

Invited Review Article

Progress in host plant resistance in wheat to Russian wheat aphid (Hemiptera: Aphididae) in North Africa and West Asia**M. El Bouhssini^{1*}, F. C. Ogonnaya¹, H. Ketata¹, M. M. Mosaad¹, K. Street¹, A. Amri¹, M. Keser¹, S. Rajaram¹, A. Morgounov³, F. Rihawi¹, A. Dabus¹ and C. M. Smith²**¹International Center for Agricultural Research in the Dry Areas (ICARDA), P.O. Box 5466, Aleppo, Syria²Department of Entomology, Kansas State University, Manhattan, KS 66506-4004, USA³International Maize and Wheat Improvement Centre (CIMMYT), 06511, Ankara, Turkey

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Abstract

Russian wheat aphid (RWA), *Diuraphis noxia* (Kurdjumov), is an important pest of wheat and barley in several countries of North Africa and West Asia, e.g., Morocco, Algeria, Tunisia, Ethiopia, Yemen, Turkey and Iran. Host plant resistance is the most economical and practical means of controlling this insect. Field and greenhouse screening of introduced and local wheat germplasm at ICARDA resulted in the identification of several sources of resistance which were subsequently incorporated into ICARDA elite wheat germplasm and distributed as RWA gene pool to NARS (National Agricultural Research Systems) in affected countries. Crosses were initiated in 1998 to introgress resistance into winter/facultative bread wheat and the segregating populations were evaluated for RWA resistance and agronomic performance at the ICARDA Experiment Station at Tel Hadya. Selected advanced lines were sent to North African, and West Asian countries for evaluation of RWA and disease resistance and agronomic adaptation under local conditions. Additional identified sources of RWA resistance are now in use in the ICARDA wheat breeding program. Haplotype analysis using molecular markers previously identified as diagnostic for *Dn* resistance genes revealed that some recently identified resistance sources are unrelated to previously described *Dn1–Dn9* genes, and may represent new genes for deployment in RWA breeding. These apparent novel resistance gene(s) could be effective against some of the more virulent biotypes and could be deployed in breeding programs to increase the diversity of available genetic resistances. The reaction of wheat differentials containing different *Dn* genes indicates that the Syrian RWA biotype is less virulent than US RWA2 biotype.

Keywords: Bread wheat, biotypes, durum wheat, *Diuraphis noxia*, resistance.**Abbreviations:** ASL: Above sea level; CWANA- North Africa West and Central Asia; ICARDA-International Center for Agricultural Research in the Dry Areas; NARS-National Agricultural Research Systems; RWA-Russian wheat aphid; UNESCO-United Nations Educational, Scientific and Cultural Organization.**Introduction**

The Russian wheat aphid (RWA), *Diuraphis noxia* (Kurdjumov) (Fig. 1), a pest of wheat and barley, is indigenous to southern Russia, Iran, Afghanistan and countries bordering the Mediterranean Sea (Hewitt et al., 1984). The pest has spread widely and is now found in all continents except Australia (Ennahli et al., 2009), and causes economic damage to wheat in many parts of the world. In Ethiopia, Miller and Haile (1988) reported 68% yield loss in wheat. In South Africa, 21–92% yield losses were reported (Du Toit and Walters 1984). Elmali (1998) reported 25–60% wheat yield losses in Turkey. Feeding damage by *D. noxia* to plant leaves results in characteristic longitudinal white, yellow or red chlorotic streaks with a convoluted rolling of the leaf. Rolling of the leaves reduces photosynthetic area, and protects aphids from contact insecticides and natural enemies (Khan et al., 2010). Host plant resistance is the most sustainable, cost-effective and environmentally safe way of controlling RWA. Ten resistance genes (*Dn1–Dn9* and *Dnx*) have been identified from *Aegilops tauschii*, rye or wheat (Liu et al., 2001; Smith et al., 2004) and used to develop varieties with RWA resistance (Souza et al., 2002; Haley et

al., 2004). The chromosome locations of nine of the genes are known. *Dn1*, *Dn2*, *Dn5*, *Dn6*, *Dn8* and *Dnx* are either allelic or comprise a closely related gene cluster on wheat chromosome arm 7DS (Liu et al., 2001, 2002, 2005), and differ from *Dn4*, located on chromosome arm 1DS, while *Dn9* is located on 1DL (Liu et al., 2001, 2002; Arzani et al., 2004). *Dn7* is derived from rye chromosome arm IRS and is present in a 1R/1B chromosome translocation located on wheat chromosome arm IRS (Lapitan et al., 2007). Biotypic variation in RWA populations exists in several parts of the world (Liu et al. 2010), and currently the US biotype 2 is the most virulent (Haley et al., 2004, Burd et al., 2006) of the eight known biotypes (Peng et al., 2007). The development of virulent RWA biotypes will likely continue to pose a serious threat to wheat production in regions where they occur. Continuous efforts are necessary to identify and introduce additional resistance genes into commercially acceptable cultivars. Microsatellite (SSR) markers are commonly used for genetic diversity, quantitative trait loci (QTL) studies and marker assisted selection (MAS) as surrogates for traits linked to genes of agronomic importance.

Table 1. List of RWA resistant bread wheat and durum wheat lines identified in the ICARDA gene bank.

Species	Accession number or cultivar	Origin	Leaf rolling	Leaf chlorosis
<i>T. aestivum</i> subsp. <i>Aestivum</i>	IG -41556	Pakistan	1.0	1.0
	IG- 41603	Pakistan	1.25	2.0
	IG- 43273	Pakistan	1.75	1.75
	IG- 107147	Iran	1.5	2.0
	IG- 138374	Uzbekistan	1.0	1.25
	IG- 138998	Iran	1.25	1.25
	IG- 41556	Pakistan	2.0	2.5
	IG-41560	Pakistan	2.0	2.5
	IG-138330	Tajikistan	2.0	2.75
	IG-138413	Azerbaijan	2.0	2.75
	IG-107166	Iran	2.0	3.0
	IG-138810	Pakistan	2.67	3.33
	<i>T. turgidum</i> subsp. <i>Durum</i>	IG- 83976	Afghanistan	2.0
IG-90154		Afghanistan	2.0	2.0
IG-90260		Afghanistan	2.0	1.75
IG-90264		Afghanistan	2.0	2.0

**Fig 1.** Adult Russian wheat aphid.

SSRs are increasingly used to infer haplotype diversity (Huang et al., 2002; Ogonnaya et al., 2007; Fofana et al., 2008) and to differentiate wheat germplasm with different sources of disease resistance (Bai et al., 2003; McCartney et al., 2004; Yu et al., 2010). This approach minimizes redundancy and ensures that germplasm with potentially novel genes are targeted for exploitation. We report on the progress in (i) the identification of sources of resistance to RWA in wheat from the ICARDA gene bank and introduced germplasm, (ii) identifying uncharacterized genes for RWA resistance in some gene bank landraces and (iii) the introgression of RWA resistance into adapted winter/facultative bread wheat cultivars.

Results and discussion

From field and plastic house screenings, a total of 16 lines (12 bread wheat and four durum wheat) showed varying levels of resistance to RWA (Table 1). The 12 bread wheat sources were selected from the ICARDA gene bank accessions using (El Bouhssini et al., 2011). A total of 7236 accessions were previously screened without success demonstrating the rarity of the trait within the germplasm pool conserved at ICARDA. The 12 resistant bread wheat lines (Fig. 3) reported here were captured in a subset of only 510 accessions, thus indicating the effectiveness of the FIGS process. This method has been successful in identifying relatively small sets of germplasm that have contained accessions carrying resistance to Sunn pest, *Eurygaster integriceps* Puton (El Bouhssini et al., 2009). It is suggested

that the FIGS process can be further refined with these results and used in future germplasm selection for RWA screenings. It is essential in any germplasm characterization with diagnostic SSR markers linked to genes of interest to know the specific SSR haplotypes tightly linked to targeted resistance genes. Liu et al., (2005) demonstrated that SSR markers *Xgwm44* and *Xgwm111* amplify specific SSR haplotypes tightly linked to RWA genes. *Xgwm44* amplified two SSR haplotypes, 180 and 200 bp, while *Xgwm111* amplified SSR haplotypes 200, 210, 220 and 225 bp, respectively, which are diagnostic for *Dn1*, *Dn2*, *Dn5*, *Dn6* and *Dn9* on 7DS depending on the germplasm. Similarly, *Xgwm106* and *Xgwm337* amplified diagnostic fragments, 125 and 175 bp, respectively, linked to *Dn4* on 1DS. In the current study, wheat landrace accessions IG-41560, IG-107147 and IG-138374 possessed the 175 bp SSR haplotype amplified by *Xgwm 337*, while accessions IG41603, IG138810, IG41578, IG107147, IG41556, IG138374 and IG107166 possessed the 125 bp SSR haplotype amplified by *Xgwm 106* on 1DS and diagnostic for *Dn4*. Landrace accessions IG-43273, IG-41556 and IG-138330 possessed the 180 bp fragment, while IG-41556 possessed the 200 bp fragment amplified by *Xgwm44* and *Xgwm111*, respectively, and tightly linked to *Dn1*, *Dn2*, *Dn5*, *Dn6* and *Dn9* on 7DS. IG-41603 and IG-138413 displayed none of the markers linked to RWA genes so far identified in bread wheat, and thus are potential novel sources of RWA resistance (Fig 4). Haplotyping wheat accessions with SSR markers that flank resistant QTLs linked to traits may provide useful information for predicting novel QTLs, by comparing

Table 2. Bread wheat lines selected for resistance to RWA and rusts (yellow and leaf), and for cold tolerance, ICARDA, 2007-2010.

Entry	Cross	SELHX
1	VORONA/HD2402//F96PY3-1828	-0AP-16AP-6AP-OAP-1AP-0AP
2	VORONA/HD2402//F96PY3-1828	-0AP-16AP-14AP-OAP-2AP-0AP
3	VORONA/HD2402//F96PY3-1828	-0AP-84AP-10AP-OAP-4AP-0AP
4	VORONA/HD2402//F96PY3-1828	-0AP-84AP-11AP-OAP-5AP-0AP
5	VORONA/HD2402//F96PY3-1828	-0AP-84AP-12A-OAP-6AP-0AP
6	TIRCHMIR1/LCO//VORONA/HD2402	-0AP-133AP-13AP-OAP-1AP-0AP
7	AU/CO652337//2*CA8055/3/UNKN/HATUSHA	-0AP-230AP-6AP-OAP-4AP-0AP
8	AU/CO652337//2*CA8055/3/UNKN/HATUSHA	-0AP-230AP-12AP-OAP-5AP-0AP
9	AU/CO652337//2*CA8055/3/UNKN/HATUSHA	-0AP-230AP-16AP-OAP-6AP-0AP
10	TIRCHMIR1/LCO//VORONA/HD2402	-133AP-15AP-OAP
11	FL302//BUC/PVN/3/RSK/CA8055//CHAM6	-27AP-36AP-OAP
12	YUMAI13=ZHENGZHOU891/F96PYN3-1828	-0AP-0AP
13	YUMAI13=ZHENGZHOU891/F96PYN3-1828	-0AP-0AP
14	YUMAI13=ZHENGZHOU891/F96PYN3-1828	-0AP-0AP
15	YUMAI13=ZHENGZHOU891/F96PYN3-1828	-0AP-0AP
16	YUMAI13=ZHENGZHOU891/F96PYN3-1828	-0AP-0AP
17	L 29-91 K 4/KS92WGRC24	-0AP-0AP

**Fig 2.** Field screening wheat for resistance to Russian wheat aphid at ICARDA's research station, Tel Hadya, Syria.

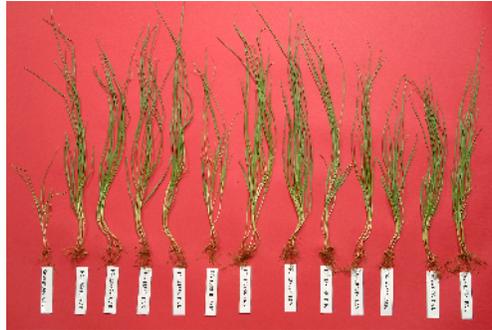
haplotypes of target accessions with known cultivars (Yu et al., 2006). The underlying assumption is that if a wheat line has the same allelic pattern for marker loci flanking the QTL as a known resistant line, then the two most likely have the same gene (Bai et al., 2003; McCartney et al., 2004; Yu et al., 2010). If a wheat line has a different allelic pattern from a known resistant line, the two lines most likely have different alleles of the gene (Yu et al., 2006). The ability to characterize genetic diversity using markers associated with RWA resistance in both cultivated and wild relatives of wheat will be an important strategy for identifying novel RWA resistance genes to diversify the sources of resistance. This study identified two landrace accessions that do not possess SSR haplotypes associated with any of the currently known sources of RWA resistance. McCartney et al., (2004) employed a similar strategy of using SSR markers to successfully infer haplotypes' diversity and to differentiate wheat germplasm with different sources of disease resistance. Recently, Peng et al., (2009) used an association-mapping approach to identify 28 SSR loci significantly associated with RWA leaf chlorosis, and eight with leaf rolling. They also identified new chromosome regions associated with biotype-2 Russian wheat aphid (RWA2) resistance, which indicated the existence of new RWA resistance genes located on chromosomes of homoeologous groups other than groups previously reported in bread wheat. However, some markers (*Xgwm111*) are multi-locus in nature; thus, it is essential that the specific allele size associated with *Dn* resistance genes be specified before being deployed in Markers Assisted Selection (MAS). It could be argued that specific SSR haplotypes associated with *Xgwm44* and *Xgwm111* for *Dn*

resistance may be due to founder effects, since both these markers are linked and may not be diagnostic and polymorphic across a wide range of germplasm for effective utilization in MAS. These limitations would be overcome as more functional-markers become readily available from ongoing wheat sequencing projects, and tightly linked markers are identified for various *Dn* resistance genes. Due to the potentially multiple sources of resistance identified, this study has opened up several possible areas for future research. These include the genetic characterization of resistance found in these accessions, the transfer of these potentially novel resistances into locally adapted elite wheat cultivars, and the pyramiding of diverse resistance genes into elite cultivars.

These areas of research will increase our knowledge about the potential usefulness of these wheat accessions and the genes they contain in breeding for RWA resistance in bread wheat. The identified sources of resistance are being used in the wheat breeding programs to develop RWA resistant germplasm for deployment in highlands of North Africa, West Asia and Central Asia (CWANA) where this insect causes economic damage (Miller and Haile 1988; Elmali 1998; El Bouhssini and Nachit 2000; Lhaloui et al., 2001). Crosses have also been made to develop mapping populations to characterize the RWA resistance in these sources. Seventeen bread wheat lines were identified from several cycles of selection for RWA and yellow rust resistance at Tel Hadya, Syria, and for RWA resistance and leaf rust and winter hardness at Edirne and Izmir, Turkey, during 2008, 2009 and 2010 (Table 2). These lines have been assembled in RWA nurseries, distributed to countries in

Table 3. Response of wheat differentials to infestation with the RWA Syrian biotype, ICARDA, 2010.

Collection ID/Variety	Dn gene	Origin	Leaf rolling	Leaf chlorosis
PI 262660 TR05/Turtsikum	2	Azerbaijan	2	2
PI 372129/Yamar (Colorado)	4	Turkmenistan	3	3
PI 294994/CO950043 (Colorado)	5	Bulgaria	3	3
Gamatoos R/94M370 (South Africa)	7	Turkey	1	1
PI 47545 TR05/CI 6501	6	Iran	1	1
RWA-MATRIX/CI 6501	6	Iran	1	1
Tukey 77 (Rye)/94M370(South Africa)	7	Turkey	1	1
ICBW-13961 (Resistant check)	Unknown	ICARDA	1	2
AYT-98-RF-9346 (Susceptible check)	0	ICARDA	3	5

**Fig 3.** Reaction of the 12 resistant bread wheat lines and the susceptible check to Russian wheat aphid, ICARDA, Syria, 2009.**Fig 4.** PCR amplification products using SSR markers *gwm106* of wheat landraces. M: molecular weight marker; 1: IG138998; 2: IG41560; 3: IG41603; 4: IG138810; 5: IG43273; 6: IG41578; 7: IG107147; 8: IG41556; 9: IG138413; 10: IG138330; 11: IG138374 and 12: IG107166

CWANA where RWA infestation is high, and will be used to select for RWA resistance in national wheat breeding programs. Cultivars carrying RWA resistance have been developed and deployed in several countries such as the USA (Souza et al., 2002; Haley et al., 2004b). The reaction of the *Dn* differentials to the Syrian biotype (Table 3) indicated high resistance in *Dn6* and *Dn7* (leaf rolling [LR] and leaf chlorosis [LC] scores of 1), good resistance in *Dn2* (LR and LC scores of 2), and moderate resistance in *Dn4* and *Dn5* (LR and LC scores of 3). Based on these results, the Syrian RWA biotype is less virulent than the recently discovered RWA 2 biotype in the USA (Haley et al., 2004a).

Materials and methods

Screening for resistance

1. Initial field screening

Since 1997, we evaluated a total of 7746 wheat accessions, 2972 durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) and 4774 bread wheat (*T. aestivum* L. subsp. *aestivum*) from the ICARDA gene bank and from introduced germplasm. While the majority of the germplasm screened at ICARDA for RWA resistance was chosen from the gene bank either at random or in such a way as to maximize geographic diversity, recent selections were made using the

Focused Identification of Germplasm Strategy (FIGS). The FIGS approach (Mackay and Street 2004) develops environmental profiles, using statistical approaches and GIS technologies, to predict where selection pressures are likely to occur for specific traits. Germplasm collection sites that fall within the appropriate environmental profile are identified and a best-bet subset of accessions is selected for screening. In this case, wheat landrace accessions were chosen from collection sites within areas where RWA is known to have existed historically, from sites classified arid, semi-arid and semi humid as per the UNESCO (United Nations Educational, Scientific and Cultural Organization) agro-climatic zone system and from altitudes between 800–2000 m above sea level. These site conditions were deemed to favor higher RWA population densities when viewed from an historic perspective. Field screening were conducted at ICARDA main experimental station, Tel Hadya, Syria (longitude 36.93, latitude 36.01, and altitude 284 m asl.). Entries were planted in hill plots, 10 seeds/hill using an augmented design. One susceptible check is used every 10 entries. At the two-leaf stage, entries were infested with a mixture of RWA nymphs and adults by placing infested leaves from the laboratory culture on the plants. Evaluations were made when symptoms of leaf rolling and leaf chlorosis were clearly visible on susceptible checks using a 1–3 scale for leaf rolling (LR), where: 1 = no rolling; 2 = trapping or curling in one or more leaves; and 3 = rolling in one or more

leaves. For leaf chlorosis (LC) a 1–6 scale was used where: 1 = no LC; 2 = < 33% of leaf area with LC; 3 = 33–66% area with LC; 4 = > 66% area with LC; 5 = necrosis in at least one leaf; and 6 = plant death (Formusoh et al., 1992). A representative wheat field screening for resistance to RWA is presented in Fig 2.

2. Confirmation of results from initial screening in the greenhouse

Selected lines from the field screening were re-evaluated in the greenhouse at 20–24°C, a light/dark photoperiod of 16/8 h, and a relative humidity of 50–60%. Seeds were planted in 53.5 cm × 35.5 cm metal flats with five seeds per hill, and thinned to three plants per hill after germination. A randomized complete block design with four replications is used, with two checks included in the test, one susceptible and one resistant.

3. Evaluation of the wheat differentials for resistance to the Syrian biotype

A set of RWA differentials with seven wheat lines containing *Dn2*, *Dn4*, *Dn5*, *Dn6* and *Dn7* were evaluated for reaction to the Syrian RWA biotype. The seeds of these differentials were provided by Kansas State University. The experimental design was as described above including the use of two cultivars as controls. The RWA used for field and greenhouse tests were originally collected from Slenfeh (longitude 36.10, latitude 35.60, and altitude 660 m asl.), Syria and were reared in the greenhouse on plants of susceptible bread wheat cultivars. Seeds are sown in a mixture of soil, sand and peat (2:1:6). Plants were infested at the 2-leaf stage with 10 adult aphids per plant. Evaluations were made 4 weeks post-infestation using the two scales described previously.

Microsatellite markers and genotyping

Using SSR markers linked to previously identified RWA resistance genes, we characterized the RWA resistance of the 12 bread wheat accessions identified in the ICARDA gene bank through the focused identification of germplasm strategy (FIGS) (Table 1). The microsatellite markers used were *Xgwm44*, *Xgwm106*, *Xgwm111* and *Xgwm337*, tightly linked to RWA resistance genes on chromosomes 1DS and 7DS, respectively (Liu et al., 2005). Other markers tested included 1RS-specific markers such as *Xrems1303* and *Xiag95* closely linked with *Dn7* in the coupling phase and 1BS-specific SSR, *Xgwm11* and *Xgwm18* closely linked with *Dn7* in the repulsion phase (Peng et al., 2007). The 12 lines were grown in the greenhouse and DNA extracted according to Ogonnaya et al., (2001). Microsatellite PCR was conducted as described by Liu et al., (2005). PCR fragments were separated by polyacrylamide gel while allele sizes were determined and scored manually.

Crosses

Four RWA resistant bread wheat cultivars, two from the US (KS92WGRC24 from Kansas State University and F96PYN3-1828 from Colorado State University) and two from ICARDA (46-RWA-94N-85 and MNCH/3/JUP/BJY//GA) were crossed to four susceptible bread wheat cultivars (Sardari, Sultan95, Pehlivan, and VORONA/3/TOB*2/

7C//BUC). Segregating F₂, F₃, and F₄ populations were evaluated for RWA resistance in the field at ICARDA under artificial infestation. Later generations were evaluated for agronomic characters and disease resistance at the main ICARDA experimental station in Tel Hadya, Syria, and at experimental sites at Edirne (longitude 26.57, latitude 41.67, and altitude 14 m asl.) and Izmir (longitude 27.09, latitude 38.25, and altitude 30 m asl.), Turkey.

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