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Phenotypic Selection and Bulked Sergeant Analysis for 1000-Kernel Weight under Heat Stress in Durum Wheat

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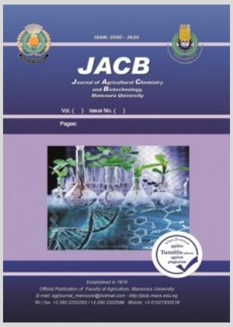

ABSTRACT

Divergent phenotypic selection for 1000-kernel weight (TKW) was performed under heat stress in a population of 120 F_7 recombinant inbred lines (RILs) of durum wheat. The direct response to selection for TKW and correlated response in grain yield per plant (GYP) were assessed under favorable and heat stress conditions. Considerable genetic variations were found among the tested RILs for TKW and GYP. Under heat stress, mean TKW of F_7 RILs selected in the high and low directions were 62.28 and 34.42g, respectively. Positive and highly significant responses to selection were obtained for TKW in the high (14.92 and 16.29%) and low (20.78 and 26.88%) directions under favorable and heat stress conditions, respectively. Selection for higher TKW produced positive and highly significant correlated response in GYP under heat stress (11.05%), whereas selection for lower TKW produced positive and highly significant correlated responses in GYP under favorable (11.13%) and heat stress (19.33%) conditions. High realized heritability estimates were obtained for TKW (0.74 and 0.75) and GYP (0.65 and 0.71) under favorable and heat stress conditions, respectively. F_8 RILs derived from selection for higher TKW showed higher heat tolerance index (averaged 1.20) than RILs selected for lower TKW (0.52), indicating the usefulness of selection for higher TKW in improving heat tolerance. Bulked segregant analysis with 40 simple sequence repeats (SSR) markers identified seven positives alleles located on 2A (1), 3B (2), 4A (1), 5A (1), 6A (1) and 7B (1) chromosomes that were associated with higher TKW as an indicator for heat tolerance.

Keywords: Durum wheat, Kernel weight, Heat tolerance, Selection, Bulked Segregant Analysis and SSRs.

INTRODUCTION

Durum wheat (*Triticum turgidum* L. var. durum) is an important cereal crop worldwide due to its nutritional value. It is mainly used to produce pasta and other semolina-based products, which are consumed in many countries of the world, including Egypt (Arriagada *et al.*, 2020). Due to its adaptation to dryland and semi-arid environments, durum wheat is mainly cultivated in the Mediterranean regions. However, it may be seriously affected by the climate change, resulting in a significant yield reduction (Dettori *et al.*, 2017).

Heat stress in wheat is a major factor caused yield reduction in many wheat-growing regions of the world including Egypt (Hassan *et al.*, 2016). The reduction resulted by high temperature is mainly due to the reduction in the rate of photosynthesis, enzymes inactivation, high respiration rate, protein denaturation, membrane injury and accelerated leaf senescence (Shah and Paulsen, 2003; Howarth, 2005).

Negative effects of heat stress are particularly severe at grain filling stage, and thus, 1000-kernel weight (TKW) is highly affected by heat stress (Saha *et al.*, 2020). High temperature affects kernel weight by reducing the duration of grain filling and inhibition of starch biosynthesis in the grains (Keeling *et al.*, 1993; Jenner, 1994), and consequently a significant reduction in kernel weight (Kumar *et al.*, 2016; Saha *et al.*, 2020).

Selection of high-yielding genotypes under heat stress conditions is one of the main goals of wheat breeding programs. However, the low heritability and presence of genotype-by-environment interactions reduce the efficiency of using grain yield as a direct selection criterion (Fellahi *et al.*, 2018). Unlike, indirect selection using highly heritable and more stable yield components traits might be more effective than direct selection for grain yield (Mohamed *et al.*, 2019). In addition, the genetic gain for grain yield was found to be correlated with different yield component traits, including 1000-kernel weight (TKW), kernel length and kernel width, which finally affect grain yield (Kumar *et al.*, 2021). Among yield components, TKW is highly heritable trait and less affected by the environment, and consequently it is more effective for indirect selection for grain yield (Xu *et al.*, 2017). Therefore, the improvement of grain yield in wheat has been successfully achieved by increasing TKW (Tian *et al.*, 2011; Zheng *et al.*, 2011; Lopes *et al.*, 2012; Aisawi *et al.*, 2015; Tshikunde *et al.*, 2019).

Since the conventional breeding for polygenic traits needs much more efforts, integrating marker-assisted selection (MAS) into conventional plant breeding programs would enhance the efficiency of improving polygenic traits (Babu *et al.*, 2004; Holland, 2004). In this regard, the association of numerous simple sequence repeats (SSR) markers with heat tolerance-related traits has been reported in wheat (Barakat *et al.*, 2011; Sun *et al.*, 2015; El-Rawy, 2016; Saha *et al.*, 2020). Bulked segregant analysis (BSA) is a rapid and highly efficient method described by Michelmore *et al.* (1991) for the detection of molecular markers in specific genomic regions using segregating populations. Since chromosomal locations of numerous SSR markers have been determined in different species, the map location of closely

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linked quantitative trait loci (QTLs) can be determined using BSA without the need for genotyping every individual in the population (Quarrie *et al*., 1999). Therefore, BSA has been successfully used for identification of SSR markers associated with important traits (Torres *et al*., 2010; Barakat *et al*., 2011; Hassan *et al*., 2016).

In the present study, divergent phenotypic selection for TKW was applied under heat stress conditions in a population of 120 F_7 recombinant inbred lines (RILs) derived from a cross between heat tolerant and susceptible durum wheat genotypes. The objectives were (1) to estimate the direct response to selection for TKW and the correlated response in grain yield per plant (GYP) under favorable and heat stress conditions; (2) to identify promising durum wheat genotypes under heat stress conditions; and (3) to detect SSR markers associated with TKW under heat stress using BSA to be used in wheat breeding programs.

MATERIALS AND METHODS

Plant materials and field evaluations

The basic plant material utilized in the present study is a population of 120 F_7 recombinant inbred lines (RILs) of durum wheat (*Triticum turgidum* L. var. durum) derived from a cross between an Egyptian durum wheat cultivar (Sohag-3) and a black-glumed landrace (WK-12-2). The parental landrace WK-12-2 was originally collected from a farmer`s field near Dandara Temple in Qena Governorate in 1993, and since then it was cultivated and allowed to self-pollinate every year to ensure its stability. Cytological analysis was also performed which confirmed the tetraploid nature of the chromosome complement (2n= 4x= 28) and the regularity of meiosis (Omara *et al*., 2006). In a previous work conducted at Faculty of Agriculture of Assiut University by Hassan and El-Rawy (unpublished data), WK-12-2 produced higher 1000-kerenl weight (TKW) than Sohag-3. However, Sohag-3 showed higher grain yield per plant (GYP) and higher tolerance to heat stress than WK-12-2.

In 2018-2019 season, seeds of the two parental genotypes (Sohag-3 and WK-12-2) and the derived 120 F_7 RILs were sown at the Experimental Farm of Faculty of Agriculture, Assiut University, Egypt on 26th November as a favorable sowing date and 31st December as a late sowing date in a randomized complete block design (RCBD) with three replications. Each genotype was represented in each block by a single row of 10 plants spaced 30cm from each other, with 50cm row spacing. The late sowing date was chosen to allow the late sown plants to be subjected to heat stress which usually develop later in the season. At the maturity, GYP (g) and TKW (g) were recorded individually for each plant of the two sowing dates.

Selection procedure

Based on the phenotypic data recorded in 2018/2019 season, a divergent phenotypic selection for TKW was employed on the 120 RILs of the late sowing date. The eight RILs with the highest TKW as well as the eight RILs with the lowest TKW were selected (an intensity of 6.7%). Equal numbers of seeds were taken from each RIL and pooled together to form the unselected bulk.

In 2019/2020 season, the highest and lowest RILs along with the unselected bulk were sown in the field on 25th November as a favorable sowing date and 30th December as a late sowing date (heat stress condition) in a RCBD with three replications. Each RIL was represented in each block by a single row of 10 plants spaced 30cm from each other, with 50cm row spacing, while four rows were used for the unselected bulk. At the maturity, GYP (g) and TKW (g) were recorded individually for each plant of the two sowing dates.

The recorded maximum air temperatures at the experimental site during March and April of 2018/2019 and 2019/2020 seasons indicated that several heat waves (above 33°C) have occurred for several days especially in April of the two growing seasons, which coincided with the post flowering stages (Fig.1).

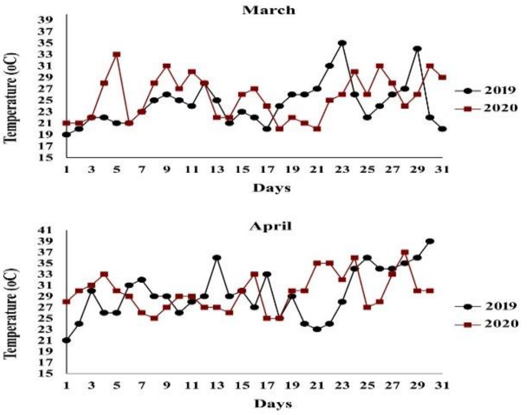

Fig.1. Maximum daily temperatures during March and April of the two growing seasons of 2018/2019 and 2019/2020 at the experimental site. (https://www.wunderground.com).

Statistical analysis

To test the significance of differences among RILs, the phenotypic data of TKW and GYP were statistically analyzed using the analyis of variance (ANOVA) for each environemt (sowing date). Combined ANOVA across the two environments (sowing dates) were also used to test the significance of differences among all genotypes and environments and the significance of genotype-by-environment interactions. Components of variance of TKW and GYP were estimated from the mean squares of the combined ANOVA and broad-sense heritability estimates were then computed. Data of TKW and GYP of the RILs selected for higher TKW and RILs selected for lower TKW as well as the unselected bulk were subjected to analysis of variance in order to test the significance of the differences between the high as well as the low selected RILs against the unselected bulk. Pearson's correlation coefficients were estimated between GYP and TKW under favorable and heat stress conditions.

The selection differential for each direction was calculated as the difference between the mean of selected F_7 RILs and the mean of the base population (120 F_7 RILs population).

The direct response to selection for TKW and correlated responses in GYP in the high and low directions were calculated for each environment as follow:

$$\text{Response \%} = \frac{\text{Mean of selected } F_8 \text{ RILs} - \text{Mean of } F_8 \text{ bulk}}{\text{Mean of } F_8 \text{ bulk}} \times 100$$

Heritability estimation

Heritability of TKW and GYP was estimated by the following two methods:

1- The realized heritability was calculated as:

$$h^2 = \frac{[\bar{H}_S - \bar{L}_S]}{[\bar{H}_B - \bar{L}_B]}$$

Where: $\bar{H}_S$ and $\bar{L}_S$ are the means of selected F_8 RILs in the high and low directions, respectively, while $\bar{H}_B$ and $\bar{L}_B$ are the means of their corresponding progenitors (F_7 RILs) selected in the high and low directions, respectively (Ibrahim and Quick, 2001).

2- The parent-offspring regression (b_{po}) was calculated by regression of the means of selected F_8 RILs on the means of their corresponding progenitor F_7 RILs.

Heat tolerance index (HTI)

Heat tolerance index of F_8 RILs selected in the high and low directions was adjusted based on TKW under favorable and heat stress conditions using the formula described by Fernandez (1992) as follow:

$$\mathbf{HTI = (Y_p * Y_s)/(\bar{Y}_p)^2}$$

Where, Y_p and Y_s are the mean TKW of a genotype under favorable and heat stress conditions, respectively and $\bar{Y}_p$ is the mean of all genotypes under favorable conditions.

Bulked segregant analysis (BSA)

To identify SSR markers associated with TKW in specific genomic regions under heat stress conditions, the F_7 RILs population was subjected to BSA (Quarrie *et al.,* 1999) with 40 SSR markers. TKW was used as an indicator of heat tolerance to perform BSA. The highest eight and lowest eight RILs selected from the F_7 RILs population based on TKW evaluated under heat stress conditions were used to construct two DNA bulks for BSA. DNA extraction of each RIL was carried out following Murray and Thompson (1980). Aliquots of DNA from each RIL in each group were mixed to produce high and low DNA bulks. A total of forty wheat microsatellites or SSR primer pairs representing all wheat chromosomes of the A and B genomes of wheat (Table 1) were selected and used for BSA (at least two SSRs were used for each chromosome).

Sequences of SSR primers, chromosomal locations and PCR conditions were obtained by the GrainGenes Database (http://wheat.pw.usda.gov). PCR amplifications were performed in a SensoQuest LabCycler with OnePCR master mix (GeneDireX, Inc.). PCR products were separated on 2.5% agarose gels in TBE buffer (0.5 X). A 100bp DNA Ladder was used to estimate the size of amplified DNA fragments (bands). Putative polymorphisms between the high and low bulks were detected for each SSR marker separately.

Table 1. Names, chromosomal locations, sequences of forward and reverse primers and annealing temperature °C (Tm) of 40 SSR markers used in this study.

Name	Forward primer (5' - 3')	Reverse primer (5' - 3')	Tm (°C)
Xgwm33-1A	GGAGTCACACTTGTTTGTGCA	CACTGCACACCTAACTACCTGC	60
Xgwm497-1A	GTAGTGAAGACAAGGGCATT	CCGAAAGTTGGGTGATATAC	55
Xgwm95-2A	GATCAAACACACACCCCTCC	AATGCAAAGTGAAAAACCCG	60
Xgwm294-2A	GGATTGGAGTTAAGAGAGAACCG	GCAGAGTGATCAATGCCAGA	55
Xgwm339-2A	AATTTTCTTCCTCACTTATT	AAACGAACAACCACTCAATC	50
Xgwm356-2A	AGCGTTCTTGGGAATTAGAGA	CCAATCAGCCTGCAACAAC	55
Xgwm155-3A	CAATCATTTCCCCCTCCC	AATCATTGGAAATCCATATGCC	60
Xwmc651-3A	CGACGACGTCCGGGTG	CATTTCCTCTCCCATATCTCTCATC	60
Xgwm160-4A	TTCAATTCAGTCTTGGCTTGG	CTGCAGGAAAAAAAGTACACCC	55
Xgwm165-4A	TGCAGTGGTCAGATGTTTCC	CTTTTCTTTCAGATTGCGCC	60
Xgwm186-5A	GCAGAGCCTGGTTCAAAAAG	CGCCTCTAGCGAGAGCTATG	60
Xgwm291-5A	CATCCCTACGCCACTCTGC	AATGGTATCTATTCCGACCCG	60
Xgwm292-5A	TCACCGTGGTCACCGAC	CCACCGAGCCGATAATGTAC	60
Xgwm293-5A	TACTGGTTCACATTGGTGCG	TCGCCATCACTCGTTCAAG	55
Xbarc186-5A	GGAGTGTCGAGATGATGTGGAAAC	CGCAGACGTCAGCAGCTCGAGAGG	60
Xgwm459-6A	ATGGAGTGGTCACACTTTGAA	AGCTTCTCTGACCAACTTCTCG	55
Xbarc113-6A	GCGCACAACAACGGACACTTAACAAT	GGGACTCATTTAGCTTCTACTCGCCATTA	50
Xgwm63-7A	TCGACCTGATCGCCCCTA	CGCCCTGGGTGATGAATAGT	60
Xgwm260-7A	GCCCCCTTGCACAAATC	CGCAGCTACAGGAGGCC	55
Xwmc273-7A	AGTTATGTATTCTCTCGAGCCTG	GGTAACCACTAGAGTATGTCCTT	50
Xwmc596-7A	TCAGCAACAAACATGCTCGG	CCCGTGTAGGCGGTAGCTCTT	60
Xwmc603-7A	ACAAACGGTGACAATGCAAGGA	CGCCTCTCTCGTAAGCCTCAAC	60
Xbarc121-7A	ACTGATCAGCAATGTCAACTGAA	CCGGTGTCTTTCCTAACGCTATG	50
Xgwm18-1B	GGTTGCTGAAGAACCTTATTTAGG	TGGCGCCATGATTGCATTATCTTC	50
Xgwm268-1B	AGGGGATATGTTGTCACTCCA	TTATGTGATTGCGTACGTACCC	55
Xgwm111-2B	GTTGCACGACCTACAAAGCA	ATCGCTCACTCACTATCGGG	55
Xgwm120-2B	GATCCACCTTCCTCTCTCTC	GATTATACTGGTGCCGAAAC	60
Xgwm389-3B	ATCATGTCGATCTCCTTGACG	TGCCATGCACATTAGCAGAT	60
Xgwm493-3B	TTCCCATAACTAAAACCGCG	GGAACATCATTTCTGGACTTTG	60
Xgwm533-3B	AAGGCGAATCAAACGGAATA	GTTGCTTTAGGGGAAAAGCC	60
Xgwm566-3B	TCTGTCTACCCATGGGATTTG	CTGGCTTCGAGGTAAGCAAC	60
Xgwm113-4B	ATTCGAGGTTAGGAGGAAGAGG	GAGGGTCGGCCTATAAGACC	55
Xgwm513-4B	ATCCGTAGCACCTACTGGTCA	GGTCTGTTCATGCCACATTG	60
Xgwm408-5B	TCGATTTATTTGGGCCACTG	GTATAATTCGTTCACAGCACGC	55
Xgwm499-5B	ACTTGTATGCTCCATTGATTGG	GGGGAGTGGAAACTGCATAA	60
Xgwm626-6B	GATCTAAAATGTTATTTTCTCTC	TGACTATCAGCTAAACGTGT	50
Xwmc398-6B	GGAGATTGACCGAGTGGAT	CGTGAGAGCGGTTCTTTG	60
Xgwm146-7B	CCAAAAAAACTGCCTGCATG	CTCTGGCATTGCTCCTTGG	60
Xgwm573-7B	AAGAGATAACATGCAAGAAA	TTCAAATATGTGGGAACTAC	50
Xgwm577-7B	ATGGCATAATTTGGTGAAATTG	TGTTTCAAGCCCAACTTCTATT	55

RESULTS AND DISCUSSION

The base population (F_7 RILs)

The separated analysis of variance of TKW and GYP for each environment (Table 2) revealed highly significant differences (*P*<0.01) among the 120 RILs under favorable and heat stress conditions for both traits. In addition, the combined ANOVA (Table 2) revealed highly significant differences (*P*<0.01) for TKW and GYP among the 120 RILs across the two environments (sowing dates). Highly significant (*P*<0.01) mean squares due to environments were also observed for TKW and GYP. However, a highly significant genotype-by-environment interaction was observed only for GYP. High broad-sense heritability estimates, calculated form mean squares of the combined ANOVA, were obtained for TKW (0.92) and GYP (0.79), indicating the occurrence of considerable genetic variations.

Table 2. Mean squares of 1000-kernel weight (TKW) and grain yield per plant (GYP) of the 120 F_7 RILs population under favorable and heat stress conditions as well as mean squares of the combined analysis of variance across the two environments and heritability estimates.

Source of variance	d.f	Mean square			
		Favorable		Heat stress	
		TKW	GYP	TKW	GYP
Replicates	2	4.42	143.55	3.01	11.23
Genotypes	119	227.3**	5591.5**	156.0**	1515.3**
Error	238	6.39	54.39	4.31	15.47

The combined ANOVA

Source of variance	d.f	Mean square	
		TKW	GYP
Environments (E)	1	21421.5**	287428**
Replicates within E	4	3.72	77.4
Genotypes (G)	119	379.3**	6423.7**
G×E interaction	119	3.95	683.1**
Error	476	5.35	34.93
Broad-sense heritability (h^2_B)		0.92	0.79

**** indicates significant differences at 0.01 level of probability.**

A wide range of variation was observed for TKW in the RILs population under both favorable and heat stress conditions, which was higher under favorable condition (S^2= 75.7) than those obtained under heat stress (S^2= 52.0). The TKW values of the F_7 RILs ranged from 38.36 to 78.63 g under favorable environment, and from 31.12 to 65.42 g under heat stress conditions. Some segregates exhibited extreme TKW which exceeded values of the parental genotypes, indicating the occurrence of transgressive segregation for TKW. The mean TKW of the F_7 RILs population reduced from 60.80 g under favorable conditions to 49.90 g under heat stress conditions (an average reduction of 17.93%). The frequency distributions of TKW in the F_7 RILs population under favorable and heat stress conditions (Fig.2) were continuous and approached normality, indicating that TKW is under the control of polygenes and amenable to selection. In accordance, it has been stated that TKW is a quantitative trait controlled by polygenes (Giura and Saulescu, 1996; Ammiraju *et al.*, 2001).

Considerable differences in spike morphology and kernel size were observed in the F_7 RILs population under heat stress condition (Fig.3). The means TKW of the F_7 RILs selected under heat stress ranged from 60.28 to 65.42 g with an average of 62.28 g in the high direction, and from 31.12 to 37.06 g with an average of 34.42 g in the low direction. The selection differential in the high TKW direction, ranged from 10.38 to 15.52 with an average of 12.39, was smaller in magnitude than those obtained in the low direction which ranged from 12.84 to 18.78 with an average of 15.48 (Table 3).

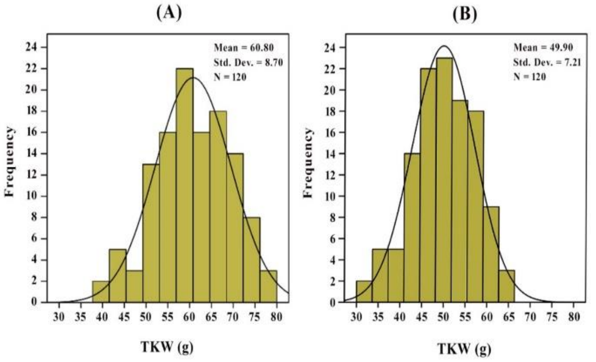

Fig.2. Frequency distribution of TKW in the F_7 RILs population under favorable (A) and heat stress (B) conditions.

Fig.3. Differences in spike morphology and kernel size in the F_7 RILs population.

Table 3. Means of TKW (g) of 120 F_7 RILs population and F_7 RILs selected for high and low TKW under heat stress condition as well as the selection differentials for the two directions.

Population Mean	High RILs		Low RILs		Selection differential	
	No.	Mean	No.	Mean	High	Low
49.90	1	61.44	1	36.34	11.54	13.56
	2	60.28	2	37.06	10.38	12.84
	3	61.09	3	31.12	11.19	18.78
	4	64.09	4	34.33	14.19	15.57
	5	60.56	5	34.62	10.66	15.28
	6	60.46	6	33.52	10.56	16.38
	7	65.42	7	35.50	15.52	14.40
	8	64.94	8	32.85	15.04	17.05
Average		62.28		34.42	12.39	15.48

Responses to selection and heritability estimates

The analysis of variance for TKW and GYP of selected F_8 RILs and F_8 bulk (Table 4) revealed highly significant differences (*P*<0.01) between the F_8 RILs selected for higher TKW and the F_8 bulk. Meantime, significant differences (*P*<0.01) were found between the F_8 RILs selected for lower TKW and the F_8 bulk. Highly significant differences (*P*<0.01) were also observed for TKW and GYP between RILs selected for higher and RILs selected for lower TKW under both favorable and heat stress conditions.

Table 4. The analysis of variance of TKW and GYP of the F_8 bulk (B) and F_8 RILs selected for high (H) and low (L) TKW under favorable and heat stress conditions.

Source of variance	d.f	Mean square			
		Favorable		Heat stress	
		TKW	GYP	TKW	GYP
High *vs* Bulk					
Replicates	2	18.43	37.31	3.65	142.32**
Genotypes	9	141.26**	7077.52**	75.32**	661.08**
H *vs* B	1	357.04**	207.53	307.58**	296.59**
Among H	7	130.61**	9048.90**	52.89**	806.11**
Among B	1	0.18	147.81	0.05	10.35
Error	18	21.48	130.99	5.20	11.56
Low *vs* Bulk					
Replicates	2	1.95	45.84	7.22*	318.75**
Genotypes	9	157.78**	2114.79**	100.37**	451.23**
L *vs* B	1	692.21**	832.48**	808.24**	907.42**
Among L	7	103.98**	2578.97**	13.57**	449.05**
Among B	1	0.05	147.81	0.05	10.35
Error	18	9.25	57.79	1.37	29.07
High *vs* Low					
Replicates	2	21.63	21.67	2.75	242.84**
Genotypes	15	1568.9**	10441.7**	1136.7**	2612.57**
H *vs* L	1	21891**	75231.7**	16585**	30402.5**
Among H	7	130.6**	9048.90**	52.89**	806.11**
Among L	7	103**	2578.97**	13.57*	449.05**
Error	30	14.20	81.37	3.54	26.89

*** and ** indicate significant differences at 0.05 and 0.01 levels of probability, respectively.**

The impact of heat stress of the late sowing date on TKW was quite remarkable with the F_8 RILs selected in the low direction, where the mean TKW reduced from 45.78 g under favorable conditions to 35.55 g under heat stress (an average reduction of 22.35%). Whereas, the mean TKW of the F_8 RILs selected for higher TKW reduced from 66.41 g under favorable conditions to 56.53 g under heat stress, with an average reduction of 14.88%.

Reduction in kernel weight between 21 and 35% due to heat stress was reported in wheat by Assad and Paulsen (2002). Accordingly, heat stress of the late sowing shortened the developmental stages of wheat which negatively affected the grain development and thus grain yield (Suleiman *et al.*, 2014). Heat stress affects kernel weight by reducing the duration of grain filling and inhibition of starch biosynthesis (Keeling *et al.*, 1993; Jenner, 1994), causing a significant reduction in kernel weight (Kumar *et al*., 2016; Saha *et al.*, 2020).

Under favorable condition, the mean TKW of the F_8 RILs selected for higher TKW ranged from 60.45 to 78.50g, with an average 66.41g. Whereas, RILs selected for lower TKW ranged from 36.80 to 56.94g, with an average of 45.78g. The direct responses to selection for TKW in the high direction were significantly positive in four of the eight selected RILs which ranged from 4.60 to 35.84%, with an average of 14.92%. However, direct selection for lower TKW resulted in significant positive responses in seven RILs which ranged from 1.47 to 36.32%, with an average of 20.78% (Table 5).

Under heat stress, the mean TKW of the F_8 RILs selected for higher TKW ranged from 50.31 to 64.05g, with an average of 56.53g. Whereas, the F_8 RILs selected for lower TKW ranged from 31.51 to 37.75g, with an average of 35.55g. Positive and highly significant direct responses to selection for higher TKW were obtained in the eight selected RILs which ranged from 3.5 to 31.76%, with an average of 16.29%. Meantime, positive and highly significant direct responses to selection for low TKW were also obtained in the eight RILs, ranging from 22.34 to 35.18%, with an average of 26.88% (Table 6).

Evidently, the observed direct responses in the low direction were higher in magnitude than those obtained in the high direction under both favorable and heat stress conditions. This result was expected because the response to selection depends on the selection differential and heritability (Falconer and Mackay, 1996), and the selection differential obtained in the present study was higher in the low direction than those obtained in the high direction. On the other hand, the observed direct responses to selection for TKW in the high and low directions were higher in magnitude under heat stress (16.29 and 26.88%, respectively) than those obtained under favorable conditions (14.92 and 20.78%, respectively), indicating the occurrence of considerable genetic advance under heat stress compared to favorable environment. This result is expected according to Falconer (1990) who demonstrated that the response to selection is maximum under the environment in which selection was conducted. In accordance, Mutawe *et al.* (2018) reported that selection for higher TKW conducted under heat stress of a late sowing date resulted in a greater response under heat stress than under favorable conditions. In addition, different estimates of genetic gains were previously reported for TKW in wheat under different environments (Beche *et al.*, 2014; El-Rawy, 2015; Zhang *et al.*, 2016; Gao *et al.*, 2017).

High realized heritability estimates were observed for TKW under favorable (0.74) and heat stress (0.75) conditions and were found to be similar to heritability estimates obtained by the parent-offspring regression under favorable (0.75) and heat stress (0.75) conditions (Table 5 and Table 6), indicating high additive gene effects. Obviously, heritability estimates obtained in the present study for TKW were higher in magnitude than realized heritability (0.28) and heritability calculated from parent-offspring regression (0.29) reported by (El-Rawy, 2015). High broad-sense (0.85) and narrow-sense (0.66) heritability estimates were also obtained for TKW under heat stress conditions by El-Rawy (2016).

Table 5. Means of TKW (g) of the F_8 bulk and F_8 RILs selected for high and low TKW as well as the observed responses to selection (%), realized heritability and parent-offspring regressions (b_{po}) under favorable condition.

Bulk Mean	High RILs		Low RILs		Response to Selection (%)	
	No.	TKW (g)	No.	TKW (g)	High	Low
57.79	1	74.00	1	46.40	28.05**	19.71**
	2	64.67	2	47.05	11.91*	18.58**
	3	67.73	3	46.65	17.20**	19.28**
	4	62.15	4	48.20	7.54	16.59**
	5	60.45	5	41.15	4.60	28.79**
	6	60.93	6	43.05	5.43	25.51**
	7	62.88	7	56.94	8.80	1.47
	8	78.50	8	36.80	35.84**	36.32**
	Mean	66.41	Mean	45.78	14.92**	20.78**
Realized Heritability			0.74			
b_{po} ± se			0.75** ± 0.11			

*** and ** indicate significant differences at 0.05 and 0.01 levels of probability, respectively.**

Table 6. Means of TKW (g) of the F$_8$ bulk and F$_8$ RILs selected for high and low TKW as well as the observed responses to selection (%), realized heritability and parent-offspring regressions (b$_{po}$) under heat stress condition.

Bulk Mean	High RILs		Low RILs		Response to Selection (%)	
	No.	TKW (g)	No.	TKW (g)	High	Low
48.61	1	57.85	1	37.75	19.01**	22.34**
	2	57.15	2	37.37	17.57**	23.13**
	3	50.31	3	31.51	3.50*	35.18**
	4	53.05	4	35.98	9.13**	25.98**
	5	57.40	5	35.73	18.08**	26.50**
	6	58.75	6	36.47	20.86**	24.97**
	7	53.65	7	36.35	10.37**	25.22**
	8	64.05	8	33.20	31.76**	31.70**
	Mean	56.53	Mean	35.55	16.29**	26.88**
Realized Heritability				0.75		
b$_{po}$ ± se				0.75** ± 0.06		

*** and ** indicate significant differences at 0.05 and 0.01 levels of probability, respectively.**

A high heritability for TKW which reached 0.88 was also observed in wheat by Ramya *et al.* (2010). In addition, different high heritability estimates were previously found for TKW, indicating the occurrence of high additive gene effects and thereby a high response to selection for this trait (Wang *et al.*, 2009; Al-Tabbal and Al-Fraihat, 2012; Wang *et al.*, 2012).

Correlated responses to selection

Highly significant and positive phenotypic correlations were found between TKW and GYP in F$_8$ RILs under favorable (r= 0.37; $P<0.01$) and heat stress (r= 0.60; $P<0.01$) conditions (Fig.4). The F$_8$ RILs selected for higher TKW produced higher GYP (g) under favorable and heat stress conditions (124.88 and 78.99, respectively) than the F$_8$ RILs selected for lower TKW (105.14 and 57.38, respectively), indicating that the impact of heat stress on mean GYP was higher with the F$_8$ RILs selected in the low direction, with an average reduction of 45.43%, than the F$_8$ RILs selected for higher TKW which had an average reduction of 36.75%. Meantime, the mean GYP (g) of the bulks reduced from 118.31 under favorable condition to 71.13 under heat stress, with an average reduction of 39.88%. Highly significant ($P<0.01$) and positive correlated response to selection for higher TKW was obtained in GYP under heat stress (11.05%), whereas non-significant correlated response (5.55%; $P>0.05$) was found under favorable conditions. However, highly significant ($P<0.01$) correlated responses to selection for lower TKW were obtained in GYP under both favorable (11.13%) and heat stress (19.33%) conditions. High realized heritability estimates were observed for GYP (g) under favorable (0.65) and heat stress (0.71) conditions. However, low heritability estimates for GYP were obtained by the parent-offspring regression under favorable (0.18) and heat stress (0.30) conditions (Table 7).

Evidently, the concurrent responses to selection for TKW obtained in GYP were greater under heat stress than those obtained under favorable conditions; this can be explained as due to the correlation between TKW and GYP being much larger under heat stress than under favorable conditions. In this regard, moderate to high correlations between TKW and GYP have been widely reported in wheat under different environmental conditions (Zheng *et al.*, 2011; Al-Tabbal and Al-Fraihat, 2012; El-Rawy, 2015; Qin *et al.*, 2015; Hassan *et al.*, 2016; Gao *et al.*, 2017), suggesting that selection of higher TKW is highly effective for improving GYP in wheat (Tshikunde *et al.*, 2019).

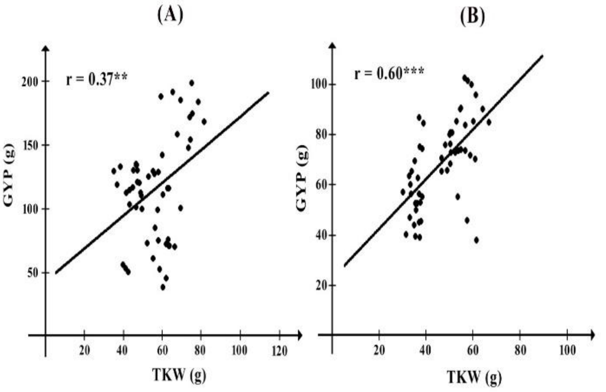

Fig. 4. Phenotypic correlation between TKW and GYP in the F$_8$ RILs under favorable (A) and heat stress (B) conditions.

Table 7. Mean GYP (g) of the F$_8$ bulk and F$_8$ RILs selected for high and low TKW as well the correlated responses to selection (%), realized heritability and parent-offspring (b$_{po}$) under favorable and heat stress conditions.

Genotype	Favorable		Heat stress	
	GYP (g)	Correlated Response (%)	GYP (g)	Correlated Response (%)
Bulk	118.31	-	71.13	-
High	124.88	5.55	78.99	11.05**
Low	105.14	11.13**	57.38	19.33**
Realized Heritability	0.65		0.71	
b$_{po}$ ± se	0.18±0.49		0.30±0.18	

**** indicates significant differences at 0.01 level of probability.**

Heat tolerance index based on TKW

In the present study, heat tolerance index adjusted based on TKW (HTI-TKW) was used as an indicator of heat tolerance for selected F$_8$ RILs (Fig.5). Obviously, the impact of heat stress of the late sowing date on TKW was quite remarkable with the F$_8$ RILs selected in the low direction being most affected with an average reduction of 22.35%, whereas the F$_8$ RILs selected for higher TKW were least affected with an average reduction of 14.88%. Consequently, HTI-TKW of the F$_8$ RILs selected in the high direction ranged from 1.05 to 1.60 (averaged 1.20), while HTI-TKW of the F$_8$ RILs selected in the low direction ranged from 0.39 to 0.66 (averaged 0.52). In this regard, Li *et al.* (2018) reported that heat tolerance of wheat genotypes can be much better defined using HTI, where genotypes with a high HTI (>1) can be considered as heat tolerant. Therefore, HTI could be used as an effective criterion for heat tolerance. Accordingly, these findings indicated that the F$_8$ RILs selected for higher TKW had a higher heat tolerance than the F$_8$ RILs selected in the low direction. Interestingly, out of eight F$_8$ RILs selected for higher TKW, the RIL-8 showed the highest TKW (64.05 g) and the largest response to selection (31.76%) under heat stress conditions. Furthermore, the RIL-8 had the highest HTI-TKW (1.60), indicating that RIL-8 could be considered as the most promising genotype under heat stress conditions to be used in wheat breeding programs.

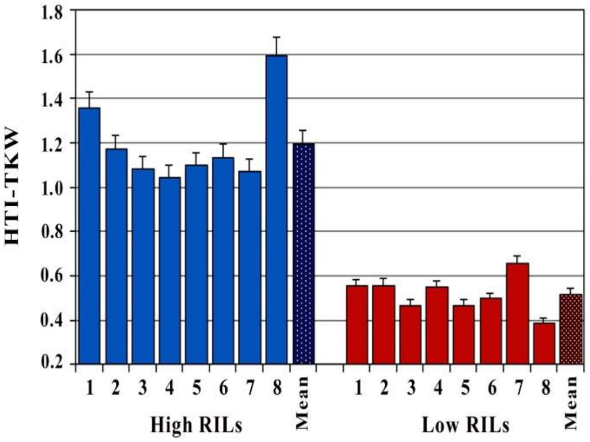

Fig.5. Heat tolerance index based on TKW (HTI-TKW) in the high and low F_8 RILs.

Bulked-segregant analysis (BSA)

In the present study, the F_7 RILs population was subjected to BSA to identify SSR markers associated with TKW as an indicator for heat tolerance. The PCR assays with 40 SSR primer pairs generated different number of DNA bands (alleles) which were depending on the primer set used, indicating the presence of allelic diversity among SSRs as reported previously by Ravi *et al*. (2003) and Ram *et al.* (2007). Such variation is due to several factors including the structure of primers and number of annealing sites in the genome (Muralidharan and Wakeland, 1993).

Out of 40 SSRs used with BSA, seven SSR markers, namely Xgwm95-2A, Xgwm155-2A, Xgwm533-3B, Xgwm165-4A, Xgwm293-5A, Xbarc113-6A and Xgwm577-7B, generated 13 polymorphic bands, which were able to distinguish the high from the low bulk (Fig.6 and Fig.7). Of which, seven positive bands (alleles) located on 2A (116 bp), 3B (109 and 179 bp), 4A (258 bp), 5A (255 bp), 6A (212 bp) and 7B (211 bp) chromosomes were associated with the higher TKW, while six alleles located on 2A (144, 228 and 348 bp), 3B (123 and 198 bp) and 4A (277 bp) chromosomes were associated with the lower TKW (Table 8). In accordance, BSA has been successfully used to identify SSR markers associated with important traits (Torres *et al.*, 2010; Barakat *et al.*, 2011; Hassan *et al.*, 2016). Moreover, the association between SSR markers and heat tolerance-related traits has been long reported in wheat (Barakat *et al.*, 2011; Sun *et al*., 2015; El-Rawy, 2016; Saha *et al*., 2020). Recently, numerous QTLs have been identified for TKW on all wheat chromosomes (Arriagada *et al*., 2020, Gupta *et al*., 2020; Ren *et al*., 2021). Of which, a total of 201 QTLs were identified for TKW on all chromosomes of durum wheat (Arriagada *et al.*, 2020). In addition, different QTLs for TKW were recently detected on chromosomes 2A, 3B, 4A, 5A, 6A and 7B (Guan *et al.*, 2018; Su *et al.*, 2018; Zhai *et al.*, 2018; Golan *et al.*, 2019; Guan *et al.*, 2019; Sakuma *et al.*, 2019; Wang *et al.*, 2019; Arriagada *et al.*, 2020; Fatiukha *et al.*, 2020; Liu *et al.*, 2020; Mir *et al.*, 2021). The association between different SSR markers and TKW as an indicator for heat tolerance has been also reported in wheat (Ramya *et al.*, 2010; Wang *et al.*, 2012; El-Rawy, 2015; Amallah *et al.*, 2016).

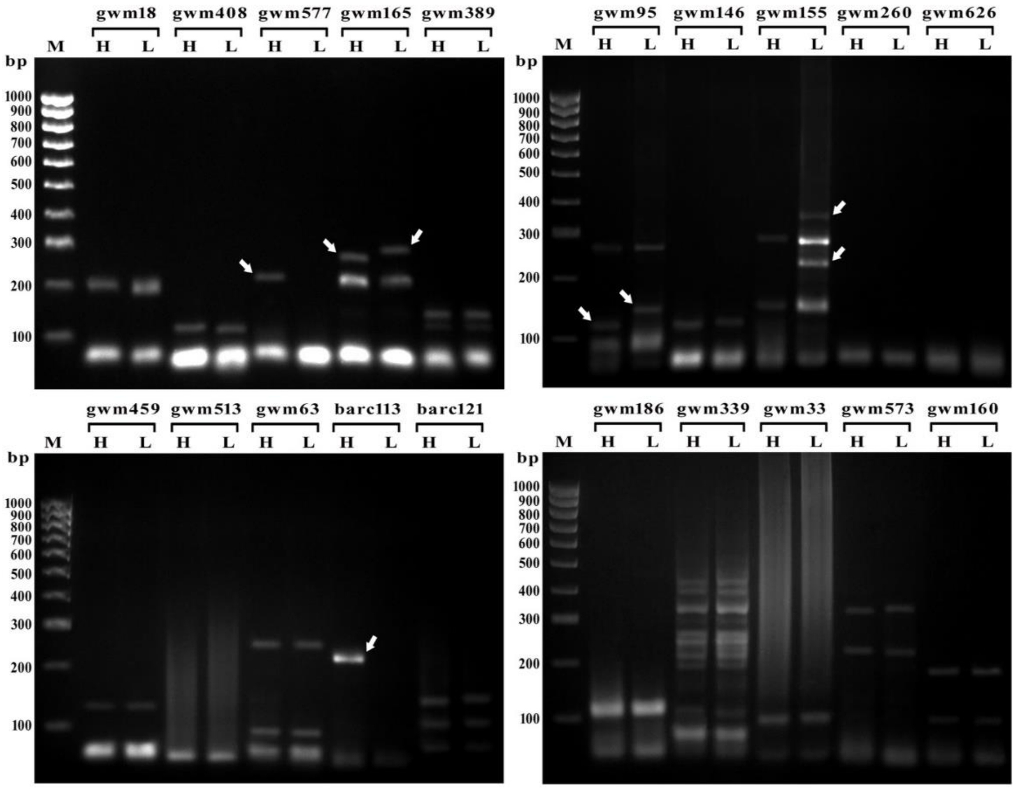

Fig.6. PCR amplifications obtained using BSA with SSR markers. M: A 100bp DNA ladder; H: high-TKW RILs bulk and L: low-TKW RILs bulk. Polymorphic bands were generated by the markers Xgwm577-7B, Xgwm165-4A, Xgwm95-2A, Xgwm155-2A and Xbarc113-6A. Arrows indicate polymorphic bands (alleles), which distinguished the high from the low bulk.

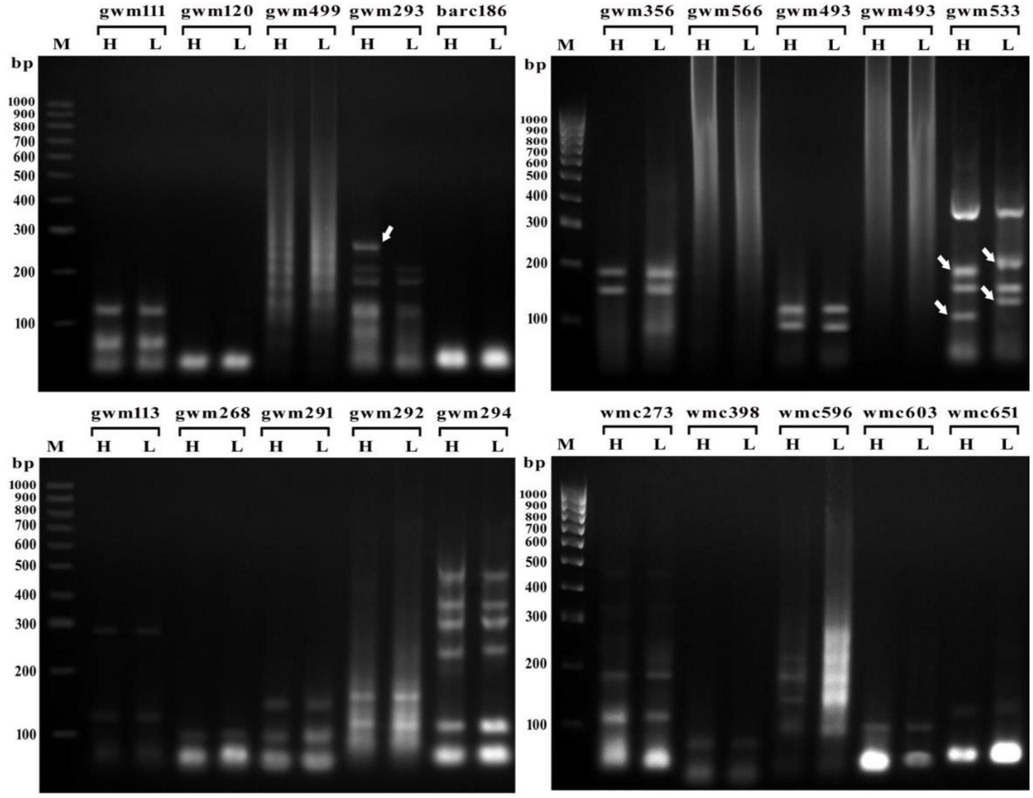

Fig.7. PCR amplifications obtained using BSA with SSR markers. M: A 100bp DNA ladder; H: high-TKW RILs bulk and L: low-TKW RILs bulk. Polymorphic bands were generated by the markers Xgwm293-5A and Xgwm533-3B. Arrows indicate polymorphic bands (alleles), which distinguished the high from the low bulk.

Table 8. Bands (alleles) detected for high and low TKW using BSA.

Marker	Chromosomal location	Size of positive alleles (bp)	
		High TKW	Low TKW
Xgwm95	2A	(+) 116	(+) 144
Xgwm155	2A	-	(+) 228 , (+) 348
Xgwm533	3B	(+) 109 , (+) 179	(+) 123 , (+) 198
Xgwm165	4A	(+) 258	(+) 277
Xgwm293	5A	(+) 255	-
Xbarc113	6A	(+) 212	-
Xgwm577	7B	(+) 211	-

(+) indicates a presence of a specific band (positive) following by its size (bp).

In conclusion, enhancing heat tolerance in wheat can be achieved by selecting a higher TKW under heat stress conditions. RILs selected for higher TKW under heat stress could be used to develop high-yield and heat-tolerant durum wheat varieties. BSA detected seven SSR markers that could be considered as markers associated with TKW under heat stress conditions. However, additional marker analysis under different environmental conditions is still required to confirm their usefulness for MAS in wheat breeding programs.

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الانتخاب المظهري وتحليل ضم الإنعزالات المتفارقة لصفة وزن الألف حبة تحت الإجهاد الحراري في القمح الصلب

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تم إجراء انتخاب مظهري ثنائي الإتجاه لصفة وزن الألف حبة تحت ظروف الإجهاد الحراري في عشيرة من القمح الصلب مكونة من ١٢٠ سلالة مرباة داخلياً ذات اتحادات وراثية جديدة في الجيل السابع. تم تقدير الإستجابة المباشرة للانتخاب لصفة وزن الألف حبة وكذلك الإستجابة المتلازمة لصفة محصول الحبوب للنبات تحت الظروف المواتية وظروف الإجهاد الحراري. وجدت اختلافات وراثية كبيرة بين السلالات المختبرة بالنسبة لصفتي وزن الألف حبة ومحصول الحبوب للنبات. كان متوسط وزن الألف حبة لسلالات الجيل السابع المنتخبة تحت ظروف الإجهاد الحراري في الإتجاه المرتفع ٦٢,٢٨ جرام وفي الإتجاه المنخفض ٣٤,٤٢ جرام. تم الحصول على إستجابة موجبة ومعنوية جداً للانتخاب لوزن الألف حبة في الإتجاه المرتفع (١٤,٩٢ و ١٦,٢٩٪) وكذلك في الإتجاه المنخفض (٢٠,٧٨ و ٢٦,٨٨٪) تحت الظروف المواتية وظروف الإجهاد الحراري، على التوالي. أدى الانتخاب لوزن الألف حبة المرتفع إلى إستجابة متلازمة موجبة ومعنوية جداً في محصول الحبوب للنبات تحت ظروف الإجهاد الحراري (قدرها ١١,٠٥٪)، بينما أدى الانتخاب لوزن الألف حبة المنخفض إلى إستجابة متلازمة موجبة ومعنوية جداً تحت كل من الظروف المواتية (١١,١٣٪) وظروف الإجهاد الحراري (١٩,٣٣٪). تم الحصول على تقديرات مرتفعة للمكافئ الوراثي المتحقق لصفة وزن الألف حبة (٠,٧٤ و ٠,٧٥) وكذلك صفة محصول الحبوب للنبات (٠,٦٥ و ٠,٧١) تحت الظروف المواتية وظروف الإجهاد الحراري، على التوالي. أظهرت سلالات الجيل الثامن الناتجة من الانتخاب لوزن الألف حبة المرتفع دليل تحمل حرارة أعلى (بمتوسط قدره ١,٢٠) من السلالات الناتجة من الانتخاب لوزن الألف حبة المنخفض (بمتوسط قدره ٠,٥٢)، مما يشير إلى أهمية الانتخاب لوزن الألف حبة المرتفع في تحسين صفة التحمل الحراري. أدى تحليل ضم الانعزالات المتفارقة باستخدام أربعين من واسمات التتابعات البسيطة المتكررة SSR إلى تحديد سبعة أليلات واقعة على كروموسومات 2A (١) و 3B (٢) و 4A (١) و 5A (١) و 6A (١) و 7B (١)، والتي كانت مرتبطة بوزن الألف حبة المرتفع كمؤشر لصفة التحمل الحراري.