Phenotypic Selection and Bulked Segregant Analysis for Flag Leaf Angle under Heat Stress in Bread Wheat (*Triticum aestivum* L.)

Hassan, M. I.^{*}; M. A. El-Rawy; A. M. Ali and M. M. El-Defrawy

Department of Genetics, Faculty of Agriculture, Assiut University, Assiut, Egypt *Corresponding author: m_hassan79@aun.edu.eg

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Abstract

Divergent phenotypic selection was performed for flag leaf angle (FLAN) under heat stress in five F₂ populations of bread wheat (*Triticum aestivum* L.). Direct responses for FLAN and correlated responses for grain yield per plant (GYP) and thousand kernel weight (TKW) were measured. FLAN was positively and significantly correlated with GYP and TKW under heat stress. Positive and highly significant (P < 0.01) responses to selection for FLAN were obtained in both directions for the five populations, which were higher in magnitude in the low direction (averaged 31.41) than those obtained in the high direction (averaged 22.0%). Selection for high FLAN produced concurrent positive and significant (P < 0.05) responses in GYP in only two populations, with an average of 5.17%, which was lower in magnitude than averaged correlated responses (8.24%) obtained in GYP for lower FLAN in four populations. Significant (P < 0.05) correlated responses to selection in TKW for higher FLAN were obtained in four populations (averaged 4.03%) and were smaller in magnitude than those obtained for lower FLAN (averaged 9.56%). Additive gene effects were found to be mainly controlling FLAN. Moderate realized heritability estimates obtained for FLAN (averaged 0.53) were similar to heritability obtained by parent-offspring regression (averaged 0.50). Bulked segregant analysis (BSA) using twelve simple sequence repeats (SSR) markers for FLAN identified three SSR markers, namely Xgwm294-2A; Xbarc113-6A and Xwmc398-6B were able to distinguish high from low bulks in at least two populations. Three bands specific for high and two specific for low FLAN were generated, that could be used in the future as markers associated with FLAN under heat stress in wheat. The information presented here could help in understanding the genetic system controlling FLAN and its relationship with grain yield under heat stress.

Keywords: Heat stress, selection, flag leaf, SSR, Triticum aestivum L.

Introduction

Wheat is the third highest produced cereal crop after maize and rice and is the leading source of plantbased protein in human food (Wu *et al.*, 2016). Heat stress in wheat is a major factor caused yield reduction in many wheat-growing regions of the world including the Mediterranean basin like Egypt. Heat stress at the time of anthesis until ripeness significantly reduces grain yield. Moreover, heat stress that began 15 days after anthesis and continued until complete ripeness caused a significant effect on kernel number and weight (Gibson and Paulson, 1999). Heat stress aggravates the process of flag leaf senescence in wheat by decreasing the levels of photosynthetic pigments and through declines in photosynthetic activity. Heat stress also damaged the ultrastructure of organelles such as chloroplasts, nuclei and mitochondria (Feng *et al.*, 2014).

Heat tolerance in wheat would be improved by selecting and developing genotypes with heat tolerance. Improvement grain yield under heat stress implies selecting genotypes for grain size and rate of grain filling (Farooq et al., 2011). Breeding for heat tolerance is further complicated since several types of abiotic stress can challenge crop plants simultaneously. A range of heat tolerance indicators including yield, morphological and physiological traits has been suggested to be used for screening wheat genotypes under heat stress conditions (El-Rawy and Youssef, 2014; Hassan et al., 2016). Genetic associations of various molecular markers including SSRs with heat tolerance have been reported in wheat (Sofalian et al., 2008; Ciuca and Petcu, 2009; Barakat et al., 2012). Moreover, quantitative and molecular characterization of heat tolerance in hexaploid wheat has also been reported (Yang et al., 2002). Therefore, integrating biotechnological tools with conventional breeding techniques will help to develop wheat varieties with better grain yield under heat stress during reproductive and grain-filling phases (Faroog et al., 2011).

In cereal crops, the top three leaves on the stem, especially flag leaf are the primary source of carbohydrates production (Sicher, 1993). The flag leaf could produce a large proportion of the carbohydrates stored in grains, and it is responsible for regulating final plant growth and yield formation in cereal crops (Biswal and Kohli, 2013; Tian *et al.*, 2015; Yang et al., 2016). Therefore, flag leaf characteristics have been considered to be important determinants of grain yield in cereals crops including wheat (Chen et al., 1995; Hirota et al., 1990; Simon, 1999). Furthermore, flag leaf traits provide a potential target for selection (Blake et al., 2007). In addition, leaf angle is more important in determining the degree of light penetration into the canopy (Williams and Kwi, 1967). However, to date, little information is available on the genetic mechanisms of flag leaf traits in wheat (Wu et al., 2016).

Therefore, in the present study, divergent phenotypic selection was applied for flag leaf angle under heat stress to five F_2 populations of bread wheat (*Triticum aestivum* L.). The objectives were (1) to estimate the response to selection for flag leaf angle and the correlated responses in both grain yield per plants and thousand kernel weight; and (2) to identify molecular markers associated with FLAN using Bulked Segregant Analysis (BSA).

Materials and Methods

The plant material and field experiment

The plant material utilized in the present study consisted of five F_2 populations derived from crosses established between six advanced lines of bread wheat (*Triticum aestivum* L.) selected for cell membrane thermostability (CMS) at the department of Genetics, Faculty of Agriculture, Assiut University, Egypt. The five crosses were: L₉ x L₁, L₇ x L₆, L₉ x L₂, L₇ x L₁₀ and L₉ x L₁₀.

In 2012 -2013 season, 150-200 seeds were taken from each of the

five F_2 populations and sown at the Experimental Farm of the Faculty of Agriculture, Assiut University, Egypt on the 3rd of December as a favorable sowing date and on the 14th of January as a late sowing date to allow the late sown plants to be subjected to the heat stress which usually develop later in the season.

Plants were arranged in rows of 10 plants spaced 30 cm apart with plants within rows set 30 cm from each other. Field observations and measurements for individual plants of each population were recorded for the following characters:

- 1. GYP: grain yield per plant (g).
- 2. TKW: thousand kernel weight (g).

3. FLAN: flag leaf angle (°) = the angle between the flag leaf and stem.

Selection procedure:

Divergent phenotypic selection for FLAN was applied to the late sown F_2 plants of each of the five populations (individual selection). The highest and lowest five plants in FLAN score were selected for each population (selection intensity ranged from 0.036 to 0.047). Equal numbers of seeds were pooled from the F_2 plants of each population to form the F_3 bulks.

In 2013-2014 season, the selected F_3 families of the five populations were sown on the 5th of January (heat stress condition) along the F_3 bulks in a randomized complete block design (RCBD) with three replications. The late sowing date was so chosen as to expose the selected plants to heat stress resulting when temperature rises late in the growing season. GYP, TKW and FLAN measurements of the selected F_3 families as well as the F_3 bulks were recorded.

Statistical analysis

Pearson's correlation coefficients among different traits and distribution of F₂ populations for FLAN under heat stress were carried out using analysis and chart tools of Microsoft Excel. To test the significance of differences among selected families in both directions as well as the bulks of each population, phenotypic data were statistically analyzed using the analyis of variance. Direct response to selection (R), the difference between the mean phenotypic value of the offspring of the selected parents and the whole of the parental generation before selection for FLAN in the high and low directions, and correlated responses to selection for GYP and TKW were obtained.

Heritability estimation

Heritability of each trait was estimated by the following two methods:

1. Realized heritability, was calculated as:

$$h^{2} = \frac{\left[\overline{H}_{S} - \overline{L}_{S}\right]}{\left[\overline{H}_{B} - \overline{L}_{B}\right]}$$

Where: \overline{H}_{S} and \overline{L}_{s} are the average of the selected F₃ families in the high and low directions, respectively while \overline{H}_{B} and \overline{L}_{B} are the average of the F₂ plants selected for that trait in the two directions (Ibrahim and Quick, 2001).

2. Parent-offspring regression (b_{po}) was calculated by regressing the means of the F₃ selected families on the values of their corresponding progenitor F₂ plants.

Bulked segregant analysis (BSA)

In order to identify molecular markers associated with flag leaf an-

gel, as an indicator for heat tolerance, in specific genomic regions, the five F₂ populations were subjected to BSA (Quarrie et al., 1999) with twelve wheat microsatellite or simple serepeats (SSR) markers, quence Xgwm291, Xgwm294, namely Xgwm356, Xgwm484, Xgwm339, Xgwm493, Xgwm577, Xwmc273, Xwmc398, Xwmc596, Xbarc113 and The highest and lowest Xbarc121. five plants selected from each of the F₂ populations were used to construct two DNA bulks for BSA. DNA extraction was carried out according to the cetyltrimethylammonium bromide (CTAB) method for plant tissues (Murray and Thompson, 1980) with some modifications. DNA quality and concentration were determined using spectrophotometer according to a Stulnig and Amberger (1994) and Khirshyat 1.0 micro-program (Youssef, 2012).

SSR markers analysis

Primers sequences and PCR conditions of SSR markers were obtained by GrainGenes Database for Triticeae and Avena (http://wheat.pw.usda.gov). PCR amplifications were performed in a Sen-LabCycler soOuest (SensoOuest GmbH, Göttingen, Germany). PCR products were separated on 2.5% agarose gels in $0.5 \times$ TBE buffer. A 100bp HyperLadder[™] was used to estimate the size of each amplified DNA fragment. Putative polymorphisms among the two bulks of the five populations were detected for each marker separately. Only strong, reproducible, and clearly distinguished bands were considered. Polymorphic markers were analyzed, and the percentage of polymorphism

for each marker was calculated by dividing the number of polymorphic bands with the total number of amplified bands.

Results

Performance of the F₂ populations

The FLAN of the five populations ranged from 8° in population-1 to 75° in population-5 with an average of 37.78° across all populations (data not shown). Correlation coefficients (Table 1) showed that FLAN was positively and significantly correlated with GYP in population-1 (r=0.23, *P*<0.05), population-2 (*r*= 0. 26, P < 0.01), population-3 (r = 0.25, P < 0.01) and population-4 (r = 0.22, P < 0.05). Whereas, positive and significant correlations were found between FLAN and TKW in population-2 (r= 0.21, P < 0.05) and population-3 (r= 0. 20, P<0.05). The frequency distribution of each of the five F₂ populations for flag leaf angle under late sowing date (Fig. 1) was continuous and approached normality, indicating that FLAN is under the control of polygenes and amenable to selection.

Means FLAN of the selected F₂ plants ranged from 49.0 in population-2 to 72.6 in population-5 in the high direction, with an average of 62.84°. Whereas, in the low direction means FLAN ranged from 8.6 in population-1 to 21.4 in population-5, with an average of 14.20°. The selection differentials in the high FLAN direction were of comparable magnitude for the five populations ranging from 22.21 in population-2 to 26.90 in population-3, with an average of 25.06%. However, the selection differential in the low direction was much smaller in magnitude in popula-

tion-2 being 15.79%, while it was higher in magnitude in population-3 (30.10%) than those obtained in the high direction in the five populations, with an average of 23.38% (Table 2).

Responses to selection and heritability estimates

The analysis of variance for FLAN (data not shown) revealed highly significant differences (P < 0.01) between the F₃ families selected for high and those selected for low FLAN in the five F_2 populations. Positive and high significant (P < 0.01) responses to selection for FLAN were obtained in both high and low directions in the F_3 families of the five populations (Table 3). The mean FLAN of the selected families reduced from 64.84 in population-4 to 31.48° in population-1. The highest reduction (46.48%) was observed in population-3 and the lowest (37.96%) was found in population-4, with an average reduction of 42.95%. The % responses ranged from 15.08% in population-1 to 26.67% in population-3 in the high direction, and ranged from 24.1% in population-5 to 35.92% in population-1 in the low FLAN direction. It is observed that, the % responses in the low direction (averaged 31.41) were higher in magnitude than those obtained in the high direction (averaged 22.0%). Moderate realized heritability values were observed for FLAN which ranged from 0.40 to 0.66 (averaged (0.53) and were found to be similar and corresponded to heritability estiby mates obtained the parentoffspring regression, ranged from 0.41 to 0.56 with an average of 0.50. **Correlated responses to selection**

The correlated response to selection in GYP in the high direction was found to be significant (P < 0.05) in population-1 (4.75%) and population-3 (5.58%). However, the correlated responses in the low FLAN dihighly significant rection was (P < 0.05) in population-2, whereas significant correlated responses (P < 0.05) were found in population-1, population-3 and population-4. The lowest significant correlated response to selection in the low direction was population-1 observed in being 3.77%, while the highest correlated response (10.45) was found in population-2, with an average of 8.24% (Table 4).

Significant (P<0.05) and low correlated responses to selection in TKW in the high direction were observed in four of the five populations and ranged from 2.98 in population-1 to 5.01% in population-5 (averaged 4.03%). However, the correlated responses in TKW for lower FLAN ranged from 5.98 in population-1 to 12.08% in population-2 and were found to be highly significant (P < 0.01) and higher in magnitude (averaged 9.56%) than those obtained in the high direction (Table 5).

Molecular marker analysis

In order to identify SSR markers associated with flag leaf angel, as an indicator for heat tolerance, the five F2 populations were subjected to BSA using SSR markers. Out of twelve SSR markers tested, four SSRs (33.3%) namely Xgwm294, Xgwm356, Xwmc398 and Xbarc113 located on chromosomes 2A, 2A, 6B and 6A, respectively, were polymorphic. A total number of 38 bands were amplified and ranged from 6 for Xwmc398-6B to 15 for Xgwm294-2A, with an average of 9.5 bands per marker. Of the 38 bands amplified with 4 SSRs, 25 bands (65.8%) were polymorphic, with an average of 6.3 polymorphic bands per marker. The lowest polymorphism (50.0%) was obtained with Xwmc398-6B, whereas the highest polymorphism (80.0%) was produced with Xgwm294-2A (Table 6).

Three out of the four polymorphic SSRs were able to distinguish high from low bulks in at least two populations. The marker Xgwm294-2A generated a specific band (404bp) for high FLAN in four populations, and another specific band (684bp) for low FLAN was present in three populations. A specific band (166bp) for high FLAN was also generated by Xbarc113-6A, but only in two populations. The marker Xwmc398-6B amplified a specific band (317bp) for high FLAN and another specific band (785bp) for low FLAN, and both bands were presented in population-1, population-4 and population-5 (Fig. 2).

Discussion

Flag leaf characteristics have been considered to be important determinants of grain yield in cereals crops including wheat (Chen *et al.*, 1995; Hirota *et al.*, 1990; Simon, 1999), and could provide a potential target for selection (Blake *et al.*, 2007). Therefore, in the present study, divergent phenotypic selection was applied for flag leaf angle under heat stress to five F_2 populations of bread wheat (*Triticum aestivum* L.). The positive and significant responses to divergent selection for FLAN obtained in the five populations used in the present study, in both directions, indicated the presence of abundant additive genetic variation among F₂ segregates allowing such responses to occur. To date, little information is available on the genetic mechanisms of flag leaf traits in wheat (Wu et al., 2016). Additive gene effects were reported to be mainly controlling FLAN in wheat (Nigam and Srivastava, 1976; Borojevic and Kraljevic-Balalic, 1984; Simon, 1999). Isidro et al. (2012) reported that multiple genes controlling leaf angle. However, Cristaldo et al. (1992) found one gene with at least three distinct alleles controlling the expression of leaf angle. Joshi and Chand (2002) reported that leaf angle was under the control of approximately three genes. The majority of studies provide evidence that leaf angle is a quantitative trait with vertical angle partially dominant over the horizontal angle (Wu et al., 1984). Moreover, the quantitative control of leaf angle is not fixed but changed as the plant matures; indicating that the genetic variation of this trait decreased through adult plant growth stages (Isidro et al., 2012).

Moderate to rather high heritability estimates obtained here for FLAN either realized (averaged 0.53) or estimated by the parent-offspring regression (averaged 0.50) were higher in magnitude than those reported by Simon (1999) for FLAN in four wheat crosses (averaged 36.1%). Positive and significant concurrent responses in GYP with selection for higher FLAN were obtained in two populations, with an average of 5.17%, which was lower in magnitude than the averaged correlated responses (8.24%) obtained in GYP for lower FLAN in four populations. The correlated responses to selection in TKW for higher FLAN obtained in four populations (averaged 4.03%) were much smaller in magnitude than those obtained for lower FLAN (averaged 9.56%).

The relationship between flag leaf angle and grain yield has been studied with variable results. Singh et al. (2008) found that flag leaf angle was positively and significantly correlated with grain yield in bread wheat. Araus and Slafer (2002) stated that stress during plant development causes changes in canopy features produces horizontal leaves. and Higher flag leaf angle should cause light influence into the canopy and, in turn, increase in grain yield (Lonbani and Arzani, 2011). It has been reported that horizontal leaves in wheat retain dew better and longer than vertically disposed leaves and, therefore, maintain a better water balance under (Henry, non-irrigated conditions 2006). However, Yap and Harvey (1972) and Winter and Ohlrogge (1973) could not detect any significant beneficial effect of leaf angle on crop growth rate in barley and maize, respectively. Advantages of erect leaves in giving increased crop growth rate and grain yield has been demonstrated in wheat (Maksimchuk, 1966; Tanner et al., 1966). Erect leaves can enhance photosynthesis and dry matter production by greater sunlight capture (Duncan, 1971; Isidro et al., 2012). However, the advantage of erect leaves is determined by the crop canopy and growing conditions (Yap and Harvey, 1972). If varieties were developed with a higher leaf area index at population density giving maximum vield and/or in environment-management situations capable of supporting a higher leaf area index, upright leaves might increase grain yield significantly (Duncan. 1971; Nigam and Srivastava, 1976). Horizontal leaves have maximum crop growth rates with leaf area index less than 4, but vertical leaves are thought to be superior with leaf area index greater than 4 (Loomis et al., 1967).

In our study, FLAN was positively and significant correlated with both GYP and TKW under heat stress condition. Optimum plant spacing for seed production was used. Moreover, the largest FLAN does not exceed 75°, which in turn does not allow shading of the lower leaf by a horizontal flag leaf directly above. In this regards, several aspects can affect leaf angle including plant densities (Pepper et al., 1976), temperature (Ledent and Moss, 1977), light intensity and wavelength (Kimura 1974, 1977). Adjustment of leaf angle improves biomass yield through light interception (Foulkes et al., 2007, 2009; Reynolds et al., 2009). Hasanuzzaman et al., (2013) reported that, high temperature can affect the degree of leaf rolling in many plants. Moreover, the amount of shading of the lower leaf, by a horizontal leaf directly above, rapidly decreased as distance between the leaves increased (Whigham, 1971).

Understanding of the genetic control of leaf angle can be furthered with quantitative trait loci (QTL) mapping to discover their chromosomal locations (Isidro *et al.*, 2012). However, there are very few investi-

gations on identifying the genomic regions controlling this trait in wheat using molecular markers. In the present study, BSA for FLAN revealed that three SSR markers (Xgwm294-2A; Xbarc113-6A and Xwmc398-6B) were able to distinguished high from low bulks in at least two populations. Three bands specific for high and two specific bands for low FLAN were generated, that could be used in the future as markers associated with FLAN under heat stress in wheat, indicating the suitability of SSR markers for identifying QTLs for FLAN on different wheat chromosomes. Similarly, by using 423 microsatellite primer pairs, Isidro et al., (2012 identified large-effect QTL for flag-leaf angle on chromosomes 2A, 2B, 3A, 3B, 4B, 5B and 7A of durum wheat. Recently, using a wheat microsatellite consensus map, Yang et al., (2016) identified a total of 55 additive and 51 pairs of epistatic QTLs on all the 21 chromosomes of bread wheat except 6D. A cytogenetic study by Li et al., (1992) in common wheat showed that chromosome 2D carried genes controlling leaf angle. In conclusion, the information presented here could help in understanding the genetic control involved in the inheritance of flag leaf angle and its relationship with grain yield under heat stress conditions in wheat. Moreover, SSR markers identified using BSA could be used as markers associated with FLAN under heat stress. Validation of these markers, by genotyping the whole F₂ populations, is still required to allow implementation of markerassisted selection (MAS) in wheat.

Traits	Pop. 1	Pop. 1 Pop. 2		Pop. 4	Pop. 5
FLAN with GYP	0.23*	0.26**	0.25**	0.22*	-0.06
FLAN with TKW	0.14	0.21*	0.20*	0.09	-0.05
TKW with GYP	0.41**	0.57**	0.53**	0.47**	0.62**

Table 1. Correlation coefficients among the studied traits in the five F₂ populations under heat stress conditions.

Table 2. Means of FLAN of the five F_2 populations under heat stress condition as well as means of the plants selected in the high and low direction.

Pop.	Base population		he selected lants	Selection differential		
No.	Mean	High	Low	High	Low	
1	30.65	56.00	8.60	25.35	22.05	
2	26.79	49.00	11.00	22.21	15.79	
3	43.10	70.00	12.00	26.90	30.10	
4	42.27	66.60	18.00	24.33	24.27	
5	46.11	72.60	21.40	26.49	24.71	
Mean	37.78	62.84	14.20	25.06	23.38	

Table 3. Observed response to selection in the F_3 families for FLAN in the high and low directions as well as realized heritability and parent-offspring regressions (bpo).

Pop. No.	Pop. 1		Pop. 2		Pop. 3		Pop. 4		Pop. 5	
	Mean	% OR	Mean	% OR						
Bulk	49.13	-	46.69	-	47.46	-	52.08	-	44.2	-
High	56.53	15.08**	56.65	21.33**	60.11	26.67**	64.84	24.50**	54.0	22.4**
Low	31.48	35.92**	31.61	32.29**	32.17	32.20**	37.73	27.55**	33.5	24.1**
Realized heritability	0.53		0.66		0.48		0.56		0.40	
b _{po} ± se	0.53** ± 0.039		0.50** ± 0.021		0.48** ± 0.074		0.56** ± 0.072		0.41** ± 0.063	

*, ** Significant at P < 0.05 and P < 0.01, respectively. % OR: the observed response to selection, b_{po} : parent-offspring regression.

Pop. No.	Pop. 1		Pop. 2		Pop. 3		Pop. 4		Pop. 5	
	Mean	% CR	Mean	% CR	Mean	% CR	Mean	% CR	Mean	% CR
Bulk	39.29	-	39.29	-	31.05	-	30.95	-	33.06	-
High	41.15	4.75*	39.50	0.53	32.78	5.58*	31.50	1.78	33.40	1.04
Low	37.81	3.77*	35.18	10.45**	28.34	8.73*	27.85	10.02*	31.72	4.03

Table 4. Correlated responses to selection in GYP in the high and low directions.

*, ** Significant at P < 0.05 and P < 0.01, respectively. % CR: the correlated response to selection.

Pop. No.	Pop. 1		Pop. 2		Pop. 3		Pop. 4		Pop. 5	
	Mean	% CR	Mean	% CR	Mean	% CR	Mean	% CR	Mean	% CR
Bulk	53.25	-	48.50	-	46.64	-	47.13	-	44.24	-
High	54.83	2.98*	50.59	4.31*	47.71	2.29	48.93	3.81*	46.46	5.01*
Low	50.06	5.98**	42.64	12.08**	42.00	9.95**	42.31	10.24**	43.52	1.63

*, ** Significant at P < 0.05 and P < 0.01, respectively. % CR: the correlated response to selection.

Table 6. Polymorphism detected between high and low bulks for FLAN in the five F₂ populations using four SSR markers.

Marker Name	CL	Sequence (5' - 3')	FR	ТВ	PB	%P
Xgwm294	2A	F: GGATTGGAGTTAAGAGAGAACCG R: GCAGAGTGATCAATGCCAGA	97-684	15	12	80.0
Xgwm356	2A	F: AGCGTTCTTGGGAATTAGAGA R: CCAATCAGCCTGCAACAAC	181-564	9	5	55.6
Xwmc398	6B	F: GGAGATTGACCGAGTGGAT R: CGTGAGAGCGGTTCTTTG	167-785	6	3	50.0
Xbarc113	6A	F: GCGCACAACAACGGACACTTAACAATT R:GGGACTCATTTAGCTTCTACTCGCCATTA	72-386	8	5	62.5
Tota	I		-	38	25	-
Average			-	9.5	6.3	65.8

Cl: Chromosol location of a marker, FR: fragment range (bp), TB: number of total bands, PB: number of polymorphic bands, %P: percentage of polymorphism.

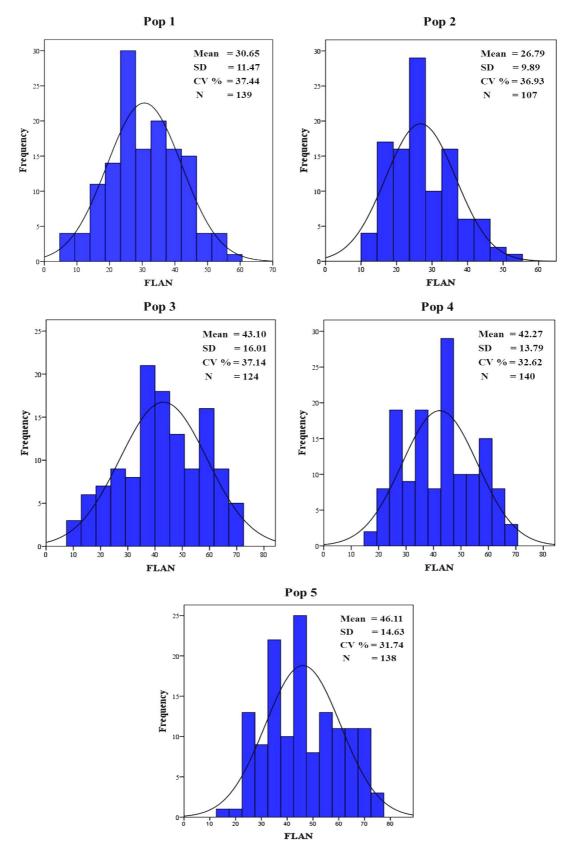
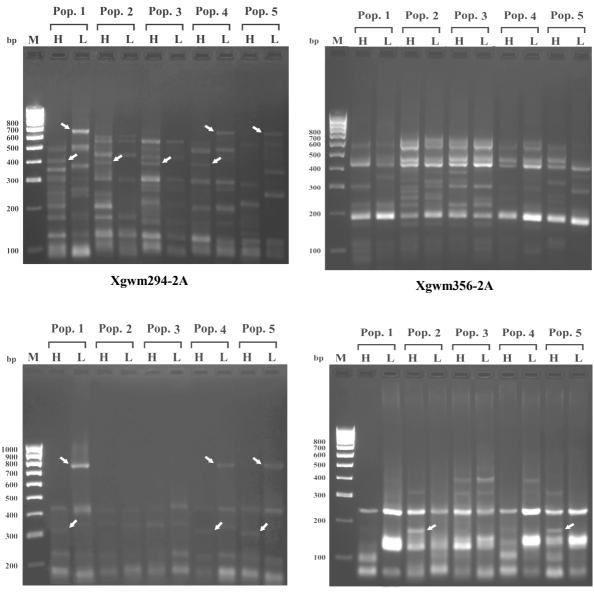


Fig. 1. Frequency distribution of the five F_2 populations for FLAN under heat stress.



Xwmc398-6B

Xbarc113-6A

Fig. 2. DNA amplification patterns obtained using BSA of FLAN in the five populations. M is the 100bp DNA ladder. Differences between the high (H) and low (L) bulks were detected using Xgwm294-2A, Xwmc398-6B and Xbarc113-6A markers. Arrows indicate polymorphic bands obtained which distinguished the high from low bulk.

References

- Araus J.L. and Slafer G.A. (2002). Plant breeding and drought in C3 cereals: what should we breed for?. Ann. Bot. 89: 225–240.
- Barakat M.N, Al-Doss A., Elshafei A.A., Moustafa A.K.A. (2012). Bulked segregant analysis to detect quantitative trait loci (QTL) related to heat tolerance at grain filling rate in wheat using simple sequence repeat (SSR) markers. Afr. J. Biotechnol. 11: 12436-12442.
- Biswal A.K. and Kohli A. (2013). Cereal flag leaf adaptations for grain yield under drought: knowledge status and gaps. Mol. Breed. 31:749–766.
- Blake N.K., Lanning S.P., Martin J.M., Sherman J.D. and Talbert L.E. (2007). Relationship of Flag Leaf Characteristics to Economically Important Traits in Two Spring Wheat Crosses. Crop Sci. 47: 491-494.
- Borojevic S. and Kraljevic-Balalic M. (1984) Inheritance of leaf architecture at different stages of wheat development. Zeitschrift für Pflanzenzüchtung 93: 89-100.
- Chen W.F., Xu Z.J. and Zhang B.L. (1995). Physiological bases of super high yield breeding in Rice. Liao Ning Science and Technology Publishing Company, Shenyang, China.
- Ciuca M. and Petcu E. (2009). SSR markers associated with membrane stability in wheat (*Triticum aestivum* L.). Romanian Agric. Res. 26: 21-24.
- Cristaldo R.M.O., De Carvalho F.I.F., Barbosaneto J.F. and Federizzi L.C. (1992). Inheritance of flag leaf angle in wheat (*Triticum aestivum* L.). Revista Brasileira de Genetica 15: 385–395.
- Duncan W.G. (1971). Leaf angles, leaf area and canopy photosynthesis. Crop Sci. 11: 482-485.

- El-Rawy M.A., Youssef M. (2014). Evaluation of drought and heat tolerance in wheat based on seedling traits and molecular analysis. J. Crop Sci. Biotech. 17: 183-189.
- Farooq M., Bramley H., Paltac J.A. and Siddique K.H.M. (2011). Heat Stress in Wheat during Reproductive and Grain-Filling Phases. Crit. Rev. Plant Sci. 30: 491-507.
- Feng B., Liu P., Li G., Dong S.T., Wang F.H., Kong L.A. and Zhang J.W. (2014). Effect of Heat Stress on the Photosynthetic Characteristics in Flag Leaves at the Grain-Filling Stage of Different Heat-Resistant Winter Wheat Varieties. J. Agron. Crop Sci. 200: 143–155.
- Foulkes M.J., Reynolds M. and Sylvester-Bradley R. (2009). Genetic improvement of grain crops: yield potentia. In: Sadras V.O. and Calderini D. (eds.). Crop physiology applications for genetic improvement and agronomy. Elsevier, Amsterdam.
- Foulkes M.J., Snape J.W., Shearman V.J., Reynolds M.P., Gaju O., Sylvester-Bradley R. (2007). Genetic progress in yield potential in wheat: recent advances and future prospects. J. Agric. Sci. 145:17
- Gibson L.R. and Paulsen G.M. (1999). Yield components of wheat grown under high temperature stress during reproductive growth. Crop Sci. 39: 1841-1846.
- Hasanuzzaman M., Nahar K., Alam M.M., Roychowdhury R. and Fujita M. (2013). Physiological, Biochemical, and Molecular Mechanisms of Heat Stress Tolerance in Plants. Int. J. Mol. Sci. 14: 9643–9684.
- Hassan M.I., Mohamed E.A., El-Rawy M.A. and Amein K.A. (2016). Evaluating interspecific wheat hybrids based on heat and drought stress tolerance. J. Crop Sci. Bio-

technol. 19: 85-98.

- Henry A. (2006). Breeding efficient crops and varieties for dryland conditions. In: Trivedi P.C. (ed.). Advances in Plant Physiology. I.K. international Publishing House Pvt. Ltd. New Delhi, India.
- Hirota O., Oka M., Takeda T. (1990). Sink activity estimation by sink size and dry matter increase during the ripening stage of barley (*Hordeum vulgare*) and rice (*Oryza sativa*) Ann. Bot. 65:349–354.
- Ibrahim A.M.H. and Quick J.S. (2001). Heritability of heat tolerance in winter and spring wheat. Crop Sci. 41: 1401–1405.
- Isidro J., Knox R., Clarke F., Singh A., DePauw R., Clarke J. and Somers D. (2012). Quantitative genetic analysis and mapping of leaf angle in durum wheat. Planta 236: 1713– 1723.
- Joshi A.K., Chand R. (2002) Variation and inheritance of leaf angle, and its association with spot blotch (*Bipolaris sorokiniana*) severity in wheat (*Triticum aestivum*). Euphytica 124:283–291.
- Kimura K. (1974) Effect of light on leaf inclination of *Triticum aestivum*.L. Monochromatic light. Ber. Ohara Inst. landw. Biol. Okayama Univ. 16: 47-56.
- Kimura K. (1977). Effect of light on leaf inclination of *Triticum aestivum*. IV. Intermittent irradiation and after effect of blue light. Ber Ohara Inst. Landw. Biol. Okayama Univ. 17:1–6.
- Ledent J. and Moss D. (1977). Spatial orientation of wheat leaves. Crop Sci. 17: 873–879.
- Li W.L., Li Z.S. and Mu S.M. (1992). Chromosomal locations of genes for erect flag leaves of common wheat variety Xiaoyan No. 6. Hereditas 19:71–75.
- Lonbani M. and Arzani A. (2011). Mor-

pho-physiological traits associated with terminal drought-stress tolerance in triticale and wheat. Agron. Res. 9: 315–329.

- Loomis R.S., Williams W.A., and Duncan W.G. (1967). Community architecture and the productivity of terrestrial plant communities. In: San Pietro, A., Greer F., and Amy T.J. (eds.). Harvesting the sun. Academic Press, New York.
- Maksimchuk G.P. (1966) Inheritance of position of leaves on yield of winter wheat. Sel. Semenovod 31 : 41-46.
- Murray M.G. and Thompson W.F. (1980). Rapid isolation of high molcular weight plant DNA. Nucleic Acids Res. 8: 4321-4325.
- Nigam S.N., Srivastava J.P. (1976). Inheritance of leaf angle in *Triticum aestivum* L. Euphytica 25: 457-461.
- Pepper G.E., Pearce R.B. and Mock J.J. (1976). Leaf Orientation and Yield of Maize. Crop Sci. 17: 883-886.
- Quarrie S.A., Lazic-Jancic V., Kovacevic D., Steed A. and Pekic S. (1999). Bulk segregant analysis with molecular markers and its use for improving drought resistance in maize. J. Exp. Bot. 50: 1299–1306.
- Reynolds M., Foulkes M.J., Slafer G.A., Berry P., Parry M.A.J., Snape J.W., Angus W.J. (2009). Raising yield potential in wheat. J. Exp. Bot. 60: 1899-1918.
- Sicher R.C. (1993). Assimilate partitioning within leaves of small grain cereals. In: Yash P.A., Prasanna M. and Govindjee D. (eds.). Photosynthesis Photoreactions to Plant Productivity. Dordrecht, the Netherlands: Kluwer Academic Publishers.
- Simon M.R. (1999). Inheritance of flagleaf angle, flag-leaf area and flagleaf area duration in four wheat crosses. Theor. Appl. Genet. 98:

310-314.

- Singh G.P., Chaudhry H.B, Yadav R. (2008). Genetics of flag leaf angle, width, Length and area in bread wheat (*Triticum aestivum*). Ind. J. Agric. Sci. 78: 436-438.
- Sofalian O., Chaparzadeh N., Javanmard A. and Hejazi M.S. (2008). Study the genetic diversity of wheat landraces from northwest of Iran based on ISSR molecular markers. Int. J. Agric. Biol. 10: 465-468.
- Stulnig T.M. and Amberger A. (1994). Exposing contaminating phenol in nucleic acid preparations. Bio-Techniques 16: 403-404.
- Tanner J.W., Gardner C.J., Stoskopf N.C. and Reinbergs E.A. (1966). Some observations on upright-leaf type small grains. Can. J. Plant Sci. 46: 690.
- Tian Y., Zhang H., Xu P. *et al.* (2015.) Genetic mapping of a QTL controlling leaf width and grain number in rice. Euphytica 202: 1–11.
- Whigham D.K. (1971). Leaf orientation and its effect on *Zea mays* L.. Retrospective Theses and Dissertations. Iowa State University, Paper 4862.
- Williams C.N. and Kwi S.N. (1967). A simple foliage model for studying light penetration. Ann. Bot. New Series 31: 783-790.
- Winter S.R. and Ohlrogge A.J. (1973).

Leaf angle, leaf area and corn (Zea mays L.) yield. Agron. J. 65: 395 397.

- Wu Q., Chen Y., Fu L. *et al.* (2016). QTL mapping of flag leaf traits in common wheat using an integrated high-density SSR and SNP genetic linkage map. Euphytica 208: 337– 351.
- Wu X.K., Xu Y.K. and Zhang F.Q. (1984) Genetic analysis of flag angle in wheat. Appl At Energy Agric 2: 1–5.
- Yang D., Liu Y., Cheng H., Chang L., Chen J., Chai S. and Li M. (2016). Genetic dissection of flag leaf morphology in wheat (*Triticum aestivum* L.) under diverse water regimes. BMC Genetics 17: 94.
- Yang J., Sears R.G., Gill B.S. and Paulsen G.M. (2002). Quantitative and Molecular characterization of heat tolerance in hexaploid wheat. Euphytica 126: 275–282.
- Yap T.C. and Harvey B.L. (1972). Inheritance of yield components and morpho-physiological traits in barley, *Hordeum vulgare* L. Crop Sci. 12: 283-286.
- Youssef M. (2012). Khirshyat 1.0: a simple micro-program for some molecular biology protocols. Genes, Genom. Genomics 6: 102-105.

الانتخاب المظهري وتحليل ضم الانعز الات المتفارقة لصفة زاوية ميل ورقة العلم تحت الاجهاد الحراري في قمح الخبز

محمد إبراهيم محمد حسن، محمود أبو السعود الراوي، علي محمد علي، محمد محمود حسيب الدفراوي قسم الوراثة – كلية الزراعة – جامعة أسيوط – جمهورية مصر العربية

الملخص

تم اجراء انتخاب ثنائي الاتجاه لصفة زاوية ميل ورقة العلم تحت ظروف الاجهاد الحراري في الجيل الثاني لخمس عشائر من قمح الخبز. وتم تقدير الاستجابة المباشرة للانتخاب لصفة زاوية ميل ورقة العلم وكذلك الاستجابة المتلازمة لصفتي محصول الحبوب للنبات الواحد ووزن الألف حبة. اظهرت زاوية ميل ورقة العلم ارتباطاً موجباً ومعنوي جداً مـع كـل مـن محصول الحبوب للنبات ووزن الألف حبة. وجدت استجابة موجبة ومعنوية جداً للانتخاب لزاوية ميل ورقة العلم في كلا الاتجاهين في الخمسة عشائر، حيث كانت الاستجابة في الاتجاه المنخفض (بمتوسط قدره ٣١,٤١%) أعلى في المقدار من الاستجابة فـي الاتجاه المرتفع (بمتوسط قدره ٢٢,٠%). وأدي الانتخاب في الاتجاه المرتفع لزاوية ميل ورقة العلم إلى استجابة متلازمة موجبة ومعنوية في محصول الحبوب للنبات الواحد في عشيرتين فقط بمتوسط قدره ٥,١٧%، والتي كانت منخفضة في مقدارها عن متوسط الاستجابة المتلازمة للانتخاب في الاتجاه المنخفض (٨,٢٤%) والذي تم الحصول عليه في أربعة عشائر. كما وجــدت اســتجابة متلازمة ومعنوية في وزن الألف حبة عند الانتخاب لارتفاع زاوية ميل ورقة العلم فـــي أربعـــة عشائر بمتوسط قدره ٤,٠٣%، والتي كانت أقل في المقدار من الاستجابة المتلازمــة التــي تــم الحصول عليها في الاتجاه المنخفض بمتوسط قدره ٩,٥٦%. كانت الآثار الجينية المضيفة هي المتحكم الرئيسي في صفة زاوية ميل ورقة العلم. كما كانت قيم المكافئ الوراثي المتحقق معتدلة (بمتوسط ٥,٥٣) ومماثلة لقيم المكافئ الوراثي التي تم الحصول عليها من خلال انحدار النيسل على متوسط الأبوين (بمتوسط ٠,٥٠). وأدى تحليل ضم الانعز الات المتفارقة لصفة زاوية ميل ورقة العلم باستخدام اثنا عشر واسم SSR إلى تحديد ثلاثة واسـمات هـــى Xgwm294-2A ، Xwmc398-6B ، Xbarc113-6A كانت قادرة على تمييز المجموعة المرتفعة بالنسبة لزاوية ميل ورقة العلم عن المجموعة المنخفضة في عشيرتين على الأقل. نتجت ثلاثة حزم خاصة بارتفاع زاوية ميل ورقة العلم وحزمتين خاصتين بانخفاضها ، والتي يمكن استخدامها في المستقبل كواسمات مرتبطة بزاوية ميل ورقة العلم تحت الاجهاد الحراري في القمح. والمعلومات الناتجة من البحث يمكن أن تساعد في فهم النظام الوراثي المتحكم في صفة زاويــة ميل ورقة العلم وعلاقتها مع محصول الحبوب تحت ظروف الإجهاد الحراري.