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Research Paper

Effect of salinity on seed germination of five mangroves from Sri Lanka: use of hydrotime modelling for mangrove germination

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Abstract

Mangroves are highly adapted to extreme environmental conditions that occur at the interface of salt and fresh water. Adaptations to the saline environment during germination are a key to mangrove survival, and thereby, its distribution. The main objective of this research was to study the effect of salinity on seed germination of selected mangrove species and the application of a hydrotime model to explain the relationship between water potential of the medium and rate of seed germination. Germination of seeds was examined at 15, 25 and 35°C in light/dark over a NaCl gradient. Germination time courses were prepared, and germination data were used to investigate whether these species behave according to the principles of the hydrotime model. The model was fitted for the germination of *Acanthus ilicifolius* seeds at 25°C. Final germination percentage was significantly influenced by species, osmotic potential and their interaction at 25°C. Moreover, temperature had a clear effect on seed germination (*Sonneratia caseolaris* and *Pemphis acidula*) which interacted with osmotic potential. Only *A. ilicifolius* seeds behaved according to the hydrotime principles and thus its threshold water potential was −1.8 MPa. Optimum germination rates for seeds of the other species occurred at osmotic potentials other than 0 MPa. The descending order of salinity tolerance of the tested species was *Aegiceras corniculatum* > *Sonneratia caseolaris* > *Acanthus ilicifolius* > *Pemphis acidula* > *Allophylus cobbe*, suggesting that the viviparous species (*A. corniculatum*) is highly salt tolerant compared with the non-viviparous species. The results revealed that seeds of the study species exhibited facultative halophytic behaviour in which they can germinate over a broad range of saline environments. Use of a hydrotime model for mangroves was limited as germination of their seeds did not meet model criteria.

Introduction

Mangroves are taxonomically diverse halophytic (salinity-tolerant) evergreen plants that dominate the intertidal zone between the land and the sea of tropical and subtropical areas. Although some authors categorize mangroves as obligate halophytes (i.e. salinity is necessary for their growth and cannot survive in freshwater permanently; Lugo and Snedaker, 1974; Clough, 1984; Wang *et al.*, 2011), the general view is that they are facultative halophytes (i.e. salinity is not necessary for their growth and they are capable of growing in freshwater; Smith and Snedaker, 1995; Parida and Jha, 2010). As mangroves live in both high and fluctuating saline habitats, they maintain mechanisms and adaptations to cope with these harsh environmental conditions (Tomlinson, 1994). Ion compartmentalization, osmoregulation, selective transport and uptake of ions and capacity to accommodate the salt influx are some of the main adaptations that plants have developed to cope with the saline conditions (Parida and Jha, 2010). These adaptations of seedlings and mature trees to tolerate saline conditions in most of the true mangroves have been widely studied (Tomlinson, 1994; Hogarth and Hogarth, 2007). However, adaptation of mangrove seeds and their germination behaviour has rarely been investigated (Baskin and Baskin, 2014). Furthermore, even the seed germination physiology of other halophytes is still mostly unknown (Khan *et al.*, 2006).

Timing of germination is an important life history trait that determines seedling survival as well as the phenotypic expression of post-germination characters (Donohue *et al.*, 2005). Germination behaviour (dormancy, dormancy-breaking requirements and germination requirements) determines the timing of germination (Baskin and Baskin, 2014). Thus, seeds should possess adaptations to their germinating environmental conditions, i.e. the seed stage is the first line of adaptation to tolerate harsh environmental conditions (Donohue *et al.*, 2005). Studying germination further unveils the strategies that mangroves have developed to cope with the harsh environmental conditions they face. Furthermore, knowledge

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on seed germination behaviour can be used in meaningful conservation of mangroves as well as mangrove ecosystems, which are greatly threatened by development (Duke *et al.*, 2007; Polidoro *et al.*, 2010; Van Lavieren *et al.*, 2012). Therefore, the main objective of this study was to examine the effect of salinity on germination of five plant species (*Acanthus ilicifolius*, *Aegiceras corniculatum*, *Allophylus cobbe*, *Pemphis acidula* and *Sonneratia caseolaris*) from mangrove plant communities in Sri Lanka.

Although the effect of salinity on seed germination of halophytes (salinity-tolerant plants) has been studied extensively (Khan *et al.*, 2000; Huang *et al.*, 2003; Duan *et al.*, 2004; El-Keblawy and Rawai, 2005), there are only a few studies that have included mangroves (Downton, 1982; Ye *et al.*, 2005; Patel and Pandey, 2009). Salinity affects germination by an osmotic effect or by specific ion toxicity (Katembe *et al.*, 1998). Salehifar *et al.* (2010) have shown that the effect of NaCl on germination is mainly an osmotic result. Although there are several other salts in mangrove water, 85% of the salt content is represented by NaCl (Scholander *et al.*, 1962). Thus, in our research we mainly focused on the osmotic effect of NaCl on seed germination of the study species. Furthermore, as the mangrove forests occur in the intertidal zone, the salt concentration can be as high as ~35 parts per thousand (3.5%) and osmotic potentials as low as approximately –2.5 MPa (Walter, 1979). Thus, in our experiments we used osmotic potentials ranging from 0 to –2.5 MPa.

Water potential of the germination medium is one of the crucial factors controlling seed germination (Baskin and Baskin, 2014). The hydrotime model describes the relationship between the water potential of the medium and the seed germination rate: $\theta_H = (\Psi - \Psi_b(g))t_g$ (Gummerson, 1986), where θ_H is a hydrotime constant (MPa h), Ψ is the water potential of the medium, $\Psi_b(g)$ is base water potential (threshold water potential) preventing radicle emergence of percentage g, and t_g is the actual time to radicle emergence of percentage g. Hydrotime models have mostly been used to examine the seed germination of salinity-sensitive plant species (Bradford, 1990; Dahal and Bradford, 1994; Toselli and Casenave, 2005; Gianinetti and Cohn, 2007; Windauer *et al.*, 2007; Zhang *et al.*, 2012). In contrast, they have scantly been used to study the seed germination of halophytes (Allen *et al.*, 2000; Zhang *et al.*, 2012). To the best of our knowledge, the model has not been used to examine the seed germination of mangroves. Thus, our study would be the first attempt to understand the effect of water potential on seed germination of mangrove species using the hydrotime model.

Materials and methods

Study species

Five species, *Aegiceras corniculatum* (L.) Blanco, *Pemphis acidula* J.R. Forst. & G. Forst., *Sonneratia caseolaris* (L.) Engl., *Allophylus cobbe* (L.) Raeusch. and *Acanthus ilicifolius* L., were selected from a mangrove plant community in Sri Lanka to study the effect of salinity on germination as preliminary studies showed that seeds of these species germinate at a range of salinities. *Acanthus ilicifoius* (Acanthaceae) is a native shrub or sprawling woody herb in Sri Lanka (Tomlinson, 1994) that is common along river banks, tidal canals and low swampy areas of mangrove forests. *Aegiceras corniculatum* (Primulaceae) is a cryptoviviparous evergreen treelet or shrub commonly distributed within the dry, intermediate and wet zone mangroves in Sri Lanka (Jayatissa and Koedam, 2002), and it often grows near estuarine banks (IUCN, 2013). *Allophylus cobbe* (Sapindaceae) is a branched shrub or small tree distributed from the coast to the hill country in Sri Lanka (Wadhawa and Meijer, 1998). It is also common in mangrove forests (Tomlinson, 1994). *Pemphis acidula* (Primulaceae) is a small tree or low shrub that grows within mangrove communities and in rocky foreshores in Sri Lanka. *Soneratia caseolaris* (Primulaceae) is a native tree species to Sri Lanka distributed in tidal estuaries, extending upstream into freshwater river banks (Macnae and Fosberg, 1981).

Collection of seeds

Fruits were collected from at least five individual plants from each species in Sri Lanka on different days (Table 1). All fruits were collected and placed into polythene bags separately, labelled and transported to the Department of Botany, University of Peradeniya, Sri Lanka. Diaspores (hereafter referred to as seeds) were extracted from the fruits and stored in plastic bottles in the laboratory for less than one week until experiments were initiated.

Effect of osmotic potential on germination of seeds

Six samples with three replicates (each containing 15–20 seeds) of *A. corniculatum*, *S. caseolaris* and *A. ilicifolius* seeds, and five samples with three replicates (each containing 15–20 seeds) of *A. cobbe* and *P. acidula*, were incubated separately in 9 cm diameter Petri dishes on tissue paper moistened with NaCl solution, representing an osmotic potential gradient of –0.1 to –2.5 MPa, or with distilled water (0 MPa treatment) at 25°C in light/dark (14 h/10 h) conditions (Table 1). In addition, germination of *S. caseolaris* and *P. acidula* seeds were tested at 15 and 35°C in light/dark (14 h/10 h) conditions over the same osmotic potential gradient; seeds of the other three species were not tested at these two temperatures due to limited seed supply. Seeds were observed for germination (radicle emergence to >1 mm) regularly and a cut test was used to check the viability of un-germinated seeds at the end of the germination test (after 60 days). Cumulative germination percentages were calculated (based on number of seeds tested) for each osmotic potential × temperature combination of each species. Germination time courses (for germination tests at 25°C) were prepared for the mean germination percentages by fitting sigmoidal logistic 4 parameter curves using the SigmaPlot statistical software (version 10.0, Systat Software GmbH, Erkrath, Germany).

Time taken for 10, 20, 30, 40, 50, 60, 70 and 80% (t_g) of the seeds to germinate in each osmotic potential (water potential) for each species (tested at 25°C) was determined using the germination time courses explained above. Germination rates (GR_g) were calculated by taking the reciprocal of the time to each germination percentage (g) of each species per water potential. Water potentials were plotted against germination rate and a linear regression curve was fitted for each species using SigmaPlot. Hydrotime modelling was performed on *A. ilicifolius* as it was the only species that had sufficiently high R^2 values when linear regression lines were fitted to osmotic potential *vs* mean germination rate for 50% germination (GR_{50}) at 25°C (Table 4). For this species, threshold water potentials (Ψ_b) for each germination percentage were determined using the x-intercept values of each linear curve and hydrotime constant (θ_H) was determined from the reciprocal of the slope. Germination percentage (g) values were probit transformed as described by Bradford (1990) and plotted

Table 1. Osmotic potential series prepared from NaCl solutions for germination tests of the mangrove study species and information on seed collection sites and dates in Sri Lanka

Species	Osmotic potential (MPa)						Collection site	Collection date
Acanthus ilicifolius	0	–0.1	–0.3	–0.5	–1.0	–2.5	Negombo & Chilaw	20 December 2013
Aegiceras corniculatum	0	–0.1	–*	–0.5	–1.0	–2.5	Kalamatiya	21 December 2012
Allophylus cobbe	0	–0.1	–0.3	–0.5	–1.0	–*	Galle	8 May 2013
Pemphis acidula	0	–0.1	–*	–0.5	–1.0	–2.5	Puttalum	10 February 2014
Sonneratia caseolaris	0	–0.1	–0.3	–0.5	–1.0	–2.5	Kalamatiya	10 June 2013

–*, not tested.

Table 2. Results of a two-way ANOVA for final germination among the five study species at different osmotic potentials at 25°C

Factor	Sum of squares	d.f.	Mean square	*F*	*P*-value
Species	9.897	4	2.474	77.500	<0.001
Osmotic potential	9.696	5	1.939	60.738	<0.001
Species × osmotic potential	3.661	17	0.215	6.746	<0.001

against the threshold water potential values for *A. ilicifolius*. Mean water potential threshold (water potential threshold at 50% germination) was determined for each species using the *x*-intercept, while the standard deviations ($\sigma_{\Psi b}$) for the threshold water potentials were determined using the slope of the curve.

Data analysis

The effect of salinity on final seed germination percentage (after 60 days) was analysed using a parametric two-way analysis of variance (ANOVA) with species and osmotic potential as factors for tests done at 25°C. Two-way ANOVAs were performed for *P. acidula* and *S. caseolaris* to examine temperature and osmotic effects on germination. Differences among means were further examined by using one-way ANOVAs followed by Tukey's multiple comparison test. All data were arcsine transformed prior to analysis.

Results

Effect of osmotic potential on seed germination at 25°C

Mean final germination percentages were significantly influenced by species, osmotic potentials and their interaction (Table 2). Germination was significantly reduced at –2.5 MPa in all species: *A. corniculatum* (50.0%) and *S. caseolaris* (8.9%) were the only species to germinate at –2.5 MPa (Table 3). Highest germination differed among the species being highly dependent on the osmotic potential when incubated at 25°C (Table 3). The optimal osmotic range (i.e. osmotic range that showed higher germination percentage) for *A. cobbe* was 0 to –0.5 MPa, for *S. caseolaris* it was 0 to –0.3 MPa, and for the other three species 0 to –1.0 MPa. Highest final germination percentage at the optimal osmotic potential for *A. ilicifolius*, *A. corniculatum*, *A. cobbe*, *P. acidula* and *S. caseolaris* was 100.0, 100.0, 91.7, 69.3 and 68.0%, respectively. Germination percentages varied significantly across species at each salinity level (Table 3, Fig. 1). *Acanthus ilicifolius*, *A. corniculatum* and *A. cobbe* showed significantly highest germination (91.7–100.0%) in distilled water, while *P. acidula* and *S. caseolaris* were the lowest (57.8–60.0%) in water. *Allophylus cobbe* was the only species that did not germinate at –1.0 MPa, while *A. ilicifolius*, *A. corniculatum*, *P. acidula* and *S. caseolaris* germinated to 100.0, 95.8, 45.3 and 37.8%, respectively. *Aegiceras corniculatum* had the highest germination (50.0%) at –2.5 MPa. Embryos of all un-germinated seeds were not viable (either they had fungal infections or had yellowish soft embryos) after 60 days of incubation.

Seeds of *A. corniculatum*, *A. cobbe*, *P. acidula* and *S. caseolaris* showed a non-significant linear relationship with low R^2 value between water potential (Ψ) *vs* germination rate for 50% germination (GR_{50}) (Fig. 2, Table 4). Conversely, a significant ($P < 0.05$) positive linear relationship with high R^2 value was observed between Ψ *vs* GR_{50} for seeds of *A. ilicifolious*. Threshold osmotic potential (Ψ_b) estimated for 50% germination ($g = 50$) was –1.8 MPa and hydrotime constant (θ_H) was 1.02 for *A. ilicifolius*. Moreover, the mean threshold water potential (Ψ_{b50}) of *A. ilicifolius* seeds was calculated to be –1.80±0.31 MPa with 0.08 standard deviation ($\sigma_{\Psi b}$) (Fig. 3).

Effect of osmotic potential on germination of S. caseolaris and P. acidula at different temperatures

Germination percentages for seeds of *S. caseolaris* and *P. acidula* differed significantly among osmotic potentials and among temperature regimes (Table 5). Germination at particular osmotic potentials was highly dependent on temperature for final germination of *P. acidula* at –0.1, –0.5 and –1.0 MPa, while germination at 0 and –2.5 MPa was not significant across temperature range (Table 6). In contrast, the effect of temperature on germination percentage of *S. caseolaris* was significant at all osmotic potentials except at –1.0 MPa. For most osmotic potentials, germination was highest at 15°C for *P. acidula*, while it was highest at 35°C for *S. caseolaris*.

Discussion

Hydrotime models have been used to predict and understand seed germination of crop species at different field water potentials (Gummerson, 1986; Bradford, 1990) and this model can also be applied to salinity-sensitive plants inhabiting extreme environments (Bradford, 1990; Dahal and Bradford, 1994; Toselli and Casenave, 2005; Gianinetti and Cohn, 2007; Windauer *et al.*, 2007; Zhang *et al.*, 2012). However, use of hydrotime models to

Table 3. Mean final germination percentage after 60 days of incubation at 25°C and the effect of salinity on five mangrove species

Osmotic potential (MPa)	*Acanthus ilicifolius*	*Aegiceras corniculatum*	*Allophylus cobbe*	*Pemphis acidula*	*Sonneratia caseolaris*	*P*-value	d.f.	*F*-value
0	100.0 ± 0.0Ab	100.0 ± 0.0Ab	91.7 ± 4.8Ab	60.0 ± 10.6Aa	57.8 ± 2.2Aa	<0.001	4	22.18
−0.1	100.0 ± 0.0Ab	100.0 ± 0.0Ab	91.7 ± 4.8Ab	68.0 ± 6.1Aa	68.0 ± 8.0Aa	<0.001	4	16.75
−0.3	–*	100.0 ± 0.0Ab	77.8 ± 5.6Aa	–*	66.7 ± 6.7Aa	<0.001	2	38.83
−0.5	96.7 ± 3.3Ac	91.7 ± 4.2Ac	91.7 ± 8.3Aab	69.3 ± 3.5Aab	51.1 ± 9.6ABa	0.002	4	9.57
−1.0	100.0 ± 0.0Ab	95.8 ± 4.0Ab	0.0 ± 0.0Ba	45.3 ± 2.3Aa	37.8 ± 14.6ABa	<0.001	4	40.42
−2.5	0.0 ± 0.0Bb	50.0 ± 19.1Ba	–*	0.0 ± 0.0Bb	8.9 ± 2.2Bab	0.021	3	5.78
P-value	<0.001	<0.001	<0.001	<0.001	0.004			
d.f.	4	4	5	4	5			
F-value	104.96	18.84	9.17	16.32	6.29			

–*, not tested. Values are means ± S.E. from three replicates, and different lower case letters in the same row or different upper case letters in the same column are significantly different at the level of 0.05.

Table 4. R^2 and *P*-values for polynomial linear regression lines fitted to osmotic potential *vs* mean germination rate for 50% germination (GR_{50}) for seeds of the study species at 25°C in light/dark (14 h/10 h) conditions

Species	R^2 value	*P*-value
Acanthus ilcifolius	0.98	0.006
Aegiceras corniculatum	0.01	0.885
Allophylus cobbe	0.03	0.819
Pemphis acidula	0.42	0.551
Sonneratia caseolaris	0.01	0.906

study the effect of water potential on seed germination on salinity-tolerant plant species (halophytes) are scanty in the literature (Allen *et al*., 2000; Zhang *et al*., 2012) especially for mangroves. *Acanthus ilicifolius* showed a significant positive linear relationship with high R^2 value (Table 4) between germination rate and osmotic potential, and parallel linear graphs among germination proportions (Fig. 2), which are the basic requirements to construct a hydrotime model as described by Bradford (1990). In contrast, the other four species had a tenuous linear relationship with low R^2 and un-parallel linear graphs. Therefore, the only species in our study that fitted the hydrotime model was *A. ilicifolius*. Furthermore, highest germination rate (GR_{50}) for *A. ilicifolius* was at 0.0 MPa and all other rates gradually decreased with an increase of salinity. Similar germination patterns have been observed in several halophytic species, e.g. *Haloxylon recurvum* (Khan and Ungar, 1996), *Salicornia rubra* (Khan *et al*., 2000) and *Prosopis strombulifera* (Sosa *et al*., 2005). According to the probit germination graph of *A. ilicifolius*, the threshold water potential value for $g = 50$ was − 1.80 MPa (Fig. 3). This threshold water potential value suggests that *A. ilicifolius* seeds can germinate even in highly saline conditions at 25°C, although the highest germination rate was observed at 0 MPa. Germination rate was highest for *A. corniculatum*, *P. acidula* and *S. caseolaris* at water potential less than 0 MPa. These species in which their seeds germinate at a higher rate when incubated at high salinity conditions than on distilled water may not fit the hydrotime model. Other non-mangrove species such as stem succulent halophytes also germinated better in highly saline conditions than in fresh water (Khan and Gul, 2006), suggesting that these species also may not follow the hydrotime model. Notably, a considerable variation observed in seed germination rates over the range of osmotic potentials in these species (Fig. 2) indicates the different behaviour of individual seeds. This may be an important germination trait that can facilitate these mangrove plants to survive in highly fluctuating salinity levels, either with the tide or diluted by fresh water.

One of the most critical stages in the life cycle of halophytes is the period of germination and establishment (Ungar, 1978). In this regard, the salinity of the soil water solution is one of the major factors that can influence germination (Baskin and Baskin, 2014). The effect of salinity on final germination percentage was significant for all species in our study. For instance, seeds of all species (except *A. corniculatum*) did not germinate or germinated to a very low percentage at −2.5 MPa, suggesting that they may have poor early germination establishment in hypersaline soil conditions (Fig. 1, Table 3). Ionic toxicity effect at a high concentration of NaCl (Baskin and Baskin, 2014) could be one of the reasons for loss of seed viability at high salinities. Similarly, there are other reports of reduced germination and viability at high NaCl concentrations in halophytes (Khan and Ungar, 1996). Khan and Ungar (1996) reported that although *Haloylon recurvum* seeds can tolerate very high salt concentrations, highest germination percentages were obtained in distilled water (0 MPa). Similarly, *A. corniculatum*, *A. ilicifolius and A. cobbe* showed highest germination percentage (but not significant) in distilled water when compared with other salinity levels. In contrast, *S. caseolaris* and *P. acidula* tended to germinate better at −0.1 and −0.5 MPa, respectively, although it was not significantly different from germination at 0 MPa (Table 3).

Germination percentages across the osmotic potential gradient differed significantly among species, suggesting different salinity tolerances (Tables 2 and 3). Considering final germination percentage, the descending order of salinity tolerance was *A. corniculatum* > *S. caseolaris* > *A. ilicifolius* > *P. acidula* > *A. cobbe* (Table 3). The highest salt tolerance was observed in seeds of *A. corniculatum*, a crypto-viviparous species, whereas all the other species in the study were non-viviparous. Consistently, in terms of the whole germination process, Ye *et al.* (2005) reported that the seeds of *A. corniculatum* (crypto-viviparous) were more salt

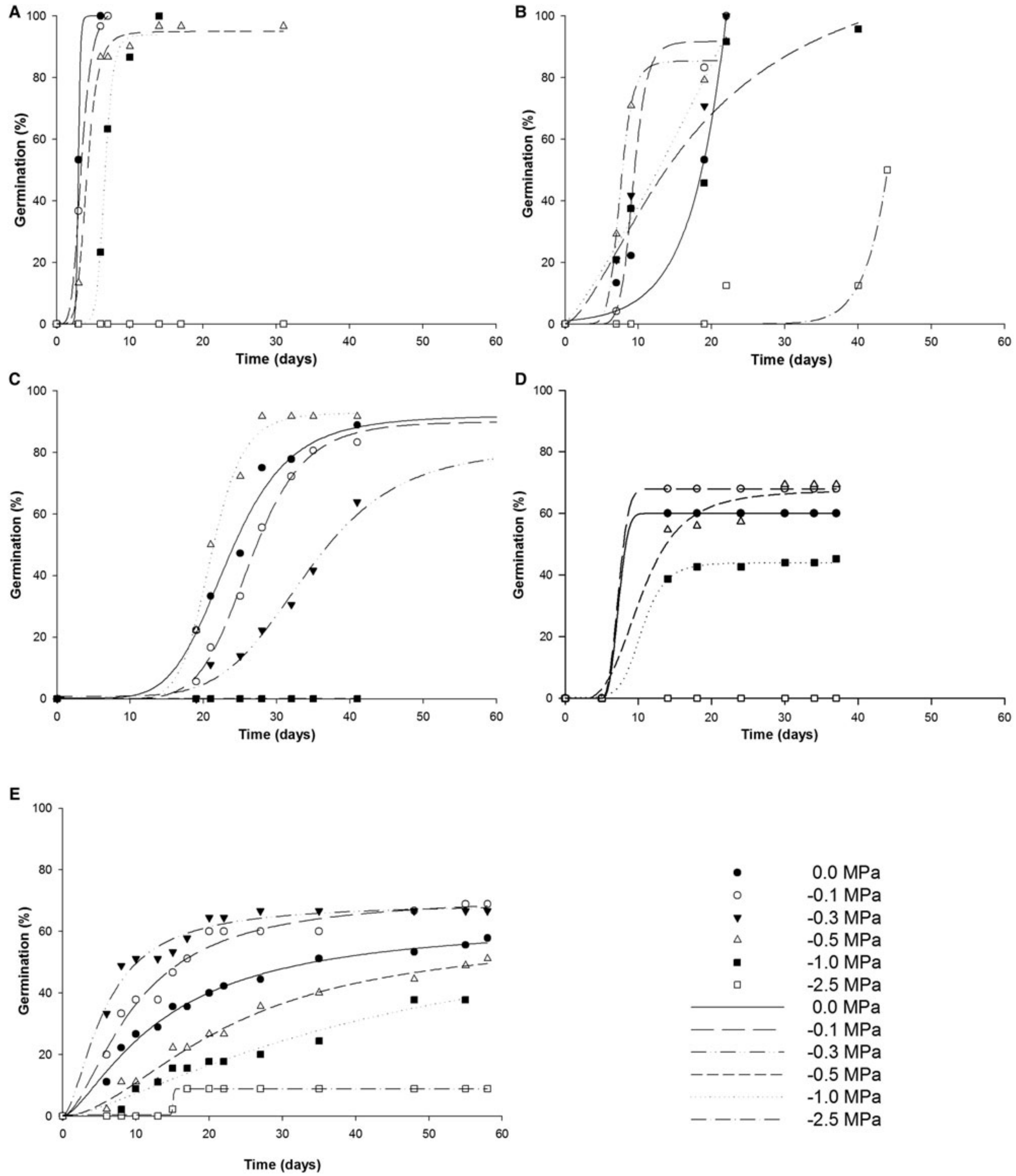

Fig. 1. Seed germination time courses for *Acanthus ilicifolius* (A), *Aegiceras corniculatum* (B), *Allophylus cobbe* (C), *Pemphis acidula* (D) and *Sonneratia caseolaris* (E) on tissue paper moistened with NaCl solutions with different osmotic potentials at 25°C in light/dark (14 h/10 h) conditions. Logistic 4 parameter curves have been fitted for the mean germination percentage at each osmotic potential to obtain the germination time courses.

tolerant than those of *A. ilicifolius* (non-viviparous). The reason for such an observation may be that in viviparous species germination and subsequent development of the propagule take place while the fruit is still attached to the mother plant (Tomlinson, 1994). Thus, the adaptation of the propagule to saline environments actually starts before dispersal (Joshi *et al.*, 1972) and gives an added advantage for them to establish even at high saline conditions.

The variation of salinity tolerance among species may reflect their ecological distribution. *Acanthus ilicifolius* and *A. corniculatum* occur in the dry, intermediate and wet mangrove zones in Sri Lanka (Jayatissa and Koedam, 2002). Thus, the high germination

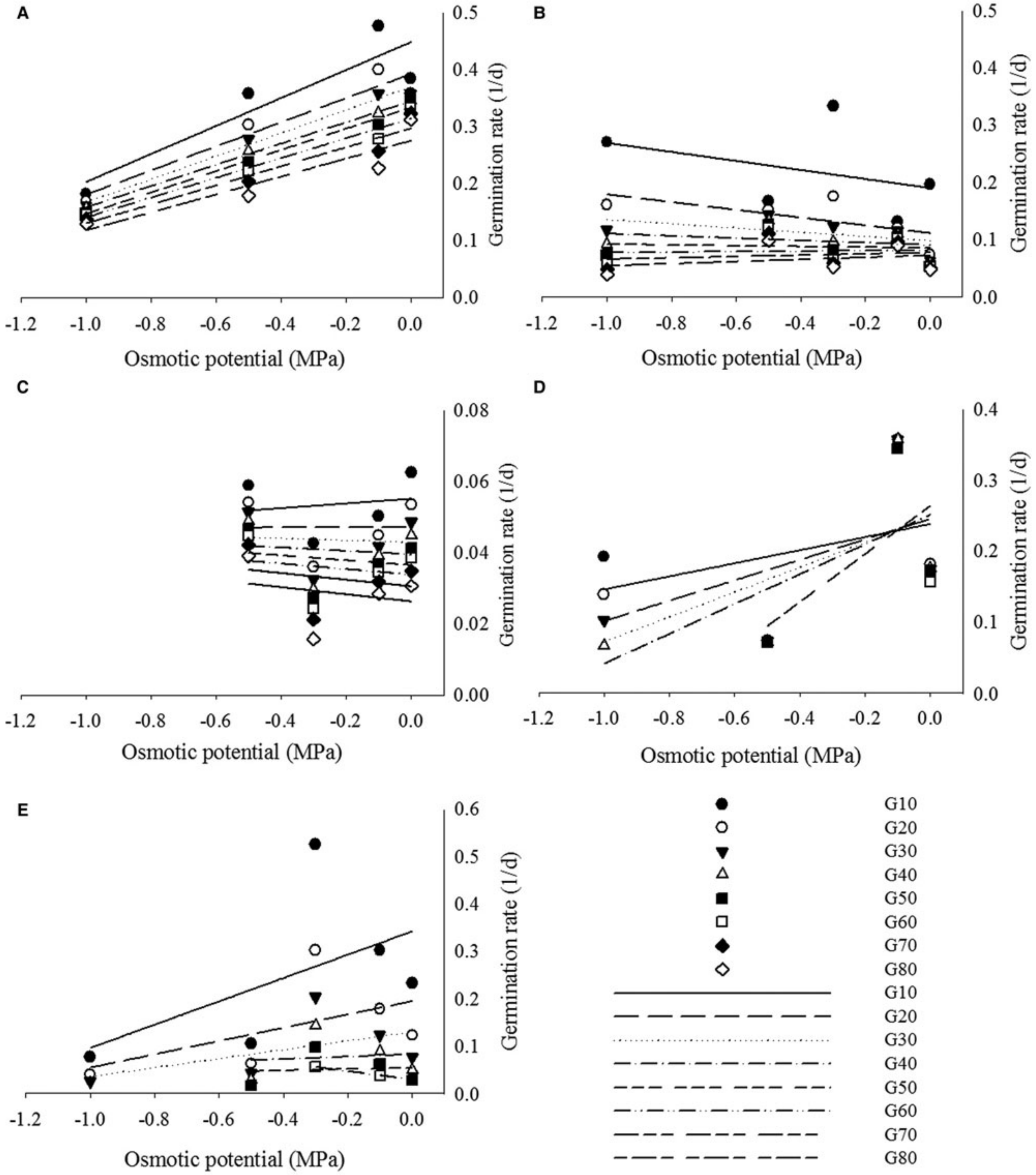

Fig. 2. Mean germination rates (GR_g) for 10, 20, 30, 40, 50, 60 and 70% germination of *Acanthus ilicifolius* (A), *Aegiceras corniculatum* (B), *Allophylus cobbe* (C), *Pemphis acidula* (D) and *Sonneratia caseolaris* (E) seeds on tissue paper moistened with NaCl solutions with different osmotic potentials at 25°C in light/dark (14 h/10 h) conditions. Simple linear regression curves were fitted to each germination percentage.

percentage of these species over a wide range of salinity levels (i.e. threshold water potential was −1.8 MPa for *A. ilicifolius*) may be an important seed trait that allows these species to have a wide ecological distribution. Although *A. cobbe* is one of the important members in the mangrove plant community, it can be found from coastal to montane zones in Sri Lanka (Wadhawa and Meijer, 1998) giving the reason why seeds of *A. cobbe* did not germinate at −1.0 MPa, but germinated well in distilled water as well as in moderate saline solutions down to −0.5 MPa.

At the range of salinity used in our study, the germination rate as well as final germination percentage of *S. caseolaris* was low to moderate at 25°C. Similar germination behaviour was observed in *P. acidula* (but seeds did not germinate at −2.5 MPa). However, germination rate as well as the final germination percentage of

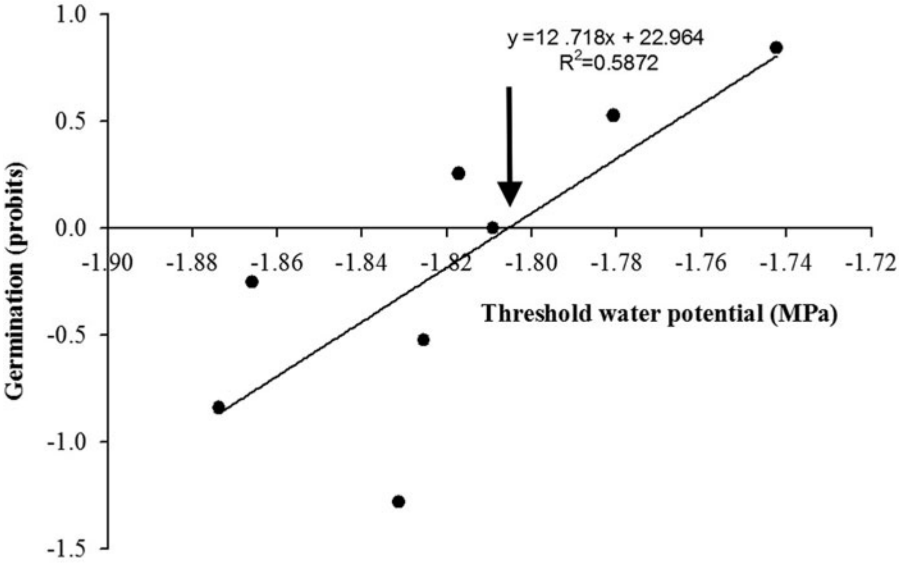

Fig. 3. Threshold water potential for different probit transformed percentages for seeds of *Acanthus ilicifolius* at 25°C in light/dark (14 h/10 h) conditions. The arrow indicates the mean threshold water potential value. A simple linear regression line was fitted to determine the relationship between water potential thresholds and probit transformed seed germination percentages.

Table 5. Results of two-way ANOVA for germination of two mangrove species seeds on different osmotic potentials at three temperatures

Species factor	Sum of squares	d.f.	Mean square	*F*-value	*P*-value
Pemphis acidula					
Temperature	1.341	2	0.670	28.152	<0.001
Osmotic potential	2.851	4	0.713	29.934	<0.001
Temperature × osmotic potential	0.811	8	0.101	4.259	0.002
Sonneratia caseolaris					
Temperature	5.146	2	2.573	86.86	<0.001
Osmotic potential	6.810	5	1.362	45.98	<0.001
Temperature × osmotic potential	3.506	10	0.351	11.836	<0.001

Table 6. Effect of temperature on seed germination of two mangrove species over a range of osmotic potentials

Species temperature	0 MPa	−0.1 MPa	−0.3 MPa	−0.5 MPa	−1.0 MPa	−2.5 MPa
Pemphis acidula						
35°C	57.3 ± 7.4a	37.3 ± 2.7a	–*	12.0 ± 8.3a	1.3 ± 1.3a	0.0 ± 0.0a
25°C	60.0 ± 10.6a	68.0 ± 6.1a	–*	69.3 ± 3.5b	44.0 ± 2.3b	0.0 ± 0.0a
15°C	70.7 ± 15.7a	73.3 ± 4.8b	–*	66.0 ± 14.0b	71.0 ± 3.4c	0.0 ± 0.0a
P-value	0.622	0.007	–*	0.008	0.001	0.422
d.f.	2	2	–*	2	2	2
F-value	0.52	12.89	–*	14.54	104.48	1.00
Sonneratia caseolaris						
35°C	100 ± 0a	100 ± 0a	95.9 ± 2.1a	82.2 ± 8.0a	35.5 ± 15.6a	2.2 ± 2.2ab
25°C	57.8 ± 2.2b	68.9 ± 8.0b	66.7 ± 6.7b	51.1 ± 9.7a	37.8 ± 14.6a	8.9 ± 2.2b
15°C	86.7 ± 6.7ab	8.9 ± 5.9c	11.1 ± 4.4c	2.2 ± 2.2b	0.0 ± 0.0a	0.0 ± 0.0a
P-value	0.005	<0.001	<0.001	0.002	0.154	0.032
d.f.	2	2	2	2	2	2
F-value	14.66	107.98	47.91	21.69	2.59	0.42

–*, not tested. Values are means ± S.E. from three replicates, and different letters in the same row are significantly different at the level of 0.05.

S. caseolaris seeds was highest at 35°C when compared with those at 25 or 15°C. Conversely, seeds of *P. acidula* germinated better at 15°C than at 25 or 35°C in most of the tested salinity levels (Table 6). Thus, temperature had a clear effect on germination rate and percentage which interacted with the salinity showing wide range of germination requirements between species. These different germination requirements among species may play a crucial role in their establishment, growth and distribution under fluctuating salinity conditions. Temperature controlling the salinity sensitivity of seeds has been reported in (non-mangrove) halophytes (Khan and Ungar, 1985, 1996; El-Keblawy and Rawai, 2005). For example, *P. juliflora* germination was higher in high saline conditions at 40°C than at low temperatures (El-Keblawy and Rawai, 2005), and Huang *et al.* (2003) reported that the halophyte *Haloxylon ammodendron* germinated well in saline conditions at 10°C than at 30°C.

Overall, our results showed that all of the study species behave as facultative halophytes when considering their seed germination behaviour. Such germination behaviour is ecologically important to establish mangrove species along the border between saline and non-saline ecosystems (Tomlinson, 1994). These species also have physiological adaptations that enable their vegetative body to cope with saline as well as non-saline conditions, making them facultative halophytes (Parinda and Das, 2005). Our study revealed that seeds of *A. corniculatum*, *A. cobbe*, *P. acidula* and *S. caseolaris* did not behave according to the hydrotime model at 25°C, suggesting that this model cannot be used to explain the effect of salinity on seed germination of these species. Instead, seeds of these species had a peak germination pattern with high germination rate at moderate saline conditions. In contrast, *A. ilicifolius* seeds behaved according to the hydrotime model at 25°C, and thus the model was used to explain the effect of salinity on its germination.

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Conflicts of interest. None

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