Development of a set of stem rust susceptible D-genome Disomic substitutions based on Rusty durum

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ABSTRACT

Stem rust (*Puccinia graminis* Pers.:Pers. f.sp. *tritici* Eriks. and Henn.) is one of the most devastating diseases of wheat (*Triticum aestivum* L.) and durum (*T. turgidum* L. ssp. *durum*). Prior to the development of molecular techniques, studies of genes for stem rust resistance genes in wheat were completed using the Chinese Spring (CS) aneuploids. However, few genes were studied in durum because the major set of durum aneuploids, Langdon D-genome disomic substitutions (LDN-DS), had limited use due to the presence of at least three genes for stem rust resistance. Thus, development of a set of stem rust susceptible durum D-genome disomic substitutions would be useful for studies of stem rust resistance in tetraploid wheat. To do this, a breeding process was initiated where the LDN-DS were backcrossed to stem rust susceptible durum line 47-1. In the BC_1 generation, double monosomic plants that were susceptible to three stem rust pathotypes were selected for backcrossing. The stem rust susceptible genotype 'Rusty' became available during this breeding process and backcrossing to 47-1 was discontinued in favour of Rusty. In each cycle, double monosomic plants were selected for backcrossing. After six backcrosses to Rusty were completed for all 14 chromosomes, double monosomic plants were selfed and disomic substitutions were selected and confirmed using molecular markers, endosperm protein markers, and conventional cytogenetic techniques. Twelve Rusty-DS lines have thus far been selected, the exceptions being 5D(5B) and 6D(6B) DS, which are presently under selection**.**

INTRODUCTION

Numerous genetic studies of common wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* L. ssp. *durum*) have been dependent upon aneuploids. This includes assignment of individual genes, markers, and linkage groups to chromosomes based on studies of aneuploids. In tetraploid wheat, a set of 14 Langdon (LDN) D-genome disomic substitution lines (LDN-DS) are the major aneuploid stocks and has been widely used in genetic studies (Joppa and Williams, 1988).

Langdon carries genes for stem rust resistance from Khapli emmer (Ausemus and Heerman, 1959). It is suggested that LDN likely carries *Sr13*, *Sr9e*, and possibly *Sr14* and *Sr7a* based on its parentage (Heyne, 1959), genetic studies (Knott, 1962; Salazar and Joppa, 1981), and gene postulations (Luig, 1983). Thus, the LDN-DS have limited use in studies of stem rust (*Puccinia graminis* Pers.:Pers. f.sp. *tritici* Eriks. and Henn.) due to their resistant reactions to a wide range of stem rust pathotypes. To facilitate stem rust studies in durum wheat, we initiated the breeding and selection of a stem rust susceptible set of D-genome disomic substitutions (DS) using the LDN DS as the chromosome donors and a near-universally susceptible durum genotype, Rusty (Klindworth et al., 2006), as the recurrent parent. So far, six backcrosses to Rusty have been completed for all 14 chromosomes and 12 Rusty-DS lines have been developed. In this paper, we summarized breeding process and updated current status in development of Rusty DS.

MATERIALS AND METHODS

Rusty had not yet been selected when this breeding process was initiated. We began using the stem rust susceptible durum genotype 47-1 as the recurrent parent (Klindworth et al., 2007). In the BC_1 generation, we inoculated seedlings following the procedures described by Klindworth et al. (2007). Chromosome pairing at metaphase I of meiosis (MI) was examined and double-monosomic (13" + 2' at MI) plants that were susceptible to stem rust pathotypes *Pgt-JCMN*, *-TPMK*, and *–LBBL* were selected for backcrossing to 47-1. Double-monosomic plants were selected and crossed to 47-1 in each succeeding backcross cycle.

After replacing 47-1 with Rusty, we completed six backcrosses to Rusty for all 14 chromosomes. In the BC_1 generation of the backcross to Rusty, we tested the lines for a second time for susceptibility to *Pgt-LBBL*. In each backcross cycle, we recorded chromosome pairing configurations and, for double-monosomic plants, seed set. For the final selection of disomic substitutions, we used endosperm protein markers (SDS-PAGE and Acid-PAGE), molecular markers, and morphological markers to identify disomic substitutions and reduce cytological examination of plants. Each selected DS was crossed to the appropriate Langdon double ditelocentric stock; and the presence of three univalents (13" + 1' + t' + t') at MI of the hybrid confirmed that the parental plant was a DS.

RESULTS AND DISCUSSION

In our second trial for stem rust susceptibility, the BC_1 generation of the backcross to Rusty was tested with pathotype *Pgt-LBBL*. There are only a few genes on

which *Pgt-LBBL* is virulent, and we found that all families tested were homozygous susceptible. This indicated that all of the major *Sr* genes from LDN had been eliminated from the breeding populations.

During the breeding process, seed set was recorded on double-monosomic plants for both crossed and open pollinated spikes. For crossed spikes, all crosses were made with Rusty as the male parent. Seed set was generally similar on both crossed and open-pollinated spikes, although seed set was higher on open-pollinated spikes for the 4D(4B) and 6D(6A) populations. This indicated that crosses were not made at the time of optimum female fertility for these two populations. The most notable problem with seed set occurred in the 6D(6B) population. Seed set per spike was only 1.9 seeds per spike on 166 crossed spikes, and 0.6 seeds per spike from 155 open-pollinated spikes. This low seed set made the 6D(6B) line difficult to maintain.

Female transmission of monosomes in double-monosomic plants was generally similar to the transmission rates observed in the development of the LDN-DS (Joppa and Williams, 1988) with only one notable exception. The frequency in which none of the monosomes was transmitted through the female (and hence the frequency of monosomic plants) was 0.566 in the 6D(6A) population; and this compared to a frequency of 0.384 in the corresponding LDN population (Joppa and Williams, 1988). Also of note is that triploid plants have not been observed in the progeny of double-monosomic 7D(7A) plants, as was observed in double-monosomic LDN 7D(7A) (Joppa et al., 1987). However, this result may merely reflect a lower fertility and lower rate of monosomic progeny in the Rusty 7D(7A) as compared to the LDN 7D(7A).

Morphological, protein and molecular markers were used to identify new DS lines. The 2D(2B) DS was selected by screening for nonglaucous spikes. The 1D(1B) DS was identified by SDS-PAGE. Rusty carries high molecular weight (HMW) alleles *Glu-A1c* (null) and *Glu-B1d* (6+8) on chromosomes 1A and 1B, respectively. Chinese Spring carries HMW allele *Glu-D1a* (2+12) on chromosome 1D. Therefore, the 1D(1B) DS line will carry only HMW-GS 2+12 (Fig. 1). In a similar fashion, we used HMW-GS to assist in selecting the 1D(1A) DS, and Acid-PAGE to screen gliadins for selection of the 6D(6A) DS. Molecular markers were also used in selecting some DS lines. For example, the 4D(4B) DS was selected using target region amplification polymorphism (TRAP) markers for primer set W22-T03-T13 (Li et al., 2006).

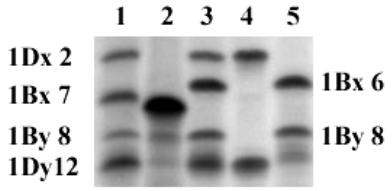

Figure 1. Use of high molecular weight (HMW) glutenin subunits to select Rusty 1D(1B) disomic substitution. Lane 1 = Chinese Spring; Lane 2 = Wells; Lanes 3-5 = banding patterns observed in the BC_6F_2 of LDN1D(1B)/*6 Rusty. Lane 4 has the HMW bands expected of a Rusty 1D(1B) DS.

There are five LDN-DS lines, 4D(4A), 5D(5A), 3D(3B), 5D(5B), and 6D(6B), in which an A- or B- genome monosome must be present in order to maintain the line (Joppa and Williams, 1988). Conventional cytogenetic techniques were more efficient than molecular or protein markers to select these five lines. Chromosome number was counted in root-tips and plants having 29 chromosomes were grown. Two progeny from each selected plant were grown and screened for the plant characteristics that would be expected of a DS line. For example, plants with 28 chromosome should be asynaptic in the 3D(3B) DS line and male-sterile in the 5D(5A) DS (Joppa and Williams, 1988).

LDN is in the parentage of Rusty, and Rusty shares several similarities with LDN (Klindworth et al., 2006). Both are similar in height. Both are late heading, though Rusty is slightly earlier than LDN. Spike morphology of the two is similar. There are nine Rusty-DS lines that have 14 bivalents at MI, and these lines can be maintained by selfing with minimal cytology. Similar to the LDN-DS lines, there are five Rusty-DS lines, 4D(4A), 5D(5A), 3D(3B), 5D(5B), and 6D(6B) where an A- or B-genome monosome must be present to maintain the line. Conventional cytogenetic or molecular marker techniques are necessary to maintain these five lines. A major difference in the two sets of aneuploids is the presence in Rusty of the black glume (*Bg)* gene on chromosome 1A. As a consequence, all Rusty-DS lines have black glumes except for 1D(1A), which has white glumes.

This new set of aneuploids should have several uses. Because they have susceptibility to stem rust, their primary use will be in genetics of stem rust resistance in tetraploid wheat. For assignment of genes to chromosomes, they will be useful for either conventional aneuploid analysis (Klindworth et al, 2007), or for assigning molecular markers to chromosomes. They will also be useful for developing chromosome substitution lines. The Rusty 5D(5B) line should be useful for chromosome engineering. For diseases other than stem rust, if it can be shown that Rusty is more susceptible than LDN to a particular disease/isolate, the Rusty-DS lines may be preferable to the LDN-DS lines for genetics of disease resistance. In general, the LDN-

DS set will be superior to the Rusty-DS set for characters other than disease resistance.

Backcrossing has been completed for all fourteen chromosomes. Rusty-DS lines have been selected and are being increased for all chromosomes except 5D(5B) and 6D(6B). We anticipate that the 5D(5B) line should be available within the next 12-18 months. Because of very low seed set in the 6D(6B) line, it is difficult to predict when this line will be available.

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