Characterization of environmental stress responses during early development of *Pringlea antiscorbutica* in the field at Kerguelen

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**Summary**

- Early development of Kerguelen cabbage (*Pringlea antiscorbutica*) was studied in the Kerguelen archipelago, its natural habitat, and under laboratory conditions. Polyamines, which are involved in developmental processes and responses to stress in several plant species, were used as markers of physiological status of *P. antiscorbutica* seedlings.
- Analysis under laboratory conditions of responses to low water availability and to salinity enabled identification of major environmental constraints restricting seedling development in the subantarctic region.
- Salt stress was found to modify polyamine distribution between seedling organs, in controlled experiments and in the field, thus indicating that polyamine responses to salt stress were functional in the field at Kerguelen. By contrast, exposure to low water availability induced different polyamine responses in controlled experiments and in the field.
- The present work thus shows that, under certain conditions, polyamine concentrations can be used as a marker of specific stress responses of seedlings in the field. Discrepancies are discussed in terms of growth conditions in the laboratory and of combined stresses in natural habitats.

**Key words:** Kerguelen cabbage (*Pringlea antiscorbutica*), polyamines, salt stress, drought, seedling development, root, environmental stress, climatic change.


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**Introduction**

Environmental factors affecting germination and early seedling growth are often primary determinants of distribution and abundance of plant populations (De Villier *et al.*, 2001; Clark & Wilson, 2003). Seedling stages are also considered to be far more sensitive than older vegetative forms to environmental stresses (Hou & Romo, 1998; Clark & Wilson, 2003). Thus, water availability, which depends on soil characteristics and salinity, drought and temperature deeply influence species distribution. The subantarctic species *Pringlea antiscorbutica* (Kerguelen cabbage) forms widespread populations in the Kerguelen archipelago (Hennion & Bouchereau, 1998). These islands are characterized by a cold oceanic climate as shown by an annual mean temperature of +4.5°C and, in the eastern part of the archipelago, an annual mean precipitation of 775 mm (Météo-France, 1951–2002 records, Port-aux-Français). Within this region, distribution and occurrence of vegetation are strongly influenced by microenvironmental conditions including salt deposition, substrate instability and exposure to wind (Jenkins, 1997). *Pringlea antiscorbutica* exhibits a wide ecological amplitude, growing and flowering in coastal areas subjected to sea flooding as well as in mountain areas (Hennion & Bouchereau, 1998). This perennial species can thus tolerate exposure to salt spray, soil salinity and freezing temperature. However, mature plants require a high and efficient water supply by roots and air moisture. Indeed, relative water content in leaves does not decrease below 83% in the field, although *P. antiscorbutica* leaves exhibit low diffusion resistance, low responsiveness of stomatal closure.
and no anatomical adaptation related to water stress (Dorne & Bligny, 1993). *Pringlea antiscorbutica*, at least at mature stage, presents physiological adaptations required to face the harsh climate characterizing the subantarctic zone (Dorne & Bligny, 1993; Hennion & Bouchereau, 1998). Mature plants of *P. antiscorbutica* exhibit several biochemical characteristics, which could be related to cold tolerance, such as the abundance, in all organs, of polyamines (Hennion & Martin-Tanguy, 2000), proline and glucose (Aubert et al., 1999a,b). Solute accumulation, observed at mature stage, might also be related to osmoregulation. Proline contents, which are highly responsive to salinity, and glucose concentrations might allow *P. antiscorbutica* to resist severe water stress (Hennion & Bouchereau, 1998).

However, responses of mature plants to environmental stress do not imply tolerance at early stages of development. Besides, climatic changes recorded over the last 40 yr at Kerguelen show a significant increase in temperature and a decrease in precipitation (Frenot et al., 1997), which induce wilting of *P. antiscorbutica* mature plants in the driest stands (Hennion & Martin-Tanguy, 2000). Moreover, responses of *P. antiscorbutica* seedlings to environmental stress have not yet been studied in their natural habitat. Seed germination occurs mainly during austral summer, mostly around mother plants. Meanwhile, seeds can disperse by floating in fresh or saline water, thus colonizing new locations through seed deposition on the shore (Chapuis et al., 2000). Subsequent seedling establishment has been observed, thus indicating that germination and early growth could occur even under conditions of regular sea flooding (Chapuis et al., 2000).

Among its physiological and biochemical characteristics, *P. antiscorbutica* exhibits high amounts of polyamines in organs from mature plants collected in the field (Hennion & Martin-Tanguy, 2000). In higher plants, polyamines, namely spermidine (Spd), spermine (Spm) and their diamine precursors, agmatine (Agm) and putrescine (Put), are generally considered as growth regulators involved in various developmental processes, such as germination and root growth, although their precise role in these processes remains unclear (Smith, 1985; Evans & Malmberg, 1989; Martin-Tanguy, 2001; Coudé et al., 2004). These small and positively charged compounds may assume complex cellular functions in relation to resistance to various abiotic stresses (Lefèvre et al., 2001). Considering their protective effects on cellular structures and processes (Smith, 1985; Lee et al., 1995), their accumulation has been associated, in several plant species, with tolerance to a variety of abiotic stresses, such as salinity (Chattopadhyay et al., 1997; Soyka & Heyer, 1999), chilling (Tajima & Kabaki, 1981; Kramer & Yang, 1989; Lee, 1997; Lee et al., 1997; Shen et al., 2000; He et al., 2002) and water stress (Pedrol et al., 2000). Polyamines are therefore useful biochemical markers of stress response in plants. In the present work, modifications of growth and variations of polyamine contents were investigated during early development of *P. antiscorbutica* in its natural habitat and under laboratory-controlled conditions of salt stress and drought. Comparison of the different conditions demonstrated that adaptive variations of polyamine concentrations and distribution between organs were functional in the field. Comparative study of seedling responses to stress treatment and cultivation under different microhabitats in Kerguelen was used to characterize the environmental constraints imposed on seedlings during their development in the subantarctic region.

Materials and Methods

Description of experimental stations

Criteria of distance to seaside and vegetation composition were used to set up four contrasted experimental stations on Australia Island (Kerguelen Archipelago, 68–70°E, 48–50°S), in sites where natural germination has been observed. These stations have been previously chosen for long-term monitoring of *P. antiscorbutica* growth and physiology (R. Douce, F. Hennion, A. J. Dorne & S. Aubert, unpubl.) as they were close enough to share the same climatic conditions. Station 1 (S1) was flat and slightly sheltered, with deep and moist organic soil and a dense vegetation cover (100%) of *Acaena magellanica* (80%) and *P. antiscorbutica* (20%). Station 2 (S2) was located on top of a gentle slope (10% slope) at 30 m from the coast, and was characterized by exposure to salt spray and by dry or lightly moist organic soil. The vegetation cover in S2 was dense (100%) and consisted of *A. magellanica* (40%), *Azorella selago* (30%) and *P. antiscorbutica* (30%). Located on top of a coastal cliff and exposed to wind, Station 3 (S3) presented drought characteristics, as shown by dry soil with many organic remains. The vegetation cover was dense (90%) and consisted of *Vulpia bromoides* (40%), *Senecio vulgaris* (20%), *P. antiscorbutica* (20%) and *A. magellanica* (10%). Station 4 (S4) was characterized by a peaty soil saturated by seawater. S4 was on a flat flooding coastal zone, with rocks, black peat soil and sparse vegetation cover (70%) consisting of *Crassula mucedo* (50%), a halophytic species (Jenkins, 1997), and *P. antiscorbutica* (20%). Unlike other stations, where soils were covered by superficial remains of vegetation, S4 was deprived of litter. In S4, natural vegetation emerged between rocks and sea flooding remains. S4 was flooded by high tide at least once during the experiment.

Plant material

Seeds of *P. antiscorbutica* R. Br. (Brassicaceae) were collected, during the austral summer in February 2002, on S1 of Australia Island. Seeds were taken from a single flowering stalk. After collection, seeds were kept dry at 4°C in the dark until further use. For field and laboratory experiments, seed germination, up to radicle emergence, was carried out under axenic conditions. Seeds were surface-sterilized for 1 min in 95% ethanol, then soaked for 2 h in 10% (w : v) calcium hypochlorite and rinsed in distilled water (Hennion & Walton, 1997).
Field experiments
Surface-sterilized seeds were plated on sterile Whatman paper in Petri dishes and were kept in dark and humid conditions at 22°C for 9 d. Seedlings, at the same stage of development, were then transferred to the 4 contrasted experimental stations in Australia Island. Plots of 1 m² were enclosed in a plank paddock covered by a net in order to prevent seedling destruction by rodent or wind exposure. In order to prevent competition with other plant species, vegetation was manually discarded from experimental plots without disturbing soil surface. One-hundred seedlings per station were sown along four sowing lines (25 by lines) to facilitate harvest. Seedlings were sown on 28 April 2002 and harvested on 5 June 2002 after 38 d under natural environmental conditions of austral autumn. Before harvest, the number of surviving seedlings was counted within each station. During harvest, seedlings were quickly frozen, in situ, in liquid nitrogen for extraction of free polyamines. Five to 10 seedlings were conserved in 75% ethanol for measurements of morphological parameters.

Microclimatic data and soil parameters
Microclimatic data were recorded every 30 min during the experiment using a permanent Delta-T Logger DL2 (Delta-T Devices Ltd, Cambridge, UK) meteorological station in station 1 (S1). Soil surface temperature (at 5 cm depth) was measured with a thermostor probe; air temperature and relative humidity of atmosphere were measured with an RHT22nL captor (Delta-T Devices Ltd) (150 cm above the ground surface). In order to compare water-holding capacity of soils at sites 1, 2 and 3, samples were collected in the first horizon (about 5 cm depth, where roots of *P. antiscorbutica* were numerous) using cylindrical cores (5 cm diameter, 5 cm high). The samples were kept moist and sent for analysis in France. They were saturated with deionized water, weighed and transferred on ceramic plates. A pressure plate extractor was used to determine water contents of samples at different pressures, expressed in hPa or in its logarithmic form, named pF (hydric potential), from saturation (pF = 0) to total desiccation by drying samples at 105°C for 4 d (pF = 7).

Cultivation and treatments in a controlled growth chamber
Surface-sterilized seeds were plated on square (14 × 14 cm) Petri dishes for germination under axenic conditions. Growth medium consisted of 0.8% (w:v) agar completed with 1× Murashige and Skoog (MS) basal salt mix (M 5519; Sigma, Saint Louis, USA), pH 5.7. Mannitol (300 mM) or NaCl (300 mM) were added to MS medium when required. Petri dishes were sealed with Parafilm (American National Can, Chicago, IL, USA) and stored vertically. Seeds were allowed to germinate, up to radicle emergence, in a temperate growth chamber at 22°C for 4 d (16 h light/8 h dark regime) (Hennion & Walton, 1997). Seedlings were then transferred to controlled growth chambers under cold conditions close to mean conditions of subantarctic summer (5°C night/10°C day, 14 h light, 1.3 MJ m⁻² d⁻¹) (Hennion & Bouchereau, 1998). Salt and drought treatments were performed for 10 d at 5°C/10°C.

Measurement of morphological parameters
At the end of experiments, primary root, hypocotyl length and lateral root number were determined. Morphological parameters are given as the mean ± standard error of the mean (SEM). Measurements were performed on at least five seedlings for field experiments and on 30 seedlings for controlled treatments.

High-pressure liquid chromatography analysis of polyamines
After collection in the field or after 10 d of treatment in controlled growth chambers, seedlings were frozen in liquid nitrogen and lyophilized. Shoots and roots were separated after lyophilization. Polyamine analysis was performed on pools of tissues, each representing at least five seedlings. Amine concentrations were calculated as the mean (± SEM) from measurements performed on three pools. In order to extract free amines, aerial parts, roots or whole seedlings were ground in 0.5 ml of 1 M HCl (Hennion & Martin-Tanguy, 2000; Hummel et al., 2002). After extraction for 1 h, samples were centrifuged at 18 000 g for 30 min, and the supernatant containing free amines, was stored frozen at −20°C until use. High-pressure liquid chromatography (HPLC) was used to separate and quantify polyamines prepared as their dansyl derivatives, as described previously (Hennion & Martin-Tanguy, 1999; Hummel et al., 2002). The amine fraction was analysed by HPLC using a LKB 2152 plus LKB 2150 chromatography system (Pharmacia LKB, Uppsala, Sweden) with a HPLC column packed with reverse phase spherisorb ODS-2 (particle size 5 µm; 4.6 × 250 mm; Waters, Milford, MA, USA). Samples (20 µl) of the dansylated amine fractions were applied to the column and eluted with a programmed methanol–water solvent gradient, changing from 60% to 95% over 23 min at a flow rate of 0.8 ml min⁻¹. Elution was completed after 7 min. For detection of dansylated amines, an excitation wavelength of 365 nm was used with an emission wavelength of 510 nm (Hennion & Martin-Tanguy, 1999). Results were standardized with equimolar (0.1 nmol) mixtures of dansylated amines. All amine standards were purchased from Sigma (Saint Louis, MO, USA).

Statistical analysis
The effects of stations or controlled treatments were tested using the Kruskal–Wallis nonparametric analysis of variance. When statistically significant differences were observed
(P < 0.05), pairwise comparisons were carried out using the Mann–Whitney U-test on ranks (P < 0.05). Statistical analyses were performed with Minitab release 13.31 statistical software (Minitab, State College, PA, USA).

Results

Characterization of environmental conditions in the field

During field experiments, climatological data were continuously recorded in the experimental station S1. Figure 1 shows mean air and soil temperatures and relative atmospheric humidity during a 24-h period. Soil temperature at 5 cm below ground level was relatively constant during 24-h periods, with a mean of 3.7 ± 0.2°C and during the period of experiments, as shown by constancy of standard error (Fig. 1). Relative atmospheric humidity was also constant during 24-h periods and during the period of experiments, with a mean of 70.7 ± 0.3%. Air temperature was higher during the light period than during the night (Fig. 1). Mean air temperature reached 4.6 ± 0.2°C during the light period and was relatively constant at 3.9 ± 0.2°C after sunset and around 3.6 ± 0.2°C during the night. Air temperature was less constant than soil temperature, reaching 11°C maximum and −0.5°C minimum during the period of experiments. Only 10 measurements from 2 d gave air temperature below 0°C. Climatological data plotted in S1 gave an estimation of environmental parameters in Australia Island. The four experimental stations were close enough (600 m maximum distance) to share common climatic parameters. Moreover, the four sites were located on the same side of the island and thus subjected to the same light conditions. However, site three was subjected to wind exposure, which could locally modify air humidity and temperature.

Soil texture and related water availability were studied in stations 1, 2 and 3 in the first horizon, in which early development and primary root growth of seedlings occur (Fig. 2). Station 4 was characterized by a massive soil structure, which resulted from presence of peat, and was saturated with water, mainly sea water. Whereas S1 and S2 showed closely similar soil textures in the first horizon, with more than 70% of clay (Fig. 2a), soil water available for plants might be more restricted in S2 than in S1. Water amount in S2 only depended on precipitation while soil in S1 was continuously well-watered by fresh water from an upstream lake. Moreover, soil surface was punctually dry in S2 while remaining wet in S1. Whereas S1 and S2 exhibited fine-textured soils, S3 was characterized by a silt-sandy soil (Fig. 2a). The relationship between water content and water availability depends mainly on structure and texture of the soil. Fig. 2b illustrates the different properties of soils from sites 1, 2 and 3, on the basis of the relationship between the water content and the hydric potential (i.e. the pressure needed to extract water from soil porosity). The water available for plants is usually defined by two limits: the field capacity (pF = 3.0) when all the macro-porosity is full of water and the permanent wilting point (pF = 4.2). The shapes of the curves shown in Fig. 2b suggest that the water is available for plants when soil moisture content is higher than 200% (of dry weight) in S1, 150% in S2 and 100% in S3. A survey of soil water content in these soils revealed that these limits are usually reached in summer at site 3, occasionally at site 2 and very rarely in site 1 (Y. Frenot, unpubl. data). These data did not give evolution of water content during the time of experiment or exact stress imposed on seedlings, but showed that S2 and S3 were more susceptible to water stress than S1.

Seedling establishment in the field and morphological characterization

Figure 3a shows the percentage of seedling survival in the four experimental stations. Whereas no difference was observed...
Fig. 2 Estimation of soil structure and water availability in three stations of Australia Island. (a) Relative percentages of clay (black), silt (unfilled) and sand (tinted) in the first horizon were measured in station 1, 2 and 3 (S1, S2, S3). (b) Curves of soil water release in the first horizon were plotted as relative soil moisture against hydric potential, within station 1 (tinted circles), station 2 (closed circles) and station 3 (open circles).

Fig. 3 Effects of cultivation in four contrasted stations of Australia Island on the development of *Pringlea antiscorbutica* seedlings. (a) Percentage of survival, (b) primary root length, (c) number of emerged lateral roots, (d) hypocotyl length and (e) phenotypes of seedlings grown in stations 1, 2, 3 and 4 (S1, S2, S3 and S4) are shown. Conservation of field samples in 75% (v : v) ethanol for transportation resulted in bleaching. In the field, seedlings from S1, S2 and S3 showed greening of aerial organs, whereas cotyledons of seedlings from S4 were chlorotic. Estimation of survival was made just before harvest. Values are the mean ± SEM of measurements on at least five seedlings. Measurements were made at the end of a 38-d growing period in the field. Statistical analysis of seedling survival was based on the data from the different sowing lines. Bars with different letters indicate significant differences (P < 0.05).
between sowing lines within an experimental station, survival rate was drastically modified in response to growth conditions. Maximal transplantation success was achieved in the well-watered station (S1). In comparison, survival percentage was significantly reduced in S2, S3 and S4 (Fig. 3a). Survival of seedlings in the natural habitats, even in absence of competition with other plant species thus drastically depended on location. These differences in transplantation success might be associated with differential responses to environmental constraints.

Figure 3 also shows morphological parameters measured on seedlings harvested in the four stations. Seedlings grown in S1 exhibited the most developed root architecture with a longer and more ramified primary root (Fig. 3b,c) and S1 was the most propitious station for seedling survival. Root development was inhibited in S4, leading to a short primary root deprived of lateral roots (Fig. 3b,c). Therefore, reduction of survival percentage to 33% in S4 could be ascribed to poor implantation through short anchorage system and poor nutrient acquisition (Fig. 3b,c). Primary root lengths were similar in seedlings from S2 and S3, but significantly shorter than those from S1 (Fig. 3b), and the number of lateral roots was decreased in S2 and S3 seedlings in comparison with S1 seedlings (Fig. 3c). However, lateral root density was similar between S1 and S2 seedlings (Mann–Whitney test, \( P > 0.05 \), ns), with 0.35 ± 0.1 and 0.32 ± 0.2 lateral roots per mm of primary root, respectively. By contrast, seedlings grown in S3, which is characterized by sandy soil, exhibited a less ramified root system with 0.12 ± 0.1 lateral roots per mm of primary root (Mann–Whitney test, \( P < 0.05 \)), thus resulting in poorly developed root architecture.

At the end of the experiment, only seedlings from S1 had developed first true leaves (Fig. 3e). Cotyledons were injured and exhibited necroses in seedling grown in S2, and showed chlorosis in seedlings from S4 (data not shown). Moreover in S4, hypocotyl length of seedling was significantly reduced (Fig. 3d). Hypocotyl elongation in S1, S2 and S3 might be a response for light foraging in stations where seedlings were sown below litter. However, lower hypocotyl length might also be ascribed to reduction of whole seedling development in S4.

Since S1 was the most favourable station for seedling establishment, as shown by both root and shoot development, S1 could be considered as control station for comparison with other locations. By contrast, S2 was not propitious for seedling survival, even if root architecture was not deeply modified in comparison with S1, since seedlings grown in S2 exhibited shoot necrosis and injuries. Growth in S3 resulted in poor root development, and growth in S4 induced overall reduction of development and resulted in both shoot damage and inhibition of root development.

In order to relate environmental conditions with the potential effects of saline and osmotic stresses, \( P. \) antiscorbutica seedlings were grown under controlled laboratory conditions and were subjected to saline and osmotic stress after germination and beginning of primary root development. Treatments were performed until seedlings grown on control medium reached the same stage of shoot development as observed during field experiments in S1 at the day of harvest. Figure 4 shows the effects of salt and osmotic stress on seedling phenotypes. Salt stress caused a threefold reduction of primary root length in comparison with control (Fig. 4a) and inhibited development of lateral roots (Fig. 4b). However, salt stress induced neither chlorotic cotyledons nor reduction of hypocotyl length (Fig. 4b). Compared with control medium, osmotic stress, which was performed by adding mannitol to the growth medium, induced reduction of root growth and delayed lateral root and shoot development. The major effect of osmotic stress on phenotypes was inhibition of lateral root growth.
development as well as absence of first true leaves compared with control medium (Fig. 4b).

Stress-induced variations of free polyamine contents in seedlings

Figure 5 shows free polyamine contents in both roots and shoots of seedlings harvested during field experiments. In all organs, Agm was the most abundant free polyamine, representing at least 66% of total polyamine pool whatever the station considered. In S1 seedlings, Agm and Put concentrations were significantly higher in shoots than in roots whereas the amounts of Spm were similar in both organs at 0.1 µmol g⁻¹ d. wt (Fig. 5). In seedlings grown in S4, Agm, Put, Spd and even Spm concentrations were significantly more abundant in roots than in shoots (Fig. 5). Thus, cultivation in S4 drastically modified polyamine distribution between shoots and roots. Concentrations of Agm, Spd and Spm, but not Put, were higher in roots of seedlings grown in S4 compared with other stations (Fig. 5). By contrast, Agm, Put and Spd contents in shoots of S4 seedlings were drastically reduced (Fig. 5). Cultivation in S2 or S3 did not significantly modify the amounts of Put, Spd and Spm in roots (Fig. 5b,c,d). Besides, Agm concentration was 30% higher in roots from seedlings grown in S1 than in S2 or S3. In shoots, more drastic differences appeared between those stations (Fig. 5). Shoots from seedlings grown in S2 were characterized by high concentrations of Agm and Spd, without accumulation of Put, as well as important interindividual differences in Agm and Spd contents, as shown by high standard error (Fig. 5). By contrast, shoots from seedlings grown in S3 were characterized by high Spd contents and a significant increase of Put concentrations (Fig. 5b,c).

Figure 6 shows polyamine contents in roots and shoots of seedlings subjected to salt or osmotic stress in a controlled growth chamber at 5°C/10°C (night/day). In these controlled experiments, Agm concentrations varied in the same range of concentration as other polyamines (Fig. 6). However, Agm

Research represented 49% of total polyamines in control shoots and up to 79% and 61% in salt-stressed shoots and roots, respectively. Nevertheless, Agm concentration was low in salt-stressed shoots (Fig. 6a). Indeed, salinity exposure induced overall reduction of polyamine concentrations in shoots in comparison with control (Fig. 6). When growth was carried out under osmotic stress, Put and Spd, but not Agm, contents in shoots were significantly reduced in comparison with control (Fig. 6a–c). In roots, salt and osmotic stress both induced Agm accumulation and Put depletion in comparison with control (Fig. 6a,b). Moreover, Spd and Spm contents in roots were drastically modified in salt-stressed roots (Fig. 6c,d). Considering control seedlings, Agm and Put amounts were significantly more abundant in shoots than in roots whereas Spd and Spm were significantly more abundant in roots (Fig. 6). The distribution of polyamines between shoots and roots was deeply modified under salt stress, since Agm, Put, Spd and even Spm concentrations were then significantly higher in roots than in shoots (Fig. 6). By contrast, under osmotic stress, Agm and Put concentrations were higher in shoots than in roots whereas Spd and Spm were twice as abundant in roots than in shoots. Therefore, one of the major effects of saline and osmotic stress was the modification of polyamine distribution between roots and shoots (Fig. 6).

Discussion

Conditions of optimal seedling development in the field and in controlled conditions

Seedling development is difficult to monitor within the harsh and fluctuating environment of subantarctic regions. The impact of stress in a homogeneous medium was therefore studied in laboratory experiments, whereas field experiments gave an ecophysiological meaning to phenotypic alterations in response to imposed stresses. In order to assess phenotypic...
and biochemical changes, polyamine concentrations were used as indicators of seedling response to stress (Smith, 1985; Lee et al., 1995; Shen et al., 2000; Lefèvre et al., 2001). Polyamine accumulation has been related to developmental processes, including root development (Couëc et al., 2004), and to the response to chilling, drought and salt stress (Bouchereau et al., 1999). Nevertheless, although polyamines show a number of properties that may be relevant to stress tolerance (Bouchereau et al., 1999), the exact protective or signaling roles of polyamines in the tolerance to each specific stress remain to be elucidated.

Polyamine concentrations greatly differed between experimental approaches (Figs 5 and 6), thus showing that, in *P. antiscorbutica* seedlings, polyamine concentrations were strongly responsive to growth conditions. Total polyamine concentrations of 7.9 ± 0.4 µmol g⁻¹ d. wt in control shoots and 3.8 ± 0.1 µmol g⁻¹ d. wt in control roots were obtained in laboratory-grown seedlings. By contrast, total polyamine pools were greater in seedlings collected in the field (Figs 5 and 6), with 17.2 ± 1 µmol g⁻¹ d. wt in S1 shoots and 9.2 ± 0.5 µmol g⁻¹ d. wt in S1 roots. Given that polyamine accumulation has been associated with tolerance to stresses (Bouchereau et al., 1999), these differences of polyamine contents may be due to harsher environmental conditions in the field than those used in the laboratory. Mean temperature observed during field experiment was lower (Fig. 1) than that used in controlled conditions, which corresponded to mean temperature regime in the subantarctic summer (Hennion & Bouchereau, 1998). Even if optimal nutrient supply in controlled experiments might also account for phenotypic and polyamine variations between field and laboratory results, temperature is highly likely to limit seedling development. Under field conditions, slow seedling development (Figs 3e and 4b) might thus be ascribed to lower temperature than under controlled conditions, especially when considering the permanent soil temperature of 3.7 ± 0.2°C. This hypothesis was also in full agreement with polyamine accumulation observed in the field and with the fact that increase in polyamine concentrations is a typical response of a number of cold-tolerant plants to low temperature (Tajima & Kabaki, 1981; Kramer & Yang, 1989; Lee et al., 1995; Lee, 1997; Shen et al., 2000; He et al., 2002). When growth was carried out in MS-Agar medium, Agm contents were decreased in roots and shoots, in comparison with seedlings grown in the field, thus clearly indicating that *P. antiscorbutica* seedlings were responsive to low temperature, and that cold tolerance of *P. antiscorbutica* seedlings involved plasticity of polyamine metabolism.

Plant species showing high accumulation of polyamines under environmental stress, such as *P. antiscorbutica*, may therefore be useful to study the mechanisms of action of polyamines in response to stress. However, stress-induced modifications of polyamine concentrations, as occurs in the response to chilling, must depend on various levels of regulation, from gene expression to metabolism and conjugation of polyamines (Bouchereau et al., 1999; Martin-Tanguy, 2001). Further work is thus needed to determine which regulations are activated in *P. antiscorbutica* seedlings in response to environmental constraints in the field.

Effects of salinity and drought on early development of *P. antiscorbutica*

Although temperature and nutrient supply could account for phenotypic and polyamine differences, root exposure to salinity was shown to give similar alterations in the laboratory and in the field (Figs 3e and 4b), with, respectively, significant reduction of root length and complete inhibition of root growth. By contrast, shoot phenotypes exhibited chlorosis only when growth was carried out in S4. On the shore, salt exposure occurred not only at root level, as in controlled experiments, but shoots were also exposed to salt spray and to some events of sea water flooding. Even mature plants of *P. antiscorbutica*, approximately 30 cm above ground, surrounding S4, presented yellowish and dry leaves, which were not observed in other stations. In several plant species, yellowing or death of older leaves are induced by long-term exposure to salinity (Munns, 2002). Therefore, injury of shoots in S4 seedlings might be ascribed to direct exposure to salt. In both field and laboratory approaches, salinity modified polyamine patterns and resulted in relative accumulation of polyamines in roots and relative depletion of polyamines in shoots.

Redistribution of polyamines induced by salinity occurred even in the presence of high polyamine contents, since S1 and S4 seedlings exhibited much higher polyamine concentrations than, respectively, controlled and salt-stressed seedlings in the laboratory. Thus, under field conditions, high polyamine accumulation in seedlings, which could be ascribed to low temperature, did not prevent responsiveness to other stresses and over-accumulation of polyamines. Salinity induced polyamine depletion in shoots even before salt-induced phenotypic injury (Figs 4 and 6). In *Atropa belladonna*, germination in the presence of salt results in similar reduction of seedling growth and similar modifications of Put patterns between organs (Ali, 2000). Given that polyamine metabolism may be responsive to salt exposure within a few hours (Lefèvre et al., 2001), the present work shows that accumulation in roots and redistribution between roots and shoots are maintained during long-term exposure, and therefore in the field.

Drought conditions result in reduction of root growth or in changes in root developmental programmes for several plant species (Vartanian et al., 1994). Low water availability, which was induced either by transplantation in dry soils, or by the addition of mannitol in growth medium, greatly reduced root and shoot development of *P. antiscorbutica* seedlings (Figs 3 and 4). However, shoot phenotypes in S2 and S3 were very different. This was in agreement with important differences of Agm contents in shoots of S2 and S3 seedlings (Fig. 5), thus strongly suggesting that environmental conditions other
than the common sensitivity to water stress prevailed in S2 and S3.

In laboratory experiments, the presence of mannitol induced overall reduction of polyamine concentrations in both roots and shoots (Fig. 6), with the exception of Agm pool enhancement in roots. Changes in polyamine concentrations in response to drought in *P. antiscorbutica* were therefore very different between laboratory and field experiments (Figs 5 and 6). The response to drought of laboratory-grown seedlings may have been affected by growth conditions, such as nutrient availability. Alternatively, it was also possible that, in the field, S2 and S3 seedlings were subjected to additional stresses interacting with the response to drought.

**Seedling survival and root development under conditions of environmental stress**

Root development of *P. antiscorbutica* seedlings is greatly modified through inhibition of polyamine metabolism under controlled conditions (Hummel et al., 2002; Dufeu et al., 2003). Whereas the Agm content in roots is positively correlated with primary root development under constant cold conditions (Hummel et al., 2002), similar Agm concentrations were associated, in the present work, with drastic reduction of root length, caused by salinity or drought (Fig. 6). By contrast, under constant cold conditions, artificial variations of polyamines were shown to induce no drastic difference in shoot development (Hummel et al., 2002). In the present work, both shoot and root phenotypes were modified by stress exposure, thus indicating that relationships between polyamine contents and developmental processes were modified. Environmental constraints would thus limit seedling development by reducing overall growth processes and favouring protective responses to stresses. An increase in polyamine concentrations appears to be a protective mechanism against salt stress in several plant species (Zapata et al., 2003). In *P. antiscorbutica* seedlings, the major effect of salinity on polyamine concentrations was redistribution of polyamines between shoots and roots. Higher investment of polyamines in roots may be a developmental response to stress. Final root length might therefore be the combined result of positive action of polyamines and of direct effects of stresses. Moreover, polyamine accumulation in roots may facilitate reinitiation of root growth whenever stress conditions become less drastic.

Salinity reduced seedling development and especially root development, which could result in reduction of implantation, with deleterious consequences for seedling survival. In addition, exposure to salt spray, or other stresses imposed on shoots, induced chlorosis or necrosis, and endangered seedling survival. However, *P. antiscorbutica* seedlings can develop and survive within a harsh environment, such as the decrease of air temperature below 0°C and low soil temperature. This tolerance seemed to be facilitated by high water availability, which was beneficial during establishment and early growth of *P. antiscorbutica* seedlings. Given that global change induces significant enhancement of mean temperature and concomitant reduction of precipitation (Frenot et al., 1997), establishment of *P. antiscorbutica* seedlings may thus be endangered or restricted to the most propitious microhabitats. The situation in S2 strongly suggests that, under certain conditions, low water availability has strong deleterious effects on seedlings. It is therefore likely that climate-related change will restrict seedling development, which in turn may hamper population renewal and long-term survival.

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