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Full Length Research Paper

Identifying leaf rust resistance gene *Lr*19 in durum wheat using simple sequence repeat (SSR) marker

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Leaf rust, caused by *Puccinia triticina* Eriks., is an important disease affecting durum wheat (*Triticum turgidum* ssp. *durum*) worldwide, particularly in the Mediterranean region. The disease can be controlled through the use of plant host resistance. Based on seedling resistance tests of 103 durum genotypes against a bulk of *P. triticina*, urediniospores were previously collected from Syria and Lebanon during 2007/08 and 2008/09 growing seasons. Percentage of resistance in the durum set tested was up to 52%. The resistant genotypes might have one of the following resistance gene(s): *Lr*15, *Lr* 19, *Lr* 24, *Lr* 25, *Lr* 27 +31, *Lr* 28 and *Lr* 29. Results revealed that the Gb/130-bp polymorphic band was linked to *Lr*19 and *Sr*25. In this study, twelve genotypes carrying resistance to *Lr*19 and *Sr*25 have been identified (Azeghar2, Rutucha1, Ammar9/Azeghar2, Ammar9/Terbol97-4, *T.polonicum*9/Ch1//Icamor-TA04-68/3/Icamor-TA04-69//(Lahn/ Ch1)2519, Arislahn5//Icamor-TA04-63/Icasyr1, *T.dicoccum*1/Ch1//Ammar8/3/Bonadur/Icamor-TA04-63, Mrb3/ *T. urartu*500651/4/Icamor-TA04-63/3/Bcr/Gro1// Mgnl1, SwAlg/Gd1-81//Ch1-48, Icamor-TA04-1//Mgnl3/Ainzen1, 319-ADDO/5/D68-1-93A-1A//Ruff/Fg/3/Mtl5/4/Lahn, and Mrf1/Stj2/3/1718/BT24//Karim). Promising results on Gb/130-bp and genotypes carrying *Lr*19 and *Sr*25 will be used in a marker assisted selection of the durum breeding programs in the Mediterranean region.

Key words: *Puccinia triticina*, durum genotypes, Syria, Lebanon, *Lr*19, *Sr*25, Gb primer.

INTRODUCTION

The leaf rust fungus, *Puccinia triticina* Eriks., is considered to be one of the most important pathogens in wheat production worldwide (Dehne and Oerke, 1998) where it causes significant yield losses every year. The disease can reduce yield by 1% for every 1% increase in the infection level (Khan et al., 1997). It also reduces the number of kernels per spike and kernel quality (Bremenkamp-Barrett et al., 2008). The level of damage inflicted by leaf rust varies with the degree of infection and host plant resistance. Under severe infection, susceptible wheat varieties can suffer yield losses (up to 40%) (Marasas et al., 2004). Fungicides can be used to control leaf rust to some extent. Using resistant cultivars is an economic and environmental friendly way of minimizing losses caused by the disease. Fifty-six leaf rust resistance genes have been designated and 51 of them have been mapped to corresponding chromosomes (Yang and Liu, 2004). *Lr*19 is one of the few widely effective genes conferring resistance to leaf rust in wheat. *Lr*19 still provides effective resistance against all leaf rust races in Syria, Lebanon and Turkey (Kassem et al., 2010). Ayala-Navarrete et al. (2007) had developed sequence-tagged-site (STS) markers for *Lr19* and *Sr25* from wheat ESTs and mapped them to chromosome 7DL (Qi et al., 2004), which was also found to be in the translocated segment of wild relative *Thinopyrum ponticum* in durum wheat (Gennaro et al., 2009). Leaf rust resistance gene Lr19 has been developed with biochemical markers (Winzeler et al., 1995), restriction fragment length poly-

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Abbreviations: STS, Sequence-tagged-site; **RFLP,** restriction fragment length polymorphism; **AFLP,** amplified fragment length polymorphism; **RH,** relative humidity; **SDS,** sodium-dodecyl-sulfate; **EtOH,** ethanol.

morphism (RFLP) (Autrique et al., 1995) and amplified fragment length polymorphism (AFLP), and were converted to STS (Prins et al., 2001). The aim of the present work was to identify a DNA marker for the leaf rust resistance gene *lr19* in resistant durum wheat genotypes.

MATERIALS AND METHODS

Sources of pathogen isolates

Wheat leaves samples infected by leaf rust (*P. triticina* Eriks.) were collected from the International Center for Agricultural Research in the Dry Areas (ICARDA) summer nursery at Terbol research station, Lebanon in 2007; and from farmers' wheat fields in Syria during the growing season 2007/08 and 2008/09. Urediniospores from each collection were used to inoculate 7-day-old seedlings of the susceptible variety (Morocco). After increasing all isolates, a bulk of urediniospores from each isolate was diluted with talcum powder. The mixture was sprayed on 7-day-old seedlings leaves of 103 durum wheat genotypes from ICARDA according to Xing et al. (2006). The inoculated seedlings were placed in plastic-covered cages as described by Wang et al. (2010). Following the inoculation, plants were transferred to mist chambers and incubated for 16 h in darkness at 18°C and approximately 100% relative humidity (RH). After misting period, plants were allowed to dry slowly for 4 h before being placed in a growth chamber at 18 to 20°C with a 16-h photoperiod according to Kolmer et al. (2007).

Scoring of leaf rust symptoms was performed 12 to 14 days after inoculation (Kolmer et al., 2009). Infection types (ITs) of *P. triticina* were quantified using a standard 0 to 4 scale, where 0 = no symptoms, 1 = small uredinia surrounded by necrosis, 2 = small uredinia surrounded by chlorosis, 3 = moderate size uredinia without chlorosis and 4 = large uredinia without chlorosis as described by Stakman et al. (1962) and Long and Kolmer (1989). Designations of + and - were used with the 0 to 4 scale to indicate larger and smaller than normal uredinia, respectively (Mebrate et al., 2008). Category 0 and 1 were considered as resistant, that of 2 and 3 were moderately resistant and moderately susceptible, respectively and that of 4 was considered highly susceptible (Chu et al., 2009).

DNA extraction

The method used to extract DNA is a sodium-dodecyl-sulfate (SDS) as described by Nachit et al. *(*2001) and Elouafi and Nachit (2004). Young leaves were collected at seedling stage (10 to 14 days old) of 55 resistant durum genotypes from ICARDA and the differential line TC*7/Tr (RL6040) carrying *Lr*19 resistance used as control, were quick frozen in liquid nitrogen and grinded. The powdered leaves were transferred to tubes and 20 to 25 ml of heated extraction buffer (65°C) (500 mM NaCl; 100 mM Tris- HCl pH 8.0; 50 mM EDTA; 0.84 SDS sodium bisulfate) at pH 8.0 was added to each tube. This mixture was incubated for 30 min at 65°C, with shaking after every 5 min. 20 ml of chloroform-isomyl alcohol (24:1) was added and the whole content was shaken vigorously to produce an emulsion. After centrifugation for 15 min at 2,800 rpm, the supernatant was recuperated, and 2 volumes of cold 95% ethanol (EtOH) (-20°C) was added. The DNA was precipitated and kept at -20°C for 30 to 60 min or for overnight. The precipitated DNA was afterward recuperated, and washed twice with cold ethanol 70% and dissolved in 500 µl of TE (10 mM Tris pH 8; 1 mM EDTA pH 8). The DNA was then treated by 2 µg/ml stock solution of RNAse for 30 min at 37°C and stored at -20°C until used. The concentration of DNA extract was determined by spectrophotometer (Amersham Biosciences- Gene Quant *pro*).

Microsatellites (SSR)

The Gb primer (Gb F 5'- CAT CCT TGG GGA CCT C -3', Gb R 5'- CCA GCT CGC ATA CAT CCA -3') was used (Prins et al., 2001). The Gb-PCR amplification was performed in a total volume of 25 µl reaction final concentrations of the reagents used in the PCR amplification 50 to 100 ng template DNA, 5 pmol of each primer, 1 unit of *Taq* DNA polymerase, 2 µl 15 mM $MgCl_2$, 2 µl 10X buffer, 2 µl 1 mM dNTPs. The PCR cycling program used was: Denaturing step, 5 min at 94°C; amplification step (40 cycles), 94°C for 30 s, 60°C for 30 s and 72°C for 1 min; extension step: 5 min at 72°C (Prins et al., 2001).

Electrophoresis

The resulting mixtures were denatured and loaded on a 5% denaturing polyacrylamide gel. After electrophoresis at 65 V constant voltages, the gels were silver-stained according to Nachit et al. (2001) and Elouafi and Nachit (2004).

RESULTS AND DISCUSSION

Results revealed a number of genotypes with resistance against the mixture of urediniospores (bulk) and the physiological races of *P. triticina* identified in Syria and Lebanon during 2007, 2008 and 2009 growing seasons. The bulk was highly virulent, with 81% of the available resistance gene(s) in the differential set being susceptible, including the resistance gene *Lr*9 which was broken in 2007/2008 season (Kassem et al., 2010). Whereas, only seven resistant gene(s) were found to still be effective against all races in Syria and Lebanon (*Lr*15, *Lr*19, *Lr*24, *Lr*25, *Lr*27+31, *Lr*28 and *Lr*29) at seedling and adult plant stages. Percentage of resistance in the ICARDA 103 durum set was up to 52% against the *Lr*-bulk collected in Syria and Lebanon. As for percentages of moderate resistance, moderate-susceptible and susceptible genotypes, they were 17.47, 14.56 and 15.53%, respectively.

Table 1 shows the genotypes resistant against the bulk of *P. triticina*. Analyzing the pedigree of these genotypes to identify the source of resistance revealed the following: Mtl5, Icamor-TA04, Icasyr1, Atlast1, Ruff and Bcr with probably major resistance genes, as the resistance was shown at seedlings and adult stage. These genotypes might have one of the resistance gene(s): *Lr*15, *Lr* 19, *Lr* 24, *Lr* 25, *Lr* 27 +31, *Lr* 28 and *Lr* 29. It is clear that some of these genotypes have more than one source of resis=-tance referred to earlier, such as genotypes Bcr/Lks4// Mrf1/Stj2/3/Icasyr1, Atlast1/961081//Icasyr1 and Gsbl1/4/ D68-1-93A-1A//Ruff/Fg/3/Mtl5, indicating accumulation of more than one resistance genes in one genotype. The program at ICARDA uses pyramiding of resistance genes (Nachit, pers. comm.). Therefore, these results show the importance of using these crossing models.

Both *Lr*19 and *Sr*25 genes were previously mapped to chromosome 7DL and positioned within confidence intervals delineated by molecular markers (Prins et al., 2001). In durum wheat, they are probably located on the

Table 1. Resistant genotypes against bulk *P. triticina*.

No	Genotype	No	Genotype	No	Genotype
1	Awalbit7	20	Icamor -TA04-1//Mgnl3/Ainzen1	39	Sbh/4/D68-1-93A-1A//Ruff/Fg/3/Mtl5
2	Rusomar3	21	Bcr/Lks4//Mrf1/Stj2/3/(Hau/Ch1)3109	40	Stk/Hau//Heca1
3	Stojocri6	22	Icamor -TA04-59/Miki3	41	Icasyr2
4	Mbar2	23	Icamor -TA04-72/Ammar7	42	Geromtel3
5	Azeghar2	24	Icamor -TA04-60/6/Terbol97-5/5/F4 13/3/Arthur71/Lahn//Blk2/Lahn/4/Quarmal	43	Icasyr1
6	Rutucha1	25	Ammar9/Terbol97-4	44	Sebatel1
7	Murlagost2	26	319-ADDO/5/D68-1-93A-1A//Ruff/Fg/3/Mtl5/4/Lahn	45	Icajihan1
8	Serene2	27	Bcrch1/DCD DW 7//Ossl1/Gdfl(1)	46	Younes1
9	Bcr/Gro1//Mgnl1/5/Mra1/4/Aus1/3/Scar/GdoVZ579//Bit	28	Bcrch1/DCD DW 7//Ossl1/Gdfl(2)	47	Marsyr3//Saadi 1989/Chan
10	Bcr/Lks4//Mrf1/Stj2/3/Icasyr1	29	Mrf1/Stj2/3/1718/BT24//Karim (1)	48	Lgmb1/Bezaiz98-1
11	Icamor-TA04-1//Icamor-TA04-63/Bicrederaa1	30	Mrf1/Stj2/3/1718/BT24//Karim (2)	49	IcalmorH5-69
12	Icamor-TA04-1//Icamor-TA04-63/Bicrederaa1	31	SwAlg/Gd1-81/Ch1- 23	50	Atlas2
13	Quabrach1/4/Icamor-TA04-62/3/Quabrach3//Vitron/Bidra1	32	SwAlg/Gd1- 81/Ch1-48	51	D68-1-93A-1A//Ruff/Fg/3/Mtl5/4/Lahn
14	*T. polonicum* 9/Ch1//Icamor-TA04-68/3/Icamor-TA04-69//(Lahn/Ch1)2519	33	Gsbl1/4/D68-1-93A-1A//Ruff/Fg/3/Mtl5	52	Beltagy3
15	Maamouri2/Cl115/5/F4 13 J.S/3/Arthur71/Lahn//Blk2/Lahn/4/Quarmal	34	*T. dicoccum*1/Ch1//Ammar8/3/Bonadur/ Icamor -TA04-63	53	Miki2
16	Geromtel1	35	Geruftel2	54	Mrb3/*T. urartu* 500651/4/ Icamor -TA04-63/3/Bcr/Gro1//Mgnl1
17	Atlast1/961081//Icasyr1	36	Icasmor-B-22	55	Aghrass1
18	Aghram	37	Atlast1/961081//Icasyr1		
19	Ammar9/Azeghar2	38	Arislahn5// Icamor -TA04-63/Icasyr1		

7A or 7B (Zhang et al., 2005). Further, the *Lr*19 is closely linked to the yellow pigment which was found to be located on chromosme 7B (Elouafi et al., 2001).

Results showed that the Gb/130-bp polymorphic band was present in both *Lr*19 and *Sr*25 differential lines, and marker is tightly linked to gene *Lr*19 and *Sr*25. Resistant genotypes have both genes *Lr*19 and *Sr*25 at Gb/130–bp, but this band in the same size was absent in another resistant genotypes which did not have *Lr*19 and *Sr*25 (Figure 1).

In this study, at least twelve genotypes were having *Lr*19 and *Sr*25 resistance in their background (Azeghar2, Rutucha1, Ammar9/Azeghar2, Ammar9/Terbol97-4, *T. polonicum*9/Ch1//Icamor-TA04-68/3/ Icamor-TA04-69//(Lahn/Ch1)2519, Arislahn5// Icamor-TA04-63/Icasyr1, *T.dicoccum*1/Ch1//Ammar8/3/Bonadur /Icamor-TA04-63, Mrb3/*T. urartu*500651/4/Icamor-TA04-63/3/Bcr/Gro1// Mgnl1, SwAlg/Gd1-81//Ch1-48, Icamor-TA04-1//Mgnl3/ Ainzen1, 319-ADDO/5/D68-1-93A-1A//Ruff/Fg/3/Mtl5/4/ Lahn and Mrf1/Stj2/3/1718/ BT24//Karim). These genotypes may also have other *Lr*-resistance genes available.

Using the above-mentioned 12 genotypes as resistant sources in a breeding program is an economic and effecttive way to minimize losses caused by leaf rust. Identification of molecular markers closely linked to resistance genes can facilitate the accumulation of other minor/ major genes into a single genotype.

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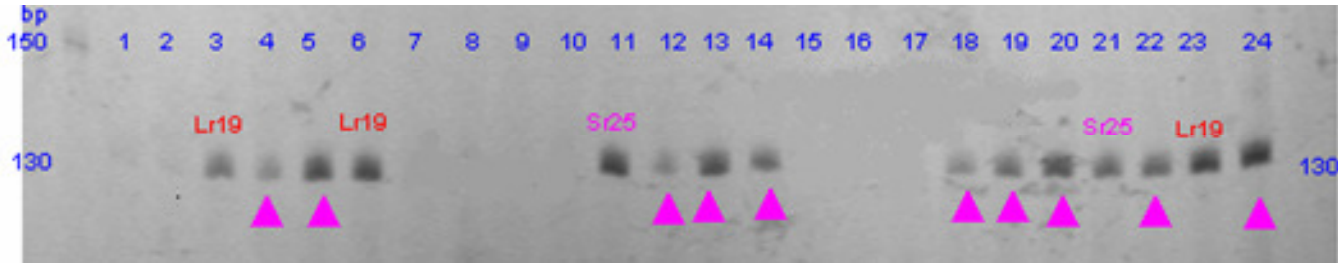

Figure 1. PCR products for *Gb* primer: 3, 6 and 23 -differential line (*Lr*19). 11 and 21 -differential line (*Sr*25). Genotypes 4, 5, 12 to 14, 18 to 20 and 22 -marker present Azeghar2, Rutucha1, Ammar9/Azeghar2, Ammar9/Terbol97-4, *T. polonicum*9/Ch1//Icamor-TA04-68/3/ Icamor-TA04-69//(Lahn/Ch1)2519, Arislahn-5//Icamor-TA04-63/Icasyr1, *T. dicoccum*1/Ch1//Ammar8/3/Bonadur/ Icamor -TA04-63, Mrb3/*T. urartu*500651/4/Icamor-TA04-63/3/Bcr/Gro1//MgnI1 and Mrf1/Stj2/3/1718/BT24//Karim, respectively, 1 - 2, 7 - 10 and 17 - 19 -marker absent.

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REFERENCES

Autrique E, Singh RP, Tanksley SD, Sorrells ME (1995). Molecular markers for four leaf rust resistance genes introgressed into wheat from wild relatives. Genome, 38: 75-83.

Ayala-Navarrete L, Bariana HS, Singh RP, Gibson JM, Mechanicos AA, Larkin PJ (2007). Trigenomic chromosomes by recombination of *Thinopyrum intermedium* and *Th. ponticum* translocations in wheat. Theor. Appl. Genet. 116: 63-75.

Chu CG, Friesen TL, Xu SS, Faris JD, Kolmer JA (2009). Identification of novel QTLs for seedling and adult plant leaf rust resistance in a wheat doubled haploid population. Theor. Appl. Genet. 2009 Jul; 119(2): 263-269. Epub 2009 Apr 25.

Bremenkamp-Barrett B, Faris JD, Fellers JP (2008). Molecular Mapping of the Leaf Rust Resistance Gene *Lr17a* in Wheat. Crop Sci. 48: 1124-1128.

Dehne HW, Oerke EC (1998). Impact of diseases and disease control on crop production. pp. 1-21.

Elouafi DHI, Nachit MM, Martin LM (2001). Identification of a microsatellite on chromosome 7B showing a strong linkage with yellow pigment in durum wheat (*Triticum turgidum* L. var. *durum*). Hereditas, 135(2-3): 255-261.

Elouafi I, Nachit MM (2004). A genetic linkage map of the durum *Triticum dicoccoides* backcross population based on SSRs and AFLP markers, and QTL analysis for milling traits. Theor. Appl. Genet. 108: 401-413.

Gennaro A, Koebner RMB, Ceoloni C (2009). A candidate for *Lr19*, an exotic gene conditioning leaf rust resistance in wheat. Functional Integr. Genomics, 9: 325-334.

Kassem M, El Ahmed A, Hakim MS, El-Khalifeh M, Nachit MM (2010). Races of *Puccinia triticina* Eriks. Identified in Syria and Lebanon During 2007. Arab Plant Prot. J. (In press).

Khan MA, Trevathan LE, Robbins JT (1997). Quantitative relationship between leaf rust and wheat yield in Mississippi. Plant Dis. 81: 769-772.

Kolmer JA, Long DL, Hughes ME (2007). Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2005. Plant Dis. 91: 979-984.

Kolmer JA, Long DL, Hughes ME (2009). Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2007. Plant Dis. 93: 538-544.

Long D, Kolmer JA (1989). A North American System of Nomenclature for *Puccinia recondita* f.sp. *tritici*. Phytopathology, 79: 525-529.

Marasas CN, Smale M, Singh RP (2004). The Economic Impact in Developing Countries of Leaf Rust Resistance Breeding in CIMMYT related Spring Bread Wheat. Mexico, DF: International Maize and Wheat Improvement Center.

Mebrate SA, Dehne HW, Pillen K, Oerke EC (2008). Postulation of Seedling Leaf Rust Resistance Genes in Selected Ethiopian and German Bread Wheat Cultivars. Crop Sci. 48: 507-516.

Nachit MM, Elouafi I, Pagnotta MA, El Saleh A, Iacono E, Labhilili M (2001). Molecular linkage map for an intraspecific recombinant inbred population of durum wheat (*Triticum turgidum* L. var. *durum*). Theor. Appl. Genet. 102(2-3): 177-186.

Prins R, Groenwald JZ, Marias GF, Snape JW, Koebner RMD (2001). AFLP and STS tagging of Lr19, a gene conferring resistance to leaf rust in wheat. Theor. Appl. Genet. 103: 618-624.

Qi LL, Echalier B, Chao S, Lazo GR, Butler GE, Anderson OD, Akhunov ED, Dvorak J, Linkiewicz AM, Ratnasiri A, Dubcovsky J, Bermudez-Kandianis CE, Greene RA, Kantety R, La Rota CM, Munkvold JD, Sorrells SF, Sorrells ME, Dilbirligi M, Sidhu D, Erayman M, Randhawa HS, Sandhu D, Bondareva SN, Gill KS, Mahmoud AA, Ma XF, Miftahudin GP, Conley EJ, Nduati V, Gonzalez-Hernandez JL, Anderson JA, Peng JH, Lapitan NLV, Hossain KG, Kalavacharla V, Kianian SF, Pathan MS, Zhang DS, Nguyen HT, Choi DW, Fenton RD, Close TJ, McGuire PE, Qualset CO, Gill BS (2004). A chromosome bin map of 16,000 expressed sequence tag loci and distribution of genes among the three genomes of polyploid wheat. Genetics, 168: 701-712.

Stakman EC, Stewart DM, Logering WQ (1962). Identification of Physiologic Races of *Puccinia graminis* var tritici. U.S. Dept. Agric. Res. Serv. E-617. p. 53.

Winzeler M, Winzeler H, Keller B (1995). Endopestidase polymorphism and linkage of the EP-Dlc null allele with the *Lr19* leaf-rust resistance gene in hexaploid wheat. Plant Breed. 114: 24-28.

Xing Y, Ichida F, Matsuoka T, Isobe T, Ikemoto Y, Higaki T, Tsuji T, Haneda N, Kuwabara A, Chen R, Futatani T, Tsubata S, Watanabe S, Watanabe K, Hirono K, Uese K, Miyawaki T, Bowles KR, Bowles NE, Towbin JA (2006). Genetic analysis in patients with left ventricular noncompaction and evidence for genetic heterogeneity. Mol. Genet. Metab. 2006 May; 88(1): 71-7. Epub 2006 Jan 19.

Yang WX, Liu DQ (2004). Advances in localization and molecular markers of wheat leaf rust resistance genes. Scientia Agricultura Sinica, 3: 770-719.

Wang Y, Peng H, Liu G, Xie C, Ni Z, Yang T, Liu Z, Sun Q (2010). Identification and molecular mapping of a leaf rust resistance gene in spelt wheat landrace Altgold. Springer Science+Business Media B.V. 2010.

Zhang W, Lukaszewski AJ, Kolmer J, Soria MA, Goyal S, Dubcovsky J (2005). Molecular characterization of durum and common wheat recombinant lines carrying leaf rust resistance (*Lr19*) and yellow pigment (*Y*) genes from *Lophopyrum ponticum*. Theor. Appl. Genet. 111: 573-582.