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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(3): 1930-1936. **OPEN ACCESS**

Molecular characterization of salt tolerance of wheat genotypes using microsatellite markers and stress tolerance index

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Salinity affects wheat (*Triticum aestivum* L) plants growth by the osmotic stress of the salt around the roots, as well as by toxicity caused by excessive accumulation of salt in leaves. Therefore, salinity has a limiting factor for wheat crop in Saudi Arabia. In this study, twenty wheat genotypes (5 genotypes from ICARDA, 7 genotypes from Pakistan, 5 Australian genotypes, one American genotype (YocoraRojo), one Egyptian genotype (Sakha 93) and one local genotype (Sama)) were subject to different salinity levels of Irrigated water (control, 4000, 8000 ppm NaCl). The trial was conducted under green house environmental condition at Qassim University Agricultural Research and Experimental Station to observe stress susceptibility index (SSI) based on grain yield plant^{-1}. The genetic variation and relationships among different wheat genotypes with different responses to salt stress were also investigated by SSR markers. The results showed that Auqab 2000, Bhan 2000 and Shaka 93 scored the lowest SSI value. These genotypes can be considered as salinity resistant varieties In addition, the result of genetic variation reveled that there are two main groups at genetic similarity at (0.69), the first group divided into four subgroups where genotype IC1 in the first subgroup and the second subgroup divided into two sub-sub-group at similarity (0.80). The first sub-sub-group included genotypes Sakha93, YocaraRojo, IC16, P7, P8, IC17, Bhan2000, Pasban90, and Auqab2000 and the second sub-sub-group included Sis27, Sis32, Inq91, and P6 and with similarity (0.85), and the third sub-group included IC96. While, the fourth sub-group included the least salt tolerance genotypes (local and IC15, P2, P9). Thus, the three wheat genotypes that show salt tolerance (Sakha93, Bhan2000 and Auqab2000) are in one group after using SSR markers that linked to salt tolerance. These genotypes can be used in wheat breeding programs for salt affected areas.

Keywords: Wheat genotypes, salt stress, stress susceptibility index, SSR markers.

INTRODUCTION

Wheat considered less tolerant to salinity comparing to other field crops. However, some studies have shown that there is a variation between wheat's stage of growth to salinity, started from germination to maturity and seed formation (Kingsbury and Epstein 1984, El-Hendawy et al.2005). On the other hand, there is a variation among wheat varieties in their ability to salinity stress. A study was published which compared a group of wheat varieties in semi-arid areas in Jordan has showed that a significant difference in salinity tolerance between the stage of growth in the varieties under the study (Abdel-ghani, 2008). Salinity tolerance is a complex trait and involves many genes (Negrão et al., 2017). Even though traditional plant breeding methods is good way to introduce new enhanced varieties for salt stress, they take longer time (it takes 8-12 years) in order to release new varieties and those varieties may not registered. Plant breeders always looking for new methods and new techniques that hasten new release, DNA markers is a technique that helps to pinpoint genes of interest and ensure that desirable genes are in the new release so there is more interest in building genetic markers in plant (Li, 2000). DNA markers targets specific DNA locations either in chromosome or mitochondria or chlorophyll (Alpha- Amylase Gene Analysis). In recent years DNA markers grow fast on different plants. In cereal for example scientist are able to accumulate high amount of DNA markers, there are more than 1.6 million primers and can be accessed through www.ncbi.nlm.gov

There are different uses for genetic markers for example genetic mapping, marker assisted selection (MAS), and variety fingerprinting. In order to enhance wheat varieties to tolerate to salt, different physiological traits can be used to identify salt tolerance varieties for example high K/Na good indicator for tolerance to salt and has been used frequently (Royo and Abio, 2003) and (Kingsbury and Epstein 1984). In recent years, scientists are using DNA markers to release salt tolerance wheat. In another study by Din et al. (2008) studied eight soft wheat varieties for salt tolerance (Lu-26, Inqilab-91, Bakhtawar, Parwaz-93, Punjab-85, Pak-81, Pashan-90 and Potohar-1) and they measure relative growth, chlorophyll content, protein content and K/Na and rank varieties for salt tolerance at 4000 ppm as follows lu-26 85 % and Punjab-85 65% and inqilab-91 at 55% and K/Na ratio and chlorophyll content was higher in Lu-26 and followed by Punjab-85 and then Inqilab-91 meanwhile, Bakhtawar, Parwaz-93, Pak-81, Pasban-90 and Potohar-1 are sensitive for salt witch hold more sodium that others. In another study using real time PCR markers scientist showed that variety "Bam" has higher genetic expression for gene TaGSK1 among nine wheat varieties which succor 71% while the lowest expression was variety "ER-Salt-85-17" which succors 39% (Bahrani et al., 2009). There are several DNA markers that are linked to salt tolerant in wheat and they are available for the plant breeding programs (Mehboob –ur-rahman et al., 2008). The objective of this research was to use DNA markers that are linked to salt tolerance in wheat and search for salt tolerant genes in selected wheat genotypes under study.

MATERIALS AND METHODS

Plant materials:

Twenty wheat genotypes from Australia, Pakistan, Egypt and local genotype (Sama) were used (Table 1).

Table 1: List of the of the 20 wheat genotypes used in this study

No	Genotype name	Genotype source
1	Auqab 2000	Pakistan
2	Inq-91	Pakistan
3	Pas ban 90	Pakistan
4	Sis 13	Pakistan
5	Sis 32	Pakistan
6	Sis 27	Pakistan
7	Bhan 2000	Pakistan
8	P2	Australia
9	P6	Australia
10	P7	Australia
11	P8	Australia
12	P9	Australia
13	IC1	ICARDA
14	IC15	ICARDA
15	IC16	ICARDA
16	IC17	ICARDA
17	IC 96	ICARDA
18	Sakha 93	Egypt
19	*YR	USA
20	**Sama	Saudi Arabia

* Yocora Rojo: - commercial genotype grows commonly in Saudi Arabia ** Sama: local genotype

Greenhouse experiment:

This study was conducted at the Experimental Farm, College of Agriculture and Veterinary Medicine, Al-Qassim. The geographical location of the farm is 26º 18' N latitude and 43º 58' E longitude, and 725m above sea level, in central Saudi Arabia. The soil type of this farm is classified as sandy, 96.3% sand, 1.8% silt and 1.9% clay. The experiments were done at the greenhouse. Three salt levels of water control, 4000 ppm and 8000 ppm were used. A split plot design was used with three replications.

Stress susceptibility index (SSI) was calculated for grain yield as described by Fischer and Maurer (1978):

$$SSI = (1- Y/Yp) / (1- X/Xp)$$

where, Y = Grain yield of genotype in a stress environment Yp = Grain yield of genotype in a stress-free environment X = Mean Y of all genotypes Xp = Mean Yp of all genotypes

(Higher SSI indicates greater susceptibility).

Statistical analyses

Analysis of variance (ANOVA) was performed using JMP Ver. 11 (SAS Institute 2013) to compare means of wheat genotypes for stress susceptibility index. Significant differences among genotypes means were calculated based on Tukey's HSD at P < 0.05.

Genetic diversity in wheat

Total genomic DNA of wheat genotypes was extracted using the method described by Saghai-Maroofet al. (1984). The quality and quantity of the DNA was determined by using UV-Spectrophotometer at wavelengths of 260 and 280nm.

SSR analysis:

SSR primers design were performed using the web-based 'SSR Primer Discovery Tool' (Plant Biotechnology Centre, La Trobe University; http://hornbill.cspp.latrobe.edu.au/ssrdiscovery.html) (Table 2).

Table 2: Sequences of SSR primers.

No.	SSR Primers	Sequence (5'-3')
1	Xgwm181 -3B F	TCATTGGTAATGAGGAGAGA (20)
	Xgwm181 -3B R	GAACCATTCATGTGCATGTC (20)
2	Xgwm247 -3B F	GCAATCTTTTTTCTGACCACG (21)
	Xgwm247 -3B R	ATGTGCATGTCGGACGC (17)
3	Xgwm299 -3B F	ACTACTTAGGCCTCCCGCC (19)
	Xgwm299 -3B R	TGACCCACTTGCAATTCATC (20)
4	Xgwm340 -3B F	GCAATCTTTTTTCTGACCACG (21)
	Xgwm340 -3B R	ACGAGGCAAGAACACACATG (20)
5	TaGSK1genes-F	CCATGAGTTGAAGGGTGTGC (20)
	TaGSK1genes-R	AAGCAGTGGTATCAACGCAGAGT (23)
6	SC1-F	TTCTGCCTCGTATTTCTGGGTC (22)
	SC1-R	CGCGGCTTGCTGTCATCTCG (20)

PCR reactions were conducted in a total volume of 20 uL, using 50 ng of buffelgrass DNA, 1X Promega MgCl2-free PCR Buffer, 2.5 mM MgCl2, 0.2 mMdNTPs, 2 mM of each primer, and 1 unit of PromegaTaq polymerase. The PCR method was included: 1) an initial denaturation at 95° C for 3 min, 2) 10 touchdown decrement cycles at 95° C for 25 sec, 64-55° C for 25 sec, and 70° C for 45 sec, 3) 36 cycles at 95°C for 25 sec, 55° C for 25 sec, and 70° C for 45 sec, 4) an elongation cycle at 70° C for 10 min, and 5) a final hold at 4° C. Electrophoresis was run using a MEGA-GEL High Throughput Vertical Unit (C.B.S. Scientific, Del Mar, CA) as described by Wang et al.(2007).

Data Handling and Cluster Analysis

Data was scored for computer analysis on the basis of the presence or absence of the amplified products for each random primer. Basically, if a product is present in a genotype, it was designated "1", but if absent it was designated "0" after excluding irreproducible bands. Pair-wise comparisons of cultivars, based on the presence or absence of unique and shared polymorphic products, was used to generate similarity coefficients based on a simple matching. The similarity coefficients was used to construct a dendogram by UPGMA (Unweighted Pair-Group Method with Arithmetical Averages) using NTSYS-PC (Rohlf, 2000).

RESULTSAND DISCUSSION

Stress susceptibility index of wheat genotypes

Stress susceptibility index (SSI) based on grain yield of different genotypes indicated a wide range in salt susceptibility among wheat genotypes (Table 3). The estimates of SSI index revealed the genotypes Auqab 2000, Bhan 2000 and Sakha 93 had the lowest SSI value (Table 3). These genotypes can be considered as salinity resistant varieties. In contrast, wheat genotypes (Local and Australian genotypes) had the highest SSI values and could be identified as highly susceptible to salt. It has been evident in literature that SSI could be a more useful index in discriminating better genotypes under salt stress condition (Goudarzi and Pakniyat, 2008; Nouri et al. 2011).

Table 3: Mean comparison of stress susceptibility index (SSI) of wheat genotypes using Tukey's HSD method.

Genotypes	2011 season	2012 season
Local	1.15 a	1.15 a
Auqab 2000	0.64 d	1.02 b
P9	1.09 a	1.06 b
Iuq-91	1.00 b	0.9 c
P2	1.13 a	1.00 b
Pas Ban 90	0.95 b	1.12 a
IC 1	1.04 b	0.87 c
P 7	0.98 b	0.91 c
Sis 13	1.13 a	1.10 b
IC 17	1.1 0 a	1.15 a
Bhan 2000	0.81 c	0.69 d
Sis 32	1.12 a	0.72 d
IC 96	1.01 b	1.21 a
YR	1.02 b	0.63 d
Sis 27	1.08 ab	0.87 c
IC 15	1.02 b	0.99 bc
P6	0.99 b	1.18 a
IC 16	1.08 ab	1.13 a
Sakha 93	0.83 c	0.61 d
P 8	1.06 ab	1.24 a

Table 4: SSR markers along with their annealing temperatures (TA), allele size, chromosome number, and number of polymorphic alleles detected (P. Allele)

No.	Locus/Marker	TA (˚C)	Location	P. Allele	Size (bp)
1	Xgwm181	50	3B	0	150-200
2	Xgwm247	55	3B	3	180-200
3	Xgwm299	52	3B	2	200-220
4	Xgwm340	60	3B	2	160
5	TaGSK1	55	2AL	4	200-250
6	SC1	52	---	2	400

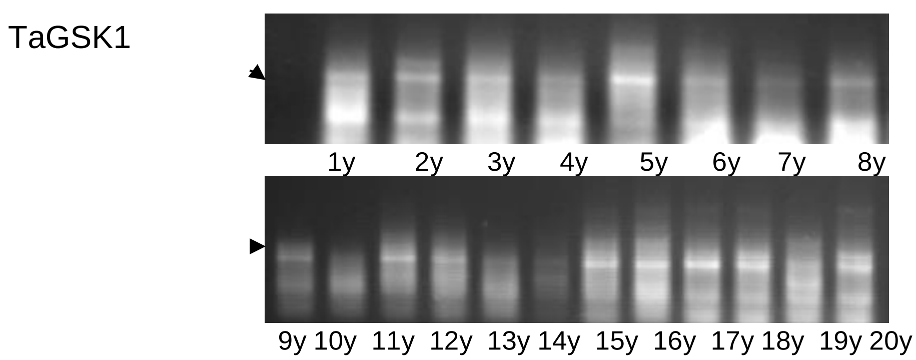

Figure 1:Polymorphism revealed using primer TaGSK1 to amplify genomic DNA purified from the tested wheat genotypes(1y to 20y)where; 1y = IC 1, 2y = Sis13, 3y = Sakha93, 4y = Yocora Rojo, 5y = IC15, 6y = IC16, 7y = P6, 8y = Sis27, 9y = Sis32, 10y = IC17, 11y = Local, 12y = P2, 13y = P7, 14y = P8, 15y = P9, 16y = INQ91, 17y = Bhan2000, 18y = Auqab2000, 19y = IC96 and 20y = Pasban90.

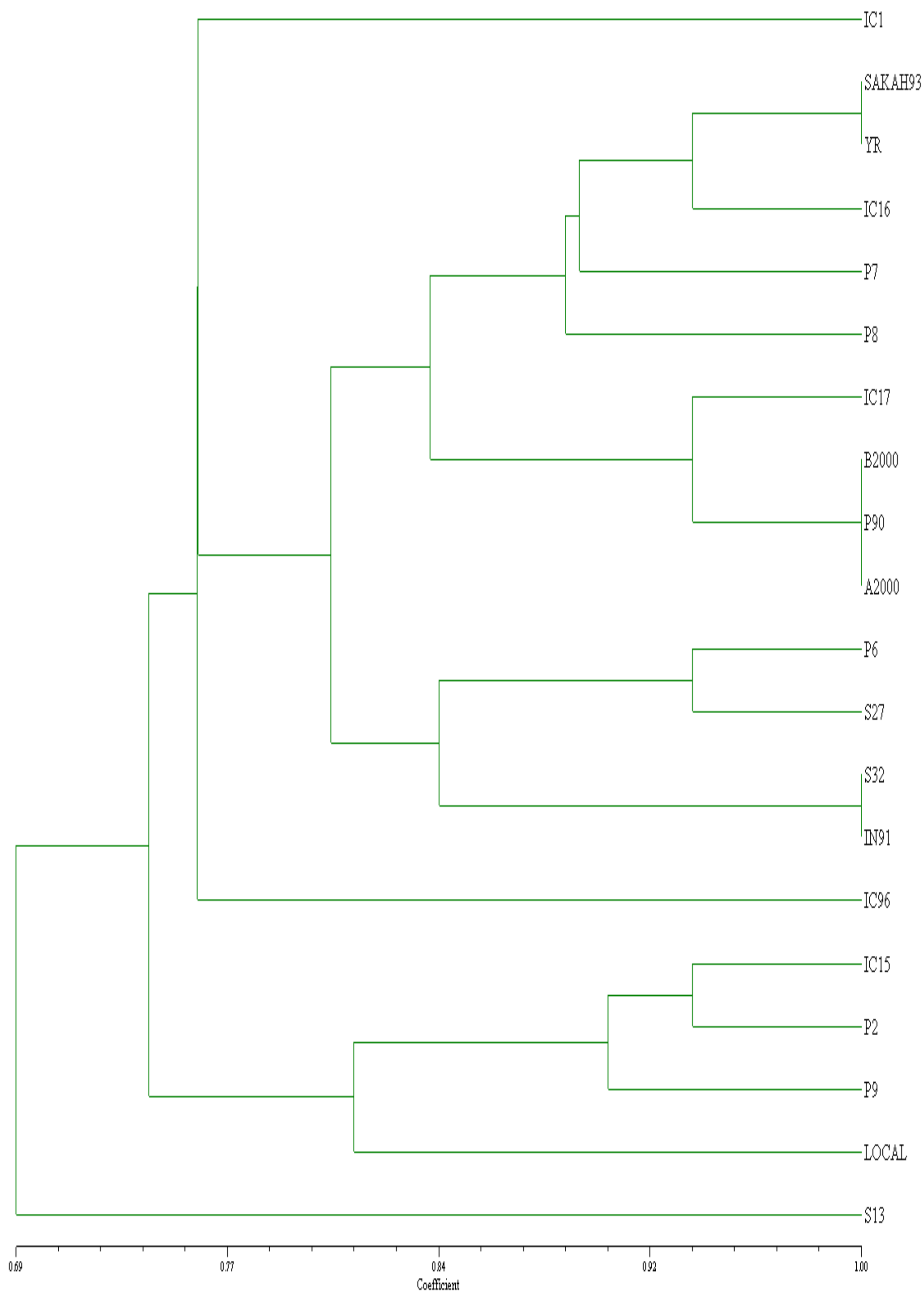

Figure 2: Dendrogram constructed from similarity coefficients and showing the clustering of the tested wheat genotypes.

Interestingly, Al-Otayk (2020) showed that wheat genotypes (Auqab 2000, Bhan 2000 and Shaka 93) had the highest yield at high level of salt, and Sis 13, P2 and Local were the least in yield. A selection based on minimum yield reduction under stress conditions in comparison with nonstress conditions (TOL) failed to identify the most tolerant genotypes (Rizza et al. 2004). Rosielle and Hamblin (1981) reported that selection based on the tolerance index often leads to selecting cultivars which have low yields under nonstress conditions. The reduction in grain yield under salt stress might have resulted from causes, such as lower Na^{+} content, produced higher grain yield under saline conditions, and loss of photosynthetic capacity (Goudarzi and Pakniyal, 2008; Thalji and Shalaldeh. 2007; Al-Otayk, 2020).

Genetic relation between wheat genotypes using SSR markers linked to salt tolerance genes

Six SSR markers were used in this study for genetic relation between wheat genotypes for salt tolerance. DNA was amplified and different bands for different genotypes were between 0-4 bands (Table 4 & Figure 1), primer TaGSK1 and primer Xgwm247 have higher bands than other primers. Similar results were shown in a study by Ali et al. (2016) which showed that Xgwm247 is responsible for absorption of potassium and gene HKT1 is exclude sodium in wheat under salt stress. In figure 2 genetic dendrogram tree for what genotypes under the study using unweighted Pair-group with UPGMA analysis showed that there are two main groups at similarities (0.69), the first main group divided into four sub-groups where IC1 located in the first sub-group and in the second sub-group divided into sub-sub-group at similarity (0.80) where sub-sub group 1 included genotypes (Sakha 93, YocaraRojo, IC16, P7, P8, IC17, Bhan2000, Pasban96, and Auqab2000) and the second sub-sub group included genotypes (Inq91, P6, Sis 27, and Sis 3), meanwhile the third sub-group included IC96 and the fourth sub-group include the least salt tolerance genotypes namely (local, P9, P2, and IC15) finally the second main group included only genotype Sis 13. We noticed that wheat genotypes that showed most tolerance to salt namely (Sakha93, Bhan2000, and Auqab 2000) were in one group based on genetic markers for absorption of potassium and rejection of sodium. Similar results in study by Bahrami (2009) when they use primer TaGSK1, they found that wheat variety ''Bam'' which is salt tolerance has more gene expressing for TaGSK1 comparing to nine other varieties. In contrast variety ER- Salt-85-17 which is the least salt tolerance showed the least expression of the gene, the other nine verities showed a different genetic expression for TaGSK1. It was between 39% for sat sensitive ER-Salt and 61% for most tolerance variety. Ali et al. (2016) and Zuang et al. (2008) studied the transporter of potassium and sodium in salt tolerance rice and wheat, and they pointed that Nax1 and Nax2 are the controller of sodium in vegetative stage. They also showed that most SSR genetic markers that related to salt tolerance are located in chromosome (1A, 1D, 2B,2D,3D,4D,6A,7B).

CONCLUSION

In this study, stress susceptibility index (SSI) was an efficient screening tool for identification of salinity-tolerance genotypes. We identified three genotypes (Sakha93, Bhan2000, and Auqab 2000) as the most salinity-tolerant genotypes with the lowest SSI in both years. Moreover, these genotypes were in one group based on SSR markers. Therefore, these genotypes can be recommended as promising genotypes for salt-affected area in Saudi Arabia.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

Thanks to the College of Agriculture and Veterinary Medicine for allowing us to use their available facilities.

AUTHOR CONTRIBUTIONS

SMA designed and performleed the experiments and also wrote the manuscript.

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