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Separating effects of clonal integration on plant growth during submergence and de-submergence

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ABSTRACT

Responses to both submergence and de-submergence are important for evaluating flood tolerance of plants. Clonal integration (resource translocation between connected ramets within clones) has been shown to increase flood tolerance of amphibious clonal plants. However, no study has truly separated the effects of clonal integration during de-submergence from those during submergence. We grew 40 clonal fragments of the amphibious plant species *Alternanthera philoxeroides*, each consisting of two ramet systems, under half-submerged conditions (one ramet system submerged and the other un-submerged) for 30 d and then de-submerged for 20 d. To evaluate the effects of clonal integration during submergence, stolon connection between the two ramet systems was either severed or not on day 1, and 10 replicates of the fragments of each treatment were harvested on day 30. To evaluate the effects of clonal integration after de-submergence, the remaining 20 clonal fragments with connected stolons during submergence were de-submerged; half of them were subjected to stolon severing on day 31 and the remaining 10 fragments were not. All the 20 fragments for de-submergence were harvested on day 50. After 30 d of submergence, stolon connection between the submerged and the un-submerged ramets significantly increased growth, biomass allocation to roots and photosynthetic capacities of the submerged ramets, and also increased growth and photosynthetic capacities of the un-submerged ramets. Consequently, clonal integration significantly increased growth of the apical ramets, the basal ramets and the whole fragments. However, after 20 d of de-submergence, stolon connection did not significantly affect growth or photosynthetic capacities of either de-submerged or un-submerged ramets. The results suggest that clonal integration plays different roles during submergence and de-submergence. It increases performance of submerged ramets during submergence by translocation of photosynthates from un-submerged ramets, but plays little roles during de-submergence.

1. Introduction

Submergence and de-submergence are common stresses encountered by riparian plants due to water level fluctuations (McGowan et al., 2011; Raulings et al., 2010). Both of them can strongly influence growth and physiological processes of plants (Striker and Colmer, 2017; Voesenek and Bailey-Serres, 2015). Submergence, especially under turbid floodwater, greatly decreases gas diffusion and light intensity underwater, leading to carbohydrate starvation and fatal injury of plants (Voesenek et al., 2006; Wang et al., 2016). After de-submergence, plant injuries developed underwater can be greatly intensified (Striker, 2012). Sudden increase in O_2 and light intensity may lead to over-accumulation of reactive oxygen species and toxic oxidative products in plants (Blokhina et al., 2003; Santosa et al., 2007). Besides, low hydraulic conductivity of roots is not able to provide enough water to meet shoot transpirational demand, causing wilting of shoots in many plant species (Holbrook and Zwieniecki, 2003). Therefore, plant injuries suffered from de-submergence can be as serious as those suffered from submergence (Malik et al., 2002; Striker, 2012).

Submergence-tolerant species often can alter morphology and anatomy of submerged shoots and roots to facilitate internal diffusion of gases such as O_2, CO_2 and ethylene (Colmer and Voesenek, 2009; Luo et al., 2016; Mommer and Visser, 2005). Some of them can also adjust

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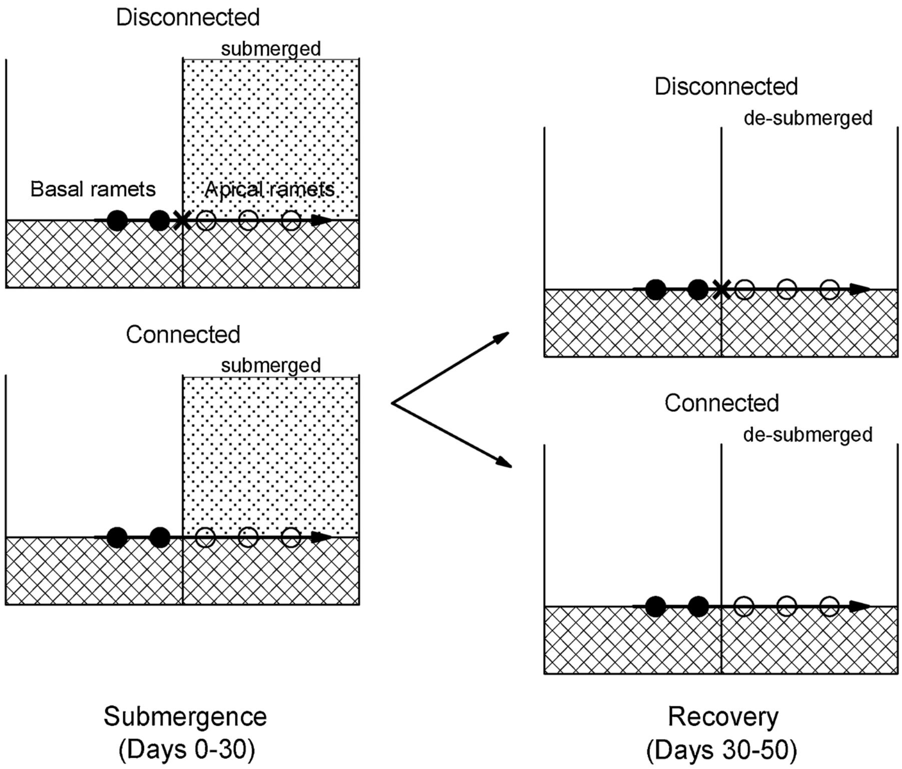

Fig. 1. Schematic representation of the experimental design. Each clonal fragment of *Alternanthera philoxeroides* consists of two basal ramets (closed circles) and three apical ramets (open circles) with a stolon apex (arrow). During days 0–30, the apical ramets were submerged and the basal ramets were un-submerged. On day 31, the apical ramets were de-submerged. The basal and the apical ramets were either connected or disconnected by severing to evaluate roles of clonal integration during submergence and de-submergence.

Table 1
The split-plot ANOVA results for effects of clonal integration (I), section (S) and their interaction on growth and chlorophyll fluorescence of *Alternanthera philoxeroides* during submergence and de-submergence.

	Submergence			De-submergence		
	I	S	I × S	I	S	I × S
Leaf biomass	2.40	**4.64***	0.98	0.01	0.55	1.01
Stem biomass	**8.83****	0.25	1.11	0.31	0.11	0.67
Root biomass	1.67	**7.42***	**4.79***	0.03	0.02	1.03
Total biomass	**4.66***	1.72	0.24	0.15	0.01	0.87
Root to shoot ratio	1.22	**4.12**$^{\#}$	**5.45***	0.11	0.40	0.21
Main stem length	**3.48**$^{\#}$	**23.73*****	0.40	0.39	**14.71****	0.30
Ramet number	**7.66***	0.47	0.01	2.22	**50.12*****	1.69
Fv/Fm	0.08	**28.18*****	0.29	0.21	2.63	0.23
ΔF/Fm'	**16.87****	**32.96*****	2.20	0.23	1.83	0.23
NPQ	1.61	**16.17****	0.27	1.03	0.60	0.09

Values are *F* and symbols show p (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; $^{\#}$, $0.05 \le p < 0.1$ and no symbols $p \ge 0.1$). Degrees of freedom for effects of I, S and I × S are (1, 18), (1, 18) and (1, 18), respectively. For the analyses, the whole clonal fragments are as main plots and the apical/basal ramets are as subplots.

metabolic pathways by switching from aerobic to anaerobic respiration to alleviate energy crisis (Gibbs and Greenway, 2003). When floodwater is subsided, some of these species have the ability of delaying chlorophyll degradation (Roiloa and Retuerto, 2016), retaining functional PSII complexes even after prolonged submergence (Herrera, 2013), or recovering growth and photosynthetic capacities quickly (Luo et al., 2011).

The ability of plants to tolerate submergence is determined not only by their performance during submergence, but also by their performance after de-submergence (Striker, 2012; Voesenek and Bailey-Serres, 2015). Several studies have shown that performance of plants after de-submergence was not necessarily positively related to that during submergence (Chen et al., 2011; van Eck et al., 2004; Striker et al., 2011). For example, species showing poor performance during submergence may not be submergence-sensitive because they can quickly resume growth by using reserved carbohydrates and/or newly-formed photosynthates after de-submergence (Akman et al., 2012; Striker et al., 2012). However, plant responses after de-submergence have often been overlooked (Malik et al., 2002; Striker, 2012). Therefore, the conclusions on plant tolerance to submergence have been mainly based on their responses during or immediately after submergence. In recent years, increasing number of studies have investigated effects of de-submergence on recovery of plant growth and photosynthetic capacities (van Eck et al., 2004; Gautam et al., 2015; Luo et al., 2011, 2014; Malik et al., 2002; Striker, 2012). However, the 'de-submergence effects' evaluated in these studies in reality tested those on the responses of plants to the integrated effects of both submergence and de-submergence.

Many amphibious species are clonal plants, which enable them to spread their asexual individuals (ramets) from terrestrial to aquatic habitats and *vice versa* (Lin et al., 2017; Liu et al., 2016; Wang et al., 2009). The connecting stolons between ramets within a clone allow the translocation of resources such as carbohydrates, water and nutrients between connected ramets, i.e., clonal integration (Roiloa et al., 2014; Song et al., 2013; Zhou et al., 2017). Many studies have shown that clonal integration increases performance of ramets suffering from various environmental stresses in both terrestrial and aquatic conditions (Liu et al., 2016; Luo et al., 2014; Roiloa et al., 2014; Wang et al., 2017a,b; Yu et al., 2004, 2008). Several studies on roles of clonal integration in resisting flooding stress have shown that clonal integration benefits submerged ramets with the translocation of photosynthates and probably also O_2 from un-submerged ramets during the submergence (Ayi et al., 2016; Luo et al., 2014; Wang et al., 2009; Xiao et al., 2010).

In this study, we used a novel design to truly separate effects of clonal integration during submergence and de-submergence with the well-studied amphibious clonal plant, *Alternanthera philoxeroides*. The main aim is to test whether clonal integration affects growth and photosynthetic capacities of *A. philoxeroides* after de-submergence. We also address whether clonal integration increases performance of *A. philoxeroides* during submergence. Our hypothesis is that clonal