Molecular Detection of the Resistance to Biotic Stress Conditions in Hellenic Bread Wheat Commercial Cultivars

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Abstract: Biotic stress conditions are the most serious obstacle in bread wheat cultivation resulting in yield reduction and consumption safety problems (poisonous toxic production). For this, the identification of resistant cultivars and their respective genes is the main prerequisite in most breeding programs. In order to exploit the benefits of molecular technology in studying their genetic background, eight Hellenic bread wheat cultivars were analyzed to determine their gene constitution at some important disease resistance loci. It was revealed that cultivar Elissavet carries genes conferring resistance to tan spot (insensitivity to toxins A and B), rusts, powdery mildew, and barley yellow dwarf virus (*Lr34/Yr18/Pm38/Sr57/Bdv1* in combination with the genes on the wheat-rye 1BL/1RS translocation*)*. Cultivar Strymonas has three genes for resistance to necrotrophic diseases. Cultivar Yecora E carries the genes conferring resistance to tan spot and rusts (*Lr34/Yr18/Pm38/Sr57/Bdv1)* but lacks the translocation. The third cultivar, i. e. (Acheron) which carries the 1BL.1RS wheat-rye chromosome translocation, also has genes for resistance to tan spot (due to insensitivity to toxin B) and Fusarium head blight but lacks the resistance allele of the *Lr34* gene. It is concluded from the results that cultivar Elissavet constitutes a remarkable combination of favorable genes and must be more extensively used as a parental line in breeding programs to developing novel wheat germplasm.

Key words: resistance, fungal diseases, bread wheat, resistance genes

1 Introduction

Biotic stressing factors and more precisely foliar diseases represent the most serious obstacle in bread wheat cultivation (Faris *et al.* 2010). In addition to decreased yields (e. g. caused by rusts), biotic stressing factors could cause safety problems to

Proceedings of the 8th International Conference on Information and Communication Technologies in Agriculture, Food and Environment (HAICTA 2017), Chania, Greece, 21-24 September, 2017.

consumers (e. g. poisonous toxins produced by *Fusarium*), or, additionally carry other pathogens in various crops (e. g. viruses transferred by aphids) (Moreno et al. 2012). For all these reasons, identifying resistant varieties and their corresponding genes is a key goal in many breeding programs (Diethelm *et al.* 2014). Modern molecular breeding with very detailed technology available can greatly contribute to this objective (Abeysekara *et al.* 2010). It is well established that the *Lr34/Yr18/Pm38/Sr57/Bdv1* gene conferring moderate resistance to biotrophic pathogens is located on chromosome 7D (Krattinger *et al.* 2009; Lagudah *et al*. 2009; Dakouri *et al*. 2010), the *Tsn1* gene, responsible for sensitivity to the toxins A of the necrotrophic fungi *Pyrenophora tritici-repentis* (Died.) Drechesтa *Stagonos poranodorum* (Berk.) E. Castell. & Germano is located on chromosome 5A (Faris *et al*. 2010), and the *TDF_076_2D* gene conferring moderate resistance to *Fusarium* head blight is located on chromosome 2D (Diethelm *et al*. 2014). The *Tsc2* gene responsible for sensitivity to the toxin B of *P. tritici-repentis* was mapped on chromosome 3B (Abeysekara *et al*. 2010). Biochemical screening of the existing germplasm could also facilitate this identification (Xynias *et al.* 2007). In a previous article we reported the presence of the 1BL.1RS wheat rye chromosome translocation in Hellenic bread wheat cultivars after applying biochemical analysis (Xynias *et al.* 2006). This presence was further confirmed by molecular markers (Peros *et al.* 2014). The 1BL/1RS translocation from the rye Petkus (2x) of the Kavkaz type, is the most widespread introgression among common wheat varieties (Rabinovich 1998). The importance of this translocation is due to certain important genes located on the small arm of 1R chromosome. The main advantages of the translocation is high yield potential of the host cultivar (Kim *et al.* 2004), and resistance to both biotic and abiotic stressing factors (e. g. disease, insect and drought resistance, Anonymous 2013; Peng *et al.* 2007; Xynias *et al.* 2007). For this, screening Hellenic germplasm, to identifying the above and/ or other resistant genes is also important, because this germplasm could be involved in crosses to transfer all the important traits to new varieties.

In the present work eight Hellenic bread wheat varieties were studied in order to find which ones carry resistance genes for some of the most serious biotic stress factors, such as rusts, *Fusarium* head blight, tan spot, powdery mildew.

2 Material and methods

2.1 Plant material

Seven commercial bread wheat cultivars produced at Cereal Institute of Thessaloniki (i. e. cvs. Yecora E, Elissavet, Xenia, Acheron, Strymonas, Louros, and Lydia), one non commercial cultivar (cv. Chios) developed at the University of Thessaloniki, Greece and the Russian cultivar KVZ/Cgn were used for the purpose of the study. The cultivar 'Chinese Spring' was used as the control for the "tr" allele of the marker *Xfcp623* (associated with the *tsn1* ToxA insensitiveness allele of the gene) (Faris *et*

al. 2010), the "tsr" allele of the marker *XBE444541* (associated with the *tsc2* PtrToxB insensitivity allele of the gene) (Abeysekara *et al*. 2010) and the allele "+" of the *Lr34* gene (presence of resistance) (Lagudah *et al*. 2009). The cultivar 'Katepwa' was used as the control for the "Ts" allele of the marker *Xfcp623* (associated with the *Tsn1* toxin sensitive dominant allele of the gene), the "Tss" allele of the marker *XBE444541* (associated with the *Tsc2* toxin sensitive dominant allele of the gene) and the allele "-" of the *Lr34* gene (absence of resistance) (http://wheatpedigree.net/sort/show/31123). For the *TDF_076_2D* gene, the cultivar Mironovskaya 808 was used as the control for the allele 2 (associated with moderate resistance to *Fusarium* head blight) and the cultivar 'Chinese Spring' – as the control for the allele 1 (Diethelm *et al.* 2014).The cultivars for the control were kindly provided by the National Center for Plant Genetic Resources of Ukraine of NAAS (Kharkiv). The marker *Xfcp623* has 2 alleles: 379 bp (associated with sensitivity, further – "Ts") and null-allele (associated with insensitivity, further – "tr") (Faris *et al.* 2010).

2.2 Method

DNA was extracted from the sample of 25-30 mg. obtained from grinding 5-7 seeds with further use of a Diatom™ DNA Prep100 DNA isolation kit (the sales representative in Ukraine is Neogene® Company) following the standard protocol. PCR was performed using GenPak® PCR Core Kits (the sales representative in Ukraine is Neogene® Company) according to the manufacturer's recommendations.
The PCR was performed in the amplifier 2720 GeneAMP System using GenPak® PCR Core kits (the sales representative in Ukraine is the Neogene® Company) according to the manufacturer's recommendations.
The marker *XBE444541-STS* has 2 alleles: 340 bp (associated with sensitivity, further – "Tss") and 509 bp (associated with insensitivity, further – "tsr", on the agarose gel electrophoresis it is masked by nonspecific bands) (Abeysekara *et al.* 2010).
To determine the allelic state of the *Lr34* gene a combination of the gene-localized marker *SNP12* and the closely linked marker *ISBP1* were used (Dakouri *et al.* 2010). The amplified fragments of 509 and 234 bp in length are associated with the Lr34+ allele and the fragments of 391 bp in length – with the Lr34- allele.
For the *TDF_076_2D* gene the intron-localized marker *INDEL1* was used (Diethelm *et al.* 2014). In case of the resistance-associated allele 2 the amplifies fragments of 212 and 221 bp in length were obtained and in case of susceptibility associated allele – only fragments of 212 bp in length.
The annealing temperature was lowered to 42°C for the primer pair flanking the marker *XBE444541*. For the combination of the markers *SNP12* and *ISBP1* the condition following conditions: dissociation/activation of the hot-start polymerase at 95°C for 7 minutes then 32 cycles with dissociation phase at 94°C for 30 s, annealing

phase at 62.5°C for 40 s and elongation at 72°C for 40 s; final elongation – for 5 m. (Karelov *et al*. 2014).Besides this PCR was performed according the literature conditions (Diethelm *et al.* 2014; Abeysekara, *et al.* 2010; Faris *et al.* 2010).
PCR results were visualized by electrophoresis in 2–2.5% agarose gel in 0.5 x TBE buffer with subsequent staining with ethidium bromide or (in case of the INDEL1 marker) – by 8% the PAAGE with subsequent staining with $AgNO_3$ and use of the gel- visualization system VISION Gel.

2.3 Genes detected

The resistance-associated allele of the *Lr34/Yr18/Pm38/Sr57/Bdv1* gene (Dakouri et al. 2010) was marked as *Lr34*+, the allele associated with absence of resistance as *Lr34*-; for the *Tsn1* gene (the marker *Xfcp623)*, the allele for insensitivity to the toxin A (Faris et al. 2010) was designated as *tr*, the allele for sensitivity as *Ts*; for the *Tsc2* gene (the marker *XBE444541-STS)*, the allele for insensitivity to the toxin B (Abeysekara *et al*. 2010) was marked as *tsr*, the allele for sensitivity as *Tss*; for the *TDF_076_2D* gene, the allele conferring *Fusarium* head blight resistance (Diethelm e al.2014) was designated as *TDF-1*, the allele for the absence of such resistance as *TDF-2*. The marker *INDEL1* of the *TDF_076_2D* gene was analyzed by the procedure described in (Diethelm *et al*. 2014; Karelov *et al*. 2015).
The presence of the wheat-rye 1BL/1RS translocation and respective resistance genes was marked as +, and the absence as - (according to Xynias *et al*. 2006).

3 Results and discussion

The results of the molecular analysis regarding the allele constitution of genes conferring resistance to biotic factors examined and are expressed in Hellenic bread wheat cultivars are presented in Table 1.

Table 1. Allele constitution of genes conferring resistance to biotic stressing factors in Hellenic bread wheat cultivars.

Cultivar	*Tsn1*	*Tsc2*	*Lr34*	*TDF_076_2D*	*1BL/1RS (Pm8, Sr31, Lr26,Yr9)*
Yecora E	Ts	trr	+	2	-
Elissavet	tr	trr	+	2	+
Xenia	Ts	trr	-	1	-
Acheron	Ts	trr	-	1	+
Strymonas	tr	trr	-	1	-
Louros	tr		-	2	-
Lydia	Ts		-	1	-
Chios	tr		-	1	+/-
KVZ/Cgn	Ts		+	1	+

Where for the *Tsn1* gene *tr* is the allele for insensitivity and *Ts* is the allele for sensitivity; for the *Tsc2* gene *tsr* is the allele for insensitivity, and *Tss* the allele for sensitivity; for the *Lr34* gene with (+) is marked the resistant allele and with (–) the non-resistant; for the *TDF_076_2D* gene 1 is the resistant and 2 is the non resistant allele; the presence of the 1BL.1RS wheat rye translocation is marked with (+) and the absence with (-).

In the majority of varieties, combinations of two or more resistance genes were revealed at the loci analyzed (Table1). Cultivar Elissavet carries genes conferring resistance to tan spot due to insensitivity to toxins A and B of *P. tritici-repentis*, resistance to rusts, powdery mildew due to the presence of the wheat-rye 1BL/1RS translocation and the gene *Lr34/Yr18/Pm38/Sr57/Bdv1*, which confers moderate race-nonspecific resistance to a number of biotrophic pathogens, including yellow dwarf virus. Cultivar Yecora E has the gene for resistance to tan spot (insensitivity to toxin B) and the gene for moderate race-nonspecific resistance to rusts and other pathogens (*Lr34/Yr18/Pm38/Sr57/Bdv1)*. It should be noted that the important gene *Lr34/Yr18/Pm38/Sr57/Bdv1* is rare among European wheats (Kolmer *et al.* 2008). Cultivar Acheron, which also carries the 1BL.1RS wheat rye translocation, has respective resistance genes as well as the gene for insensitivity to *P. tritici-repentis* (tanspot) toxin B and moderate resistance to Fusarium head blight. The cultivar Strymonas is characterized by three genes conferring resistance to nectrotrophic

pathogenes (tan spot and Fusarium head blight). At least two important disease resistance genes were detected in cultivars Xenia, Chyos, and KVZ.

4 Conclusion

It can be concluded from the above results that cultivar Elissavet, which carries the 1BL.1RS wheat rye translocation, represents a good combination of favorable genes and for this it must be extensively used as parental line in breeding programs for producing new wheat germplasm. Other varieties can also be used as sources of important resistance genes in marker-assisted selection.

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