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ROMANIAN AGRICULTURAL RESEARCH, NO. 29, 2012
Print ISSN 1222-4227; Online ISSN 2067-5720

PRELIMINARY IDENTIFICATION OF SOURCES OF RESISTANCE TO THE GREENBUG, *SCHIZAPHIS GRAMINUM* RONDANI (*HEMIPTERA: APHIDIDAE*) AMONG A COLLECTION OF TUNISIAN BREAD WHEAT LINES

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ABSTRACT

The greenbug, *Schizaphis graminum* (Rondani), is a major pest of wheat worldwide. In this research, 14 Tunisian bread wheat genotypes were evaluated for resistance to *S. graminum*. Eight cultivars were scored as susceptible with an average injury level varying between 7.7 and 9. Three cultivars were moderately resistant (4.0 - 6.0) and three expressed high levels of resistance (1.2 - 2.0). A previously described SSR marker (*Xwmc634*) cosegregating with the *Gb*3 greenbug resistance gene in wheat was identified in 'Ariana 66' cultivar, suggesting the presence of *Gb*3 in this cultivar. Based on these results, we conclude that 'Ariana 66' should be incorporated in wheat breeding programs for resistance against greenbug, in Tunisia, as a local adapted source of *Gb*3. Additionally, cultivars 'Soltane 73' and 'EAP63A', which expressed high levels of resistance and did not express the SSR marker associated with *Gb*3, could be taken into consideration for diversifying the genetic basis of greenbug resistance in wheat breeding programs.

Key words: Aphid; Cereals; Resistance genes; Marker-Assisted Selection (MAS).

INTRODUCTION

The greenbug, *Schizaphis graminum* (Rondani) is an economically important aphid pest of small grain crops in many parts of the world, and is a damaging pest of durum (*T. turgidum* ssp. *durum* Desf.) and bread wheat (*Triticum aestivum* L.) in Tunisia (Boukhris-Bouhachem et al., 2007). Damage to wheat occurs as a result of greenbug feeding on the phloem. Greenbug saliva causes physiological reactions that lead to chlorosis in the wheat plant (Ryan et al., 1990). This pest also damages wheat by vectoring several viruses, such as the Barley Yellow Dwarf Virus (BYDV, Fauquet et al., 2005), Maize Mosaic Dwarf Virus (MMDV, Nault and Bradley, 1969) and Sugarcane Mosaic Virus (SCMV, Ingram and Summers, 1938).

The greenbug is especially problematic because of frequent changes in biotypes. Over 20 greenbug biotypes have been recognized (Nuessly et al., 2008), most commonly on the basis of their ability to overcome different sources of plant resistance and/or utilize different cereal hosts. The most effective and environmentally-safe strategy for controlling this aphid species is breeding for resistance in wheat varieties.

Indeed, the use of resistance genes in plant cultivars is cost-effective and avoids frequent insecticide applications that are expensive to apply, damage ecosystems and destroy non-targeted beneficial insects (Dogimont et al., 2010).

Seven (*Gb*1–*Gb*7) greenbug resistance genes have been identified in wheat relatives and transferred into the wheat genome (Weng

Received 25 November 2011; accepted 16 March 2012

et al., 2005; Lu et al., 2010). The greenbug resistance gene *gb*1 was identified in durum wheat (Lu et al., 2010). *Gb*2 and *Gb*6, located on wheat chromosome arm 1RS (Hollenhorst and Joppa, 1983; Porter et al., 1991), were transferred to wheat from rye (*Secale cereale* L.). *Gb*3, *Gb*4 and *Gb*7 are derived from *Aegilops tauschii* Coss. and located on wheat chromosome arm 7DL (Weng and Lazar 2002; Zhu et al., 2005; Weng et al., 2005). A chromosome segment, containing *Gb*5 from *Aegilops speltoides* L., was transferred into wheat *via* a translocation involving 7AL (Dubcovsky et al., 1998).

The *Gb*3 gene confers resistance to a wide spectrum of greenbug biotypes, including C, E, H, I, J, K, WWG, TX4, TX5, KS2, TX6, TX7, KS3 and TX10 (Nuessly et al., 2008). Therefore, for this important gene to be incorporated into commercial wheat cultivars, more or less complex breeding programs should be implemented. In addition to infestation assays, molecular genetic markers tightly linked to or cosegregating with *Gb*3 are required to assist the breeding process. Indeed, the availability of tightly linked molecular (PCR-based) markers makes possible to infer the plant resistance gene by the marker, without depending on the natural pest or pathogen occurrence or waiting for its phenotypic expression (Najimi et al., 2003).

Weng et al. (2005) constructed a microsatellite map of *Gb*3 in a mapping population from 'Largo' x 'TAM 107' and identified a marker (*Xwmc634*) co-segregating with *Gb*3 and four markers (*Xbarc76*, *Xgwm037*, *Xgwm428* and *Xwmc824*) closely linked to *Gb*3. These markers should be a valuable tool for marker-assisted selection of *Gb*3-conferred greenbug resistance in wheat breeding.

The objectives of the present study were to (1) assess the level of resistance to *S. graminum* in a collection of bread wheat genotypes from Tunisia; and (2) screen bread wheat material for the presence of *Xwmc634* *Gb*3-cosegregating marker.

MATERIAL AND METHODS

Greenbug resistance assessment in Tunisian bread wheat lines

Fourteen Tunisian bread wheat cultivars, kindly provided by the Centre Régional de Recherches sur les Grandes Cultures (CRRGC, Béja, Tunisia) were used in this investigation (Table 1), in addition to the barley cultivar 'Custer' (susceptible control) and the bread wheat 'Largo' (positive control carrying *Gb*3). Greenbug samples used in infestation assays were collected during may 2009, from private wheat fields in the region of Béja (36°43'42.3'' N, 9°11'32.3" E), in the North of Tunisia. These insects were reared for three generations on the susceptible barley cultivar 'Custer', in a growth chamber, under standard conditions (22-5°C, 50% relative humidity and 16L: 8D photoperiod) (Shufran et al., 1992).

The greenhouse test followed the infestation method described by Starks and Burton (1977): 10 seeds of each entry were planted (1.8 cm deep) in hills spaced 5 cm apart within rows and 4.5 cm between rows (replicated four times) in a flat (90 x 50 x 10 cm) containing a mixture of sandy loam soil, sand, and peat (1:1:1 ratio). There was a total of 64 hills in the flat (16 entries with 4 replications) in a randomised complete block design. Each seedling was infested by ten adult *S. graminum*, immediately after emergence (7 d after planting). A composite damage rating (1: no damage, to 9: dead plant) was recorded on each group of 10 seedlings per entry, 21 days after infestation, when the susceptible check was rated 9.0 (i.e. dead plant). Characterization of damage scores was as follows: 1-3, resistant (R); 4-6, moderately resistant (MR); 7-9, susceptible (S). Data from the test were subjected to analysis of variance (ANOVA), and mean values were compared with Tukey's honestly significant difference (HSD) test at the 0.05 level.

Screening for *Xwmc634* *Gb*3-cosegregating marker

'Largo' cultivar (*T. durum* Langdon/*A. tauschii* PI 268210) carrying the *Gb*3 gene (Joppa and Williams, 1982) was used in this

study, as positive control. This cultivar was developed by the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) in cooperation with the North Dakota Agriculture Experimental Station, and kindly provided by the USA National Small Grains Collection (http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1080418). In addition, 'TAM107' cultivar (TAM 105 x 4/Amigo), carrying only *Gb*2 greenbug resistance gene (Porter et al., 1987) was used as negative control. This cultivar was developed cooperatively by the Texas Agriculture Experimental Station and the USDA-ARS, and kindly provided by the USA National Small Grains Collection (http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1390530). Wheat genomic DNA was isolated from each Tunisian bread wheat line and from the *Xwmc634* positive and negative control cultivars, 'Largo' and 'TAM107', according to the DNA extraction procedure reported by Doyle and Doyle (1987). *Xwmc634* screening was conducted through microsatellite amplification, as described by Weng et al. (2005). Reactions were carried out in 25 µl final volume, containing 50 ng of DNA, 1 unit of Taq polymerase (Promega, USA), 1X PCR buffer (Promega, USA), 1.5 mM $MgCl_2$, 200µM of dNTPs (dATP, dGTP, dTTP and dCTP) and 0,5 mM of each primer (WMC634-F: 5'-AGC GAG GAG GAT GCA TCT TAT T-3' and WMC634-R: 5'-GAC ATA CAC ATG ATG GAC ACG G-3'). PCR reactions were performed in a 2720 thermal cycler (Applied Biosystems, USA), with an initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C, hybridization at 50°C and elongation at 72°C each for 1 min and a final extension at 72°C for 10 min.

Table 1. List of Tunisian bread wheat cultivars tested for resistance to *Schizaphis graminum* and their characteristics (after Déghaïes et al., 2007)

Species	Type	Line	Pedigree	Breeding institute / region of origin	Year of registration – Year of cancellation of registration
Triticum aestivum L. (bread wheat)	Old cultivars	Richelle Hative 110	-*	INRAT	1912-1920
		INIA 66	Lerma Rojo 64/ Sonara64	CIMMYT / INRAT	1970-1974
		Ariana 66	Kenya 338/ Etoile de Choisy	SCAP/ INRAT	1970-1995
		EAP63A	P26/Florence 46C3	INRAT	1954-1970
		Mahon 73	-	Algeria	1910-1953
		Baroota 52	-	INRAT	1923-1958
		Soltane 73	Son64/Kl.Rend II19975-68-1J-6Y-1J-3Y-0TU	CIMMYT	1974-1977
		FATH	-	INRAT	-
		FXA	-	INRAT	-
	Modern cultivars	Vaga92	47778*2//Fkm/Gb/3/Vee#5/4/Buc "S " / Pun "S" CM66684-B-1M-6Y-2M-2Y-1M-0Y-0Bj	CIMMYT / INRAT	1992 - .
		Salambo 80	Pato//Corre Camminos/Inia CM1021-7MB-14BJ-4BJ-0BJ	CIMMYT / INRAT	1980 - .
		Utique 96	ND/VG 9144// Kal/ Bb/ 3/ Yaco/ 4 / Vee#5/ CM85836-50Y-0M-0Y-3M-0Y-0Bj	CIMMYT	1996 - .
		Haidra 99	Bow ''S'' / Dougga 74 T83-89-0SBj-0Kf-0E-26Kf-16Bj-0Bj	INRAT	2003 - .
		Tebica	Seri/Buc "S"	INRAT	1985 - .

* data not available.
CIMMYT: The International Maïze and Wheat Improvement Centre (Mexico).
SCAP: Central Station of Plant Breeding (Versailles, France).
INRAT: National Institute of Agronomic Research (Tunisia).

Amplification products were first visualized under UV light on 1.5% agarose gel to check for amplification, then loaded into 6% denaturing polyacrylamide gel (Weng et al., 2005).

RESULTS AND DISCUSSION

Resistance of Tunisian bread wheat lines to *S. graminum*

Results of tests with *S. graminum* and bread wheat lines are summarized in Table 2. 'Custer' was susceptible in all assays conducted, whereas "Largo" displayed a high level of resistance (1.0). The response of Tunisian bread wheat lines to infestation was heterogeneous. 'Vaga 92', 'Richelle Hative 110', 'FATH', 'Salambo 80', 'Tebica', 'Mahon 73', 'FXA' and 'Baroota' were scored as susceptible, with an average injury level varying between 7.7 and 9.0. 'INIA 66', 'Utique 96' and 'Haidra 99' were moderately resistant (4.0 - 6.0). Finally, 'Ariana 66' (1.2), 'Soltane 73' (1.5) and 'EAP63A' (2.0) expressed high levels of resistance.

In all wheat breeding programs, attention is paid to obtaining a good yield performance in conjunction with pest resistance. Cultivars 'Ariana 66', 'Soltane 73' and 'EAP63A', which showed a highly resistant behaviour against greenbug, are all old cultivars whose recommendation to farmers was cancelled, either because they were replaced by more productive ones (e.g. 'Ariana 66' and 'EAP63A'), or because of their susceptibility to fungal diseases (e.g. 'Soltane 73', highly susceptible to *Puccinia striiformis*, Déghaïes et al., 2007). Results obtained here, showed that these 3 cultivars might be promising in use as donor parents in breeding programs, in order to transfer their resistance to the greenbug towards highly productive modern cultivars, preferably cultivars such as 'Utique 96' and 'Haidra 99', which already have a moderate resistance. Before that, 'Ariana 66', 'EAP63A' and 'Soltane 73' should be further studied to identify precisely the source of resistance and check for the stability of resistance expression in various abiotic conditions and against selected biotypes.

Table 2. Damage ratings in response to infestation by *S. graminum* and *Xwmc634* microsatellite marker (208 bp) genotyping, in 14 Tunisian bread wheat lines

Line	Mean injury level	Score	*Xwmc634* Genotype (bp)
Custer (susceptible control)	9.0 d	S	-
Vaga 92	8.5 d	S	214/214
Richelle Hative 110	9.0 d	S	212/212
FATH	8.5 d	S	214/214
INIA66	4.0 b	MR	218/218
Salambo 80	9.0 d	S	218/218
Ariana 66	1.2 a	R	208/208
Tebica	9.0 d	S	214/214
EAP63A	2.0 a	R	218/218
Mahon 73	9.0 d	S	212/212
FXA	8.0 d	S	212/212
Utique 96	4.0 b	MR	218/218
Haidra 99	6.0 c	MR	214/214
Baroota	7.7 d	S	214/214
Soltane 73	1.5 a	R	218/218
Largo (*Gb*3-positive control)	1.0 a	R	208/208
TAM107 (*Gb*3-negative control)	-	-	210/210

Within each column, mean values followed by the same letter, are not significantly different (P = 0.01), based on Tukey's HSD test.

Identification of *Xwmc634*

Screening for *Xwmc634* microsatellite marker (208 bp), in the studied collection of Tunisian bread wheat lines, as well as in 'Largo' and 'TAM107', revealed different alleles and genotypes (Table 2). Thirteen out of the 14 Tunisian lines, did not display the 208 bp allele, which was present only in 'Ariana 66' and the positive control 'Largo' (Table 2), indicating that 'Ariana 66' harbours *Gb*3 gene. Given the importance of *Gb*3 as a multiple resistance source against a wide spectrum of greenbug biotypes (Nuessly et al., 2008), we, therefore, suggest that 'Ariana 66' should be the first choice for use within a wheat breeding strategy, consisting in the sequential deployment of single resistance genes, combined with monitoring of greenbug biotypes (Porter et al., 2000).

Additionally, cultivars 'Soltane 73' and 'EAP63A', which expressed high levels of resistance and did not express the SSR marker associated with *Gb*3, could be taken into consideration for diversifying the genetic basis of greenbug resistance in wheat breeding programs.

CONCLUSIONS

Results obtained, here, showed that 'Ariana 66' is a promising source of resistance that could be used as recurrent parent in breeding, in order to transfer *Gb*3-conferred resistance towards highly productive modern cultivars.

For an optimal efficiency of wheat-breeding programs, we are continuing the present study by the identification of greenbug biotypes in Tunisia. In fact, field and molecular tests are to be conducted permanently, as biotype shifts may occur, rendering previously efficient genes, susceptible to the new biotypes.

Acknowledgement

The authors thank Dr. Abdennour Sébéi (Centre Régional de Recherches sur les Grandes Cultures, Béja, Tunisia) for providing Tunisian bread wheat lines. We also gratefully acknowledge the USA National Small Grains Collection, for providing 'Largo' and 'TAM 107' cultivars. This work was funded by PRF project between the Ministry of Higher Education and Scientific Research (Tunisia) and the Ministry of Agriculture (Tunisia).

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