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Vanıllylacetone Improves the Defense of Wheat Agaınst Salt Stress

Hüseyin BULUT

Erzincan Binali Yıldırım University Vocational School of Health Services, 24100 Erzincan / Turkey (Corresponding author)

Abstract: ***Among abiotic stresses, salinity is the most important factor causing loss of yield after drought. As a result of global warming, chemical fertilizers, pesticides, and environmental pollution caused by anthropogenic activities, the salinity of the soil increases. Plants are developing evolutionary adaptations to deal with stresses. In this study, the contribution of vanilla to wheat defense mechanism against salt stress was evaluated. Genetic change in 0, 3, 6, g / l, 9, 12 and 15 g / l salt-related and salt stress and vanillacetone was used by molecular tests. The average of polymorphism value formed by Inter Retrotransposon Amplified Polymorphism (IRAP) analysis is determined as 31.28%. In these examples, the realization of the Genomic Template Stability (GTS) value was 68.72%. In addition to salt stress, the average polymorphism of the applied samples of vanilla was determined as 26.89% and the average of the GTS value was 73.11%. It was determined from the study results that vanillyacetone relieves salt stress in wheat and contributes to defense regulation.***

Keywords: IRAP, Retrotransposone, Salt stress, *Triticum aestivum* L., Vanillylacetone

1. Introduction

Among the abiotic stresses, drought most restricts the production of agricultural products, and the salinity factor is followed. Soil salinity harms 50% of the cultivated areas and 20% of the agricultural lands all over the world (Munns and Tester, 2008; Sun et al., 2016). While inefficient areas cover 2% of the surface area in our country, 74% of these barren areas are made up of salty soils (Kendirli et al., 2005). It is anticipated that the salinity problem will worsen in the coming years as an increase in soil salinity is expected due to climate change (AbdElgawad et al., 2016).

Approximately 82% and 85% of the world population's protein and basic calorie requirement is based on wheat (Chaves et al., 2013). In Turkey, 72 million square meters in wheat sowing is done, production is 20 million tons (TSI, 2019).

Wheat cultivation constitutes 65% of the cultivation area and 36% of the production amount among the main agricultural products (Caverzan et al., 2016). Although wheat is grown in tropical, subtropical regions, rain fed and irrigated areas due to its high compatibility, it is significantly affected by environmental stresses such as salt stress. Salt stress affects plants in three ways. First, it decreases the usability of water by increasing the osmotic pressure of the soil. Second, high concentrations of Na + and Cl - ions can cause specific toxic effects. Third, it is the antagonistic effect of some nutrients (Na + and Cl -) with important nutrients that cause nutritional imbalance (Grattan and Grieve, 1999).

The formation of toxic ions due to excess salt in the plant structure caused an ionic imbalance that triggered the production of reactive oxygen species (ROS) that damage membrane lipids, nucleic acids and proteins (Chaparzadeh et al., 2004). Throughout evolution, plants have developed several strategies to deal with excessive salt and control their homeostasis (Munns and Tester, 2008; Roy et al., 2014). Cellular metabolism keeps the amount of these ROS under normal conditions with antioxidants and various protection systems (Breusegem et al., 2001). The activity of antioxidant systems decreases under the influence of environmental stress factors and these conditions cause their accumulation by promoting the synthesis of ROS (Mittler, 2002). Increased ROS under stress conditions cause damage to DNA and RNA. Excessive ROS production under oxidative stress causes oxidative damage in lipids, protein and DNA, leading to cell death (Apel and Hirt, 2004). Different plant extracts and extracts in different organisms have been used in many studies to determine the protective effects of natural antioxidants. For example, turmeric (Hosseini and Hosseinzadeh, 2018), black cumin (Tavakkoli et al., 2017), milk thistle (Fanoudi et al., 2018), cinnamon (Dorri et al., 2018), measles (Mohammadzadeh et al., 2017) and ginger (Lee et al., 2018) antioxidant effects were investigated.

Ginger forms a very rich group with its plant diversity and active compounds. It is known that there are more than 60 active ingredients in ginger, which is widely divided into volatile and non-volatile compounds. Ginger extracts and components gingerol, shagaol, zingerone, and zerumbone have been reported to effectively improve the toxicity and ROS production of different factors in in vivo and in-vitro preclinical models (Kim et al., 2007; Guahk et al., 2010; Thongrakard et al. ., 2014; Kamel et al., 2017; Yang et al., 2018).

Different plant types such as *Allium cepa*, *Triticum aestivum*, *Zea mays*, *Hordeum vulgare*, *Vicia faba*and *Arabidopsis thaliana* have been used by researchers as a model organism for the detection of genotoxic effect in recent years (Liu et al., 2005). Study of importance to wheat production in Turkey *Triticum aestivum* L. germination period caused by different concentrations of salt stress and ginger vanillylacetone component is intended to determine the

protective effect against stress.

2. Materials and Methods

2.1 Material

Triticum aestivum L. seeds, which were obtained purely from Atatürk University Faculty of Agriculture, were washed in a 5% sodium hypochlorite (NaClO) solution for 10 minutes to ensure surface sterilization. Seeds were rinsed with distilled water and dried. Seeds were placed in petri dishes with sterile filter papers, and 5 ml of 0, 3, 6, 9, 12 and 15 g / l NaCl (Ekmekci et al., 2005) were added to germination. At the same doses, 500 ppmvanillacetone was added to other group petri dishes, and the control group was germinated with 0 g / l NaCl. Samples from experimental and control groups were stored at -80 ° C for use.

2.2 DNA isolation and determination of concentrations

DNA were isolated from plant samples for analysis by IRAP methods. DNA isolation was performed with minor modifications in the protocol of Shagai-Maroof et al. (1984). Total DNA concentrations were measured by ACTGene Spectrophotometer (ACTGene UVIS-99, NJ, USA) to determine A260 / 280 O.D. Were taken. Based on these re-sults, the total DNA of all samples was adjusted to 0,5µg.

2.3 IRAP analysis

Differentiation in the genome from retrotransposon mobility can be analyzed by IRAP technique. IRAP is a marker system based on mobile elements discovered by Kalendar et al. (1999) to generate DNA fingerprints. It targets a group of retrotransposons containing direct LTRs ranging in size from 100 to 5,000 bp (Atienzar, 1999) In this technique, DNA segments between the two LTR sequences are amplified and the primers are linked to these regions. PCR was performed with 6 primers in IRAP analyzes. The names, sequences and annealing temperatures of the primers used are given in Table1.

Table 1: Details of primers used in IRAP-PCR analysis

Primer name	***Sequence 5'→3'***	***T.M. (^{0}C)***
SUKKULA	GATAGGGTCGCATCTTGGGCGTGAC	63.3
3LTR-5	TGTTTCCCATGCGACGTTCCCCAACA	64.6
LTR 6150	CTGGTTCGGCCCATGTCTATGTATCCACACATGTA	64.4
NIKITA E2647-	ACCCCTCTAGGCGACATCC	58.7
5LTR1	TTGCCTCTAGGGCATATTTTCCAACA	58.4
LTR 6149 -5	CTCGCTCGCCCACTACATCAACCGCGTTTATT	65.9

For IRAP PCR, at ratios 2.0 µl of 10 × PCR buffer, 0.5 µl dNTPs (10 nM), 1.25 µl Magnesium Cloride (25 mM), 1.0 µl primer (5 mM), 1.0 µl Taq DNA polymerase enzyme (5 units) 13.25 µl ultra-pure water, 1.0 µl Genomic DNA was prepared for each sample and primer. The prepared master mix mixtures were placed in a PCR apparatus in PCR tubes with a volume of 500 µl. PCR protocol 95 °C for 2 minutes, 95 °C for 30 seconds 2 cycles, Primer T.M. Temperature 1 minute, 72 oC 2 minutes, 95 oC 30 seconds 41 cycles, 35 oC 1 minute, 72 oC 2 minutes, 72 oC 5 minutes, 4 oC infinite.

2.4 IRAP PCR electrophoresis protocol

The resulting PCR products were run on agarose gel. For this, 0.7 g Agarose 10 ml TBE (Tris / Borate / EDTA) pH 8 was added and purified to 100 ml with distilled water and heated in a micro-wave oven and 2 µl Ethidium Bromide was added. Samples were run for 100 minutes at 90 volts ta electrophoresis and visualized with a UV camera.

2.5. IRAP analyzes and determination of GTS

For each primer, the presence and absence of amplified DNA bands were determined by negative control IRAP profiles, and the decrease and increase of band intensities were determined by agarose gel imager and Total LAB TL 120 (Nonlinear Dynamics) software. Genomic mold stability (%) for all primer products; 100 x 1-a / b.
In the formula;
a; The IRAP polymorphic profiles determined for each application sample,
n; Was selected as the total number of bands of DNA obtained in the corresponding primer negative control group. According to the polymorphism negative control group observed in the IRAP profiles belonging to the application groups, a new band does not occur or the existing band dis-appears.

3. Results

3.1 IRAP analysis results

319 bands were obtained from 6 primers used for IRAP analysis. Shows the tape image obtained from the primer 5LTR1 in Figure 1. The sizes of these bands vary between 76 and 1.523 bp. Details of IRAP analysis results are given in Table 2 and Table 3.

Table 2: IRAP-PCR analysis results of samples exposed to salt stress

Primer name	*Control*	*NaCl 3 g/l*	*NaCl 6 g/l*	*NaCl 9 g/l*	*NaCl 12 g/l*	*NaCl 15 g/l*
LTR6150	4	+1.086	+1.121	+1.156 +572	+1.138 -688	+1.182 +596 -688
LTR6149-5	4	-468	+612	+646 -468	+692 -468	+704 -468
5LTR1	7	+512	+564 -452	+572 -452	+722 +596	+768 +603
3LTR-5	3	+812	+826	+868	+904 -356	+918 -356
NIKITA-E2647-	5	+624	+632	+647	-232	+694 -232
SUKKULA	6	+356	+374	+382	+401 -744	+446 -744
Number of bands	29	6	7	9	11	13
Polymorphism value		20.68	24.13	31.03	37.93	41.37
GTS value		79.32	75.87	68.97	62.07	58.63

Most polymorphic bands were obtained from LTR6150 primer with 9 pieces. Polymorphism was detected in all samples exposed to salt stress compared to the control group. It was observed that polymorphism values increased in direct proportion with the increase in salt dose. Polymorphism values vary between 20.68% and 41.37%, the highest polymorphism is determined in the germinated sample with 41.37% and the lowest polymorphism value in the germinated dose with 20.68% and 3 g/l salt dose. GTS values of seeds germinated under salt stress decreased due to increased salt stress. GTS values varied between 58.63% and 79.32%. The highest GTS value was obtained as 79.32% from the lowest dose salt application, 3 g / l.

Table 3: IRAP-PCR analysis results of salt stress-Vanillylacetone applied samples

Primer name	*Control*	*NaCl 3 g/l +500 ppm Vanillylacetone*	*NaCl 6 g/l +500 ppm Vanillylacetone*	*NaCl 9 g/l +500 ppm Vanillylacetone*	*NaCl 12 g/l + 500 ppm Vanillylacetone*	*NaCl 15 g/l +500ppm Vanillylacetone*
LTR6150	4	-386	-386	+812 -386	+868 -386	+892 +792
%LTR6149-5	4	-814	-814	+436	+475 -814	+924 -814
5LTR1	7	….	+468	+476 -364	+492 -364	+504 -364
3LTR-5	3	+634	+644	+656	+674 -212	+691 -212
NIKITA-E2647-	5	….	+610	+613	+632	+648
SUKKULA	6	+294	+299	+311	+318	+376 -620
Number of bands	29	4	6	8	10	11
Polymorphism value		13.79	20.68	27.58	34.48	37.93
GTS value		86.21	79.32	72.42	65.52	62.07

The polymorphism-GTS values obtained from samples germinated only under salt stress and the dose-related changes of these values are given in Figure 2.

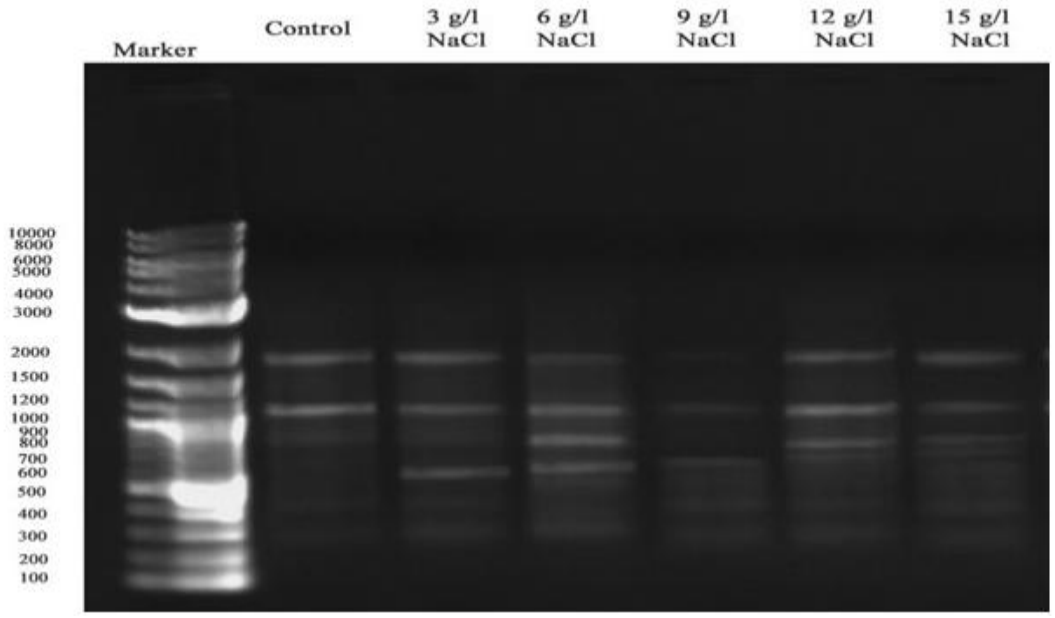

Figure 1: Band image from primer 5LTR1

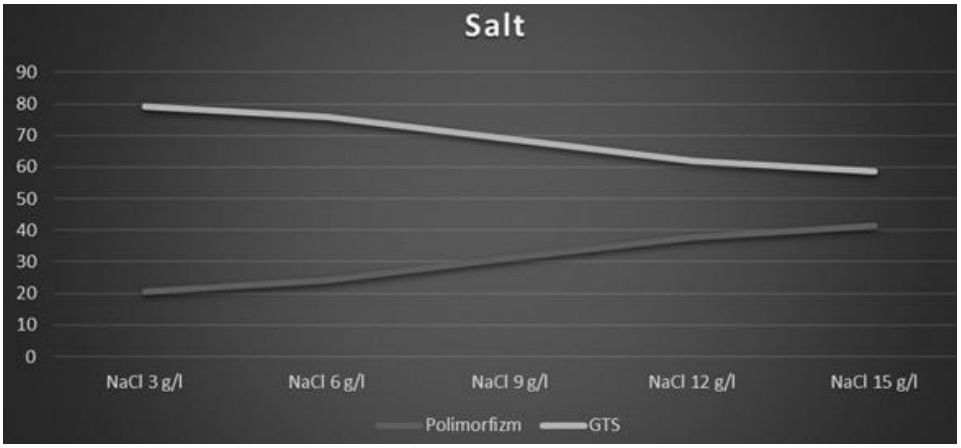

Figure 2: Effect of salt stress dose on polymorphism and change in GTS value

In salt stress applied by adding vanillylacetone, polymorphism values decreased in the samples germinated at all dose levels. The polymorphism values obtained from these samples were found to vary between 13.79% and 37.93%. In these examples, a positive correlation was found between salt dose and polymorphism. GTS values, on the other hand, were found to be higher than those applied only to salt stress. GTS values were found to vary between 62.07% and 86.21%. The polymorphism-GTS values obtained from Vanillylacetone applications added with salt stress and the dose-related changes of these values are shown in Figure 3.

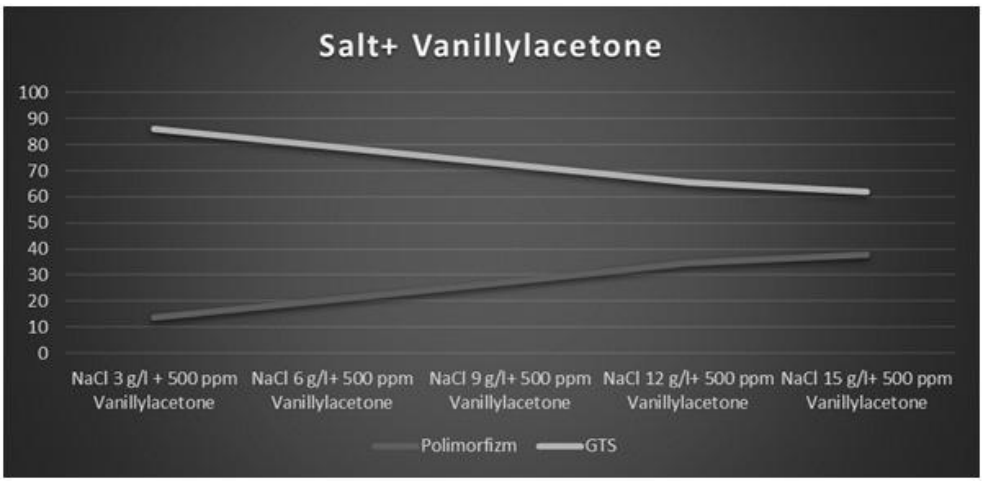

Figure 3: Polymorphism and change in GTS values obtained by application of salt stress-vanillacetone

4. Discussion

The molecular effect of salt stress on wheat, which has such an important place in grain production, and the protective properties of vanillylaceton, the ginger extract against this stress, have been studied. It has been determined that salt stress applied to wheat causes polymorphism at all dose levels. The reason for the occurrence of polymorphism may be the changes in the genome profile as a result of retrotransposon mobility changing the primary binding points. This may cause new bands to form or bands not to form. In addition, it is seen that GTS values, which are the expression of genomic stability, decrease due to increasing salt stress. The decrease in GTS value can be expressed as salt stress affects the stability of the genome. In addition to salt stress and those exposed to salt stress, vanillylacetone applied samples showed significant positive changes in polymorphism values. For example, the average polymorphism value of wheat samples exposed only to salt stress has decreased from 31.28% to 26.89%. At the same time, the GTS value increased from 68.72% to 73.11%. It is understood from the polymorphism and positive change in GTS values that Vanillylacetone has a protective effect against wheat salt stress in different studies. Vanillylacetone, an important ingredient found in ginger root, has anti-mutagenic and anti-carcinogenic activities, often associated with anti-oxidative and anti-inflammatory activities. A decrease in polymorphism and an increase in GTS value were detected in all doses administered with vanillylacetone in this study. It can be interpreted that vanillylaceton decreases ROS production, preventing genomic instability. Different types of evolutionary defense mechanisms have been developed to deal with metabolism stress. In this study, it was found that the translation of defense proteins whose gene expression was evaluated against salt stress increased.

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