



Stripe rust resistance and dough quality of new wheat - *Dasypyrum villosum* translocation lines T1DL•1V#3S and T1DS•1V#3L and the location of HMW-GS genes

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ABSTRACT. The transfer of agronomically useful genes from wild wheat species into cultivated wheat is one of the most effective approaches to improvement of wheat varieties. To evaluate the transfer of genes from *Dasypyrum villosum* into *Triticum aestivum*, wheat quality and disease resistance was evaluated in two new translocation lines, T1DL•1V#3S and T1DS•1V#3L. We examined the levels of stripe rust resistance and dough quality in the two lines, and identified and located the stripe rust resistant genes and high molecular weight glutenin subunit (HMW-GS) genes *Glu-V1* of *D. villosum*. Compared to the Chinese Spring (CS) variety, T1DL•1V#3S plants showed moderate resistance to moderate susceptibility to the stripe rust races CYR33 and Su11-4. However, T1DS•1V#3L plants showed high resistance or

immunity to these stripe rusts. The genes for resistance to stripe rust were located on 1VL of *D. villosum*. In comparison to CS, the dough from T1DS•1V#3L had a significantly shorter developing time (1.45 min) and stable time (1.0 min), a higher weakness in gluten strength (208.5 FU), and a lower farinograph quality index (18). T1DL•1V#3S had a significantly longer developing time (4.2 min) and stable time (5.25 min), a lower weakness in gluten strength (53 FU) and a higher farinograph quality index (78.5). We also found that T1DS•1V#3L had reduced gluten strength and dough quality compared to CS, but T1DL•1V#3S had increased gluten strength and dough quality. The results of SDS-PAGE analysis indicated that *Glu-V1* of *D. villosum* was located on short arm 1VS and long arm 1VL. These results prove that the new translocation lines, T1DS•1V#3L and T1DL•1V#3S, have valuable stripe rust resistance and dough quality traits that will be important for improving wheat quality and resistance in future wheat breeding programs.

Key words: Stripe rust resistance; Dough quality; HMW-GS; Wheat; *Dasypyrum villosum*

INTRODUCTION

The transfer of genes for agronomically useful traits from wild wheat species into the genomes of cultivated varieties is one of the most effective ways for improving wheat quality (Gradzielska, 2006). *Dasypyrum villosum* (syn. *Haynaldia villosum*) is an allogamous annual diploid grass that has been found to carry many important agronomic traits, especially those for high resistance to wheat diseases, such as rust (Hyde, 1953; Chen et al., 2002), powdery mildew (Qi et al., 1993; Chen et al., 1995; Li et al., 2005), spindle streak mosaic viruses (Zhang et al., 2005), wheat curl mite (Chen et al., 2002; Li et al., 2002), and eyespot. Genes for high salt and drought tolerance (Qualset et al., 1981; Blanco et al., 1983; Zhong and Dvorak, 1995), high efficiency of zinc use (Schlegel et al., 1998), and increased grain protein content have also been identified. *D. villosum*, therefore, offers a valuable gene resource for wheat resistance and quality improvement.

D. villosum is also regarded as an important genetic resource for wheat seed storage protein improvement (Shewry et al., 1987; Blanco et al., 1991) because its grain protein content can reach 26% due to the action of alleles of high-molecular weight glutenin subunit (HMW-GS) (Zhong and Qualset, 1993). The loci for HMW-GS (*Glu-V1*), which is orthologous to *Glu-A1*, *Glu-B1* and *Glu-D1* (Montebove et al., 1987; Blanco et al., 1991), for sulfur-poor (ω -type) and sulfur-rich (γ -type) monomeric prolamins (*Gli-V1*), which are orthologous to *Gli-1*, and low molecular weight polymeric prolamins (*Glu-V3*) of wheat (Shewry et al., 1987; Blanco et al., 1991) are located on chromosome 1V of *D. villosum*. The *Glu-V1* and *Gli-V1/Glu-V3* alleles on chromosome 1V of *D. villosum* have been found to have large positive effects on quality in wheat (De Pace et al., 2001).

Genes for resistance to rust diseases are also located on the 1V chromosome of *D. villosum* (De Pace et al., 2001). However, these genes have not yet been fine mapped. In order to be able to exploit the genetic resources of *D. villosum*, many chromosome addition lines, sub-

stitution lines and translocation lines have been constructed for use in the transfer of beneficial genes into cultivated wheat (Chen et al., 1995, 2002; Zhang et al., 2005; Gradzielewska, 2006).

Zhao et al. (2010) developed two new compensating Robertsonian translocation lines, T1DL•1V#3S and T1DS•1V#3L. The T1DL•1V#3S line significantly increased the Zeleny sedimentation value, while the T1DS•1V#3L line significantly decreased the Zeleny sedimentation value compared to Chinese Spring (CS). In this study, the resistance to stripe rust and the farinograph characteristics for dough quality of the CS cultivar were compared with those of the T1DL•1V#3S and T1DS•1V#3L lines; the HMW-GS genes *Glu-V1* of *D. villosum* were also identified and located.

MATERIAL AND METHODS

Plant materials

The following plant resources were used in this study: wheat variety Chinese Spring (CS), *D. villosum* accession No. TA10220, CS-*D. villosum* translocation lines T1DL•1V#3S and T1DS•1V#3L developed by Zhao et al. (2010), six CS-*D. villosum* 1V, 2V, 4V-7V disomic addition lines, DA1V, DA2V, DA4V, DA5V, DA6V, DA7V, and the 3V monosomic addition line MA3V.

Resistance to stripe rust

The levels of adult plant resistance to stripe rust were investigated in CS and the CS-*D. villosum* translocation lines T1DL•1V#3S and T1DS•1V#3L grown in the field at Yangling, Shaanxi Province, China, during 2008-2011 (three cropping seasons) by the College of Plant Protection, Northwest A&F University. Each genotype was grown in 2-4 rows at 25 cm spacing, 1 m long, with three replicates. The epidemic stripe rust races CYR33 and Su11-4 (the main stripe rust races affecting local wheat production) were separately used for artificial inoculation of rust at the jointing stage. The adult plant responses to stripe rust were recorded on a 0-4 scale at anthesis according to the Roelfs and Martens (1988) method with some modification. Infection type 0 indicated immunity, 1 was high resistance, 2 was moderate resistance, 3 was moderate susceptibility, and 4 was high susceptibility.

Evaluation of dough quality

To analyze wheat dough quality in the translocation lines, seeds of CS, and the homozygous T1DL•1V#3S and T1DS•1V#3L translocation lines were planted in 2008-2010 (two cropping seasons) in a randomized complete block design, with three replicates, in the field at Yangling, Shaanxi Province, China. Each plot had 4 (2008-2009) or 8 (2009-2010) rows with 2m length and 0.25-m spacing between rows and 0.05-m spacing between plants within rows. A fertilizer mixture of 300 kg N and 240 kg P₂O₅ per hectare was applied at the sowing stage. Field management followed local practices. The seeds from each plot were harvested and used for gluten property analysis. The farinograph characteristics of the gluten were performed according to the AACC (1995) method (Li et al., 2007). Variance analyses were performed on the original farinograph data with the DPS TMv11.50 software designed by Hangzhou Refine Information Tech. Co. Ltd. of Zhejiang University (<http://www.chinadps.net>).

HMW-GS analysis

The HMW-GS of CS, TA10220, T1DL•1V#3S, T1DS•1V#3L, DA1V, DA2V, MA3V, DA4V, DA5V, DA6V and DA7V was extracted from mature seeds, and separated by SDS-PAGE as described by Montebve et al. (1987). HMW-GS was identified and analyzed according to the previously published methods for wheat species and subspecies (Payne and Lawrence, 1983).

RESULTS

Resistance to stripe rusts

The responses to stripe rust inoculation in CS, T1DL•1V#3S and T1DS•1V#3L for three years are shown in Table 1. Both CS and T1DL•1V#3S showed infection type 2 or 3 for the two races of rust in the three year trial. These plant varieties therefore showed moderate resistance or moderate susceptibility to CYR33 and Su11-4 stripe rust races. By contrast, the T1DS•1V#3L translocation line had an infection type of 0 for CYR33 race and 0-1 for Su11-4 race, indicating that this line was either immune or had high resistance to CYR33 and Su11-4.

Table 1. Resistance to stripe rust (CRY33, Su11-4) of adult CS, T1DL•1V#3S and T1DS•1V#3L plants in 2008-2011.

Genotype	CYR33			Su11-4		
	2008-2009	2009-2010	2010-2011	2008-2009	2009-2010	2010-2011
CS	2-20-100 ^a	2-5-10	2-1-10	2-10-100	2-5-100	3-20-100
T1DL•1V#3S	3-40-100	2-10-100	2-40-20	3-10-100	2-20-100	3-20-100
T1DS•1V#3L	0	0	0	0	0-10-10	1-10-20

^aStripe rust resistance level was scored according to infection type (first number), severity level (second number), and proportion (%) of infected plants (third number).

Dough quality

The dough qualities were assessed for developing time, stable time, and weakness in gluten strength, and the farinograph quality index was also determined. These various dough characteristics showed significant differences among the three genotypes in two cropping seasons. For translocation line T1DS•1V#3L, the mean developing time (1.45 min) and stable time (1.0 min) were significantly shorter than those of CS (3.45 and 3.25 min respectively). The mean gluten weakness (208.5 FU) was significantly higher in T1DS•1V#3L than CS (117 FU), and farinograph quality index (18) was significantly lower than CS (51.5). However, compared with CS, the developing time (4.2 min), stable time (5.25 min) and farinograph quality index (78.5) were significantly higher and weakness in gluten strength (53 FU) was significantly lower in T1DL•1V#3S (Table 2). These results showed that the translocation line T1DS•1V#3L had a reduced gluten strength and grain quality, whereas the translocation line T1DL•1V#3S increased gluten strength and enhanced quality.

Table 2. Dough quality characteristics of CS, T1DL•1V#3S and T1DS•1V#3L lines in 2008-2010.

Genotype	Developing time (min)	Stability (min)	Weakness (FU)	Farinograph quality index
T1DL•1VS	4.20 [†]	5.25 ^a	53 ^c	78.5 ^a
CS	3.45 ^b	3.25 ^b	117 ^b	51.5 ^b
T1DS•1VL	1.45 ^c	1.0 ^c	208.5 ^a	18 ^c

[†]Within columns, means followed by the same letter were not significantly different according to least-significant difference ($P < 0.05$).

Localization of HMW-GS

On SDS-PAGE, the HMW-GS of DA2V, MA3V, DA4V, DA5V, DA6V and DA7V showed four bands (2, 7, 8 and 12) identical to CS. Bands 2, 7, 8 and 12 were also identified from T1DL•1V#3S and DA1V. One HMW-GS band of *Glu-V1* from *D. villosum* accession No. TA10220 was also found in the latter two lines and located between bands 7 and 8 of CS. The HMW-GS of translocation line T1DS•1V#3L showed bands 7, *Glu-V1* and 8. From these results, the HMW-GS gene *Glu-V1* of *D. villosum* was located on both arms 1VS and 1VL, and the expression level of *Glu-V1* on T1DL•1VS was higher than in the T1DS•1VL line (Figure 1).

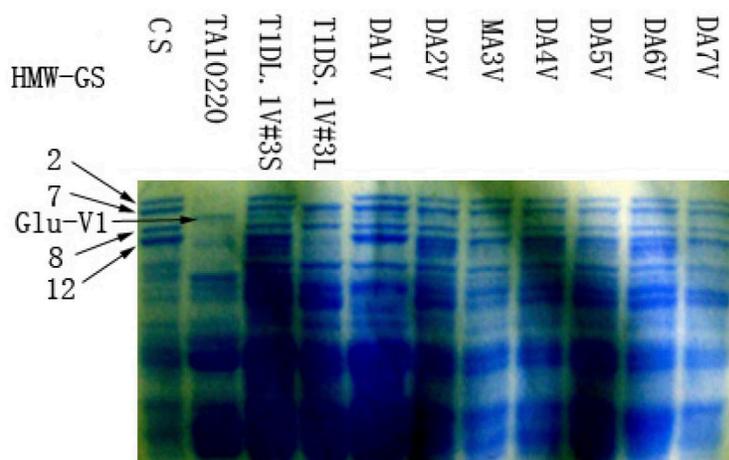


Figure 1. SDS-PAGE of total seed proteins from CS, *Dasyphyrum villosum* accessions TA10220, CS-*D. villosum* translocation lines T1DL•1V#3S, T1DS•1V#3L, and CS-*D. villosum* addition lines 1V-7V. CS = Chinese spring; HMW-GS = high molecular weight glutenin subunit.

DISCUSSION

De Pace et al. (2001) located genes for resistance to wheat rust on chromosome 1V. In the present study, we found that the levels of resistance to CYR33 and Su11-4 races of wheat stripe rust were significantly different in the two translocation lines compared to CS. The translocation line T1DL•1V#3S had moderate resistance to moderate susceptibility to the two stripe rust races, while the severity of infection was greater than in CS. However, the T1DS•1V#3L translocation line was immune to CYR33 and highly resistant or immune to

Su11-4, and the severities of infection were lower than in CS. These results indicate that the stripe rust resistance genes might locate on chromosome 1VL.

The large positive effects on dough quality in wheat chromosome addition lines with *D. villosum* chromosome 1V might be due to the HMW-GS alleles *Glu-V1* and *Gli-V1/Glu-V3* (De Pace et al., 2001). In this study, we found that, compared to CS, the dough quality of T1DS•1V#3L showed a significantly shorter developing time and stable time, a higher weakness in gluten strength and a lower farinograph quality index. However, the translocation line T1DL•1V#3S had a significantly longer developing time and stable time, lower weakness in gluten strength and higher farinograph quality index than CS. The results indicated that T1DS•1V#3L decreased gluten strength with no enhancement to grain quality; in contrast, T1DL•1V#3S increased gluten strength and enhanced quality. We found that there were significant differences in the gluten quality between T1DS•1V#3L and T1DL•1V#3S. These conclusions are in agreement with the results predicted by Zhao et al. (2010).

The composition and relative content of HMW-GS, which are closely related to the quality of the wheat dough, are influenced by genes located on the long arm of homologous group 1 chromosomes (Payne et al., 1987). Halford et al. (1992) reported that the characteristics of wheat gluten could be improved by increasing the amount of HMW-GS. The SDS-PAGE separation of the HMW-GS of T1DS•1V#3L and T1DL•1V#3S showed that the former had five HMW-GS bands 2, 7, *Glu-V1*, 8, and 12, whereas the latter had three bands, 7, *Glu-V1*, and 8. The reason for this difference may be that the T1DS•1V#3L translocation line has lost 1DL, in which bands 2 and 12 are located (Payne et al., 1980). The results proved that the *Glu-V1* genes were located on chromosomes 1VS and 1VL, but the expression of *Glu-V1* in the T1DL•1VS line was stronger than in the T1DS•1VL line. Therefore, the difference in HMW-GS composition and content of the dough of T1DS•1V#3L and T1DL•1V#3S lines was because of the differences in dough quality. Additionally, dough quality was also influenced by the *Gli-V1* and *Glu-V3* genes located on 1VS (Zhang, 2010).

The dough quality of T1DS•1V#3L was significantly better than that of T1DS•1V#3L. This observation provides important information for improving wheat quality through hybrid breeding in the future. The results of this study proved that the new translocation lines, T1DS•1V#3L and T1DS•1V#3L, possess genes with value for stripe rust resistance and dough quality. These genes have been shown to be presented in *D. villosum*, which will be useful in future attempts to improve wheat quality and resistance and for the analyses of gene functions in *Triticum*.

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