Abstract

A dwarf, multi-pistil and male sterile dms mutant was previously reported by us. However, the genetic changes in this dms are unclear. To examine the genetic changes, single nucleotide polymorphism (SNP) association, chromosome counting, and high-resolution chromosome fluorescence *in situ* hybridization (FISH) techniques were employed.

By comparing tall plants (T) with dwarf plants (D) in the offspring of dms mutant plants, SNP association analysis indicated that most SNPs were on chromosome 2A. There were 3 types in offspring of dms plants, with 42, 41 and 40 chromosomes respectively. High-resolution chromosome painting analysis demonstrated that T plants had all 42 wheat chromosomes; the medium plants (M) had 41 chromosomes, lacking one chromosome 2A; while D plants had 40 wheat chromosomes, and lacked both 2A chromosomes. These data demonstrated that dms resulted from a loss of chromosome 2A. There were 23 chromosome 2A specific genes involved in the development of stamens or pollen grains. Transcript analysis showed that most of the v-myb avian myeloblastosis viral oncogene homolog (MYB) transcription factor genes were expressed at a lower level in the stamens of D plants. Because MYBs were involved in exine formation, their lower expression might lead to the shrunken pollen grains of D plants. These results lay a solid foundation for further analysis of the molecular mechanisms of wheat male sterility. Because D plants can be used as a female parent to cross with other wheat genotypes, dms is a unique germplasm for any functional study of chromosome 2A and wheat breeding specifically targeting genes on 2A.

Keywords Wheat (*Triticum aestivum* L.), dms, Chromosome, Nullisomic line, Pollen development

Introduction

Allohexaploid wheat (*Triticum aestivum* L., 2n = 6x = 42, genomic constitution AABBDD) has a genome from three diploid species: *Triticum urartu* Thum. (the source of the A genome), *Aegilops speltoides* (Tausch) Gren. or a closely related species (the source of the B genome), and *Aegilops tauschii* Coss. (the source of the D genome) (Huang et al., 2002). Because allohexaploid wheat has high level of functional redundancy, it can host a range of diverse whole-chromosome aneuploids (Zhang et al., 2013; Zhang et al., 2017). There are various types aneuploid variations available in wheat, such as nulli-tetrasomic lines, nullisomic lines, monosomic lines, ditelosomic lines, as well as chromosome fragmental deletion lines (Endo & Gill, 1996; Qi et al., 2003). The aneuploid stocks are useful in wheat gene mapping, and especially, genes can

Deleted: novel natural mutant of
Deleted: what
Deleted: have occurred
Deleted: was
Deleted: settle this issue
Deleted: analysis
Formatted: Font: Italic
Deleted: in this study
Deleted: the
Deleted: the
Deleted: Later, chromosome counting confirmed that t
Deleted: of chromosome numbers
Deleted: the
Deleted: ed
Deleted: 2A
Deleted: was
Deleted: 2A
Deleted: proprietary
Deleted: were
Deleted: and
Deleted: developments
Deleted: tome
Deleted: less
Deleted: less
Deleted: wizened
Deleted: id
Deleted: the
Deleted: study
Deleted: in future
Deleted: about
Deleted: design
Formatted: Font: Italic
Deleted: The a
Formatted: Font: Italic
Deleted: was contributed by
Formatted: Font: Italic
Formatted: Font: Italic
Formatted: Font: Italic
Deleted: repertoire
Deleted: have been obtained
Deleted: al
Deleted: a certain

be located to a small segment using a series of chromosome fragmental deletion lines (Endo & Gill, 1996; Qi et al., 2003). A set of homozygous chromosome deletion lines were obtained in cv. Chinese Spring (Endo & Gill, 1996). Most of the homozygous chromosome 2A short arm deletion lines are sterile; genes involved in male development are located on the chromosome 2A short arm (Endo & Gill, 1996). Presently, at least five stable genic male sterility (GMS) genes are known in bread wheat. They are *ms1* on 4BS (Wang et al., 2017), *Ms2* on 4DS (Xia et al., 2017), *Ms3* on 5AS (Maan et al., 1987), *Ms4* on 4DS (Maan & Kianian, 2001) and *ms5* on 3AL (Pallotta et al., 2019), but the molecular regulation mechanisms of the male sterility lines are largely unknown. Anther and pollen development are complex biological processes, determining wheat male fertility. Pollen development starts from a single cell layer, which undergoes a series of cell divisions and differentiation to form the innermost meiocytes encased within four somatic anther cell layers; from inner to outer these are the tapetum, middle layer, endothecium and epidermis (Zhang & Yang, 2014). The tapetum serves as a nutritive tissue, providing metabolites, nutrients, and cell wall precursors for the development of pollen grains (Goldberg, Beals & Sanders, 1993). The regulatory genes involved in pollen exine patterning are known in Arabidopsis and rice (Pearce et al., 2015; Lin et al., 2017). For example, AtMS1 (Male Sterility 1) (Wilson et al., 2001), AtDRL1 (Dihydroflavonol 4-Reductase-Like 1) (Tang et al., 2009), AtLAP3 (Less Adherent Pollen 3) (Dobritsa et al., 2009), AtLAP5 (Less Adherent Pollen 5) (Dobritsa et al., 2010) in Arabidopsis, and OsGAMYB (GA, gibberellin; MYB, v-myb avian myeloblastosis viral oncogene homolog) (Aya et al., 2009), OsNP1 (No Pollen 1) (Liu et al., 2017), OsDPW2 (Defective Pollen Wall 2) (Xu et al., 2017) in rice are involved in the biosynthesis of sporopollenin, which is a major constituent of exine in the outer pollen wall. However, pollen developmental studies in wheat have lagged behind these plants. Previously, we reported a mutant of dwarf, multi-pistil and male sterile dms in wheat (Duan et al. 2015; Zhu et al. 2016). Pollen vigor and hybridization tests demonstrated that dms mutant was male sterile. Male sterility and male fertility followed a segregation ratio of 1:3 [D:(T+M) =1:3] (Duan et al. 2015). However, the genetic basis of this mutant is unknown. The present study is to discover the genetic basis at the cytological and molecular levels. We also identified a set of genes playing potential key roles accounting for male sterility on chromosome 2A.

Deleted: in

Deleted: fragment

Deleted: have been

Deleted: from

Deleted: . Critical

Deleted: surely

Deleted: in

Deleted: Up to now

Deleted: have been identified

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Deleted: . Although some wheat male sterile germplasms have been reported,

Deleted: remained

Deleted: s

Deleted: have been identified

Deleted: the

Deleted: novel natural wheat

Deleted: was reported by us

Deleted: The p

Deleted: The

Deleted: m

Deleted: e

Deleted: completely

Deleted: its

Deleted: variation

Deleted: variation