Research article

Genome-wide identification and expression analysis of *SWI1* genes in *Boechera* species

Fatih Sezer[1], Gözde Yüzbaşıoğlu[1], Aslıhan Özbilen[1], Kemal M. Taşkin*,[1]

Canakkale Onsekiz Mart University, Department of Biology, 17100 Canakkale, Turkey

ABSTRACT

As a mode of reproduction in plants, apomixis leads to the generation of clones via seeds. Apomictic plants form viable diploid female gametes without meiosis (apomeiosis) and produce embryos without fertilization (parthenogenesis). Apomeiosis, as a major component of apomixis, has recently been reported in some *Arabidopsis thaliana* mutants; *dyad* mutants of *SWI1* showed developmental processes common to apomeiosis, such as producing functional diploid gametes. However, the orthologs of *SWI1* genes in natural apomicts has not been previously reported. To identify the relationship between the *SWI1* gene and the apomeiosis process, we isolated and sequenced *SWI1* orthologs from *Boechera* species, including apomictic and sexual species. *Boechera* species are close relatives of *A. thaliana* and thus are advantageous model species for apomixis research. The *SWI1* cDNAs were obtained by RT-PCR from apomictic and sexual *Boechera* young flower buds. We sequenced partial *SWI1* transcripts that were 650 bp for *B. holboellii* and 684 bp for *B. stricta*. These *SWI1*-like sequences showed 86% similarity for *B. holboellii* and 92% for *B. stricta* to the *A. thaliana SWI1* transcript. We also used available genome data and amplified genomic sequences for *SWI1* orthologs in *B. holboellii* and *B. stricta*. The predicted proteins contain a phospholipase C domain and a nuclear localization signal. Sequence analysis did not show significant mutations related to apomixis, and phylogenetic analysis showed that *SWI1*-like sequences were common across plant families, regardless of the presence of a sexual or apomictic reproduction system. We also investigated the expression levels of *SWI1* mRNA in the *B. holboellii* and *B. stricta* young unopened flower buds and found that relatively high levels of expression occurred in apomicts.

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1. Introduction

In most angiosperm species, seeds are produced by sexual reproduction, although in apomixis, as an exception of this mode of reproduction, embryo formation occurs without paternal genome contribution (fertilization) of the egg. It has already been reported that three common components are observed in apomicts: generation of an unreduced embryo sac that contains an egg cell capable of forming an embryo without prior meiosis (apomeiosis), the spontaneous, fertilization-independent development of the embryo (parthenogenesis) and the capacity to either produce endosperm autonomously or to use an endosperm derived from fertilization (Bicknell and Koltunow, 2004). Because the progeny of apomictic plants are genetically identical to the mother plant,

apomixis would provide a new tool to maintain hybrid vigour in plant breeding (Koltunow and Grossniklaus, 2003). Apomixis is not common in economically important plants; therefore, its introduction into sexual plants holds great promise. The molecular mechanisms underlying apomixis are not known. In *Arabidopsis thaliana*, a sexual plant, several meiotic mutants, including *osd1* and *switch1/dyad*, have phenotypes that are capable of apomeiosis and produce viable, unreduced gametes that have been identified (Agashe et al., 2002; d'Erfurth et al., 2009; Ravi et al., 2008). The functional investigation of the *Arabidopsis* SWITCH1/DYAD protein revealed that it is functional in prophase I for sister chromatid cohesion and recombination during both male and female meiosis (Agashe et al., 2002; Boateng et al., 2008; Mercier et al., 2001). Four different mutations have been reported for *SWI1*, including *swi1-1* and *dyad*, which only result in defects in female meiosis (Motamayor et al., 2000; Siddiqi et al., 2000), and *sw1-2*, which results in defects in both female and male meiosis (Mercier et al., 2001, 2003). These mutations cause a defective prophase I in which the separation of sister chromatids leads to the formation of univalents instead of bivalents (Agashe et al., 2002; Mercier et al.,

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* Corresponding author.
  E-mail addresses: fsezerfatih@gmail.com (F. Sezer), gozde.nisli@yahoo.com
(G. Yüzbaşıoğlu), aslihanozbilen@hotmail.com (A. Özbilen), kmtaskin@gmail.com
(K.M. Taşkin).
  [1] All authors contributed equally to this work.

2001). As a result, sister chromatids are equally separated to opposite poles in a single division within each unreduced female gamete and most female gametophytes are not viable in these mutants. However, in *dyad* mutants, a small number of unreduced (diploid) female gametes are functional and can produce 1–10 seeds per plant.

Apomictic *Boechera* (*Brassicaceae*) species are attractive model species that are used to study the molecular biology of apomixis because they are close relatives of *A. thaliana* (Dobeš et al., 2006; Dobes et al., 2007; Schranz et al., 2005, 2006; Sharbel et al., 2004). They have been reported as facultative apomicts and, in common with many apomicts, require fertilization to generate an endosperm (Moore 1952; Naumova et al., 2001; Schranz et al., 2006). Diplosporous apomixis has been previously described in *Boechera* species (Böcher 1951; Naumova et al., 2001; Sharbel et al., 2005; Taskin et al., 2004). *Boechera* has seven chromosomes, and we previously observed 21 univalents instead of seven trivalents during late prophase I (or metaphase I) in a triploid apomict *B. holboellii*. This phenotype resembles meiosis I in *dyad* mutants (Ravi et al., 2008). We also found a high frequency of unreduced (2n) gamete formation in the triploid apomict *B. holboellii*, but reduced (n) formation in the anthers of a diploid sexual *B. stricta* (Taskin et al., 2009). These results suggest that the unreduced (2n) male gametes in triploid apomictic *B. holboellii* are produced via apomeiosis, are viable and may be able to overcome the endosperm ratio problem.

The present study aims to characterize the *SWI1* orthologs in triploid and diploid apomictic and sexual *Boechera* spp. We report the genomic and partial *Boechera* cDNA sequences of *SWI1* and describe the phylogenetic tree of these sequences and expression studies.

2. Materials and methods

2.1. Plants

In this study, we used triploid and diploid apomictic and diploid sexual *Boechera* species. Seeds for the triploid apomict *Boechera holboellii* (Hornemann) (A. Löve & D. Löve plants) (Taskin et al., 2004, 2009) were originally collected from North America and obtained from Dr. Bitty Roy (University of Oregon) and Prof. Dr. Rod J. Scott (University of Bath, UK). The triploid apomict *B. holboellii* authenticated voucher specimens (CBB 00000346) were deposited in the Herbarium of Canakkale Botanic Garden in Canakkale Onsekiz Mart University. Diploid sexual *B. stricta* (ES:6) seeds (ES ID no., species, HEID ID no.; haplotype; GenBank accession no. 6, *B. stricta*, 500206; DG; DQ013050) were obtained from Dr. Eric Schranz (Schranz et al., 2005, 2006). Plants were grown in a growth chamber under long-day conditions under a regime of 16 h light: 8 h dark (21 °C) on a peat:perlite mix (1:4) for 28 days. Plants were then vernalized at 5–10 °C for six weeks and transferred to the growth chamber (Schranz et al., 2005). The plants started to flower four weeks following transfer.

2.2. RNA isolation

Total RNA isolated from various tissues included young unopened buds of inflorescences at early development stages, mature flowers, leaves and siliques. Tissues were stored in RNAlater solution (Ambion – #AM7020) to prevent RNA degradation and dissected with sterile scalpels under a stereomicroscope. RNA isolations were carried out using a Purelink RNA Mini Kit (Invitrogen – 12183-018A). The RNA concentrations were quantified with an Invitrogen Qubit Fluorometer. RNA samples were stored at −80 °C.

2.3. PCR amplification of *Boechera* SWI1 *orthologs*

Reverse Transcriptase-PCR was used to clone the *Boechera SWI1* orthologs. Total RNA (0.1–1 μg) was used to make cDNA with a First Strand cDNA Synthesis kit (Fermentas – #K1622). PCR reactions were performed in a total volume of 25 μl containing approximately 0.5 ng of cDNA, 4 pm of each primer, 1 unit of High Fidelity PCR Enzyme mix (Fermentas), 2.5 μl of 10× High Fidelity PCR buffer, 2 μl of 25 mM $MgCl_2$ and 0.5 μl of 100 mM dNTP mix. PCR amplification was performed with cDNA templates using a primer pair specific to the *AT5G51330* gene (Table 1). The D1 primer pair was used for *B. stricta* and D2 for *B. holboellii* to amplify partial SWI1 transcripts. A house keeping gene, *ACTIN2 (At3g18780)*, was used as a positive control. The reaction conditions were as follows: 2 min at 94 °C, followed by 35 cycles of 20 s at 94 °C, 30 s at 60 °C and 2.5 min at 72 °C and a final extension at 72 °C for 7 min. The PCR products were visualized by 1% agarose gel electrophoresis.

2.4. Sequence analysis

DNA sequence analysis was performed by REFGEN (Ankara, TURKEY) with an Applied Biosystems ABI 310 DNA Sequencer (BC-ABI310) using the Sanger technique-based Double Strand Primer Walking Method. The Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used for a homology search against known plant genes.

2.5. Bioinformatics analysis

The reference sequences of *SWI1* (*AT5G51330*) obtained from the TAIR website (www.arabidopsis.org) were used to identify homologues in *Boechera*. We performed a BLAST search of *SWI1* in the diploid sexual *B. stricta* genome in the Phytozome 10.2 database (Goodstein et al., 2012) using *AT5G51330* as a query. Additionally, we downloaded raw sequencing data in fastq format of the apomict *B. holboellii* cv Panther genome from SRA (http://trace.ncbi.nlm.nih.gov/Traces/study/?acc=SRP004021). Then, we used the Geneious R8 (http://www.geneious.com, Kearse et al., 2012) software "map to references" function to obtain homologous genes and their -1000-bp upstream region. At the mapping step, we trimmed the low quality reads and used iterative fine tuning to improve the results. We also predicted the putative amino acid sequences for the coding regions of *SWI1* homologues using Geneious R8 software.

For phylogenetic analyses, the homologous amino acid sequences of *SWI1* proteins were obtained by Blast (Altschul et al., 1997) using the Phytozome 10.2 database (Goodstein et al., 2012). The sequences were aligned with Clustal Omega using its default parameters. For each gene alignment, the phylogenetic tree was constructed based on the full-length amino acid sequences of *SWI1* genes and estimated by maximum parsimony (MP) using PAUP (Swofford, 1993).

We predicted the molecular weights and isoelectric points of SWI1 proteins using Expasy's ProtParam server (http://web.expasy.org/protparam/) (Gasteiger et al., 2005). The conserved

Table 1
Nucleotide sequences of the primers used in PCR reactions.

Primer	Sequence
D1 Reverse	5′-TCATCCTTCACTCTAGAACTCTCCTC-3′
D1 Forward	5′-GGAACGAAGATTATCGAGAGCA-3′
D2 Reverse	5′-GTCACCAAGCTTCCAACCAG-3′
D2 Forward	5′-TCAAGTGGGGAAGGAGATTG-3′
Actin 2 Forward	5′-TGGTGAAGGCTGGATTTGC-3′
Actin 2 Reverse	5′-TCGGTAAGAAGAACAGGGTGC-3′