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RESEARCH ARTICLE

Defective callose walls and cell plates during abnormal meiosis cause male-sterility in the oat mutant *zbs1*

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Abstract

During meiosis in flowering plants, degradation of the callose wall in tetrads releases newly produced microspores, which develop into mature pollen grains. In this study, we identified *zbs1*, a male-sterile mutant of naked oat (*Avena nuda* L.) that displayed complete spikelet sterility due to inviable mature pollen. The abnormal pollen grains originated from microspores with a defective callose wall and cell plate during meiosis. The defective callose wall and cell plate of the *zbs1* mutant were detected by the labeling of cell wall epitopes (β-1,3-glucan) with immunogold during meiosis, and an abnormal chromosome configuration was observed by propidium iodide staining. The mature pollen grains of the *zbs1* mutant were irregular in shape, and abnormal germination was observed by scanning electron microscopy. Together, our results indicate that the cause of male sterility in *zbs1* is abnormal meiosis.

Keywords: callose, male sterility, meiosis, microspore, naked oat

1. Introduction

Meiosis plays a crucial role in the sexual reproduction of higher plants. However, related reports of meiosis in Avena are still limited, though oats are one of the major crops for human nutrition, livestock feeding and cosmetic application. Several studies have been published on meiosis in *Arabidopsis thaliana*, maize (*Zea mays*; Murphy *et al*. 2014; Wang *et al*. 2014; Zhou and Pawlowski 2014) and rice (*Oryza sativa*; Mercier and Grelon 2008). Rice and oat are monocots, and rice is a popular model species for developmental studies of monocotyledonous plants (Itoh *et al*. 2005).

Heterosis refers to the phenomenon in which the progeny of diverse varieties of a species or an interspecific cross have an increased biomass, developmental rate and fertility rate compared to the parents (Birchler *et al*. 2010). The development and utilization of hybrid rice varieties have contributed greatly to food security in China and other Asian countries (Sparks 2009). Interspecific F_1 hybrids of wild-type (WT) species often show high levels of sterility; this is partly attributed to defects in the homologous pairing of chromosomes during meiosis (Brar and Khush 1997). Research on meiosis in rice will help us to reduce reproductive barriers between different rice species. Already, several meiosis-related genes in rice, including *MEL1*, *MEL3*, *MER3*,

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PAIR1–3, *DMC1*, *RAD21-4*, *MSH4*, *MSH5*, and *RAD51C*, have been isolated and functionally characterized (Jenkins *et al*. 2008). *MEL1* encodes a novel ARGONAUTE family protein that functions in mitosis and meiosis (Nonomura *et al*. 2007), while *MER3* encodes a protein that is essential for normal meiosis (Wang *et al*. 2009). *PAIR1*, *2* and *3* are essential for homologous chromosome pairing during meiosis I (Caryl *et al*. 2000; Armstrong *et al*. 2002; Nonomura *et al*. 2004a, b, 2006). Similarly, rice *DMC1A* and *DMC1B*, which are homologs of yeast *DMC1*, are required for the pairing of homologous chromosomes (Ding *et al*. 2001; Kathiresan *et al*. 2002; Deng and Wang 2007). *RAD21-4* is essential for efficient mitosis (Zhang *et al*. 2006), while *MSH4* and *MSH5* encode interacting factors that promote crossover formation during meiosis in rice (Zhang *et al*. 2014). The *rad51c* mutant exhibits complete male and female sterility, indicating that *RAD51C* is required for synaptonemal complex assembly (Abe *et al*. 2005; Kou *et al*. 2012). However, despite this progress, our knowledge of the regulatory mechanisms on controlling meiosis in rice is fragmentary.

Callose, β-1,3-glucan, is widespread in various organs and diverse development processes of higher plants, the synthesis and deposition of callose play important roles in the process of reproductive development. In Petunia, it has been identified that the timely formation and degradation of callose plays a key role in the process of microsporogenesis by the study of several related mutants (Izhar and Frankel 1971; Warmke and Overman 1972). In transgenic tobacco, the expression of callose synthase gene was improved in the tapetum cells and the callose wall was dissolved prematurely, which affecting pollen development resulting in male sterility (Worrall *et al*. 1992). *AtGSL10*, a *Callose Synthase like 5* gene in *Arabidopsis*, participates in the process of mitotic division, the knock-out of *AtGSL10* lead to aberrant asymmetric microspore division (Huang *et al*. 2009). A study of *cals5* in *Arabidopsis*, a *callose synthase like 5* mutant, indicated that callose accumulates in the cell plate of dividing cells before the formation of the cell wall (Hong *et al*. 2001). Both *AtGSL11* and *AtGSL12* related to the formation of callose wall in microsporocytes and play redundant roles in the microsporogenesis (Enns *et al*. 2005). In *Arabidopsis*, GSL8 functions in the process of cytokinesis and cell patterning, a *gsl8* mutant exhibits abnormal cell wall and cell division of microsporocytes during *Arabidopsis* development (Chen *et al*. 2009). In rice, the study of a *gsl5* mutant identified that the callose synthesized by GSL5 plays a key role in the microsporogenesis, the knock-out of *GSL5* lead to the formation of defective callose wall and cell plate, which caused the reduced panicle seed setting rate (Shi *et al*. 2015).

The use of male-sterile lines in breeding eliminates the need for emasculation; moreover, such lines are ideal for use as female lines in hybrid seed production. Male-sterile lines have been used to study autogamous crops, including wheat (*Triticum aestivum* L.) (Whitford *et al*. 2013), maize (Shull 1908), cotton (Weider *et al*. 2009), rice (Dias 2010; Huang *et al*. 2013), and other gamogenetic crops (Silva 2010). In a previous study, a spontaneous dominant male-sterile mutant oat line (*zbs1*) was discovered that did not exhibit any differences in vegetative growth when compared to WT plants. However, the callose wall of *zbs1* microspore mother cells was defective at the dyad and tetrad stages during meiosis, and the microspores released from the dyads were irregular in shape. Observation of the chromosome configuration during microsporogenesis revealed that the chromosome distribution was abnormal during meiosis and that most of the newly released microspores contained 1–3 micronuclei after meiosis. Furthermore, the mature pollen grains of *zbs1* were defective and exhibited multiple abnormal morphologies.

2. Results

2.1. Immunolocalization of the microspore mother cell wall epitopes

Callose deposition in the microspore mother cells of WT and *zbs1* mutant oat during meiosis was quantitatively analyzed by the labeling of callose wall and cell plate epitopes using immunogold reagent. Compared with the callose wall in WT oat (Fig. 1-A), less immunogold labeling was observed in *zbs1* microspore mother cell s (Fig. 1-B and C); the density of gold particles in each square array was 6–11% of that for the WT microspore mother cells (1 μm^2 area; Fig. 1-G). Moreover, the density of gold particles in the *zbs1* cell plates was 4–7% of that in the WT cell plates. Immunolocalized signals corresponding to anti-callose (anti-β-1,3-glucan) antibodies were significantly more abundant at the callose wall and cell plate than at other microspore mother cell regions, and there were significant differences in the number of immunogold particles between the callose wall and cytoplasm in WT and *zbs1* mutant oat (Fig. 1-A–F). These findings indicate that much more callose was present in the callose wall of the WT microspore mother cells compared to the *zbs1* mutant. In addition, the callose wall and cell plate of the *zbs1* microspore mother cells were defective at the tetrad stage during meiosis.

2.2. Cross-sectional analyses of microspores after meiosis and of the tapetum at the microspore mother cell stage

To further examine the characteristics of the free micro-