Genetic Mapping of ms1s, Wheat

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The utilization of heterosis is an important way to improve wheat yield, and the production of wheat hybrid seeds mainly relies on male-sterile lines. Male sterility in line 15 Fan 03 derived from a cross of 72,180 and Xiaoyan 6 is controlled by a single recessive gene. The gene was mapped to the distal region of chromosome 4BS in a genetic interval of 1.4 cM and physical distance of 6.57 Mb between SSR markers Ms4BS42 and Ms4BS199 using an F2 population with 1205 individuals. Sterile individuals had a deletion of 4.57 Mb in the region presumed to carry the Ms1 locus. The allele for sterility was therefore named ms1s. Three CAPS markers were developed and verified from the region upstream of the deleted fragment and can be used for ms1s marker-assisted selection in wheat hybrid breeding. This work will enrich the utilization of male sterility genetic resources.

Keywords: genic male sterility ; genetic mapping ; CAPS marker ; Triticum aestivum

1. Introduction

As a major food crop, common wheat (*Triticum aestivum* L., AABBDD) plays a vital role in ensuring the food security of human beings. Development of wheat semi-dwarf varieties during the 1950s and 1960s improved lodging resistance and permitted significant increases in yield that led to a stable global food supply and eliminated the threat of hunger. These events became known as the “Green Revolution”. However, with arable land currently decreasing due to urbanization, environmental degradation, and rising population levels, there is an emerging need to further increase wheat yield. Heterosis, a common biological phenomenon widely used in maize (*Zea mays* L.) and rice (*Oryza Sativa* L.), offers a way to increase wheat yield [1][2]. Despite decades of research, the use of male sterility for the production of hybrid wheat has achieved only limited success. Wilson and Ross (1962) introduced the nucleus of common wheat into the cytoplasm of *Triticum timopheevii* to develop a common wheat T-type male-sterile line, and fertility restorer lines (T-type restorer lines) were obtained by breeding and selection [3]. Thus, a three-parent hybrid system was devised. Subsequently, new types of sterility systems, e.g., K- and V-type cytoplasmic male sterility systems, a photo-thermal sterility system, and chemical male sterilizing agents were described. However, critical biological and economic problems hindered each system, and hybrid wheat as a commercial enterprise never exceeded 0.2% of the total production [4][5].

In 1931, Bovicini first discovered the phenomenon of nuclear male sterile in wheat, and, to date, five nuclear male sterility loci have been identified, i.e., *Ms1*, *Ms2*, *Ms3*, *Ms4*, and *Ms5* [6][7][8][9][10]. Sterility alleles *ms1* and *ms5* are recessive and located on chromosome 4BS and 3AL, respectively [11][12], whereas *Ms2*, *Ms3*, and *Ms4* are dominant. *Ms2* and *Ms4* are located on chromosomes 4DS, whereas *Ms3* is on chromosome 5AS [7][8][10][13][14]. Many sterile alleles of the *Ms1* gene have been described. These presumably arose as natural mutants, such as Pugsley’s male sterile (*ms1a*, [6][15]) and LZ (*ms1g*, [16]); as X-ray-induced mutants, such as Probus male sterile (*ms1b*, [17]) and Cornerstone (*ms1c*, [18]); or as EMS induced mutants, including FS2 (*ms1d*), FS3 (*ms1e*), and FS24 (*ms1f*) [10][11]. Recently, 12 isomorphic variants of *Ms1* gene, i.e., *ms1d.1*, *ms1d.2*, *ms1h*, *ms1i*, *ms1j*, *ms1k*, *ms1l*, *ms1m*, *ms1n*, *ms1o, ms1p,* and *ms1q*, from the spring wheat variety Ningchun 4 were identified following EMS treatment [19]. Many scientists believe that nuclear male-sterile lines are ideal lines for the production of hybrid varieties, but a lack of stable male-sterile maintainers prohibits their widespread application. Although a “two-line system” [20] and an “XYZ hybrid” system using the nuclear male-sterile mutant “Cornerstone” were proposed, neither proved successful [21][22]. Huang et al. (1988, 1991) discovered a nuclear male-sterile mutant in the offspring of a cross of wheat varieties 72,180 and Xiaoyan 6 and created a blue-aleurone nuclear male-sterile maintainer system (BM system) by introducing a 4E chromosome from *Agropyron elongatum* containing a dominant blue endosperm gene and a fertility restorer gene [23][24]. The seeds of the wheat 4E monomer alien addition line are light blue. This line can produce seeds normally by selfing, and the offspring can separate about 64% non-blue seeds (nuclear male sterile), 16% light blue seeds, 16% medium blue seeds, and 4% deep blue seeds (fertile). All plants grown from non-blue seeds were male sterile, whose fertility could be restored well in F_1 by almost any variety of bread wheat. The light blue seed is used as a maintainer for male sterility. Zhou et al. (2006) also introduced the same 4E chromosome into Lanzhou Mutant 257A (*ms1g*) and established a recessive nuclear male-sterile 4E-ms system [25]. This solved the

difficulties in obtaining stable maintainer lines and producing homozygous male-sterile seeds in large quantities. It was a significant step forward in the applied research of wheat nuclear male sterility.

2. Discussion

Application of nuclear male sterility is one way to study heterosis in wheat. Although there are many reports on nuclear male sterility in wheat, only 5 loci *Ms1-Ms5* have been identified and physically mapped. Sterility-causing alleles of *Ms1* and *Ms5* are recessive, and those of *Ms2*, *Ms3*, and *Ms4* are dominant. Three of them have been isolated and cloned. The dominant *Ms2* sterility allele in *Taigu* contains an originally unexpressed “orphan” gene in its promoter region, which is activated and specifically expressed in anthers causing male sterility [13]. Tucker et al. (2017) and Wang et al. (2017) cloned the wildtype allele of *Ms1* and found that it encodes a lipid transport protein expressed only in microspores [19][26]. Pallotta et al. (2019) demonstrated that *Ms5* encodes a lipid transfer protein anchored by glycosylphosphatidylinositol, which is required for normal development of the pollen outer wall [12].

Here, we studied the wheat nuclear male-sterile line 15 Fan 03 and compared it with Mian 07-374 and generated an F_2 population. The sterility gene was mapped between the SSR markers *Ms4BS42* and *Ms4BS199* physically positioned at 10.61 and 17.18 Mb, respectively. Sterility was caused by deletion of a chromosome 4BS fragment, detectable by chromosomal FISH. Based on IWGSCWGA v1.0, the deleted region included *TraesCS4B01G017900* identified as the *Ms1* allele positioned at 13.126 Mb. Among reported *Ms1* mutants, *ms1a* (Pugsley’s mutant) and *ms1c* (Cornerstone) mutants are terminal deletions of chromosome 4BS [18][27]; *ms1b* (Probus mutant) and *ms1g* (LZ, [25]) are interstitial deletions [17][19]; and *ms1d* (FS2), *ms1e* (FS3), and *ms1f* (FS24) are nucleotide variants [10]. Nucleotide variant alleles in a series of EMS-induced sterile mutants in variety Ningchun 4 were named *ms1h*, *ms1i*, *ms1j*, *ms1k*, *ms1l*, *ms1m*, *ms1n, ms1o, ms1p,* and *ms1q* [19]. At the same time, a nucleotide variant allele in a Qual 2000 TILLING population was named *ms1h*. This allele has been re-designated as *ms1r* (RA McIntosh, personal communication).

The flowering phenotype of sterile plants in 15 Fan 03 differs from that in the fertile plants of Mian 07-374, as the glume remains open for a longer time at a wider angle, and at the tri-nuclear stage, the pollen grains degrade and the pollen activity is lost. However, genetically, the sterile line 15 Fan 03 was similar to *ms1b* and *ms1g* mutants in possessing interstitial deletions of 4BS chromosome segments bearing the *Ms1* locus.

Wheat nuclear male-sterile lines have many advantages. For example, they are easily restored by most common wheat varieties, with significant heterosis, and without the adverse effect of exogenous cytoplasm. Furthermore, it is easy to select excellent hybrid combinations. The main problem with this type of male-sterile line is that it is difficult to obtain a stable male-sterile maintainer line; that is, it is difficult to produce a large number of homozygous male-sterile seeds. However, using the blue-aleurone nuclear male-sterile maintainer system (BM system), we can easily obtain wheat male-sterile lines (white grains, such as 15 Fan 03) and maintainer lines (light blue grains). We obtained 11 combinations of BM systems with modern main varieties. Moreover, white and blue seeds can be separated at the seed stage, so it is easier to obtain pure male-sterile lines for the production of hybrid wheat [23][24]. This solves the difficulties in obtaining stable male sterile maintainer lines and producing a large number of homozygous male sterile seeds. Moreover, the easy restoration of these lines by common wheat varieties has opened up a new direction for the application of and research into wheat nuclear male sterility.

Because the genome of common wheat is large, complex, and heterogeneous, many chromosomal variations are preserved in the species. These chromosomal abnormalities may also alter the plant phenotype. For example, the absence of the end of chromosome 5AL causes reduced plant height [28]. In this study, a deleted chromosomal fragment was found in sterile plants, and the positions of some markers were in opposite orientations in genetic and physical maps (**Figure 1**), indicating that chromosomal inversion might have occurred in the parental material. The upstream position of the deletion was between *TraesCS4B02G015400LC* and marker *MS41*, whereas the downstream position was between *TraesCS4B021300* and *TraesCS4B02G021600.* Using this information and the wheat660K SNP chip data, three CAPS markers were developed and validated on the F_2 pooled DNA and heterozygous F_2 individuals, as well as 12 widely grown modern wheat varieties. These CAPS markers can be used to genotype individuals in segregating populations at an early stage of growth. Since an SNP marker is usually developed from a sequence polymorphism between different cultivars, it is background genotype specific. We are currently exploring other types of molecular markers that might be more suitable for marker-assisted selection of *ms1s*.

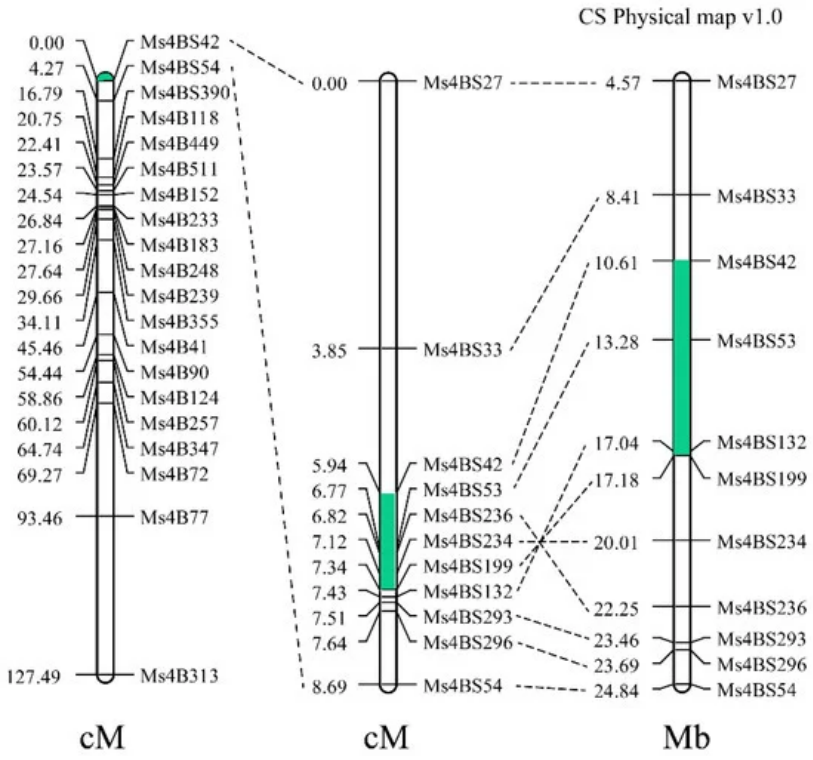

Figure 1. Linkage maps for locus *Ms1*. Green sectors portray the deleted chromosome 4BS segment, and the putative inverted segment is indicated.

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