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Early responses of *Bassia diffusa* (Thunb.) Kuntze to submergence for different salinity treatments

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ABSTRACT

Early responses of the salt marsh succulent *Bassia diffusa* (Thunb.) Kuntze to combined salinity and submergence were studied in a laboratory experiment aimed at determining the pattern of the response of photosynthetic pigments, membrane stability, oxalic acid and water relations to these stressors. Three key stages in the response were identified. A drop in chlorophyll $a+b$ within 6 h (4.2 ± 0.2 to 2.4 ± 0.3 mg g^{-1} DM) with a corresponding increase in carotenoid concentration (0.6 ± 0.1 mg g^{-1} DM) indicated an immediate response to submergence. Oxalic acid concentration was highest on Day 4 (1.7 g g^{-1} DM) as opposed to control levels, indicative of its role in submergence tolerance, thus Day 4 may be the peak of positive acclimation. The third phase was marked by a sharp increase in electrolyte leakage to $47.5\pm2.6\%$ on Day 10, from $9.4\pm1.4\%$ on Day 7, with a corresponding decrease in total dissolved solutes between Days 7 and 10. Results suggest that oxalic acid accumulates under submergence possibly as a stabilising osmolyte. The threshold for tolerance of the species under submergence is 7 days with membrane damage thereafter. *B. diffusa* would not survive prolonged submergence (>7 days) but could survive submergence of short duration (<7 days) through continuous underwater photosynthesis, accumulation of osmolytes such as oxalic acid and carotenoid, and maintenance of relative water content and succulence within control levels. These data show that this upper intertidal salt marsh plant would be sensitive to prolonged inundation as a result of sea level rise or due to estuary mouth closure and a subsequent rise in water level.

1. Introduction

Fringe ecosystems like salt marshes and the plants therein are susceptible to wide fluctuations in abiotic stressors. Among the most important of these stressors are the hydrology and salinity regimes. An increase in flood events has been predicted in low-lying salt marshes as a result of climate change (Engelbrecht et al., 2009; Jury and Levey, 1993; Mather, 2007; Mather et al., 2009), or following mouth closure in temporarily open/closed estuaries (TOCEs) (Riddin and Adams, 2008). Increased flooding would result in significant hypoxic/anoxic conditions (Adkins et al., 1990; Colmer and Flowers, 2008; Felle, 2005; Weisner et al., 1993), changes in internal ion homeostasis of plant cells (Zhu, 2002) as well as increased membrane damage through reactive oxygen species (ROS) action, and osmotic effects (Bohnert and Jensen, 1996; Jithesh et al., 2006). One effect of anoxia under submerged conditions is shortage of available ATP, which in turn limits survival and increases die-back of intertidal estuarine macrophytes (Bailey-Serres and Voesenek, 2008; Riddin and Adams, 2008; Voesenek et al., 2006). In addition, predicted increase in sea levels along the South African coastline (Mather, 2007; Mather et al., 2009) would shift the tidal front and result in larger submerged areas and habitat alteration beyond the areas directly under tidal inundation. For upper intertidal salt marsh species which would be inundated more often under predicted climate change conditions, a rapid increase in the concentration of reactive oxygen species, and consequently membrane lipid peroxidation would be of greater significance especially in the short term (Bohnert and Jensen, 1996; Jithesh et al., 2006; Rawyler et al., 1999). Physiologically, tolerant species respond to submergence through either low oxygen escape responses or low oxygen quiescence responses which consist of down-regulating metabolism, increased synthesis of antioxidant enzymes, and up-regulation of compatible osmolytes (Bailey-Serres and Voesenek, 2008; Benschop et al., 2005; Colmer and Voesenek, 2009; Hassine et al., 2008; Jackson, 2008; Marcum and Murdoch, 1992; Naidoo and Kift, 2006; Tabot and Adams, 2012; Voesenek et al., 2003). Considerable research has been done on regulation of metabolism and mediating ROS action under submergence (Apel and Hirth, 2004; Bailey-Serres and Chang, 2005; Bailey-Serres and Voesenek, 2008; Bohnert and Jensen, 1996; Jithesh et al., 2006; Voesenek et al., 2006). However, the direct effects of submergence on photosynthetic pigments have received scant attention. For plants to photosynthesise efficiently the integrity and concentration of photosynthetic and accessory pigments is indispensable to light capture (Jaleel et al., 2009) especially under attenuated light conditions

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that would occur under submergence. Such experiments have been reported for rice cultivars and species of *Rumex* (Das et al., 2008; Fukao et al., 2006; Mommer et al., 2005) but not for salt marsh plants. In addition, determining the direct osmotic effects on membranes of submerged plants is essential in understanding response patterns. Electrolyte leakage can be used as an indication of membrane integrity or resilience under submergence (Bajji et al., 2001; Blokhina et al., 2003; Pang et al., 2003). Also, while the role of glycinebetaine and proline as compatible solutes in halophytes has been well established in the literature (Ashraf and Foolad, 2007; Chen and Jian, 2010; Chen and Murata, 2008; Hanson et al., 1994; Naidoo et al., 2008; Tabot and Adams, 2012) there is renewed but weak evidence that oxalic acid could play a more significant role in ionic balance in some species (Ma et al., 2011). Indeed, preliminary results showed that oxalic acid responds to salinity variation under tidal conditions, hence it was important to determine its role in *B. diffusa* under short term combined salinity and submergence treatments, alongside established compatible solutes like proline and glycinebetaine.

Although not explicitly considered in most climate change predictions, changes in water level in salt marshes are invariably linked with changes in the salinity to which salt marsh plants are exposed, yet there is very little information on the physiological responses of salt marsh plants to combined flooding and salinity. Studies on *Sarcocornia perennis* (Mill.) A. J. Scott and *Spartina maritima* (Curtis) Fernald did not find any significant interactive effect of salinity and submergence (Adams and Bates, 1994, 1995), but did find a significant impact of submergence on growth of the species. In addition, most studies of plant responses to submergence are long term, although the success of longer-term strategies depends on the effectiveness of immediate responses.

B. diffusa (Thunb.) Kuntze of the family Chenopodiaceae is a perennial herb about 15 cm tall, which predominantly occurs at high tide limits of some estuaries in South Africa and Mozambique, and reproduces both vegetatively, and sexually through seed production (Wright, 1912). In most instances it occurs in homogenous stands, and due to the low species diversity and low redundancy levels in salt marshes (Elliott and Whitfield, 2011), its response to predicted submergence is of great importance. This study investigated the effects of submergence, combined with salinity of 0, 8, 18, 35 and 45 psu on chlorophyll *a* and *b*, total carotenoid and compatible solutes concentrations (oxalic acid, glycinebetaine, proline, malonic acid, malic acid and citric acid concentrations), membrane integrity and water relations on shoots of the species over 10 days, and at 6 hour intervals over 42 h, on the species. The following were hypothesised:

1 Upon submergence at the different salinities, the concentration of chlorophyll *a*, *b*, $a+b$, and total dissolved solutes in leaves would be reduced, as has been shown for other salt marsh species (Armstrong et al., 1999; Tabot and Adams, 2012).
2 Concurrently, there would be an increase in electrolyte leakage, succulence, compatible solutes and total carotenoids concentration, as hypoxic/anoxic conditions would result in increased membrane damage either through ROS activity or osmotic effects (Bohnert and Jensen, 1996; Jithesh et al., 2006), and compatible solutes are upregulated for ROS scavenging and other stabilising functions (Gill and Tuteja, 2010 and references therein).

We suggest that the extent of these responses would determine tolerance limits for the species. These results are ecologically significant for better management of *B. diffusa* populations in salt marshes susceptible to flooding, and add to the growing knowledge on the impacts of submergence on photosynthetic pigments and oxalic acid responses. This short term study is of particular ecological significance in estuaries in which upper intertidal ranges are dominated by a single or few susceptible species due to low redundancy levels in salt marshes (Elliott and Whitfield, 2011).

2. Materials and methods

2.1. Experimental layout

2.1.1. Site of sample collection

Seedlings for the study were collected from a single cohort at the Swartkops Estuary, a permanently open estuary situated approximately 15 km north of Port Elizabeth, South Africa, at Lat 33°52′S and Lon 25°38′E (Baird, 2001; Potgieter, 2008). The estuary is approximately 16.4 km long and permanently connected to the Indian Ocean through Algoa Bay (Scharler and Baird, 2003). It is the third largest intertidal salt marsh area along the South African coastline (Colloty et al., 2000). In the estuary temperature fluctuates between 13.5 °C in winter and 28 °C in summer, with mean annual precipitation of approximately 636 mm (Baird, 2001). The estuary has six different plant community types, including supra- and intertidal salt marsh (Colloty et al., 2000) which covers about 165 ha, with a clear zonation of species in most areas. The soils are typically sandy-clayey. In the upper intertidal ranges, soil redox potentials (first 5 cm) are highly variable, ranging from −158 to 268 mV, the depth to ground water ranges from 50 to 85 cm, and porewater salinity ranges from 22 to 44 psu (Adams, unpublished data). In this zone, *B. diffusa* (Thunb.) Kuntze is dominant, mainly occurring in homogenous stands and at times in mixed stands with *Limonium linifolium* (L.f.) Kuntze.

2.1.2. The tank submergence system

A tank system was set up in the glasshouse of the Botany Department of the Nelson Mandela Metropolitan University. The transparent glass tanks measured 50 × 30 × 30 cm and were used for the submergence treatments. Five tanks were used, one for each salinity + submergence combination. Each tank contained 3 growing trays, each measuring 22 × 15 × 5 cm, and filled with estuarine soil. Air was passed automatically from air taps in the glasshouse, through each tank for the duration of the experiment to simulate natural conditions and maintain uniform salinity in the water. Within the glasshouse, ambient light conditions at midday ranged from 551 to 841 μmol m^{-2} s^{-1}, and temperature peaked at 19 °C (12–19 °C) for the month of July 2011.

2.1.3. Seedling collection and establishment

Seedlings were collected from the Swartkops Estuary in Port Elizabeth (33°52′8.43″S, 25°38′34.81″E) in March 2011and grown in trays for 3 months in estuarine soil collected from the same site, before application of treatments. Seedlings were raised from the same cohort to reduce intrinsic differences, and in order for treatment effects to be comparable. Estuarine soil collected at the same site as the plants was used to simulate natural conditions, reduce variability in soil media, provide certainty that there are suitable nutrients for the plants, and for uniformity so that the most important variables would be those imposed by the treatments.

2.1.4. Experimental design

The experiment consisted of completely submerged conditions, within which five salinities were combined, hence investigating the combined effect of salinity and submergence as would naturally occur under different scenarios. Because submergence was constant across treatments, salinity effects could be teased out, and the different parameters were measured over time, hence schematically, the study could be conceived as a '(submergence + salinity) × time' experiment.

2.1.5. Treatment application and monitoring

In July 2011, established plants (3 months old) at approximately the same height were submerged with water of 0, 8, 18, 35 and 45 psu salinity. Salinities below 35 psu were obtained by diluting seawater with distilled water and the hypersaline salinity treatment by addition of research-grade Reef Aquarium Sea salts (Reef Aquatics, Cape Town) to seawater. The salts were free of nitrates and phosphates but contained