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Tissue-specific transcriptomic profiling provides new insights into the reproductive ecology and biology of the iconic seagrass species *Posidonia oceanica*

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ABSTRACT

Seagrasses form extensive meadows in shallow coastal waters and are among the world's most productive ecosystems. Seagrasses can produce both clonally and sexually, and flowering has long been considered infrequent, but important for maintaining genetically diverse stands. Here we investigate the molecular mechanisms involved in flowering of the seagrass *Posidonia oceanica*, an iconic species endemic to the Mediterranean. We generated a de novo transcriptome of this non-model species for leaf, male and female flower tissue of three individuals, and present molecular evidence for genes that may be involved in the flowering process and on the reproductive biology of the species. We present evidence that suggests that *P. oceanica* exhibits a strategy of protogyny, where the female part of the hermaphroditic flower develops before the male part, in order to avoid self-fertilization. We found photosynthetic genes to be up-regulated in the female flower tissues, indicating that this may be capable of photosynthesis. Finally, we detected a number of interesting genes, previously known to be involved in flowering pathways responding to light and temperature cues and in pathways involved in anthocyanin and exine synthesis. This first comparative transcriptomic approach of leaf, male and female tissue provides a basis for functional genomics research on flower development in *P. oceanica* and other seagrass species.

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1. Introduction

Seagrasses are flowering monocot plants, fully adapted to develop their life cycle completely submerged in the marine realm (Larkum et al., 2007). During their evolutionary history, the four independent seagrass lineages that colonized the marine environment from terrestrial counterparts developed independent, but often converging adaptive strategies (Eckert et al., 2016; Olsen et al., 2016; Wissler et al., 2011). The complex adaptation of seagrasses involved important reproductive and life-history traits, including sexual reproduction, i.e. the presence of submerged flowers and filamentous pollen in most of the species and, above all, hydrophilous pollination (Du and Wang, 2014; Philbrick and Les, 1996). Marine plants

reproduce both sexually and asexually (clonally), and the important contribution of sexual reproduction for standing genetic variation of a population is generally acknowledged. However, the way the relative occurrence of reproductive strategies affects demographic and genetic structure of populations is still poorly understood (e.g. Procaccini et al., 2007; Kendrick et al., 2012, 2016). Seagrasses also display a wide variety of mating systems (Ackerman, 2006), which interact and affect a wide range of genetic and ecological factors before and after pollination. Among them, the spatial separation of male and female reproductive structures (i.e. dichliny) and the phenological separation between pollen release and pollen reception (dichogamy) are common in marine plants (McConchie and Knox, 1989a; Ruckelshaus, 1995; Les et al., 1997). These strategies should enhance outcrossing by reducing the probability of self-pollination either within the same flower (autogamy) or between separate flowers of the same genet (geitonogamy).

The investment in sexual reproduction is energetically costly and the reproductive success in seagrasses is low. The frequency and intensity of flowering seems to be the main bottleneck (Marbà and Walker, 1999; Hemminga and Duarte, 2000). Very little is known about the genetic basis of flowering in this group of marine plants,

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