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

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Heat stress is a major abiotic stress factor for wheat production worldwide negatively affecting plant 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). So, the development of wheat genotypes with high yield under heat stress conditions is needed. And it has been clear that 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 develop 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 be inbred to create genetically stable recombinant inbred 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 different environmental conditions. Thus, identification of stable molecular markers can be used in breeding to increase the selection efficiency for traits that are difficult and costly costly to phenotype. Among various DNA based markers which have been used successfully with wheat cultivars is simple sequence repeat (SSR) marker that was used in many studies because of it is simplicity, high polymorphism and locus specificity. SSR markers are useful tools to differentiate wheat cul-

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tivars 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) Develop a set of wheat RILs that could be used in the future in wheat breeding programs. 2) Develop 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 two bread wheat cultivars namely ;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 F_1 seeds. The F_1 seeds were grown in 2012 winter season and were allowed to self-pollinate to produce F_2 seeds. The F_2 seeds were grown in 2013 winter season and allowed to self-pollinate until F_6 lines by SSD method. Then, seeds from the individual plants were bulked to create 100 advanced lines of bread wheat (100 F_7 RILs).

Field experiments

The parental genotypes and 100 F_7 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-palnts/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 procedures of Saghai-Maroof *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 according to 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 dates are presented in Table (3). High variation was observed between the two parents regard to 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 variation was observed in the RILs regarding 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, NKS and SL. However, it was higher than genotype-environment interaction variance for GYS, TKW, SL and NKS. Broad sense heritability estimated ranged from 0.30 for TKW to 0.68 for NKS. However, intermediate broad sense heritability values were estimated 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 estimated in previous studies. However, contradicting with our results, other authors estimated 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 investigated 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 primers 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 (Fig. 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 generated by markers Xgwm210 and Xwmc626, respectively. These variation in bands number and molecular weight which generated by the tested markers are resulted from 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 are similar to those observed in another studies on wheat using SSR markers (Hassan, 2016; Rathi *et al.*, 2018; Sonmezoglu and Terzi, 2018; Poudel *et al.*, 2019). Out of five SSR markers tested, only one marker (Xwmc626) which located on chromosome 1B 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 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 reported 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. These suggest that, the specific band which generated by Xwmc626 marker in the present study 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 tested markers could not generate polymorphic patterns between the contrasted parents in the present study. So, these SSR markers were not used further to differentiated 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 high yielding parent. These RILs are suitable material to 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 highest parent (Shandaweel-1) and the highest RILs regard to GYS under heat stress conditions, suggesting that this band could be considered as SSR marker associated with GYS under heat stress conditions in wheat.

SUMMARY

In the present study, we developed a set of bread wheat RILs using SSD method to be used in the future in wheat breeding programs. To achieve that, a cross between the two parents Giza-168 and Shandaweel-1 was employed in 2011 winter season to produce F_1 seeds. The F_1 seeds were grown and allowed to self-pollinate until F_6. Then, seeds from the individual plants were bulked to create 100 advanced lines of bread wheat (100 F_7 RILs). All genotypes were then grown in two cultivation times (under normal and heat stress conditions) in 2018 winter season, and the yield components were eval-

uated. All yield components were decreased under heat stress conditions in all tested genotypes, however, GYS for some RILs was higher than those of the highest parent (Shandaweel-1) under heat stress conditions. On the other hand, five SSR primers pairs were used to develop SSR markers for the high 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 with molecular weight of 420 bp only in the highest parent (Shandaweel-1) and the highest RILs regard to GYS under heat stress conditions, suggesting that this band could be considered as SSR marker associated with GYS under heat stress conditions in wheat.

Running title: SSR for wheat grain yield

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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): Primers codes and sequences used in the present study.

Primer	Sequence		Annealing temperature
Xgwm371	F	5' GACCAAGATATTCAAACTGGCC-3'	60 °C
	R	5' AGCTCAGCTTGCTTGGTACC-3'	
Xgwm219	F	5' GATGAGCGACACCTAGCCTC-3'	60 °C
	R	5' GGGGTCCGAGTCCACAAC-3'	
Xgwm210	F	5' TGCATCAAGAATAGTGTGGAAG-3'	60 °C
	R	5' TGAGAGGAAGGCTCACACCT-3'	
Xwmc626	F	5' AGCCCATAAACATCCAACACGG-3'	61 °C
	R	5' AGGTGGGCTTGGTTACGCTCTC-3'	
Xwmc596	F	5' TCAGCAACAAACATGCTCGG-3'	61 °C
	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			
			Mean	Min	Max			Mean	Min	Max	
GYS	2.60	4.00	3.23	2.6	4.00	1.63	2.26	1.56	1.36	2.86	0.397
TKW	43.40	55.40	39.60	46.60	55.04	34.31	49.22	40.64	34.31	49.22	5.527
SL	11.66	12.33	11.65	10.30	12.30	10.30	12.30	10.26	9.60	12.30	1.275
NKS	81.00	81.60	78.06	72.60	85.00	37.30	52.30	34.93	29.60	52.30	2.439

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**	0.04*	0.47
TKW	10.88**	22.91*	1.95*	0.30
SL	0.32**	0.23**	0.14*	0.46
NKS	10.22**	4.46**	0.22**	0.68

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

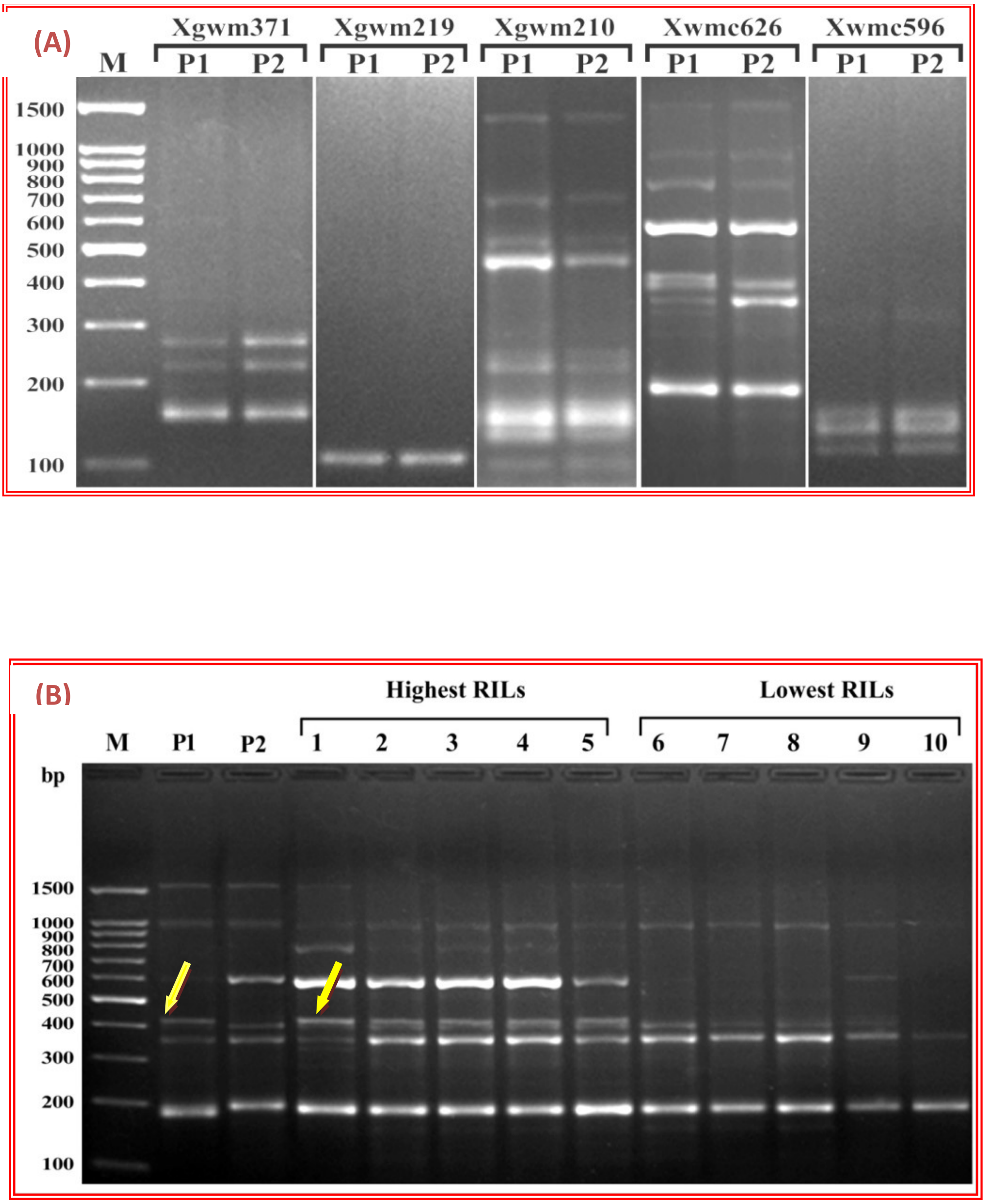

Fig. 1: A) Banding profile of wheat parents generated by SSR primers; B) Banding profile of wheat parents and the RILs generated by Xwmc626 primer, where P1: Shandaweel-1 and P2: Giza-168.