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# Isolation and characterization of a *SEPALLATA*-like gene, *ZjMADS1*, from marine angiosperm *Zostera japonica*

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## ABSTRACT

In flowering plants, floral homeotic MADS-box genes, which constitute a large multigene family, play important roles in the specification of floral organs as defined by the ABCDE model. In this study, a MADS-box gene, *ZjMADS1*, was isolated and characterized from the marine angiosperm *Zostera japonica*. The predicted length of the ZjMADS1 protein was 246 amino acids (AA), and the AA sequence was most similar to those of the *SEPALLATA* (*SEP*) subfamily, corresponding to E-function genes. Southern blot analysis suggested the presence of two *SEP3*-like genes in the *Z. japonica* genome. *ZjMADS1* mRNA levels were extremely high in the spadices, regardless of the developmental stage, compared to other organs from the reproductive and vegetative shoots. These results suggest that the *ZjMADS1* gene may be involved in spadix development in *Z. japonica* and act as an E-function gene in floral organ development in marine angiosperms.

## 1. Introduction

Marine angiosperms (seagrassesa) are a polyphyletic group of monocotyledonous angiosperms consisting of 60 species in four families (Posidoniaceae, Zosteraceae, Hydrocharitaceae, and Cymodoceaceae) belonging to a single order (Alismatales) (Omori, 2000; Anderson and Janβen, 2009; Wissler et al., 2011). Molecular dating methods and the fossil record suggest that the Alismatales diverged from terrestrial monocots 131 million years ago (Ma), and all living lineages of Alismatales diverged from each other 128 Ma (Anderson and Janβen, 2009; Wissler et al., 2011). In addition, phylogenetic analysis based on plastid genes indicates that the return to the marine environment occurred at least three times independently through parallel evolution from a common aquatic ancestor of terrestrial origin. Generally, seagrasses superficially resemble some features of terrestrial grasses of the family Poaceae, which have horizontal creeping rhizomes and long-narrow leaves originating from a basal meristem. In addition, floral systems of seagrasses are generally conservative and reflect their terrestrial heritage. However, living submerged in the marine environment imposes some challenges to seagrasses requiring morphological adaptations that are distinct from terrestrial angiosperms. For instance, seagrasses share morphological traits such as reduced stamens and corollas, and elongated pollen for hydrophilous pollination (De Cock, 1980; Philbrick and Les, 1996).

The intertidal seagrass *Zostera japonica*, which belongs to the Zosteraceae, is a marine angiosperm endemic to East-Asia and distributed along the North Pacific coastline from North Japan to the Philippines and Viet Nam (Aioi and Nakaoka, 2003; Fortes, 2003). *Z. japonica* primarily grows on intertidal sandy and muddy bottoms at a depth of approximately 1 m (Nakaoka and Aioi, 2001; Aioi and Nakaoka, 2003). In addition, *Z. japonica* is one of the major components of *Zostera* beds which play important roles in the intertidal ecosystem, supplying spawning and growing grounds for fish and shellfish, primary production, and seawater clarification by nutrient uptake (Harrison and Bigley, 1982; Larned, 2003; Uede, 2007). *Z. japonica* is perennial and its reproduction consists of both asexual and sexual mechanisms as in some terrestrial plants (Soros-Pottruff and Posluszny, 1995; Philbrick and Les, 1996; Abe et al., 2009a, b) (Fig. 1A). In general, *Z. japonica* grows vegetatively throughout most of the year, and develops new lateral shoots through the creeping rhizome. With rising water temperature in the summer, *Z. japonica* develops reproductive shoots, forms spadices as inflorescence, and

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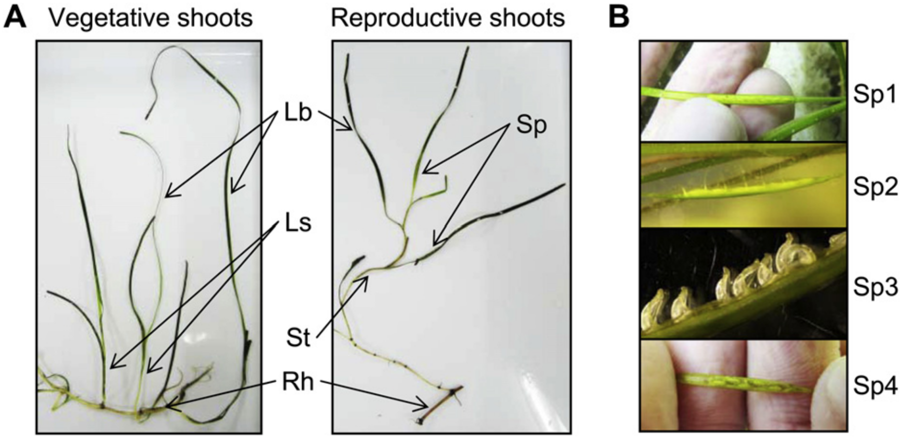

**Fig. 1.** Vegetative and reproductive shoots of *Z. japonica* (A) and developmental stages of the spadix (B). In vegetative growth, *Z. japonica* develops new lateral shoots consist of leaf-sheaths (Ls) and leaf-blades (Lb) through the rhizome (Rh). In sexual reproduction, *Z. japonica* develops reproductive shoots with elongated stems (St) and forms spadices (Sp) followed by the flowering, pollination, fruiting, and seed release. The spadix can be separated into four developmental stages: immature spadices (Sp1), spadices with erect styles in pistils (Sp2), spadices releasing pollen from thecae (Sp3), and spadices with developing seeds (Sp4).

releases seeds (De Cock, 1980; Soros-Pottruff and Posluszny, 1995; Philbrick and Les, 1996). The spadix development, including flowering and seed production, can be divided into four stages (Fig. 1B): formation of immature spadices including thecae and pistils (male and female flowers, respectively) enveloped by single spathe (Sp1), flowering of the gemale flowers (Sp2), flowering of the male flowers (Sp3), and development of seeds after pollination (Sp4). During the development of the *Z. japonica* spadix, the primordia of the different organs appear in the following temporal sequence: spathe, stamens, and pistils. As in terrestrial plants, both asexual and sexual reproductions are important to *Z. japonica* population growth. Seawater temperature and time of exposure to air in low tide affects growth and development of the vegetative and reproductive shoots (Morita et al., 2010). These environmental factors serve as the primary cues for the reproductive shoot formation and subsequent induction of seed production. However, molecular information related to the transition from vegetative to reproductive growth in *Z. japonica*, as well as in other seagrasses, including development of the reproductive shoots, induction of spadix formation, and seed production, has received little attention. In order to study the molecular mechanism of reproductive development in *Z. japonica*, we focused on floral homeotic MADS-box genes in the context of higher plant phylogeny and evolution.

Floral organ specification can be defined by the ABCDE model, which is mainly derived from molecular genetic studies of *Arabidopsis* and *Antirrhinum* mutants (Coen and Meyerowitz, 1991; Weigel and Meyerowitz, 1994; Pelaz et al., 2000; Honma and Goto, 2001). According to the ABCDE model, A-function genes alone specify sepal formation, whereas the combinations of A, B, and E-function genes and of B, C, and E-function genes specify petal and stamen formation, respectively. In addition, C and E-function genes work together to specify carpel formation, and C, E, and D-function genes determine ovule identity. In *Arabidopsis thaliana*, A-function is provided by *APETALA1* (*AP1*) and *AP2*, B-function by *AP3* and *PISTILLATA* (*PI*), C-function by *AGAMOUS* (*AG*), D-function by *SEEDSTICK* (*STK*, formerly known as *AGAMOUS-LIKE11*), and E-function by *SEPALLATA* (*SEP*, formerly known as *AGAMOUS-LIKE2*) genes (Theiβen, 2001; Becker and Theiβen, 2003; Liu et al., 2010). Except for some A-function genes such as *AP2* from *A. thaliana*, all floral organ identity genes encode proteins of the MADS-box transcription factor family and are called MADS-box genes. The MADS-box protein family which plays important roles in floral organ identity is characterized as "MIKC-type" because of the modular structure consisting of four functional domains: the highly conserved MADS domain (M-domain) required for DNA-binding and protein dimerization; the less conserved intervening domain (I-domain) and keratin-like domain (K-domain), which mediate protein heterodimerization; and the variable C-terminal domain (C-domain) involved in transcriptional activation and enhancing and/or stabilizing interactions mediated by the K-domain (Kaufmann et al., 2005). The MIKC-type genes have been isolated not only from various dicot species but also from monocot species (Becker and Theiβen, 2003; Kofuji et al., 2003; Zhao et al., 2006a; Adam et al., 2006, 2007a, b; Liu et al., 2010; Song et al., 2010), suggesting that the ABCDE model seems to be widely applicable among angiosperms, including marine species such as *Z. japonica*.

As a first step toward understanding the molecular mechanisms for development of reproductive and floral organs in marine angiosperms, the present study was undertaken to clone and sequence the cDNA and genomic DNA (gDNA) of a *Z. japonica* MADS-box gene, *ZjMADS1*. In addition, in order to understand the role of the *ZjMADS1* gene in the specification of floral organs as defined by the ABCDE model, the gene was analyzed based on the sequence similarities and the level of transcript accumulation was investigated.

## 2. Materials and methods

### 2.1. Materials

Vegetative and reproductive shoots of *Z. japonica* were harvested from *Zostera* beds by the Tategami-ura (34°17′N, 136°47′E) in Ago Bay, Mie Prefecture, central Japan, from April to August 2009. The harvested shoots were immediately washed with sterile seawater to remove all debris on the surface of the shoots. Various organs such as leaf-blade, leaf-sheath, stem, rhizome, and spadix were dissected from the shoots. The spadices of the reproductive shoots were divided into 4 groups corresponding to developmental stages: immature spadix, spadix with erecting styles of pistils, spadix releasing pollens from thecae, and spadix with developing seeds. After washing with sterile water, the separated organs were immediately frozen with liquid nitrogen and stored at −80 °C until RNA and DNA extraction.

### 2.2. cDNA and genomic DNA cloning

For isolation of cDNA fragments encoding *Z. japonica* ZjMADS proteins, 3′ rapid amplification of cDNA ends (3′ RACE) was used.