UDC 575.630
https://doi.org/10.2298/GENSR2002751N
*Original scientific paper*

# GENETIC AND PHYSIOLOGICAL ANALYSIS OF COTTON CULTIVARS UNDER SALT STRESS

Zahra NOORMOHAMMADI[1*], Elham TORABIZADEH[1], Farah FARAHANI[2], Omran ALISHAH[3], Masoud SHEIDAI[4]

[1]Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Department of Microbiology, Qom Branch, Islamic Azad University, Qom, Iran
[3] Cotton Research Institute of Iran, Agricultural Research, Education and Extension Organization (ARREO), Gorgan, Iran
[4] Faculty of life Sciences and Bioechnology, Shahid Beheshti University, Tehran, Iran

Noormohammadi Z., E. Torabizadeh, F. Farahani, O. Alishah, M. Sheidai (2020). *Genetic and physiological analysis of cotton cultivars under salt stress*.-Genetika, Vol 52, No.2, 751-764.

The salinity caused changes in plant metabolic processes. Cotton is one of the important fiber products which may be affected by salinity stress in Iran. In the present study, the effects of salinity stress and salicylic acid (SA) as improvement of abiotic stress were examined on biochemical traits and retrotransposon based markers profile (IRAP and REMAP) in cotton cultivars. Cotton seeds of two cultivars (GT40 and NNC) were cultivated in pots and different treatments; control plants with normal irrigation, 0.5 and 1mM SA, 100 and 150mM NaCl treatments and, combination of 0.5mM SA and 150mM NaCl were performed. Physiological, morphological, cellular and, genetic diversity parameters were measured after treating and following seven days of irrigation. Activity of antioxidant enzymes; catalase, peroxidase and proline in 150mM NaCl treated plants were more than other treatments. In comparison to two cultivars, GT40 cultivar had a higher activity of antioxidant enzymes. Based on retrotransposon based markers analyses, the highest heterozygosity was shown in 0.5mM SA treatment of GT40 cultivar as well as 0.5 mM SA, 150mM NaCl treatments in NNC cultivar. Totally, GT40 showed the highest genetic variation in retrotransposon profiles. The Salt stress and salicylic acid treatment provided discriminate profiles from retrotransposon movement.

*Keywords:* Cotton, retrotransposon, salinity stress, salicylic acid.

Corresponding authors*:* Zahra Noormohammadi, Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran, Tel/Fax Number: +98 44865939**;** E-mail address: marjannm@yahoo.com z-nouri@srbiau.ac.ir

## INTRODUCTION

Environmental stresses such as salinity directly impact crop growth, which in turn can affect the world food supply and societal prosperity. It is estimated that over 800 million hectares of land throughout the world are salt-affected. In arid and semi-arid regions, salt concentration can be close to that in the seawater. Hence, there are intensive efforts to improve plant tolerance to salinity and other environmental stressors (MIMOUNI *et al.,* 2016).

Salt-tolerant crops are desirable for areas facing soil salinity. Therefore, screening plant crop germplasm for identifying salt tolerant cultivars and performing breeding strategies leading to production of salt tolerant accessions are of importance to human (SHEIDAI *et al.,* 2018). The genetic potential of plants for their adaptability is one of the solutions against salinity. Genetic variability for salt tolerance has been observed among and within plant species (HOSSEINI *et al.,* 2012; TURAN *et al.,* 2012).

Improving salinity tolerance in crops is a complex process in which various traits are involved like, ion exclusion, osmotic tolerance and tissue tolerance (KAMARAN *et al.,* 2016).

Salicylic acid (SA) has a regulatory role on plant growth, development, flowering and yield of plants under different environmental conditions and control stomatal conductivity (RIVAS-SAN VICENTE and PLASENCIA, 2011).

Salicylic acid (SA) is a signaling molecule known to participate in defense responses against the variety of environmental stresses including salinity. However, the specific knowledge on how SA signaling propagates and promotes salt tolerance in plants remains largely unknown. It has been reported that SA increases cv. Marmande tomato growth by improving photosynthesis, regulation and balance of osmotic potential, induction of compatible osmolyte metabolism, and alleviating membrane damage. Therefore, SA might be considered as a potential growth regulator to improve tomato plant salinity stress resistance, in the current era of global climate change (MIMOUNI *et al.,* 2016). Cotton is a dual purpose crop, used as fiber and oil source. It has been considered as a moderately salt-tolerant or relatively salt tolerant plant species with salinity threshold level 7.7 dS m -1 (HOSSEINI and THENGANE, 2007). The plant growth inhibition still occurs when the plant is exposed to saline stress. Different cotton cultivars vary in their salinity tolerance (DOGAN *et al*., 2012), and recent investigation (SHEIDAI *et al.,* 2018) revealed that these relatively tolerant cottons vary in their genetic and agronomic characteristics.

Various molecular markers have been used to evaluate genetic variations and association studies in different crop plants including cotton (SHEIDAI *et al*., 2007; KANTARTZI *et al.,* 2010; NOORMOHAMMADI *et al.,* 2013, 2016). Retrotransposons (RTs) are the main mobile elements in plant genome which are activated under biotic and abiotic stress conditions. For instance, UV radiation, Jasmonic acid and salicylic acid treatments of barley, elevated OARE-1 retrotransposon expression (KIMURA *et al*., 2001), while heat stress increased the GBRE-1 expression in cotton species *G. hirsutum* and *G. barbadense* (CAO *et al.,* 2015; NOORMOHAMMADI *et al.,* 2018).

The retrotransposon-microsatellite amplified polymorphism (REMAP), and inter-retrotransposon amplified polymorphism (IRAP), are commonly used PCR retrotransposon-based molecular markers to study genetic variations in plant genomes (SANTANA *et al.,* 2012; SZUĆKO and ROGALSK, 2015).

The main aims of the present study were 1- To investigate biochemical, morphological and cellular changes under salinity and salicylic acid treatments in two cotton cultivars, namely NNC (salinity resistant cultivar), and GT40 (salinity sensitive cultivar), and 2-To investigate association between REMAP and IRAP molecular markers and cotton response to salinity stress. Data obtained may be useful in future hybridization and breeding of cotton varieties with abiotic stress resistant.

## MATERIAL AND METHODS

*Plant material and treatments*

Healthy cotton seeds of two tetraploid cultivars, NNC a salinity resistant cultivar and GT40 a salinity sensitive cultivar were sterilized with 1 % sodium hypochlorite for 15 min followed rinsed with distilled water. NNC and GT40 cultivars are the result of intra-species (*G. hirsutum* L.) and inter-species (*G. barbadence* × *G. hirsutum*) hybridization. Both cultivars have been named and introduced by Gorgan Cotton Research Center, Iran. The seeds were sown in plastic pots (volume 500 mL), filled with fin pitte and perlite (31) soil. The pots were maintained in a growth chamber with 25/15 °C and 16/8h photoperiod. Five healthy plants with 4-5 true leaves were selected and irrigated with NaCl and salicylic acid (SA) solution every day for one week. For irrigation 50 mL per day was used for each pot.

The following treatments based on Liu et al. (2014), were used in present investigation 1- 0 mM NaCl + 0 mM SA (control); 2- 0 mM NaCl + 0.5 mM SA (T1); 3- 0 mM NaCl + 1 mM SA (T2); 4- 100 mM NaCl + 0 mM SA (T3), and 5- 150 mM NaCl + 0 mM SA (T4) + 150 mM NaCl + 0.5 mM SA (T5). Treated plants (5-8 leaves of each treatment) were used for further studies.

*Physiological and enzyme measurements*

The leaf epidermal cell size was determined in 5 marginal and 5 central leaf cells. Similarly, the mean leaf size and fresh leaf weight were also measured in plant samples after one and two weeks of irrigation.

The catalase and peroxidase enzymes were extracted by using 1 ml extraction buffer (1.2gr Tris, 2gr ascorbic acid, 3.8 borax, 2gr EDTA and 50 gr PEG) for one gram of the homogenized fresh leaves. The homogenate solution was kept at 4°C for 24 hours then centrifuged for 15 min at 15000×g. The supernatant was used for enzyme activity assay (EBERMANN and STICH, 1982).

*Assays of antioxidant enzyme activities*

The samples were prepared as described by MUKHERJEE and CHOUDHURI (1983). A leaf sample (0.5 g) was frozen in liquid nitrogen and grounded by using a porcelain mortar and pestle. The frozen powder was added to 10 ml of 100 mM phosphate buffer (KH2PO4/K2HPO4) pH 7.0, containing 0.1 mM Na2EDTA and 0.1 g of polyvinylpyrrolidone. The homogenate was centrifuged at x 15,000 *g* for 10 min at 4°C., and the resulted supernatant was collected and stored at 4°C for CAT and POD assays (AZOOZ *et al.*, 2012).

The catalase (EC 1. 11. 1. 6) activity was estimated by the decrease of absorbance at 240 nm for 1 min as a consequence of H2O2 consumption (HAVIR and MELLATE, (1987).

The peroxidase (EC 1. 11. 1. 7) activity was determined by the oxidation of guaiacol in the presence of H2O2. The increase in absorbance due to formation of tetraguaiacol was recorded at 470 nm (MAEHLY and CHANCE, 1954). The enzyme activity unit is Δ OD /Min/mg protein.

*Determination of proline content*

The leaf proline content was determined according to BATES *et al*. (1973). The dry weight (0.1 g) of leaves was extracted in 3% sulfosalicylic acid (10 mL) and kept for over-night. The extract was centrifuged at 3000g for 10 min. The supernatant was mixed equal volume of fresh acid ninhydrin solution and glacial acetic acid for each reaction and kept for 1 h at 100 °C. The mixture was extracted with 4 mL toluene after terminating reaction in an ice bath. The extract was stirred for 20 s vigorously. The chromophore-containing toluene was separated from the aqueous phase. The absorbance was measured at 520 nm. The standard cure was used to calculate proline content.

*IRAP and REMAP analyses*

Three to five fresh leaves of each treatment after first and second week of treatment were collected and genomic DNA was extracted based on KRIZMAN *et al*. (2006). The quantity and quality of DNA extraction were checked by Nano photo spectrometer and running on 0.8% agaros gel electrophoresis following GelRed™ Nucleic Acid Gel Staining.

The six single outward-facing LTR primers Nikita, LTR6149, LTR6150,5´LTR1, 5´LTR2 and 3´LTR (TEO *et al.*, 2005) were used for IRAP analysis (Table 1). We also used 36 different combinations (hetero-primer) of outward-facing LTR pair primers.

*Table 1. Cotton LTR and SSR primers and their sequences based on Teo et al. [29] and cotton BLN source available at http//www.cottonmarker.org/project/cm*

| primer name | Sequence Oligo |
|---|---|
| Nikita | 5´-CGCATTTGTTCAAGCCTAAACC |
| LTR6149 | 5´-CTCGCTCGCCCACTACTACAACCGCGTTTATT- |
| LTR6150 | 5´-CTGGTTCGGCCCATGTCTATGTATCCACACATGTA |
| 5´LTR1 | 5´-TTGCCTCTAGGGCATATTTCCAACA |
| 5´LTR2 | 5´-ATCATTCCCTCTAGGGCATAATTC |
| 3´LTR | 5´-TGTTTCCCATGCGACGTTCCCCAACA |
| SSR-BNL-0598F | 5´-TATCTCCTTCACGATTCCATCAT |
| SSR-BNL-0598R | 5´-AAAAGAAAACAGGGTCAAAAGAA |
| SSR-BNL-1162F | 5´-GCGCAAGCGTAGGAGTTTAC |
| SSR-BNL-1162R | 5´-TTGAACGATGAAAGGGAAGG |

For REMAP analysis, 10 different combinations of six IRAP primers and four cotton SSR primers (obtained from BNL sources http//www.cottonmarker.org/project/cm, Table 1) were used. The hetero-primer combinations were as following 3´LTR-BNL0598F, 5´LTR2-BNL0598F, Nikita- BNL0598F, 3´LTR-BNL0598R, Nikita- BNL1162F, 3´LTR- BNL1162F, 5´LTR2-BNL1162F, 3´LTR-BNL1162R, 5´LTR2-BNL1162R and Nikita-BNL1162R.

IRAP-PCR and REMAP were performed in a total volume of 20 µl, containing 50 ng template DNA,1X PCR buffer (10 mM Tris-HCl buffer, pH 8, 50 mM KCl), 3.0 mM $Mg^{2+}$, 200 µM dNTP mix, 0.6 µM of each primer (Bioron, Germany), and 1 U Smart *Taq* polymerase (Cinnagen, Iran).

We used the Bio-Rad thermocycler (Bio-Rad Laboratories Inc., Hercules, CA, USA) for both IRAP and REMAP marker reactions by the similar program as following; an initial denaturation for 3 min at 95°C, followed by 40 cycles in three segments 45 s at 95°C, 45s at 47°C and 90s at 72°C. Final extension was performed at 72°C for 8 min. PCR productions were visualized by 2% agarose gel electrophoresis following GelRed™ Nucleic Acid Gel Staining. Band size was assessed by using a 100 bp molecular size ladder (Vivantis, Malaysia).

*Data analyses*

Morphological and physiological characters as well as protein and enzyme activity assays were studied in two cotton cultivars in all treatments. ANOVA (Analysis of variance), followed by Turkey's test was used to study significant differences among the samples.

IRAP and REMAP profiles obtained were scored as binary characters. Genetic diversity parameters were analyzed in each treatment and cultivar. These parameters were Nei's gene diversity (He), Shannon information index (I), number of effective alleles, and percentage of polymorphism (WEISING *et al.*, 2005; FREELAND *et al.*, 2011). For grouping of the regenerated plant samples, UPGMA clustering was performed after 100 times bootstrapping/ permutations (PODANI, 2000; FREELAND *et al.*, 2011). Significant genetic difference among the studied samples was determined by AMOVA (Analysis of molecular variance) with 1000 permutations by using GenAlex 6.4 (PEAKALL and SMOUSE, 2006).

## RESULTS

*Morphology, enzyme and protein assays in NCC cotton cultivar*

The plants treated with SA after 7 and 14 days, showed significant increase in the leaf length, the leaf width and also the fresh leaf weight. Moreover, the plants treated with 0.5 mM SA, had increased cell size compared to those of the other treated plants (Fig.1).

The plants treated with 0.5 mM SA +150mM NaCl medium, showed significant ($P<0.05$) increase in the leaf size and the fresh leaf weight compared to those in plants treated by only NaCl (Fig.1).

Treated cotton plants with 150 mM NaCl for 7 and 14 days, showed significant increase in the activity of catalase and peroxidase enzymes as well as the proline content. However, cotton plants treated with 0.5 mM SA showed the lowest activity of enzyme content among the studied treatments (Fig. 2).

*Morphology, enzyme and protein assays in GT40 cotton cultivar*

ANOVA revealed significant difference ($P<0.05$) for morphological traits and cell size in all treated GT40 cotton plants. The plants of 0.5 mM SA treatment showed an increase in leaf length, leaf width and cell size compared to the other treated plants (Fig.1). The Salicylic acid besides 150mM NaCl treatment was also protective against salt stress as they increased the leaf and cell sizes (Fig.1).

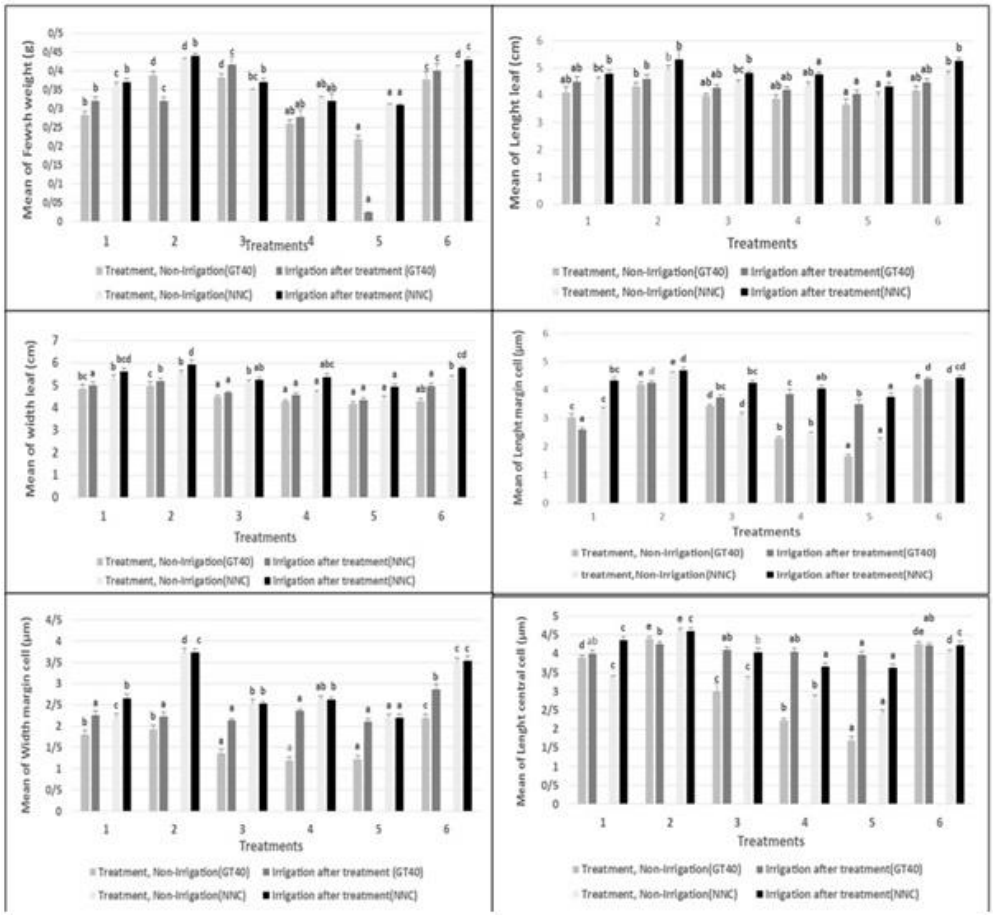


Fig.1. Histogram graphs for morphological and cellular characters in NNC and GT40 cultivars in different treatments. 1) Control samples, 2) 0.5 mM SA, 3) 0.1 mMSA, 4) 100mM NaCl, 5) 150mM NaCl and 6) 150mM Na Cl+0.5 mMSA. Letters on columns are based on Duncan test

The activity of catalase, peroxidase and proline enzymes were highest in 150 mM NaCl treated plants while the lowest activity occurred in 0.5 mM NaCl treatment (Fig.2). All treated

plants showed significant differences between one week-and two- week irrigation both in leaves and roots ($P<0.05$).

In total, 0.5 mM SA treated plant in both cotton cultivars showed the highest values for morphological and cellular characteristics. While 150mM NaCl treated plants of NNC and GT40 cultivars showed the lowest values in the same characters (Fig.1).

In enzyme activity assay, both cultivars had the highest values in 150 mM NaCl treatment, while 0.5 mM SA treated samples showed the lowest activity of enzymes' activity in NCC and GT40 plants (Fig. 2).

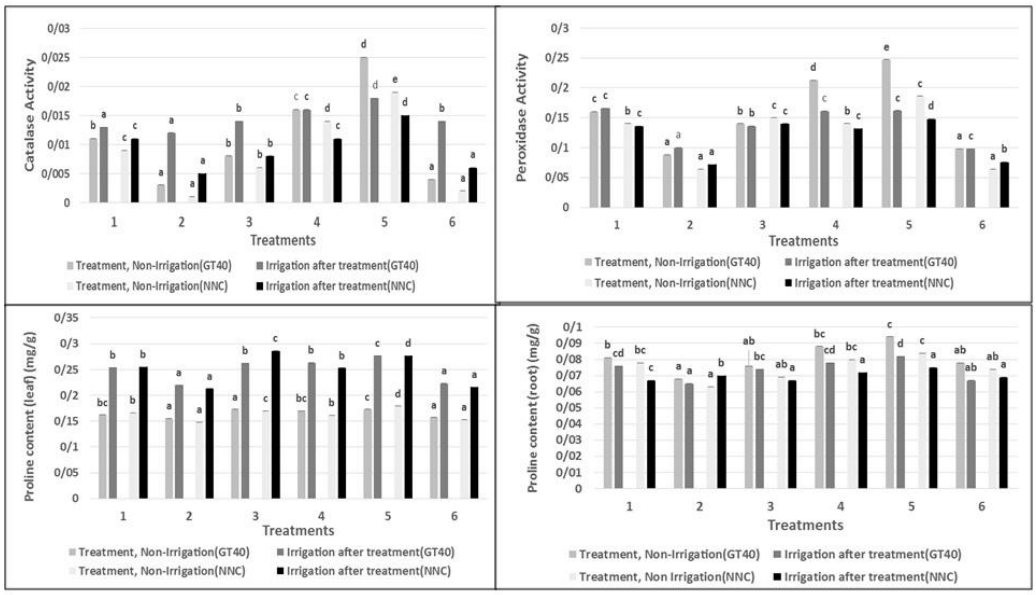


Fig.2. Histogram graphs for Catalase and peroxidase activities as well as proline amount in NNC and GT40 cultivars in different treatments. 1) control samples, 2) 0.5 mM SA, 3) 0.1 mMSA, 4) 100mM NaCl, 5) 150mM NaCl and 6) 150mM Na Cl+0.5 mMSA .

The NNC cultivar showed significant increases ($P<0.05$) in morphology and cell size in different treatments as well as after one week of irrigation compared to GT40 cultivar. While, the activity of catalase, peroxidase enzymes and proline were significantly higher in GT40 compared to those in NNC treated plants. The control plants in both cultivars showed no significant difference in activities of enzymes studied in one and two week's irrigations.

*Retrotransposon profiles assay*

In NNC plants, the highest number of effective allele, Shannon index and Nei's gene diversity occurred in control plants, while, the highest number of different alleles was observed in 0.5 mM SA and 150 mM NaCl.

In GT40 treated plants, the highest values in Shannon index, number of effective allele, number of different allele and Nei gene diversity occurred in 0.5 mM SA treated plants.

AMOVA produced significant genetic difference (PhiPT = 0.956, P = 0.010) among the treated samples (Table 2). It revealed that 96% of genetic variability was due to among groups genetic difference, while 4% of total variance was due to within groups' genetic variability.

*Table 2. AMOVA analysis based on IRAP and REMAP data between treated groups of NNC cotton samples. df= degree of freedom, SS= sum of square, MS= mean of squar, Est. Var= estimated variance, Var%= percentage of variance.*

| source | df | SS | MS | Est. Var. | Var% |
|---|---|---|---|---|---|
| Among groups | 11 | 2956.042 | 268.731 | 79.875 | 96% |
| Within groups | 28 | 103.333 | 3.690 | 3.690 | 4% |
| Total | 39 | 3059.375 | | 83.566 | 100% |
| PhiPT | 0.956 | P= 0.001 | | | |

AMOVA for GT40 treated plants also showed significant genetic difference (PhiPT = 0.990, P = 0.010, Table 3). It revealed that 99% and 1% genetic variations due to among and within treated groups' difference, respectively.

UPGMA clustering of the treated plants based on combined IRAP and REMAP data (Fig.3), separated treated plants in four major distinct clusters. It therefore indicates that differently treated cotton plants have different genetic content. This may be a direct result of stress conditions over retrotransposons profiles in cotton.

However, within each major cluster, we observed some degree of genetic variability as also evidenced by AMOVA.

*Table 3. AMOVA analysis based on IRAP and REMAP data between treated groups of GT40 cotton samples. df= degree of freedom, SS= sum of square, MS= mean of squar, Est. Var= estimated variance, Var%= percentage of variance*

| Source | df | SS | MS | Est. Var. | Var% |
|---|---|---|---|---|---|
| Among groups | 11 | 3397.933 | 308.903 | 92.811 | 99% |
| Within groups | 28 | 26.267 | 0.938 | 0.938 | 1% |
| Total | 39 | 3424.200 | | 93.749 | 100% |
| PhiPT | 0.990 | | P= 0.001 | | |

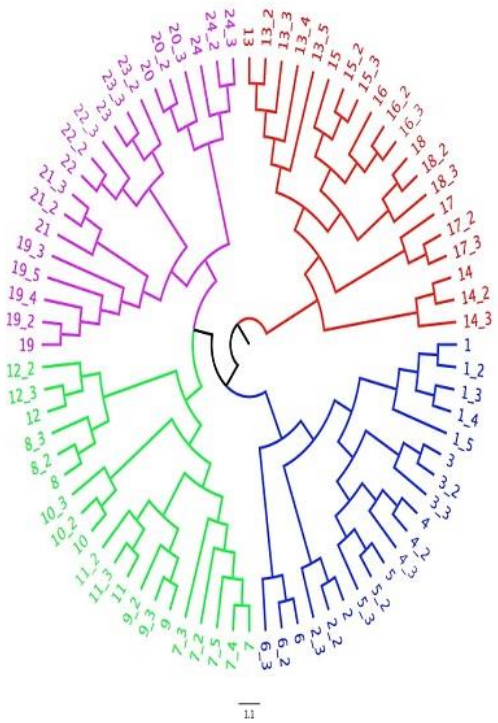


Fig.3. UPGMA clustering of treated cotton plants based on combined IRAP-REMAP data.
(Cotton plants 1-6 are 1)control samples, 2) 0.5 mM SA, 3) 0.1 mMSA, 4) 100mM NaCl, 5) 150mM NaCl and 6) 150mM Na Cl+0.5 mMSA GT40 treated plants (in blue). 7) control samples, 8) 0.5 mM SA, 9) 0.1 mMSA, 10) 100mM NaCl, 11) 150mM NaCl and 12) 150mM Na Cl+0.5 mMSA. GT40 treated plants (in green). 13) control samples, 14) 0.5 mM SA, 15) 0.1 mMSA, 16) 100mM NaCl, 17) 150mM NaCl and 18) 150mM Na Cl+0.5 mMSA NNC treated plants (in red). 19) control samples, 20) 0.5 mM SA, 21) 0.1 mMSA, 22) 100mM NaCl, 23) 150mM NaCl and 24) 150mM Na Cl+0.5 mMSA NNC treated plants (in purple).

## DISCUSSION

The severity and duration of salinity stress may cause different changes in physiological and metabolic processes in plants and affect plant products. The osmotic salt stress causes the accumulation of toxic ions (GUPTA and HUANG, 2014).

During the early stage of salinity, water absorption capacity is reduced in the root system, nutrient imbalance occurs and impaired ability to detoxify the reactive oxygen species (ROS) is the result (GUPTA and HUANG, 2014).

The changes in antioxidant enzymes and photosynthetic activity are among the other effects of salinity stress. One of the harmful effects of salinity is the accumulation of $Na^+$ and $Cl^-$ ions in plant tissues which cause severe ion imbalance. Salt also causes oxidative damage and ultimately ends the vital cellular functions (GUPTA and HUANG, 2014).

In present study, the salinity stress decreased the leaf length, the leaf width and the fresh leaf weight while, these characteristics were increased in the presence of salicylic acid (0.5 mM). It is known that the salicylic acid at relatively low concentrations (less than 100 lM) can enhance the plant growth; while at high concentrations (more than 1 mM) may adversely decrease it (RIVAS-SAN VICENTE and PLASENCIA, 2011). The SA brings about the accumulation of inorganic and organic osmolytes, which in turn increase the water absorption and may raise biomass volume in plant tissues (SZEPESI *et al.*, 2005).

We observed that the fresh weight of both NNC and GT40 cotton cultivars were decreased with NaCl (150mM) treatment. The reason may stem from salt effect on growth, reproduction and development of plant (TAVILI and BINIAZ, 2009).

We observed an increase in the proline content in leaves and roots of cotton plants which were under salt stress with 150 mM NaCl concentration. This is in agreement with findings in Arya-Anubam cotton cultivar (DESINGH and KANAGARAJ, 2007).The proline enzyme acts as an osmotic balance between the cytoplasm and the vacuole. In addition, it is a reservoir of carbon and nitrogen resources and protects cells against free radical damages (MATYSIK *et al.*, 2002).

We observed reduced peroxidase activity in the early growth stage for NNC cotton cultivar (salt resistance), and increased enzyme activity in all growth stages for GT40 cotton cultivar (salinity sensitive cotton). These results are in agreement with similar studies in Siokra (salt resistance), and Sahel (salinity sensitive) cotton cultivars (REZAEE *et al.*, 2006).

Antioxidant enzymes are responsible for the accumulation and detoxification of hydrogen peroxide. Their activities could control the production of reactive oxygen species and prevent to generate the oxidative stress in plant cells (GAPINSKA *et al.*, 2008). Salicylic acid has been reported to have a differential influence on the activities of antioxidative enzymes. It showed an increase in superoxide dismutase, peroxidase and ascorbate peroxidase activities in salt treatment in maize, while decreased the catalase activity (FAHAD *et al.*, 2015).

The earlier investigations in cotton have shown that ectopic expression of cotton CBL-interacting protein kinase gene (GhCIPK6) and SnRK2 could enhance abiotic stress tolerance (ZHANG *et al.*, 2016). Furthermore, over-expressing the vacuolar location AtNHX1 gene in cotton can also improve salt tolerance (RIVAS-SAN VICENTE and PLASENCIA, 2011). Evidence from transgenic plants has demonstrated the important role of transcription factors under salt stress in cotton. Such as GhWRKY39-1 and GhDREB1 enhance abiotic stress tolerance in transgenic plant (RONTEIN *et al.*, 2002) and over-expression of the rice NAC gene, SNAC1, could also improve salt tolerance in transgenic cotton (LIU *et al.*, 2014a, b). Over-expression ROS scavengers, such as GhSOD1, GhCAT1, and GhMT3a, showed high salt stress tolerance in cotton (ZHANG *et al.*, 2016).

IRAP and REMAP markers used revealed a higher degree of genetic variation in GT40 (salt sensitive) cotton plants compared to those of NNC (salt resistant) plants. High genetic variability in GT40 cotton cultivar was also reported under drought stress condition (ZAHRAEE *et al.*, 2014).

GT40 is a hybrid genotype (*G. barbadense x G. hirsutum*), which may be the reason for its higher degree of genetic variation compared to NNC cultivar.

Retrotransposon activities have been reported in plant genomes and examined largely so far, possible movements in genomes have been identified specially under environmental and genomic stresses as well as inter-specific hybridization (WESSLER, 1996; HU *et al.*, 2010; ALZOHAIRY *et al.*, 2014). However, the merging of two diverged regulatory systems in hybrids may acts as interference of epigenetic suppression of transposable elements (HU *et al.*, 2010).

In our results the highest degree of gene diversity was observed in 0.5 mM SA treated plants. These plants were grouped together and were separated from the other treated plants in UPGMA dendrogram. It is supposed that salicylic acid acts as an internal signal and induces the retrotransposon promoters (KIMURA *et al.*, 2001).

## CONCLUSION

Our finding showed the effects of 0.5 mM SA on morphological, physiological and cellular behaviors under salt stress in the studied cotton cultivars. It is suggested that SA activates the anti-oxidant plant defense system, and increases the tolerance of cotton against oxidative stress. Finally, salt stress produced different RTs profiles and cotton cultivar GT40 showed a higher degree of genetic heterozygosity compared to the cultivar NNC.

Received, March 09th, 2019
Accepted January 18th, 2020

## REFERENCES

ALZOHAIRY, A.M., J.S.M., SABIR, G., GYULAI, A., BAHIELDIN (2014): Environmental Stress Activation of Plant LTR-Retrotransposons Function. Plant Biology, *41*: 557-567.

AZOOZ, M.M., M.F., ABOU-ELHAMD, M.A., AL-FREDAN (2012): Biphasic effect of copper on growth, proline, lipid peroxidation and antioxidant enzyme activities of wheat (*Triticum aestivum* cv. Hasaawi) at early growing stage. Australian Journal of Crop Science, *6*:688-694.

BATES, L.S., R.P., WALDERN, I.D., TEARE (1973): Rapid determination of free proline for water stress studies. Plant Soil, *39*: 205–207.

CAO, Y., Y., JIANG, M., DING, S., HE (2015): Molecular characterization of a transcriptionally active Ty1/copia-like retrotransposon in Gossypium. Plant .Cell. Rep., *34*: 1037-1047.

DESINGH. R., G., KANAGARAJ (2012):Influence of salinity stress on photosynthesis and antioxidative systems in two cotton varieties. Genet. Appl. Plant Physiol., *33*: 221-234.

DOGAN, I., G., KEKEC, I., OZYIGIT, M.S., SAKCALI (2007): Salinity induced changes in cotton (*Gossypium hirsotum* L.). Pak. J. Bot., *44*: 21-25.

EBERMANN, R., K., STICH (1982): Peroxidase and amylase isoenzymes in the sapwood and heartwood of trees. Phytochem., *21*: 2401-2402.

FAHAD, S.H., S., HUSSAIN, A., MATLOOB, F.A., KHAN, A., KHALIQ, S.H., SAUD, S.H., HASSAN, D., SHAN, F., KHAN, N., ULLAH, M., FAIQ, M.R., KHAN, A.K., TAREEN, A.B., KHAN, A., ULLAH, N., ULLAH, J., HUANG (2015): Phytohormones and plant responses to salinity stress a review. Plant Growth Regul., *75*: 391–404.

FREELAND, J.R., H., KIRK, S.D., PETERSON (2011): Mol Ecol,second ed. UK Wiley-Blackwell.

GAPINSKA, M., M., SKLODOWSKA, B., GABARA (2008): Effect of short- and long-term salinity on the activities of antioxidative enzymes and lipid peroxidation in tomato roots. Acta. Physiol. Plant., *30*: 11–18.

GUPTA, B., B., HUANG (2014): Mechanism of salinity tolerance in plants physiological, biochemical, and molecular characterization. Intel. J. Genomics, 4920-4924.

HAVIR, E.A., N.A., MELLATE (1987): Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. Plant Physiol., *84*: 450-455.

HOSSEINI, G.H., R.J., THRNGANE (2007): Salinity tolerance in cotton (*Gossypium hirsutum* L.) genotypes. Inter. J. Bot., *3*: 48-55

HOSSEINI, S.J., Z., TAHMASEBI SARVESTANI, H., PIRDASHTI (2012):Analysis of Tolerance Indices in Some Rice (*Oryza sativa* L.) Genotypes at Salt Stress Condition. Intel. Res. J. Appl. Basic Sci., *3*: 1-10.

HU, G., S.J., HAWKINS, C.E., GROVER, J.F., WENDEL (2010): The history and disposition of transposable elements in polyploid *Gossypium.* Genome, *53*: 599–607.

KAMARAN, S., T.M., KHAN, A., SHAKEEL, A., AHMED, A., ZEB (2016): Genetics of salt stress tolrrance in upland cotton (*Gossypium hirsutum* L.). Inter. J. Eng. Inventions., *5*: 31-37.

KANTARTZI, S.K, M., ULLOA, E., SACKS, J.M.C.D., STEWART (2010): Assessing genetic diversity in *Gossypium arboreum* L. cultivars using genomic and EST-derived microsatellites. Genetica, *139*:141-147.

KIMURA, Y., Y., TOSA, S., SHIMADA, R., SOGO, M., KUSABA, T., SUNAGA, S., BETSUYAKU, Y., ETO, H., NAKAYASHIKI, S., MAYAMA (2001): OARE-1, a Ty1-copia retrotransposon in oat activated by abiotic and biotic stresses. Plant Cell Physiol., *42*: 1345–1354.

KRIZMAN, M., J., JAKSE, D., BARICEVIC, B., JAVORNIK, P., MIRKO (2006): Robust CTAB-activated charcoal protocol for plant DNA extraction. Actaagricul. Slav., *87*: 427 – 433.

LIU, G., X., LI, S., JIN, X., LIU, L., ZHU, Y., NIE, X., ZHANG (2014): Overexpression of Rice NAC Gene SNAC1 Improves Drought and Salt Tolerance by Enhancing Root Development and Reducing Transpiration Rate in Transgenic Cotton. PLoS ONE, *9*: e86895.

LIU, S.H., Y., DONG, L., XU, J., JING KONG (2014): Effects of foliar applications of nitric oxide and salicylic acid on salt-induced changes in photosynthesis and antioxidative metabolism of cotton seedlings. Plant Growth.Regul., *73*: 67–78.

MAEHLY, A.C., B., CHANCE (1954):The assay of catalase and peroxidase. Methods Biochem. Analysis (D. Glick, ed.). *1*: 357-424.

MATYSIK, J., B., BHALU, P., MOHANTY (2002): Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. Curr. Sci., *82*: 525-532.

MIMOUNI, H., S., WASTI, A., MANAA, E., GHARBI, A., CHALH, B., VANDOORNE, S., LUTTS, H., BEN AHMED (2016): Does Salicylic Acid (SA) Improve Tolerance to Salt Stress in Plants? A Study of SA Effects on Tomato Plant Growth, Water Dynamics, Photosynthesis, and Biochemical Parameters. OMICS, *20*: 180-90.

MUKHERJEE, S.P., M.A., CHOUDHURI (1983): Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. Plant Physiol., *58*: 166-170.

NOORMOHAMMADI, Z., N., IBRAHIM-KHALILI, M., SHEIDAI, O., ALISHAH (2018): Genetic fingerprinting of diploid and tetraploid cotton cultivars by retrotransposon-based markers. Nucleus, *61*: 137-143.

NOORMOHAMMADI, Z., Y., HASHEMINEJAD-AHANGARANI-FARAHANI, M., SHEIDAI, S., GHASEMZADEH-BARAKI, O., ALISHAH (2013):Genetic diversity analysis in Opal hybrids by multiple molecular approach SSR, ISSR, and RAPD combined method. Genet. Mol. Res., *12*: 256-269.

NOORMOHAMMADI, Z., N., IBRAHIM-KHALILI, S., GHASEMZADEH BRAKI, M., SHEIDAI, O., ALISHAH (2016): Genetic screening of diploid and tetraploid cotton cultivars by retrotransposon based markers. Aneles de Biol., *22*: 115-117.

PEAKALL, R., P.E., SMOUSE (2006): GENALEX 6 genetic analysis in Excel Population genetic software for teaching and research. Mol .Ecol. Notes, *6*: 288-295.

PODANI, J. (2000): Introduction to the Exploration of Multivariate Data [English translation], Leide, Netherlands Backhuyes.

REZAEE, M., D., KHAVARINEJAD, R., FAHIMI (2006): The effect of natural salinity of soil on peroxidase activity of two cotton cultivars. Basic Sciences of Islamic Azad University. 62, 79-89 (in Persian).

RIVAS-SAN VICENTE, M., J., PLASENCIA (2011): Salicylic acid beyond defence its role in plant growth and development. J. Exp. Bot., *62*: 3321–3338.

RONTEIN, D., G., BASSET, A.D., HANSON (2002): Metabolic engineering of osmoprotectant accumulation in plants. Metabol. Engin., *4*: 49-56.

SANTANA, M.F., E.F., DE ARAÚJO, J.T., DE SOUZA, E.S.G., MIZUBUTI, M.V., DE QUEIROZ (2012): Development of molecular markers based on retrotransposons for the analysis of genetic variability in Moniliophthoraperniciosa. Europ. J .Plant. Pathol., *134*: 497-507.

SHEIDAI, M., M., RIAZIFAR, A., HOORDADIAN, O., ALISHAH (2018): Genetic finger printing of salt- and drought-tolerant cotton cultivars (*Gossypium hirsutum*) by IRAP-REMAP and SRAP molecular markers. Plant Gene, *14*: 12-19 .

SHEIDAI, M., Z.H., SHAHRIARI, H., ROKNIZADEH, Z., NOORMOHAMMADI (2007): RAPD and Cytogenetic study of some Tetraploid cotton (*Gossypium hirsutum* L.) cultivars and their Hybrids. Cytologia, *72*: 77-82.

SZEPESI, A., J., CSISZÁR, S., BAJKÁN, K., GÉMES, F., HORVÁTH, L., ERDEI, I., TARI (2005): Role of salicylic acid pre-treatment on the acclimation of tomato plants to salt-and osmotic stress. Acta. Biol. Szeged, *49*: 123-125.

SZUĆKO, I., S.M., ROGALSK (2015): Application of ISSR-PCR, IRAP-PCR, REMAP-PCR, and ITAP-PCR in the assessment of genomic changes in the early generation of triticale. Biol. Plantarum, *59*: 708-714.

TAVILI, A., M., BINIAZ (2009): Different salts effects on the germination of *Hordeum vulgare* and *Hordeum bulbosum*. Pak. J. Nutrit., *8*: 63-68.

TEO, C.H., S.H., TAN, C.L., HO, Q.Z., FARIDAH, Y.R., OTHMAN, J.S., HESLOP-HARRISON, R., KALENDAR, A.H., SCHULMAN (2005): Genome constitution and classification using retrotransposon-based markers in the orphan crop banana. J. Plant. Biol., *48*: 96-105.

TURAN, S., K., CORNISH, S., KUMAR (2012): Salinity tolerance in plants Breeding and genetic engineering. Aust. J. Crop. Sci., *6*: 1337-1348.

WEISING, K., H., NYBOM, K., WOLFF, G., KAHL (1996): DNA Fingerprinting in Plants, second ed. Boca Rayton, Fl, USA.

WESSLER, S.R. (1996): Turned on by stress. Plant retrotransposons. Current Biology, *6*: 959–961.

ZAHRAEE, B.H., Z., NOORMOHAMMADI, F., FARAHANI, M., SHEIDAI (2014): Analysis of induced genetic variation in cotton cultivars under drought stress. Gene Conserv., *13*: 18-37.

ZHANG, F., G., ZHU, L., DU, X., SHANG, C.H., CHENG, B., YANG, Y., HU, C., CAI, W., GUO (2016): Genetic regulation of salt stress tolerance revealed by RNA-Seq in cotton diploid wild species, Gossypium davidsonii. Sci. Rep., *6*: 20582.

# GENETSKA I FIZIOLOŠKA ANALIZA KULTIVARA PAMUKA U USLOVIMA STRESA SOLI

Zahra NOORMOHAMMADI[1*], Elham TORABIZADEH[1], Farah FARAHANI[2], Omran ALISHAH[3], Masoud SHEIDAI[4]

[1]Departman za biologiju, nauku i istraživanje, Islamic Azad Univerzitet, Teheran, Iran
[2]Departman za mikrobiologiju, Islamic Azad Univerzitet, Qom, Iran
[3] Istraživački institut Irana za pamuk, Poljoprivredna istraživačka, obrazovna i savetodavna organizacija (ARREO), Gorgan, Iran
[4] Fakultet za prirodne nauke i biotehnologiju, Shahid Beheshti Univerzitet, Teheran, Iran

Izvod

Salinitet je uzrokovao promene u metaboličkim procesima u biljci. Proizvodnja pamuka je veoma važna u Iranu i na nju značajno može uticati stres soli. U ovoj studiji, ispitivani su uticaji stresa soli i salicilne kiseline (SA) na biohemijske osobine i profil markera zasnovanih na retrotranspozonu (IRAP i REMAP) kod sorti pamuka. Dve sorte pamuka (GT40 i NNC) gajane su u saksijama sa različitim tretmanima; kontrolne biljke sui male normalno navodnjavanje, tretmani su imali 0,5 i 1 mM SA, 100 i 150 mM NaCl tretmani i kombinaciju 0,5 mM SA i 150 mM NaCl. Fiziološki, morfološki, ćelijski i genetski parametri diverziteta su mereni nakon tretmana I narednih sedam dana tokom navodnjavanja. Aktivnost antioksidativnih enzima; katalaza, peroksidaza i prolin u biljkama tretiranim sa 150 mg NaCl bili su viši nego u drugim tretmanima. Kultivar GT40 imao je veću aktivnost antioksidativnih enzima. Na osnovu retrotransposonskih analiza markera, najviša heterozigotnost je pokazana u 0,5mM SA tretmanu GT40 kultivara, kao i u 0,5 mM SA, 150mM NaCl tretmanima u NNC kultivaru. Ukupno, GT40 je pokazao najveću genetsku varijaciju u retrotransposonskim profilima. Tretman sa stresom soli i salicilnom kiselinom pokazao je diskriminirajući profil u odnosu na retrotransposone.

Primljeno 09.II.2019.
Odobreno18.I.2020

.