Tracking of Powdery Mildew and Leaf Rust Resistance Genes in *Triticum boeoticum* and *T. urartu*, Wild Relatives of Common Wheat

Nelli A. HOVHANNISYAN[1], Mohammad Ehsan DULLOO[2], Aleksandr H. YESAYAN[1], Helmut KNÜPFFER[3] and Ahmed AMRI[4]

[1]Department of Ecology and Nature Protection, Faculty of Biology, Yerevan State University, Yerevan, Armenia; [2]Bioversity International, Rome, Italy; [3]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany; [4]International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria

Abstract: Wild *Triticum* and *Aegilops* species are increasingly used in wheat breeding programmes around the world as donors of genes conferring resistance to biotic and abiotic stresses, as well as of genes that contribute to the improvement of grain quality. In the present study, thirty-nine accessions of diploid species with the A genome (*Triticum boeoticum* and *T. urartu*) were evaluated for the presence of the genes conferring resistance to powdery mildew (*Blumeria graminis*) and leaf rust (*Puccinia recondita)* using both inoculation tests and sequence tagged sites (STS) marker analyses in order to find correspondence between STS markers and resistance as a trait. The most resistant entries were *T. boeoticum* accessions. All the marked *Lr* and *Pm* resistance genes (*Pm1*, *Pm2*, *Pm3*, *Lr10*, *Lr47*, *Lr25* and *Lr28*) were identified in the check *T. aestivum* cultivar Bezostaya 1. The resistance to powdery mildew in the material studied was conferred by the combination of the *Pm1* gene with either *Pm2* or *Pm3*. The *Pm1* and *Pm3* markers appeared to be suitable for tracking these powdery mildew resistance genes, while the *Pm2* gene marker cannot be considered as usable in various genetically different wheat accessions. The presence of the genes *Lr25*, *Lr28* and *Lr47* seems to be particularly useful for obtaining leaf rust resistance in *T. boeoticum* and *T. urartu* species.

Keywords: *Blumeria graminis*; inoculation test; *Puccinia recondita*; resistance genes; STS marker; *Triticum boeoticum*; *Triticum urartu*

Powdery mildew and rusts (leaf, stem, and stripe) belong to the most important diseases that significantly affect wheat production. Powdery mildew, caused by *Blumeria graminis* (DC.) E.O. Speer f.sp. *tritici* em. Marchal (syn. *Erysiphe graminis* DC. ex Merat f.sp. *tritici* Marchal), is distributed worldwide in areas with cool or warm and humid climates (Priestley & Bayles 1998; Hua *et al.* 2009). Powdery mildew is observed every year and causes considerable yield losses if effective protective measurements such as elimination of conducive conditions by altering planting density, crop rotation with non-host plants, or chemical control are not applied.

Leaf rust, caused by *Puccinia recondita* Dietel & Holw. f.sp. *tritici* (Erikss. & E. Henn.) D.M. Henderson, is a foliar disease of wheat occurring more regularly and more widely than stem rust

or stripe rust. Currently, new races of wheat leaf, stripe and stem rust are spreading in different regions, causing large problems.

The preferred approach to counteract rust epidemics is the development of genetic resistance. So, the presence of resistance genes in wheat cultivars is the most effective way to prevent infection and to avoid yield and grain quality losses. However, the use of cultivars with single-gene resistance permits the selection of mutations at a single locus to render the resistance effective in a relatively short time. The pathogens are dynamic, and their races are constantly changing. Particularly the widespread cultivation of modern disease-resistant cultivars and the decrease of their genetic diversity lead to a rapid development of more virulent pathotypes and counteract the efforts of breeders to develop wheat cultivars with durable resistance (Todorovska *et al.* 2009). This is observed for powdery mildew and for the three rusts of wheat.

For example, wheat stem rust caused by *Puccinia graminis* Pers. f.sp. *tritici* Erikss. & Henn, a major disease of wheat, has been controlled by the resistance gene complex *Sr2* and other alien derived resistance genes such as *Sr24*, *Sr26,* and *Sr31* for more than 35 years to the extent that it was thought that the fungus was beaten for good. However, the new virulent race Ug99 on *Sr31* detected in Uganda in 1999 is now threatening wheat in major wheat growing areas of Northeast Africa, and Western and Central Asia (MacKenzie 2007; Stokstad 2007; FAO 2008; Vurro *et al.* 2010).

In recent years, 32 loci and 48 genes/alleles for resistance to powdery mildew have been identified and located on various chromosomes (Liu *et al.* 2002; Zeller *et al.* 2002; Hsam *et al.* 2003). Several genes conferring resistance to different *Blumeria graminis* isolates have also been identified in common wheat and in species closely related to wheat. Some of them have been exploited in commercial wheat cultivars, but these genes were often overcome by new pathogen races, i.e. the resistance was not durable (Bennett 1984).

At present, more than 60 different leaf rust resistance genes derived from wheat and related species have been described, many of which are utilized in wheat breeding programmes (McIntosh *et al.* 2003). However, molecular markers, particularly STS, SCAR, CAPS and SSR markers, have been developed only for 21 of these genes, namely *Lr1*, *Lr10*, *Lr13*, *Lr16*, *Lr19*, *Lr20*, *Lr21*, *Lr24*, *Lr25*, *Lr26*, *Lr28*, *Lr29*, *Lr34*, *Lr35*, *Lr37*, *Lr39*, *Lr46*, *Lr47*, *Lr50*, *Lr51*, and *LrW* (Chełkowski *et al.* 2003; Cherukuri *et al.* 2003, 2005; Błaszczyk *et al.* 2004; Prabhu *et al.* 2004; Hiebert *et al.* 2005; Mago *et al.* 2005; Obert *et al.* 2005; http://maswheat.ucdavis.edu). The most frequent leaf rust resistance genes in European wheat cultivars are: *Lr1*, *Lr3a*, *Lr3ka*, *Lr10*, *Lr11*, *Lr13*, *Lr14a*, *Lr17b*, *Lr20*, *Lr26*, and *Lr37* (Pathan & Park 2006).

For the Caucasus region, including Armenia, the most effective resistance genes against powdery mildew and leaf rust have not been identified yet.

The species of *Triticum* and *Aegilops* carry ancestral or homoeologous genomes to those of common wheat (*Triticum aestivum* L.) (Gill *et al.* 2006). Many of these species are being used in wheat breeding (see e.g. Knüpffer 2009). *Triticum urartu* is distributed in Armenia, Iran, Iraq, Lebanon, and Turkey and is considered mostly susceptible to fungal diseases (Dorofeev *et al.* 1979; Knüpffer *et al.* 2002). *Triticum boeoticum* is known to have good resistance to fungal diseases, including powdery mildew and leaf rust; it is distributed in Transcaucasia, the Balkan Peninsula and Western Asia (Dorofeev *et al.* 1979).

Triticum boeoticum Boiss. and *T. urartu* Tumanian ex Gandilyan have the A genome similar to that in bread wheat (*T. aestivum*) and durum wheat (*T. durum* Desf.). Thus, they are a part of the primary gene pool that can supply genes for improvement of bread wheat. Direct introgression through crosses and backcrosses is frequently used to transfer genes from wild and distant species into cultivated wheat (Qi *et al.* 2007). For example, *T. boeoticum* has been reported as a valuable source of desirable genes conferring higher protein quality and amino-acid content, and resistance to biotic stresses (Bahraei & Jaradat 1998; Anker & Niks 2001).

Armenia is a part of the centre of origin of cereals (Zhukovsky 1971; Gandilyan & Avagyan 2001), and bread wheat plays a major role in Armenia, not only as food, but also as an element of culture and customs (Haykazyan & Pretty 2006). Some of them are known to be drought-resistant, which is particularly important in Armenia due to its dry climate and frequent water shortages. Others may possess resistance to wheat leaf rust and powdery mildew and can be used to create new disease-resistant cultivars, thus contributing to stabilizing yields and minimizing the use of chemicals (Gill *et al.* 2006).

The detection of molecular markers linked to the *Pm* and *Lr* genes is only a starting point of the implementation of markers in marker-assisted selection (MAS), and successful application of DNA markers in breeding programmes depends on the degree of correspondence between a marker and a trait, on the effectiveness of a marker in different genetic backgrounds and a high reproducibility and reliability of a marker across laboratories (Gupta *et al.* 1999).

Some STS markers can be directly used, and facilitate the tracking and pyramiding of the genes *Lr* and *Pm*. The identified markers are valuable for breeding wheat possessing durable leaf rust resistance based on combining race-specific and race nonspecific resistance genes.

The aim of this study was to identify the genes of resistance to *Blumeria graminis* and *Puccinia recondita* in selected accessions of the wild relatives of wheat *T. boeoticum* and *T. urartu* by using both inoculation tests and STS marker analyses and to find correspondence between STS markers and resistance as a trait.

MATERIALS AND METHODS

The research was mainly carried out at the Yerevan State University, but part of the STS analysis was conducted at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany, to compare the results obtained (Table 1, Figure 1).

Plant material. Twenty-nine accessions of *T. boeoticum*, 10 accessions of *T. urartu* and the control cultivar Bezostaya 1 were chosen for inoculation test and STS marker analyses. The material was obtained from the seed bank of the Armenian State Agrarian University, the International Center for Agricultural Research in the Dry Areas (ICARDA), and the Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany (IPK), and included samples from Armenia and Turkey collected in the wild (Table 1, Figure 1).

The *Triticum* accessions were screened for resistance to powdery mildew and leaf rust. Resistance to powdery mildew was first evaluated in an infected field, where a suspension of mixtures of spores applied to the susceptible wheat Michigan Amber was used as a spreader row. A more detailed evaluation of resistance to powdery mildew was performed in the greenhouse under controlled conditions. The inoculum was composed of three different virulent isolates (isolate 4: virulence/avirulence formulae *Pm4b*, *Pm13*, *Pm21*/*Pm1c*, *Pm3c*, *Pm2a*; isolate 10: virulence/avirulence formulae *Pm13*, *Pm6c*, *Pm8*/*Pm1c*, *Pm3a*, *Pm2a*; isolate 25: virulence/avirulence formulae *Pm13*, *Pm6c Pm8*/*Pm1c*, *Pm2a*, *Pm21*), collected from several locations in the Republic of Armenia.

Seedlings (10 plants of each accession), grown in plastic pots containing sterile soil, were dusted with mixtures of spores at the three-leaves stage, then covered with a glass cylinder for 48 h and evaluated 10 days after inoculation. Reactions to infection were scored on a scale of 0–4, where 0 = immune, no visible signs of infection; 1 = highly resistant, increasing from no necrosis to large necrotic areas, increasing from no mycelium to little mycelium; 2 = moderately resistant, necrotic areas changing to chlorotic areas, increasing in amounts of mycelium and conidiospore production; 3 = moderately susceptible, decreasing from chlorotic areas to no chlorosis, increasing in amounts of mycelium and conidiospore production, and 4 = susceptible, increasing in amounts of mycelium and conidiospore production to complete susceptibility (Moseman *et al.* 1984; Ovesná *et al.* 2002). For the evaluation of leaf rust reaction, 5–10 seedlings from each accession were inoculated using the urediniospore mixture oil-suspension inoculation and plant-growth methods as described by Browder (1971). Urediniospores were composed of three virulent isolates collected from the South of Armenia (isolate 2: virulence/avirulence formulae *Lr1*, *Lr10*, *Lr13*, *Lr15*, *Lr21*/*Lr17*, *Lr19*, *Lr47*, *Lr25*, *Lr28*; isolate 9: virulence/avirulence formulae *Lr1*, *Lr47*, *Lr10*, *Lr13*, *Lr15*, *Lr21*/*Lr17*, *Lr19*, *Lr2a*, *Lr2b*, *Lr25*, *Lr28*; isolate 15: virulence/avirulence formulae *Lr1*, *Lr2a*, *Lr2b*, *Lr15*, *Lr21*/*Lr17*, *Lr19*, *Lr47*, *Lr25*, *Lr28*). After 10 days of inoculation under controlled conditions, the leaf rust infection types (IT) were coded according to the system of Browder and Young (1975) on a scale of 0–9, where 0 = no sporulation, tiny necrotic flecks and 9 = large urediosori, abundant sporulation, and no host tissue reaction around the sori. A rating of 0–4 was considered resistant (R), 5–7 moderately resistant (MR), and 8–9 as susceptible (S).

DNA isolation. DNA extraction was carried out at the Department of Ecology and Nature Protection (Plant Biodiversity Conservation Laboratory) of the Yerevan State University in Armenia. DNA

Table 1. List of the studied diploid wheat (*Triticum boeoticum* and *T. urartu*) accessions; IG accession numbers refer to ICARDA, TRI numbers to IPK Gatersleben; for *T. boeoticum* and *T.urartu* collected in the wild, longitude, latitude and altitude (m a.s.l.) are provided where known

No.	Species	Accession No.	Origin	Longitude	Latitude	Altitude
1	Bezostaya 1 control cultivar	Seed collection of Agrarian State University	Soviet Union	–	–	–
2	*T. boeoticum*	IG 126246	Armenia (Yerevan)	E4436	N4010	1225
3	*T. boeoticum*	IG 126257	Armenia (Yerevan)	E4437	N4011	1515
4	*T. boeoticum*	IG 131174	Armenia (Yerevan)	E4446	N4009	1490
5	*T. boeoticum*	Erebuni Reserve	Armenia			1350
6	*T. boeoticum*	IG 44938	Armenia (Kotayk)	E4438	N4017	1300
7	*T. boeoticum*	IG 44939	Armenia (Kotayk)	E4438	N4012	1400
8	*T. boeoticum*	IG 137335	Armenia (Kotayk)	E04436	N400904	1093
9	*T. boeoticum*	IG 140979	Armenia (Kotayk)	E443617	N400009	1093
10	*T. boeoticum*	IG 44941	Armenia (Ararat)	E4450	N3950	1250
11	*T. boeoticum*	IG 126381	Armenia (Ararat)	E445943	N394902	1740
12	*T. boeoticum*	IG 126286	Armenia (Ararat)	E450001	N394913	1770
13	*T. boeoticum*	IG 137477	Armenia (Ararat)	E04500	N394929	1765
14	*T. boeoticum*	IG 137392	Armenia (Ararat)	E04455	N395209	1427
15	*T. boeoticum*	IG 126380	Armenia (Vayots Dzor)	E450949	N3945	1075
16	*T. boeoticum*	IG 137409	Armenia (Vayots Dzor)	E04507	N394918	1556
17	*T. boeoticum*	IG 44819	Turkey (Kayseri)	E3630	N3848	1510
18	*T. boeoticum*	IG 44823	Turkey (K. Maras)	E3735	N3823	1780
19	*T. boeoticum*	IG 44824	Turkey (Elazig)	E3904	N3837	1160
20	*T. boeoticum*	IG 44858	Turkey (Canakkale)	E2637	N4017	30
21	*T. boeoticum*	IG 44864	Turkey (Balikesir)	E2801	N4003	60
22	*T. boeoticum*	IG 44867	Turkey (Bursa)	E2906	N4020	250
23	*T. boeoticum*	IG 44870	Turkey (Tagirdag)	E2802	N4108	100
24	*T. boeoticum*	IG 44878	Turkey (Ankara)	E3228	N3927	850
25	*T. boeoticum*	IG 44881	Turkey (Konya)	E3303	N3717	700
26	*T. boeoticum*	IG 44882	Turkey (Kayseri)	E3559	N3829	1140
27	*T. boeoticum*	IG 44887	Turkey (Sivas)	E3715	N3949	970
28	*T. boeoticum*	IG 44892	Turkey (Urfa)	E3849	N3711	660
29	*T. boeoticum*	IG 44907	Turkey (Van)	E4330	N3833	2100
30	*T. boeoticum*	IG 44908	Turkey (Malatya)	E3813	N3834	675
31	*T. urartu*	TRI 6735	Armenia			–
32	*T. urartu*	IG 45213	Armenia	–	–	–
33	*T. urartu*	IG 45219	Armenia	–	–	
34	*T. urartu*	–	Armenia (Erebuni)	–	–	1350
35	*T. urartu*	IG 116203	Iran (East Azerbaijan)	E371054	N355515	1240
36	*T. urartu*	IG 46066	Turkey (Gaziandep)	E3730	N3658	870
37	*T. urartu*	IG 116192	Turkey (Gaziandep)	E371349	N364220	700
38	*T. urartu*	IG 45107	Turkey (Gaziandep)	E4006	N3713	465
39	*T. urartu*	IG 45108	Turkey (Mardin)	E3946	N3713	600
40	*T. urartu*		Turkey (Urfa)			600

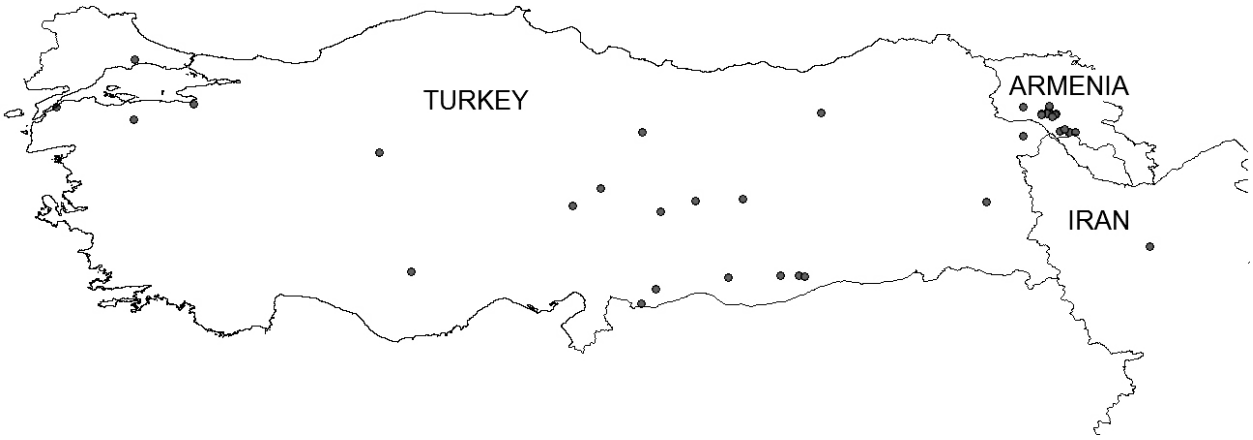

Figure 1. Collection sites of the studied accessions of *Triticum boeoticum* and *T. urartu*

from young, seven-days-old seedlings was extracted by the application of a modified Doyle protocol with addition of activated charcoal (Doyle & Doyle 1990). Fresh leaves (five plants of each accession, 5 mg) were harvested to 2 ml tubes, frozen at –20°C at least one day before the day of extraction. After grinding the material with the pestle, 700 µl extraction buffers with 1 w/v activated charcoal (warmed to 65°C) were added. The samples were incubated at 65°C in water bath for 35 min. After incubation, 700 µl of a mixture of chloroform and isoamyl alcohol (25:1/vol.) (Sigma-Aldrich, Taufkirchen, Germany) were added and shaken strongly. After centrifugation (10 min at 8000 rpm), the top phase was transferred to a new Eppendorff tube. The chloroform–isoamyl alcohol extraction was repeated once again. The supernatant was then transferred to a new Eppendorff tube and added with 85 µl of acetate mix (6 parts of 3M NaOAc solution and 5 parts of 10M NH_4OAc solution) and 500 µl isopropanol. The precipitate was centrifuged at 13 000 rpm for 30 min, the supernatant was removed and the DNA was washed with 1 ml of 70% ethanol, and then centrifuged for 10 min at 13 000 rpm. The pellet was air-dried and resolved with 100 µl TE buffer. The extracts were stored at –20°C until use.

PCR amplification and gel electrophoresis. PCR amplification and gel electrophoresis were performed at the Department of Ecology and Nature Protection (Plant Biodiversity Conservation Laboratory) of the Yerevan State University (Armenia) and at the Plant Genome Resources Centre (PGRC) of IPK (Germany). Each sample contained 0.05 µl of Taq polymerase (PGRC of IPK, Germany, and Evrogen, Russia), 2.5 µl PCR buffer, 10µM of forward/reverse primers (Table 2), 2.5mM of each dNTP and about 50 ng of plant DNA (Evrogen Moscow, Russia; Eurofins MWG Operon, Huntsville, USA). PCR conditions [Biokom PCR amplifier (Armenia), Geneamp PCR system 9700 and SansQest labcycler (Germany)] were used and the primer sets are listed in Table 3 (Stepień *et al.* 2002). Molecular STS analysis protocols for screening wild accessions of *Triticeae* for genes of disease resistance were optimized.

PCR amplified STS DNA fragments were electrophoresed in 2% agarose gels at 110 V for about 2 h, stained with ethidium bromide, visualized under UV light and photographed. Gel images were captured in TIFF files and analyzed. In all cases the amplified PCR fragments were clearly identified and results found to be comparable and reproducible.

Products corresponding to seven STS markers of the resistance genes were amplified successfully from DNA of the *T. boeoticum* and *T. urartu* accessions studied. The sizes of the marker fragments were: *Pm1* – 400 bp, *Pm2* – 600 bp, *Pm3* – 610 bp; *Lr10* – 310 bp, *Lr47* – 282 bp, *Lr28* – 380 bp, *Lr25* – 1800 bp.

RESULTS AND DISCUSSION

The marker of the powdery mildew resistance gene *Pm1* located on the 7A chromosome was found in almost all accessions studied and was confirmed in bread wheat Bezostaya 1 used as a positive control (Figure 2a). Markers for the *Pm2* gene were identified in ten out of 29 accessions of *T. boeoticum*, among which seven accessions are from Armenia (Yerevan – 3, Erebuni, Kotayk – 2, and Vayots Dzor), and three are from

Table 2. Sequences, marker sets and reference for primers used to identify resistance genes in accessions of *Triticum boeoticum*

Gene	Original marker	Gene STS marker set	Sequence of primers 5'-3'	Annealing temperature (°C)	Product size (bp)	Reference
Pm1	RAPD UBC 320	C320-1 C320-2	fwd: CCG GCA TAG ATC GAG AAT AG rev: CCG GCA TAG AAC TTT AAG CG	60	420	Hu *et al.* (1997)
Pm2	RFLP Whs 350	Whs 350-1 Whs 350-2	fwd: AGC TGT TTG GGT ACA AGG TG rev: TCC CCT GTG CTA CTA CTT CTC	60	598	Mohler and Jahoor (1996)
Pm3	RFLP	SSR	fwd: TCC CGC CAT GAG TCA ATC rev: TTG GGA GAC ACA TTG GCC	60	610	McIntosh *et al.* (1995)
Lr10	RFLP MWG2245	F1.2245 Lr10-6/r2	fwd: GTG TAA TGC ATG CAG GTT CC rev: AGG TGT GAG TGA GTT ATG TT	57	310	Schachermayr *et al.* (1997)
Lr28	RAPD OPJ 01	Lr28-01 Lr28-02	fwd: CCC GGC ATA AGT CTA TGG TT rev: CAA TGA ATG AGA TAC GTG AA	50	378	Naik *et al.* (1998)
Lr25	RAPD/ DGGE	Lr25F 20 Lr25R19	fwd: CCA CCC AGA GTA TAC CAG AG rev:CCA CCC AGA GCT CAT AGA A	58	1800	Procunier *et al.* (1995), Procunier (s.a.)
Lr47	CAPS	PS10R PS10L	GCT GAT GAC CCT GAC CGG T TCT TCA TGC CCG GTC GGG T	55	282	Dubcovsky *et al.* (1998), Dubcovsky (s.a.)

Table 3. PCR amplification programmes used for different primer combinations of STS marker sets

STS marker	PCR programme
Pm1	94°C – 4 min, 35 cycles (94°C – 1 min, 60°C – 1.5 min, 72°C – 2 min), 72°C – 5 min
Pm2	94°C – 4 min, 35 cycles (94°C – 1 min, 60°C – 2 min 72°C – 1 min), 72°C – 5 min
Pm3	94°C – 4 min, 35 cycles (92°C – 1 min, 60°C – 1 min, 72°C – 2 min), 72°C – 5 min
Lr47	94°C – 3 min; 7 cycles (94°C – 0.5 min; 70°C – 64°C – 0.5 min; 72°C – 0.5 min); 35 cycles (94°C – 0.5 min; 64°C – 0.5 min; 72°C – 0.5 min), 72°C – 10 min
Lr25	95°C – 2.5 min; 7 cycles (94°C – 45 s; 62 – 56°C – 45 s; 72°C – 45 min); 72°C – 10 min
Lr10	94°C – 3 min, 35 cycles (94°C – 45 s, 57°C – 45 s, 72°C – 30 s), 72°C – 3 min
Lr28	94°C – 6 min, 35 cycles (94°C – 1 min, 50°C – 1 min, 72°C – 2 min), 72°C – 5 min

Turkey (Elazig, Canakkale, and Baliksesir). The marker for the *Pm2* gene was also identified in seven out of 10 accessions of *T. urartu* (Figure 2b), as well as in the control, Bezostaya 1. According to McIntosh *et al.* (1998), the *Pm2* gene is located on the wheat chromosome 5D. In our experiments, the STS marker for the *Pm2* gene was identified in *T. boeoticum* and *T. urartu* accessions (Figure 2b). According to Stepień *et al.* (2001) this marker is not reliable in wheat accessions of diverse genetic backgrounds due to lack of strong linkage to resistance genes.

Gene markers for *Pm3* (located on wheat chromosome 1A) have been identified in ten accessions of *Triticum boeoticum* and one *T. urartu* accession collected from Iran (Figure 2c). All *T. boeoticum* accessions collected from the Ararat valley carry the *Pm3* gene. This marker has been identified in accessions collected from different sites of Turkey located far from each other, viz. Konya, Tagirtak and Bursa.

A marker for the gene *Lr10* located on chromosome 1A was identified in eight accessions of *T. boeoticum* collected from Armenia and Turkey, and in seven *T. urartu* accessions (Figure 2d).

A marker for the gene *Lr28* located on chromosome 4A was identified in 16 accessions of *T. boeoticum*, out of which only three accessions were collected in Armenia (Figure 2e). According to our results, this gene is mostly distributed in Turkey. A marker for the gene *Lr28* has also been identified in four *T. urartu* accessions from Turkey and Iran, but not in Armenian material (ICARDA and IPK genebanks). The presence of markers for the genes *Lr10* and *Lr28* was confirmed in the control, Bezostaya 1 (Figures 2d, e).

Markers for the gene *Lr25* located on chromosome 1A were identified in twenty accessions of *T. boeoticum* collected from Armenia and Turkey, but not in *T. urartu* accessions (Figure 2f).

Markers for the gene *Lr47* located on chromosome 7A were identified only in eight accessions of *T. boeoticum* collected in Armenia and Turkey as well as in three accessions of *T. urartu* from the Gaziandep region in Turkey. The presence of markers for the genes *Lr25* and *Lr47* was confirmed in the control, Bezostaya 1 (Figures 2f, g).

Most of the studied accessions were resistant to powdery mildew and to leaf rust. The results of inoculation tests showed that accessions with only the *Pm1* gene were susceptible or moderately susceptible to powdery mildew (Table 4).

Accessions where the *Pm2* gene was identified in combination with the *Pm1* gene marker were resistant to a mixture of powdery mildew isolates. So, the resistance to *B. graminis* in our experiments can be attributed to the *Pm2* gene. The results showed that in *T. boeoticum* and *T. urartu* accessions, the presence of *Pm3* and *Pm1* markers was effective in conferring resistance to a mixture of *B. graminis* isolates. In the studied accessions the resistance to powdery mildew is conferred to the presence of a combination of *Pm1* with *Pm2* or *Pm3* genes. The gene markers for *Pm1* and *Pm3* appear to be useful for tracking the resistance genes, but the *Pm2* gene marker cannot be reckoned as specific and tightly linked to resistance genes.

The results of the evaluation of resistance to *Puccinia recondita* show that two accessions of *T. urartu* (39 and 40) were susceptible, while others were moderately to highly resistant. The susceptible or moderately resistant accessions of *T. urartu* were identified as carriers of the single *Lr10* gene. The accessions of *T. urartu* collected from Turkey were identified with a marker for the *Lr10* gene, but were susceptible to a mixture of Armenian isolates of *Puccinia recondita*. Simultaneously the accessions of *T. urartu* collected from Armenia were moderately resistant to the studied isolates and were identified with *Lr10* gene markers. For the susceptible accessions of *T. urartu*, the possible explanation might be the virulence of the isolates from Armenian ecosystems. For moderately resistant accessions identified with the *Lr10* gene marker, it can be assumed that the *Lr10* gene in Armenian accessions is partially suppressed, or its product synthesis is inhibited at a certain stage, or that resistance genes are present that have not been tested in this study. This explanation is in compliance with literature. It is known that the resistance gene expression is dependent on the genetics of host-parasite interaction, temperature conditions, plant developmental stage, and interaction between resistance genes with suppressors or other resistance genes in the wheat genomes (Kolmer 1996). The presence of the gene-specific suppressors for resistance in the A and AB genomes was discussed by different authors (Ma *et al.* 1997; Singh *et al.* 2007). So, the results obtained show the importance of further investigations of this gene and its alleles for local and regional breeding programmes. Resistance to leaf rust could be obtained by variable combinations of the resistance genes studied.

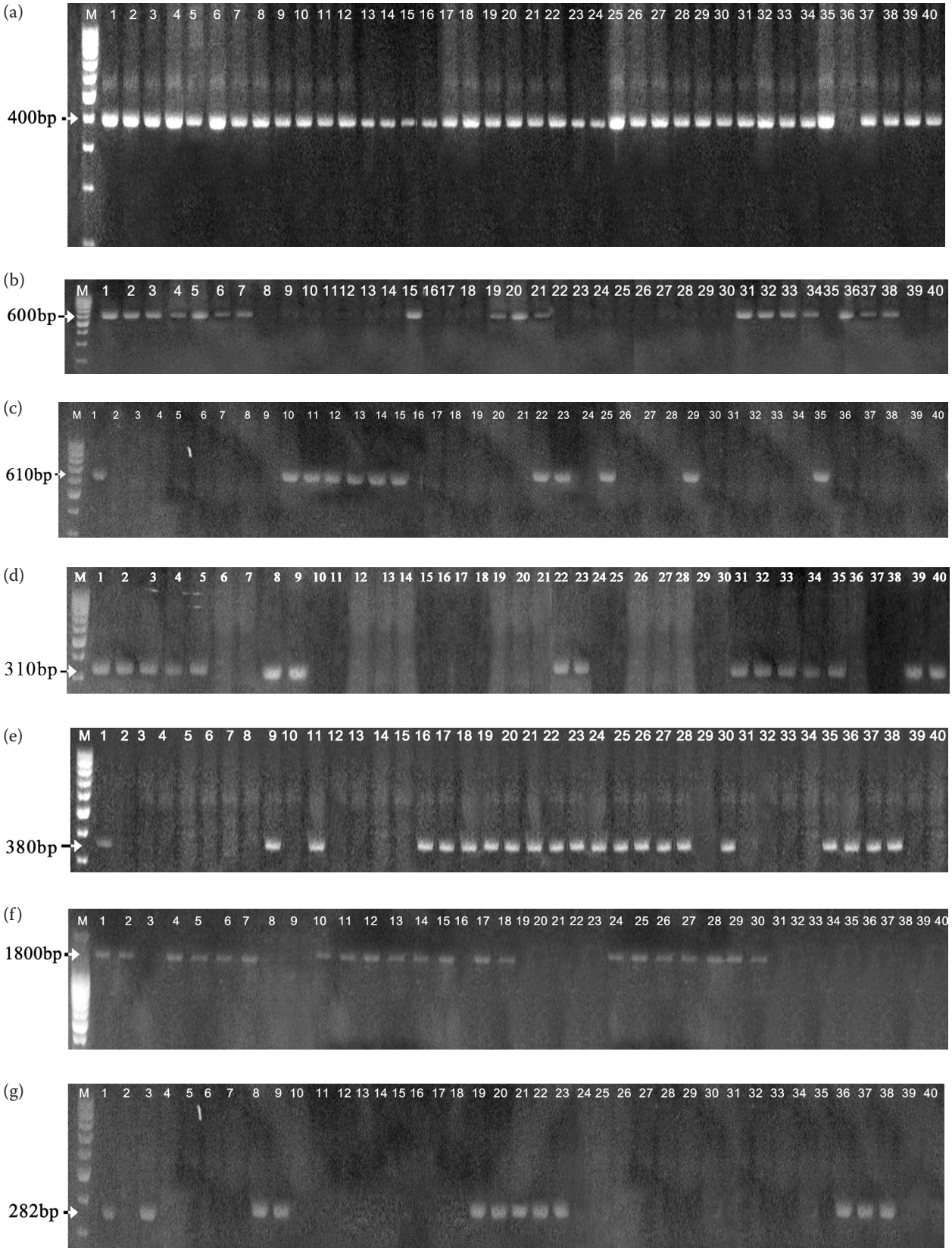

Figure 2. STS gene markers for resistance genes in the studied accessions of wild diploid *Triticum* species (cf. Table 1): (a) *Pm1* gene, (b) *Pm2* gene, (c) *Pm3* gene, (d) *Lr10* gene, (e) *Lr28* gene, (f) *Lr25* gene, (g) *Lr47* gene; M = weight marker (100 bp ladder – Fermentas)

Table 4. Results of inoculation experiments and DNA STS analyses of the wild *Triticum* accessions; for accession numbers and origin information of the material see Table 1

No.	Field resistance to *Blumeria graminis* isolates mixture	Resistance to isolates mixture		*Pm1*	*Pm2*	*Pm3*	*Lr10*	*Lr47*	*Lr25*	*Lr28*
		Blumeria graminis	*Puccinia recondita*							
1	R	R	R	+	+	+	+	+	+	+
2	R	R	R	+	+	–	+	–	+	–
3	R	R	R	+	+	–	+	+	–	–
4	R	R	R	+	+	–	+	–	+	–
5	R	R	R	+	+	–	+	–	+	–
6	R	R	R	+	+	–	–	–	+	–
7	R	R	R	+	+	–	–	–	+	–
8	MS	MS	R	+	–	–	+	+	–	–
9	MS	S	R	+	–	–	+	+	–	+
10	R	R	R	+	–	+	–	–	+	–
11	R	R	R	+	–	+	–	–	+	+
12	R	R	R	+	–	+	–	–	+	–
13	MR	R	R	+	–	+	–	–	+	–
14	R	R	R	+	–	+	–	–	+	–
15	R	R	R	+	+	+	–	–	+	–
16	MS	MS	R	+	–	–	–	–	–	+
17	S	S	R	+	–	–	–	–	+	+
18	S	S	R	+	–	–	–	–	+	+
19	R	R	R	+	+	–	–	+	–	+
20	R	R	R	+	+	–	–	+	–	+
21	R	R	R	+	+	–	–	+	–	+
22	R	R	R	+	–	+	+	+	–	+
23	MR	MR	R	+	–	+	+	+	–	+
24	S	S	R	+	–	–	–	–	+	+

Table 4 to be continued

No.	Field resistance to *Blumeria graminis* isolates mixture	Resistance to isolates mixture		*Pm1*	*Pm2*	*Pm3*	*Lr10*	*Lr47*	*Lr25*	*Lr28*
		Blumeria graminis	*Puccinia recondita*							
25	R	R	R	+	–	+	–	–	+	+
26	MS	S	R	+	–	–	–	–	+	+
27	MS	S	R	+	–	–	–	–	+	+
28	S	S	R	+	–	–	–	–	+	+
29	R	R	R	+	–	+	–	–	+	–
30	S	S	R	+	–	–	–	–	+	+
31	R	R	MR	+	+	–	+	–	–	–
32	R	R	MR	+	+	–	+	–	–	–
33	R	R	MR	+	+	–	+	–	–	–
34	R	R	MR	+	+	–	+	–	–	–
35	R	R	R	+	–	+	+	–	–	+
36	MR	R	R	–	+	–	–	+	–	+
37	R	R	R	+	+	–	–	+	–	+
38	R	R	R	+	+	–	–	+	–	+
39	S	S	S	+	–	–	+	–	–	–
40	S	S	S	+	–	–	+	–	–	–

Blumeria graminis: R = resistant (0–1), MR = moderately resistant (2), MS = moderately susceptible (3), S = susceptible (4); *Puccinia recondita*: R = resistant (0–4), MR = moderately resistant (5–7), S = susceptible (8–9)

Triticum boeoticum accessions with markers for the *Lr25* gene have been identified. No such markers were found in *T. urartu*. The results of inoculation analyses have shown that *T. boeoticum* accessions with the *Lr25* gene were resistant to leaf rust. The accessions carrying one of the following combinations of markers: *Lr10* + *Lr25*, *Lr10* + *Lr28*, *Lr10* + *Lr47*, *Lr25* + *Lr28*, *Lr47* + *Lr28*, *Lr10* + *Lr28* + *Lr47*, were resistant to leaf rust. It can be concluded for the material studied that the *Lr25* gene is effective in conferring resistance to leaf rust in *T. boeoticum* accessions, while in *T. urartu* accessions the combinations *Lr28* and *Lr47* can be effective in conferring the resistance to leaf rust. The *Lr10* gene is effective in combination with *Lr25* or *Lr28.* The gene markers for *Lr25*, *Lr28* and *Lr47* and their combinations seem to be useful for the identification of leaf rust resistance genes.

The selection of genotypes with combinations of non-race specific resistance genes defining durable resistance as well as race-specific genes at seedling stage is a task of prime importance for molecular marker-assisted breeding. As demonstrated in this paper, the use of STS markers can help to identify desirable genes more precisely and will facilitate the transfer of resistance genes into wheat cultivars. However, for an effective application of DNA gene markers, further research is needed to confirm their reliability in diverse genetic backgrounds, which is especially important in wheat due to the complex structure of its hexaploid constitution.

Acknowledgements. The study was supported by an International Foundation for Agricultural Research (IFAR) fellowship programme through Bioversity International. The authors would like to thank ICARDA and IPK Gatersleben, Germany, for providing samples of the genebank accessions for this study. We thank Dr. M.S. Röder, Gatersleben, for valuable scientific and technical assistance.

References

Anker C.C., Niks R.E. (2001): Prehaustorial resistance to the wheat leaf rust fungus, *Puccinia triticina*, in *Triticum monococcum* (s.s.). Euphytica, **117**: 3–12.

Bahraei S., Jaradat A.A. (1998): Diversity in seed storage proteins of *T. boeoticum* and *T. urartu*. In: Jaradat A.A. (ed.): Proc. 3rd Int. Triticeae Symposium Triticeae III. Aleppo, 237–243.

Bennett F.G. (1984): Resistance to powdery mildew in wheat: a review of its use in agriculture and breeding programmes. Plant Pathology, **33**: 279–300.

Błaszczyk L., Chełkowski J., Korzun V., Kraič J., Ordon F., Ovesná J., Purnhauser L., Tar M., Vida G. (2004): Verification of STS markers for leaf rust resistance genes of wheat by seven European laboratories. Cellular and Molecular Biology Letters, **9**: 805–817.

Browder L.E. (1971): Pathogen specialization in cereal rust fungi, especially *Puccinia recondita* f.sp. *tritici*: Concepts, methods of study and application. USDA Agricultural Research Service Technical Bulletin No. 1432.

Browder L.E., Young H.C. (1975): Further development of an infection-type coding system for the cereal rusts. Plant Disease Reporter, **59**: 964–965.

Chełkowski J., Golka L., Stepień L. (2003): Application of STS markers for leaf rust resistance genes in near-isogenic lines of spring wheat cv. Thatcher. Journal of Applied Genetics, **44**: 323–338.

Cherukuri D.P., Gupta S.K., Charpe A., Koul S., Prabhu K.V., Singh R.B., Haq Q.M.R., Chauhan S.V.S., Weber W.E. (2003): Identification of a molecular marker linked to an *Agropyron elongatum*-derived gene *Lr19* for leaf rust resistance in wheat. Plant Breeding, **122**: 204–208.

Cherukuri D.P., Gupta S.K., Charpe A., Koul S., Prabhu K.V., Singh R.B., Haq Q.M.R. (2005): Molecular mapping of *Aegilops speltoides* derived leaf rust resistance gene *Lr28* in wheat. Euphytica, **143**: 19–26.

Dorofeev V.F., Filatenko A.A., Migushova E.F., Udaczin R.A., Jakubziner M.M. (1979): Wheat. In: Dorofeev V.F., Korovina O.N. (eds): Flora of Cultivated Plants, Vol. 1. Kolos, Leningrad. (in Russian)

Doyle J.J., Doyle J.L. (1990): Isolation of plant DNA from fresh tissue. Focus, **12**: 13–15.

Dubcovsky J. (s.a.): Disease resistance. Leaf rust. *Lr47.* MAS Wheat. Available at http://maswheat.ucdavis.edu/protocols/Lr47/index.htm (accessed March 15, 2011)

Dubcovsky J., Lukaszewski A.J., Echaide M., Antonelli E.F., Porter D.R. (1998): Molecular characterization of two *Triticum speltoides* interstitial translocations carrying leaf rust and greenbug resistance genes**.** Crop Science, **38**: 1655–1660.

FAO (2008): Wheat Rust Disease Global Programme. Resource Document. Available at ftp://ftp.fao.org/docrep/fao/011/i0378e/i0378e.pdf (accessed August 16, 2010)

Gandilyan P.A., Avagyan W.A. (2001): Erebuni Reservation. Tigran Mets, Yerevan.

Gill B.S., Friebe B., Raupp W.J., Wilson D.L., Cox T.S., Sears R.G., Brown-Guedira G.L., Fritz A.K.

(2006): Wheat Genetics Resource Center: the first 25 years. Advances in Agronomy, **85**: 73–135.

Gupta P.K., Varshney R.K., Sharma P.C., Ramesh B. (1999): Molecular markers and their applications in wheat breeding. Plant Breeding, **118**: 369–390.

Haykazyan V., Pretty J. (2006): Sustainability in Armenia: New Challenges for the Agricultural Sector. University of Essex, Colchester.

Hiebert C., Thomas J., McCallum B. (2005): Locating the broad-spectrum wheat leaf rust resistance gene *Lr52* (*LrW*) to chromosome 5B by a new cytogenetic method. Theoretical and Applied Genetics, **110**: 1453–1457.

Hsam S.L.K., Lapochkina I.F., Zeller F.J. (2003): Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.). 8. Gene *Pm32* in a wheat-*Aegilops speltoides* translocation line. Euphytica, **133**: 367–370.

Hu X.Y., Ohm H.W., Dweikat I. (1997): Identification of RAPD markers linked to the gene *Pm1* for resistance to powdery mildew in wheat. Theoretical and Applied Genetics, **94**: 832–840.

Hua W., Liu Z., Zhu J., Xie C., Yang T., Zhou Y., Duan X., Sun Q., Liu Z. (2009): Identification and genetic mapping of *Pm42*, a new recessive wheat powdery mildew resistance gene derived from wild emmer (*Triticum turgidum* var. *dicoccoides*). Theoretical and Applied Genetics, **119**: 223–230.

Knüpffer H. (2009): *Triticeae* genetic resources in *ex situ* genebank collections. In: Feuillet C., Muehlbauer G. (eds.): Genetics and Genomics of the *Triticeae*. Plant Genetics and Genomics: Crops and Models 7, Springer Science+Business Media, LLC, 31–79.

Knüpffer H., Morrison L.A., Filatenko A.A., Hammer K., Morgounov A., Faberová I. (2002): English translation of the 1979 Russian taxonomic monograph of *Triticum* L. by Dorofeev *et al.*: project progress report. In: Hernández P., Moreno M.T., Cubero J.I., Martin A. (eds): Proc. 4th Int. Triticeae Symposium Triticeae IV. September 10–12, 2001, Córdoba, 49–55.

Kolmer J.A. (1996): Genetics of resistance to wheat leaf rust. Annual Review of Phytopathology, **34**: 435–55.

Liu Z., Sun Q., Ni Z., Nevo E., Yang T.M. (2002): Molecular characterization of a novel powdery mildew resistance gene *Pm30* in wheat originating from wild emmer. Euphytica, **123**: 21–29.

Ma H., Singh R.P., Mujeeb-Kazi A. (1997): Resistance to stripe rust in durum wheats, A-genome diploids, and their amphiploids. Euphytica, **94**: 279–286.

MacKenzie D. (2007): Billions at risk form wheat superblight. New Scientist, **194**: 6–7.

Mago R., Miah H., Lawrence G.J., Wellings C.R., Spielmeyer W., Bariana H.S., McIntosh R.A., Pryor A.J., Ellis J.G. (2005): High-resolution mapping and mutation analysis separate the rust resistance genes *Sr31*, *Lr26*, and *Yr9* on the short arm of rye chromosome 1. Theoretical and Applied Genetics, **112**: 41–50.

McIntosh R.A., Hart G.E., Gale M.D. (1995): Catalogue of gene symbols for wheat. In: Li Z.S., Xin Z.Y. (eds): Proc. 8th Int. Wheat Genetics Symposium. Beijing, 1993, 1333–1500.

McIntosh R.A., Hart G.E., Devos K.M., Gale M.D., Rogers W.J. (1998): Catalogue of gene symbols for wheat. In: Proc. 9th Int. Wheat Genet. Symposium Saskatchewan.

McIntosh R.A., Appels R., Devos K.M., Dubcovsky J., Rogers W.J., Yamazaki Y. (2003): Catalogue of gene symbols for wheat. In: Proc. 10th Int. Wheat Genet. Symposium Roma, 1–34.

Mohler V., Jahoor A. (1996): Allele-specific amplification of polymorphic sites for the detection of powdery mildew resistance loci in cereals. Theoretical and Applied Genetics, **93**: 1078–1082.

Moseman J.G., Nevo E., Morshidy M.A., Zohary D. (1984): Resistance of *Triticum dicoccoides* to infection with *Erysiphe graminis tritici*. Euphytica, **33**: 41–47.

Naik S., Gill K.S., Prakasa Rao V.S., Gupta V.S., Tamhankar S.A., Pujar S., Gill B.S., Ranjekar R.K. (1998): Identification of a STS marker linked to the *Aegilops speltoides*-derived leaf rust resistance gene *Lr28* in wheat. Theoretical and Applied Genetics, **97**: 535–540.

Obert D.E., Fritz A.K., Moran J.L., Singh S., Rudd J.C., Menz M.A. (2005): Identification and molecular tagging of a gene from PI 289824 conferring resistance to leaf rust (*Puccinia triticina*) in wheat. Theoretical and Applied Genetics, **110**: 1439–1444.

Ovesná J., Kučera L., Bocková R., Holubec V. (2002): Characterisation of powdery mildew resistance donors within *Triticum boeoticum* accessions using RAPDs. Czech Journal of Genetics and Plant Breeding, **38**: 117–124.

Pathan A.K., Park R.F. (2006): Evaluation of seedling and adult plant resistance to leaf rust in European wheat cultivars. Euphytica, **149**: 327–342.

Prabhu K.V., Gupta S.K., Charpe A., Koul S. (2004): SCAR marker tagged to the alien leaf rust resistance gene *Lr19* uniquely marking the *Agropyron elongatum*-derived gene *Lr24* in wheat: a revision. Plant Breeding, **123**: 417–420.

Priestley R.H., Bayles R.A. (1998): The contribution and value of resistant cultivars to disease control in

cereals. In: Clifford B.C., LesteR E. (eds): Control of Plant Disease-costs and Benefits. Blackwell, Oxford, 53–65.

Procunier J.D. (s.a.): Disease resistance. Leaf rust resistance. *Lr29 – Lr25*. MAS Wheat. Available at http://maswheat.ucdavis.edu/protocols/lr29/index.htm (accessed March15, 2011)

Procunier J.D., Townley-Smith T.F., Fox S., Prashar S., Gray M., Kim W.K., Czarnecki E., Dyck P.L. (1995): PCR-based RAPD/DGGE markers linked to leaf rust resistance genes *Lr29* and *Lr25* in wheat (*Triticum aestivum* L.). Journal of Genetics and Breeding, **49**: 87–92.

Qi L., Friebe B., Zhang P., Gill B.S. (2007): Homoeologous recombination, chromosome engineering and crop improvement. Chromosome Research, **15**: 3–19.

Schachermayr G., Feuillet C., Keller B. (1997): Molecular markers for the detection of the wheat leaf rust resistance gene *Lr10* in diverse genetic backgrounds. Molecular Breeding, **3**: 65–74.

Singh K., Chhuneja P., Ghai M., Kaur S., Goel R.K., Bains N.S., Keller B., Dhaliwal H.S. (2007): Molecular mapping of leaf and stripe rust resistance genes in *Triticum monococcum* and their transfer to hexaploid wheat. In: Buck H., Nisi J.E., Solomon N. (eds): Wheat Production in Stressed Environments. Developments in Plant Breeding, Springer, Netherlands, **12**: 779–786.

Stepień L., Chen Yu., Chełkowski J., Kowalczyk K. (2001): Powdery mildew resistance genes in wheat: verification of STS markers. Journal of Applied Genetics, **42**: 413–423.

Stepień L., Holubec V., Chełkowski J. (2002): Resistance genes in wild accessions of *Triticeae* – inoculation test and STS marker analyses. Journal of Applied Genetics, **43**: 423–435.

Stokstad E. (2007): Plant pathology: Deadly wheat fungus threatens world's breadbaskets. Science, **315**: 1786–1787.

Todorovska E., Christov N., Slavov S., Christova P., Vassilev D. (2009): Biotic stress resistance in wheat – breeding and genomic selection implications. Biotechnology & Biotechnological Equipment, **23**: 1417–1426.

Vurro M., Bonciani B., Vannacci G. (2010): Emerging infectious diseases of crop plants in developing countries: impact on agriculture and socio-economic consequences. Food Security, **2**: 113–132.

Zeller F.J., Kong L., Hartl L., Mohler V., Hsam S.L.K. (2002): Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.). 7. Gene *Pm29* in line Pova. Euphytica, **123**: 187–194.

Zhukovsky P.M. (1971): Crop Plants and their Wild Relatives. Kolos, Leningrad. (in Russian)

Received for publication December 5, 2010
Accepted after corrections April 13, 2011

Corresponding author:

Dr. Nelli Hovhannisyan, PhD., Yerevan State University, Faculty of Biology, Department of Ecology and Nature Protection, Alex Manoogian Str. 1, 0025 Yerevan, Armenia
e-mail: bionellibiotech@yahoo.com