions every week.

All experiments were carried out in controlled environment chambers (W.H., O' Gorman Ltd. Farseley, Leeds, U.K.). There were 56 warm white fluorescent tubes (65 / 80 W) and four tungsten bulbs (60 W) were used as a source of red light, giving a photosynthetically active radiation (PAR) of 28 Wm^{-2} . The temperature inside the growth chamber was 20±2°C, while the relative humidity ranged between 70 to 80 %.

Measurements

All measurements in these experiments were carried out using fully expanded leaf 6, since this leaf started to initiate after 8 to 10 days from sowing the seed [13] which means that this leaf could be exposed to the salt effect during its initiation and growth.

Shoot growth

The number of tillers and total leaf area were determined in both cultivars. The area of leaves was measured using Lambda Portable Area Meter, model LI 3000 (Crump Scientific Ltd., Essex, U.K.).

Water relations

Water potential (Ψ_w) was measured using the pressure chamber technique, and solute potential (Ψ_s) was measured using the Fisons 'Advance' osmometer 3w (Advanced Instruments Inc., Massachusetts, USA).

Ion content

Leaves were dried at 85°C for 3-4 days, and then ground using a Cyclotec sample Mill (Tecator AB, Sweden). Wet digestion with a mixture of nitric and perchloric acids was used to prepare solutions for the determination of K, Ca, and P. The acids were removed by volatilization at 180-200 ° C and the soluble constituents were dissolved in hydrochloric acid [14, 15]. The concentrations K^+ were determined using a flame photometer (Corning 400, U.K.), and the concentrations of Ca^{2+} were determined using an atomic absorption spectrophotometer (Perkin-Elmer Model 272, USA). Phosphorus concentration was determined at 400 nm wavelength using a Pye Unicam SP6 spectrophotometer (Pye Unicam, U.K.).

Proline determination

The proline concentration was determined in leaves aeter, Bates *et al.* [16].

Results

Shoot growth

As the osmotic potential of NaCl, PEG and Na_2SO_4 decreased the number of tillers and total leaf area decreased (Figures 1 and 2). The osmotic agents seemed to have similar effects on these variables. However, a small but statistically significant difference was found between NaCl and Na_2SO_4 in experiment 2. Sodium chloride suppressed the number of tillers and total leaf area more than sodium sulfate.

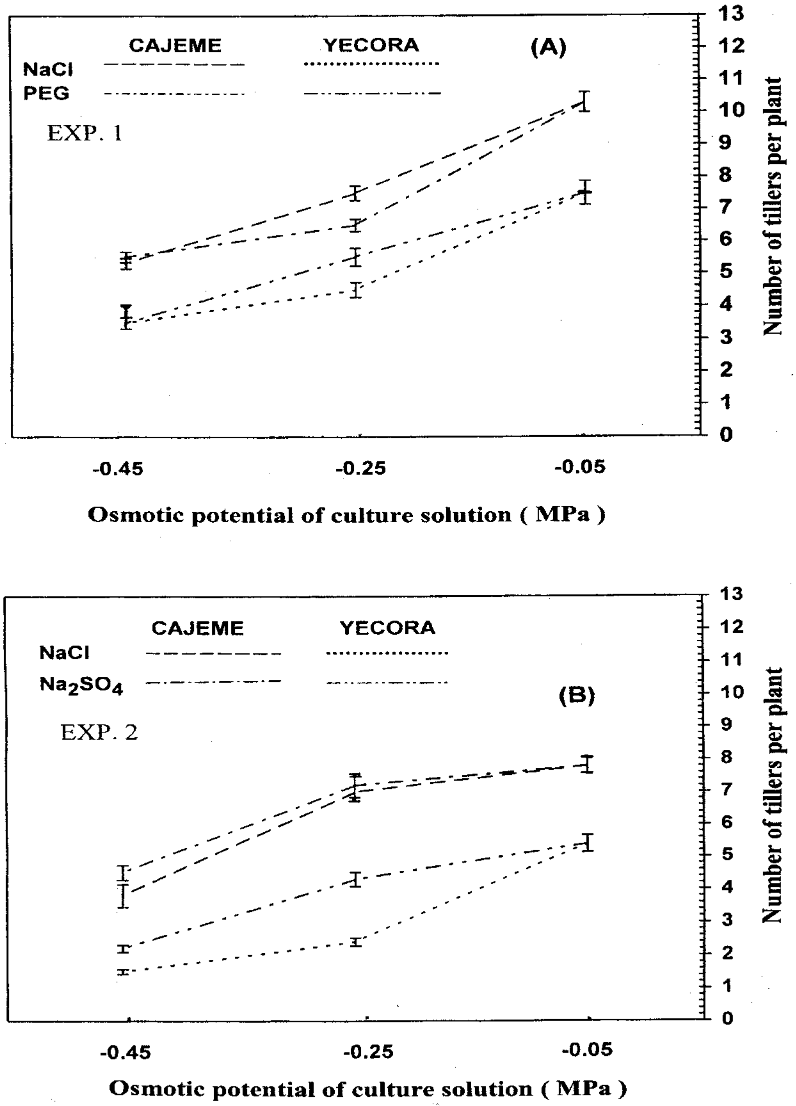
Water status

The water potential (Ψ_w) of leaf 6 in both cultivars decreased significantly as the osmotic potential of NaCl, PEG and Na_2SO_4 in the culture solution became more negative (Figure 3). The ionic agents, NaCl and Na_2SO_4, were more effective in lowering the water potential than PEG. The solute potential (Ψ_s) of leaves in both cultivars was almost similar due to these agents, however, it was less negative in PEG than in NaCl and Na_2SO_4 especially at – 0.45 MPa (Figure 4).

Ionic content

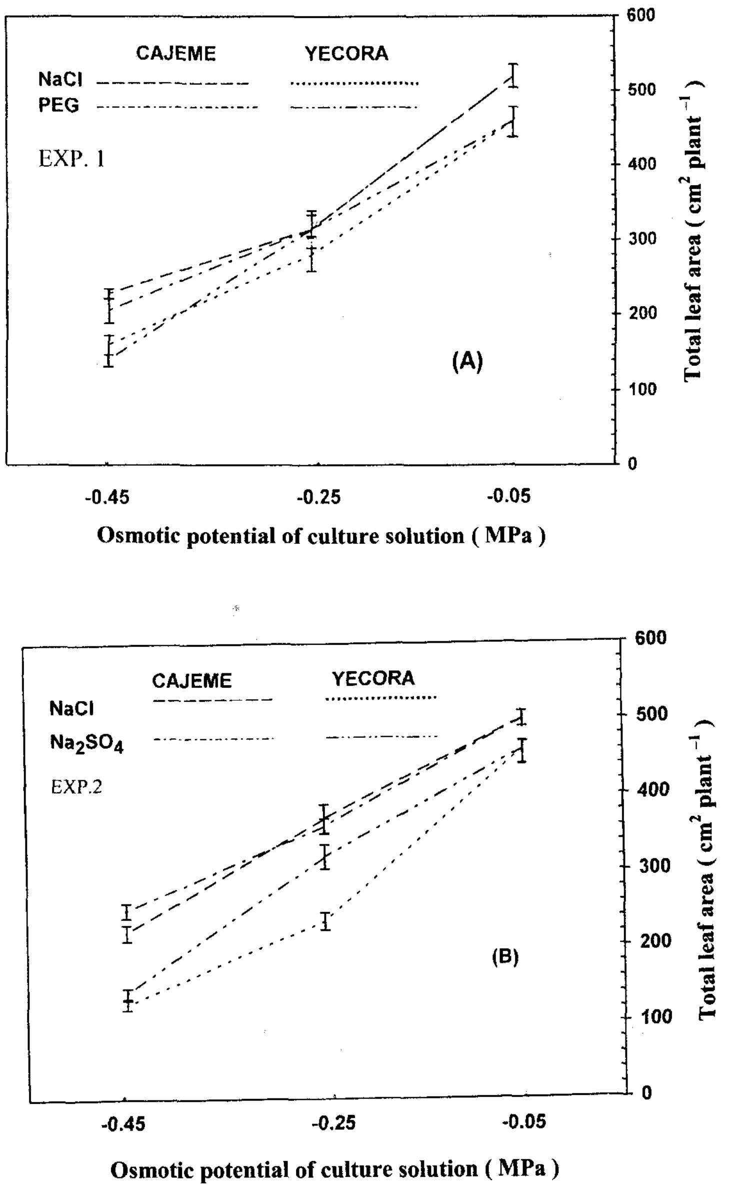
Phosphorus concentration increased quite significantly in the leaves of both cultivars as the osmotic potntial of NaCl, PEG and Na_2SO_4 became more negative in the culture solution (Figure 5).

However, the effect of these agents was not similar as shown from the statistical analysis; NaCl was found to have a pronounced effect in promoting phosphorus accumulation in both cultivars followed by Na_2SO_4 while PEG had the least effect. Potassium was slightly reduced by PEG, while both NaCl and Na_2SO_4 lowered its concentration substantially as the osmotic potential of these agents became more negative (Figure 6). This can be explained only by the specific effects of these agents. It seems that the osmotic stress imposed by PEG had little effect on K^+ accumulation in leaves of both cultivars. On the other hand, Na_2SO_4, reduced K^+ content more than NaCl, especially at osmotic potential of -0.45 MPa. The specific effect of these osmotic agents was more pronounced on the calcium content in the leaves. Figure 7 shows that Ca^{2+} concentration was changed only slightly by PEG, whereas both NaCl and Na_2SO_4 reduced Ca^{2+} substantially as the osmotic potential decreased in the culture solution. On the other hand, Na_2SO_4 decreased Ca^{2+} more significantly than did NaCl. The order of the effect of these osmotic agents was Na_2SO_4 > NaCl > PEG.



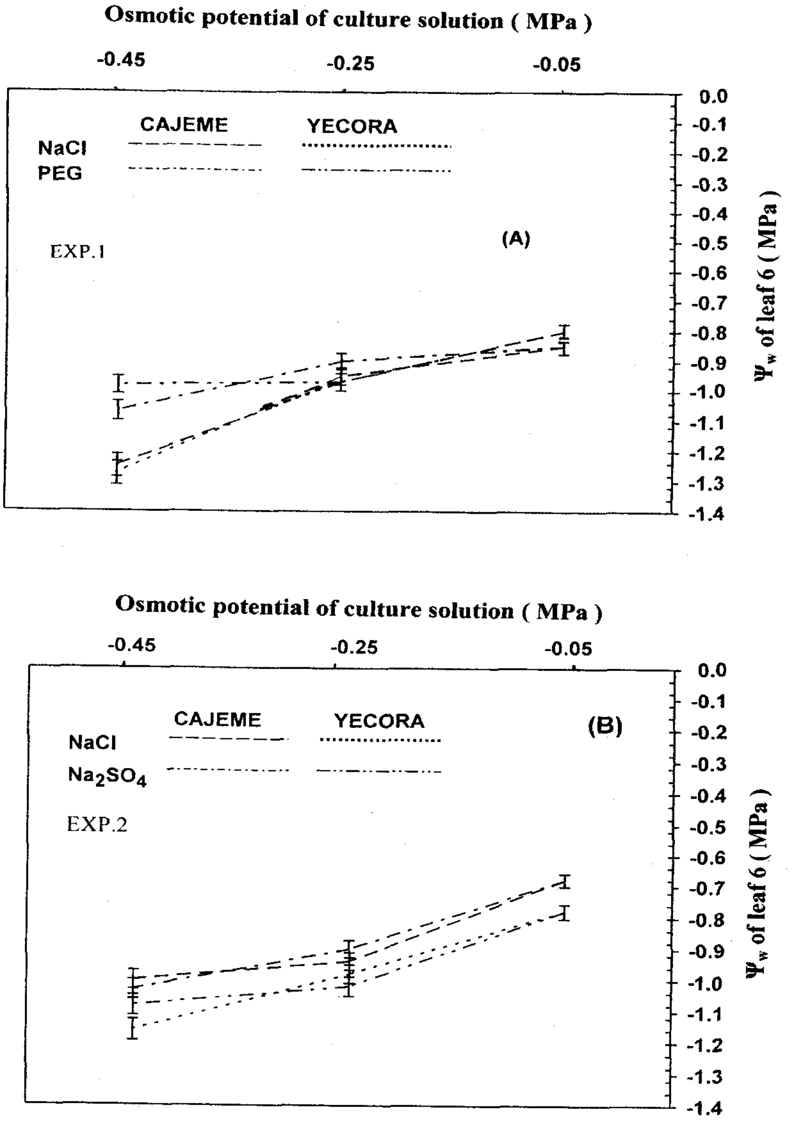
	Experiment 1	Experiment 2
Cultivars	$p < 0.001$	$p < 0.001$
Osmotic agents	n.s.	$p < 0.05$
Osmotic potentials	$p < 0.001$	$p < 0.001$

Figure 1: The effect of isosmotic potentials of NaCl and PEG (Experiment 1), NaCl and Na_2SO_4 (Experiment 2) on the number of tillers per plant of the two wheat cultivars. (Vertical bars represent the standard errors for the means).



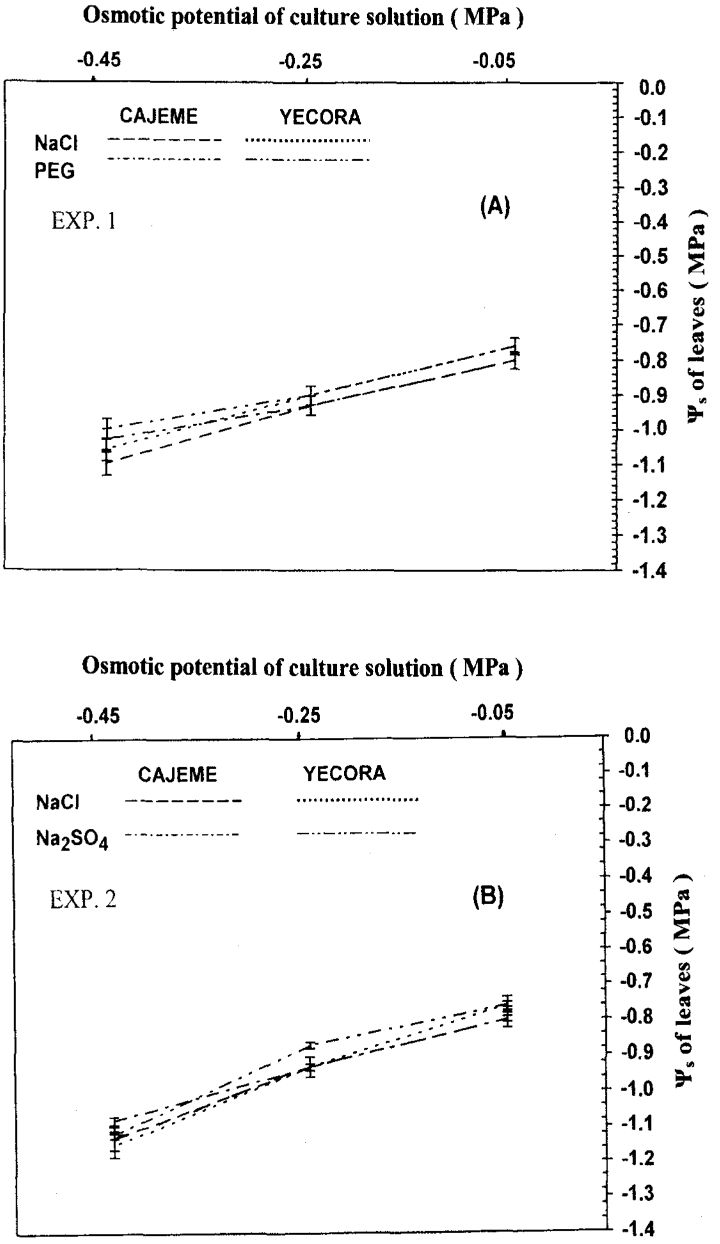
	Experiment 1	Experiment 2
Cultivars	$p < 0.01$	$p < 0.001$
Osmotic agents	n.s.	$p < 0.05$
Osmotic potentials	$p < 0.001$	$p < 0.001$

Figure 2: The effect of isosmotic potentials of NaCl and PEG (Experiment 1), NaCl and Na_2SO_4 (Experiment 2) on the total leaf area of the two wheat cultivars. (Vertical bars represent the standard errors for the means).



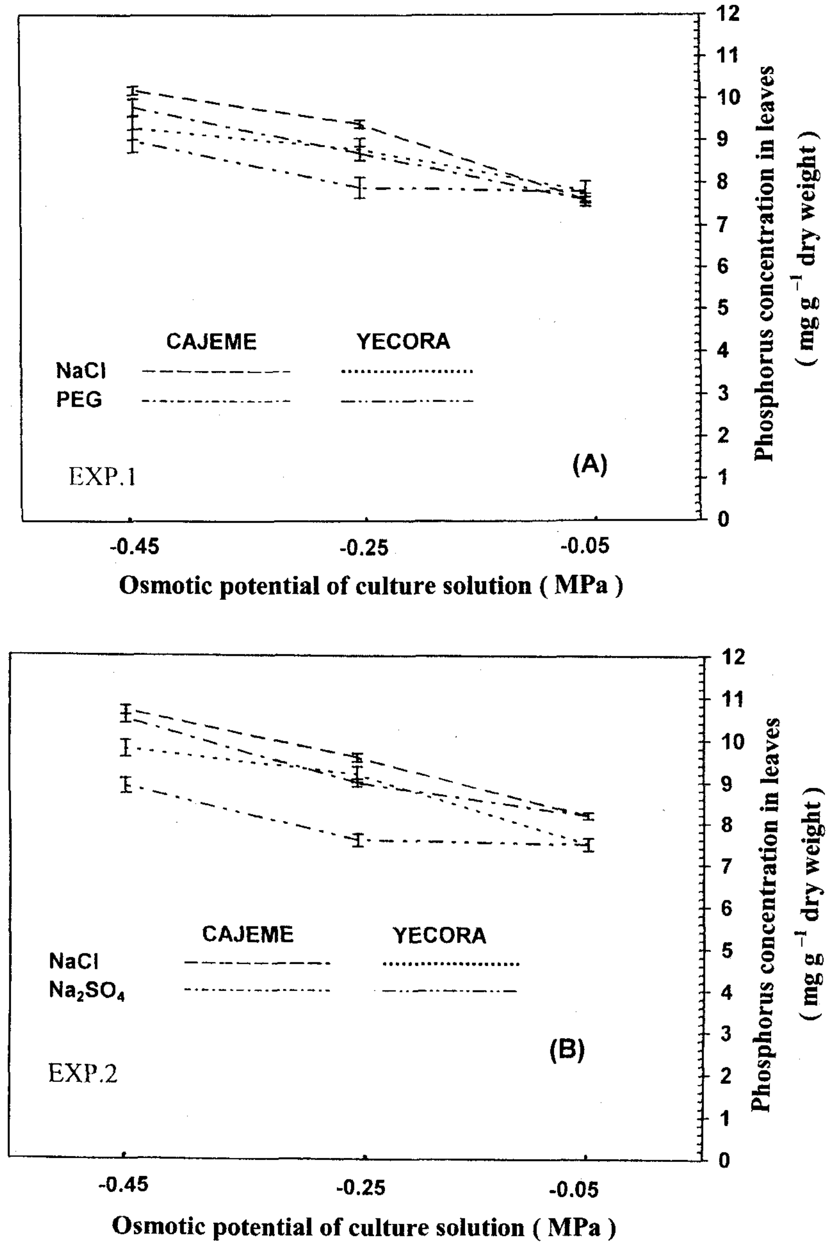
	Experiment 1	Experiment 2
Cultivars	n.s.	$p < 0.001$
Osmotic agents	$p < 0.001$	n.s.
Osmotic potentials	$p < 0.001$	$p < 0.001$

Figure 3: The effect of isosmotic potentials of NaCl and PEG (Experiment 1), NaCl and Na_2SO_4 (Experiment 2) on the water potential of leaf 6 of the two wheat cultivars. (Vertical bars represent the standard errors for the means).



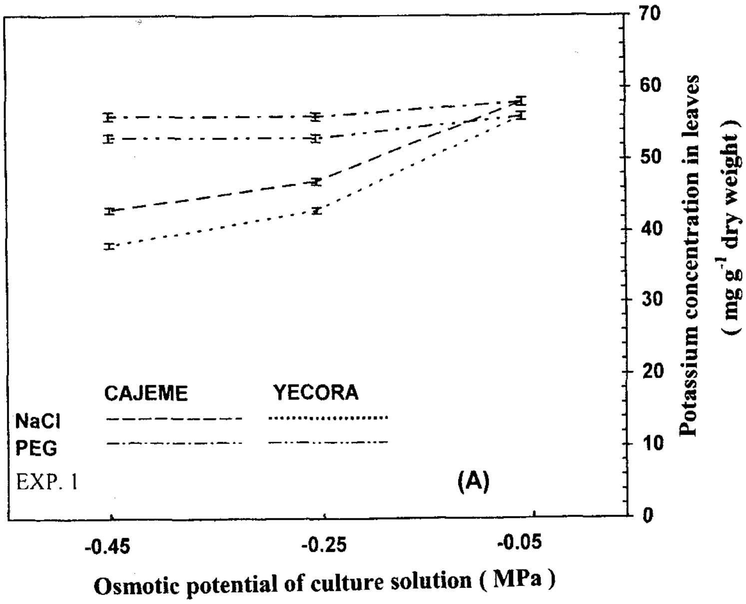
	Experiment 1	Experiment 2
Cultivars	n.s.	n.s.
Osmotic agents	n.s.	n.s.
Osmotic potentials	$p < 0.001$	$p < 0.001$

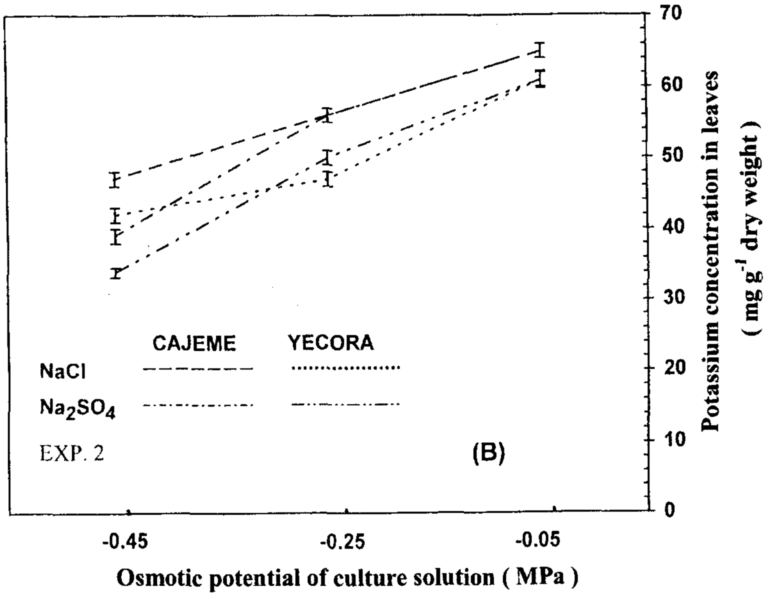
Figure 4: The effect of isosmotic potentials of NaCl and PEG (Experiment 1), NaCl and Na_2SO_4 (Experiment 2) on the solute potential of leaves of the two wheat cultivars. (Vertical bars represent the standard errors for the means).



	Experiment 1	Experiment 2
Cultivars	$p < 0.01$	$p < 0.001$
Osmotic agents	$p < 0.05$	$p < 0.01$
Osmotic potentials	$p < 0.001$	$p < 0.001$

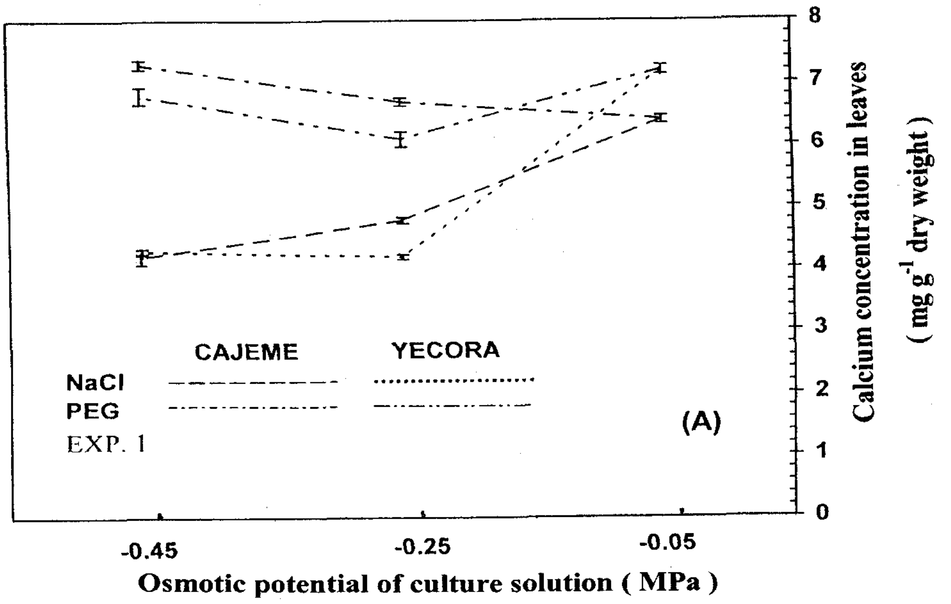
Figure 5: The effect of isosmotic potentials of NaCl and PEG (Experiment 1), NaCl and Na_2SO_4 (Experiment 2) on the phosphorus content of leaves of the two wheat cultivars. (Vertical bars represent the standard errors for the means).

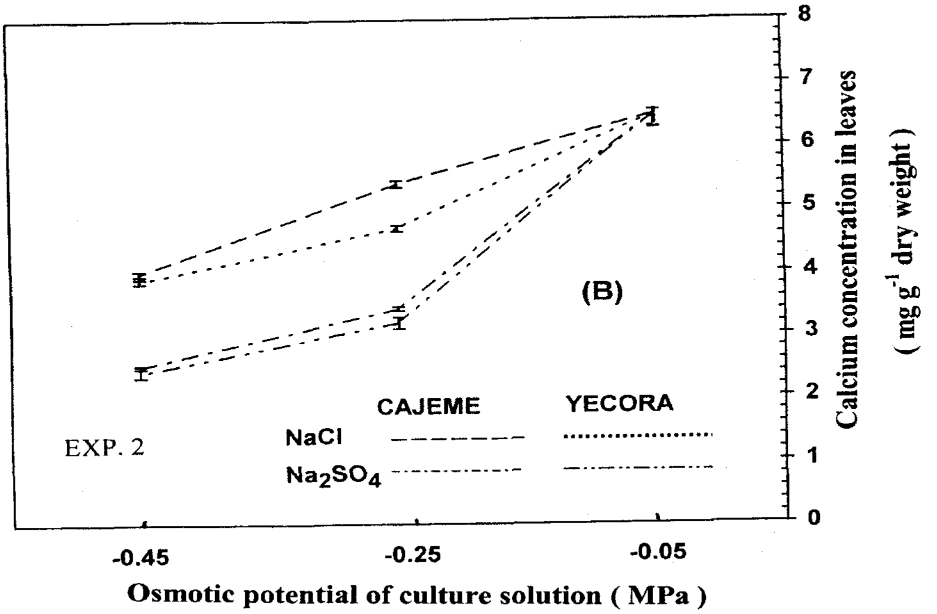




	Experiment 1	Experiment 2
Cultivars	$p < 0.01$	$p < 0.001$
Osmotic agents	$p < 0.001$	$p < 0.01$
Osmotic potentials	$p < 0.001$	$p < 0.001$

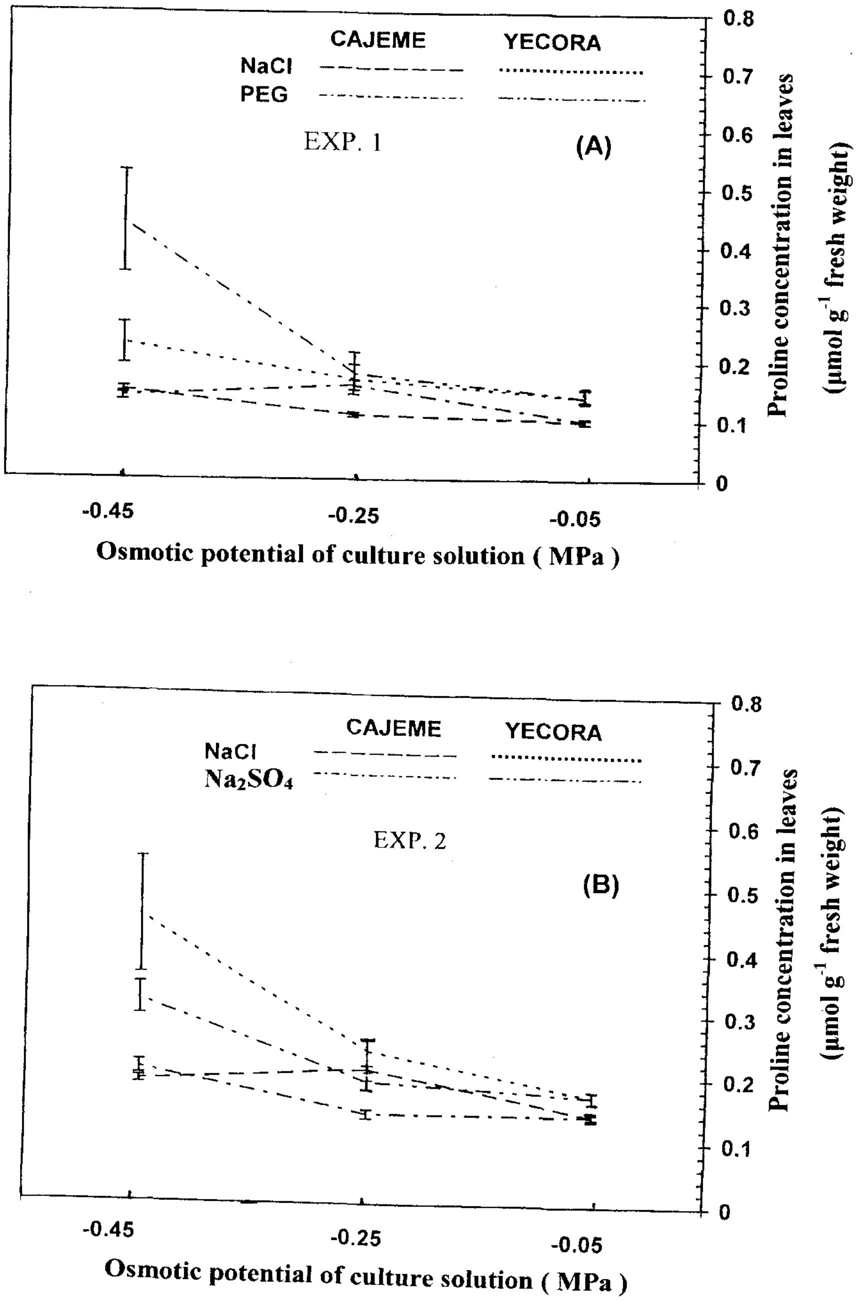
Figure 6: The effect of isosmotic potentials of NaCl and PEG (Experiment 1), NaCl and Na_2SO_4 (Experiment 2) on the potassium content of leaves of the two wheat cultivars. (Vertical bars represent the standard errors for the means).





	Experiment 1	Experiment 2
Cultivars	n.s.	n.s.
Osmotic agents	$p < 0.001$	$p < 0.001$
Osmotic potentials	$p < 0.001$	$p < 0.001$

Figure 7: The effect of isosmotic potentials of NaCl and PEG (Experiment 1), NaCl and Na_2SO_4 (Experiment 2) on the calcium content of leaves of the two wheat cultivars. (Vertical bars represent the standard errors for the means).



	Experiment 1	Experiment 2
Cultivars	$p < 0.01$	$p < 0.1$
Osmotic agents	$p < 0.1$	n.s.
Osmotic potentials	$p < 0.001$	$p < 0.05$

Figure 8: The effect of isosmotic potentials of NaCl and PEG (Experiment 1), NaCl and Na_2SO_4 (Experiment 2) on the proline content of leaves of the two wheat cultivars. (Vertical bars represent the standard errors for the means).

Proline accumulation

Proline accumulation increased significantly in leaves of both wheat cultivars as the osmotic potential of NaCl, PEG and Na_2SO_4 became more negative in the culture solution (Figure 8). However, the effect of osmotic potential was very highly significant ($p < 0.001$) in experiment 1, and only significant ($P < 0.05$) in experiment 2. At the osmotic potential of –0.45 MPa, the effect of the osmotic agents became more pronounced. PEG promoted the accumulation of proline to balance the osmotic potential between the plant and the culture solution. Both NaCl and Na_2SO_4 had almost similar effects on proline accumulation in the leaves. It is interesting to note that Yecora accumulated higher amounts of proline as compared to Cajeme.

Discussion

The results revealed two possible mechanisms for the effects of NaCl on the growth and physiology of wheat plants namely, osmotic effects, and specific ion effects. The response of growth of both cultivars to the isosmotic concentrations of single salts (NaCl and Na_2SO_4) and PEG indicated that the reduction in growth due to osmotic stress in the nutrient solution was linear [9]. All these osmotic agents had similar effects on the growth of the two wheat cultivars. However, a slight but significant difference was found between NaCl and Na_2SO_4 in experiment 2. NaCl suppressed the number of tillers and total leaf area more than Na_2SO_4. Some reports indicated that chloride salts caused more reduction in growth of plants than sulfate salts [17]. The great reduction in the solute potential of leaves due to the decrease in the osmotic potential of the nutrient solution could be a result of physiological drought stress [18]. In addition, the accumulation of the excessive ions and organic compounds such as proline in the leaves of plants could be a consequence of physiological drought [18, 19, 20, 21]. Accumulation of ions and organic compounds helps the plant to decrease the solute potential [22, 23, 24, 25], thereby decreasing Ψ_w which became low enough to maintain positive water balance between the nutrient solution and leaves [9].

On the other hand, there is some evidence for specific ion effects of NaCl on some physiological aspects. The data indicated that NaCl caused an unusual ionic balance, since wheat plants may have suffered from mineral deficiency (Figures 6, 7). Similar results were found for wheat and tomato plants [3, 9], sunflower [26] and in many other plants [5, 6, 7, 8, 27]. On the other hand, PEG, had little effects on K^+ and Ca^{2+} accumulation in comparison with NaCl. Phosphorus concentration in the leaves was not increased as much by PEG as it was with NaCl or Na_2SO_4. It is interesting to emphasize that both K^+ and proline have been considered as important physiological and biochemical variables respectively involved in salt stress mechanism, and are playing crucial roles in osmoregulation [28]. These solutes showed different responses to different osmotic agents in different cultivars. Yecora, for example, (a salt sensitive cultivar) accumulated much proline and less K^+ than Cajeme (a salt tolerant cultivar) [9].

The mechanisms and molecular basis for such differences in various osmotic agents and the varietal differences need further investigation [29]. However, these efforts may include the effect of sodium salts on various transporters and genes associated with proline metabolism [30].

Finally, it appeared that the main effect of NaCl on plants tested was osmotic in nature involving both reduced Ψ_s and reduced expansive growth as manifested by reduced tillering and reduced leaf area. However, the observed reduced growth may also involve effects of mineral nutrition imbalance as manifested by reduced leaf content of some mineral elements such as potassium. It can, therefore, be concluded that despite the lack of morphological symptoms of mineral deficiency the effect of NaCl involved both osmotic and mineral nutrition imbalance.

REFERENCES

[1] Rains D. W., Croughan T. P. and Stavarek S. J. (1980). Selection of salt-tolerant plants using tissue culture. In: D. W. Rains, R. C. Valentine and A. Hollaender (eds.), Genetic engineering of osmoregulation: impact on plant productivity for food, chemical and energy, pp. 279-292, Plenum press, New York.

[2] Bernstein L. (1975). Effects of salinity and sodicity on plant growth. *Amer. Rev.Phytopathol.* **13**: 295–312.

[3] Cerda A. and Bingham F. T. (1978). Yield, mineral composition and salt tolerance of tomato and wheat as affected by NaCl and P nutrition. *Agrochemica.* **22** (2): 140–149.

[4] Maas E. V. and Nieman R. H. (1978). Physiology of plant tolerance to salinity. In: Crop tolerance to suboptimal land conditions, ASA, Special publication number 32, American Society of Agronomy, Madison, USA.

[5] Wyn Jones R. G. (1981). Salt tolerance. In: Physiological processes limiting plant productivity, Butterworths, London.

[6] Yeo A .R. (1983). Salinity resistance: Physiologies and prices. *Physiol. Plant.* **58**: 214–222.

[7] Badger K. S. and Ungar I. A. (1990). Effects of soil salinity on growth and ion content of the inland halophyte *Hordeum jubatum*. *Bot. Gaz.* **151**(3): 314–321.

[8] Poss J. A., Grattan S. R., Grieve C. M. and Shannon M. C. (1999). Characterization of leaf borob injury in salt – stressed Eucalyptus by image analysis. *Plant and Soil* **206**: 237–245.

[9] Yasseen B. T. (1983). An analysis of the effects of salinity on leaf growth in Mexican wheat, Ph.D. Thesis, The University of Leeds, UK.

[10] Torres – B C. and Bingham F. T. (1973). Salt tolerance of Mexican wheat. I. Effect of NO_3 and NaCl on mineral nutrition, growth and grain production of four wheats. *Soil Sci. Soc. Amer. Proc.* **37**: 711–715.

[11] Torres – B. C., Bingham F. T. and Oertli J. (1974). Salt tolerance of Mexican wheat. II. Relation to variable sodium chloride and length of growing season. *Proc. Amer. Soc. Soil Sci.* **38**: 777–780.

[12] Hewitt E. J. (1966). Sand and water culture methods used in the study of plant nutrition, Commonwealth Agricultural Bureau.

[13] Williams, R. F. (1975). The shoot apex and leaf growth, Cambridge University Press.

[14] Chapman H. D. and Pratt P. F. (1961). Methods of analysis for soils, plants and waters, Div. Agr. Sci., Univ. Calif. Berkeley, California.

[15] Technical Bulletin No.27 (1973). The analysis of agricultural materials, Ministry of Agriculture, Fisheries and Food, H. M. S. O., London.

[16] Bates L. S., Waldren R. P. and Teare I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil.* **39**: 205–207.

[17] Levitt J. (1980). Responses of plants to environmental stresses, Vol. II, 2nd Ed., Academic Press, New York.

[18] Yasseen B. T. (1992). Physiology of water stress in plants, University Press, Mosul University, Mosul, Iraq.

[19] Paleg L. G. and Aspinall D. (1981). The physiology and biochemistry of drought resistance in plants, Academic Press, Sydney, New York, London.

[20] Yasseen B. T. (2001). Preliminary assessment of the effect of saline water used in irrigation on growth of the local barley cultuvar (Harma). *Qatar Univ. Sci. J.* **21**:55–64.

[21] Hare P. D., Cress W. A. and Van Staden J. (1998). Dissecting the role of osmolyte accumulation during stress. *Plant, Cell and Environment.* **21**: 535–553.

[22] Munns R. and Weir R. (1981). Contribution of sugars to osmotic adjustment in elongating and expanded zones of wheat leaves during moderate water deficits of two light- levels. *Aust. J. Plant Physiol.* **8**: 93-105.

[23] Timpa J. D., Burke J. J., Quisenberry J. E. and Wendt C. W. (1986). Effect of water stress on the organic acid and carbohydrate compositions of cotton plants. *Plant Physiol.* **82**: 724-728.

[24] Alhadi F. A., Yasseen B. T. and Al-Dubaie A. S. (1997). Changes in carbohydrate and nitrogen fraction during germination of fenugreek (*Trigonella foenum– graecum* L.) seeds presoaked in GA3, growing under different osmotic potentials, *Qatar Univ. Sci. J.* **17**:271–279.

[25] Alhadi F. A., Yasseen B. T. and Jabr M. (1999). Water stress and gibberellic acid effects on growth of fenugreek plants. *Irrig. Sci.* **18**: 185–190.

[26] Pakroo N. and Kashirad A. (1981). The effect of salinity and iron application on growth and mineral uptake of sunflower. *J. Plant Nutrition.* **4**: 45–56.

[27] Montero E., Cabot C., Poschenrieder CH. and Barcelo J. (1998). Relative importance of osmotic-stress and ion-specific effects on ABA - mediated inhibition of leaf expansion growth in *Phaseolus vulgaris. Plant, Cell and Environment.* **21**: 54–62.

[28] Lutts S., Kinet J-M. and Bouharmont J. (1996). Effects of salt stress on growth, mineral nutrition and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Plant Growth Regul.* **19**: 207–218.

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

[30] Orcutt D. M. and Nilson E. T. (2000). Physiology of Plants under Stress, Soil and Biotic Factors, Chapter 5, pp. 177–235, John Wiley & Sons Inc., New York.