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Research Article
QTL Analysis of Grain Yield-Related Traits for Terminal Heat Stress Tolerance in Wheat Using SSR Markers

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Abstract

Background and Objective: Late sowing of wheat exposes the anthesis and grain filling stages of the crop to a terminal heat temperature stress Therefore, detecting putative QTL associated with grain yield and its attributes and identifying the most tolerant genotypes to terminal drought and heat stress across environments will be beneficial in wheat breeding programs. **Materials and Methods:** In the present study, among 49 CIMMYT wheat lines evaluated for yield and stability in eight environments, we selected the highest ten high-yielding (HYL) and the lowest ten low yielding lines (LYL) along with three wheat check cultivars (CC) for screening with eighteen previously published SSR molecular markers associated with drought and heat stress tolerance. **Results:** Two SSR markers (BARC126 and BARC11) on 7D were associated with delay heading dates under normal and late sowing dates. Likewise, the SSR markers WMC396, GWM537 and XGWM577 which were mapped on 7B, were significantly linked with grain yield-related traits under one/or both sowing dates, most of them showed desirable effects, indicating terminal heat stress tolerance. Different SSR markers viz., BARC11, XGWM132 and GWM537 showed pleiotropic effects. **Conclusion:** The SSR markers BARC186-5A, XGWM132-6B, WMC396-7B, XGWM577-7B and GWM165-4B were more prominently associated with heat tolerance by showing a desirable performance of grain yield-related traits under late sowing or across environments, some of these desirable alleles were corresponding to previously QTL in various genotypes that could be valuable in breeding for high-yield in wheat.

Key words: Heat stress, climate change, wheat, SSR markers, QTL, association mapping

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Several reports warn of the passive impacts of climate change on crop productivity and consequently on food security worldwide, particularly, in the Mediterranean region which is predicted to raise temperatures in this region and reduce rainfall[1,2]. The ambient temperature is likely to increase by 6°C by the end of the 21st century and the frequency and duration of dry spells and heat waves are also expected to increase in dryland areas[3,4]. More interestingly, Egypt is one of the countries vulnerable to climate change, because of its geographical position and its reliance on climate-sensitive economic sectors as reviewed by Kassem *et al.*[5].

Wheat plays a crucial role in food security in Egypt and worldwide as well where it contributes about 30% of world grain production, therefore it is considered as a staple food for more than 40 countries of the world[6,7]. As of 2020, Egypt produces approximately 8.9 million metric tons, which represented about 40% of local consumption and imports the other percentage. Therefore, Egypt is being one of the largest wheat importers in the world. The last projections refer that Egypt will demand wheat triple by the end of the century because of the continuous annually growing of population[6]. To be a self-sufficient country of wheat production, therefore, Egypt needs to increase and enhance wheat production by both increasing the agricultural cultivated area (horizontal expansion) and developing new tolerant wheat varieties to unfavorable environmental conditions (vertical expansion), especially high temperatures and drought.

The anthesis and grain filling stages are the most plant phases that are influenced by terminal heat temperature and drought stress due to climate changes and may cause a severe reduction in grain yield[8,9]. Sehgal *et al.*[10] reported that seed filling in food crops is highly affected by both drought and heat stresses. Both stresses are complex phenomena controlled by multiple genes associated with different morphological and physiological traits[11]. Therefore, dissecting and understanding the genetic bases of crop plants' responses to heat and drought stress is a prerequisite for breeding future genotypes, especially under late sowings in the arid and semi-arid areas. Additionally, many characteristics are efficiently associated with wheat improvement under harsh stresses and the genetic gains were also studied in several environments[12,13].

Marker assisted selection (MAS) is considered an effective approach to improve plant stress tolerance because of the general complexity of abiotic stress tolerance and the difficulty in phenotypic selection for tolerance[14]. However, this approach requires the determination of molecular marker(s) associated with QTL responsible for stress tolerance. More than 854 QTLs for high temperature and drought stresses tolerance traits in wheat have been reported in the last two decades[15]. They detected 66 m-QTL genomic positions for 81 different traits linked to high temperature and drought stresses tolerance.

The molecular markers are powerful tools in studying quantitative traits like heat, drought and salinity tolerance through quantitative trait loci (QTLs) mapping, which may reduce problems resulting from genotype×environment (G×E) interactions[16]. Marker-assisted selection in improving drought responses in wheat was reported by Quarrie *et al.*[17]. There were numerous marker techniques have been used in genetic mapping studies of economically important traits in wheat[18,19]. SSRs are the most used molecular markers for the DNA analysis of plants. SSR markers are co-dominant inheritance, multi-allelic markers, have high polymorphism ratio, high reproducibility, their assay method is simple and are widely located along the genome[20]. Tomar *et al.*[21] used the SSR markers to detect the genomic regions associated with morphological and agronomic characters under drought stress conditions and they concluded a phylogenetic relationship among 31 wheat genotypes. Many different QTLs were reported for morphological, physiological and agronomical traits and mapped by using the SSR markers[22]. Additionally, many reports stated that chromosome 7 is associated with drought stress tolerance in wheat[18,23].

This study aimed to use the SSR markers to: (1) Study the genetic diversity in two sets of CIMMYT wheat lines along with three check cultivars to terminal drought and heat stress tolerance across multiple environments, (2) Detect the most associated QTL with grain yield and its attributes as indicators for terminal drought and heat stress tolerance and (3) Identify the most tolerant genotypes to terminal drought and heat stress across environments that could be valuable in wheat breeding programs.

MATERIALS AND METHODS

Plant material and field experiments: Twenty CIMMYT wheat lines (CWL), obtained from CIMMYT (International Maize and Wheat Improvement Center, Mexico), were grouped as ten high-yielding lines (HYL) and ten low-yielding lines (LYL) along with three local wheat check cultivars (CC), i.e. Misr 2, Giza 171 and Gemiza 11 (Table 1) were selected to be used in this study depending on the study of Sayed *et al.*[24]. In brief, the CWL wheat lines and the local cultivars were evaluated at Assiut

Table 1: CIMMYT wheat lines and local check cultivars used in the study and their yield responses to late sowing date across environments according to Sayed *et al.*[24]

No.	Genotypes	Name and selection history	Origin	Yield response
L33	G234	BECARD//ND643/2*WBLL1, CMSS08B00422S-099M-099NJ-5RGY-0B	CIMMYT	High yield
L42	G243	SUP152*2/KENYA SUNBIRD, CMSS08B00798T-099TOPY-099M-099NJ-11RGY-B	CIMMYT	High yield
L8	G209	KENYA SUNBIRD/KACHU, CMSS08Y00235S-099Y-099M-099NJ-3RGY-0B	CIMMYT	High yield
L28	G229	BABAX/LR42//BABAX*2/3/SHAMA/4/WAXWING*2/KRONSTAD F2004, CMSS08B00256S-099M-099NJ-099NJ-26RGY-0B	CIMMYT	High yield
L26	G227	BABAX/LR42//BABAX*2/3/SHAMA/5/PRL/2*PASTOR/4/CHOIX/STAR/3/HE1/3*CNO79//2*SERI, CMSS08B00254S-099M-099NJ-099NJ-7RGY-0B	CIMMYT	High yield
L30	G231	BONSU, CMSS08B00259S-099M-099NJ-30RGY-0B	CIMMYT	High yield
L40	G241	ND643/2*WBLL1/4/CHIBIA//PRLII/CM65531/3/MISR 2/5/BECARD, CMSS08B00776T-099TOPY-099M-099NJ-099NJ-21RGY-0B	CIMMYT	High yield
L22	G223	WBLL1*2/BRAMBLING/4/BABAX/LR42//BABAX*2/3/SHAMA, CMSS08B00196S-099M-099NJ-099NJ-11RGY-0B	CIMMYT	High yield
L20	G221	WBLL1/KUKUNA//TACUPETO F2001/3/BERKUT//PBW343*2/KUKUNA, CMSS08B00153S-099M-099Y-13M-0RGY	CIMMYT	High yield
L12	G213	TUKURU//BAV92/RAYON/3/ND643/2*WBLL1, CMSS08Y00351S-099Y-099M-099NJ-099NJ-4RGY-0B	CIMMYT	High yield
L50	Gemiza 11	BOW"S"/KVZ"S"//7C/SER182/3/GIZA 168/SAKHA61, GM7892-2GM-1GM-2GM-1GM-0GM	Egypt	Check variety
L51	Giza 171	Sakha 93 / Gemmeza9 S.6-1GZ-4GZ-1GZ-2GZ-0S	Egypt	Check variety
L52	Misr 2	SKAUZ/BAV92. CMSS96M03611S-1M-010SY-010M-010SY-8M-0Y-0EGY	Egypt	Check variety
L3	G204	KLEIN CACIQUE, -0ARG	CIMMYT	Low yield
L6	G207	MUTUS//ND643/2*WBLL1, CMSS08Y00224S-099Y-099M-099NJ-099NJ-4RGY-0B	CIMMYT	Low yield
L4	G205	KENYA HEROE, -0KEN	CIMMYT	Low yield
L31	G232	PFAU/WEAVER*2//TRANSFER#12,P88.272.2/4/BABAX/LR42//BABAX*2/3/SHAMA, CMSS08B00269S-099M-099Y-12M-0RGY	CIMMYT	Low yield
L17	G218	WBLL1*2/BRAMBLING//TAM200/TUI/3/VILLA JUAREZ F2009, CMSS08Y00912T-099TOPM-099Y-099M-099Y-2M-0RGY	CIMMYT	Low yield
L19	G220	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1*2/4/NIINI #1, CMSS08Y00924T-099TOPM-099Y-099M-099NJ-099NJ-12RGY-0B	CIMMYT	Low yield
L14	G215	CHIBIA//PRLII/CM65531/3/FISCAL*2/4/NIINI #1, CMSS08Y00851T-099TOPM-099Y-099M-099NJ-8RGY-0B	CIMMYT	Low yield
L18	G219	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1*2/4/NIINI #1, CMSS08Y00924T-099TOPM-099Y-099M-099NJ-099NJ-8RGY-0B	CIMMYT	Low yield
L5	G206	FRANCOLIN #1/BLOUK #1, CMSS06B00010S-0Y-099ZTM-099NJ-099NJ-9RGY-0B-8BMX-0RGY	CIMMYT	Low yield
L1	G202	VOROBEY, CMSS96Y02555S-040Y-020M-050SY-020SY-27M-0Y	CIMMYT	Low yield

(Faculty of Agricultural Farm with clay loam soil, Assiut University, Assiut, Egypt) and Nubariah (National Research Center Farm with sandy loam soil, Agricultural Research Center, Nubariah, Egypt) under two sowing dates trials (normal (N) on 25th November and late (L) on 25th December) for two seasons 2017/2018 and 2018/2019. Both sites represent different agro-edaphic and ecological environments in Egypt[25]. Assiut is located approximately in the middle of Egypt and is characterized as a hot and dry environment with an average annual temperature of 24.0°C and precipitation is about 1 mm per year with clay loan soil. Nubariah is in the north of Egypt and is characterized as a moderate-temperature and semi-rainfall environment with an average annual temperature of 20.9°C and annual rainfall of 62 mm. In each environment, the tested genotypes were raised in a Randomized Complete Block Design (RCBD) with three replications. The experimental unit, the trials management and the agronomic practices were presented in detail in Kumar *et al.*[26].

Data collection and statistical analysis: The heading date (HD) for each genotype represents the number of days required for the heading of 50% plants in a plot from the date of sowing was recorded. Observations on grain yield and its attributes were recorded from the middle rows per plot. At maturity time, Plant Height (PH, cm) was measured as an average of randomly five middle plants per genotype in each plot. At harvest, the number of spikes/m^2 (SN) was counted on the middle-squared meter in each plot. Biological yield ha^{-1} (BYH, t ha^{-1}), grain yield ha^{-1} (GYH, t ha^{-1}) were measured on the whole field plot basis. Finally, Thousand Kernel Weight (TKW, g), 1000 grains from each genotype were weight and recorded in grams. Harvest Index (HI) was calculated as the ratio of grain yield to biological yield. A combined analysis of variance (ANOVA) of a combination of two locations, two years and two sowing dates as eight environments was performed using PROC GLM of SAS according to Moore and Dixon[25].

Table 2: SSR primer pairs polymorphism and their QTL chromosomal locations related to drought and heat stress tolerance in wheat

		Amplified fragments								
Primer	Sequences	Total	Size (bp)	P.	PIC (%)	QTL	CL	Pos (cM)	PS (bp)	References
BARC11	F-5' GCGATGCGTGTAAAGTCTGAAGATGA 3' R-5' GCGTCCATGGAGCTCTGTTTTATCTGA 3	8	80-300	5	78.1	Drought tolerance*	2D	4	80	He *et al.*[34]
BARC68	F-5' CGATGCCAACACACTGAGGT 3' R-5' GCCGCATGAAGAGATAGGTAGAGAT 3'	4	80-170	2	62.5	Chlorophyll content	3B	66	120	Kumar *et al.*[26]
BARC101	F-5' GCTCCTCTCACGATCACGCAAAG 3' R-5'GCGAGTCGATCACACTATGAGCCAATG 3'	5	60-200	3	72	Canopy temperature	3B	99	100	Kumar *et al.*[26]
BARC126	F-5' GCG CCG TGT AAA TAG TTT TGT TTA3' R-5' CTTGCACAGCCAAATAGTGTGGATAA3'	4	200-250	2	62.5	Drought tolerance*	7D	9.1	250	Pinto *et al.*[27]
BARC186	F-5'GTGCTTGCTGAGCTATGAGTC3' R-5' GTGCCACGTGGTACCTTTG 3'	6	200-250	5	77.8	Days to anthesis	5A	57	170	Pinto *et al.*[27]
GDM93	F-5'AAAAGCTGCTGGAGCATACA3' R-5' GGAGCATGGCTACATCCTTC3'	3	120-190	2	66.7	Normal difference vegetation index	2A	93	120	Liu *et al.*[35]
GWM111	F-5' TCTGTAGGCTCTCTCCGACTG3' R-5' ACCTGATCAGATCCCACTCG3'	6	130-250	6	83.3	Drought tolerance*	7D	89	250	Liu *et al.*[35]
GWM165	F-5' TGCAGTGGTCAGATGTTTCC 3' R-5' CTTTTCTTTCAGATTGCGCC 3'	6	180-290	5	78.8	Drought stress	4B	32	200	Quarrie *et al.*[19]
GWM190	F-5'GGAGTGTCGAGATGATGTGGAAAC3' R-5' CGCAGACGTCAGCAGCTCGAGAGG 3'	8	70-500	4	65.6	Heat stress	5D	9	150	Liu *et al.*[35]
GWM428	F-5' AGC GTT CTT GGG AAT TAG AGA3' R-5' CCA ATC AGC CTG CAA CAA C3'	5	180-350	4	80	Heat stress (grain filling)	7D	11	200	Barakat *et al.*[8]
GWM537	F-5' AAGAGATAACATGCAAGAAA3' R-5' TTCAAATATGTGGGAACTAC3'	3	200-280	2	66.7	Drought tolerance*	7B	50.4	200	Gupta *et al.*[36]
WMC83	F-5' TGGAGGAAACACAATGGATGCC3' R-5' GAGTATCGCCGACGAAAGGGAA3'	9	70-350	9	88.9	Drought tolerance*	7A	119.4	120	Jaiswal *et al.*[37]
WMC121	F-5' GGCTGTGGTCTCCCGATCATTC3' R-5' ACTGGACTTGAGGAGGCTGGCA3'	4	250-400	3	75	Drought tolerance*	7D	86	250	Jaiswal *et al.*[37]
WMC396	F-5' TGCACTGTTTTACCTTCACGGA3' R-5' CAAAGCAAGAACCAGAGCCACT3'	6	70-190	4	77.8	Drought tolerance*	7B	68	170	Jaiswal *et al.*[37]
WMC488	F-5' AAAGCACAACCAGTTATGCCAC3' R-5' GAACCATAGTCACATATCACGAG3'	5	100-190	4	80	Drought tolerance*	7A	176.4	190	Singh *et al.*[38]
WMC525	F-5' GTTTGACGTGTTTGCTGCTTAC3' R-5' CTACGGATAATGATTGCTGGCT3'	8	100-300	8	87.5	Drought tolerance*	7A	140	140	Jaiswal *et al.*[37]
XGWM132	F-5' TAC CAA ATC GAA ACA CAT CAG G3' R-5' CAT ATC AAG GTC TCC TTC CCC3'	3	70-250	3	66.7	Heat stress (grain filling)	6B	36.6	120	Barakat *et al.*[8]
XGWM577	F-5' ATG GCA TAA TTT GGT GAA ATT G3' R-5' TGT TTC AAG CCC AAC TTC TAT T3'	5	70-250	4	80	Heat stress (grain filling)	7B	6.1	160	Barakat *et al.*[8]

*According to Cattivelli *et al.*[18], Galiba[23] and Quarrie *et al.*[19], P: Polymorphic, PIC (%): Polymorphic information content, QTL: Quantitative trait loci, CL: Chromosomal location, Pos(cM): Allele position in centimorgan, PS: Allele product size

SSR markers analysis: The SSR marker analysis was performed at Plant Molecular Biology Lab (PMBL), Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City (USC), Minoufiya, Egypt. DNA was extracted from seedlings of the wheat genotypes using i-genomic Plant DNA Extraction Mini Kit (iNtRON Biotechnology Inc., Korea) according to their manufacturer instructions. The extracted DNA solutions were adjusted at 25 ng μL^{-1} and stored at -20 until use. Eighteen previously published SSR primer pairs specific for wheat were used in this study which was associated with abiotic stress tolerance including drought and heat stress tolerance in wheat according to[8,26-30] (Table 2).

SSR analysis was conducted using a reaction mixture volume of 15 µL contained: 7.5 µL of 2× PCR Master mix solution (i-Taq, iNtRON Biotechnology Inc., Korea), 0.15 µL from each primer (100 µM mL^{-1}), 4 µL DNA and 3.2 µL ddH_2O. The PCR program was performed for 35 cycles of the following steps: Denaturation at 95°C for one 50 sec, annealing at 48°C for 40 sec and extension at 72°C for one minute. The previous PCR program was preceded with denaturation step at 95°C for five min and followed by final extension step at 72°C for 3 min. After completing the PCR reaction, samples were separated on 1.5% agarose gel electrophoresis. The total and a polymorphic number of amplified fragments produced from SSR analysis were calculated. Polymorphic information content (PIC) was calculated using the following simplified equation according to Abdellatif and Khidr[31]:

$$PIC_i = 1-\Sigma p_{ij}\ 2$$

where, p_{ij} is the frequency of the jth allele for SSR primer, ith summed across all SSR alleles for the locus.

The simple matching algorithm (SM) was used to calculate the similarity matrix coefficient for the SSR markers and the unweighted pair-group method with arithmetical algorithms averages (UPGMA) method was used to construct the dendrogram[32]. All the above-mentioned analyses were performed using the NTSYS PC2.1 software[33].

QTL detection: The association between SSR markers and the investigated traits in each environment (as QTL by environment interaction) and across all environments (as QTL main effect) was performed with stepwise regression analysis and Tukey's multiple comparison test. We employed Tukey's test to compare the difference between each pair of least squares means of SSR markers with appropriate adjustment for the multiple testing and was done overall environments. Both methods were performed using the Statistical Analysis System (SAS) software ver. 9.2[27]. The magnitude of the marker associated with trait phenotypic effect was described by the F value and the coefficient of determination (R^2).

RESULTS

Phenotypic assessment of wheat genotypes: The phenotypic data of grain yield and its attributes of the twenty CIMMYT wheat lines along with the three check cultivars were obtained from the field trials under normal sowing and late sowing at two different locations over two continuous years (as such, eight environments). The analysis of variance revealed highly significant differences among genotypes and within each group for all studied traits, except in very few cases (Table 3). In addition, there were highly significant differences between environments and also in their interaction with genotypes for all studied traits. In most cases, the low-yielding group was more different than the high yielding and check groups. The eight environments displayed a high contribution to the total sum of squares (TSS) by values ranging between 24.6 (SN) and 89.6% (HD). The genotypes contributed to the TSS by values varied from 3.7 (HD)-21.8% (HI), whereas G×E interaction contributed to the TSS by values ranging between 3.5 (HD) and 41.0% (SN). Interestingly, the contribution of the low-yielding group by environments interaction was higher than that of high-yielding and check cultivars in most cases. This result indicates that locations and sowing dates had significant impacts on grain yield and its attributes of all genotypes, in particular low-yielding lines. The coefficients of variation (CV%) were low and ranged between 3.0 (HD) and 11.9% (GY). In contrast, coefficients of determination (R^2) were high and varied from 85.9 (TGW)-96.8% (HD), Table 3.

Table 3: Combined analysis of the studied traits showing components of genotype by environment interaction (GEI) and the contribution to the total sum of squares (cont. %)

S.O.V.	DF	HD	Cont. (%)	PH	Cont. (%)	SN	Cont. (%)	BYH	Cont. (%)	GYH	Cont. (%)	HI	Cont. (%)	TGW	Cont. (%)
Genotypes (G)	22	157.4**	3.7	316.4**	11.0	33447.2**	21.0	76.9**	18.3	9.6**	16.2	249.4**	21.8	106.4**	14.3
HYL	9	102.5**	1.0	296.1**	4.2	9609.6**	2.5	17.6**	1.7	2.6**	1.8	70.2**	2.5	53.0**	2.9
LYL	9	202.1**	1.9	270.5**	3.9	8577.0**	2.2	92.0**	9.0	0.6	0.4	353.8**	12.7	156.1**	8.6
CC	2	53.8**	0.1	172.5**	0.5	34339**	2.0	7.2	0.2	5.8**	0.9	180.2**	1.4	131.5**	1.6
Residual	2	307.1**	0.7	757.9**	2.4	251740**	14.4	346.2**	7.5	86.0**	13.1	655.0**	5.2	97.9**	1.2
Envi. (E)	7	11984.8**	89.6	5207.8**	57.7	122955.**	24.6	483.4**	36.6	83.1**	44.4	948.6**	26.4	1081.4**	46.1
GEI	154	21.6**	3.5	102.0**	24.9	9306.0**	41.0	21.6**	36.0	2.4**	27.9	61.8**	37.9	27.2**	25.5
HYL×E	63	17.0**	1.1	60.5**	6.0	4019.0**	7.2	16.2**	11.1	2.1**	10.1	48.1**	12.1	18.3**	7.0
LYL×E	63	14.5**	1.0	127.8**	12.7	6196.1**	11.2	26.2**	17.9	1.9**	9.4	82.9**	20.8	31.8**	12.2
CC×E	14	54.3**	0.6	122.2**	3.4	17462.2**	15.6	21.7**	3.8	3.4**	4.8	39.9**	2.8	51.8**	1.9
Residual	14	41.0**	3.2	153.0	6.4	38935.9	13.3	25.0	9.1	4.5	11.5	49.8**	13.8	21.8	14.1
Error	368	8.2		11.0		1267.4		2.3		0.4		9.4		6.3	
Total SS	551	93677.5		63168.0		3496060.2		9241.0		1309.9		25112.1		16408.0	
R^2		96.8		93.6		86.7		90.9		88.5		86.2		85.9	
CV%		3.0		3.9		9.2		9.8		11.9		8.7		6.3	

HD: Heading date, PH: Plant height, SN: Number of spikes/m^2, BYH: Biological yield/ha t ha^{-1}, GYH: Grain yield/hat ha^{-1}, HI: Harvest index (%), TGW: Thousand kernel weight (g), **Significant at 1% level of probability, Total SS: Total sum of squares, R^2: Coefficient of determination and CV%: Coefficient of variation

Table 4: Averages of the high-yielding lines (HYL), the low-yielding lines (LYL) and check cultivars (CC) for the studied traits at each environment

Location	Environment	Groups	HD	PH	SN	BYH	GYH	HI	TGW
Assiut	Control	CC	103.0	91.5	278.2	13.1	4.5	34.6	45.8
	A_1	HYL	100.2	91.5	439.5	19.1	6.7	35.3	44.5
		LYL	104.4	93.1	368.6	17.7	5.4	32.1	42.8
		Mean	102.4	92.2	387.6	17.7	5.8	33.8	43.9
	Late	CC	72.2	85.3	307.3	12.5	3.5	27.0	38.3
	A_2	HYL	72.8	80.8	407.3	15.6	4.9	31.4	34.5
		LYL	74.9	76.0	274.0	12.0	3.2	27.7	35.2
		Mean	73.6	79.3	336.3	13.6	4.0	29.2	35.3
	Control	CC	103.1	94.9	318.9	11.2	4.9	43.6	48.2
	A_3	HYL	103.9	95.2	469.4	16.4	6.9	42.1	47.0
		LYL	106.7	94.3	405.1	15.0	5.6	38.8	44.9
		Mean	105.0	94.8	421.8	15.1	6.1	40.8	46.2
	Late	CC	83.0	77.1	299.8	10.8	3.4	30.6	38.8
	A_4	HYL	76.7	80.8	416.8	13.7	4.8	35.0	34.8
		LYL	78.6	77.6	274.2	10.5	3.1	31.0	35.6
		Mean	78.4	79.0	339.5	12.0	3.9	32.7	35.6
Nubariah	Control	CC	103.1	99.5	455.1	16.7	7.1	42.6	41.1
	N_1	HYL	107.2	96.3	447.6	18.7	7.1	38.8	41.1
		LYL	107.9	91.7	383.1	17.8	6.3	36.6	41.4
		Mean	107.0	94.7	420.6	18.1	6.8	38.3	41.2
	Late	CC	91.3	87.2	374.0	14.0	6.0	42.7	39.2
	N_2	HYL	88.8	75.9	359.3	15.4	5.8	38.1	38.1
		LYL	91.2	71.1	354.7	12.9	4.5	36.2	38.2
		Mean	90.1	75.3	359.2	14.1	5.3	37.9	38.3
	Control	CC	105.3	92.3	463.1	17.1	6.0	34.9	41.5
	N_3	HYL	106.4	88.4	451.9	19.8	6.7	34.2	40.5
		LYL	108.1	89.6	448.1	19.9	6.3	31.8	41.9
		Mean	107.0	89.4	451.7	19.5	6.5	33.3	41.3
	Late	CC	90.0	78.8	381.1	13.4	5.5	40.5	38.1
	N_4	HYL	89.5	75.1	368.3	14.5	5.2	35.6	37.9
		LYL	91.6	73.3	363.4	12.8	4.2	33.0	36.0
		Mean	90.5	74.8	367.9	13.6	4.8	35.1	37.1

HD: Heading date, PH: Plant height, SN: Number of spikes/m2, BYH: Biological yield/ha t ha^{-1}, GYH: Grain yield/ha t ha^{-1}, HI: Harvest index (%), TGW: Thousand kernel weight (g), A_1 and A_3 are normal sowing dates at Assiut in the first and the second season, respectively, second season, respectively, N_1 and N_3 are normal sowing dates at Nubariah in the first and the second season, respectively and N_2 and N_4 are late sowing dates at Nubariah in the first and the second season, respectively

Means of all studied traits for the high-yielding lines (HYL), low-yielding lines (LYL) and wheat check cultivars (CC), at each environment were presented in Table 4. The HYL performed well under normal sowing dates in both locations and relatively gave close GYH, whereas the LYL gave higher yields under normal sowing dates at the Nubariah location compared to the Assiut location. This result may be due to that these genotypes did not show tolerance to the terminal heat stress that occurs in the grain filling stage. In contrast, under late sowing dates, both HYL and LYL gave higher yields under Nubariah compared to Assiut. The HYL showed better performance at the Assiut location under both sowing dates compared to check cultivars, which displayed the contrast in the Nubariah location under both sowing dates as well. This result indicated that the HYL was adapted well to both locations, while CC was adapted to Nubariah conditions. These findings were confirmed by stress (heat) tolerance index (STI) based on grain yield (Table 5). For the HYL, the STI ranged between 0.81 (L12) and 1.13 (L33) with an average of 0.90. For LYL, the STI ranged between 0.48 (L3) and 0.69 (L14) with an average of 0.57. While for CC, the STI ranged between 0.54 (L50) and 0.80 (L52) with an average of 0.65.

SSR polymorphisms and molecular pattern: The result of the SSR marker revealed that 98 fragments were amplified from eighteen primer pairs. The total number of amplified fragments from each primer pair varied and ranged from three fragments (for primer pairs GDM93, GWM537 and XGWM132) to nine fragments (for primer pair WMC83, Table 2). The number of polymorphic bands for the SSR primer pairs ranged from two to eight and the Polymorphic Information Content (PIC) ranged from 62.5 (for primer pairs BARC68 and BARC126) to 88.9% (for primer pairs GDM93, GWM537 and WMC83). The targeted amplified allele size differed also

Table 5: Means of the high-yielding lines (HYL), the low-yielding lines (LYL) and check cultivars (CC) for the studied traits across eight environments

Groups	Line	HD	PH	SN	BYH	GYH	HI	TGW	STI
CC	L50	92.5	86.8	317.4	13.0	4.7	35.1	41.0	0.54
	L51	93.6	86.8	371.3	13.7	5.0	35.9	43.9	0.61
	L52	95.5	91.4	390.3	14.1	5.6	40.2	39.3	0.80
	Average	93.9	88.3	359.7	13.6	5.1	37.1	41.4	0.65
HYL	L12	93.5	89.4	404.0	15.4	5.7	37.3	41.7	0.81
	L20	97.2	82.4	401.7	17.3	5.7	33.5	38.2	0.81
	L22	93.6	84.3	420.3	15.2	5.7	38.3	38.6	0.81
	L26	92.5	82.0	411.7	16.6	6.0	36.5	39.2	0.90
	L28	92.2	80.9	444.9	15.9	6.1	38.0	39.6	0.92
	L30	93.1	87.9	440.7	17.0	5.8	34.4	38.3	0.85
	L33	91.0	83.9	437.7	17.7	6.8	38.3	41.4	1.13
	L40	93.2	90.0	414.5	17.0	5.8	34.6	42.4	0.83
	L42	90.0	90.1	439.1	17.4	6.3	35.8	39.5	0.95
	L8	95.6	84.2	385.6	17.1	6.2	36.4	39.2	0.97
	Average	93.2	85.5	420.0	16.7	6.0	36.3	39.8	0.90
LYL	L1	93.7	82.8	384.1	18.4	5.0	27.6	43.0	0.61
	L14	93.2	84.3	373.8	15.5	4.9	32.2	36.1	0.69
	L17	93.1	77.2	343.3	13.7	4.9	35.4	40.9	0.57
	L18	94.5	84.8	377.7	14.3	4.9	34.9	36.4	0.58
	L19	96.3	86.0	353.1	16.2	4.9	30.2	41.6	0.59
	L3	100.0	84.0	326.3	12.4	4.8	37.8	41.0	0.48
	L31	94.1	86.9	368.1	12.9	4.8	38.1	42.2	0.57
	L4	100.8	86.4	363.4	17.2	4.5	27.7	36.9	0.56
	L5	96.1	77.7	363.8	14.1	5.0	35.6	38.2	0.57
	L6	92.5	83.3	335.6	13.4	4.6	34.8	38.7	0.52
	Average	95.4	83.3	358.9	14.8	4.8	33.4	39.5	0.57

HD: Heading date, PH: Plant height, SN: Number of spikes/m2, BYH: Biological yield/ha t ha^{-1}, GYH: Grain yield/ha t ha^{-1}, HI: Harvest index (%), TGW: Thousand kernel weight (g) and STI: Stress (heat) tolerance index

and ranged from 80 (for primer pairs BARC11)-250 bp (for primer pairs BARC126, GWM111 and WMC121, Table 2). Most of the amplified primers showed differences in the amplified fragments. The differences in the pattern could be noticed especially in the pattern of the primer pairs WMC83, WMC525, GWM111, GWM190 and XGWM577 (Fig. 1).

Cluster analysis for SSR markers: The results of cluster analysis of SSR data showed that wheat genotypes were distributed into five clusters (from up to down of the dendrogram). The first cluster (from the above) consisted of genotypes (L33, L28, L42 and L8) while the second cluster included the genotypes (L40, L22, L20, L12 and L52 (Misr2)). L51 (Giza171) genotype was separated apart from this cluster (Fig. 2). The third cluster contained genotypes (L14, L5, L1 and L18) and the fourth cluster consisted of the genotypes (L26 and L30). The genotypes L19 and L17 have clustered separately apart from the previous clusters. The fifth cluster included the genotypes (L50 (Gemmiza11, L3, L6 and L4). The genotype L31 was clustered separately apart from the fifth cluster (Fig. 2). The most related genotypes according to the SSR analysis were L5 (G206) and L1 (G202) lines followed by L12 (G213) and L52 (Misr2), (Fig. 2).

SSR markers by trait associations: Table 6 shows the summary statistics of detected QTL that were associated with all studied traits and showed QTL by environment interaction and main effects through stepwise regression and least-square means comparisons. Two statistical methods, stepwise regression and least-square means comparisons, were performed in the current study to detect the significant markers associated with grain yield and its attributes of the selected CIMMYT lines that showed high and low yields evaluated in eight environments. A total of 33 QTL effects were obtained by the stepwise regression analysis. Among these, twenty QTL showed marker × environment interactions, five displayed marker main effects and eight QTL showed both effects. Whilst least-square means comparisons overall environments revealed fifteen significant QTL which was associated with all traits except HD.

Heading date (HD): The SSR markers BARC126 and BARC11 were associated with delay HD under both sowing dates in Assiut and Nubariah sites with estimated parameters ranging between 2.4 and 3.3 days. Both markers were mapped on chromosome 7D. BARC126 was presented in 7 HYL, 1 LYL and 1 CC while BARC11 has existed in 7 HYL, 2 LYL and 3 CC (Fig. 1). As average overall environments, the presence of

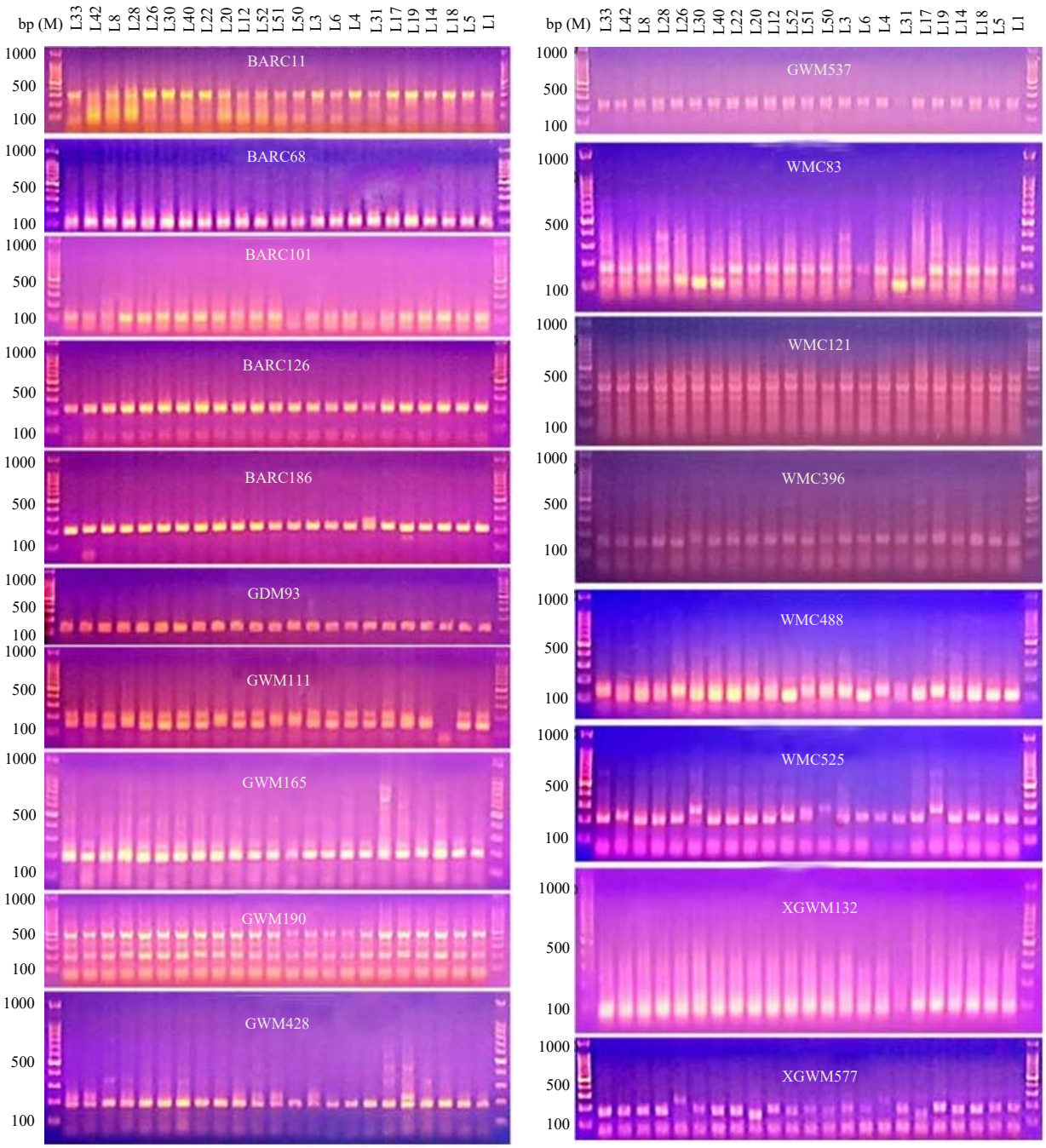

Fig. 1: PCR products of 18 SSR primer pairs of 23 bread wheat genotypes separated on 1.5% agarose gel electrophoresis

their bands (250 and 80 bp, respectively) was associated with delay HD by 2.6 and 6.5 days, with R^2 values of 15.9 and 16.8%, respectively. The least-square means (Ls-means) comparisons were not-significant.

Plant height (PH): The QTL analysis revealed four markers, WMC525, BARC126, XGWM132 and GWM165 which were associated with PH and mapped on chromosomes 7A, 7D, 6B and 4B, respectively. The markers WMC525 (7A, 140 cM, 140 bP) and BARC126 (7D, 9.1 cM, 250 bp) were associated with shortening plants under both sowing dates by estimated values varied from -5.9 to -10.6 cm as confirmed by the stepwise regression and LS-means comparisons methods. These markers displayed R2 values ranging between 11.2 and 42.5%. As average overall environments, both markers led to reducing PH by values of -5.8 and -5 cm, respectively. The

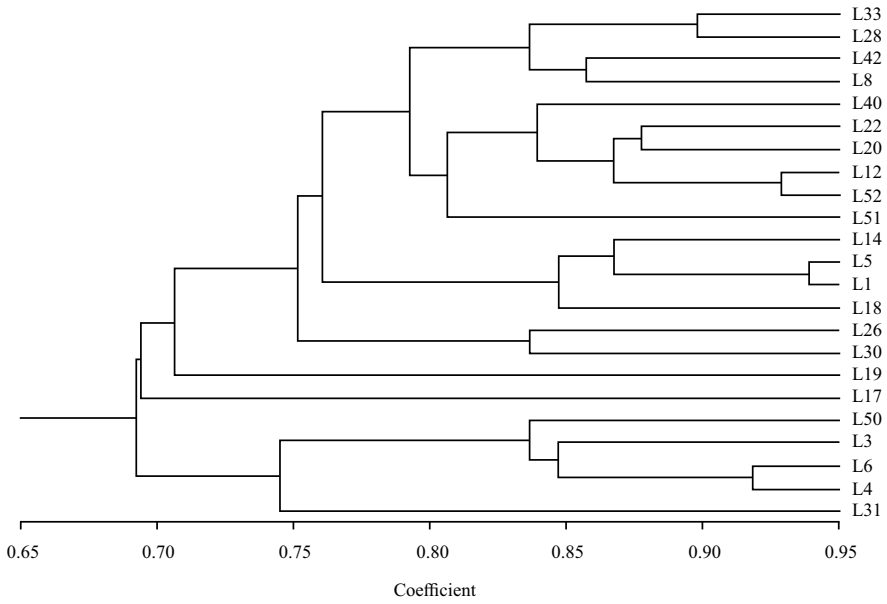

Fig. 2: Cluster analysis of wheat genotypes using the simple matching coefficient of SSR data and UPGMA clustering method

marker XGWM132 (6B, 36.6 cM, 120 bp) associated with reducing PH under late sowing date in Nubariah with the value of -8.7 cm, while it increased PH by a value of 3.2 cm overall environments. The marker GWM165 (4B, 32 cM, 200 bP) was correlated with an increase in PH under late sowing date by 7.2 cm at Nubariah with an R^2 value of 13.6% and overall, by 5.9 cm. This primer was presented in 6 HYL, 6 LYL and one CC (Fig. 1).

Number of spikes (NS): Seven QTL were identified for NS and distributed on chromosomes 2A, 5A, 7A, 3B, 6B and 7D. Three out of seven showed marker main effect, three marker×environment interactions and one showed both effects. The markers XGWM132 (6B, 36.6 cM, 120 bp) and WMC525 (7A, 140 cM, 140 bP) were linked to increasing NS under normal sowing date at Assiut by estimated value of 69.8 and 87.1 spike/m^2, respectively. While the marker locus BARC68 was linked to a decrease NS by an estimated value of -62.5 spike/m^2 at the same conditions. The markers GDM93 and WMC396 exhibited undesirable performance by reducing NS by estimated values of -75.5 and -51.4 spike/m^2. Additionally, the LS-means analysis revealed five highly significant QTL and two markers BARC186 (5A, 57 cM, 170 bp) and XGWM132 (6B, 36.6 cM, 120 bp) showed desirable effects overall environments by increasing NS by values of 70 and 80.2 spike/m^2, respectively.

Biological yield per hectare (BYH): The stepwise regression revealed five QTL for BYH which are located on chromosomes 7A, 3B, 6B, 7B and 5D. The marker GWM190 was associated with reducing BYH under normal and late sowing dates at the Assiut location and it also led to reducing BYH overall environments by a value of -1.8 t ha^{-1}. The marker XGWM132 (6B, 36.6 cM, 120 bp) showed a desirable correlation with BYH under normal and late sowing date at Assiut location as well as overall environments by the value of 8.3 t ha^{-1}. The markers BARC101 (3B, 99 cM, 100 bp) and GWM537 (7B, 50.4 cM, 200 bp) were exhibited unfavourable performance by reducing BYH under normal conditions at Nubariah as well as overall environments.

Grain yield per hectare (GYH): Only two QTL were detected for GYH by stepwise regression analysis and located on chromosomes 2A and 7D. Both markers showed undesirable effects by reducing GYH under both sowing dates at the Nubariah location. The marker GDM93 (2A, 93 cM, 120 bp) was correlated to reducing GYH overall environments by a value of -1.9 t ha^{-1} as revealed by the LS-means comparisons method.

Harvest index (HI): The QTL analysis revealed three QTL for HI and mapped on chromosomes 2A, 7B and 7D. The markers GDM93 and WMC396 showed only main effects and gave estimated values of -3.9 and 2.9% across environments.

Table 6: Summary statistics of detected QTL that were associated with all studied traits

		QTL by environment Interaction effects													
		Assiut		Nubariah		F value		R^2					LS means of band effect		
Traits	Marker	Normal	Late	Normal	Late	Min.	Max.	Min.	Max.	F value	QTL main effect	R^2	Presence	Absence	Diff.[¥]
Heading date	BARC126		2.4[€1]	2.9		8.46	12.75	11.2	15.9				92.1	89.4	2.6
	BARC11			2.8	3.3[€1]	8.55	12.93	11.31	16.8				94.0	87.5	6.5
Plant height	WMC525	-5.8[€2]			-9.2[€1]	21.51	30.74	24.3	29.73	-3.5	26.78	4.64	82.4	88.2	-5.8**
	BARC126		-8.0[€1]	-10.6[€1]	-5.9[€1]	12.79	49.61	11.24	42.54	-3.2	10.64	1.81	82.8	87.8	-5.0*
	XGWM132				-8.7[€2]	16.8	22.45	11.91	25.1				86.8	83.7	3.2
	GWM165				7.2[€1]	14.66		13.61					88.2	82.3	5.9**
Number of spikes	BARC186									36.7	16.4	2.9	409.9	339.9	70.0**
	XGWM132	69.8[€2]				12.42	13.13	15.64	16.39				415.0	334.8	80.2**
	WMC525	87.1[€2]				17.35	18.21	17.40	18.24				369.7	380.1	-10.3
	BARC68	-62.5[€2]				14.7	15.32	12.20	12.63	-34	15.05	2.52	330.6	419.2	-88.6**
	GDM93		-75.51[€1]			35.66		34.73					357.0	392.8	-35.8
	BARC126		-51.4[€1]			16.85		13.28					345.5	404.3	-58.8**
	WMC396									47.3	8.16	1.42	387.9	361.8	26.1
	BARC11									-33.3	9.87	1.69	349.5	400.2	-50.7**
Biological yield	GWM190	-3.7[€1]	-2.8[€1]			12.23	14.21	15.43	17.5	-1.3	14.26	2.53	11.0	12.8	-1.8
	XGWM132	3.5[€1]	3.1[€1]			14.87	16.16	15.17	16.63	1.2	10.51	1.83	16.0	7.8	8.3**
	BARC101			-2.4[€1]		11.91		15.09					10.6	13.2	-2.6
	BARC68				-0.41[€2]	14.17	16.05	17.45	19.33				13.9	9.9	3.9
	GWM537			1.9[€1]		15.07		18.36					10.6	13.2	-2.6
Grain yield	GDM93			-0.93[€1]	-0.91[€1]	19.32	24.39	22.28	26.68	-0.43	10.35	1.85	3.7	5.6	-1.9**
	BARC126				-1.06[€1]	13.18		12.92					4.3	5.0	-0.6
Harvest index	GDM93									-3.9	25.08	4.36	36.9	40.2	-3.3
	WMC396									2.9	16.35	7.13	39.5	37.7	1.8
	BARC11			-5.65[€1]		16.17		19.44		-1.6	8.16	1.36	36.3	40.9	-4.6**
1000-grain weight	WMC396	-3.5[€1]		-2.5[€1]	2.6[€2]	12.33	29.6	7.07	30.64	-1.5	10.01	1.79	40.1	45.4	-5.2**
	GWM190			-4.0[€1]		23.27		25.78					42.6	42.9	-0.2
	GWM428			2.7[€1]		18.72		16.4					43.9	41.6	2.3**
	GWM537			1.8[€1]		28.27		14.53					45.1	40.4	4.8**
	XGWM577			2.1[€1]	4.1[€1]	14.99	25.53	6.32	27.59				44.2	41.3	2.9**
	WMC525			-1.4[€1]		12.25		4.38					41.2	44.2	-3.0**
	BARC101			2.6[€1]		28.17		29.6					46.1	39.3	6.8**
	BARC11				-2.7[€1]	26.28		20.62					41.2	44.3	-3.0
	GWM165				-3.7[€1]	23.37		9.92					44.4	41.0	3.4

Showed QTL by environment interaction and main effects through stepwise regression and least-square means comparisons. [¥]Significant difference at 0.01 probability level. [€]Detected QTL was effective in one or two environments under normal and late sowing dates in both locations. All variables left in the model of the stepwise regression are significant at the 0.001 level. F value and R^2 are the statistical F-value and the coefficient of determination obtained from the stepwise regression analysis, respectively. Min. and max are the minimum and maximum values of F-value and R^2. Presence and absence of the bands of the primers in the scanned genotypes

Whereas the marker BARC11 was associated with reducing HI under normal sowing date at Nubariah by an estimated value of -5.6% and across environments by a value of -4.6%.

Thousand grain weight (TGW): The stepwise regression revealed nine QTL for TGW which are located on chromosomes 7A, 4B, 7B, 5D and 7D. The marker WMC396 was associated with reducing TGW under normal sowing dates at both locations and increased TGW under late sowing dates by an estimated value of 2.6 g at Nubariah, whereas it reduced TGW across environments by a value of -5.2 g. The markers GWM428, GWM537, BARC101 and XGWM577 were associated with increasing TGW under normal sowing date at Nubariah by values ranging between 1.8 and 2.7 g and as well as across environments by values ranging from 2.3-6.8 g. In contrast, the markers BARC11 and GWM165 were linked to reducing TGW under late sowing date at Nubariah by values of -2.7 and -3.7 g, respectively.

Co-location of QTL for grain yield and its attributes: The QTL analysis revealed eleven QTL which showed pleiotropic effects governing two or more traits. The BARC126 marker was linked to HD, PH, NS and GYH under the late sowing date. The marker BARC11 was associated with HD, NS, HI and TGW. The WMC525 marker was co-located with PH, NS and TGW under normal sowing date. The XGWM132 was controlled PH, NS and BYH under both sowing dates. The GWM165 was linked to PH and TGW under the late sowing date. The BARC68 was correlated to NS and BYH, while GDM93 was associated with NS, HI and GYH under both sowing dates. The marker WMC396 overlapped NS, HI and TGW overall environments. The markers GWM190 and BARC101 were linked to BYH and TGW overall environments and under normal sowing dates, respectively. Finally, the marker GWM537 overlapped BYH and TGW under normal conditions.

DISCUSSION

After the recent repercussions of global warming and climate change, the investigation of the effects of climate change on food security has become a global hot spot. Egypt is one of the countries that is a fall in wheat yield production projected in the coming years due to rising temperatures[39]. Therefore, the most effective strategy to improve tolerance to abiotic stresses including terminal heat stress and drought is producing and growing tolerant cultivars under these environments[40-42]. According to Kumar *et al.*[26], among 49 CIMMYT Lines (CLs) evaluated for grain yield and its attributes and stability across eight environments (a combination of two sites, normal and late (as heat stress) sowing dates and two years), ten HYL and ten LYL along with three check cultivars (CC) were screened by eighteen previously published SSR markers. The current study aimed at examining if the scanned SSR markers are present in both investigated groups of CLs and check cultivars at each environment and across all environments. Additionally, detection of the most significant QTL associated with grain yield and its attributes under terminal heat stress due to late sowing over locations and years.

We found highly significant differences between genotypes for each HYL, LYL and CC for all studied traits as main effects and as interaction with environments, indicating the variation in the performance of each group from one environment to another environment. The investigated environments significantly affected LYL by a high degree, this may indicate that these lines did not tolerant of late sowing and were affected by the terminal heat stress at the end of the season. Consistent with our findings, Mondal[43] and Ali and El-Sadek[44], found significant variation between CIMMYT wheat lines evaluated under different environments. Sayed *et al.*[45] evaluated fourteen wheat cultivars at different sowing dates under Assiut conditions and found a remarkable variation between cultivars in response to the late sowing tolerance. Sowing wheat genotypes in two locations at two different sowing dates (normal and late sowing date) provided ample opportunity to see the difference for stress (heat) tolerance index of the investigated traits (Table 5), which indicated a better grain filling under terminal heat stress. Paliwal *et al.*[46] reported a reduction in grain yield and TGW due to terminal heat stress. Similarly, Tahmasebi *et al.*[47] evaluated a wheat recombinant inbred line population for grain yield and its attributes under a combination of well-watered, drought, late planting (heat) environments and found large variation among genotypes for heat and drought tolerance.

The SSR markers are effective in detecting the genetic diversity in wheat as well as in other crop plants. The differences among the SSR primer pairs in the total generated bands as well as in both the polymorphic bands and the polymorphic information content were observed in this study. Such results have been reclaimed by other researchers such as[48,49]. They reported differences in both amplified polymorphism and PIC number in the SSR pattern of wheat genotypes. The dendrogram generated by SSR markers divided the wheat genotypes into five clusters in this study. Comparable results were reported by other researchers. For example, El-Rawy[50] reclaimed that SSR dendrogram showed that the studied cultivars were grouped into two main clusters. The first main cluster contained bread wheat

genotypes and the second cluster contained durum wheat genotypes. Five clusters were generated from the SSR dendrogram of 26 wheat genotypes according to a study by Saha *et al.*[49].

Interestingly, the high yielding CLs L20, L26, L28 and L22 contained most of the bands of the SSR markers used in the current study that ranged between 13 and 16 bands out of 18. This result indicates that these lines contain the QTL alleles of the SSR markers associated with abiotic stress tolerance. Additionally, these lines amongst the lines had greater stability and high yielding genotypes[26]. Similarly, the CLs L4 and L6 showed the lowest GYH across all environments and contained 4 and 6 bands out of 18, respectively. This may be due to the effective SSR markers alleles did not exist completely. Sharing bands between tolerant and susceptible genotypes in wheat to abiotic stresses were reported in many studies. For instance, Eid[48] found that Wmc396 marker produced sharing bands between the cultivar Sahel1 (drought tolerant) and the cultivar Gemmiza (drought susceptible) at 173pb and Wmc517 amplified sharing bands between the cultivar Sahel1 (drought tolerant) and the cultivar Sakha93 (drought tolerant) at loci 206 pb. Generally, the reason why the tolerant and susceptible genotypes share alleles may be, that the tolerant genotypes may have different physiological and morphological tolerance mechanisms like canopy temperature, desiccation- and heat-tolerant enzymes, osmotic adjustment, superior photosynthesis and root system architecture each governed by a different set of genes[51]. El-Rawy *et al.*[52] used twenty-eight SSR primers for screening twenty-one bread wheat genotypes under control and drought conditions and found that three bands produced by SSR primers Xgwm596-7A (507 bp), Xgwm497-1A (556 bp) and Xgwm174-5D (409 bp) were presented in all tolerant genotypes to drought.

The heading time of a crop is crucial for sustainable productivity and ensuring high yield and quality. Two SSR markers BARC126 and BARC11 on 7D were associated with delay HD under both sowing dates. Liu *et al.*[53] detected four markers mapped on chromosome 7D including BARC126 which were associated with HD. Additionally, we found that the SSR markers BARC126, BARC11, GWM190 and GWM428 which were distributed on genome D, were associated significantly with grain yield and its attributes at multiple environments, indicating the significant contribution of the genome D to wheat production and adaptation[53]. Likewise, the SSR markers WMC396, GWM537 and XGWM577 which were mapped on 7B were significantly linked with grain yield-related traits such as NS, BYH, HI and TGW under one of the two sowing dates or both. Most of the detected QTL of these markers showed desirable effects. Many genes associated with drought and heat stress tolerance-related traits as well as under normal conditions were reported on 7B[19,54]. The SSR marker BARC186 (5A, 57 cM) was linked with increasing NS across environments, indicating heat tolerance. Sehgal *et al.*[10] found that the SSR marker BARC186 was associated with heat tolerance QTL of days to heading. Furthermore, in previous studies, several SSR markers were reported to be associated with heat tolerance. Rai *et al.*[9] used the regression analysis and detected highly significant associations between the SSR markers Xgwm132, Xgwm577 and Xgwm617 and the heat tolerance-related traits in wheat. Paliwal *et al.*[46] detected three significant genomic regions associated with heat tolerance on 2B (23.0 cM), 7B (3.6 cM) and 7D (3.1 cM).

The co-location of QTL for various characteristics implies the likely presence of pleiotropic or closed linkage between the QTL governs the characteristics. Among the eleven QTL which showed pleiotropic effects, the marker XGWM132 was co-located with shortening PH, increasing both NS and BYH under both sowing dates. Similarly, the marker GWM537 showed a desirable effect and overlapped increasing each of BY and TGW under normal conditions. Most of the detected QTL by stepwise regression displayed high F value and R^2, coupled with co-location may strongly indicate the presence of significant QTL in the current study. Guo *et al.*[55] detected nine SSR loci, including Xgwm186-5A and Xgwm132-6B, were significantly associated with two or more traits across environments. In general, various co-localized QTL for yield and its attributes have been studied in wheat under constraints conditions such as drought and heat stresses under temperate irrigated conditions[27,46,56]. The SSR markers BARC186-5A, XGWM132-6B, WMC396-7B, XGWM577-7B and GWM165-4B were more prominently associated with heat tolerance by showing a desirable performance of grain yield-related traits under late sowing or across environments, some of these desirable alleles were corresponding to previously QTL in various genotypes that could be valuable in breeding for high-yield in wheat.

CONCLUSION

In this study, it was revealed that some of the SSR markers used were common between high and low yields genotypes, indicating that there are additional factors controlling terminal heat stress tolerance across multiple environments. These factors may govern morphological and physiological mechanisms associated with heat stress tolerance. Interestingly, the SSR markers BARC186-5A, XGWM132-6B,

WMC396-7B, XGWM577-7B and GWM165-4B were more prominently associated with heat tolerance by showing a desirable performance of grain yield-related traits under late sowing or across environments. Few SSR markers were validated with previous published QTL for heat tolerance in the current study such as Xgwm132, Xgwm577 and BARC186. Moreover, the high yielding lines showed high stress (heat) tolerance index and surpassed the check cultivars. The CIMMYT wheat lines L33, L8 and L42 were the highest yielding, terminal heat stress-tolerant and stable across multiple environments which could be used to enhance heat and drought tolerance in wheat breeding programs.

SIGNIFICANCE STATEMENT

The current study showed the importance of QTL in analyzing wheat grain yield and its attributes for terminal heat and drought stresses as a result of late cultivation across different regions and years in Egypt. In addition, recommend the best genotypes that can serve in the wheat breeding programs to harsh environmental conditions tolerance.

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