EVALUATION OF SALINITY TOLERANCE IN SOME BREAD WHEAT RECOMBINANT INBRED LINES USING MICROSATELLITES MARKERS

AYA-TU-ALLAH E. A. MANCY[1*], F.M. ABDEL-TAWAB[1], A. H. ABODOMA[1], MAHMOUD MAGDY[1], THARWAT M. ELAMEEN[2]

1 Department of Genetics, Faculty of Agriculture, Ain Shams University, Shoubra El- Kheimah, 11241, Egypt

2 Department of Agricultural Botany, Faculty of Agriculture, South Valley University, Qena, 83523, Egypt

** Corresponding Author email :ayaeezzat38@agr.asu.edu.eg*

Wheat (*Triticum aestivum* L.) is the most essential cereal crop cultivated around the world and is a member of the Poaceae (*Gramineae*) grass family (Mahmud *et al.*, 2018). About 95% of wheat grown today is hexaploid and used for the preparation of bread and other baked products. It has total production of 9 million tons in Egypt (FAO, 2021); and 775.83 million tons in the world, and its global production is predicted to reach 780.28 million tons by 2022 (USDA, 2021). In Egypt, there is a significant gap between wheat agricultural output and its consumption. However, Egypt is the world's largest wheat importer; with wheat imports for the 2019/2020 marketing year were estimated at 12.5 million tons, increasing about 15% above the average of the last five years. As a result, it is critical to improve this crop in order to tackle this issue (El-Rawy, 2020). The problem of salinization in the Nile Delta is one of the obstacles to narrow the gap between wheat production and consumption in Egypt (Elshafei *et al.*, 2019). Salt stress is the primary cause of hormonal instability, nutrient uptake fluctuations, and oxidizing agent over-production in wheat varieties (Ilyas *et al.*, 2020). Enhancing the salinity-tolerant ability of crops is one of the most efficient and sustainable approaches to reduce the negative impacts of salinity on agricultural productivity (Tao *et al*., 2021). The presence of genetic cultivars in wheat is essential for determining the contrasting parents for traditional breeding (Budak *et al.*, 2015). The single seed descent technique (SSD) in combination with *in vitro* growth of embryos dissected from immature seeds can be used to decrease the breeding cycle. Starting with F_2, the SSD technique entails selecting one seed at random from each individual plant in each generation. All seeds from individual plants are gathered in F_6 and later generations, and the progeny of a single plant is treated as an SSD line (Watson *et al.*, 2018). Wheat genotypes, characteristics, and genes related to abiot-

Egypt. J. Genet. Cytol.,51: 147-163, July, 2022
Web Site (*www.esg.net.eg*)

ic stress adaptability will enable breeders to adapt wheat to various environmental circumstances. As a result, breeding can be used along with stable molecular markers to improve the efficiency of selection for features that are difficult and expensive to characterize (Mohamed and El-Ameen, 2019). Due to high efficiency, reproducibility, easy-to-use, co- dominance and multi-allelic nature, high degree of polymorphism, relative abundance and good genome coverage, microsatellite markers are widely- used as molecular markers for fingerprinting germplasm to assess genetic diversity, pedigree analysis, evolutionary studies and genome mapping (Mohammadi-Nejad *et al.,* 2008). The over-abundance of the amino acid proline (Pro), which acts as a compatible solute, an osmo-protectant, and plays a vital role in cytosolic enzymes and cellular organelles protection in a wide range of higher plant species, is well known as one of the most common responses to water deficit and saline environments. Furthermore, Pro is a nitrogen supply that can help with stress recovery and growth restoration. Salt-induced Pro buildup is generally a late reaction, manifesting only after cell damage has occurred, and increased levels of Pro persist even after stressed tissues have returned to normal osmotic conditions (Jiménez- Bremont *et al.,* 2006). The objectives of the present study were to 1) evaluate and screen wheat recombinant inbred lines (RILs) -used in the study- for salinity tolerance; 2) understand the response generated at morphological and molecular levels following salt stress treatment; 3) validate microsatellite markers for salt tolerance by marker-trait association analysis; and 4) select promising lines for future wheat breeding experiments. The information generated from the study can be utilized for wheat improvement in the future.

MATERIALS AND METHODS

Plant materials and experimental setup

Twelve F7 RILs derived from a cross between two bread wheat cultivars, namely, Shandaweel-1 (moderate salt-tolerant cultivar shown as P_1) and Giza-168 (a local salt-sensitive cultivar shown as P_2) (El-Moneim *et al.,* 2020), *via* single seed descent (SSD) method and their parents, were used in this study (Table 1). The SSD method of RILs' production used in this study was described by Mohamed and El-Ameen, (2019). First, seeds were sterilized by 1% hypochlorite for 15 min, and then washed by distilled water. Then, for testing viability, germination trials were carried out in sterilized Petri dishes containing a sheet of soaked paper and moistened with distilled water. Each petri dish contained 15 seeds. After five days, the uniformly germinated seeds were transferred to plastic pots (20 cm in diameter) containing a mixture of equal quantities of peatmoss and sand that was previously sterilized by HCL (0.1 N) and washed several times with distilled water. The experiment was conducted in a growth chamber with a 16 h light/8 h dark photoperiod, 25ºC temperature, 70% humidity and a photon flux density of

300000 lux. After 25 days from sowing, the pots were divided into two groups for each genotype. The first group with three replicates (six seedlings per replicate) was irrigated with tap water and served as the control pots, and the second group with also three replicates (six seedlings per replicate) was irrigated with salt water (102 mM sodium chloride, which is equivalent to 6000 ppm) and served as the treated pots and all pots were arranged in a randomized block design (RBD). After the plants had been kept at 102 mM NaCl for 30 days, shoots and roots were separately collected as bulked samples for each genotype within treatment. Leaves were ground to a fine powder in liquid nitrogen using a mortar and pestle and stored at -80ºC for further molecular genetics and biochemical analyses.

Calculation of the salt injury index (SII)

After the plants had been kept at 102 mM NaCl for 30 days, a salt injury index (SII) was calculated according to Zhu *et al.* (2008). The parental lines and their RILs were classified for their salt tolerance by visual appearance; classification of the standard and calculation of the salt injury index followed the method of Zhang *et al.* (2003).

Phenotypic traits measurements

To assess the salt tolerance of the 12 RILs and their parents, three phenotypic traits were measured, these traits were plant height (cm), number of tillers/plant, number of leaves/plant and used as a phenotypic parameters for the effects of salt stress.

Salt tolerance trait index (STTI)

Salt tolerance trait index (STTI) was calculated following Ali *et al.* (2007), salt tolerance index (STI) was calculated as the mean of STTIs for plant height, number of tillers/plant and number of leaves/plant.

Phenotypic traits statistical analyses

Two statistical analyses were used to analyze the data of the three studied phenotypic traits under the control and salt stress conditions to determine the most tolerant and most sensitive lines using classical analysis and stress-percentages analysis. Classical analysis depends on analysis of variance (ANOVA) and multiple comparisons using SPSS statistical software. Analysis of variance and multiple comparisons using Dunnett's test were done three times, one for each phenotypic trait measured.

Biochemical analysis estimation of proline concentration

Proline concentration was estimated using a ninhydrin colorimetric method of Troll and Lindsley (1955) modified by Ouwendijk *et al*. (1996).

Molecular genetic analysis

The total genomic DNA was isolated from leaves using a method described by Infante (2002), with some mod-

ifications. As shown in Table (2), a total of 12 wheat microsatellite markers were chosen for salt stress tolerance screening. Depending on their close correlation to genome polymorphism, and according to information available in the GrainGenes database, SSRs markers for salt tolerance in wheat were selected. To perform polymerase chain reaction (PCR), 10 µl master mix (i-Taq ™), 1µl DNA, 0.5 µl forward primer, 0.5 µl reverse primer and 8 µl H_2O for 20 µl total volume were used. The PCR conditions for all primers were the same except for annealing temperature as the following: initial denaturation at 94ºC for 5 minutes only one time, denaturation step at 94ºC for 1 minute, annealing temperature was variable with primer, extension step at 72ºC for 1 minute, 35 cycles, finally the final extension was at 72ºC for 10 minutes using thermal cycler machine (TurboCycler TCST-9622). The PCR products were separated using 1.5 % agarose in TAE buffer at 100 V for 30 minutes, stained with ethidium bromide, visualized by UV, and photographed using Bio-Rad gel documentation system (gel doc 2000). The gel images were analyzed using the Total lab (TL) 120 software to determine the molecular sizes of the amplified fragments. The generated bands were scored as binary system present (1) or absent (0).

RESULTS AND DISCUSSION

3.1 Salt injury index (SII) values

Salt injury index was calculated for the 12 RILs and their parents, according to Zhu *et al.* (2008) and recorded in Table (3). Comparing with the mean value of SII values (28.11%), there was a remarkable difference in the salt injury index between the two parents due to NaCl treatment, where SII value of Shandaweel-1 (salt-tolerant parent P_1) was lower than that of Giza-168 (salt-sensitive parent P_2) , indicating that the salt tolerance of P_1 was higher than that of P_2. Moreover, SII values of the highest RILs group varied from a range of 19.28% for RIL1 to 14.26% for RIL10. However, SII values of the lowest RILs group varied from a range of 38.60% for RIL4 to 32.35% for RIL6.

3.2. Phenotypic trait measurements

Plant growth was determined by plant height, number of tillers/plant and number of leaves/plant. Considerable variations were observed between the two parents regard to the three investigated phenotypic traits. Moreover, the results of phenotypic response of wheat RILs to salinity stress indicated the varied genotypic responses. Therefore, the RILs were classified into two groups, each represented of six lines, where (RIL1, RIL5, RIL9, RIL10, RIL11 and RIL12) showed high performance regarding the three measured phenotypic traits and they represented the higher group, the other six lines (RIL2, RIL3, RIL4, RIL6, RIL7 and RIL8) represented the lower group regarding their phenotypic performance under salt stress. The mean values of phenotypic data collected from the two parents (Giza-168 and Shandaweel-1) and their RILs under control and treatment are presented in Table (4).

3.3 Salt tolerance trait index (STTI)

Salt tolerance trait index (STTI) was calculated following Ali *et al.* (2007). Salt tolerance index (STI) was calculated as the mean of STTIs to be 89.20, 82.16 and 76.45% for plant height, number of tillers/plant and number of leaves/plant, respectively, and the values were recorded in Table (5).

As shown in Table (5) STTI of values of Shandaweel-1 were 90.64, 93.02 and 92.85%, at 102 mM NaCl stress, which were markedly higher than that of Giza- 168 as 75, 62.50 and 67.85 % for plant height, number of tillers/plant and number of leaves/plant, respectively. These remarkable differences indicate that the salt tolerance of P_1 was higher than that of P_2. Difference between the range of STTI for the studied RILs was divided into two equal groups, namely, salt tolerant (STTI $\geq$ 89.20, 82.165 and 76.45%) for plant height, number of tillers/plant and number of leaves/plant, respectively, and that group represented the highest group of RILs which included (RIL1, RIL5, RIL9, RIL10, RIL11and RIL12) and its values ranged from 94.73 to 98% for plant height, 93.02 to 100% for number of tillers/plant and 93.33 to 100% for number of leaves/plant. The second group of RILs could be considered as salt sensitive (STTI $\leq$ 89.20, 82.165 and 76.45%) for plant height, number of tillers/plant and number of leaves/plant, respectively, and that group represented the lowest group of RILs which included (RIL2, RIL3, RIL4, RIL6, RIL7 and RIL8) and its values ranged from 77.46 to 89.02% for plant height, 67.44 to.75.0% for number of tillers/plant and 56.25 to 66.66% for number of leaves/plant. Overall, salt tolerant RILs showed greater STTI values regard to the three investigated phenotypic traits than sensitive RILs.

Two statistical analyses were used to analyze the data of the three studied phenotypic traits under the control and salt stress conditions to determine the most tolerant and most sensitive lines; classical analysis and stress-percentages analysis. Classical analysis depends on: analysis of variance (ANOVA) and multiple comparisons using SPSS statistical software. Analysis of variance and multiple comparisons using Dunnett's test were done three times, one for each phenotypic trait measured. From the classical method of statistical analysis, the RILs (1, 5, 9, 10, 11 and 12) were the most tolerant for salt stress (6000 ppm), whereas, RILs (2, 3, 4, 6, 7 and 8) were the most sensitive. Therefore, it is suggested to consider the stress- percentages analysis as an alternative way to classical statistical analysis for abiotic stress experiments because it is easier, quicker, more informative and considers all traits and gives the same results (Rashed *et al.*, 2006).

3.4. Phenotypic traits statistical analyses

Two statistical analyses were used to analyze the data of the three studied phenotypic traits under the control and salt stress conditions to determine the most

tolerant and most sensitive lines; classical analysis and stress-percentages analysis. Classical analysis depends on: analysis of variance (ANOVA) and multiple comparisons using SPSS statistical software. Analysis of variance and multiple comparisons using Dunnett's test were done three times, one for each phenotypic trait measured. From the classical method of statistical analysis, the RILs (1, 5, 9, 10, 11 and 12) were the most tolerant for salt stress (6000 ppm), whereas, RILs (2, 3, 4, 6, 7 and 8) were the most sensitive. Therefore, it is suggested to consider the stress- percentages analysis as an alternative way to classical statistical analysis for abiotic stress experiments because it is easier, quicker, more informative and considers all traits and gives the same results (Rashed *et al.,* 2006).

3.5. Proline content under salinity stress

A large variation could be observed for proline accumulation among the 12 wheat RILs and between their parents as shown in Fig. (1). Salt stress resulted in proline accumulation in both parents, but much higher in Shandaweel-1. As observed in Table (6), values of proline accumulation under salt stress varied from 938.4567 µg.g-I.FW for Shandaweel-1 P_1 to 478.3456 µg.g-I.FW for Giza-168 P_2. The results also showed noticeable differences among the studied RILs. The highest amounts of proline in leaves were observed for the highest RILs group, the minimum increase was 710.5252 µg.g-I.FW for RIL10 and the maximum increase was 1322.085 µg.g-I.FW for RIL12. Nevertheless, the lowest amount of Pro in leaves was observed for the lowest RILs group, and ranged from 232.9502 µg.g-I.FW for RIL6 to 506.2397 µg.g-I.FW for RIL4. According to our data, there must be a relationship between salt tolerance mechanisms and proline accumulation in wheat.

Proline accumulation under different environmental stress has been associated with stress tolerance in many plant species, and its concentration has indeed been found to be significantly higher in stress-tolerant plants than in stress- susceptible plants (Kumar *et al.,* 2003; Misra and Gupta 2005; Tani and Sasakawa 2006 and Ashraf and Foolad, 2007). Ilyas *et al.* (2020) reported that proline content has been investigated as salt stress indicator for screening of wheat lines under salt stress in several studies.

3.6 SSR markers analysis

3.6.1 Evaluation of the parental genotypes

3.6.2 Evaluation of RILs using the differential microsatellite markers

The 12 SSRs primer pairs used in this study were used first to screen the parental genotypes as shown in Fig. (2), and then the polymorphic primers were used to screen the studied RILs. The total number of generated bands was 17 bands with an average of 1.4 bands per primer pair; all primers generated the lowest number of bands (one band), except cfd49 primer located on chromosome 6D, wmc432 marker located on chromosome

1D and gwm88 marker located on chromosome 6B generated two bands, whereas gwm213 marker located on chromosomes 5B generated the highest number of bands (three bands). The molecular size (MS) of generated bands ranged from 100 bp which generated by gwm55 primer to 361 bp generated by gwm88 primer. The Data of SSRs including fragment size (bp), number of alleles, number of polymorphic bands, number of monomorphic bands and polymorphism percentage were summarized in Table (7).

These variations in bands number and molecular sizes produced by the tested primers have been resulted from the primers sequences and the number and location of their complementary sequences in the genome of the tested genotypes, respectively. Moreover, this range of number and size of generated bands in the present study are similar to those observed in another study on wheat using SSR markers by Shahzad *et al.* (2012). Out of the 12 SSR markers tested, three markers could generate polymorphic patterns between the two parents and successfully generated unique bands in Shandaweel-1 with a size of 295 bp in wmc432 marker, 361 bp in gwm88 marker and 313 bp in gwm213 marker. These unique bands which were amplified only in the tolerant parent indicated that its amplification is associated with salt tolerance in bread wheat.

Interestingly, when the three primers that differentiated between the parental genotypes namely, wmc432, gwm88 and gwm213, were used further to evaluate the studied RILs, they successfully generated these unique specific bands only in the six highest RILs regarding the three measured phenotypic traits and that accumulated high amounts of proline under salt stress conditions, as shown in Fig. (3). The banding profile and molecular sizes of wheat RILs generated by the three differential primer are present in Fig. (3) and showed that the six highest RILs, as their tolerant parent, amplified a unique band with MS of 295 bp. Whereas, this band was absent in the lowest six RILs and their sensitive parent. Furthermore, following our findings, Ghaedrahmati *et al.* (2018) reported that gwm88 located on chromosome 6B is associated with dry shoot weight under stress conditions. This suggests that, the specific bands generated by wmc432, gwm88 and gwm213 markers in the present study could be used as useful markers for salt tolerance in wheat genotypes. However, further marker validations are still needed using additional wheat genotypes to confirm the usefulness of these primers for marker-assisted selection in wheat breeding programs.

CONCLUSIONS

In the present study, 12 bread wheat RILs were evaluated and screened under salt stress conditions and some of these RILs showed high performance regarding the studied phenotypic traits under salt stress conditions. Shandaweel-1, a salt-tolerant cultivar, and the highest RILs group (RIL1, RIL5, RIL9, RIL10, RIL11 and RIL12) have a superior defense

mechanism against oxidative damage than Giza-168, a salt-sensitive cultivar, and the lowest RILs group (RIL2, RIL3, RIL4, RIL6, RIL7 and RIL8) by maintaining a higher proline levels. Additionally, wmc432, gwm88, and gwm213 markers used in the present study successfully generated unique specific bands which were amplified only in the tolerant parent (Shandaweel-1) and the highest RILs, suggesting that these markers could be considered as useful markers associated with salt tolerance in bread wheat.

SUMMARY

In most countries worldwide, including Egypt, bread wheat is essential among cereals crops. However, soil salinity is a global issue that has a negative impact on plant growth, development, and productivity. Therefore, salt tolerance is an important feature that must be improved in wheat genotypes. Identifying informative and highly differential molecular markers is critical for developing salt-tolerant genotypes that could tolerate excessive salts in the soil. Twelve bread wheat recombinant inbred lines (RILs) derived from a cross between Shandaweel-1 and Giza-168, were evaluated in pots following completely randomized design (CRD) for salinity tolerance. All genotypes were assessed under control (10 mM NaCl) and salt stress (102 mM NaCl). Some phenotypic traits including plant height, number of tillers/plant and number of leaves/plant were measured. The three phenotypic traits were positively correlated with salt tolerant trait index (STTI), and negatively correlated with the salt injury index (SII). Out of 12 microsatellites markers (SSRs) used to evaluate salt tolerance in wheat genotypes, three primers (wmc432, gwm88 and gwm213) revealed genetic polymorphism between parental genotypes and among the studied RILs. Large variations could be observed for proline accumulation among the 12 wheat RILs and between their parents, and the results of estimation of proline content confirmed the results obtained on the morphological and the molecular levels and indicated that there must be a relationship between proline accumulation and salt tolerance mechanisms in wheat. Due to their high performance under salt stress conditions, amplifying a polymorphic band within three primers associated with salt tolerance and accumulating the highest amounts of proline content under salt stress, six RILs out of the 12 studied could be considered as promising materials for improving bread wheat in breeding programs in the future.

ACKNOWLEDGMENT

Great thanks to Prof. Dr. Tharwat M. El-Ameen, South Valley University, Qena, Egypt, for providing RILs' grains. The authors are thankful for the technical support provided by Dr. Shaimaa Ahmed, Lecturer of Genetics, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

REFERENCES

Ali Z., Salam A., Azhar F. M. and Khan I. A. (2007). Genotypic variation in salinity tolerance among spring and

winter wheat (*Triticum aestivum* L.) accessions. South Afr. J. Bot., 73(5): 70-75.

Ashraf M. F. M. R. and Foolad M. R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany, 59(2): 206-216.

Budak H., Hussain B., Khan Z., Ozturk N. Z. and Ullah N., (2015). From genetics to functional genomics: Improvement in drought signaling and tolerance in wheat. Frontiers in Plant Science, 6(11): 1-13.

El-Moneim D. A., Alqahtani M. M., Abdein M. A. and Germoush M. O., (2020). Drought and salinity stress response in wheat: physiological and TaNAC gene expression analysis in contrasting Egyptian wheat genotypes. Journal of Plant Biotechnology, 47(1): 1-14.

El-Rawy M., (2020). Assessment of genetic diversity for some Egyptian wheat varieties based on morphological characters and SSR markers. Scientific Journal of Agricultural Sciences, 7(9): 86-98.

Elshafei A. A., Afiah S. A. E. A., Al-Doss A. A. and Ibrahim E. I., (2019). Morphological variability and genetic diversity of wheat genotypes grown on saline soil and identification of new promising molecular markers associated with salinity tolerance. Journal of Plant Interactions, 14(1): 564-571.

FAO - Food and Agriculticure Organization (2021) FAO GIEWS Country Brief on Egypt. https://www.fao.org/giews/countrybrief/country.jsp?code=EGY.

Ghaedrahmati M., Mardi M., Naghavi M. R., Majidi Haravan E., Nakhoda B., Azadi A. and Kazemi M., (2018). Mapping QTLs associated with salt tolerance related traits in seedling stage of wheat (*Triticum aestivum* L.). J. Agr. Sci. Tech., 16(5): 1413-1428.

Ilyas, N., Amjid M. W., Saleem M. A., Khan W., Wattoo F. M., Rana R. M., Maqsood H. R., Zahid A., Shah G. A., Anwar A., Ahmad M. Q., Shaheen M., Riaz H. and Ansari M. J., (2020). Quantitative trait loci (QTL) mapping for physiological and biochemical attributes in a Pasban90/Frontana recombinant inbred lines (RILs) population of wheat (*Triticum aestivum* L.) under salt stress condition. Saudi Journal of Biological Sciences, 27(1): 341-351.

Infante D., (2002). A rapid and simple method for small-scale DNA extraction in Agavaceae and other tropical plants. Plant Molecular Biology Reporter, 20(4): 299-301.

Jiménez-Bremont J. F., Becerra-Flora A.,

Hernández-Lucero E., Rodríguez-Kessler M., Acosta-Gallegos J. A. and Ramírez- Pimentel J. G., (2006). Proline accumulation in two bean cultivars under salt stress and the effect of polyamines and ornithine. Biologia Plantarum, 50(4): 763-766.

Kumar S. G., Reddy A. M. and Sudhakar C., (2003). NaCl effects on proline metabolism in two high yielding genotypes of mulberry (*Morus alba* L.) with contrasting salt tolerance. Plant Science, 165(6): 1245- 1251.

Mahmud R., Kabir M. R., Hoque M. E. and Yousuf Akhond M. A., (2018). Assessment of some genetic attributes in wheat (*Triticum aestivum* L.) using gene-specific molecular markers. Agriculture and Natural Resources, 52(1): 39-44.

Misra N. and Gupta A. K., (2005). Effect of salt stress on proline metabolism in two high yielding genotypes of green gram. Plant science, 169(2): 331-339.

Mohamed E. A. and El-Ameen T. M., (2019). SSR marker for grain yield under heat stress coditions in bread wheat. J. Genet. Cytol., 48(5): 205-116.

Mohammadi-Nejad G., Arzani A., Rezai A. M., Singh R. K. and Gregorio G. B. (2008). Assessment of rice genotypes for salt tolerance using microsatellite markers associated with the saltol QTL. African Journal of Biotechnology, 7(6): 730-736.

Ouwendijk J., Moolenaar C. E., Peters W. J., Hollenberg C. P., GinselL. A., Fransen J. A. and Naim H. Y., (1996). Congenital sucrase- isomaltase deficiency. Identification of a glutamine to proline substitution that leads to a transport block of sucrase-isomaltase in a pre-Golgi compartment. The Journal of clinical investigation, 97(3): 633-641.

Rashed M. A., Sallam M. A. and Ahmed N. E., (2006). Molecular markers for iron deficiency stress. J. Biol. Chem. Environ. Sci., 1 (2): 147-158.

Shahzad A., Ahmad M., Iqbal M., Ahmed I. and Ali G. M., (2012). Evaluation of wheat landrace genotypes for salinity tolerance at vegetative stage by using morphological and molecular markers. Genetics and Molecular Research: GMR, 11(1): 679-692.

Tani C. and Sasakawa H., (2006). Proline accumulates in Casuarina equisetifolia seedlings under salt stress. Soil science and plant nutrition, 52(1): 21-25.

Tao R., Ding J., Li C., Zhu X., Guo W., screening of agro-physiological indices for salinity stress tolerance in wheat at the seedling stage. and M. Zhu (2021). Evaluating and Frontiers in Plant Science, 12(3): 1-12.

Troll W. and Lindsley J., (1955). A photometric method for the determination of proline. Journal of Biological Chemistry, 215(2): 655- 660.

USDA - United States Department of Agriculture (2021), forecasts records for world wheat in 2021-2022. https://www.ers.usda.gov/webdocs/outlooks/104470/whs-22h.pdf?v=8189.

Watson A., Ghosh S., Williams M. J., Cuddy W. S., Simmonds J., Rey M., Smith D. and Hickey L. T., (2018). Speed breeding is a powerful tool to accelerate crop research and breeding. Nature plants, 4(1): 23- 29.

Zhang Y.Q., Liu S.Q., Yang F. J. and Li D., F. (2003). Study on screening of salt-tolerant watermelon stock and mechanism of salt-tolerance. Acta Agric. Boreali-occidentalis Sin, 23(12): 105-108.

Zhu J., Bie Z. and Li Y., (2008). Physiological and growth responses of two different salt-sensitive cucumber cultivars to NaCl stress. Soil Science and Plant Nutrition, 54(9): 400-407.

Table (1): List of wheat (*Triticum aestivum* L.) parental genotypes and their RILs used in the present study.

No.	Name*	Code
1	Shandaweel-1 (tolerant parent)	P_1
2	Giza-168 (sensitive parent)	P_2
3	153	RIL 1
4	1600	RIL 2
5	160	RIL 3
6	116	RIL 4
7	189	RIL 5
8	137	RIL 6
9	148	RIL 7
10	190	RIL 8
11	169	RIL 9
12	151	RIL 10
13	147	RIL 11
14	181	RIL 12

* Naming of RILs according to the source

Table (2): List of SSRs primers, chromosomal location, locus, primer sequence pairs and TM temperature.

No.	Chromosomal Location	Locus	Primer sequence 5′ → 3′	TM
1	3D	cfd 9	F: TTGCACGCACCTAAACTCTG R: CAAGTGTGAGCGTCGG	60
2	5D	cfd 18	F: CATCCAACAGCACCAAGAGA R: GCTACTACTATTTCATTGCGACCA	60
3	7D	cfd 46	F: TGGTGGTATAGTCGTTGGAGC R: CCACACACACACACCATCAA	60
4	6D	cfd 49	F: TGAGTTCTTCTGGTGAGGCA R: GAATCGGTTCACAAGGGAAA	60
5	5D	cfd 183	F: ACTTGCACTTGCTATACTTACGAA R: GTGTGTCGGTGTGTGGAAAG	60
6	2D	wmc 18	F: CTGGGGCTTGGATCACGTCATT R: AGCCATGGACATGGTGTCCTTC	61
7	1D	wmc 432	F: ATGACACCAGATCTAGCAC R: AATATTGGCATGATTACACA	51
8	2D	wmc 503	F: GCAATAGTTCCCGCAAGAAAAG R: ATCAACTACCTCCAGATCCCGT	61
9	6B	gwm 626	F: GATCTAAAATGTTATTTTCTCTC R: TGACTATCAGCTAAACGTGT	50
10	6B	gwm 88	F: CACTACAACTATGCGCTCGC R: TCCATTGGCTTCTCTCTCAA	60
11	2B	gwm 55	F: GCATCTGGTACACTAGCTGCC R: TCATGGATGCATCACATCCT	60
12	5B	gwm 213	F: TGCCTGGCTCGTTCTATCTC R: CTAGCTTAGCACTGTCGCCC	60

Table (3): Salt injury index (SII) values of the studied RILs and their parents under salt stress (102 mM NaCl).

No.	Genotype	SII%
1	P_1	22.25%
2	P_2	34.56%
3	RIL 1	19.28%
4	RIL 2	35.67%
5	RIL 3	34.45%
6	RIL 4	38.60%
7	RIL 5	17.60%
8	RIL 6	32.35%
9	RIL 7	37.32%
10	RIL 8	34.25%
11	RIL 9	16.78%
12	RIL 10	1426%
13	RIL 11	1554%
14	RIL12	1734%
Mean		2811%

Table (4): Mean values of phenotypic trait values for parents and the 12 RILs measured under the control (C) and salinity treatment (T).

Genotype	Traits					
	Plant height (cm)		No. of tillers/ plant		No. of leaves/plant	
	C	T	C	T	C	T
P1	101.6	92.10	4.3	4.0	28	26
P2	95.60	71.70	4.0	2.5	28	19
RIL 1	94.30	91.30	4.0	4.0	16	15
RIL 2	90.50	78.70	4.0	3.0	16	9
RIL 3	96.40	82.80	4.0	2.8	15	10
RIL 4	91.06	72.30	4.0	3.0	14	8
RIL 5	93.06	91.20	4.0	4.0	16	16
RIL 6	94.50	73.20	4.0	2.8	15	10
RIL 7	95.60	85.10	4.0	2.7	15	9
RIL 8	95.90	84.20	4.3	2.9	16	10
RIL 9	95.60	92.40	4.3	4.0	16	16
RIL 10	95.40	92.50	4.3	4.3	16	15
RIL 11	98.80	93.60	5.0	4.8	15	15
RIL 12	97.10	93.30	4.6	4.5	15	14

Table (5): Salt tolerance trait index (STTI) values of the studied RILs and their parents under salt stress (102mM NaCl).

		STTI		
No.	Genotype Code	Plant Height	No. of tillers	No. of leaves
1	P_1	90.64	93.02	92.85
2	P_2	75.00	62.50	67.85
3	RIL 1	96.82	100.0	93.75
4	RIL 2	86.96	75.00	56.25
5	RIL 3	85.89	70.00	66.66
6	RIL 4	79.48	75.00	57.14
7	RIL 5	98.00	100.0	100.0
8	RIL 6	77.46	70.00	66.66
9	RIL 7	89.02	67.50	60.00
10	RIL 8	87.79	67.44	62.50
11	RIL 9	96.65	93.02	100.0
12	RIL 10	96.96	100.0	93.75
13	RIL 11	94.73	96.00	100.0
14	RIL12	96.08	97.82	93.33
	Mean	89.20	82.16	76.45

Table (6): Values of proline content of the studied RILs and their parents under the control and salt stress treatment.

Genotype	Proline Content (µg/g FW)	
	Trait	
	Control	Treatment
P1	261.19	938.45
P2	330.81	478.34
RIL 1	302.07	857.58
RIL 2	428.68	362.67
RIL 3	207.08	298.00
RIL 4	265.46	506.23
RIL 5	202.40	876.76
RIL 6	218.14	232.95
RIL 7	202.70	310.48
RIL 8	197.83	435.03
RIL 9	92.802	768.93
RIL 10	117.67	710.52
RIL 11	111.52	728.92
RIL 12	234.48	1322.0

Table (7): Data of SSRs including fragment size (bp), no. of alleles, no. of polymorphic bands, no. of monomorphic bands and polymorphism percentage.

S.N.	Chromosomal Location	Primer name	Fragment size (bp)	No. of alleles	No. of polymorphic bands	No. of monomorphic bands	Polymorphism %
1	3D	cfd 9	200	1	0	1	0
2	5D	cfd 18	212	1	0	1	0
3	7D	cfd 46	200	1	0	1	0
4	6D	cfd 49	220,162	2	0	2	0
5	5D	cfd 183	198	1	0	1	0
6	2D	wmc 18	237	1	0	1	0
7	1D	wmc 432	200,295	2	1	1	50
8	2D	wmc 503	298	1	0	1	0
9	6B	gwm 626	130	1	0	1	0
10	6B	gwm 88	172, 361	2	1	1	50
11	2B	gwm 55	100	1	0	1	0
12	5B	gwm 213	134,183, 313	3	1	2	33.33
Total				17	3	14	

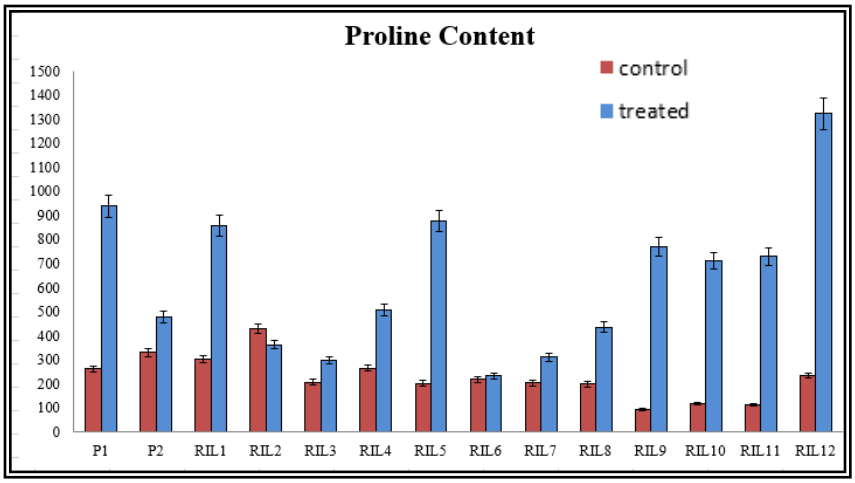

Fig. (1): Large variations in proline accumulation among the 12 wheat RILs and between their parents under the control and salt stress (102mM NaCl) conditions.

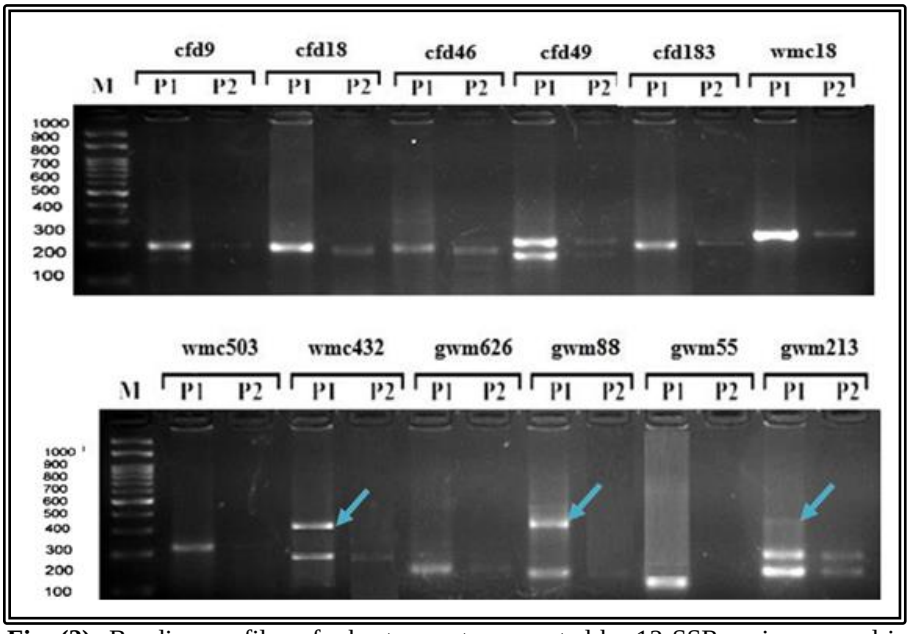

Fig. (2): Banding profiles of wheat parents generated by 12 SSRs primers used in the present study, where P1: Shandaweel-1 and P2: Giza-168. The arrows refer to the different "polymorphic" bands.

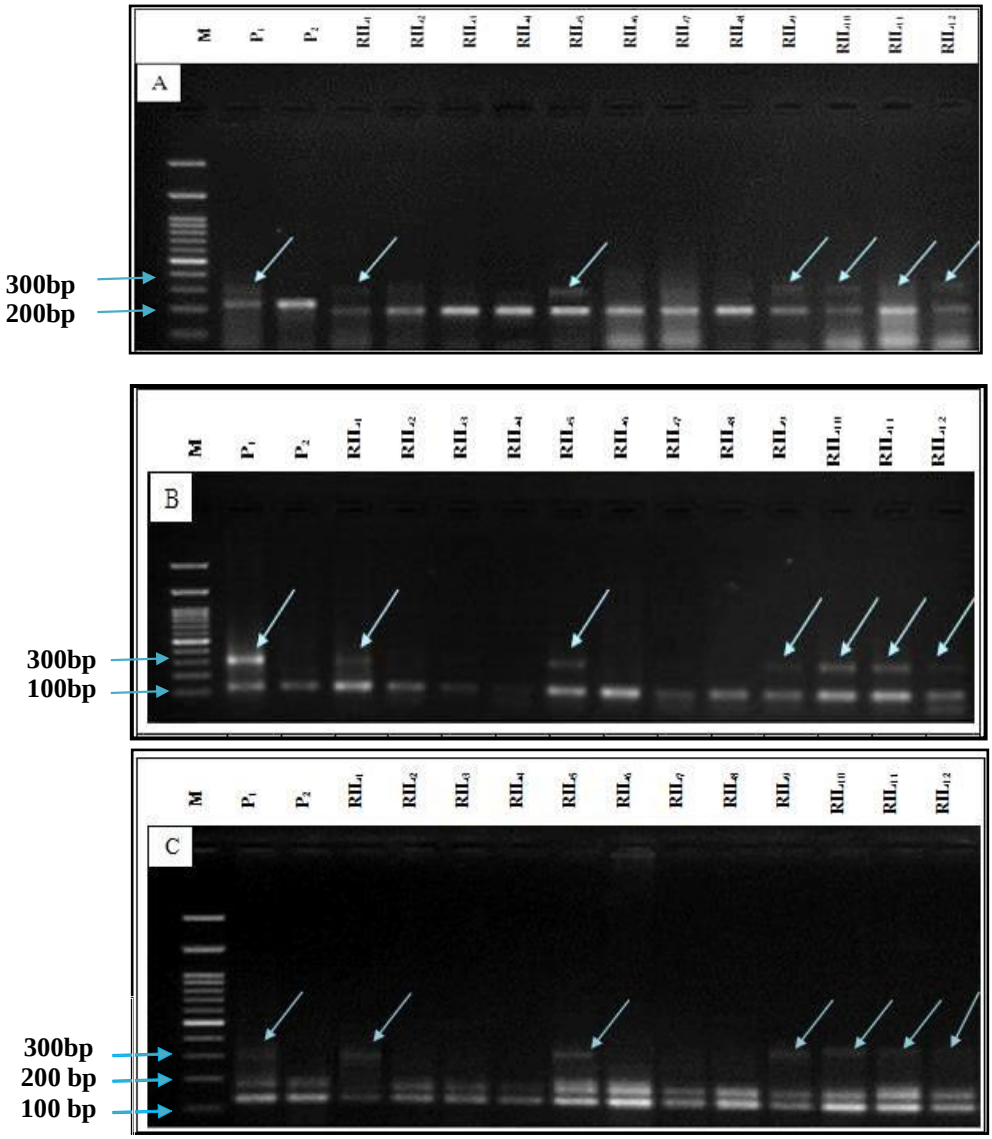

Fig. (3): Banding profile and molecular sizes of wheat RILs and their parents generated by the three differential SSRs primers, where A) wmc432 primer, B) gwm88 primer and C) gwm213 primer. The arrows refer to the different "polymorphic" band.