Published March 15, 2018

RESEARCH

Dn10, a New Gene Conferring Resistance to Russian Wheat Aphid Biotype 2 in Iranian Wheat Landrace PI 682675

Genqiao Li, Xiangyang Xu,* Brett F. Carver, Peiguo Guo, and Gary Puterka

ABSTRACT

Russian wheat aphid [RWA, *Diuraphis noxia* (Mordvilko)] is a serious pest of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) that causes heavy yield losses in many countries, and RWA biotype 2 (RWA2) is virulent to most RWA resistance genes. The objective of this study was to characterize a gene for resistance to RWA2 in Iranian landrace PI 682675, a single-plant selection from PI 624151. $F_{2:3}$ and $F_{3:4}$ families derived from cross PI 682675 × Zhengyou 6 were used to map the resistance gene. PI 682675 carries a dominant resistance gene, *Dn10*, flanked by simple sequence repeat markers *Xgwm437* and *Xwmc488* on chromosome 7DL. Physical mapping indicated that *Dn10* resides in bin 7DL 0.1 to 0.77, whereas *Dn2401* and *Dn626580* that also confer resistance to RWA2 were physically mapped to 7DS. Allelism tests showed that *Dn10* was located ~17.4 cM from *Dn2401* and 22.2 cM from *Dn626580*. *Dn10* is a new resistance gene that should be used widely in wheat breeding.

G. Li, X. Xu, and G. Puterka, Wheat, Peanut and Other Field Crops Research Unit, USDA-ARS, Stillwater, OK 74075; B.F. Carver, Plant and Soil Sciences Dep., Oklahoma State Univ., Stillwater, OK 74078; P. Guo, International Crop Research Center for Stress Resistance, College of Life Sciences, Guangzhou Univ., Guangzhou 510006, China. Received 31 Oct. 2017. Accepted 18 Dec. 2017. *Corresponding author (xiangyang.xu@ars.usda.gov). Assigned to Associate Editor Eric Olson.

Abbreviations: PCR, polymerase chain reaction; RWA, Russian wheat aphid; SSR, simple sequence repeat.

Russian wheat aphid [RWA, *Diuraphis noxia* (Mordvilko)], a serious pest of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), is indigenous to southern Russia, Iran, Afghanistan, and countries around the Mediterranean Sea (Hewitt et al., 1984). The recent occurrence of RWA in Australia suggests that RWA has spread to all continents (Australia Government Grains Research and Development Corporation, 2017). Heavy yield losses caused by RWA, ranging from 21 to 92% in South Africa (Du Toit and Walters, 1984) and from 25 to 60% in Turkey (Elmali, 1998), were previously reported. In the United States, RWA was first detected in Texas in 1986 and rapidly became a major pest of wheat and barley, causing over US$1 billion in damage and control costs in western regions from 1986 to 1997 (McIntosh et al., 1998).

Host plant resistance is preferred in managing RWA in wheat. Sixteen RWA resistance genes, including *Dn1* to *Dn9* plus *Dnx*, *Dny*, *Dn2401*, *Dn2414*, *Dn1818*, *Dn626580*, and *Dn100695*, have been reported and located in different regions of the wheat genome. Of these, *Dn1*, *Dn2*, *Dn5*, *Dn6*, and *Dnx* were mapped to the proximal region of chromosome 7DS (Liu et al., 2001, 2002, 2005; Tonk et al., 2016), and allelism tests indicated that these genes are either the same or different alleles at the same locus (Saidi and Quick, 1996; Liu et al., 2005). More recently,

Published in Crop Sci. 58:1219–1225 (2018).
doi: 10.2135/cropsci2017.10.0649

Dn100695, which confers resistance to a RWA population in Turkey, was also mapped in the proximal region of 7DS (Tonk et al., 2016). In addition, *Dn8* and *Dn9* were identified in PI 264994, in which *Dn5* was identified. *Dn8* was mapped to the distal region of 7DS, and *Dn9* was located on 1DL (Liu et al., 2001). Of the known RWA resistance genes, *Dn4* on chromosome 1DS in PI 372129, and *Dnγ*, originating from Afghanistan landrace PI 220350, were successfully deployed in commercial cultivars to reduce yield losses caused by RWA (Haley et al., 2004b; Quick et al., 1996, 2001a, 2001b, 2001c, 2001d).

Wild species are also important sources of RWA resistance. Of the known RWA resistance genes, *Dn7*, identified in rye cultivar Turkey 77 and transferred to a 1BL/1RS translocation segment in bread wheat line 93M370 (Marais et al., 1994), exhibited the highest level of resistance to all known RWA biotypes (Puterka et al., 2006; Mornhinweg 2012). The RWA resistance gene in STARS 02RWA2414-11, *Dn2414*, was also mapped to a 1RS segment. *Dn2414* and *Dn7* are either identical or different alleles at the same locus (Peng et al., 2007). Another RWA resistance gene, *Dn1881*, was identified in *T. turgidum* L. line 1881 and was mapped to chromosome 7BS (Navabi et al., 2004). In addition, *Dn3* was identified in *Aegilops tauschii* Coss. line SQ24 (Nkongolo et al., 1991), but this recessive gene has not been mapped.

The occurrence of RWA biotype 2 (RWA2) in Colorado in 2003 rendered all available RWA resistance genes except *Dn7* ineffective, including cultivars in commercial production with *Dn4* and *Dnγ* (Haley et al., 2004a). Later, another six RWA biotypes (RWA3–8) were identified in the United States (Burd et al., 2006; Weiland et al., 2008). Puterka et al. (2014) compared virulence profiles of these biotypes and found that RWA3, 4, 5, and 7 were similar. Thus they consolidated US RWA populations into five biotypes: RWA1, 2, 3/7, 6, and 8. Of these, RWA2 is the predominant and most virulent biotype in the United States (Puterka et al., 2006, 2014). Similarly, two new RWA biotypes, RWASA2 and RWASA3, were identified in South Africa. Both biotypes are virulent to *Dn1*, *Dn2*, *Dn3*, and *Dn9*, and RWASA3 is also virulent to *Dn4* and *Dnγ* (Tolmay et al., 2013).

Although *Dn7* offers high resistance to all RWA biotypes in the United States, linkage drag caused by the *Sec1* gene in the 1RS chromosomal segment makes it difficult to commercially deploy *Dn7*, as *Sec1* encodes monomeric secalins that cause sticky dough and reduce dough strength, leading to undesirable bread-making quality (Martin and Stewart 1990; Graybosch et al., 1993). Thus identification and utilization of RWA2 genes void of alien chromosomal segments are of great importance. Collins et al. (2005) evaluated responses of a set of 781 wheat accessions to RWA2 infestation and identified some resistance sources. Among identified accessions, CI 2401 carries resistance genes *Dn2401* on chromosome 7D and *Dn4* on 1DS (Liu et al., 2002). *Dn4* and *Dn2401* confer resistance to RWA1 and RWA2, respectively (Fazel-Najafabadi et al., 2015). In addition, Valdez et al. (2012) identified RWA2 resistance gene *Dn626580* in PI 626580 and mapped it to chromosome 7D.

More recently, Xu et al. (2015) screened 1149 wheat accessions for resistance to RWA2, and PI 682675 (formerly PI 624151-1-2), a selection from an Iranian landrace, exhibited high resistance. Phenotypic discrepancy between PI 682675 and another three RWA2 resistance sources—Yumar (with *Dn7*), CI 2401, and PI 626580—was observed (Puterka, unpublished data, 2017), suggesting that PI 682675 may carry a new RWA2 resistance gene. The objective of this study was to determine the chromosomal location of the RWA2 resistance gene in PI 682675.

MATERIALS AND METHODS

Plant Materials

PI 682675 (formerly PI 624151-1-2) is a single-plant selection from Iranian wheat landrace PI 624151 (Xu et al., 2015). A total of 224 F_2 plants derived from cross PI 682675 × Zhengyou 6 were used in this study. Zhengyou 6 is an elite wheat cultivar grown in the Huang-Huai Facultative Winter Wheat Zone of China. All 224 F_2 plants were selfed to produce 224 $F_{2:3}$ families for genetic analysis. To confirm the genetic mapping results from $F_{2:3}$ data and validate gene location, a set of 124 $F_{3:4}$ families were developed by selfing an F_3 plant from each of 124 randomly chosen F_3 families.

Two additional F_2 populations derived from PI 682675 × CI 2401 (hereafter the CI 2401 population) and PI 682675 × PI 626580-1 (hereafter the PI 626580 population), consisting of 1577 and 460 plants, respectively, were used for allelism tests. PI 626580-1 is a RWA2-resistant single plant selection from the Iranian landrace PI 626580. PI 682675, CI 2401, and PI 626580-1 carry *Dn10*, *Dn2401*, and *Dn626580*, respectively.

'Chinese Spring' nullisomic-tetrasomic lines N7AT7B, N7BT7A, and N7DT7A, as well as seven Chinese Spring chromosome 7D deletion lines, were used to physically locate the resistance gene in PI 682675. The aneuploidy stocks were provided by the Wheat Genetics Resource Center at Kansas State University.

Evaluation of RWA2 Resistance

The 224 $F_{2:3}$ families, parental lines, and 21 F_1 plants were evaluated for response to RWA2 infestation at the USDA-ARS Wheat, Peanut, and Other Field Crop Research Unit in 2016. A randomized complete block design with two replicates was used.

A previously described evaluation protocol was used in this study (Xu et al., 2015). Briefly, 12 seeds of each $F_{2:3}$ family were planted in three cells (4 seeds cell^{-1}) of 73-cell growing trays (Growing Systems) in each of two replicates. The susceptible check, 'Yuma', was grown in two cells, and a resistant check, 'Yumar' with *Dn7*, in one cell in each tray. Aphids were increased on barley (Xu et al., 2015), and tested seedlings were infested 7 d after planting by placing heavily infested barley

leaves close to each row of test plants. Test plants were evaluated at 3 wk after infestation using a 1-to 6 scale representing highly resistant, resistant, moderately resistant, moderately susceptible, susceptible, and highly susceptible responses, respectively (Xu et al., 2015). An additional confirmation test was performed to reevaluate those F_3 families showing discrepancies between replicates. F_2 genotypes were inferred from F_3 phenotypic data.

The same experimental design and protocol were used to evaluate RWA2 resistance of 124 $F_{3:4}$ families, as well as the CI 2401 and PI 626580 allelism test populations, in 2017.

DNA Isolation and Marker Analysis

Leaf tissues were collected from each of the 224 F_2 plants, as well as the 124 F_3 plants, and stored at −20°C. Genomic DNA was extracted using a protocol described by Dubcovsky et al. (1994).

Equal amounts of DNA from each of 10 F_2 homozygous resistant and 10 homozygous susceptible plants were pooled to build resistant and susceptible bulks, respectively. DNA from bulks and parental lines was used to screen preselected simple sequence repeat (SSR) markers evenly distributed across the wheat genome for polymorphism. Polymerase chain reaction (PCR) was performed in 2720 thermal cyclers (Applied Biosystems) in volumes of 10 μL containing ∼50 ng of genome DNA, 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleotide, 1× PCR buffer, and 0.25 U *Taq* DNA polymerase. The PCR was performed at 95°C for 5 min, followed by 39 cycles of 95°C for 30 s, 50 to 60°C (depending on the primers' annealing temperatures) for 30 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. The PCR products were separated in 6 to 10% nondenaturing polyacrylamide gels with a 29:1 acrylamide/bisacrylamide ratio and visualized with ethidium bromide. For PCR products that were not satisfactorily separated in nondenaturing polyacrylamide gels, a tailed PCR primer was synthesized by adding a 19-base M13 oligo sequence (M13 tail) to the 5′ end of each forward SSR primer and was used in PCR amplification. In such cases, three primers were used for PCR amplification, including the forward primer having an attached M13 sequence tail, the reverse primer, and an infrared fluorescence dye-labeled M13 primer with the same sequence as the tail sequence attached to the forward primer. The PCR products were separated using 6.5% KB plus polyacrylamide gel solution on a LI-COR 4300 DNA Analyzer (LI-COR Biosciences), using a protocol described by Xu et al. (2005).

The SSR markers polymorphic between two bulks were used to genotype the F_2 population, leading to identification of SSR markers associated with RWA2 resistance. Given the genomic locations of these markers, additional SSR markers previously mapped to the target region were selected to genotype the F_2 mapping population. The SSR markers flanking the RWA2 resistance gene were also used to genotype 124 F_3 plants in a subsequent confirmation experiment.

Linkage Analysis and Genetic Mapping

Chi-squared (χ^2) tests were performed to test the null hypotheses that PI 682675 carries a dominant RWA2 resistance gene. Mapmaker 3.0b (Lincoln et al., 1993) was used to map the RWA2 resistance gene using the Kosambi function (Kosambi 1943), and a logarithm of the odds score of 3.0 was set as the threshold to declare linkage. The linkage map was drawn using MapDraw (Liu and Meng, 2003).

We used molecular markers flanking the gene to genotype three Chinese Spring nullisomic-tetrasomic lines, N7AT7B, N7BT7A, and N7DT7A, as well as a set of seven chromosome 7D deletion lines, to determine the physical location of the RWA2 resistance gene in PI 682675. We also selected molecular markers flanking *Dn2401* and *Dn625810* from previous studies to genotype these stocks to physically map them to chromosome 7D and compare their locations with the resistance gene in PI 682675.

RESULTS

Inheritance of RWA Resistance in PI 682675

All PI 682675 × Zhengyou 6 F1 plants were resistant, and the $F_{2:3}$ families were genotyped as 56 homogeneous resistant, 114 segregating, and 54 homozygous susceptible, fitting an expected 1:2:1 ratio for single-gene inheritance ($\chi^2 = 0.05$, $P_{1:2:1} = 0.98$).

Linkage Analysis and Genetic Map

We intended to screen a large set of SSRs for informative markers showing polymorphism between the resistant and susceptible bulks. Two polymorphic SSR markers, *Xcfd14* and *Xwmc702*, were identified on chromosome 7D. Another 37 SSR markers previously mapped in the genomic region of *Xcfd14* and *Xwmc702* (Somers et al., 2004; USDA-ARS, 2018) were then assayed. Nine of these showed polymorphism between two parents and were used to genotype the entire F_2 population. Four SSR markers—*Xgwm437*, *Xbarc214*, *Xpsp3113*, and *Xwmc488*—were mapped to the target region in chromosome 7D, whereas the other five SSR markers— *Xgwm473*, *Xwmc438*, *Xwmc473*, *Xgwm121*, and *Xgwm111*—showed no linkage with this group. Linkage analysis assigned the resistance gene, designated *Dn10*, to the interval between *Xgwm437* and *Xwmc488*. *Dn10* was 29.1 cM proximal to *Xwmc488* and 9.0 cM distal to *Xgwm437* (Fig. 1a).

To verify the results, we randomly selected single F_3 plants from each of 124 F_3 families, among which 32, 35, and 57 families were scored as homozygous resistant, homozygous susceptible, and segregating, respectively, to derive F_4 lines. Linkage analysis based on the $F_{3:4}$ data also assigned the *Dn10* gene to the genomic region flanked by *Xgwm437* and *Xwmc488*, with genetic distances of 11.3 cM to *Xgwm437*, and 35 cM to *Xwmc488* (Fig. 1b).

Physical Bin Mapping of *Dn10*, *Dn2401*, and *Dn626580*

To determine the physical location of *Dn10*, we used flanking markers *Xgwm437* and *Xwmc488* to genotype the parental lines, Chinese Spring, three nullisomic-tetrasomic lines, and seven chromosome 7D deletion lines (Fig. 2). As expected, the target bands were amplified from N7AT7B and N7BT7D, but not from N7DT7A, confirming that

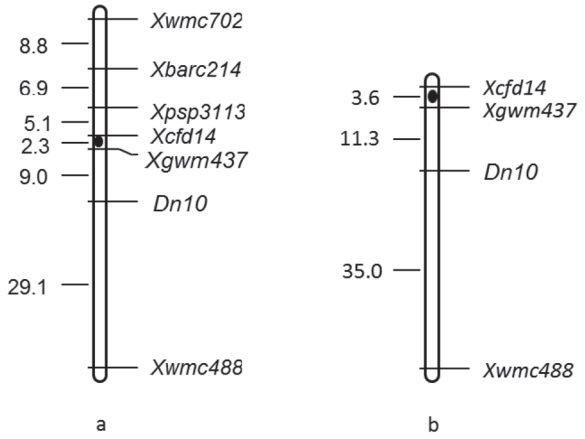

Fig. 1. Linkage maps of wheat chromosome 7D containing *Dn10* constructed using (a) $F_{2:3}$ data and (b) $F_{3:4}$ data.

these markers are located on chromosome 7D. *Xgwm437* amplified the target band from 7DL-5, 7DL-2, 7DL-8, and 7DL-3, but not from 7DL-6. Given that the breakpoint fraction lengths of 7DL-6, 7DL-5, 7DL-2, 7DL-8, and 7DL-3 were 0.1, 0.3, 0.61, 0.7, and 0.82, respectively, *Xgwm437* was located in bin 7DL 0.1 to 0.3. Similarly, *Xwmc488* was located in bin 7DL 0.61 to 0.77. Therefore, *Dn10* resides in the composite bin 7DL 0.1 to 0.77.

Resistance gene *Dn2401* was previously mapped to an interval flanked by *Xbarc214* and *Xcfd14* on chromosome 7D (Fazel-Najafabadi et al., 2015). As shown in Fig. 2, both *Xbarc214* and *Xcfd14* amplified the target bands from 7DS-4, and not from 7DS-1. The breakpoint fraction length values of 7DS-4 and 7DS-1 are 0.61 and 0.37, respectively. Thus, we assigned *Xbarc214* and *Xcfd14*, and presumably *Dn2401*, to bin 7DS 0.37 to 0.61. *Dn626580* was 1.8 cM distal to *Xbarc214* on chromosome 7DS in a previous study (Valdez et al., 2012). Given that *Xbarc214* resides in bin 7DS 0.37 to 0.61, *Dn626580* is likely present in the same or a more distal bin. Further data are needed to precisely locate *Dn626580*. In addition, *Xgwm111*, which is tightly linked to *Dn1*, *Dn2*, *Dn5*, *Dn6*, and *DnX*, was assigned to bin 7DS 0.61 to 1.0 (Fig. 2).

Allelism Tests

Using the CI 2401 and PI 626580 allelism test (F_2) populations, we determined allelic relationships of *Dn10* and the RWA2-effective resistance genes *Dn2401* and *Dn626580* on 7D. We identified 22 and 10 susceptible or highly susceptible F_2 plants in the CI 2401 and PI 626580 populations, respectively. There were 1577 plants in the CI 2401 population, and 460 plants in the PI 626580 population. Thus, the *Dn10* locus is different from both *Dn2401* and *Dn626580*, and the estimated genetic distances were 17.4 cM between *Dn10* and *Dn2401,* and 22.2 cM between *Dn10* and *Dn626580* (Fig. 3b). The inferred genetic distance between

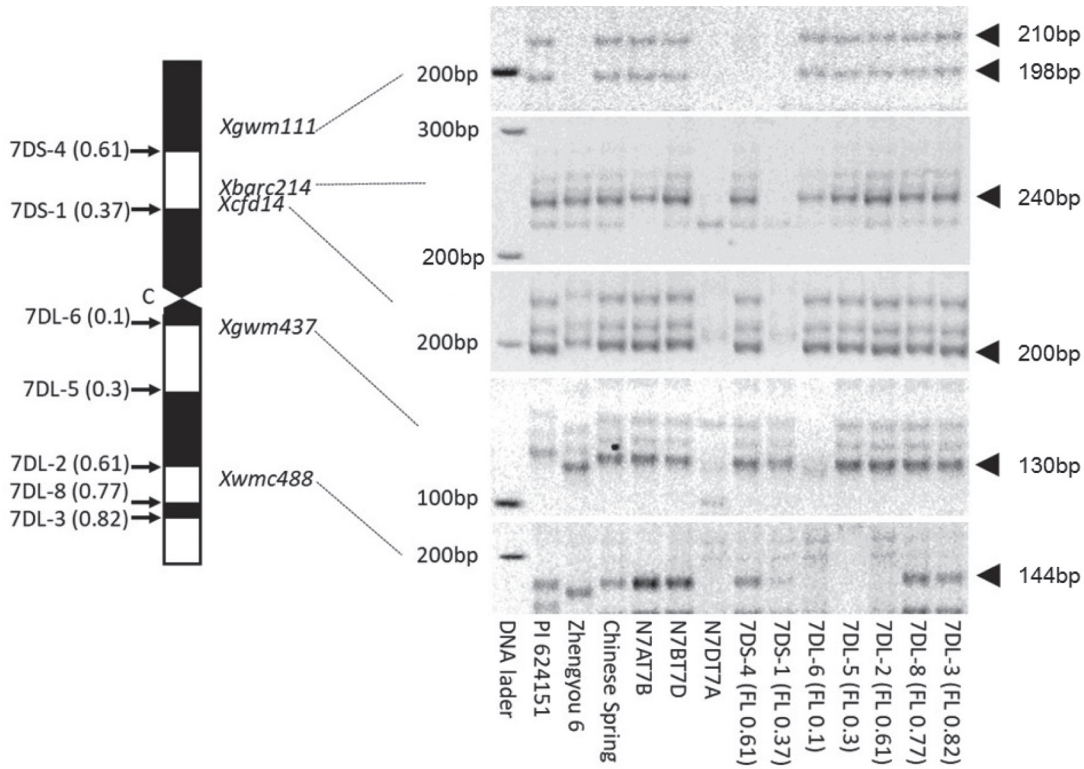

Fig. 2. Genomic locations of Russian wheat aphid resistance genes determined by genotyping three Chinese Spring nullisomic-tetrasomic lines and seven deletion lines with simple sequence repeat markers associated with each gene, including *Xgwm111* (*Dn1*, *Dn2*, *Dn5*, *Dn6*, and *DnX*), *Xbarc214* (*Dn2401* and *Dn626580*), *Xcfd14* (*Dn2401*), *Xgwm437* (*Dn10*), and *Xwmc488* (*Dn10*). The two parental lines used in this study and Chinese Spring were also included in the genotyping panel. The breakpoint fraction length value of each deletion line is given in parentheses, and the target bands are indicated by arrows.

Dn626580 and *Dn2401* was 4.8 cM (Fig. 3b), although the relationship should be confirmed by a designed allelism test between *Dn2401* and *Dn626580*.

DISCUSSION

Dn10 is a New RWA2 Resistance Gene

Wheat chromosome 7D harbors most known RWA resistance genes, including *Dn1*, *Dn2*, *Dn5*, *Dn6*, *Dn8*, *Dn10*, *Dnx*, *Dn626580*, *Dn2401*, *Dn100695*, and *Dn10* (Ma et al., 1998; Liu et al., 2001, 2002; Valdez et al., 2012; Fazel-Najafabadi et al., 2015; Tonk et al., 2016). Apart from *Dn10*, all others were mapped to 7DS. Although *Dn2* and *Dn5* were initially mapped to 7DL (Du Toit, 1987; Marais and Du Toit 1993; Du Toit et al., 1995; Ma et al., 1998), a later study assigned them to 7DS (Liu et al., 2001). *Dn8* was mapped to the distal region of 7DS, whereas *Dn1*, *Dn2*, *Dn5*, *Dn6*, and *DnX* were tightly linked to SSR marker *Xgwm111* in the proximal region, with genetic distances ranging from 1.52 to 3.85 cM. Further allelism tests suggested that *Dn1*, *Dn2*, *Dn5*, *Dn6*, and *Dnx* are either allelic or tightly linked to each other (Marais and Du Toit, 1993; Saidi and Quick, 1996; Liu et al., 2005).

RWA2 is virulent to *Dn1* through *Dn6* plus *Dn8*, *Dn9*, and *Dny*, whereas *Dn7*, *Dn2401*, *Dn626580*, and *Dn10* confer resistance to RWA2. As mentioned above, linkage drag associated with *Dn7* makes it less attractive for use in wheat breeding (Xu et al., 2015). On the contrary, *Dn2401*, *Dn626580*, and *Dn10* can be preferentially used to improve resistance to RWA2. In this study, we mapped *Dn2401* and *Dn626580* to 7DS and *Dn10* to 7DL using deletion mapping. Allelism tests suggested that *Dn10* was ~17.4 and 22.2 cM from *Dn2401* and *Dn626580*, respectively. *Dn10* is a new RWA2 resistance gene.

Xbarc214 was 1.8 cM proximal to *Dn626580* and 1.1 cM distal to *Dn2401* in previous studies (Valdez et al., 2012; Fazel-Najafabadi et al., 2015). Thus, the inferred genetic distance between *Dn626580* and *Dn2401* is 2.9 cM, which is similar to the inferred genetic distance based on allelism tests in this study. An additional allelism test is needed to determine whether *Dn626580* and *Dn2401* are different genes. In addition, *Dn100695* was also identified in 7DS. *Dn100659* confers resistance to Turkish RWA biotype 2. More studies are needed to evaluate this gene in response to US RWA biotypes, and to determine its relationship with other RWA resistance genes on 7DS.

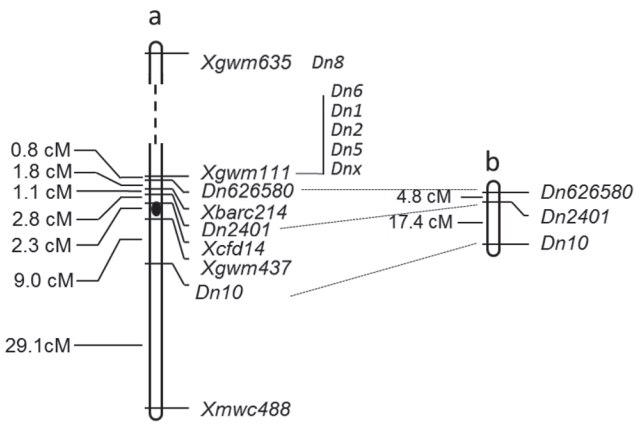

Fig. 3. A comparative map of chromosome 7D containing *Dn1*, *Dn2*, *Dn5*, *Dn6*, *Dn8*, *DnX*, *Dn626580*, *Dn2401*, and *Dn10* based on (a) literature (Liu et al. 2001, 2002; Valdez et al. 2012; Fazel-Najafabadi et al. 2015) and mapping results of this study, and (b) a map inferred from allelism tests.

Development of RWA-Resistant Wheat Cultivars

Russian wheat aphid in the United States is an invasive pest derived from a limited founder population that diverged genetically after introduction (Shufran et al., 2007; Shufran and Payton, 2009; Liu et al., 2010; Swanevelder et al., 2010). Puterka et al. (1992) collected RWA populations from Eurasia, South Africa, and the United States, found considerable differences in their virulence to a set of differential lines, and thus classified them as biotypes. United States RWA populations appeared biotypically uniform from 1986 through the late 1990s (Shufran et al., 1997). In 2003, a new biotype, detected in Colorado and designated RWA2, severely devastated cultivars with *Dny* and *Dn4* (Haley et al., 2004a), and new biotypes were later reported in different states, including RWA3 and RWA4 in Texas, RWA5 in Wyoming, and RWA6, RWA7, and RWA8 in Colorado (Burd et al., 2006; Weiland et al., 2008). Biotypic diversity was also reported in South Africa (Tolmay et al., 2013), where newly discovered RWA biotypes RWASA2 and RWASA3 overcame resistance conferred by *Dn1*, *Dn2*, *Dn3*, *Dn4*, *Dn9*, and *Dny* (Tolmay et al., 2013). A more recent study established that RWA can, with rare occurrence, reproduce sexually. Over 30 new biotypes were identified in a sexually reproducing population at one site, suggesting that biotypic diversity in RWA populations is extensive and will be a continuous threat to developing RWA-resistant wheat cultivars (Puterka et al., 2012). Therefore identification of diverse resistance genes is required to mitigate the effects of RWA on sustainable wheat production.

Landraces conserve abundant genetic variation and are valuable sources of RWA resistance. RWA resistance genes were identified in landraces collected from Iran (*Dn1*, *Dn6*, *Dn10*, *Dn626580*), Tajikistan (*Dn2401*), and Afghanistan (*Dnx*, *Dny*) (Liu et al., 2001, 2002; Valdez et al., 2012; Fazel-Najafabadi et al., 2015). Russian wheat aphid resistance genes were also discovered in commercial germplasm, including *Dn2* in Azerbaijan cultivar PI 262660, *Dn4* in Turkmenistan cultivar PI 372129, and *Dn5*, *Dn8*, and *Dn9* in Bulgarian cultivar PI 294994 (Ma et al., 1998; Liu et al., 2001, 2002). Currently, only four known genes confer resistance to RWA2, the most virulent biotype in the United States, and three of them—*Dn10*, *Dn2401*, and *Dn626580*—were identified in landraces. Given that

the fourth gene *Dn7* is associated with an undesirable *Sec1* gene, these landrace-derived RWA2 resistance genes should play an important role in US wheat breeding. A previous study showed that *Dn2401* also confers resistance to other biotypes existing in the United States (Weiland et al., 2008). Further study is needed to evaluate responses of *Dn10* and *Dn626580* to other RWA biotypes.

Wheat accessions resistant to RWA1 and RWA2 have been identified (Collins et al., 2005; Xu et al., 2015), but most of them have not been characterized. Genetic analysis of these RWA resistance sources will greatly facilitate their utilization in wheat breeding. Moreover, the biotypic diversity in RWA populations necessitates a continuous search for RWA resistance to different biotypes. It is useful, but time consuming and labor intensive, to perform comprehensive screening of wheat germplasm available in the USDA-ARS National Small Grain Collection. An alternative strategy is to reevaluate previously identified RWA1- and RWA2-resistant accessions using newly discovered biotypes and to undertake genetic analyses using different biotypes. Advances in wheat genomics make it feasible to identify molecular markers associated with the underlying RWA resistance genes to eventually develop and strategically deploy cultivars carrying multiple resistance genes.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgments

We thank Dr. Robert McIntosh of Sydney University for reviewing this paper. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. The USDA is an equal opportunity provider and employer.

References

Australia Government Grains Research and Development Corporation. 2017. Tips & tactics: Russian wheat aphid. Aust. Gov. Grains Res. Dev. Corp. https://grdc.com.au/TT-RWA (accessed 18 Jan. 2018).

Burd, J.D., D.R. Porter, G.J. Puterka, S.D. Haley, and F.B. Peairs. 2006. Biotypic variation among North American Russian wheat aphid (Homoptera: Aphididae) populations. J. Econ. Entomol. 99:1862–1866. doi:10.1093/jee/99.5.1862

Collins, M.B., S.D. Haley, F.B. Peairs, and J.B. Rudolph. 2005. Biotype 2 Russian wheat aphid resistance among wheat germplasm accessions. Crop Sci. 45:1877–1880. doi:10.2135/cropsci2004.0730

Du Toit, F., and M.C. Walters. 1984. Damage assessment and economic threshold values for chemical control of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) on winter wheat. In: M.C. Walters, editor, Progress in Russian wheat aphid (*Diuraphis noxia*) research in the Republic of South Africa. Tech. Comm. 191. Dep. Agric., Pretoria, Republic of South Africa. p. 58–62

Du Toit, F. 1987. Resistance in wheat (*Triticum aestivum*) to *Diuraphis noxia* (Hemiptera : Aphididae). Cereal Res. Commun. 15:175–179.

Du Toit, F., W.G. Wessels, and G.F. Marais. 1995. The chromosome arm location of the Russian wheat aphid resistance gene, *Dn5*. Cereal Res. Commun. 23:15–17.

Dubcovsky, J., A.F. Galvez, and J. Dvořák. 1994. Comparison of the genetic organization of the early salt-stress-response gene system in salt-tolerant *Lophopyrum elongatum* and salt-sensitive wheat. Theor. Appl. Genet. 87:957–964. doi:10.1007/BF00225790

Elmali, M. 1998. Russian wheat aphid in Konya province. Euphytica 100:69–76. doi:10.1023/A:1018351805644

Fazel-Najafabadi, M., J. Peng, F.B. Peairs, H. Simkova, A. Kilian, and N.L.V. Lapitan. 2015. Genetic mapping of resistance to *Diuraphis noxia* (Kurdjumov) biotype 2 in wheat (*Triticum aestivum* L.) accession CI 2401. Euphytica 203:607–614.

Graybosch, R.A., C.J. Peterson, L.E. Hansen, D. Worrall, D.R. Shelton, and A. Lukaszewski. 1993. Comparative flour quality and protein characteristics of 1BL/1RS and 1AL/1RS wheat-rye translocation lines. J. Cereal Sci. 17:95–106. doi:10.1006/jcrs.1993.1010

Haley, S.D., F.B. Peairs, C.B. Walker, J.B. Rudolph, and T.L. Randolph. 2004a. Occurrence of a new Russian wheat aphid biotype in Colorado. Crop Sci. 44:1589–1592. doi:10.2135/cropsci2004.1589

Haley, S.D., J.S. Quick, J.J. Johnson, F.B. Peairs, J.A. Stromberger, S.R. Clayshulte, et al. 2004b. Registration of 'Ankor' wheat. Crop Sci. 44:1025–1026. doi:10.2135/cropsci2004.1025a

Hewitt, P.H., G.V. Niekerk, M.C. Walters, C.F. Kriel, and A. Fouche. 1984 Aspects of the ecology of the Russian wheat aphid *Diuraphis noxia*, in the Bloemfontein District. I. The colonization and infestation of sown wheat, identification of summer hosts and cause of infestation symptoms. In: M.C. Walters, editor, Progress in Russian wheat aphid (*Diuraphis noxia*) research in the Republic of South Africa. Tech. Comm. 191. Dep. Agric., Pretoria, Republic of South Africa. p. 3–13.

Kosambi, D.D. 1943. The estimation of map distance. Ann. Eugen. 12:172–175. doi:10.1111/j.1469-1809.1943.tb02321.x

Lincoln, S., M. Daly, and E. Lander. 1993. Mapping genes controlling quantitative traits using MAPMAKER/QTL Version 1.1: A Whitehead Institute technical report. 2nd ed. Whitehead Inst. Biomed. Res., Cambridge, MA.

Liu, R.H., and J.L. Meng. 2003. MapDraw: A Microsoft Excel macro for drawing genetic linkage maps based on given genetic linkage data. Hereditas 25:317–321.

Liu, X., J.L. Marshall, P. Stary, O. Edwards, G. Puterka, L. Dolatti, et al. 2010. Global phylogenetics of *Diuraphis noxia* (Hemiptera: Aphididae), an invasive aphid species: Evidence for multiple invasions into North America. J. Econ. Entomol. 103:958–965. doi:10.1603/EC09376

Liu, X., C. Smith, and B.S. Gill. 2002. Identification of microsatellite markers linked to Russian wheat aphid resistance genes *Dn4* and *Dn6*. Theor. Appl. Genet. 104:1042–1048. doi:10.1007/s00122-001-0831-y

Liu, X.M., C.M. Smith, B.R. Friebe, and B.S. Gill. 2005. Molecular mapping and allelic relationships of Russian wheat aphid–resistance genes. Crop Sci. 45:2273–2280. doi:10.2135/cropsci2004.0704

Liu, X.M., C.M. Smith, B.S. Gill, and V. Tolmay. 2001. Microsatellite markers linked to six Russian wheat aphid resistance genes in wheat. Theor. Appl. Genet. 102:504–510. doi:10.1007/s001220051674

Ma, Z.Q., A. Saidi, J.S. Quick, and N. Lapitan. 1998. Genetic mapping of Russian wheat aphid resistance genes *Dn2* and *Dn4* in wheat. Genome 41:303–306. doi:10.1139/g98-013

Marais, G.F., and F. Du Toit. 1993. A monosomic analysis of Russian wheat aphid resistance in the common wheat PI 294994. Plant Breed. 111:246–248. doi:10.1111/j.1439-0523.1993.tb00636.x

Marais, G.F., M. Horn, and F. Du Toit. 1994. Intergeneric transfer (rye to wheat) of a gene(s) for Russian wheat aphid resistance. Plant Breed. 113:265–271. doi:10.1111/j.1439-0523.1994.tb00735.x

Martin, D.J., and B.G. Stewart. 1990. Dough stickiness in rye-derived wheat cultivars. Euphytica 51:77–86. doi:10.1007/BF00022895

McIntosh, R.A., Porter, D.R., C.A. Baker, and J.A. Webster. 1998. Inheritance of Russian wheat aphid resistance in PI 140207 spring wheat. Plant Breed. 117:293–294. doi:10.1111/j.1439-0523.1998.tb01944.x

Mornhinweg, D.W. 2012. Biotic stresses in barley: Insect problems and solutions. In: S. E. Ullrich, editors, Barley production, improvement, and uses. Blackwell Publications, West Sussex, UK. p. 282–306.

Navabi, Z., B. Shiran, and M.T. Assad. 2004. Microsatellite mapping of a Russian wheat aphid resistance gene on chromosome 7B of an Iranian tetraploid wheat line: Preliminary results. Cereal Res. Commun. 32:451–457.

Nkongolo, K.K., J.S. Quick, A.E. Limin, and D.B. Fowler. 1991. Sources and inheritance of resistance to Russian wheat aphid in *Triticum* species amphiploids and *Triticum tauschii*. Can. J. Plant Sci. 71:703–708. doi:10.4141/cjps91-103

Peng, J., H. Wang, S.D. Haley, F.B. Peairs, and N.L.V. Lapitan. 2007. Molecular mapping of the Russian wheat aphid resistance gene in wheat. Crop Sci. 47:2418–2429. doi:10.2135/cropsci2007.03.0137

Puterka, G.J., J.D. Burd, and R.L. Burton. 1992. Biotypic variation in a worldwide collection of Russian wheat aphid (Homoptera: Aphididae). J. Econ. Entomol. 85:1497–1506. doi:10.1093/jee/85.4.1497

Puterka, G.J., J.D. Burd, D.W. Mornhinweg, S.D. Haley, and F.B. Peairs. 2006. Response of resistant and susceptible barley to infestations of five *Diuraphis noxia* (Homoptera: Aphididae) biotypes. J. Econ. Entomol. 99:2151–2155. doi:10.1093/jee/99.6.2151

Puterka, G.J., R.W. Hammon, J.D. Burd, F.B. Peairs, T.L. Randolph, and W.R. Cooper. 2012. Cyclical parthenogenetic reproduction in the Russian wheat aphid (Hemiptera: Aphididae) in the United States: Sexual reproduction and its outcome on biotypic diversity. J. Econ. Entomol. 105:1057–1068. doi:10.1603/EC11338

Puterka, G.J., S.J. Nicholson, M.J. Brown, W.R. Cooper, F.B. Peairs, and T.L. Randolph. 2014. Characterization of eight Russian wheat aphid (Hemiptera: Aphididae) biotypes using two-category resistant-susceptible plant responses. J. Econ. Entomol. 107:1274–1283. doi:10.1603/EC13408

Quick, J.S., G.E. Ellis, R.M. Normann, J.A. Stromberger, J.F. Shanahan, F.B. Peairs, et al. 1996. Registration of 'Halt' wheat. Crop Sci. 36:210. doi:10.2135/cropsci1996.0011183X003600010046x

Quick, J.S., S.D. Haley, J.A. Stromberger, S. Clayshulte, B. Clifford, J.J. Johnson, et al. 2001a. Registration of 'Prowers 99' wheat. Crop Sci. 41:929. doi:10.2135/cropsci2001.413929x

Quick, J.S., J.A. Stromberger, S. Clayshulte, B. Clifford, J.J. Johnson, F.B. Peairs, et al. 2001b. Registration of 'Prairie Red' wheat. Crop Sci. 41:1362–1363.

Quick, J.S., J.A. Stromberger, S. Clayshulte, B. Clifford, J.J. Johnson, F.B. Peairs, et al 2001c. Registration of 'Prowers' wheat. Crop Sci. 41:928–929. doi:10.2135/cropsci2001.413928x

Quick, J.S., J.A. Stromberger, S. Clayshulte, B. Clifford, J.J. Johnson, F.B. Peairs, et al. 2001d. Registration of 'Yumar' wheat. Crop Sci. 41:1363–1364. doi:10.2135/cropsci2001.4141363x

Saidi, A., and J.S. Quick. 1996. Inheritance and allelic relationships among Russian wheat aphid resistance genes in winter wheat. Crop Sci. 36:256–258. doi:10.2135/cropsci1996.0011183X003600020007x

Shufran, K.A., J.D. Burd, and J.A. Webster. 1997. Biotypic status of Russian wheat aphid (Homoptera: Aphididae) populations in the United States. J. Econ. Entomol. 90:1684–1689. doi:10.1093/jee/90.6.1684

Shufran, K.A., L.R. Kirkman, and G.J. Puterka. 2007. Absence of mitochondrial DNA sequence variation in Russian wheat aphid (Hemiptera: Aphididae) populations consistent with a single introduction into the United States. J. Kans. Entomol. Soc. 80:319–326. doi:10.2317/0022-8567(2007)80[319:AOMDSV]2.0.CO;2

Shufran, K.A., and T.L. Payton. 2009. Limited genetic variation within and between Russian wheat aphid (Hemiptera: Aphididae) biotypes in the United States. J. Econ. Entomol. 102:440–445. doi:10.1603/029.102.0157

Somers, D.J., P. Isaac, and K. Edwards. 2004. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 109:1105–1114. doi:10.1007/s00122-004-1740-7

Swanevelder, Z.H., A.K.J. Surridge, E. Venter, and A.M. Botha. 2010. Limited endosymbiont variation in *Diuraphis noxia* (Hemiptera: Aphididae) biotypes from the United States and South Africa. J. Econ. Entomol. 103:887–897. doi:10.1603/EC09257

Tolmay, V.L., A. Jankielsohn, and S.L. Sydenham. 2013. Resistance evaluation of wheat germplasm containing *Dn4* or *Dny* against Russian wheat aphid biotype RWASA3. J. Appl. Entomol. 137:476–480. doi:10.1111/jen.12008

Tonk, F., D. İştipliler, M. Tosun, F. Turanli, H. İlbi, and M. Çakir. 2016. Genetic mapping and inheritance of Russian wheat aphid resistance gene in accession IG 100695. Plant Breed. 135:21–25. doi:10.1111/pbr.12339

USDA-ARS. 2018. GrainGenes, a database for Triticeae and Avena. USDA-ARS. https://wheat.pw.usda.gov/GG3/ (accessed 18 Jan. 2018).

Valdez, V.A., P.F. Byrne, N.L.V. Lapitan, F.B. Peairs, A. Bernardo, G. Bai, and S.D. Haley. 2012. Inheritance and genetic mapping of Russian wheat aphid resistance in Iranian wheat landrace accession PI 626580. Crop Sci. 52:676–682. doi:10.2135/cropsci2011.06.0331

Weiland, A.A., F.B. Peairs, T.L. Randolph, J.B. Rudolph, S.D. Haley, and G.J. Puterka. 2008. Biotypic diversity in Colorado Russian wheat aphid (Hemiptera: Aphididae) populations. J. Econ. Entomol. 101:569–574. doi:10.1093/jee/101.2.569

Xu, X., G. Bai, B.F. Carver, G.E. Shaner, and R.M. Hunger. 2005. Molecular characterization of slow leaf-rusting resistance in wheat. Crop Sci. 45:758–765. doi:10.2135/cropsci2005.0758

Xu, X., G. Bai, B.F. Carver, K. Zhan, Y. Huang, and D. Mornhinweg. 2015. Evaluation and reselection of wheat resistance to Russian wheat aphid biotype 2. Crop Sci. 55:695–701. doi:10.2135/cropsci2014.07.0473