High phenotypic plasticity of *Suaeda maritima* observed under hypoxic conditions in relation to its physiological basis

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**Background and Aims**

Phenotypic plasticity, the potential of specific traits of a genotype to respond to different environmental conditions, is an important adaptive mechanism for minimizing potentially adverse effects of environmental fluctuations in space and time. *Suaeda maritima* shows morphologically different forms on high and low areas of the same salt marsh. Our aims were to examine whether these phenotypic differences occurred as a result of plastic responses to the environment. Soil redox state, indicative of oxygen supply, was examined as a factor causing the observed morphological and physiological differences.

**Methods**

Reciprocal transplantation of seedlings was carried out between high and low marsh sites on a salt marsh and in simulated tidal-flow tanks in a glasshouse. Plants from the same seed source were grown in aerated or hypoxic solution, and roots were assayed for lactate dehydrogenase (LDH) and alcohol dehydrogenase, and changes in their proteome.

**Key Results**

Transplanted (away) seedlings and those that remained in their home position developed different morphology characteristic of the home or away site. Shoot Na\(^+\), Cl\(^-\) and K\(^+\) concentrations were significantly different in plants in the high and low marsh sites, but with no significant difference between home and away plants at each site. High LDH activity in roots of plants grown in aeration and in hypoxia indicated pre-adaptation to fluctuating root aeration and could be a factor in the phenotypic plasticity and growth of *S. maritima* over the full tidal range of the salt marsh environment. Twenty-six proteins were upregulated under hypoxic conditions.

**Conclusions**

Plasticity of morphological traits for growth form at extremes of the soil oxygenation spectrum of the tidal salt marsh did not correlate with the lack of physiological plasticity in the constitutively high LDH found in the roots.

**Key words:** Hypoxia, waterlogging, redox potential, *Suaeda maritima*, phenotypic plasticity, reciprocal transplant, halophyte, lactate dehydrogenase, proteomics.

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**INTRODUCTION**

The salt marsh is an important environment for providing the genetic material for understanding and improving plant responses to both salinity and flooding. Previous studies have largely concentrated on effects of salinity, but tolerance of flooding (see Colmer and Flowers, 2008) is an important trait with consequences for agriculture. Populations of *Salicornia europaea* from upper and lower marsh areas at Stiffkey, Norfolk, UK, showed differences in phenotype that were maintained in reciprocal transplants, indicating genetic differentiation between the two populations with respect to growth (Jeffries et al., 1981). On the other hand, Richards et al. (2010) found that all traits related to salt tolerance were highly plastic in response to salinity treatments in the salt marsh plant *Borrichia frutescens.*

Phenotypic plasticity is the property of a genotype to express different phenotypes in different environments (Bradshaw, 1965; Pigliucci, 2001). Pigliucci et al. (2006) reviewed the role of phenotypic plasticity in evolution and concluded that plasticity is a developmental process on which the evolutionary mechanism of natural selection can act and which may result in environmentally induced phenotypic variation becoming constitutively fixed, i.e. ‘genetic assimilation’/‘phenotypic accommodation’. Surveys of species and populations have shown that plasticity, the so-called ‘instability’ of a genotype, can be selected to fit species to demands of different environments – important for plants that cannot show the behavioural responses of animals (Bradshaw, 2006). Phenotypic plasticity is genetically controlled and is therefore of potential importance to species evolution. Empirical evidence for recent micro-evolutionary response to climate change reviewed by Gienapp et al. (2008) concluded that many adaptive responses to a changing environment could be due to plastic responses.

Phenotypic plasticity for morphological and fitness-related traits was demonstrated in *Spartina anglica* in a UK salt marsh, but also retention of some morphological variation across the range of environment indicated some genetic basis for the differences in growth between clones from high and low areas.
low marsh positions (Thompson et al., 1991). Reciprocal transplants of Scirpus americanus and S. maritimus in an intertidal marsh in Canada indicated that different genotypes in the high and low marsh populations showed similar phenotypes when grown in a common environment (Karagatzides and Hutchinson, 1991). Intraspecific phenotypic variation in relation to environmental variables was a general phenomenon shown in 12 species of south-eastern USA coastal salt marshes (although not including any species of Suaeda), with negative relationships between plant traits such as height, leaf size and number, and the environmental variables of salinity and waterlogging (Richards et al., 2005). The conclusion was that plant responses to field environmental gradients are likely to be multivariate and complex.

Upper and lower regions of salt marshes differ in soil aeration. Soil oxygen status depends both on gaseous exchange between the atmosphere and the soil and on microbial activity. Plants in well-drained, high marsh soil that has infrequent tidal inundation will generally have stable and good soil aeration due to rapid exchange between air and soil, whereas waterlogged low marsh soil will have reduced availability of oxygen due to the much (10^4 times) slower diffusion of gases in water than in air (Ponnamperuma, 1984). The result is a soil that becomes anaerobic as plant roots and soil organisms use up oxygen faster than it can be replaced (Ponnamperuma, 1972; Armstrong and Drew, 2002; Colmer and Voesenek, 2009). Reducing conditions develop due to anaerobic decomposition through a sequence of secondary oxidants with build up of potentially toxic metabolic products (Stumm and Morgan, 1981; Chester, 2000). Thus salt marsh soils will fluctuate from a drained—aerated to waterlogged—anaerobic state at different elevations and at different times.

When oxidative phosphorylation ceases in anoxic tissues, fermentative pathways can lead to the production of ethanol or lactate, although in the absence of oxygen the major end-product of fermentation in plant tissues is normally ethanol (Gibbs and Greenway, 2003). Roots of plants of all species studied so far possess both alcohol dehydrogenase (ADH) and lactate dehydrogenase (LDH), and thus have the potential for lactate metabolism (Rivoal and Hanson, 1994). Evidence that lactate can be an important pathway in some plants was shown by sustained lactate metabolism for several hours in roots of some species of the halophytic genus Limonium when plants were transferred from normoxic to anoxic conditions (Rivoal and Hanson, 1993). Lactate dehydrogenase has been shown to be inducible in some species under anoxic stress: LDH activity increased greatly in roots of barley and other cereals subjected to several days of severe hypoxia (Hoffman et al., 1986). Aleurone layers of Hordeum vulgare showed inducible LDH as well as ADH, and low initial LDH activity increased greatly under nitrogen-induced hypoxia (Hanson and Jacobsen, 1984). The roots of S. maritima might be expected to have evolved an efficient anaerobic fermentation pathway to maintain the ATP production needed for control of ion exchange in the fluctuating waterlogged conditions of the intertidal zone.

Suaeda maritima is a widely occurring halophyte of salt marshes of the northern hemisphere. Initial observations of two salt marshes in Sussex, Southern England, showed that S. maritima plants growing on adjacent areas of upper and lower salt marsh germinated at the same time, reached maturity and produced seeds at the same time (Wetson, 2008; Wetson et al., 2008). However, high marsh and low marsh S. maritima plants developed with different morphology (Wetson and Flowers, 2010): mature low marsh plants (uniform shoot height around 15 cm at both sites) were smaller and less branched than high marsh plants (shoot height around 30 cm) growing with the shrubby perennial Atriplex portulacoides. Plants at the extreme high tide mark where they grew on sheltered banks without other species were >30 cm tall with many lateral branches; those on trampled areas of path showed a much-branched prostrate morphology.

The objectives of this study were firstly to determine whether observed differences in growth of a salt marsh species, S. maritima, at different tidal elevations were examples of morphologically plastic traits by comparing growth of plants reciprocally transplanted between high and low marsh sites on the salt marsh; and, secondly, to compare these data with growth of plants from the same seed source transplanted between ‘high’ and ‘low’ marsh positions in a simulated tidal-flow system in a glasshouse where other environmental factors could be controlled and optimized and competition from other species eliminated. Thirdly, LDH and ADH activity and proteomic changes were assayed in roots of plants grown from the same high and low marsh source in aerated and hypoxic liquid culture in a controlled environment. We hypothesized that an annual plant with non-directional seed dispersal that lives in a highly challenging and variable environment would have evolved a plastic response to environmental variation in root hypoxia caused by variation in tidal flooding.

MATERIALS AND METHODS

The annual halophyte Suaeda maritima (L.) Dumort., family Amaranthaceae (or in some classifications Chenopodiaceae), was investigated at two UK salt marshes. Reciprocal transplants were carried out in one salt marsh in the estuary of the River Adur, Shoreham-by-Sea, West Sussex (TQ206060), and seed was collected both from this site and from a salt marsh at Cuckmere Haven, East Sussex (TQ515978) for growth experiments in a glasshouse and in a controlled-environment chamber.

Reciprocal transplants in Adur estuary salt-marsh

Suaeda maritima germinates in mid-March when the soil temperature reaches 15 °C (Wetson, 2008). Reciprocal transplants of dense groups of newly germinated seedlings 1 cm in height were carried out in mid-March in the salt marsh of the estuary of the River Adur. Patches of seedlings from low marsh (low tide region) and high marsh (high neap-tide region) which differed in elevation by 0.6 m were removed in blocks with an area of 10 cm^2 and depth of 3 cm to enclose all roots and the mud in which they were growing. Plants of any other species present were removed at the time of transplanting. Ten patches were removed from high and ten from low marsh positions. Five from each height on the marsh were replaced in their original sites and five were transplanted to the other tidal height (see Supplementary Data Figs S1 and S2). The number and height of S. maritima seedlings
was recorded in each patch on the day of the transplant (early March), in early June, 2 months after transplanting, and again at the beginning of August, 4 months after transplanting. Tidal flooding of low marsh sites submerged all plants twice daily for ≥ 8 h, whereas plants in high marsh sites grew in drained soil for all except about an hour at each of the two daily ‘high tides’.

Soil aeration in flooded low marsh and drained high marsh soil was assessed by redox potential in the close vicinity of the transplants using a combined redox electrode with a platinum rod joined to a calomel reference electrode ORP meter (CMPTR11/DWG1806, Thermo Electron Corporation, Fife, UK) attached to a portable microcomputer/pH meter (HI 9025 HANNA Instruments, Leighton Buzzard, UK) (Wetson, 2008; Wetson and Flowers, 2010). Readings were taken at 30 and 60 mm depth in the mud immediately after the tide had receded from each position; mean soil pH was 7.8 (low marsh), 7.7 (high marsh) and 7.9 for seawater.

Reciprocal transplants in simulated tidal flow in glasshouse tanks, plants grown from Adur estuary seed and plants grown from Cuckmere Haven seed

Seeds collected in autumn from plants growing on the high and low marsh of the Adur estuary and Cuckmere Haven salt marshes were germinated the following spring in trays of sand in a controlled-environmental chamber (Weiss 2400E/ + 5 JU-Pa-S growth cabinet; Weiss Technik, Gmbh, Reiskirchen-Lindstruth, Germany) with a 16 h photoperiod and photosynthetically active radiation (PAR) at 200 μmol m\(^{-2}\) s\(^{-1}\). Conditions during light and dark periods, respectively, were 22°C at 60% relative humidity and 17°C at 70% relative humidity. Seedlings were transplanted, one per pot, (plastic, 9 cm deep/9 cm top diameter, with a basal drainage hole) at around 14 d from germination. The growth medium was estuarine mud from the low tide site of seed collection, mixed with sand at a ratio 1:1 (v/v) to facilitate drainage (mud/sand mixture). Pots were randomly assigned by computer-generated random numbers into ‘high marsh’ or ‘low marsh’ positions in six replicate tanks (Supplementary Data Figs S3 and S4; Wetson, 2008; Wetson and Flowers, 2010).

By growing plants in tanks in a glasshouse, phenotypic plasticity was investigated under controlled conditions and without the natural harshness of the tidal salt marsh environment and the confounding factors of competition between S. maritima and other species. Tidal flow was simulated in gravel-filled tanks by flooding twice a day to simulate tides according to the method of Wetson (2008) and Wetson and Flowers (2010). Plants were grown in pots on top of the gravel at simulated high marsh ‘high tide’ (HT) or sunk in the gravel at simulated low marsh ‘low tide’ (LT). The plants positioned at LT had permanently flooded roots. Their shoots became submerged during ‘tidal inflow’ when the HT plant roots became flooded for 2 h in every 24 h. The tidal-flow tank system used filtered seawater collected locally and diluted to half-strength – shown to be the optimum salinity for growth of S. maritima (Flowers, 1972) – with added nutrients (Stout and Arnon, 1939) 6 mm KNO\(_3\), 4 mm Ca(NO\(_3\))\(_2\), 2 mm MgSO\(_4\), 1 mm KH\(_2\)PO\(_4\), 4 μm H\(_2\)BO\(_3\), 0.7 μm ZnSO\(_4\), 0.3 μm CuSO\(_4\), 7 μm MnSO\(_4\), 10 μm MoO\(_3\), 20 μm NH\(_4\)VO\(_3\), 10 μm CrCl\(_3\), 20 μm NiSO\(_4\), 20 μm Co(NO\(_3\))\(_2\), 5 μm Na\(_2\)WO\(_4\), 0.027 mm NaFeEDTA.

Treatments were: (a) plants grown from seed collected from plants growing on the high marsh and now growing in the tanks at ‘low marsh’ position (H-L); (b) plants from low marsh seed now growing at ‘low marsh’ position (L-L); (c) plants from low marsh seed now growing at ‘high marsh’ position (L-H); and (d) plants from high marsh seed now growing at ‘high marsh’ position (H-H). A first experiment using seedlings from Adur estuary seed was carried out to provide data that could be compared with data from the field transplant experiment in the Adur estuary. A second, larger scale, experiment was then carried out using seedlings from Cuckmere Haven seed. Plants of the first and second experiments were harvested after 28 and 42 d treatment, respectively. Shoot height and root length were recorded. Separated shoots were washed twice in distilled water and oven dried at 80°C for 48 h for dry weights. In the second experiment, shoot ion analysis was also carried out: ions were extracted in 10 mL of deionized water per sample at 80°C for 2 h. Sodium and potassium were determined by flame emission spectrophotometry (Eppendorf and Pye Unicam SP 90 A). Chloride was determined using a chloride combination electrode (CI 800, Wissenschaftlich-Technische-Werkstätten GmbH).

Redox potentials in the flooded and drained pots were determined at three depths in the pots (approx. 10, 40 and 80 mm below the surface), before and after flooding. Salinity of the tank seawater was checked weekly by measuring electrical conductivity (EC), and losses from evaporation were replaced with deionized water. Glasshouse temperature ranged between 17 and 25°C. Lights over the tanks (high pressure sodium lamps SON-T400W(I)) provided a minimum PAR of about 250 μmol m\(^{-2}\) s\(^{-1}\) at plant height.

Growth of plants for LDH and ADH assay

Seeds from Cuckmere high and low marsh plants were germinated in the controlled-environmental chamber as described above and transplanted at 14 d into nutrient solution (Stout and Arnon, 1939) in half-strength artificial seawater (Harvey 1966) in tubular, black plastic pots designed for gas-flushing treatments (Wetson and Flowers, 2010) or into black plastic tubs containing 0.1% (w/v) agar-nutrient solution – ‘stagnant agar’ (Wiengweera et al., 1997; Wetson and Flowers, 2010). Thus plants grown from high marsh seed and from low marsh seed had four treatments with different degrees of aeration imposed for 14 d: (1) stagnant agar (SA); (2) bubbled with N\(_2\) gas (N); (3) semi-stagnant without agar (SS); and (4) bubbled with air (A). The oxygen concentration was recorded (HI 9142 oxygen meter, HANNA Instruments) in all plant containers on every second day. Containers were topped up to a constant level as necessary with deionized water to replace evapotranspirational losses. At harvest, shoots were dried for ion analysis as described above and roots were rinsed twice in distilled water, blotted gently and around 1 g f. wt (five plants) immediately frozen in liquid nitrogen.

Extraction and assay of enzymes and proteomic analysis

For enzyme assays, each frozen root sample (around 1 g) was ground using a mortar and pestle over ice in 2 mL of
RESULTS

Reciprocal transplants in Adur estuary salt-marsh

Survivorship at high and low marsh sites was equally good with similar percentage losses of reciprocally transplanted plants from both areas. At the time of trans- and replanting between high and low marsh positions in the salt marsh there were about twice as many seedlings of \textit{S. maritima} per unit area at the higher than at the lower elevation (Fig. 1A). Two months after transplanting, the percentage loss of \textit{S. maritima} seedlings was 64.7 ± 6.5 \% (mean ± s.e.) from all 10 cm² areas and not significantly different between sites: $F_{3,18} = 0.9$, $P = 0.5$. When plants were 4 months old, losses were between 79 and 86 \% (mean 83.0 ± 6.5 \%) again with no significant difference in losses ($F_{3,18} = 0.3$, $P = 0.9$) between plants in the home or away sites (Fig. 1B). Mean losses from replants (i.e. from blocks removed and replaced in their original sites) were 81.8 ± 6.5 \% compared with 82.7 ± 5.5 \% losses from transplanted blocks. Similarly high losses were recorded from plants that were not moved at all (data not presented).

Mean shoot height of plants growing at high and low marsh levels was very different at the time of transplanting. High marsh plants (mean 3.0 ± 0.1 cm) were over twice as tall as low marsh plants (mean 1.4 ± 0.1 cm). Two months after transplanting, however, all plants growing at the high marsh position (whether home or away) had similar shoot heights (mean 6.3 ± 0.3 cm); the average height of plants (home or away) at the low marsh level was lower (mean 4.0 ± 0.2 cm), but again with no significant difference in height of home and away plants at the same location on the marsh ($F_{3,17} = 16.8$, $P < 0.001$). The difference in height on the high and low marsh was maintained and clearly defined in plants that were 4 months old. All plants now growing at low marsh positions, whether home or away, were of similar, shorter height (mean 18.6 ± 0.7 cm) and all plants now growing at high marsh were of similar, taller height (mean 26.5 ± 1.3 cm) ($F_{3,15} = 4.6$, $P = 0.02$; Fig. 2A, B).

Positive redox potential values (± s.e.) recorded in high marsh regions of both sites at 30 mm depth (Adur 367 ± 5 mV, Cuckmere 406 ± 5 mV) and 60 mm depth (Adur
313 ± 3 mV, Cuckmere 326 ± 7 mV) indicated oxygenated soil (Stumm and Morgan, 1981). Lower values at low marsh at 30 mm depth (Adur 123 ± 12 mV, Cuckmere 142 ± 10 mV) and at 60 mm depth (Adur 44 ± 12 mV, Cuckmere 50 ± 6 mV) indicated that the waterlogged mud was severely hypoxic or anoxic (Wetson and Flowers, 2010). Simulated tidal flooding in glasshouse pots gave falling redox values with degree of flooding and with depth in each pot (Wetson and Flowers, 2010). These values, together with the sulfidic smell of the waterlogged medium, indicated that reducing conditions developed in hypoxic waterlogged mud/sand mixture.

**Reciprocal transplants in simulated tidal-flow glasshouse tanks**

**Plants grown from Adur estuary seed.** Seedlings were of uniform height (mean height 5 ± 0.1 cm at 21 d) at transplanting and start of treatments. After 28 d of flooding/draining treatment, all plants growing in drained ‘high marsh’ (‘high tide’ HT) pots showed no significant difference in height and root length, irrespective of whether they had originated from ‘high marsh plant seed’ (H-H) or been transplanted there from ‘low marsh plant seed’ (L-H) ($F_{1,19} = 68.8, P < 0.001$, root length $F_{1,19} = 64.6, P < 0.001$). All plants growing in flooded ‘low marsh’ (‘low tide’ LT) pots (L-L and H-L) were of similar height, about half that of those in drained pots, irrespective of the seed source. Root systems of all plants in flooded ‘low marsh’ pots were markedly reduced in mass and in length (4–5 times shorter) than those of plants in drained ‘high marsh’ pots. Root to shoot length ratios in drained pots of 1:1 (H-H) and 0:9 (L-H) were very similar, but root length was shorter than shoot height in plants in flooded pots, giving ratios of 0:4 in both (L-L) and (H-L) treatments (Fig. 3A).

Flooding significantly reduced not only plant height, but root length, shoot fresh weight, shoot dry weight and root dry weight. Shoot dry weights of drained plants were...
between three and five times greater than those of flooded plants (Fig. 3B). Similar differences were seen in root dry weights (Fig. 3B). The origin of the seed had no significant effect on any of the above plant parameters. Mean shoot water content was found to be similar but slightly higher in flooded plants than in plants exposed to anoxic conditions.

**Plants grown from Cuckmere seed.** Plants germinated from Cuckmere seed and grown in drained, ‘high marsh’ pots (both H-H and L-H) were taller and heavier with longer lateral branches than plants growing in flooded ‘low marsh’ pots (L-L and H-L; Fig. 4A). Dry weight of HT plants was 4–5 times that of LT plants (Fig. 4B). There was no significant effect of the interaction between seed source and flooding. Fresh weights showed a similar pattern to that for dry weights, and mean percentage water in shoots was 90.5 ± 0.1% irrespective of the flooding regime at which plants were grown or the reciprocal transplant position. Reciprocal transplanting of seedlings between HT and LT tank positions supported the findings from field reciprocal transplants. There was no significant difference in shoot ion concentrations in home or away plants, but, as previously found (Wetson 2008; Wetson and Flowers 2010), flooding significantly increased both sodium and chloride and decreased potassium ion concentrations (Fig. 5).

**LDH and ADH assay and proteomic analysis**

Mean oxygen concentrations in the bulk nutrient solution were maintained at 7 ± 0.4 μM in N, 163 ± 1.0 μM in SS and 226 ± 4.0 μM in A. Stagnant agar provided a simulation of almost anoxic mud of the low marsh. Normoxic (SS) and aerated (A) solutions simulated the generally aerated drained soil of the high marsh. Seed source (from plants growing at high or low marsh positions) did not affect any plant parameters. The most interesting finding was that the plants had similarly high LDH activity in roots when grown under conditions from severe hypoxia to full aeration (Fig. 6). Mean values for enzyme activity (μmol min⁻¹ mg⁻¹ protein) in plants from high and low marsh were 66.2 ± 4.9 (SA), 78.4 ± 7.5 (N), 68.2 ± 3.0 (SS) and 66.5 ± 6.7 (A). There was no significant difference in LDH activity expressed per mg of protein in roots with seed source (F₁,₂₄ = 0.02, P = 0.90) or with hypoxia (F₃,₂₄ = 0.4, P = 0.74). Mean values for ADH activity were 0.03 ± 0.003 (SA), 0.02 ± 0.004 (N), 0.01 ± 0.003 (SS) and 0.01 ± 0.002 (A) μmol min⁻¹ mg⁻¹ protein. The values were at the limits of detection and too small to show reliable differences between the treatments (<6% of the LDH activity).

Repeating the LDH analyses on plants from the Cuckmere high marsh seed in 2010 gave comparable high values of 79.7 ± 5.1 μmol min⁻¹ mg⁻¹ protein in hypoxic (SA) and 87.3 ± 1.9 μmol min⁻¹ mg⁻¹ protein in aerated (SS) roots.

Analysis of the protein pattern of *S. maritima* roots after aerobic and anaerobic treatments showed 26 protein spots which were affected by anoxia as shown in Table 1 (a two-dimensional image of an *S. maritima* root proteome is shown in Supplementary Data Fig. S5). All these proteins were upregulated under hypoxic conditions by a factor >2 compared with aerobic conditions. These proteins consist of H⁺-ATP subunits (6, 23) and an ATPase regulating 14-3-3 protein (22). Some reductases such as ferredoxin reductase (21), cytochrome b₅ reductase (24), quinine reductase (25), ascorbate reductase (12) and two peroxidases (27, 2) were also shown to be upregulated under anoxia, as well as an enolase (8), a phosphoglycerate kinase and a pyruvate dehydrogenase (15, 20), four malate dehydrogenases (16, 17, 18, 19), a UDP-glucose-uridylyltransferase (5), four S-adenosylmethionine synthases (9, 11, 13, 14), a succrose synthase (3), two transcription factors (10, 17) and a NOD 18 protein (26) (numbers in parentheses refer to Table 1 and Supplementary Data Fig. S5).
This study of plasticity in S. maritima found that transplants in the field assumed the growth form of the ‘home’ plants of the high or low marsh position into which the seedlings had been moved, indicating high plasticity of plant height and mass. These data agreed with the responses shown in glasshouse transplants of S. maritima grown from seed collected from plants at extreme high and low marsh sites - morphology was typical of the away site, the site in which a plant had been placed, irrespective of the tidal height from which the seed had originated.

The finding that plants grew larger in drained ‘high marsh’ pots than in flooded ‘low marsh’ pots suggests that for S. maritima the degree of flooding is a determining factor for growth pattern and correlates with field observations. Observed differences in morphology and biomass of S. maritima on the high and low marsh areas of the same salt marsh may be explained not only by differences in the above-ground habitat, such as competition with other species for light (Wetson and Flowers, 2010), but also by differences in the below-ground habitat of the upper and lower marsh.

A major factor influencing the shorter, less branched growth form of S. maritima on the open mudflat of the lower marsh is likely to be the reduced oxygen available to the roots in the mud. The very different redox values in soil of the high marsh (>300 mV at 30 mm depth and only 50 mV at 60 mm depth) and low marsh (<150 mV at 30 mm depth and only 50 mV at 60 mm depth) showed that the infrequently flooded upper marsh had generally aerobic soil but frequent tidal flooding produced a waterlogged low marsh soil that was severely hypoxic. Growth of the lower marsh plants would be reduced by reduced root respiration and energy provision as a low ATP supply is likely to have affected ion homeostasis, as suggested by the higher Na and Cl but lower K concentrations in flooded as opposed to aerated pots (Yeo, 1983; Colmer and Flowers, 2008). The low porosity of the roots of S. maritima (Wetson, 2008) would be a factor in reducing oxygen availability to root cells. Additionally, growth may have been affected by toxic ions such as Mn$^{2+}$, Fe$^{2+}$ and S$^{-}$ that build up in waterlogged soil.

DISCUSSION

This study of plasticity in S. maritima found that transplants in the field assumed the growth form of the ‘home’ plants of the high or low marsh position into which the seedlings had been moved, indicating high plasticity of plant height and mass.
The novel finding is that *S. maritima* plants had constitutively high LDH activity (as high in roots grown exclusively in aerated media as in those grown in severely hypoxic media) and not the inducible and low increase in activity during hypoxia that has been demonstrated in other halophytes and glycophytes (Supplementary Data Table S1) where hypoxia can be seen to be 0.002–2-fold between aerobically grown control plants and those grown in severely hypoxic media). As the presence and metabolism of lactate as well as the increase in the protein in anaerobic as compared with aerobic conditions; kDa is the molecular mass; pH is the isoelectric point fold change, i.e., the increase in the protein is 36.7, 8.0 and not the inducible and low increase in activity during anaerobic conditions as in those grown in severely hypoxic media (Table 1, nos 6, 23 and 22). Survival of cells depends strongly on maintaining or adjusting the activity of V-ATPase, the vacuolar H\(^+\)-ATPase subunit B (Table 1, no. 2), and a 14-3-3-like protein, G-box-binding factor (Table 1, no. 18), i.e., those proteins increasing by >2-fold between aerobically grown control plants and those growing under hypoxia. In this study we detected change under anoxia in an H\(^+\)\_ATPase subunit B and a 14-3-3-like protein (Table 1, nos 6, 23 and 22). Survival of cells depends strongly on maintaining or adjusting the activity of V-ATPase, an enzyme that is indispensable for plant growth under normal conditions due to its role in energizing secondary transport, in maintenance of solute homeostasis and, possibly, in facilitating vesicle fusion (Dietz et al., 2001). However, the precise role of

### Table 1. Compilation of information on 26 protein spots that were affected by anoxia in *Suáeda maritima* roots

<table>
<thead>
<tr>
<th>No.</th>
<th>Acc. no.</th>
<th>Protein name</th>
<th>Origin</th>
<th>Fold</th>
<th>kDa, pH theore.</th>
<th>kDa, pH exp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>gi2942043</td>
<td>Vacuolar H(^+)-ATPase subunit B</td>
<td><em>Suáeda salsa</em></td>
<td>3.6</td>
<td>54.3, 4.89</td>
<td>52, 4.6</td>
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<td>23</td>
<td>gi99014557</td>
<td>Vacuolar H(^+)-ATPase</td>
<td><em>Chenopodium rubrum</em></td>
<td>2.9</td>
<td>20.4, 9.1</td>
<td>28, 4.0</td>
</tr>
<tr>
<td>22</td>
<td>gi3025187</td>
<td>14-3-3-like protein, G-box-binding factor</td>
<td><em>Mesembryanthemum crystallinum</em></td>
<td>4.4</td>
<td>29.9, 4.8</td>
<td>30, 4.4</td>
</tr>
<tr>
<td>21</td>
<td>gi4257103</td>
<td>ATPFRN2, root ferredoxin-NADP(^+) reductase/oxidoreductase</td>
<td><em>Arabidopsis thaliana</em></td>
<td>3.1</td>
<td>35.7, 6.67</td>
<td>35, 7.2</td>
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<tr>
<td>24</td>
<td>gi25556959</td>
<td>NADH-cytochrome b5 reductase</td>
<td><em>Ricinus communis</em></td>
<td>3.1</td>
<td>35.4, 8.9</td>
<td>29, 7.2</td>
</tr>
<tr>
<td>25</td>
<td>gi1263911</td>
<td>V-type proton ATPase subunit E</td>
<td><em>Spinacia oleracea</em></td>
<td>2.0</td>
<td>26.4, 6.85</td>
<td>21, 1.5</td>
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<td>12</td>
<td>gi1586541</td>
<td>Monodehydroascorbate reductase</td>
<td><em>Mesembryanthemum crystallinum</em></td>
<td>2.8</td>
<td>51.7, 6.38</td>
<td>47, 5.3</td>
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<td>27</td>
<td>gi729219</td>
<td>Peroxidase</td>
<td><em>Spinacia oleracea</em></td>
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<td>35.2, 5.43</td>
<td>36, 4.6</td>
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<td>2</td>
<td>gi20653322</td>
<td>Peroxisomal ascorbate peroxidase</td>
<td><em>Salicornia brachiata</em></td>
<td>2.6</td>
<td>31.3, 6.5</td>
<td>27, 7.5</td>
</tr>
<tr>
<td>28</td>
<td>gi3023714</td>
<td>2-Phospho-D-n-glycerol hydrolyase (enolase)</td>
<td><em>Mesembryanthemum crystallinum</em></td>
<td>2.2</td>
<td>48.4, 5.62</td>
<td>49, 5.1</td>
</tr>
<tr>
<td>29</td>
<td>gi1087071</td>
<td>14-3-3-like protein, G-box-binding factor</td>
<td><em>Spinacia oleracea</em></td>
<td>2.0</td>
<td>42.1, 5.49</td>
<td>39, 5.6</td>
</tr>
<tr>
<td>3</td>
<td>gi7007263</td>
<td>Sucrose synthase</td>
<td><em>Chenopodium rubrum</em></td>
<td>2.9</td>
<td>92.2, 5.88</td>
<td>91, 6.2</td>
</tr>
<tr>
<td>20</td>
<td>gi28465343</td>
<td>Pyruvate dehydrogenase E1 alpha subunit</td>
<td><em>Beta vulgaris</em></td>
<td>2.7</td>
<td>43.8, 8.34</td>
<td>41, 6.8</td>
</tr>
<tr>
<td>16</td>
<td>gi1113601</td>
<td>Cytoplasmic malate dehydrogenase</td>
<td><em>Beta vulgaris</em></td>
<td>3.3</td>
<td>35.4, 5.89</td>
<td>40, 5.3</td>
</tr>
<tr>
<td>6</td>
<td>gi4668487</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td><em>Nicotiana tabacum</em></td>
<td>3.2</td>
<td>36.7, 7.7</td>
<td>38, 6.0</td>
</tr>
<tr>
<td>17</td>
<td>gi1113601</td>
<td>Putative cytoplasmic malate dehydrogenase</td>
<td><em>Beta vulgaris</em></td>
<td>3.0</td>
<td>35.4, 5.89</td>
<td>39, 5.3</td>
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<tr>
<td>18</td>
<td>gi1113601</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td><em>Beta vulgaris</em></td>
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<td>36.6, 6.8</td>
<td>38, 6.0</td>
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<tr>
<td>19</td>
<td>gi1113601</td>
<td>Cytoplasmic malate dehydrogenase</td>
<td><em>Beta vulgaris</em></td>
<td>3.0</td>
<td>35.4, 5.9</td>
<td>41, 6.1</td>
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<tr>
<td>2</td>
<td>gi4539543</td>
<td>Putative cytosolic malate dehydrogenase</td>
<td><em>Beta vulgaris</em></td>
<td>2.8</td>
<td>35.5, 5.9</td>
<td>38, 6.0</td>
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<tr>
<td>5</td>
<td>gi21599</td>
<td>UTP-glucose-1-phosphate uridylyltransferase</td>
<td><em>Solanum tuberosum</em></td>
<td>2.0</td>
<td>51.8, 5.39</td>
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<td>9</td>
<td>gi28863905</td>
<td>ATP-glucose 6-dehydrogenase</td>
<td><em>Mesembryanthemum crystallinum</em></td>
<td>2.6</td>
<td>53.2, 5.57</td>
<td>53, 5.7</td>
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<td>11</td>
<td>gi6094228</td>
<td>S-Adenosyl-l-homocysteine hydrolase</td>
<td><em>Glycine max</em></td>
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<td>52.9, 7.74</td>
<td>45, 5.2</td>
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<td>13</td>
<td>gi22635326</td>
<td>UDP-glucose 6-dehydrogenase</td>
<td><em>Arabidopsis thaliana</em></td>
<td>2.4</td>
<td>43.2, 5.99</td>
<td>45, 5.2</td>
</tr>
<tr>
<td>14</td>
<td>gi22635326</td>
<td>S-Adenosylmethionine synthase</td>
<td><em>Arabidopsis thaliana</em></td>
<td>2.4</td>
<td>43.2, 5.99</td>
<td>45, 5.2</td>
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<tr>
<td>10</td>
<td>gi75309266</td>
<td>S-Adenosylmethionine synthase</td>
<td><em>Suáeda salsa</em></td>
<td>2.7</td>
<td>43.0, 7.56</td>
<td>44, 6.0</td>
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<tr>
<td>15</td>
<td>gi1192267</td>
<td>S-Adenosylmethionine synthase</td>
<td><em>Nicotiana tabacum</em></td>
<td>2.6</td>
<td>46.9, 5.38</td>
<td>40, 5.1</td>
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<td>7</td>
<td>gi24371057</td>
<td>S-Adenosylmethionine synthase</td>
<td><em>Suaeda maritima</em></td>
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<td>49.4, 9.2</td>
<td>49, 4.6</td>
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<td>26</td>
<td>gi46454886</td>
<td>NOD18 protein</td>
<td><em>Sonneratia alba</em></td>
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<td>12.0, 5.8</td>
<td>12, 5.6</td>
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<td>4</td>
<td>gi297734943</td>
<td>Unnamed protein product</td>
<td><em>Vitis vinifera</em></td>
<td>2.8</td>
<td>58.4, 5.27</td>
<td>57, 4.4</td>
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</table>

No. is the number of the protein spot according to Supplementary Data Fig. S5; Acc. no. is the GenBank accession number of the protein (http://www.ncbi.nlm.nih.gov/protein); Origin is the plant species of the detected polypeptides for identification in GenBank; Fold is fold change, i.e., the increase in the protein.
the increase in ATPase in the hypoxic roots and of the regulatory 14-3-3 protein is not clear unless it represents a mechanism for sequestering increased entry of Na\(^+\) and Cl\(^-\) that occurred under hypoxic conditions (Fig 5); others have reported an increase in ATPase in roots under anoxia (Bunney et al., 2001).

In a recent study of muskmelon, Liu et al. (2010) found that under hypoxic conditions, activities of LDH, ADH, PDC, superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), and the malondialdehyde (MDA) content were significantly higher than in plants grown under normoxic conditions. Here we did not see any change in LDH levels between plants grown under different oxygen concentrations, presumably reflecting the constitutive expression of this protein.

The levels of free radicals and activities of antioxidative enzymes are influenced by exposure to stresses, including hypoxia (e.g. Garnczarska et al., 2004). In the present study, we found that the levels of three peroxidases and a quinone reductase (Table 1, nos 27, 2, 12 and 25) of S. maritima roots increased under hypoxia. Excess reactive oxygen species that are induced by hypoxia may result in cell injury and dysfunction. The release of cytochrome c (Table 1, no. 24) was also monitored under lack of oxygen in wheat roots and thus was discussed as initiating the programmed cell death cascade (Virolainen et al., 2002). The need to maintain an active fermentative metabolism for production of ATP by fuelling the glycolytic pathway with fermentable carbohydrate is probably greater in hypoxic than in non-hypoxic roots. We confirmed this by the detection of a higher level of sucrose synthase and pyruvate dehydrogenase in hypoxically grown roots of S. maritima than roots from plants grown under non-hypoxic conditions (Table 1, nos 3 and 20). In maize roots tips suffering hypoxia, cytoplasmic acidosis is one of the first responses, accompanied by an increase in malate levels (Roberts et al., 1992). One of the ways of controlling this acidosis is by malate consumption by malate dehydrogenase, the activity of which was shown to increase 6-fold early under hypoxia (Edwards et al., 1998). Four malate dehydrogenases were found to be increased in S. maritima roots under hypoxia (Table 1, nos 16–19). The role of hypoxia in the expression of S-adenosylmethionine synthase (Table 1, nos 9–14) in Suaeda roots remains unclear. S-Adenosyl-L-methionine synthase catalyses the biosynthesis of S-adenosyl-L-methionine, a universal methyl group donor, and is expressed in all cell types and plant organs, but the greatest abundance of S-adenosylmethionine synthase gene transcripts and proteins occurred in roots (Sánchez-Aguayo et al., 2004). These authors showed that this enzyme is stress inducible in tomato plants where increased S-adenosylmethionine synthase activity correlated with a greater deposition of lignin in the vascular tissues and could be associated with changes in roots that occur in S. maritima under hypoxia (Wetson 2008).

The study of environmentally induced phenotypic responses in wild plants, with the selection and assimilation of traits into the genotype, may increase our understanding and ability to improve crop plants. The assumption that plasticity is adaptive and confers fitness in that environment is often made, but this is difficult to test. It requires manipulations of the plants to show that plants that do not show the response are at a disadvantage (Dudley and Schmitt 1996) – difficult in our study of LDH where plants of both extremes of the environmental range showed the same enzyme activity. Demonstrating that plasticity is adaptive necessitates phenotypic selection analysis as shown for shade-induced plasticity in plants (Schmitt et al., 1999; Callahan and Pigliucci, 2002) or relating fitness and trait plasticity across environments (Tucic et al., 1998). Ideally genetic manipulation of traits is also needed.

We conclude that morphological differences in S. maritima are caused by environmental variation at the site of study. Our data allow us to document this halophyte as the first to show constitutively high LDH activity. We suggest that environmentally induced physiological variation in LDH activity to the fluctuating soil hypoxia of the salt marsh environment may have been selected for and has, by the process of phenotypic accommodation (genetic assimilation), become constitutive.

**SUPPLEMENTARY DATA**

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Figure S1: photograph of a 10 cm\(^2\) patch of S. maritima transplanted in March 2007 from high to low tide position on the Adur estuary salt marsh. Figure S2: photograph of a 10 cm\(^2\) patch of S. maritima removed in March 2007 from high and replaced at high tide position on the Adur estuary salt marsh. Figure S3: photograph showing S. maritima plants growing in pots on top of, or sunk into, gravel in the tanks of the glasshouse tidal-flow tank system. Figure S4: photograph showing drained and flooded pots of S. maritima in the glasshouse tidal-flow tank system. Figure S5: two-dimensional polyacrylamide gel of Suaeda root proteome as affected by hypoxia in comparison with the aerobic control. Table S1: activities of lactate dehydrogenase measured in crude (unpurified) extracts of plant tissues subject to hypoxia.

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**LITERATURE CITED**


