

SSR MARKER FOR GRAIN YIELD UNDER HEAT STRESS CONDITIONS IN BREAD WHEAT

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Heat stress is a major abiotic stress factor for wheat production worldwide affecting growth, productivity and reducing grain yield and quality (Stone and Nicolas, (1995); Lobell and Asner, (2003). Climate changes are predicated to cause a shift in many wheat production areas especially those with heat stressed environments (Ortiz *et al.*, (2008). Rising temperatures exacerbates water deficits and affects crop development before anthesis, as well as grain filling and in general there is an agreement that winter wheat is being exposed to rising air temperatures as a result of climate changes (Braun *et al.*, (2010). The development of wheat genotypes with high yield under heat stress conditions is needed. The genetic improvement of wheat tolerance to abiotic stress is basically depending on the genetic variability presented in wheat genotypes and the heritability of the concerned traits under stress and non-stress conditions. However, the genetic control of yield in wheat is quantitative and is strongly influenced by the environment and genotype-environment interaction. In this regard, single seed descent (SSD) method is commonly used to de-

velop segregating populations of self-pollinated crops with variable and superior performance and also this method is very effective with the low and moderate heritability traits (Kanbar *et al.*, (2011; Collard *et al.*, 2017). Moreover, the segregated populations generated via SSD can undergo inbreeding to create genetically stable recombinant lines (RILs) which are very important for genetic mapping and validation of quantitative trait loci (QTLs) (Khatibani *et al.*, 2019; Zhang *et al.*, (2019).

On the other hand, identification of wheat genotypes, traits and genes associated with adaptation to abiotic stress will assist breeders in adapting wheat to adverse environments. Thus, identification of stable molecular markers can be used in breeding to increase the selection efficiency for traits that are difficult and costly. Among many DNA based markers which have been used successfully with wheat cultivars is simple sequence repeat (SSR) marker used in many studies because of its simplicity, high polymorphism and locus specificity. SSR markers are useful tools to differentiate wheat cultivars and could be used for further genetic analysis,

identifying QTLs and marker-assisted selection (Erayman *et al.*, 2016).

The objectives of this study were: 1) Development a set of wheat RILs that could be used in the future in wheat breeding programs. 2) Development SSR markers for grain yield under heat stress conditions in wheat.

MATERIALS AND METHODS

Plant materials:

The initial plant materials used in the present study consisted of Giza-168 and Shandaweel-1 which represent wide range of grain yield under normal and heat stress conditions as parental genotypes. To develop recombinant inbred lines (RILs) from these parents, a cross between the two parents was employed in 2011 winter season at the experimental farm of South Valley University to produce F₁ seeds. The F₁ seeds were grown in 2012 season and allowed to self-pollinate to produce F₂ seeds. The F₂ seeds were grown in 2013 season and allowed to self-pollinate until F₆ lines by SSD method. Then, seeds from the individual plants were bulked to create 100 advanced lines of bread wheat (100 F₇ RILs).

Field experiments

The parental genotypes and 100 RILs were sown in the field at experimental farm of South Valley University in 2018 winter season. All genotypes were sown under optimal (30 November) and late sown (30 December) conditions, where the late sowing date was used to

subject lines into heat stress during reproductive development.

The experimental design used in this study was randomized complete block design (RCBD) with three replications for the tested genotypes in the two sowing dates. Each genotype was represented in each block by 10-plants/row with row spaced 50 cm apart and plants within row at 25 cm from each other.

Phenotypic traits measurements

Details of traits, their symbols and methods of measurements are presented in Table (1).

Statistical analysis

Significance of differences between means for each genotype under normal and heat stress conditions and among genotypes means in each condition were compared using the least significant difference (LSD) at 5% probability level. A combined analysis of variance (ANOVA) across environments was conducted for all traits separately to estimate the significance of genotype (G), environment (E) and G X E interaction. Phenotypic (σ^2P), environmental (σ^2E), genetic (σ^2G) and GXE interaction (σ^2GE) components of variance were calculated and broad sense heritability (h^2_B) was established according to Fehr (1987) where $h^2_B = \sigma^2G/\sigma^2P \times 100$.

Molecular analysis

DNA extraction

The highest and lowest five RILs regarding their performance according to GYS under heat stress conditions were

selected for molecular analysis. DNA was extracted from leaf samples of the two parents and the ten RILs using the CTAB method following the procedure of Saghai-Marouf *et al.*, (1984). Then, DNA quality and concentration were determined using agarose gel electrophoresis and spectrophotometer, respectively.

SSR-PCR amplification

Five SSR primers pairs were selected and used to screen the parents and their selected RILs in the present study. The primers codes and sequences are presented in Table (2). PCR amplifications were performed following Hassan (2016).

RESULTS AND DISCUSSION

Phenotypic analysis

The mean values of phenotypic data collected from the two parents (Giza-168 and Shandaweel-1) and their RILs under normal and late sowing date are presented in Table (3). High variation was observed between the two parents regarding GYS and TKW. Average GYS was highest in early sowing date which ranged from 2.60 g (Giza-168) to 4.00 g (Shandaweel-1) and the range of GYS in RILs ranged from 2.60 g to 4.00 g. However, the GYS was reduced significantly in all genotypes tested under late sowing date which represents heat stress conditions and ranged from 1.63 g (Giza-168) to 2.26 g (Shandaweel-1) in parents and from 1.36 g to 2.86 g in the RILs. Interestingly, under heat stress conditions, high variability was observed in the RILs re-

garding GYS where some of RILs showed GYS higher than those of Shandaweel-1 (2.86) while other RILs showed GYS lower than those of Giza-168 (1.36). The superior performance of these RILs that exceeded the best yielding parent may be due to accumulation of favorable additive alleles brought by both parents, and/or the complementary interactions between alleles of different origins (Tefera *et al.*, (2003). Also, both of TKW and NKS were significantly reduced under late sowing date conditions as compared to optimal sowing date conditions in all genotypes tested. The effect of heat stress conditions on GYS, TKW and NKS reduction in the present study was similar to those observed by other authors (Hossain *et al.*, (2012); Wahid *et al.*, (2017); Balla *et al.*, (2019); Verma *et al.*; (2019) and these effects of heat stress could be caused by accelerated senescence, increased respiration, reduced photosynthesis and accelerated phasic development (Wahid *et al.*, 2017).

Variance components and broad sense heritability:

Generally, genetic variance was higher compared to environmental and genotype-environment interaction variances (Table 4). The genetic variance was higher than environmental variance for GYS and NKS, however it was higher than genotype-environment interaction variance for GYS, TKW and SL. Broad sense heritability estimated ranged from 0.30 for TKW to 0.68 for NKS. However, intermediate broad sense heritability was

observed for SL and GYS (0.46 and 0.47, respectively). In agreement with our results, low values of heritability for TKW (Kaya and akcura, (2014); Elbashier *et al.*, 2019); moderate values of heritability for grain yield (Rahman *et al.*, (2016); Mansouri *et al.*, 2018) and SL (Mesele *et al.*, (2016) and high value of heritability for NKS (Gashaw *et al.*, (2010) were reported. However, contradicting with our results, other authors reported low heritability values for grain yield (Akcura, (2009) and for SL and NKS (Yaqoob, (2016). Moreover high heritability values were also estimated for the studied traits by Arya *et al.*, (2018). The differences among heritability values estimated for the studied traits in the present study and those in the previous reports might be due to the different genetic materials used and the different climatic conditions under which the studies were undertaken (Yaqoob, (2016).

SSR markers analysis

In the present study, five SSR primer pairs were selected from wheat genome database for molecular analysis to identify SSR molecular markers associated with grain yield under heat stress conditions in wheat. These five SSR markers were used first to screen the parental genotypes, and then the polymorphic markers were used to screen the selected RILs (. 1). The total number of generated bands was 26 bands with an average of 5.2 bands per marker, where Xgwm219 marker located on chromosome 6B generated the lowest number of bands (1 band) and

Xgwm210 marker located on chromosomes 2A, 2B and 2D generated the highest number of bands (10 bands). The molecular weight of generated bands ranged from 100 to 1530 bp which was generated by markers Xgwm210 and Xwmc626, respectively. These variations in bands number and molecular size which were generated by the tested markers could be the result of the primers sequences and the number and location of their complement sequences in the genome of the tested genotypes, respectively. However, these range of number and size of generated bands in the present study agreed with those observed in other studies on wheat using SSR markers (Hassan, 2016; Rathi *et al.*, 2018; Sonmezoglu and Terzi, 2018; Poudel *et al.*, (2019). Out of the five SSR markers tested, only one marker (Xwmc626) located on chromosome 1 B could generate polymorphic pattern between the two parents and successfully generated a unique band in Shandaweel-1 with a size of 420 bp. Interestingly, when this marker (Xwmc626) was used further with the selected RILs (the highest and lowest five RILs regard to GYS under heat stress conditions), it successfully generated this unique specific band only in the highest five RILs for GYS under heat stress conditions. In accordance with our findings, Zhang *et al.*, (2009) reported that Xwmc626, Xbarc120.3 and Xwmc406 markers which are located on the same linkage group on chromosome 1B are associated with grain filling duration, chlorophyll a and heading and flowering dates in wheat, respectively. Also,

they found that all these traits were significantly correlated with grain yield under three different environmental conditions. Furthermore, other QTLs associated with grain yield (Qaseem *et al.*, 2019) and grain filling duration (Yang *et al.*, 2002) under heat stress conditions were detected on chromosomes 1B. It suggests that, this specific band generated by Xwmc626 marker could be used as a potent marker for high yield under heat stress conditions. However, further marker validations are still needed using additional wheat genotypes to confirm the usefulness of this marker for marker-assisted selection in wheat breeding programs. On the other hand, the other four markers tested could not generate polymorphic patterns between the tested parents in the present study. So, these SSR markers were not used further to differentiate between the tested RILs.

Finally, in the present study by using SSD method, we successfully developed variable RILs representing wide range of GYS under heat stress conditions and some of these RILs exceeded the best yielding parent. These RILs could be used for improving grain yield in wheat breeding programs in the future. Additionally, Xwmc626 marker used in the present study successfully generated unique specific band which was amplified only in the higher parent (Shandaweel-1) and the highest RILs regard to GYS under heat stress conditions, suggesting that this band can be considered as SSR marker associ-

ated with GYS under heat stress conditions in wheat.

SUMMARY

In the present study, we developed a set of bread wheat RILs to be used in the future in wheat breeding programs using SSD method. To achieve this, a cross between the two parents Giza-168 and Shandaweel-1 was employed in 2011 winter season to produce F₁ seeds. The F₁ seeds were grown and allowed to self-pollinate until F₆. Then, seeds from the individual plants were bulked to create 100 advanced lines of bread wheat (100 F₇ RILs). All genotypes were then sown under normal and heat stress conditions in 2018 winter season and the yield components were evaluated. All yield components were decreased under heat stress conditions in all tested genotypes, however, GYS for some RILs was higher than those of the higher parent (Shandaweel-1) under heat stress conditions. On the hand, five SSR primers were used to develop SSR markers for grain yield under heat stress conditions using the two parents and the highest and lowest five RILs regard to their performance according to GYS under heat stress conditions. Only one marker (Xwmc626) successfully generated unique specific band (420 bp) only in the higher parent (Shandaweel-1) and the highest RILs regard to GYS under heat stress conditions, suggesting that this band can be considered as SSR marker associated with GYS under heat stress conditions in wheat. There is no conflict of interest with any financial organization regarding this study.

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Table (1): Field environments and phenotypic traits evaluated on Giza-68 X Shandaweel-1 and their RILs populations.

Trait	Trait symbol	Measurements	Date
Grain yield per spike*	GYS	grams	S 2018-1 and S 2018-2
Number of kernels per spike	NKS	number	
Thousand kernel weight	TKW	grams	
Spike length	SL	cm	

S 2018-1, sown on 30 November 2018 and S 2018-2, sown on 30 December 2018

* Grain yield per spike estimated from the grain yield for the main spike

Table (2): SSR Primers codes and sequences used in the present study.

Primer	Sequence	
Xgwm371	F	5' GACCAAGATATTCAAACCTGGCC-3'
	R	5' AGCTCAGCTTGCTTGGTACC-3'
Xgwm219	F	5' GATGAGCGACACCTAGCCTC-3'
	R	5' GGGGTCCGAGTCCACAAC-3'
Xgwm210	F	5' TGCATCAAGAATAGTGTGGAAG-3'
	R	5' TGAGAGGAAGGCTCACACCT-3'
Xwmc626	F	5' AGCCCATAAACATCCAACACGG-3'
	R	5' AGGTGGGCTTGGTTACGCTCTC-3'
Xwmc596	F	5' TCAGCAACAAACATGCTCGG-3'
	R	5' CCCGTGTAGGCGGTAGCTCTT-3'

Table (3): Means of trait values for parents and RIL measured under early and late sowing date conditions in 2017 season:

Trait	Early sowing date					Late sowing date					L.S. D
	Giza-168	Shandaweel-1	RILs			Giza-168	Shandaweel-1	RILs			
			Mea n	Min	Max			Mea n	Min	Ma x	
GYS	2.60	4.00	3.23	2.6	4.00	1.63	2.26	1.56	1.36	2.86	0.39 7
TK W	43.4	55.4	39.6 0	46.6 0	55.0 4	34.31	49.22	40.6 4	34.3 1	49.2 2	5.52 7
SL	11.6 6	12.3 3	11.6 5	10.3	12.3 0	10.30	12.30	10.2 6	9.60	12.3 0	1.27 5
NKS	81.0 0	81.6 0	78.0 6	72.6	85.0 0	37.30	52.30	34.9 3	29.6 0	52.3 0	2.43 9

Table 4): Variance components and broad-sense heritability estimates for the studied traits:

Traits	Variance components			h ² (B)
	G	E	GE	
GYS	0.13**	0.11**	0.02*	0.47
TKW	10.08**	22.91*	1.95*	0.30

SL	0.32**	1.22**	0.14*	0.46
NKS	10.22**	4.46**	12.16**	0.68

*= Significant at $P < 0.05$ probability level. **= Significant at $P < 0.01$ probability level

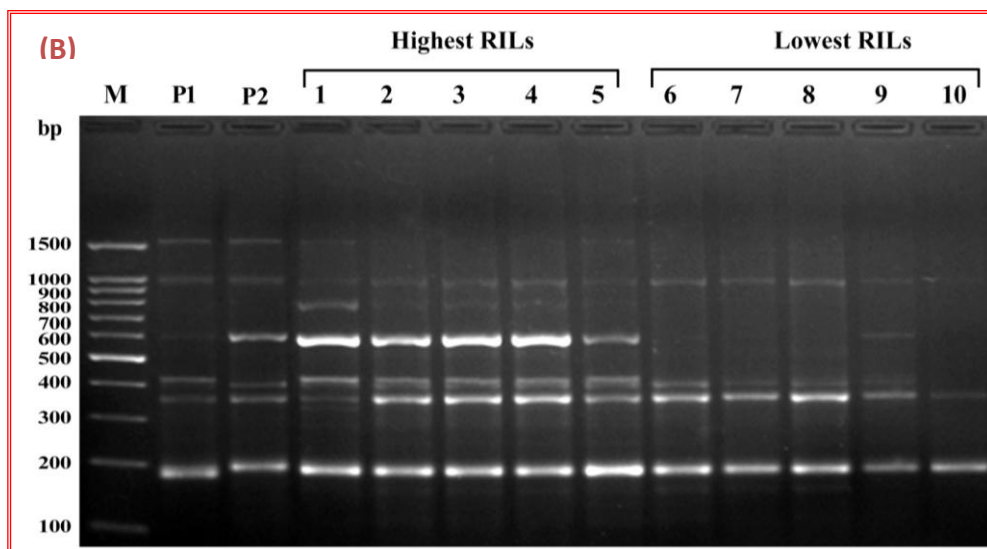
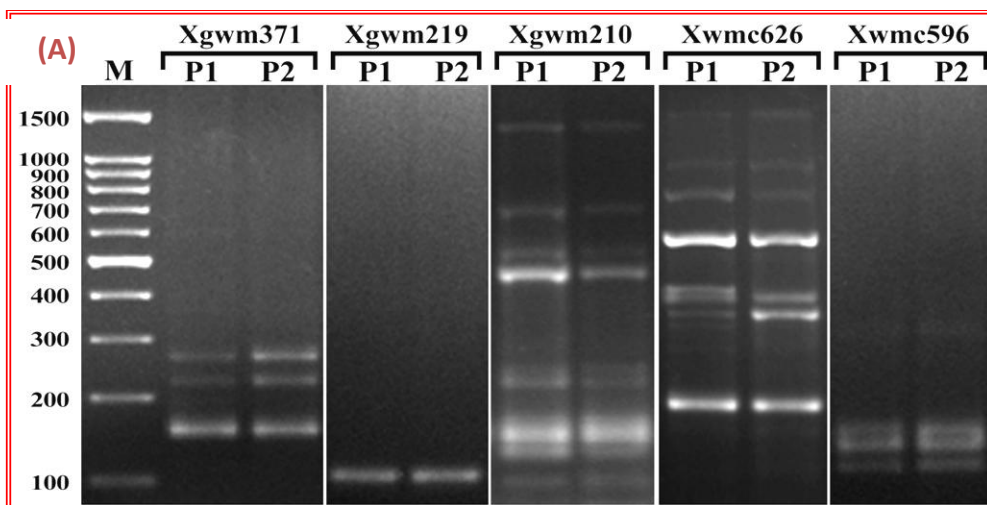


Fig. 1: A) Banding profile of wheat parents generated by SSR primers; B) Banding profile of wheat parents and the RILs generated by Xwmc626 primer, where P1: Shandaweel-1 and P2: Giza-168.