Differences in the physiological responses of two clones of Eucalyptus microtheca selected for their salt tolerance

Domenico D. Morabito, Yves Jolivet, D. Prat, Pierre Dizengremel

ELSEVIER

Plant Science 114 (1996) 129–139

PLANT SCIENCE

Differences in the physiological responses of two clones of *Eucalyptus microtheca* selected for their salt tolerance

Domenico Morabito*[a], Yves Jolivet[a], Daniel Prat[b], Pierre Dizengremel[a]

[a]*Laboratoire de Biologie Forestière associé INRA, Université Henri Poincaré, Nancy 1, BP 239 54506 Vandoeuvre lès Nancy, France*

[b]*Station d'Amélioration des Arbres Forestiers, INRA, 45160 Ardon, France*

Received 18 May 1995; revision received 20 December 1995; accepted 20 December 1995

Abstract

Two *Eucalyptus microtheca* clones (clones 42 and 43) suspected to be salt tolerant were rooted and grown in a greenhouse. Five-month old cuttings were watered for 2 months with and without 200 mM NaCl. During the salt stress period, mineral (Na, Ca, K and Cl) and organic (soluble amino acids and proline) compounds were determined in roots, stems and leaves. Changes in protein profiles induced by salt stress were investigated after 2 weeks of salt treatment. Clone 42 demonstrated a delayed growth during salt stress while clone 43 showed a complete inhibition of shoot length. Salinity had a significant effect on mineral compounds: whatever the duration of the salt treatment, the uptake of sodium in roots was 2.5 times higher in the more tolerant clone 42 than in clone 43. Regarding potassium and calcium contents in roots, clone 42 demonstrated an increase in its amount whereas clone 43 showed a decrease. The increase in soluble amino acids induced by the stress in the different organs of the two clones was not significantly different whereas a higher content of proline was determined in clone 42 relative to clone 43. Salinity had significant effects on the content of one predominant polypeptide with an apparent molecular weight of 18 kDa which was specifically induced under salt stress in roots of clone 43. In clone 42, this polypeptide was present in low amounts in control conditions and salt treatment increased its synthesis.

Keywords: *Eucalyptus microtheca*; Clones; NaCl stress; 2D-electrophoresis; Organic compounds; Ions; Osmotic adjustment

1. Introduction

The improvement of arid and semi-arid areas implies the selection of salt-tolerant species for afforestation. Many Eucalypts are found to be such salt tolerant species, particularly *Eucalyptus microtheca*, characterized as adapted to high NaCl concentrations [1,2]. Information on physiological mechanisms of salt tolerance of Eucalyptus species are still limited. For many glycophytes species, salt sensitivity is due to the absorption of relatively high amounts of Cl^- and Na^+ in the upper parts of the plants [3]. Eucalyptus species did not

* Corresponding author, Tel.: 33 83 91 25 66; Fax: 33 83 91 25 64.

0168-9452/96/$15.00
PII S0168-9452(96)04325-7

derogate to this pattern. Consequently, a suitable trait to select salt tolerant Eucalypts species could be the level of sodium accumulated in the root and in the shoot [2]. On the other hand, it is interesting to investigate eventual changes in cellular metabolism in relation to salt tolerance. Previous studies mentioned that modification of enzymes properties remained scarcely presented as a salt adapted mechanism [4,5]. However, regardless of the intracellular ions compartmentation during salt stress, the formation of a salt-tolerant isoenzyme of the tonoplast ATPase has been mentioned in NaCl-adapted tobacco cells [6]. Other polypeptides have been pointed out to be important for salt tolerance of plants [7,8]. Concerning salt associated proteins, few studies have demonstrated that protein-like osmotin [9], LEA proteins (Late Embryogenesis Abundant) [10] or germin-like polypeptide [11] were involved in salt resistance and that their quantity was associated with the level of the plant osmotic adaptation [12]. So far these polypeptides have been characterized mainly in herbaceous plants and only one salt associated protein has been mentioned in a woody plant (*Citrus sinensis*) [13].

Eucalyptus microtheca provenance Marree, native of the lowest rainfall area in Australia has been selected to be the most salt tolerant provenance within *Eucalyptus microtheca* species [2]. Considering the broad genetic variability within *Eucalyptus microtheca* provenances [2], the aim of this paper is to determine if differences in salt tolerance occurred between clones of the same provenance and are associated with changes in mineral and organic solutes content in the different organs of the two clones. The protein patterns in the roots of two clones under NaCl (200 mM) treatment were also examined.

2. Materials and methods

2.1. Plant material and culture conditions

Young shoots, 15 cm in length taken from *Eucalyptus microtheca* trees (clones 42 and 43) provenance Marree in south Australia (137°39′E, 29°40′S, altitude 85 m) were excised and nodal segments 4 cm in length were rooted in perlite using indolebutyric acid powder. One-month old cuttings were replanted in individual pots filled with perlite in a greenhouse and received daily, for the first 4 months, a dilute PLANT-PROD nutrient solution 0.01% (w/v). The environmental conditions were 20°C at night and 27°C during the day, 75% relative humidity, and an irradiance of 300 mmol m^{-2} s^{-1} was provided during 16 h/day by phytoClaude lamps 400 W. After 120 days, the treated trees were watered by a saline solution (NaCl 200 mM added to the nutrient solution). For the control treatment, only the nutrient solution was used.

Before and during the salt stress, the length of the trees was regularly measured. The plants were harvested at 8, 12, 24 and 61 days after the salt treatment and divided into 3 groups per treatment (2 plants for each one) to make 3 replications. The plants were cut into roots, stems and leaves. The fresh weight, the dry weight (after drying at 70°C for 48 h) and the water content of the organs were determined for each group. For the analysis of the mineral and the organic solutes, the samples were lyophilised.

2.2. Analysis of mineral and organic solutes

The lyophilised plant material was ground using a mortar in order to obtain a fine powder.

The powder was homogenized in distilled water and the inorganic compounds were extracted twice for 5 min in boiling water. Na^+, K^+ and Ca^{2+} were determined with a CORNING-EEL flame photometer and chloride content was measured by ionic chromatography with a DIONEX DX 300.

The organic solutes were extracted from lyophilised material with 70% ethanol in water by grounding in a mortar. The amino acids were measured according to the method of Moore and Stein [14] and the proline according to the method of Troll and Lindsley [15].

2.3. Protein extraction and analysis

Total proteins from roots of 5-month old plants treated or not by 200 mM NaCl for 2, 12 and 24 days were extracted with the acetone-TCA precipitation method described by Damerval et al.

[16]. For each salt treatment, 4 different root systems per clone were collected and crushed separately in liquid nitrogen in order to obtain 4 individual replicates. To precipitate the proteins, the powder was immediately homogenized in a cold acetone solution containing 10% (v/v) trichloroacetic acid and 0.07% (v/v) β-mercaptoethanol and kept at −20°C for 1 h. The acetone solution was centrifugated at 35 000 × *g* for 30 min at 4°C. The pellet containing the protein was washed twice with cold acetone containing 0.07% (v/v) β-mercaptoethanol and centrifugated at 35 000 × *g* for 30 min at 4°C. The final pellet was dried and solubilized in a solution containing (9.5 M urea), 5 mM K_2CO_3, 1.25% (v/v) SDS, 0.5% (w/v) DTT, 6% (v/v) Triton X-100 and 2% (v/v) Pharmalytes pH 3.5–9.5. The first dimension isoelectrofocusing (IEF) was done on cylindrical gels (1.5 mm in diameter, 16 cm long) containing 4% (w/v) acrylamide, 2% (v/v) Triton X-100, 2.5% (v/v) pharmalytes pH 5–6, 7.5% (v/v) pharmalytes pH 5–8 and 9.2 M urea. The protein samples were loaded on gel and run for 21 kV/h. The gels were then equilibrated with 62.5 mM Tris (pH 8.8) containing 2.3% (w/v) SDS and 0.3 M sucrose for 20 min. Separation in the second dimension was then carried out by SDS-PAGE according to the method described by Damerval et al. [16]. Slab gels (1.5 mm thick) containing 11% (w/v) acrylamide, 0.5 M Tris pH 8.8, 0.15% (w/v) SDS and 1% (w/v) sucrose were run at 40 mA per gel. The molecular mass of the polypeptides was estimated using standard proteins. Following electrophoresis, gels were fixed in 40% ethanol containing 10% acetic acid and were silver stained according to the method of Heukeshoven and Dernick [17].

3. Results

Before salt stress, clone 43 demonstrated a better length growth than clone 42 (Fig. 1). NaCl 200 mM was applied 120 days after the cutting state. During the first 14 days of salt stress the total shoot length of the two clones was not affected (Fig. 1). After 14 days of exposure to salt, clone 43 demonstrated a complete inhibition of its shoot

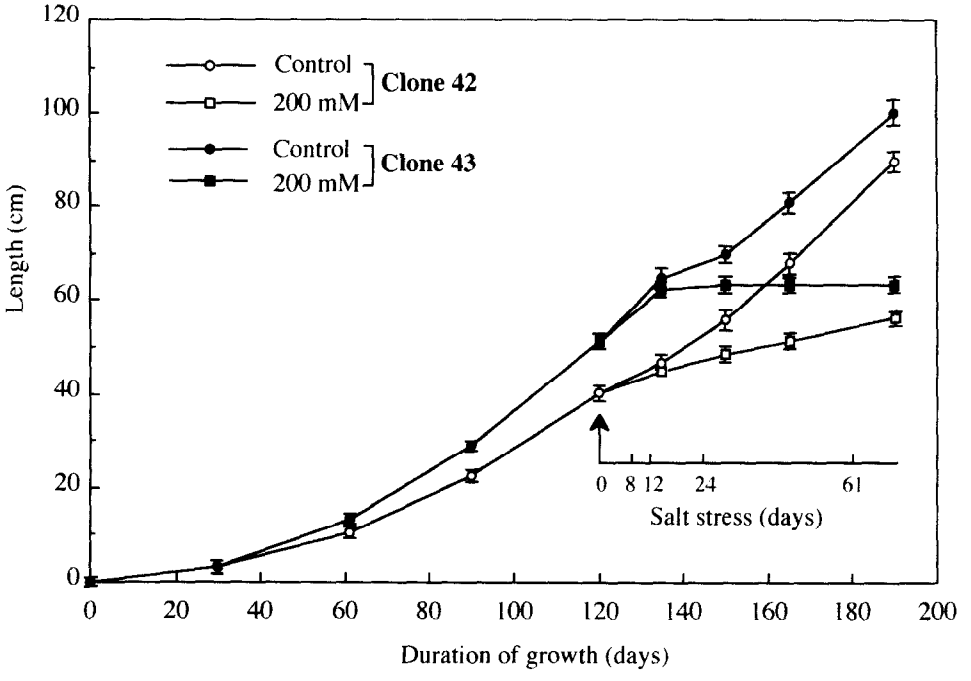

Fig. 1. Growth length of two clones of *Eucalyptus microtheca* before and after exposure to 200 mM NaCl. Vertical bars represent S.E. values.

length while clone 42 showed a delayed growth. During the salt treatment the daily growth of clone 42 was 3 times lower relative to that of control. In control conditions the fresh and dry weights were higher in clone 43 than in clone 42. After 61 days of salt stress, the fresh weights of the different organs of the two clones were markedly lower than in control plants (Table 1). The leaves of treated plants were more affected than the roots and the reduction of the fresh matter production was more pronounced for the clone 43 (82% and 75% for clone 43 and clone 42, respectively). However the differences between clones are not statistically different. The production of dry matter was reduced to the same extent. Consequently no differences in water content were noticed (Table 1).

Changes in ion content were followed in roots, stems and leaves of the two clones (Fig. 2). Sodium content (Fig. 2A and B) and chloride content (Fig. 2C and D) rapidly increased in roots of treated plants of the two clones, with a more elevated amount of sodium compared to chloride. Whatever the duration of the salt treatment, the uptake of sodium in roots was 2.5 times higher in clone 42 (Fig. 2A) than in clone 43 (Fig. 2B). In shoots of the treated plants the amount of sodium was lower than in roots and no differences were noticed between both clones till 24 days of treatment.

At 61 days, the sodium content was higher in leaves and stems of clone 42 than clone 43. However, considering the ratio of sodium content in leaf to sodium content in root, it was always much higher in clone 43 than in clone 42. Thus, after 8 days this ratio was nearly 2 times higher in clone 43 (0.25) than in clone 42 (0.13), and this factor was maintained over the time of the salt treatment. Concerning the chloride content in the different organs, no significant differences were determined between clones (Fig. 2C and D). For the levels of potassium and calcium in roots of treated plants, a different behaviour was determined between clones (Fig. 2E, F, G and H). For clone 42 the salt treatment induced an increase of potassium and calcium uptake, respectively equal to 5 and 6 times the value of the control after 61 days (Fig. 2E and G). By contrast, in clone 43 the potassium content decreased in response to salt treatment whereas no significant changes were determined for calcium (Fig. 2F and H). In the upper part of the treated clones a diminution of potassium content was mainly localized in stems (Fig. 2E and F), whereas the calcium content was not significantly different upon salt treatment (Fig. 2G and H).

In roots of the two clones, the soluble amino acids content increased progressively during the salt treatment (Fig. 3). For clone 43 the maximum value was obtained after 24 days and at 61 days it diminished to reach a value similar to that of control (Fig. 3B). In stems of clone 43 a net increase of the soluble amino acids content was noticed after 8 days of salt treatment. Afterwards, this

Table 1
Fresh weight (FW), dry weight (DW) and water content of different organs of two clones of *Eucalyptus microtheca* exposed during 61 days to 0 or 200 mM NaCl (± S.E.)

		Clone 42		Clone 43	
		Control	200 mM	Control	200 mM
FW (g/plant)	Roots	1.60 ± 0.10	0.66 ± 0.02	3.00 ± 0.15	1.74 ± 0.08
	Stems	6.95 ± 0.14	1.40 ± 0.18	7.18 ± 0.30	1.32 ± 0.15
	Leaves	15.74 ± 0.10	4.02 ± 0.40	29.01 ± 0.31	5.20 ± 0.48
DW (g/plant)	Roots	0.37 ± 0.01	0.15 ± 0.01	0.58 ± 0.02	0.25 ± 0.01
	Stems	2.92 ± 0.07	0.58 ± 0.08	2.85 ± 0.14	0.51 ± 0.06
	Leaves	5.47 ± 0.03	1.44 ± 0.14	9.48 ± 0.11	1.73 ± 0.17
Water content (%)	Roots	76.5	77.0	80.0	85.5
	Stems	58.0	58.6	60.0	61.1
	Leaves	65.2	64.2	67.3	66.7

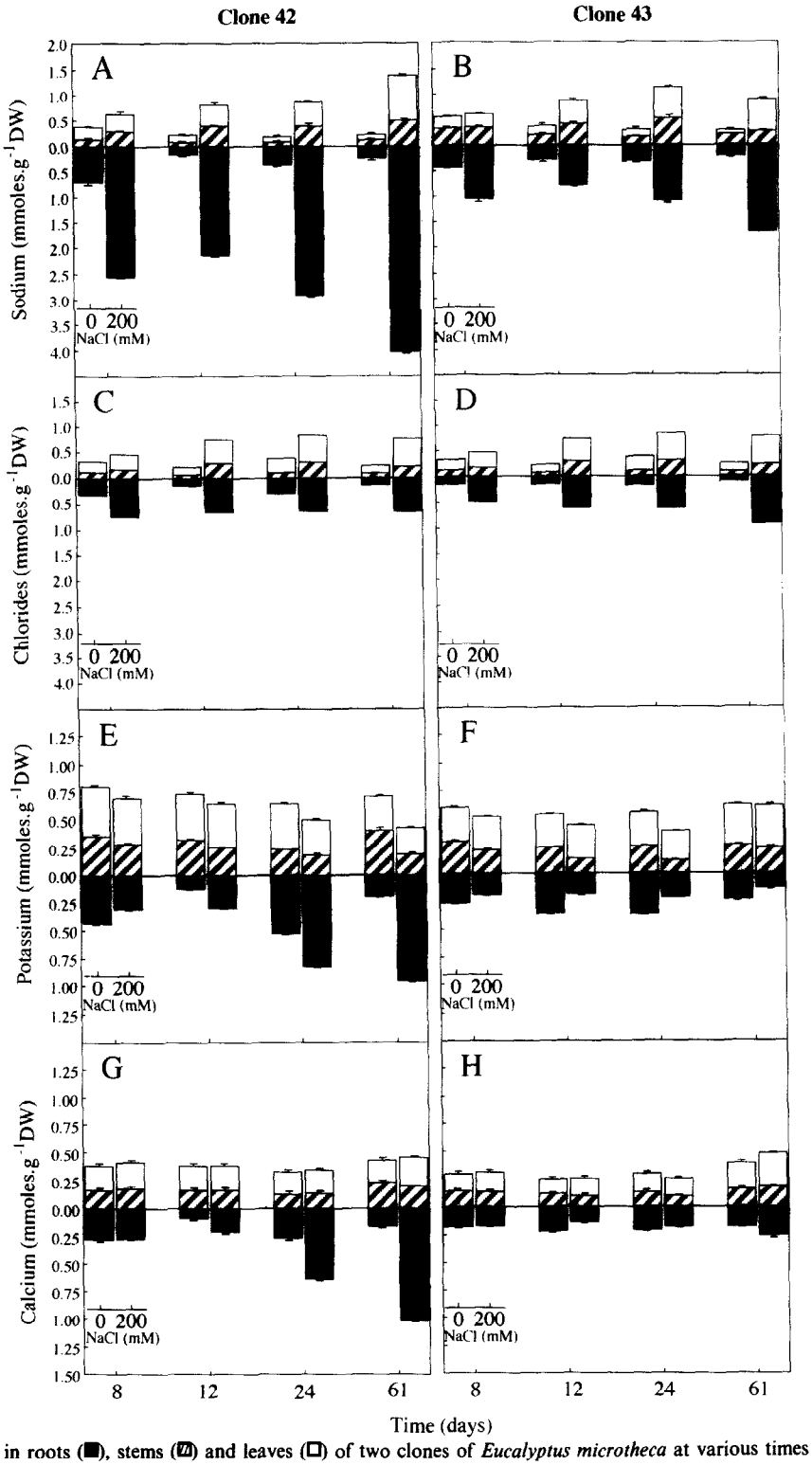

Fig. 2. Ion contents in roots (■), stems (▨) and leaves (□) of two clones of *Eucalyptus microtheca* at various times after exposure to 0 or 200 mM NaCl. Vertical bars represent S.E. values.

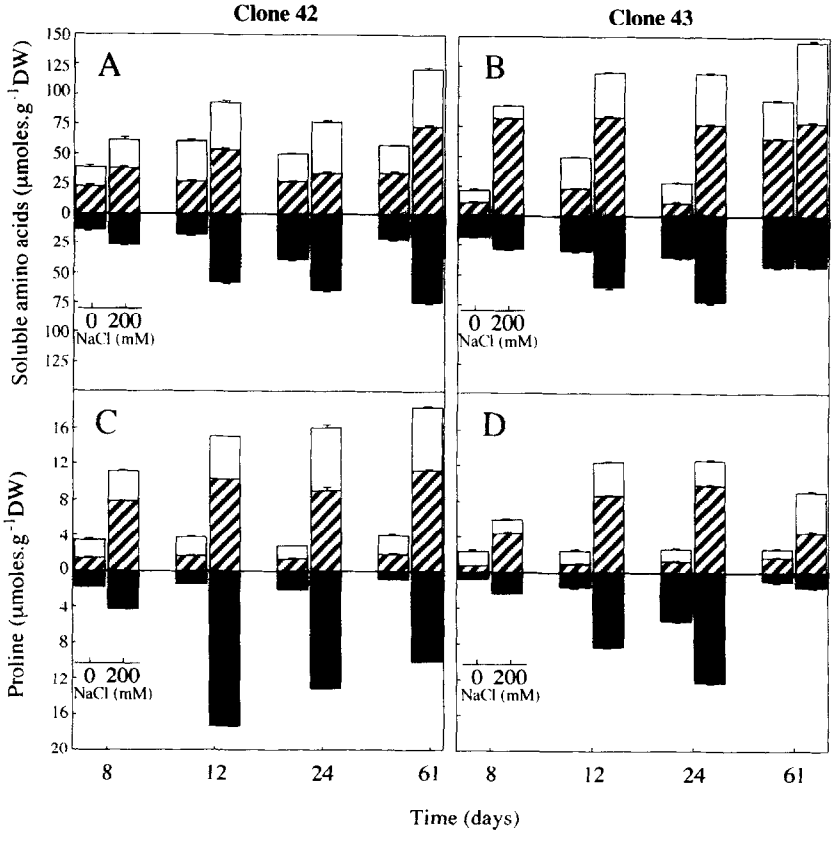

Fig. 3. Amino acids and proline contents in root (■), stem (▨) and leaf (□) of two clones of *Eucalyptus microtheca* at various times after exposure to 0 or 200 mM NaCl. Vertical bars represent S.E. values.

value remained constant during the treatment. In the leaves of this clone, the soluble amino acids level progressively raised and after 61 days it reached the same value as in the stems. In treated shoots of clone 42 an increase in soluble amino acids content took place both in stems and in leaves (Fig. 3A). In stems of this clone, this increase was lower than for clone 43, however after 61 days the two clones had the same amino acid levels in this organ. In leaves the amino acid content was quite similar to that determined in clone 43.

In response to salt treatment, proline content rapidly increased in roots and stems of the two clones (Fig. 3C and D). In clone 42 the higher level was obtained after 12 days (30% of the total soluble amino acids), whereas for clone 43 it was reached only after 24 days (18% of the total soluble amino acids). At 61 days, clone 42 maintained a high level of proline in roots and stems of the treated plants, but for clone 43 the differences with control values were less important. In leaves of both clones the proline content progressively increased with the duration of salt treatment and a higher level was determined for clone 42 (around 15% of the total soluble amino acids).

The effect of NaCl on the root protein composition has been examined on 5-month old plants after 2, 12 and 24 days of salt treatment. Until 12 days, the growth of the two clones were not significantly reduced. Total proteins were extracted from the roots and analyzed by 2D-PAGE (Fig. 4). Each gel revealed that at least 400 polypeptides were separated by the 2D gel system.

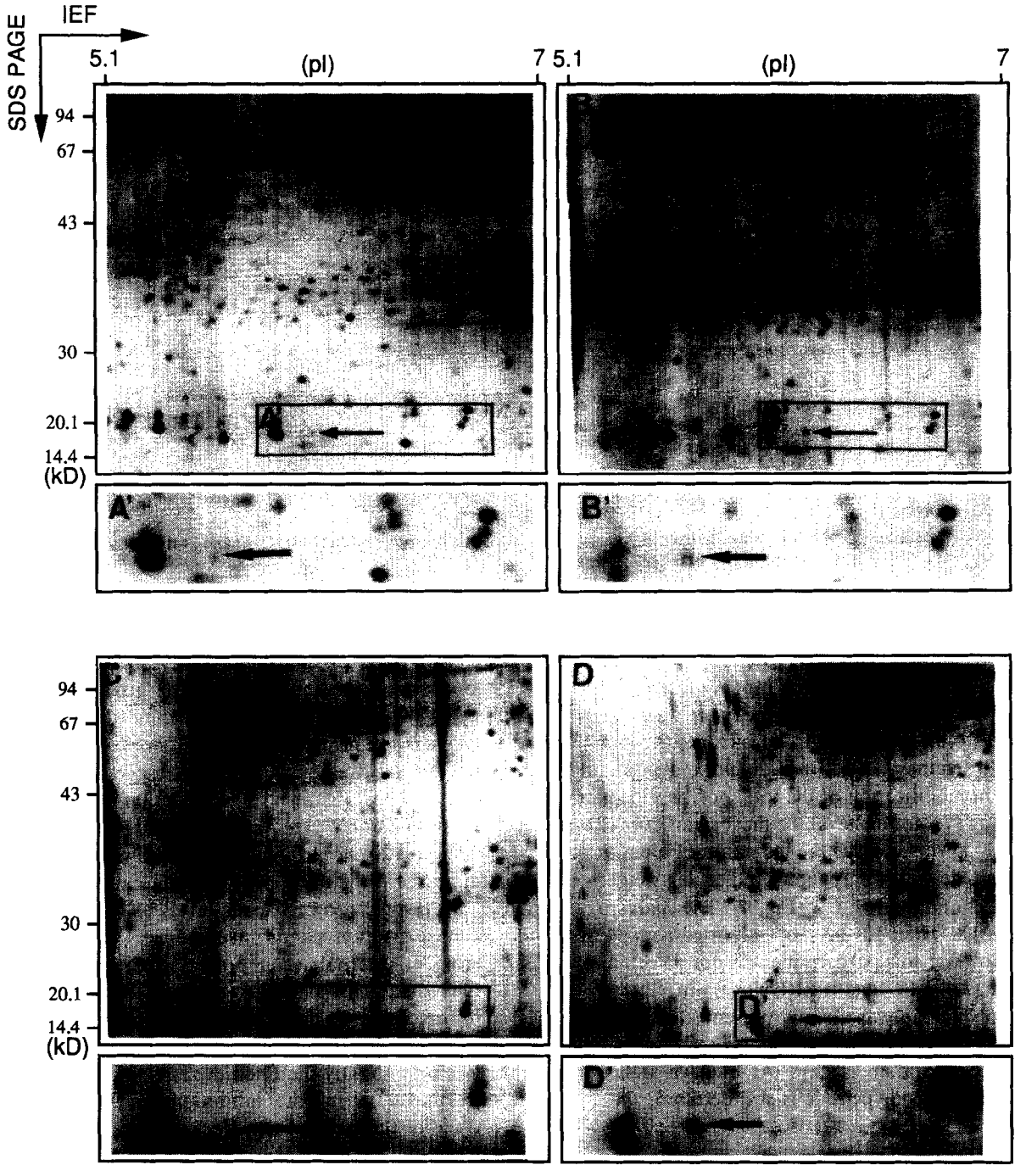

Fig. 4. Patterns of silver stained proteins extracted from roots of *Eucalyptus microtheca* clone 42 grown during 12 days with 0 mM NaCl (A), 200 mM NaCl (B) and clone 43 grown during 12 days with 0 mM NaCl (C), 200 mM NaCl (D). The arrow points to the 18 kDa polypeptide. The A′, B′, C′ and D′ gel sections represent the region containing the 18 kDa protein.

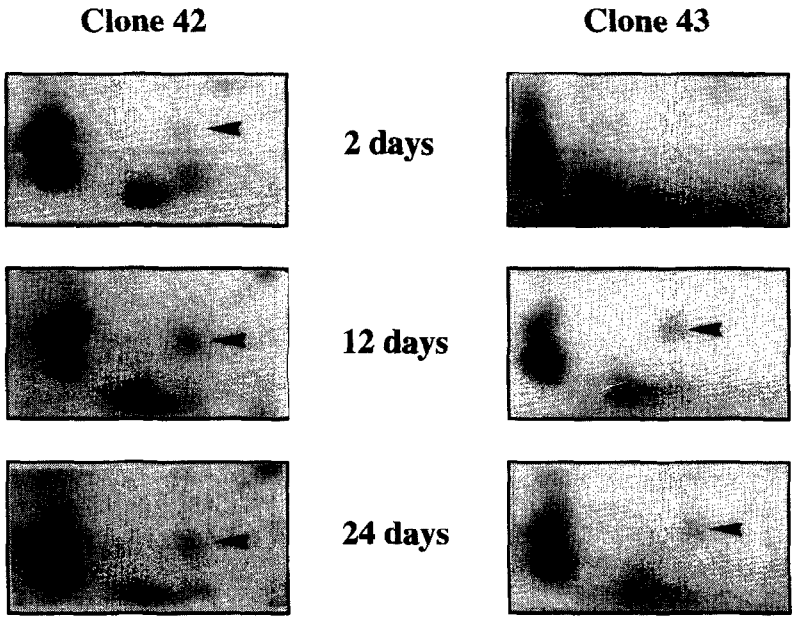

Fig. 5. Detailed portions of two-dimensional gels from roots of *Eucalyptus microtheca* clones after 2, 12 and 24 days of salt treatment (200 mM). The arrows point to the 18 kDa polypeptides.

The polypeptides were mainly distributed from 30–67 kDa and had p*I*s ranging from 5–7, a less important density of polypeptides were determined from 14–30 kDa. In both clones (42 and 43), a salt treatment of 200 mM did not induce an important modification of the total polypeptide pattern. However for clone 43 the most significant effect of 200 mM NaCl was the induction of a major polypeptide with an apparent molecular weight of 18 kDa and a p*I* of 6.2 (Fig. 4). This induction occurred after 12 days of salt treatment (Fig. 5). However this polypeptide was not detected after 2 days of salt treatment (Fig. 5). In clone 42 this polypeptide was already present at a low level in control conditions and its synthesis was slightly amplified after 12 days. At 24 days of salt treatment this polypeptide is still detected.

4. Discussion

An elevated concentration of NaCl (200 mM) resulted in a reduction of the growth of the two tolerant clones. However, differences in the intensity of the growth reduction have been noticed. Concerning the shoot length, the growth of the clone 42 organs carried on with a slower rate whereas the growth of clone 43 stopped after 24 days of salt treatment. In other respects, after 61 days of NaCl treatment, the reduction in dry matter production was more pronounced for clone 43. Thus, mainly according to height growth, clone 42 appeared to be more tolerant than clone 43.

In response to increasing levels of NaCl in the culture medium, plants have to cope with the lowering of external water potential. Consequently, the reduction of plant growth is usually attributed to osmotic stress [18]. Based on the water content of the organs, the hydric state of the two clones was only slightly modified in response to the salt treatment. Then, the inhibition of the growth could be the result of the effect of ions, particularly sodium and chloride, likely to be absorbed into the plant to adjust their hydric potential in response to the external solution. Thus, unlike halophytes, glycophyte species seemed unable to compartmentalize ions which became toxic for the

cellular metabolism [3]. Eucalyptus species did not depart from this behaviour [19] and the salt tolerance of this species appeared to be related to a low level of salt accumulation in the shoot compared to the root level [2]. In our experiment, sodium and chloride contents mainly increased in roots of the two clones, but higher levels of sodium were determined in clone 42. No data are available to determine if this mechanism of salt tolerance is the consequence of a higher sodium retention in this organ or if a mechanism of sodium efflux from the upper part was effective [20]. Compared to clone 42, sodium ions were less absorbed in clone 43. Moreover, the ratio of sodium content in leaf to sodium content in root was always much higher in clone 43 than in clone 42. Consequently, in response to an increase of NaCl in the medium, clone 42 absorbs ions, but to avoid sodium toxicity in the upper part of the plant, these ions were mainly restricted to the roots. This strategy is an 'excluder type' commonly found in tolerant glycophytes [28]. For clone 43, this strategy also takes place to a lesser extent with a limited absorption of ions. Such a difference between the two clones could explain the higher tolerance of clone 42.

Concerning the level of potassium, it only decreased in roots and stems of the treated plants. It is commonly accepted that competition exists between Na^+ and K^+ and the level of internal potassium could be reduced at high external NaCl concentrations. Thus, the roots of clone 43 exposed to 200 mM NaCl reached a level of potassium (expressed as % of K^+ in dry matter) of 0.6–1.0%, near the critical level resulting in perturbations of the metabolic process, and affecting growth [21]. Elsewhere, based on cell culture, a number of experiments have shown that a higher level of internal K^+ could be correlated with a higher level of salt tolerance [22–25]. Thus, considering the higher content of potassium in roots of clone 42, potassium may play an important role in determining the capacity for growth. The protective role of calcium could also be implied in the higher tolerance of this clone. A number of previous studies focused on the protective effect of calcium at high NaCl concentrations [26,27]. A more elevated content of calcium in the roots of clone 42 exposed to NaCl could allow the maintenance of membrane integrity and avoid leakage of K^+. By contrast, slight changes in calcium levels in the organs of the clone 43 would be insufficient to obtain a beneficial effect of this ion.

As a result of salt treatment, the soluble amino acid content increased similarly in the two clones. However a higher content of proline was determined in clone 42 relative to clone 43. In glycophytes exposed to salt treatment it has been considered that the increase in soluble amino acid levels, concomitant to an increase in nitrogen content, should avoid a toxic amount of ammonium [29]. The occurrence of a higher ratio of proline/amino acids in clone 42, the most NaCl tolerant, suggests a more specific role of this amino acid in regard to salt tolerance. An osmotic role could be excluded considering the relatively low level of proline in treated tissues. Moreover an elevated sodium/proline ratio seems to be inconsistent with proline as an osmotic compound [30]. By contrast, increasing amounts of proline in response to salt treatment could involve a protective effect of enzyme activities [31], the storage of nitrogen and reducing power [32]. A possible role of proline in maintenance of protein hydration has been evoked in response to hydric stress [33]. However this role was probably less important in this experiment considering the low fluctuation of the hydric status of the eucalyptus plant submitted to NaCl treatment.

The synthesis of a polypeptide of 18 kDa with $pI = 6.2$ was induced in roots of clone 43 after 12 days of salt treatment. This polypeptide was not detected after 2 days of salt treatment. For clone 42, the 18 kDa polypeptide was already present in low quantities in control conditions and its synthesis was amplified after 12 days of salt treatment. A study on citrus [34] had shown that salt stress only induced a very minor difference on protein patterns. The main difference was a more intense synthesis of a polypeptide of 25 kDa which was already present in control conditions in salt-tolerant cells, whereas in the salt-sensitive cells this polypeptide was less induced by salt stress but was not present in the control condition. It does not appear that the 18 kDa polypeptide corresponds to a salt shock protein because its synthesis is not stimulated during the first days of salt stress, at least

for clone 43. In comparison to other studies on salt-associated proteins it appears that the 18 kDa polypeptide is not generally the most prevalent protein associated with salt stress. The low molecular weight of the salt associated polypeptide in this study can be compared with a polypeptide of 18 kDa which was found to be stimulated by salt [7] in tobacco cells. This polypeptide may play a role in salt tolerance since it was already present in roots of clone 42 in control conditions. The presence of this polypeptide before salt treatment can assist the plant to defend against salt stress, whereas for clone 43 the absence of this polypeptide before the stress could contribute to a delay in the response of the plants to stress. The similarity in molecular weight between the 18 kDa protein stimulated during salt stress in roots of *Eucalyptus microtheca* and dehydrins proteins, the synthesis of which is stimulated during drought stress [35], may implicate this protein as a response to water deficit. At the moment this polypeptide is being characterized in order to confirm if there is any correlation with polypeptides known to be associated with abiotic stress on other plant species.

References

[1] T.A. Omran, Salinity effects on growth and mineral content of some Eucalyptus species. Alex. J. Agric. Res., 31 (1986) 449–459.

[2] D. Prat and R.A. Fathi, Variation in organic and mineral components in young Eucalyptus seedlings under saline stress. Physiol. Plant., 79 (1990) 479–486.

[3] H. Greenway and R. Munns, Mechanisms of salt tolerance in nonhalophytes. Annu. Rev. Plant Physiol., 31 (1980) 149–190.

[4] T.J. Flowers, P.F. Troke and A.R. Yeo, The mechanism of salt tolerance in halophytes. Annu. Rev. Plant Physiol., 28 (1977) 89–121.

[5] R. Munns, H. Greenway and G.O. Kirst, Halotolerant eukaryotes, in: O.L. Lange, P.S. Nobel, C.B. Osmond and H. Zeigler (Eds.), Encyclopedia of Plant Physiology, New Series, Vol. 12C, Springer Verlag, 1983, pp. 59–135.

[6] M. Reuveni, A.B. Bennet, R.A. Bressen and P.M. Hasegawa, Enhanced H^+ transport capacity and ATP hydrolysis activity of the tonoplast H^+-ATPase after NaCl adaptation. Plant Physiol., 94 (1990) 524–530.

[7] N.K. Singh, A.K. Handa, P.M. Hasegawa and R.A. Bressan, Proteins associated with adaptation of cultured tobacco cells to NaCl. Plant Physiol., 79 (1985) 126–137.

[8] Z. Zhao, J.M. Heyser and H.J. Bonnert, Gene expression in suspension culture cells of the halophyte *Distichlis spicata* during adaptation to high salt. Plant Cell Physiol., 30 (1989) 861–867.

[9] N.K. Singh, C.A. Bracker, P.M. Hasegawa, A.K. Handa, S. Buckel, M.A. Hermodson, E. Pfankovh, F.E. Regnier and R.A. Bressan, Characterization of osmotin. A thaumatin-like protein associated with osmotic adaptation in plant cells. Plant Physiol., 85 (1987) 529–536.

[10] K. Skiver and J. Mundy, Gene expression in response to abscissic and osmotic stress. Plant Cell, 2 (1990) 503–512.

[11] W.J. Hurkman, H. Peggy Tao and C.K. Tanaka, Germin-like polypeptides increase in barley roots during salt stress. Plant Physiol., 97 (1991) 366–374.

[12] P.C. Larosa, N.K. Singh, P.M. Hasegawa and R.A. Bressan, Stable NaCl tolerance of tobacco cells is associated with enhanced accumulation of osmotin. Plant Physiol., 91 (1989) 855–861.

[13] G. Ben Hayyim, Z. Faltin, S. Gepstein, L. Camoin, A.D. Strosberg and Y. Eshdat, Isolation and characterization of salt-associated protein in *Citrus*. Plant Sci., 88 (1993) 129–140.

[14] S. Moore and W.H. Stein, A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem., 211 (1954) 907–913.

[15] W. Troll and J. Lindsley, A photometric method for the determination of proline. J. Biol. Chem., 215 (1955) 655–660.

[16] C. Damerval, P. de Vienne, M. Zivy and H. Thiellement, Technical improvement in two-dimensional electrophoresis increase the level of genetic variation detected in wheat-seedling protein. Electrophoresis, 7 (1986) 52–54.

[17] J. Heukeshoven and R. Dernick, Simplified method for silver staining of proteins in polyacrylamde gels and the mechanism of silver staining. Electrophoresis, 6 (1985) 103–112.

[18] E.V. Maas and R.H. Nieman, Physiology of land tolerance to salinity, in: G.A. Yung (Ed.), Crop Tolerance to Suboptimal Land Conditions, American Society of Agronomy Special Publication, Crop Science Society of America, Madison, WI, 1978, pp. 277–299.

[19] A.L. Rogers, Foliar salt in *Eucalyptus* species. Aust. For. Res., 15 (1985) 9–16.

[20] O. Wolf, R. Munns, M.L. Tonnet and W.D. Jeschke, Concentration and transport of solutes in xylem and phloem along the leaf axis of NaCl treated *Hordeum vulgare*. J. Exp. Bot., 41 (1990) 1133–1141.

[21] R.A. Leigh and R.G. Wyn Jones, A hypothesis relating critical potassium concentrations for growth to the distribution and functions of this ion in plant cell. New Phytol., 97 (1984) 1–13.

[22] T.P. Croughan, S.Y. Staverek and D.W. Rains, Selection of a NaCl tolerant line of cultured alfalfa cells. Crop Sci., 18 (1979) 959–963.

[23] A.A. Watad, L. Reinhold and H. Lerner, Comparison between a stable NaCl-selected cell line and the wild type. Plant Physiol., 73 (1973) 624–629.

[24] G. Ben-Hayyim, P. Spiegel-Roy and H. Neumann, Relation between ion accumulation of salt-sensitive and isolated stable salt-tolerant cell lines of *Citrus aurantium*. Plant Physiol., 78 (1985) 144–148.

[25] G. Ben-Hayyim, U. Kafkafi and R. Ganmore-Neumann, Role of internal potassium in maintaining growth of cultured *Citrus* cells on increasing NaCl and $CaCl_2$ concentrations. Plant Physiol., 85 (1987) 434–439.

[26] G.R. Cramer, A. Läuchli and V.S. Polito, Displacements of Ca^{2+} by Na^+ from the plasmalemma of root cells. A primary response to salt stress? Plant Physiol., 79 (1985) 207–211.

[27] J. Wieneke and A. Lauchli, Effects of salt stress on distribution of sodium ions and some other cations in two soybean varieties differing in salt tolerance. Z. Pflanzenernachr. Bodenkol, 143 (1980) 55–67.

[28] H. Marschner, Mineral nutrition of higher plants, Academic Press, London, 1986, pp. 477–542.

[29] I. Zidan, A. Shaviv, I. Ravina and P.M. Neumann, Does salinity inhibit maize leaf growth by reducing tissue concentrations of essential mineral nutrients? J. Plant Nutr., 15 (1992) 1407–1419.

[30] W.A. Torello and L.A. Rice, Effects of NaCl stress on proline and cation accumulation in salt sensitive and tolerant turfgrasses. Plant Soil, 93 (1986) 241–247.

[31] A. Pollard and R.G. Wyn Jones, Enzyme activities in concentrated solutions of glycinebetaine and other solutes. Planta, 144 (1979) 1310–1315.

[32] Y. Bellinger and F. Larher, Proline accumulation in higher plants: a redox buffer? Plant Physiol., 6 (1987) 23–27.

[33] B. Schobert and H. Tschesche, Unusual solution properties of proline and its interaction with proteins. Biochem. Biophys. Acta, 541 (1978) 270–277.

[34] G. Ben-Hayyim, Y. Vaadia and G.B. Williams, Protein associated with salt adaptation in *Citrus* and tomato cells: involvement of 26 kDa polypeptides. Physiol. Plant., 77 (1989) 332–340.

[35] T.J. Close, R.D. Fenton and F. Moonan, A view of plant dehydrins using antibodies specific to the carboxy terminal peptide. Plant Mol. Biol., 23 (1993) 279–286.