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Original Research Article

Gene effects of resistance to *Septoria tritici* in durum wheat under three water stress level

Running title: Relationship between gene effects and water stress level in resistance to septoria tritici in durum

Abstract

Gene effects and narrow-sense heritability estimates for components of resistance to *Septoria tritici* Blotch assessed under three water stress levels were evaluated using two populations derived from partial-resistant durum wheat inbred lines Agili and Jneh Kottifa and susceptible inbred lines Karim 80 and Razzek by generation means analysis. Populations included the resistant parents, susceptible parents, F_1 and F_2 generations and backcross BC_1Pr and BC_1Ps generations. The presence of an epistatic effect was dependent upon water stress level. In absence of water stress, only additive and dominance effects were induced for the two populations and the two isolates. Therefore, in presence of water stress levels, the variation of generation means was adequately explained by additive, dominance and digenetic epistatic effects for the two populations. The variation in the mode of the gene effect of *S*TB resistance inheritance with water stress level complicated the procedure of incorporating genes of resistance in agronomically acceptable cultivars. In the absence of water stress, selection would be simpler, because only additive and dominance effects were implicated. However, the genetic mechanism will not be stable when the plant experiences water stress. For cultivars designed for exploitation in a wide range of environmental conditions, selection under water stress is suggested for stability of resistance to STB. Narrow-sense heritability estimates were consistent between combinations of crosses–isolates–water stress levels and ranged from 51 to 72%.

Keywords: durum wheat, efficient selection, gene effects, stability, water stress

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1. Introduction

Septoria tritici Blotch (STB) caused by the ascomycete *Mycosphaerella graminicola* (Fuckel) J. Schröt in Cohn.(anamorph: *Septoria tritici* Roberge in Desmaz), is an important disease in many areas where wheat is grown (Shipton et al., 1971; King et al., 1983; Eyal et al., 1987). It can cause considerable yield losses of up to 50% in the wheat growing areas with severe infections (Eyal and Levy, 1987). In Tunisia, durum wheat is the primary cultivated crop and occupies more than half of the area dedicated to cereal crops (Bnejdi and El Gazzah, 2008), with biotic and abiotic stresses the major factors seriously affecting crop production.

Resistance to STB is of great relevance in wheat breeding; and is quantitatively inherited and controlled by multiple genes (Schnurbusch et al., 2003; Uphaus et al., 2007; Kelm et al., 2011). Both additive and dominance effects (Van Ginkel and Scharen, 1987) play an important role in the expression of resistance, and epistasis also has a substantial effect (Bnejdi et al., 2011b).The environmental problem caused by continuous use of chemicals for control makes breeding for resistance to STB an essential component in controlling this important foliar disease of wheat. The genetic architecture of complex traits consists of not only the action of genes in single loci, but also the inter-locus interactions and gene × environment interactions. The complexity of the genetic architecture can be largely attributed to epistasis, which plays a significant role in heterosis, inbreeding depression, adaptation, reproductive isolation and speciation (Yang and Zhu, 2005). The efficiency of any methodology of selection is associated with the unbiased estimates of the additive genetic effect (Viana, 2000).The lack of empirical work on epistasis, in part, reflects the difficulty in quantification of epistasis (Whitlock et al., 1995; Fenster et al., 1997). Nonetheless, many studies that attempt to identify the genetic basis of quantitative traits ignore the possibility that loci interact (Carlborg and Haley, 2004), as well as the effects of environment on the expression of gene effects and their interactions with the genetic background. It is important

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to note that most models of inheritance are simplifications of complex genetic phenomena. Epistasis is a significant component in the inheritance of quantitative characters, and geneticists must face the reality of complex genetic systems. A better understanding of the relative importance of water stress in genetic components of resistance to STB would potentially lead to more efficient development of resistant varieties and deployment of germplasm resources. Therefore, the objective of the present research was to evaluate the role of water stress in the variation of gene effects in resistance to STB in durum wheat.

2. Materials and methods

2.1 Plant materials

Parental lines were selected depending on their differential reactions to STB. The partially resistant parents (Pr) were Agili and Jneh Kottifa, and susceptible parents (Ps) were Karim 80 and Razzek. F_1 and F_2 generations and BC_1Pr and BC_1Ps backcross generations from the crosses of Karim 80 × Agili and Razzek × Jneh Kottifa were used in this study. Plants were grown in a randomized complete block design with two replications. Two seeds of each genotype were sown in 1-L pots containing loam substrate with three water stress levels (LS0, LS1 and LS2 representing respectively 100%, 70% and 50% of field capacity and placed in a growth chamber at 20 ± 2°Cwith a 12-h photoperiod. Pots were weighed and water was added to bring the pots to the required saturation, if the water level was more than 10% below the required level.

2.2Pathogen production and disease evaluation

Two isolates (ISTB1 and ISTB2) of STB were collected from infected durum wheat plants from the northwest of Tunisia. Conidia of each isolate were streaked on PDA (39 g of potato dextrose agar per 1 L) Petri plates. Then the plates were placed in the growth chamber at 20 ± 2°C with a 12-h photoperiod. When the edge of the pink colony began to darken, the conidia

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were ready to harvest. A conidial suspension was prepared using equal quantities of the two isolates, filtered through two layers of cheesecloth and adjusted to 10^6 conidia mL^{-1} using a haemocytometer. Plants (two-leaf stage) were inoculated evenly by spraying the fungal conidia suspensions (40 mL/100 plants) onto leaves with an atomizer. After inoculation, the plants were covered with black polyethylene film for 72 h; and 21 d later the disease symptoms were assessed.

Disease ratings of all leaves of each plant were recorded on a 1–9 scale, based on the infection response (amount of necrosis and chlorosis) and on density of pycnidia (Zhang et al., 1999). The scale was defined as follows: (1) no visible symptoms, and the leaves remained green and healthy; (2) a few chlorotic lesions were present, and the infection site was a tan-coloured spot; (3) extensive chlorotic lesions were present, lesions occasionally had necrosis at the infection; (4) extensive chlorotic lesions merged with each other and individual lesions were identified by initial infection sites, and necrosis and chlorosis both existed on the leaf; (5) lesions were fully merged, and more than half of the leaf was desiccated by necrosis; (6) the entire leaf was desiccated, and water-soaked lesions occupied the entire leaf; (7) a few pycnidia were visible on the infected sites, and < 30% of the leaf was occupied by pycnidia-covered lesions that remain dry and green; (8) pycnidia occupied 50–70% of the leaf, and infected sites were dry yet still green; and (9) the entire leaf was covered by pycnidial lesions, and the leaf was dry yet still green. The number of plants evaluated varied depending on the generation and was greater in generations with greater segregation, such as F_2, BC_1Pr and BC_1Ps.

2.3 Statistical analyses

Prior to analysis, transforming the data by log, square root, arc-sine and arc-sine of square root of the variable had no effect on data distribution or in removing epistatic effects. Analysis of variance by population, stress level and isolate using general linear model (GLM)

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procedures (SAS, 1990) indicated that the replication and generation × replication effects were not significant. Therefore, generation means analysis was conducted without adjusting data for replication.

2.3.1 Gene effects

Generation means analysis was determined separately for each combination of cross–isolate–water stress level as follows: Weighted least squares regression analyses were used to solve for mid-parent [m] pooled additive [d], pooled dominance [h] and pooled digenic epistatic ([i], [l] and [j]) genetic effects, following the models and assumptions described in Mather and Jinks (1982). A simple additive-dominance genetic model containing only the m, d and h effects was tested first, using the joint scaling test described in Rowe and Alexander (1980). Adequacy of the genetic model was assessed using a chi-square goodness-of-fit statistic derived from deviations from this model. If statistically significant at $p < 0.05$, the genetic models containing digenic epistatic effects were then tested until the chi-square statistic was non-significant.

2.3.2 Heritability

Homogeneity of variances of non-segregating generations was tested using Bartlett's test (Bartlett, 1937), and when the variances were heterogeneous, the environmental variance (V_E) was replaced by an adequate number of separate parameters and pooled to produce a single environmental variance. Additive, dominance and environmental variance components were estimated using the maximum likelihood method with the observed variance of the six basic generations being used as the initial weights [$df/(2\times S^2)$]until the chi-squared test value reached a minimum (Lynch and Walsh, 1998). Narrow-sense heritability was calculated as follows: $h^2_n = V_A/(V_A+V_D + VE)$. Where V_A is the additive genetic component of variance,

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V_D the dominance genetic component of variance and V_E the environmental variance (Kearsey and Pooni, 1996).

3. Results

Comparison of means using Duncan's multiple range test (α =0.05), indicated significant differences between generations in all combinations of cross–isolate–water stress level. As expected, the two parental lines in each cross were consistently different for the three experimental water levels (Table I).

The results of generation means analysis revealed that in absence of water stress (LS0), the three-parameter model was sufficient to explain genetic variation of generation means of resistance to STB in the four combinations of cross–isolate and the probability of goodness-of-fit varied from 34 to 89%. Additive (d) type effect was significant, negative and more pronounced than dominance (h) type effect. However, in the presence of water stress (LS1 and LS2) the additive-dominance model did not adequately fit for all combinations and so the digenic epistatic model was applied and found appropriate. The probability of goodness-of-fit of these models ranged from 27 to 99%. In the majority of cases, dominance (h), additive × additive (i) and dominance × dominance (l) types' effects (when significant) contributed more in the control of resistance than additive effects. In all cases additive and dominance effects (when significant) were negative. The additive × dominance (j) effect was significant only in the cross Karim 80 × Agili with isolate ISTB1, where the additive ×additive (i) effect was not significant (Table II).

Variance components were estimates and used to calculate narrow-sense heritability (h^2_n) for both crosses and isolate–water stress level combinations. For all cases the additive variance effect was significant and ranged from 2.15 to 11.45 in the cross Karim 80 × Agili, and from 3.45 to 7.33 in the cross Razzek × Jneh Kottifa. The dominance variance effect was smaller than additive variance and ranged from 0.8 to 4.13 in the cross Karim 80 × Agili, and from

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0.55 to2.86 in cross Razzek × Jneh Kottifa. Environmental variance estimates were smaller than additive and dominance variances estimates and ranged from 0.36 to 1.12 in the two crosses. Under the three levels of water stress, h^2_n was consistent between crosses and isolates with a range from 51 to 72% (Table III).

4. Discussion

Significant differences were observed among generation means for resistance to STB in all combinations of cross–isolate–water stress level. This indicates that the magnitude of differences in the two populations was sufficient for selecting genotypes to improve resistance to STB in presence and absence of water stress. Plant genetic adaptability is of primary importance in many aspects of genetic research and is a special priority in the study of major crops grown in a wide range of environments. Therefore, the variation of inheritance of quantitative traits with environmental conditions has been infrequently studied and there is little knowledge on this topic. Recently Bnejdi et al. (2011a) reported that inheritance of resistance to salt on durum wheat at germination stage was dependent upon salt stress level. The variation of gene effects with water stress levels indicated that the mechanism of genetic control was not stable and was dependent upon environmental conditions. In the absence of water stress (LS0), only additive and dominance effects were implicated in the genetic control of resistance to STB. Therefore, both additive and non-additive (i.e. dominance and epistasis) effects were induced under water stress levels (LS1 and LS2). The results from our experiments indicate that epistasis contributed consistently and substantially in resistance to STB under water stress. Significance of epistatic effects were reported for resistance to yellowberry in durum wheat (Bnejdi and El Gazzah, 2008), for resistance to *Phytophthora nicotianae* in pepper (Bnejdi et al., 2009) and for resistance *Puccinia coronata* in oats (Bnejdi et al., 2010).

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The moderate to high h^2_n values indicate that genetic effects were larger than environmental effects. A similar result was reported by Bastam et al. (2010). The general stability of h^2_n noted between water stresses levels indicated that resistance to STB was not under separate genetic control. However, a genetic interaction was evident only when plants were cultivated under water stress. Wolf et al. (2000) reported that epistasis contributes to a variety of evolutionary phenomena, including adaptation, outbreeding depression, heterosis, the evolution of sex, reproductive isolation, founder effects and hybrid zone dynamics.

Breeding for resistance to STB in the absence of water stress is simple and efficient. In fact, the absence of epistatic effects and the preponderance of additive (d) compared to dominance (h) type effects facilitates the fixation of favourable genes of resistance in the productive cultivars desired. In the presence of water stress the breeding for resistance to STB is complicated by the presence of epistasis. So, the fixation of genes of resistance and suitable interactions such as additive × additive (i) type effects requires an appropriate schema of selection. Evolution research has established that the assembly and maintenance of favourable epistatic combinations adapted for a specific environment is a major mechanism of adaptation in various plant species (Allard, 1996).

5. Conclusion

Our results suggest that inheritance of resistance to STB in durum wheat depended upon water stress level. Thus, for the improvement of resistance of cultivars designed for exploitation in a wide range of environmental water stress, the choice of the environment in which the selection is made is very important. Indeed, in the presence of water stress the genetic interactions are solicited and are considered by breeders. However, in the absence of water stress, genetic interactions are not induced and not considered in the procedure of selection. Thus, the stability of selected cultivars in different environmental conditions is not guaranteed. To conclude, the higher estimates of h^2_n indicated that genetic effects were larger

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than environmental effects. Therefore, breeding of resistance to STB requires an adequate choice of parent. Also, the choice of environmental conditions in which the genetic components of resistance are incorporated in agronomically acceptable cultivars is suggested for the efficiency of any breeding strategy.

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Table I. Leaf means scores ± SE for two isolates of STB (ISTB1 and ISTB2) in parents and offspring populations from two crosses (Karim 80 × Agili and Razzek × Jneh Kottifa) assessed at three water stress levels (LS0, LS1 and LS2 representing respectively 100%, 70% and 50% of field capacity).

		Population					
Cross	**Water stress level/isolate**	**Ps(20)[y]**	**BC1Ps(30)**	**F1(20)**	**F2(50)**	**BC1Pr(30)**	**Pr(20)**
Karim 80 (Ps) × Agili (Pr)	**LS0/ISTB1**	**3.84±1.35a**	**3.90±2.14a**	**3.00±1.43ab**	**3.42±2.65a**	**2.1±1.19bc**	**1.44±0.53c**
	LS1/ISTB1	**6.10±2.39a**	**6.00±1.84a**	**4.46±1.30bc**	**5.06±2.27b**	**3.76±1.48c**	**2.55±0.61d**
	LS2/ISTB1	**7.05±0.64a**	**6.58±2.62ab**	**4.5±1.09c**	**5.93±2.19b**	**4.34±1.09c**	**3.90±0.62c**
	LS0/ISTB2	**4.07±1.36a**	**3.87±1.82a**	**2.90±1.37b**	**3.19±2.26ab**	**1.91±1.04c**	**1.22±0.38c**
	LS1/ISTB2	**6.42±0.45a**	**5.82±1.65ab**	**4.58±0.64c**	**5.14±1.88bc**	**3.44±0.87d**	**2.47±0.18e**
	LS2/ISTB2	**7.08±0.64a**	**5.99±2.13b**	**5.06±1.54c**	**5.95±2.13b**	**4.21±0.98c**	**3.23±0.60d**
Razzek (Ps) × Jneh Kottifa (Pr)	**LS0/ISTB1**	**4.43±0.76a**	**4.04±1.68a**	**2.98±1.33b**	**2.83±1.99b**	**2.18±1.72b**	**1.48±0.45c**
	LS1/ISTB1	**6.90±0.43a**	**6.22±2.34ab**	**4.86±2.23cd**	**5.64±2.78bc**	**4.06±1.28de**	**3.65±0.70e**
	LS2/STB1	**7.62±0.75a**	**7.05±2.56a**	**4.93±1.43bc**	**5.86±2.34b**	**5.41±1.25b**	**4.02±0.65c**
	LS0/ISTB2	**5.16±0.82a**	**4.27±1.41b**	**3.17±0.96c**	**3.43±1.83c**	**2.25±1.18d**	**1.36±0.31e**
	LS1/ISTB2	**7.03±0.39a**	**5.77±1.65b**	**4.93±1.48c**	**5.18±1.94bc**	**3.45±0.71d**	**2.73±0.14d**
	LS2/ISTB2	**7.77±0.81a**	**6.28±2.27b**	**5.18±1.26c**	**5.92±2.20bc**	**4.14±1.01d**	**3.27±0.50d**

For each cross, means within a line, bearing different letters differ (P<0.05)

[Y] *Number of plants evaluated in each generation*

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Table II. Estimates of additive, dominance and epistatic effects (± SE) for resistance to two isolates of STB (ISTB1 and ISTB2) in two crosses (Karim 80 × Agili and Razzek × Jneh Kottifa) evaluated at three water stress levels(LS0, LS1 and LS2 representing respectively 100%, 70% and 50% of field capacity).

C ross	water stress level/isolate	Best-fit model						
		m	d	h	i	l	j	P value
Karim 80 (Ps) × Agili (Pr)	LS0/ISTB1	2.7±0.1**	-1.3±0.1**	0.4±0.3	-----	-----	-----	0.34
	LS1/ISTB1	-5.1±1.6**	-1.9±0.2**	0.2±3.8	-0.7±0.1**	-0.9±0.1**	-----	0.31
	LS2/ISTB1	7.3±0.6**	-1.5±0.1**	-2.8±0.7**	-----	-1.8±0.6**	-1.32±0.9*	0.99
	LS0/ISTB2	2.9±0.1**	-1.5±0.1**	0.1±0.2	-----	-----	-----	0.73
	LS1/ISTB2	7.7±1.8**	-1.6±0.1**	-5.6±3.1*	-2.5±1.1*	2.7±1.4*	-----	0.29
	LS2/ISTB2	4.1±1.6**	-1.7±0.1**	6.0±3.8*	1.6±0.6**	-5.2±2.3**	-----	0.77
Razzek (Ps) × Jneh Kottifa (Pr)	LS0/ISTB1	2.7±0.2**	-1.5±0.2**	0.2±0.3	-----	-----	-----	0.42
	LS1/ISTB1	6.9±1.3**	-2.0±0.1**	-4.7±2.8*	-2.4±1.2**	2.38±1.7*	-----	0.27
	LS2/ISTB1	8.4±1.4**	-1.9±0.1**	-6.5±3.2**	-3.3±1.4**	3.14±2.2*	-----	0.73
	LS0/ISTB2	3.3±0.1**	-1.2±0.1**	-0.04±0.2	-----	-----	-----	0.89
	LS1/ISTB2	7.4±1.3**	-2.2±0.1**	-6.2±2.8**	-2.47±1.3*	3.85±1.7**	-----	0.63
	LS2/ISTB2	8.2±1.4**	-2.2±0.1**	-6.2±3.1**	-2.7±1.4**	3.2±2.0**	-----	0.82

*m, d, h, i, l, j = mean, additive, dominance, additive × additive, dominance × dominance and additive × dominance genetic effects, respectively. P = probability of adequateness of the model. *p<0.05 and **p<0.01 compared to zero.*

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Table III. Estimates of additive (V_A), dominance (V_D) and environmental variances (V_E) with ± SE and narrow-sense heritability (h^2_n) for resistance to two isolates of STB (ISTB1 and ISTB2) in two durum wheat crosses (Karim 80 × Agili and Razzek × Jneh Kottifa) assessed at three water stress levels.

Cross	Water stress level / isolate	V_E	V_A	V_D	X^2(3 df)	h^2_n
Karim 80 (Ps) × Agili (Pr)	**LS0/ISTB1**	**1.02±0.18****	**10.37±3.00****	**4.13±1.65**	**ns**	**0.66**
	LS1/ISTB1	**1.12±0.27****	**5.16±2.49****	**0.80±1.6****	**ns**	**0.72**
	LS2/ISTB1	**0.76±0.23****	**6.63±2.17****	**2.37±1.27****	**ns**	**0.67**
	LS0/ISTB2	**0.53±0.09****	**2.15±0.02****	**1.49±1.41**	**ns**	**0.51**
	LS1/ISTB2	**0.46±0.20****	**11.45±3.30****	**4.01±1.82***	**ns**	**0.71**
	LS2/ISTB2	**0.96±0.25****	**7.01±2.52****	**2.17±1.51***	**ns**	**0.69**
Razzek (Ps) × Jneh Kottifa (Pr)	**LS0/ISTB1**	**0.85±0.24****	**7.33±2.28****	**2.86±1.31**	**ns**	**0.66**
	LS1/ISTB1	**0.36±0.19****	**4.94±1.69****	**1.53±1.03***	**ns**	**0.72**
	LS2/ISTB1	**0.75±0.22****	**6.45±2.05****	**2.45±1.2****	**ns**	**0.66**
	LS0/ISTB2	**0.56±0.21****	**3.45±1.62****	**0.55±1.05**	**ns**	**0.75**
	LS1/ISTB2	**0.44±0.19****	**5.94±1.72****	**2.40±0.99****	**ns**	**0.67**
	LS2/ISTB2	**0.79±0.23****	**6.98±2.16****	**2.72±1.25****	**ns**	**0.66**

df = degrees of freedom, calculated as the number of generations minus the number of estimated variance parameters
*ns = non-significant. *p<0.05 and **p<0.01 compared to zero.*