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Genetic analysis of preharvest sprouting tolerance in bread wheat (*Triticum aestivum* L. emend. Thell.)

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Abstract: Rains during grain ripening in wheat (*Triticum aestivum* L.) can cause preharvest sprouting (PHS), which drastically affects the grain yield and the baking quality of flour. In the present study, to screen 7 cultivars and 4 lines of spring bread wheat for PHS, germination tests were conducted with seeds obtained from wet spikes harvested immediately after natural rainfall. Germination tests were carried out as 3 different treatments: 1) seeds immediately germinated after hand-threshing on sampling day (T_1), 2) seeds germinated 1 week later after hand-threshing (T_2), and 3) 10 intact spikes kept on paper under laboratory conditions at room temperature, hand threshed and then put to germinate 7 days later (T_3). Red-grained cultivars Sagitario and Pandas, red-grained line F6 0314.76/Mrl, and white-grained cultivar Sunlin showed the higher PHS tolerance, while other wheat cultivars and lines were found to be susceptible to PHS under all treatment conditions. To evaluate the mode of inheritance and combining ability of PHS tolerance, a diallel cross was made between 3 white-grained (susceptible) and 2 red-grained wheat (resistant) cultivars. Analysis of variance for combining ability showed the predominance of an additive gene effect for PHS tolerance, as the variance of the general combining ability was higher than the specific combining ability for both percent germination and germination index. Pandas and Sagitario showed positive contributions toward increasing PHS tolerance in the F_1 progenies. Results presented in this study will provide useful information for wheat breeders about PHS tolerance or dormancy level in commonly grown wheat cultivars in the Mediterranean region of Turkey, and help them in the development of white wheat cultivars with an inherently higher sprouting tolerance.

Key words: Preharvest sprouting, germination index, diallel cross, genetic parameters, bread wheat, Mediterranean region

Ekmeklik buğdayda (*Triticum aestivum* L. emend. Thell.) başakta çimlenmeye toleransın genetik analizi

Özet: Buğdayda (*Triticum aestivum* L.) dane olum süresince devam eden yağışlar, hasat öncesi başakta çimlenmeye (HÖBÇ) neden olduğu için dane verimini ve unun pişme kalitesini olumsuz etkileyebilmektedir. Bu çalışmada, yedi yazlık buğday çeşidi ve dört yazlık buğday hattının başakta çimlenme durumunu incelemek için, doğal yağmurdan hemen sonra hasat edilen ıslak başaklardan elde edilen tohumlarla çimlendirme testleri sürdürülmüştür. Çimlendirme testleri, başakların hasat edildiği gün elle harmanlanan tohumların çimlendirilmesi (T_1), elle harmanlanan tohumların laboratuvarda oda sıcaklığında bir hafta bekletildikten sonra çimlendirilmesi (T_2) ve hasattan sonra bir hafta

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laboratuvarda oda sıcaklığında bekletilen başaklardan elle harmanlanan tohumların çimlendirilmesi (T_3) olmak üzere üç farklı uygulama şeklinde yapılmıştır. Kırmızı daneli buğday çeşitleri olan Sagitario, Pandas ile kırmızı daneli buğday hattı F6 0314.76/Mrl ve beyaz daneli çeşit olan Sunlin en yüksek düzeyde başakta çimlenmeye dayanıklılık göstermesine karşın, geriye kalan tüm beyaz daneli buğday çeşit ve hatları tüm uygulamalarda başakta çimlenmeye duyarlı bulunmuşlardır. Başakta çimlenme dayanıklılığının uyum yeteğini ve kalıtım biçimini belirlemek için, üç beyaz (duyarlı) ve iki kırmızı (dayanıklı) daneli buğday çeşidi diallel olarak melezlenmiştir. Uyum kabiliyeti için yapılan varyans analizi, hem çimlenme yüzdesi hem de çimlendirme indeksi için genel uyum kabiliyeti varyansının, özel uyum kabiliyeti varyansından daha yüksek olması nedeniyle başakta çimlenmeye dayanıklılıkta eklemeli gen etkisinin daha üstün olduğu ortaya koymuştur. Pandas ve Sagitario, F_1 döllerinde başakta çimlenmeye dayanıklılığı arttırma yönünde olumlu katkılar göstermişlerdir. Bu çalışmanın sonuçları, Türkiye'nin Akdeniz bölgesinde yetiştirilen buğday çeşitlerinin başakta çimlenmeye karşı dayanıklılığı veya dormansi düzeyleri hakkında buğday ıslahçılarına faydalı bilgiler sağlayabileceği gibi, genetik olarak daha yüksek başakta çimlenme dayanıklılığına sahip olan beyaz daneli buğday çeşitlerinin geliştirilmesinde onlara yardımcı da olacaktır.

Anahtar sözcükler: Başakta çimlenme, çimlenme indeksi, diallel melezleme, ekmeklik buğday, genetik özellikler, Akdeniz bölgesi

Introduction

Preharvest sprouting (PHS) in wheat is a worldwide problem. PHS is the condition where germination of grains occurs in the spike before harvest. Rainfall and high humidity contribute to premature germination of grains in the spike before harvest. PHS can not only result in a great decrease in wheat yield, but also dramatically degrade the nutritional and processing quality of the seeds (Jiang and Xiao 2005). PHS negatively affects yield, seedling vigor, seed viability, test weight, flour milling yield, and milling and baking quality of harvested grain, due to increased enzyme activity and changes in total protein, sugars, and amino acid composition (Morad and Rubenthaler 1983; Hagemann and Ciha 1984; Paterson et al. 1989; Chastain et al. 1994; Mares 2001; Trethowan 2001; Groos et al. 2002; Himi et al. 2002; Jiang and Xiao 2005). The flour of grain affected by PHS is downgraded and becomes unsuitable for baked foods, noodles, or intended purpose (Kottearachchi et al. 2006). Groos et al. (2002) reported that flour obtained from sprouted wheat will produce products that are porous, sticky, off-color, of poor quality, and difficult to market, especially in Asian countries. Mansour (1993) similarly reported that breads baked from sprouted wheat will have a smaller volume and a compact interior. The main cause of the loss of quality of bread wheat due to PHS is high α-amylase activity.

Several factors can contribute to increased PHS tolerance, such as reduced levels of α-amylase activity in grains, the presence of inhibitors of germination, reduced water absorption by the grains, and others (Morad and Rubenthaler 1983; Derera 1989; Chastain et al. 1994; Mares 2001; Groos et al. 2002). The resistance to PHS is mainly associated with an adequate level of seed dormancy, i.e. the ability of the physiologically mature seed to withstand sprouting under conditions that are otherwise favorable for germination (Mares 1983; Morris and Paulsen 1985; McCaig and DePauw 1992). PHS is greatly dependent on both genetic and environmental conditions, i.e. temperature, rainfall, grain coat, protective structures of the spike, erectness of the spike, awn, and agronomic factors like fertilization (Morris and Paulsen 1985; Derera 1989; Gubler et al. 2005). Therefore, it is very difficult to phenotype PHS in the field. Several artificial methods were developed to measure dormancy level or resistance to PHS under laboratory conditions, such as visual examination of seeds, germination tests using either threshed seeds or intact spikes in sand or on blotting paper, and physiological analysis of seeds for enzymatic changes (Masi and Zeuli 1999; Wu and Carver 1999; Shorter et al. 2005). Germination tests using either threshed seeds or intact spikes provide a direct measurement of seed dormancy, controlled by the embryo (Wu and Carver 1999). Shorter et al. (2005) used a germination index (GI) to screen various New Zealand wheat cultivars for their PHS tolerance and reported that the use of a GI to assess grain dormancy was consistent

across years, the most reliable predictor of PHS tolerance, and recommended for use in the commercial breeding program. A strong correlation between increasing grain germinability and increasing PHS tolerance was reported among hard white wheat genotypes in Australia.

Grain color has long been known to influence resistance or susceptibility to PHS, with red kernel wheat types more resistant than white kernel types (Torada and Amano 2002). Therefore, the red grain color is used as a traditional marker for sprouting resistance in wheat. Generally, white-grained wheat is preferred by millers because of its higher flour extraction rate than red-grained wheat (McCaig and DePauw 1992), while there are very few white cultivars with the desired tolerance to PHS for marketing (Jiang and Xiao 2005). Because of PHS, farmers receive lower prices for sprouted grain and, in severe cases, their harvest may be downgraded to animal feed (Trethowan 2001), causing large financial losses in many countries. For this reason, development of PHS-tolerant white-grained wheat cultivars should be one of the main objectives in any wheat breeding program.

The Çukurova region is one of the major wheat producers, with a harvested area of 7.7% and production of 12.3%, in Turkey (Turkish Statistical Institute 2008). This region has a Mediterranean climate and rainfall during the grain ripening period occurs more frequently, resulting in PHS and causing serious damage to wheat production, which in turn significantly affects the value of the wheat in the market. Thus, improvement of wheat cultivars with PHS tolerance has become an imperative breeding objective. The diallel analysis as presented by Griffing (1956) is useful for studying genetic variations of specific traits and identifying crosses likely to produce superior segregants. Genetic information on preharvest sprouting is needed for an efficient breeding plan to improve bread wheat with a moderate level of dormancy for resistance to preharvesting sprouting under the Mediterranean conditions of the Çukurova region. Therefore, these experiments were run with an aim to 1) screen 7 cultivars and 4 lines of spring bread wheat to determine their level of dormancy required to give adequate protection from PHS, 2) study heritability and combining ability of 5 bread wheat cultivars and identify the best combining parent by crossing them in a diallel fashion, and 3) provide information to wheat breeders for development of PHS-resistant cultivars.

Materials and methods

Plant material, experimental design, and assessment of PHS tolerance

Experiment 1

In this study, 7 bread wheat cultivars and 4 advanced lines were used as plant material. Among the selected genotypes, Genç99, Balatilla, Adana99, Pandas, and Sagitario are widely grown as commercial cultivars in the Çukurova region of Turkey, while the others were selected lines from the International Maize and Wheat Improvement Center (CIMMYT). All of the cultivars or lines selected for this experiment were spring-type. Among these genotypes, Sagitario, Pandas, and F6 0314.75/Mrl were red-grained, while the other cultivars and lines were white-grained. The cultivar Sunlin, with white grain and an awnless spike, was included as the control for PHS tolerance. The field experiment was carried out at the Research and Implementation Area of the Department of Field Crops, Faculty of Agriculture, Çukurova University, Adana, in the eastern Mediterranean region of Turkey during 2004-2005. The experimental design was a randomized complete block design with 3 replications. The plot size was 6 m^2, consisting of 8 rows, 5 m in length with 0.15 m of interrow spacing. This study was established due to occurrences of heavy rainfall (about 65 mm) from May 30 to June 1 in 2005, just before the time of harvest. The total precipitation of the growing season (from sowing to physiological maturity) was 347 mm (Turkish State Meteorological Service 2005).

Preharvest sprouting tolerance of bread wheat cultivars and lines was assessed by a germination test conducted with seeds obtained from wet spikes harvested immediately after natural rainfall. According to the procedure of Paterson et al. (1989), 40 spikes were randomly hand-harvested from each plot by cutting the peduncle about 10 cm below the base of the spike. Germination tests were carried out in 3 different modes: i) 10 of 40 spikes were hand-

threshed and put to germinate on the sampling day (T_1), 2) seeds of the other 10 spikes were hand-threshed and kept for 1 week on blotting paper and then germinated (T_2), and 3) 10 intact spikes were kept on paper under laboratory conditions at room temperature and after 7 days hand theshed and put to germinate (T_3).

Experiment 2

Based on the results of Experiment 1, 5 bread wheat cultivars, Genç99, Balatilla, Adana99, Sagitario, and Pandas, were selected due to their different levels of preharvest sprouting tolerance. These cultivars were crossed in all possible combinations, including reciprocals, to determine their combining ability and heritability. All possible 20 F_1 hybrids with 5 parents were planted on December 5, 2006, according to a randomized complete block design with 3 replications at the Research and Implementation Area of the Department of Field Crops, Faculty of Agriculture, University of Çukurova, Adana (37°21′N, 35°10′E), in the eastern Mediterranean region of Turkey. Plots of F_1 hybrids and their parents consisted of 3 rows, 1 m in length, containing 10 plants that were spaced 10 cm within the rows. Spacing between rows was 20 cm. The total precipitation of the growing season, from sowing to physiological maturity, was 294 mm (Turkish State Meteorological Service 2006). Agronomic and plant protection measures were kept standard for the entire experiment.

For determining PHS tolerance, 10 intact spikes at physiological maturity were collected from each genotype/cross from each replication. After being transferred to the laboratory, the sampled spikes were sterilized with a 1% solution of bleaching powder for 10-15 min, then soaked in water in a rectangular plastic container covered with a piece of plastic paper for 4 h, according to the procedure described by Jiang and Xiao (2005) with some modifications. After soaking, spikes were transferred to a dry paper, threshed, and bulked, and a germination test was conducted to observe their PHS tolerance.

Germination test

Germination tests for both experiments were set up in petri dishes that were cleaned with double distilled water and 70% ethanol to prevent fungal and bacterial growth. The germination tests for Experiment 1 were carried out in 2005, and that for Experiment 2 in 2007. According to a completely randomized design with 3 replications as described by Hagemann and Ciha (1984), 25 seeds from each of the genotypes (Experiment 1) and from the F_1 hybrids and their parents (Experiment 2) were placed on a filter paper (8.3 cm in diameter) in a petri dish (9.0 cm in diameter). Then 6 mL of distilled water was added to each petri dish, and these were placed in PGR Conviron incubators at 20 °C, 75% humidity, 16 h light, and 8 h dark. The number of germinated seeds was counted and discarded on days from 1 to 7 for each sample. Germination was defined as coleoptiles emerged, according to the sprouting scale-3 of Hagemann and Ciha (1984).

The results were expressed as a germination index (dormancy level) (GI) and a percent germination (PG). The GI was calculated for each genotype (Experiment 1) and F_1 hybrids and their parents (Experiment 2) using the following formula, as described by Himi et al. (2002): $GI = (7 \times n_1 + 6 \times n_2 + 5 \times n_3 + 4 \times n_4 + 3 \times n_5 + 2 \times n_6 + 1 \times n_7) \times 100/(7 \text{ (day)} \times 25 \text{ (total number of seeds)}$, where n_1, n_2, … n_7 are the number of seeds that germinated on the first, second, and subsequent days until the seventh day, respectively. PG was defined as (the total number of seeds with coleoptiles as long as the seed, counted on days 1-7)/25 × 100, as described by Hagemann and Ciha (1984).

Data analysis

The variance heterogeneity of PG and GI was reduced by using arcsine square root transformation. Analysis of variance for PG and GI was performed using the general linear model procedure (PROC GLM) of the Statistical Analysis System software 9.0 (SAS 2002). Means were compared with Duncan's Multiple Range Test (SAS 2002). The analyses of variance for combining abilities were carried out according to Griffing's (1956) Method 1 and Model 1 (including parents, F_1, and reciprocals) by using the TarPopGen Statistical Package Program developed by Özcan (1999). The diallel analysis of Jinks and Hayman (1953) was used to calculate variance (Vr) and covariance (Wr), and relationships were plotted to make a Wr-Vr graph.

Results

Screening of genotypes for PHS tolerance

Results of the variance analysis for percent germination (PG) and germination index (GI) for the 11 genotypes used in this experiment are given in Table 1. There were statistically significant differences among genotypes (G) and treatments (T) for both PG and GI. The G × T interaction was statistically significant only for GI. Mean PG and GI values for 11 bread wheat genotypes are illustrated in Table 2.

Wide-ranging and significant differences among genotypes for both characters were observed. Mean values of PG and GI in T_3 were higher than in T_2, but there was no significant difference between the treatments. PG ranged from 48%-90%, 64.7%-90%, and 68.7%-90%, while GI ranged from 29.7%-57%, 37.7%-57%, and 37%-57% for T_1, T_2, and T_3, respectively. Among the genotypes, Sagitario, Pandas, Sunlin, and F6 0314.76/Mrl showed lower PG and GI values than the other genotypes under T_1, T_2, and T_3. Higher PG and GI values were found for Genç99, Balatilla, and Thb//Maya/Nac/3/Rabe/4/Milan.

Combining ability

Results of analysis of variance done for dormancy level to assess PHS tolerance for diallel crosses are summarized in Table 3. Parents and their F_1 hybrids were highly significantly different ($P \leq 0.01$) for both PG and GI. Mean values of all parents and their F_1 hybrids are given in Table 4. Analysis of variance for combining abilities showed that general combining ability (GCA), specific combining ability (SCA), and their reciprocal effects (REs) were statistically significant for both PG and GI (Table 5). Based on the estimation of the expected mean square, variance of GCA was higher than that of SCA. PG and GI were controlled by additive gene action in accordance with the GCA to SCA ratio (Table 6). This indicated the relative importance of additive gene action in controlling the genetic architecture of the traits studied. Estimates of GCA and SCA are illustrated in Table 6. Among all 5 parents, Sagitario and Pandas had a significant negative GCA effect on both PG and GI, whereas the other 3 parents, Genç99, Balatilla, and Adana99, had a significant positive GCA effect. Analysis of SCA revealed that cross combinations of Genç99 × Pandas (1 × 4), Balatilla × Pandas (2 × 4), Sagitario × Adana99 (3 × 5), and Pandas × Adana99 (4 × 5) had significant negative SCAs, whereas cross combinations of Genç99 × Adana99 (1 × 5), Balatilla × Sagitario (2 × 3), Balatilla × Adana99 (2 × 5), and Sagitario × Pandas (3 × 4) had positive SCA values for PG and GI.

Estimates of reciprocal effects (REs) of germination (Table 6) showed that cross combinations of Balatilla × Genç99 (2 × 1), Sagitario × Balatilla (3 × 2), Pandas × Sagitario (4 × 3), and Adana99 × Sagitario (5 × 3) had negative REs, whereas the rest of the crosses had positive values for PG and GI. A Wr-Vr graph was plotted to see the degree of dominance

Table 1. Analysis of variance of percent germination and germination index of 11 spring wheats screened for preharvest sprouting.

Source of variance	Degree of freedom	Mean squares	
		Percent germination	Germination index
Replication	2	92.818	28.071
Genotype (G)	10	1093.202**	499.006**
Error	20	27.863	5.315
Treatment (T)	2	321.909**	173.162**
G × T	20	46.987ns	12.173**
Error	44	35.510	4.914
CV (%)		7.39	4.41

**: Significant at $P \leq 0.01$ probability level,
ns: non-significant, CV: coefficient of variation.

Table 2. Mean percent germination and germination index of 11 spring wheat genotypes screened for preharvest sprouting.

Genotypes	Percent germination (%)				Germination index (%)			
	T1	T2	T3	Mean	T1	T2	T3	Mean
Genç99	90.0 a*	90.0 a	90.0 a	90.0 a	56.3 ab	57.0 a	56.7 ab	56.7 a
Balatilla	90.0 a	90.0 a	90.0 a	90.0 a	57.0 a	56.7 a	57.0 a	56.9 a
Thb//Maya/Nac/3/Rabe/4/Milan	90.0 a	90.0 a	86.3 ab	88.8 a	53.7 a-d	56.7 a	56.3 abc	55.6 ab
Vorona/Cno//79	86.2 ab	90.0 a	86.3 ab	87.4 a	54.7 abc	56.7 a	58.0 a	56.4 a
Inqlab91	82.7 ab	90.0 a	90.0 a	87.6 a	50.0 d	56.3 a	55.7 abc	54.0 bc
Adana99	84.5 ab	90.0 a	84.7 ab	86.4 a	52.7 bcd	56.3 a	53.0 bcd	54.0 bc
Pfau/Milan	79.0 bc	86.3 ab	90.0 a	85.2 a	51.3 bcd	52.7 a	52.7 cd	52.2 c
F6 0314.76/Mrl	72.9 cd	77.3 bc	76.7 bc	75.7 b	42.3 e	48.0 b	50.0 d	46.8 d
Sunlin	70.3 d	73.0 cd	76.0 bc	73.1 b	41.7 e	45.3 bc	49.3 d	45.4 d
Sagitario	53.3 e	65.0 d	68.7 c	62.3 c	29.7 g	37.7 d	37.0 f	34.8 f
Pandas	48.0 e	64.7 d	68.7 bc	60.4 c	35.0 f	41.3 cd	45.0 e	40.4 e
Mean	77.0 *B*	82.4 *A*	82.5 *A*	80.6	47.7 *B*	51.3 *A*	51.9 *A*	50.3

*: Values sharing common letters in a column are not statistically different according to Duncan's test at $P \leq 0.01$.

Table 3. Analysis of variance for percent germination and germination index in a 5 × 5 diallel cross of bread wheat.

Source of variance	Degree of freedom	Mean squares	
		Percent germination	Germination index
Replication	2	162.998	11.806
Genotype	24	2259.063**	845.180**
Error	48	69.327	10.750
CV (%)		15.86	9.95

**: Significant at $P \leq 0.01$ probability level, CV: coefficient of variation.

for PG and GI (Figures 1a and 1b). The regression coefficients for both traits were 0.909 and 1.044, respectively.

Discussion

PHS in wheat is one of the major factors that affect the production and quality in many major wheat producing areas, especially in Mediterranean climates, where spring rainfall and high humidity prevail (Trethowan 2001). PHS results in considerable damage to wheat grain and causes lower yields due to decreased test weight, and it limits end-use applications for wheat products due to decreased grain quality and nutritional degradation. Reduced grain quality coupled with decreased yield can result in substantial financial losses for farmers and food processors.

Tolerance to PHS or the dormancy level of wheat grain is classically measured by PG and GI (dormancy level) (Biddulph et al. 2008). PG is highly correlated with the dormancy of seed. If the seed germination percentage is higher, it means the seed possesses low dormancy, and vice versa. On the other hand, GI is a

Table 4. Mean percent germination and germination index of parents and their F_1 progeny in a 5 × 5 diallel cross of bread wheat.

Parents and crosses	Percent germination (%)	Germination index (%)
Genç99 (1)	90.0 a*	56.2 a
Balatilla (2)	90.0 a	58.7 a
Sagitario (3)	18.8 jk	11.8 l
Pandas (4)	24.4 h-k	17.4 ijk
Adana99 (5)	74.8 bc	47.5 c
1 × 2	86.2 ab	54.8 ab
1 × 3	41.5 efg	27.9 f
1 × 4	23.5 ijk	16.4 jkl
1 × 5	90.0 a	49.6 bc
2 × 1	82.3 ab	53.5 ab
2 × 3	62.3 cd	39.3 d
2 × 4	26.3 h-k	17.9 ijk
2 × 5	83.2 ab	49.8 bc
3 × 1	53.0 de	34.2 de
3 × 2	45.4 ef	29.5 ef
3 × 4	36.0 f-i	25.1 fgh
3 × 5	24.6 h-k	15.7 jkl
4 × 1	38.0 fgh	26.5 fg
4 × 2	42.2 efg	21.9 ghi
4 × 3	29.3 g-k	18.4 ij
4 × 5	28.7 g-j	12.8 kl
5 × 1	90.0 a	53.5 ab
5 × 2	84.5 ab	53.5 ab
5 × 3	16.1 k	11.3 l
5 × 4	31.6 g-j	20.4 hij
Mean	52.5	32.9

*Values sharing common letters in a column are not statistically different according to Duncan's Multiple Range Test at P ≤ 0.01.

Table 5. Analysis of variance for combining ability of percent germination and germination index in a 5 × 5 diallel cross of bread wheat.

Source of variance	Degree of freedom	Mean squares	
		Percent germination	Germination index
GCA	4	3490.364**	1295.390**
SCA	10	359.838**	137.666**
Reciprocal	10	51.267**	20.322**
Error	48	23.109	3.583
GCA/SCA		9.70	9.41

** Significant at P ≤ 0.01 probability level.

Table 6. Estimation of GCA, SCA, and REs in the F_1 generation for percent germination and germination index in a 5 × 5 diallel cross of bread wheat.

Parents and crosses	Percent germination (%)	Germination index (%)
GCA		
Genç99 (1)	15.994**	9.935**
Balatilla (2)	16.734**	10.819**
Sagitario (3)	-17.921**	-10.443**
Pandas (4)	-22.073**	-13.528**
Adana99 (5)	7.315**	3.217**
gi[a] (0.05)	2.666	1.049
gi (0.01)	3.400	1.338
SCA		
1 × 2	-0.951	0.441
1 × 3	-3.266	-1.370
1 × 4	-15.629**	-7.903**
1 × 5	14.238**	5.474**
2 × 3	2.538	1.086
2 × 4	-12.946**	-10.351**
2 × 5	7.331**	4.688**
3 × 4	20.148**	12.798**
3 × 5	-21.571**	-12.223**
4 × 5	-7.610**	-6.063**
si[b] (0.05)	5.494	2.164
si (0.01)	7.008	2.760
RE		
2 × 1	-1.923	-0.608
3 × 1	5.743	3.168*
4 × 1	7.275*	5.097**
5 × 1	0.001	1.968
3 × 2	-8.472*	-4.862**
4 × 2	7.958*	1.983
5 × 2	0.640	1.873
4 × 3	-3.327	-3.330*
5 × 3	-4.247	-2.210
5 × 4	1.452	3.782**
ri[c] (0.05)	6.662	2.624
ri (0.01)	8.498	3.348

[a]: Critical differences between GCA effects of parents.
[b]: Critical differences between SCA effects of the *ij*th F_1 hybrid.
[c]: Critical differences between reciprocal effects of the *ji*th F_1 hybrid.
*: Significant at $P \leq 0.05$, **: significant at $P \leq 0.01$.

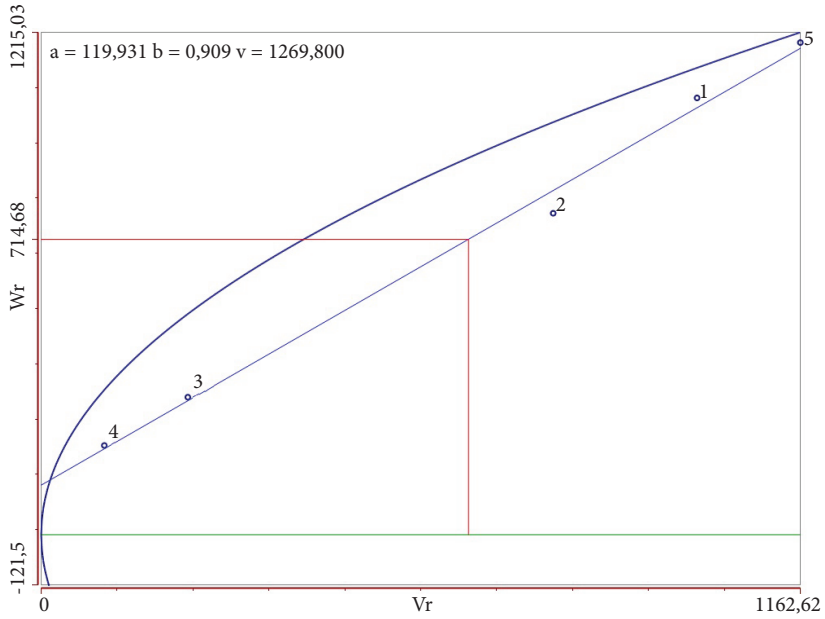

Figure 1a. Variance and covariance (Wr/Vr) regression graph of the 5 × 5 diallel cross for percent germination. Parents: 1, Genç99; 2, Balatilla; 3, Sagitario; 4, Pandas; 5, Adana99.

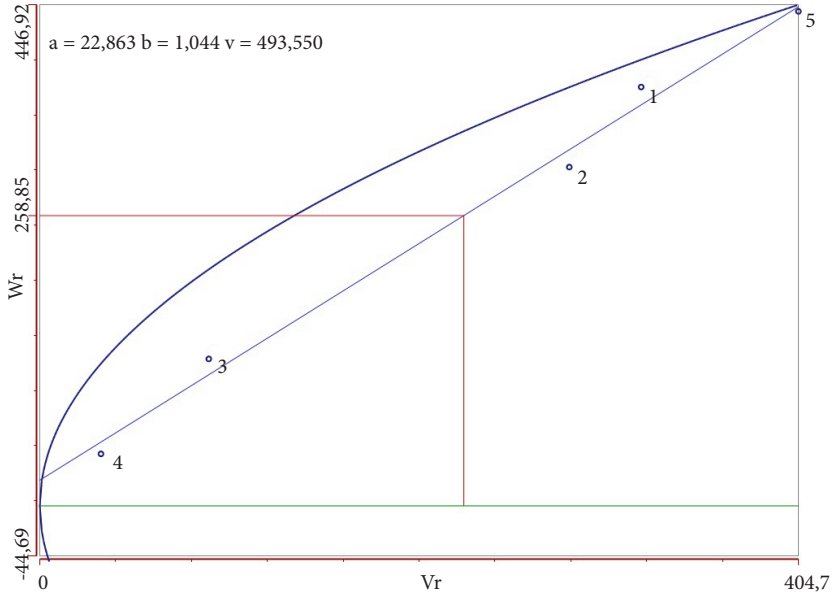

Figure 1b. Variance and covariance (Wr/Vr) regression graph of the 5 × 5 diallel cross for germination index. Parents: 1, Genç99; 2, Balatilla; 3, Sagitario; 4, Pandas; 5, Adana99.

weighted index that gives maximum weight to grain germinating early and progressively less weight to grain germinating later (Reddy et al. 1985). GI may be an important indicator of PHS tolerance when relatively small rainfall events, which will result in a slower germination rate, ultimately lower the

proportion of germinated grain, and hence cause greater sprouting tolerance. This method is easy, quick, and simple to carry out for measuring the susceptibility of preharvest sprouting. Hagemann and Ciha (1984) reported that the germination test is better and less laborious for determining the tolerance to preharvest sprouting. Wu and Carver (1999) also explained that PG is strongly correlated with field measurement of sprout damage and showed high repeatability.

In 2005, there was 65 mm of rainfall between May 30 and June 1, after plant maturity, with a daily mean temperature of 20.0 °C and a relative humidity of 87% during 3 consecutive days in Adana province, Turkey (Turkish State Meteorological Service 2005). Anderson et al. (1993) reported that PHS tolerance of wheat can be evaluated under stimulated or natural rainfall conditions or by exposure of grain to high humidity before harvest. Thus, we formed a hypothesis that the above mentioned climatic conditions in Adana could be suitable for testing the PHS tolerance of some wheat genotypes under natural rainfall conditions of the Mediterranean region. Therefore, to study PHS tolerance in 11 bread wheat genotypes, we collected wet spikes immediately after a natural rainfall and conducted a germination test under laboratory conditions to determine their PGs and GIs. Among treatments, on average, PG and GI values for T_2 and T_3 were comparatively higher than T_1 (Table 2). These differences in germination percentages might be due to accumulated temperature (increment in daily mean temperature after each degree-day) in the grain, because germination tests were conducted for T_2 and T_3 1 week later than for T_1. Likewise, Mares (1993) did find that germination percentage increased with the increase in accumulated temperature. In this study, seeds were also kept at room temperature as threshed and unthreshed for 1 week to compare the effects of wet awns on sprouting. We observed that there was not a significant difference in PG or GI between seeds kept 1 week after hand-threshing (T_2) and seeds kept with the awn (unthreshed) for 1 week (T_3) under laboratory conditions at room temperature (Table 2). Our findings are in contradiction with the results of Harrington (1949), who reported that unthreshed seeds had dormancy 20 days longer than threshed seeds. Paterson et al. (1989) suggested that sprouting susceptibility in wetted spikes may be influenced by seed dormancy and protective structures such as awns and club spikes. The other mechanisms affecting sprouting susceptibility might be ear type, the chaff, spike orientation, water uptake of the seed, drying rate of the ear, the rate of imbibitions, germination inhibitors in seeds, smooth wax and glossy surfaces, starch sensitivity, α-amylase synthesis, response to gibberellic acid, soil properties, day length, drought, and intensity of light (Fenner 1991; King and Wettstein-Knowles 2000; Rodriguez et al. 2001; Gatford et al. 2002; Himi et al. 2002). Nyachiro et al. (2002) stated that the dormancy level of a seed depended on the genotype, germination temperature, and moisture content of the seed. Rodriguez et al. (2001) reported that a 24 h imbibition period may increase germinated seeds by more than 30%, and if rainy conditions and low temperatures occur together in the field, the rate of germination and sprouting damage may increase by decreasing temperature during grain imbibitions.

Genotypes used in this study showed a high variation in PG and GI. Red-grained wheat genotypes Sagitario, Pandas, and F6 0314.76/Mrl, and white-grained wheat cultivar Sunlin showed lower GIs and PGs as compared with the other genotypes tested. Sagitario did not germinate in the first 3 days, therefore having a low GI but high PG compared with Pandas. The genotypes Genç99, Balatilla, Thb//Maya/Nac/3/Rabe/4/Milan, Vorona/Cno//79, Adana99, Inqlab91, and Pfau/Milan have a white grain color and showed higher PGs and GIs compared with red-grained genotypes (Table 2). Inqlab91 has been found to have high PG but a moderate level of GI. The average PG values in white- and red-grained genotypes were 84%, 87%, and 87%, and 58%, 69%, and 74% for T_1, T_2, and T_3, respectively, whereas the average GI values in white- and red-grained genotypes were 52%, 55%, and 55%, and 36%, 42%, and 44% for T_1, T_2, and T_3, respectively. The PG and GI values of red-grained genotypes were significantly lower than those of white-grained genotypes. A similar trend was also reported by Ogbonnaya et al. (2007), who stated that red-grained wheat genotypes were found to be more tolerant to PHS than white-grained wheat genotypes because of their genetic

backgrounds. Groos et al. (2002) reported that white-kernel wheat is usually more susceptible to PHS than red wheat due to the lack of pigmentation. They stated that association between PHS tolerance and red pigmentation is likely due to pleiotropic effects of the genes controlling grain color or a genetic linkage between pigmentation genes and genes affecting PHS, which were detected on the short arm of chromosome 5A of wheat. In this study, our results are consistent with the generally accepted link between red seed coat and PHS tolerance. Bread wheat cultivars such as Pandas, F6 0314.76/Mrl, Sunlin, and Sagitario showing PHS tolerance can provide breeding material for developing white wheat cultivars tolerant to PHS. However, in this study, the number of cultivars evaluated was insufficient, and therefore further study is needed to screen more Turkish cultivars and landraces, and, if possible wild wheats, for PHS tolerance and to make a strategy for their effective use in breeding programs.

We selected 3 white-grained wheat cultivars, Genç99, Balatilla, and Adana99 (susceptible), and 2 red-grained wheat cultivars, Sagitario and Pandas (tolerant), from the above results and crossed them in a diallel manner in order to evaluate the mode of inheritance and the combining ability for PHS tolerance in the F_1 progeny. In this study, additive and nonadditive gene effects on the controlling of the studied traits were found; however, additive gene action was more prominent, because the variance of GCA was higher than that of SCA. Various previous research (Barnard et al. 2005; Jian and Xiao 2005; Herrmann 2007) also explained that the genetic architecture of the traits associated with preharvest sprouting in cereals are mainly controlled by genes that are additive in nature.

The positive combining ability effect is an indication of an increase in a specific character evaluated and vice versa (Barnard et al. 2005). In our case, PHS tolerance of a genotype was measured in terms of PG and GI. According to these characters, any genotype with low PG and GI has a dormancy effect, which means it is tolerant to PHS. Therefore, in our case, a low or negative value of combining ability indicates a high tolerance to PHS, and a high or positive value of combining ability is an indication of poor tolerance or the susceptibility of a genotype to PHS.

Parents to be introduced into multiple breeding programs for improvement of any trait should have a good combining ability for transmitting desirable genes into the progeny (Yücel et al. 2009). Anderson et al. (1993) reported that one of the major considerations in adapting a selection strategy in a breeding program of wheat is the proportion of additive genetic variation for the trait studied. Pandas and Sagitario were the best general combiners due to their significant negative GCA values for both traits. For self-pollinated crops like wheat, SCA based on heterotic effects is likely to have a small contribution to the improvement of any particular trait (Yücel et al. 2009). The progenies with higher sprouting tolerance (negative SCA values) were Sagitario × Adana99 (3 × 5), Genç99 × Pandas (1 × 4), Balatilla × Pandas (2 × 4), and Pandas × Adana99 (4 × 5). Significant reciprocal effects for PG and GI ($P \leq 0.01$) demonstrated the strong cytoplasmic effect on both parameters. Crosses of Balatilla × Genç99 (2 × 1), Sagitario × Balatilla (3 × 2), Pandas × Sagitario (4 × 3), and Adana99 × Sagitario (5 × 3) showed negative values of reciprocal effect. Crosses such as Sagitario × Genç99 (3 × 1), Pandas × Genç99 (4 × 1), Pandas × Balatilla (4 × 2), and Adana99 × Pandas (5 × 4) had positive values of reciprocal effect; this indicated that Pandas should be exploited as a male and female parent. Crosses having high negative SCAs had also mid-parent heterosis (negative heterosis), and moreover, one of the parents involved in these crosses had negative general combining ability (Pandas and Sagitario), indicating that these combinations would yield desirable transgressive segregants. Pandas and Sagitario proved to be the best parents for both GCA and SCA. Cross combinations of Balatilla × Sagitario (2 × 3) and Sagitario × Pandas (3 × 4) had positive SCAs, which indicated that both tolerant and susceptible parents had a significant influence on the F_1 generation.

The Wr-Vr graphs for PG and GI were plotted to see the gene action in more detail (Figures 1a and 1b). The regression line intercepted the Wr-axis above the origin, indicating that PG and GI were controlled by additive gene actions. As regression coefficients were close to unity for PG and GI (b = 0.909 and 1.044, respectively), there were small nonallelic interactions or epistasis of genes. Cultivars Pandas and Sagitario

possessed more dominant genes for dormancy, whereas cultivars Genç99, Balatilla, and Adana99 had recessive genes for dormancy. The information derived from the Wr-Vr graphs also supported the results of combining ability (Tables 5 and 6).

PHS susceptibility is determined mainly by genetic factors, and it may also be influenced by environmental conditions during seed development (Rodriguez et al. 2001; Gubler et al. 2005; Biddulph et al. 2008). Breeding for PHS tolerance in wheat (*Triticum aestivum* L.) is difficult owing to the low heritability of the trait, expressed as a quantitatively inherited character (Lawson et al. 1997), and selection is limited to one generation per year (Anderson et al. 1993), as the trait is strongly affected by environmental factors (Zanetti et al. 2000). The landraces and wild wheats can be used as good tolerance sources for sprouting tolerance in white-grained wheat breeding programs according to the suggestions of Jiang and Xiao (2005). Various previous researchers reported that *Ae. tauschi* (the D-genome donor of bread wheat) is characterized by a high variation for PHS tolerance (Liu et al. 1998; Lan et al. 2005; Ren et al. 2008). Similarly, Lan et al. (2002) also reported that *Ae. tauschi* had 0% germination of both threshed seeds and intact spikes at the hard dough stage.

The results from the diallel crosses showed the positive contribution of Pandas and Sagitario toward increasing PHS tolerance in the progeny. Pandas, a red-grained wheat cultivar, has thicker and larger grains (a larger grain area) with good yield potential (Yücel et al. 2009) and wide adaptation, whereas wheat cultivar Sagitario has red grain, good quality, and moderate yield. These cultivars can be used for developing varieties with moderate dormancy and tolerance to PHS in wheat breeding programs.

Recent mapping studies based on DNA molecular markers in wheat have indicated that the genetic control of grain dormancy is a multigenic trait controlled by R genes (genes for red testa pigmentation) and other quantitative trait loci (QTLs) located on all 21 wheat chromosomes (Anderson et al. 1993; Groos et al. 2002; Kulwal et al. 2005; Ren et al. 2008; Kumar et al. 2009). Some of the most significant QTLs for PHS tolerance have been located on chromosomes of the third and fourth homologous groups of bread wheat (Groos et al. 2002; Kulwal et al. 2005; Mares et al. 2005; Mori et al. 2005; Liu et al. 2008; Ogbonnaya et al. 2008; Ren et al. 2008; Kumar et al. 2009; Munkvold et al. 2009).

In Turkey, unfortunately, selection for PHS tolerance in white-grained wheat has received no attention. Because PHS tolerance is generally linked with genes controlling the red color of grain, it is not easily usable for genetic improvement of PHS tolerance in white-grained wheat. Therefore, one possible approach is to identify and target QTLs for PHS tolerance in white wheat cultivars that will facilitate genetic improvement for PHS tolerance in white-grained wheat cultivars. Recently, Ogbonnaya et al. (2008) found 2 QTLs, namely QPhs.dpivic.4A.1 and Qphs.dpivic.4A.2, for PHS tolerance in white-grained wheat. These QTLs were located on the long arm of chromosome 4A of wheat. They also identified 2 new microsatellites, namely *Xgwm937* and *Xgwm894*, linked to major QTLs for PHS tolerance, and these markers showed strong associations with seed dormancy and PHS tolerance in a range of white-grained wheat genotypes. They stated that these 2 microsatellite markers could be used in marker-assisted selection (MAS) for incorporating QTLs responsible for PHS tolerance into elite white-grained wheat genotypes susceptible to PHS.

In conclusion, the results presented in this study will provide information to wheat breeders about the PHS tolerances or dormancy levels of some Turkish bread wheat cultivars and help them to make selections that can be used in the development of white wheat cultivars with an inherently higher sprouting tolerance and moderate level of dormancy.

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