EVALUATION OF GENOTOXICITY AND EXPRESSION PATTERN OF GENES ASSOCIATED WITH TERPENES IN LEMON (*CITRUS LIMON* L.) PLANTS UNDER THE INFLUENCE OF SALINITY STRESS

AL KASHGRY, N. A.

Department of Biology, College of Science, Taif University, Taif 21944, Saudi Arabia
(e-mail: n.takth@tu.edu.sa; phone: +966-503-047-263)

(Received 6th Dec 2023; accepted 27th Feb 2024)

Abstract. Salinity is a significant abiotic stress that adversely affects growth, the content of secondary metabolites, and can even cause cell genotoxicity. Lemon (*Citrus limon* L.) plants are sensitive to levels of NaCl salinity. To ascertain the response of *C. limon* plants to NaCl salinity stress, two distinct genetic techniques were used. The genotoxic effects of NaCl on tissue-cultured plants of *C. limon* was investigated using the comet test. Moreover, the expression of terpene genes was also investigated. Results revealed that NaCl 200 mM caused high severity levels DNA destruction (15.62%) in *C. limon* plants, whereas the level of 100 mM NaCl scored a tail length of 10.65 μm, the tail moment was 0.77 units. *RtME* gene expression peaked at a ratio of 2.29 when the plants were exposed to NaCl 100 mM, while collecting transcripts for the *RtLS* was 2.21 in plants treated with 100 mM NaCl. Conclusively, comet analysis technique provides a suitable technique to identify DNA damage and terpene genes possessing post-transcriptional control in *C. limon* salinity stress on plant cells. Prospective investigation is necessary to assess the applicability of comet assay and gene expression methods using additional genes that exhibit sensitivity to various stressors on lemon plus additional plants.
Keywords: *in vitro, NaCl, gene expression, DNA damage, lemon*

Introduction

Lemon (*Citrus limon* L.) tree has evergreen leaves and tasty yellow fruits. The fruit, especially the juice and essential oil extracted from it, serves as the primary raw material for *C. limon,* they are used to manufacture and package the juice and extract the volatile oils found in the peels, which are used in many food industries as safe alternatives to preservatives. They are also considered natural flavor and aroma additives used in many industries (Goetz, 2014; Russo et al., 2015). Numerous modern scientific research has supported this widespread application of *C. limon*, which has been recognized since antiquity. In traditional medicine, lemon juice is also used to treat high blood pressure, common cold, and irregular periods. Additionally, the application of *C. limon* essential oil is a well-known treatment for coughs (Papp et al., 2011; Bhatia et al., 2015). According to Riaz et al. (2014) and Otang and Afolayan (2016), *C. limon* is known for its diverse chemical composition, which includes treatments for antibacterial, antifungal, anti-inflammatory, anticancer, hepatoregenerating, and cardioprotective actions. Phenolic acids, coumarins, carboxylic acids, amino acids, and vitamins, in addition to flavonoids and other substances are also a major class of secondary metabolites found in the fruit. Monoterpenoids are the main constituents of the essential oil. *C. limon* holds a significant position in the food and cosmetics industries thanks to these useful chemical components (Abad-García et al., 2012; Russo et al., 2015).

The concept of salinity is a serious issue, in particular for citrus plants, which are among the most significant and salt-sensitive horticulture crops worldwide. Numerous researches were carried out to assess the efficacy of *C. lemon* rootstock at various salt

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_18671883

levels (Salwa, 2018). Salt-sensitive plants are those in the citrus and closely related genera, even though cultivar-specific differences in salt tolerance have been documented (Storey and Walker, 1999). In contrast to many other species, the harmful impact of salt on citrus appears to be brought on by chloride ions (Bañuls et al., 1997). These ions gradually build up in plant tissues, causing well-reported adverse outcomes (Gómez-Cadenas et al., 1998). An essential asexual technique for creating virus-free rootstock plants is micropropagation (Roistacher et al., 1976). Various citrus species and explant sources have been the focus of methods for cell culture and micropropagation (Duran-Vila et al., 1992; Hassanein and Azooz, 2003). The majority of recent research has been directed at determining the ideal cultural media composition for a given set of environmental parameters. The tissue culture of this plant has received very little attention. Cell culture methods have frequently been utilized through a study program utilizing in vitro chosen NaCl-tolerant plants is a possible strategy to elucidate the molecular mechanisms behind salt tolerance. Some researchers have avoided cultivating hole seedling by using tissue culture of plants as an alternative since growth conditions can be readily controlled in plant cell cultures (Davenport et al., 2003; Gu et al., 2004).

The comet assay techniques in regard to plants for pertinent distinctions, such as the restricted occurrence of juvenile buds and the strong cell wall present in plant cells those are typical (for example, the concentration of cells that divide quickly in the root apex). Detailed technical reviews of plant comet tests in various tissues and genera have been conducted by Gichner et al. (2009). The comet assay's basic idea is based on how DNA is arranged spatially in the nucleus: loops are created when a linear molecule attaches itself to the nuclear matrix periodically, and additional double helix wrapping around protein cores forms nucleosomes. Because of this arrangement, the DNA maintains its compact, supercoiled shape even after the proteins are eliminated during the assay's lysis phase. Nevertheless, the supercoiling of the loops relaxes in the presence of a DNA SB. Under a fluorescent microscope, this relaxation causes these loops, which are still connected to the nuclear matrix, to be dragged toward the anode, where they form the distinctive "comet tail" (Collins et al., 2023). The proportion of total DNA in the tail indicates how frequently breaks occur. In genotoxicity testing, the comet assay is a widely accepted technique for identifying DNA damage and few toxicological investigations with a small number of model plants, as *Allium cepa*, *Nicotiana tabacum*, *Vicia faba*, and *Arabidopsis thaliana*, for almost ten years (Ventura et al., 2013). A variety of DNA damages can be found using technique variations (Angelis et al., 1999; Collins et al., 2008). Contrasting the cellular tests, which isolate and analyze the nuclei of non-stressed plant cells, cellular assays process the nuclei of plants that have been subjected to suspected genotoxicants. This is followed by comet assay analysis. In a nutshell, an alkaline treatment (also known as A/A) was investigated (Azqueta et al., 2011a). Along with strategy and scoring, there is still discuss over the ideal scoring criteria (Azqueta et al., 2011b). However, regardless of the method and scoring, it is widely agreed that this technique enables the collection of data appropriate for thorough statistical analyses. The outcomes multiple challenges have been investigated in relation to botanical genotoxicity. The results of the comet test have shown that a potential tool to detect DNA damage and repair despite capacity specific to individual cells salinity's status as one of the most important abiotic factors controlling DNA and reason genotoxicity (Gichner et al., 2000). This investigation will examine the most recent information about this method's efficacy usage as a great strategy for checking into how salinity stress conditions affecting the genotoxicity of lemon plants. The extraordinary differences of essential oils can be

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_18671883

attributed to environmental elements like cultural practices, soil, insects, maturity stage, and weather (Azizi et al., 2009). Responses of seven citrus cultivars to stress caused by varying salt levels were previously examined in vitro and in vivo (Salwa, 2018). Genes related to a single volatile oil, or group of volatile oils, were also discovered. Crucially, the transcription factors and pathways identified in this investigation point to the possibility that, depending on the situation, essential oils might either induce cell death or boost cell growth and self-defense. The biological activity of essential oils in many cell types requires further validation and verification by follow-up investigations (Plant and Stephens, 2015).

Transcriptome study could offer a substantial, thorough, and diverse molecular regulatory network of citrus fruit during developmental life stages and under various situations to offer in-depth and priceless resources for the molecular networks and genes important for oils production (Feng et al., 2019). In a different study, 24 samples of four different citrus tissue types from six different developmental phases were used to build 72 libraries in total. The examinations of the transcriptomes produced encouraging results (Feng et al., 2019). Transcriptional activity and the mode of action of the genes related to volatile oil production may be clarified by genome study (Ashour et al., 2010). As a result, it might be able to look at choosing genes that might be for this biological goal. The important enzyme farnesyl diphosphate synthase *RtFPPS* is involved in the production of isoprenoids. It provides sesquiterpene precursors for a number of types of vital metabolites, such as proteins' farnesylation and geranylgeranylation substrates, as well as sterols, dolichols, ubiquinones, and carotenoids. It facilitates the entire picture condensation of dimethylallyl diphosphate and two isopentenyl diphosphate molecules in a sequential manner. The enzyme is made up of homodimers of its subunits, each of which has a couple of bind sites, one for isopentenyl diphosphate and one for allylic diphosphate (Anna and Danuta, 2005). Speeding up the conversion of Hexoses that require ATP to be turned into hexose 6-phosphates is the glycolytic enzyme hexokinase synthase *RtHK* (Jang et al., 1997). Linalool accumulation and linalool synthase gene transcript levels are strongly correlated with the linalool synthase enzyme *RtLS* (Lane et al., 2010).

RtGPPS, a monoterpene biosynthesis enzyme that is known to be plastid localized, is present in plastids (Tholl et al., 2004). The path's second enzyme, 1-deoxy-D-xylulose-5-phosphate reductoisomerase *DXR*, uses Mg^{2+} and NADPH as cofactors to catalyze the reduction and isomerization of *DXP* to 2-Cmethyl-D-erythritol-4-phosphate (*MEP*). Fosmidomycin, a potential inhibitor of *DXR* and an organic antibiotic, has already been found; preclinical research and clinical trials have shown that it possesses antimalarial properties (Jomaa et al., 1999; Oyakhirome et al., 2007). These results suggest that *DXR* is a realistic goal, and that apicomplexan's chemotherapy might benefit from using inhibitors of this enzyme. Aminotransferases play a significant part in a variety of metabolic processes, including as amino acid catabolism and anabolism, vitamin production, carbon and nitrogen uptake, and additional metabolism, and gluconeogenesis, among others (Liepman and Olsen, 2004; Oyakhirome et al., 2007). The purpose of the research was to determine the genotoxicity of salinity on *C. limon* plants using a comet assay and to utilize the transcriptome technology for the expression of genes associated with terpenes in tissue-cultured plants exposed to different salinity levels.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_18671883

Materials and Methods

Plant components and salinity in vitro testing

The seeds of *C. limon* were obtained from Taif, Saudi Arabia's Al-Shafa region, and disinfected by rinsing with 70% ethanol alcohol and a few droplets of Tween 20 for thirty seconds. Seeds were then washed using three rounds of distilled water, followed by a ten-min immerse in 1% sodium hypochlorite (Market Clorox solution, 10%) for 10 minutes in sterile circumstances within a hood with laminar air flow. After that, sterile distilled water was used to rinse them five times. The nutritious (Murashige and Skoog, 1962) medium (MS), which contains 3% sucrose and 0.7% agar for solidification, was aseptically inoculated with three sterilized seeds per jar. After dividing the media into growth jars (30 mL each), it was autoclaved at 121 °C for 20 minutes to sanitize it after being adjusted to a pH of 5.8. Seed cultures were grown at 25 °C in the absence of light for 10 days. Every photoperiod, cultures were relocated under 2,000 Lux of 16 hours of constant cool white fluorescent lighting. Following germination, explants were subcultured twice weekly in uncontaminated circumstances making use of identical MS media with varying numerous NaCl concentrations (0, 50, 100, and 200 mM), maintaining the previously stated culture parameters. Growth characteristics and the impact of genotoxicity were assessed after being preserved fourteen days of salt exposure using the protocol outlined below.

Genotoxicology test

Leaf explants isolated from the seedlings were maintained in a petri dish using Sörensen buffer (50 mM sodium phosphate), 0.1 mM of EDTA, or ethylene diamine tetra acetic acid, 0.5% DMSO stored on ice, and a 6.8 pH value. The transplanted leaf tissue was carefully sliced, and then the final product was repeatedly submerged in the freezing cold Sörensen's reserve. After filtering the released nuclei suspension using a disposable 30 µm filter (Partec, Münster, Germany) to get rid of most of the contaminants, it went through a centrifuge for five minutes at 4 °C at 550 g.

Nuclear separation

The leaves were put in a petite Petri plate with 200 µL of 400 mM Tris-HCl (on ice) solution that was cool and pH 7.5. A "fringe" formed by the softly split leaves under yellow light to allow atoms to enter the buffer. It has been observed that using this method to isolate nuclei is the most efficient way to obtain cells from control samples with little DNA damage. At the first, nuclear suspension (1%) produced using phosphate-buffered saline was immersed in low melting point (LMP) agarose, then the slide had a layer of 1% agarose with a normal melting point (NMP) and dried at 40 °C prior to getting coated in a slip. The coverslip was taken off the slide after at least five minutes on the ice. After reinstalling the coverslip, 0.5% LMP agarose in 110 µL were carefully overflowed the top. The coverslip was gradually removed after five minutes of being placed on ice. The mutagen solutions were applied before being washed three times in cold, distilled water for five minutes each time, for two hours, slides utilized in single cell gel electrophoresis (SCGE) were heated at 26 °C. Slides used in a horizontal gel electrophoresis containing plant cell nuclei container stuffed newly made (300 mM NaOH, 1 mM EDTA, pH > 13) chilled electrophoresis solution kept for 15 minutes. Electrophoresis procedure included the following settings: 16 V, 300 mA, and 4 °C for 30 minutes. In previous research, the electrophoresis settings used in this experiment were appropriate since they produced

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_18671883

negligible amounts of DNA toxicity in control cells and a linear level-response for the formation of comets following chemical mutagenic treatment. Ethidium bromide (20 g/mL) staining was applied to the gels for five minutes having been three times 400 mM Tris-HCl (pH 7.5) neutralized the reaction. Gels then colored, immediately immersed within icy distilled water, and inspected. 50 cells from each slide were randomly examined using a fluorescence microscope covered with a 546 nm a stimulus filter and a 590 nm gate filter using a photo processing computer (Komet Version 3.1., Kinetic Imaging, Liverpool, UK). DNA (TD %) and the Tail moment (TM) were used as measures for DNA damage (Juchimiuk et al., 2006).

RNA extracting and expression of genes

Extraction of RNA

After extraction, total RNA was obtained from samples of the *C. limon* plant (MacRae, 2007). Five hundred µL of triazole were added to 0.5 g of each treatment. The material had been ground finely, together with Chloroform, 100 µL. Centrifuge for five minutes at 10,000 rpm. The top layer was gently scraped off and it was mixed with 250 µL of isopropanol. The resulting mixture was ultimately five minutes of centrifuging at a speed of 10,000 rpm. Once the little ball has formed, the filtrate must be carefully removed. After mixing 500 µL of 75% ethanol, and DEPC water 25%, the mixture was centrifuged for 5 minutes. Once the minuscule RNA particles have dried, discard the filter, and allow them 15 minutes at 55–60 °C in a water bath to dissolve before adding 50 µL of DEPC water. Using 1% agarose in an agarose gel electrophoresis, the purity of the extracted RNA was confirmed. To prepare it, 1X TBE buffer was used to dissolve the agarose. The extracted RNA was examined using a Biometra UV star 15 transilluminator for UV light.

Reaction of cDNA first-strand synthesis

Thermoscientific Recert Kit for First Strand cDNA Synthesis, Lithuania, was used to create cDNAs by completing a 20 µL final reaction volume with 2 µL of total RNA as directed by the manufacturer. The cDNA synthesis method includes the following steps, the following ingredients were added: 20 µL of RiboLock RNase inhibitor, 2 µL of 10 mM dNTP, 1µL (dT) 18 Primer mix, 200 µL Revert Aid RT, 4 µL of 5X Reaction Buffer, and 9 µL of nuclease-free water. Finally, 20 µL of treated water containing diethylpyrocarbonate (DEPC) was added, mixed, and incubated for 60 minutes at 42 °C. After that, the mixture was heated to halt the process for five minutes at 70 °C. The cDNAs were refrigerated for at least 3-5 minutes after receiving them.

The PCR technique

Transcribing in reverse was done using the Promega High Capacity Access RT-PCR System on whole RNA aliquots that had been isolated in accordance with the directions provided by the manufacturer. Previous studies have been taken into consideration when selecting the primer, the genes' coding sequences the supplementary metabolites *RtME, RtIDH, RtFPPS, RtLs, PAL* and *RtDxR* (*Table 1*). The primary PCR reaction blend was made as follow: overall volume of the master mix was up to 20 U with the addition of ddH2 12.8 U and the use of a detrimental control. The original quantities were 0.6 U primer, 4 U PCR, and 2 U cDNA. RT-PCR assays that were semi-quantitative were conducted using a Thermo Scientific PXE 0.5 thermocycler. They were cycles according to: Step 1 lasts for 2-4 minutes at 94 °C; Step 2 (40 Cycles) lasts for 30 s at 94 °C; 1

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_18671883

minute at 61.1 °C; Step 3 lasts for 7 minutes at 68 °C; and Step 4 lasts for 4 °C. The SqRT-PCR data were examined via conventional Electrophoresis on agarose gel.

Table 1. *PCR amplification primers*

Genes	Forward primer 5′– 3′	Reverse primer 5′– 3′	Gene pathways for terpene biosynthesis	References
RtME	GCCACTGATAAGTTTGAGATGT	CCATCTCATCACTACCAATGTTC	mannose-3′, 5′ -epimerase	Doubnerová and Ryslavá, 2011
RtIDH	CCGATCTAATGTTGGTCCAGAGA	CATCTGCACCAATATCCTTTGCAA	L-idonate 5-dehydrogenase	Lemaitre and Hodges, 2006
RtFPPS	GGCACTAGAACTTTCAAACGAA	CTTGCTCTCGTACTCCATAAATG	Farnesyl pyrophosphate synthase	Zhou and Zhu, 2020
RtLs	CTTTCGACTTCTCAGACAACAAG	CAGCCTCTTCAAGTACTCTATCT	Linalool synthase	Zerihun et al., 2011
PAL	CCAAGATGATCGAGAGAGAGATCAA	TAGCTCAGAGAATTGAGCGAAGAGA	Phenylalanine ammonia-lyase	Zhou and Zhu, 2020
RtDxR	GTTGCGGTAAGAAATGAGTCAT	GCAACCTACTATCCCTGTAACTA	Deoxy-D-xylulose 5-phosphate reductoisom	Zhou and Zhu, 2020
Actin	GTTCTCAGTGGTGGCTCAACTATGT	GAGGAGCAACCACCTTAATCTTCAT	House keeping	Nicot et al., 2005

Agarose gel separation

As previously mentioned, PCR samples were investigated using 1.5% gel agarose that was generated for 90 minutes at 100 V. UV transelements were used to photograph and display the samples. The sizes of reaction byproducts included computed due to the 100-1500 bp DNA molecular size range. GelPro32, version 4.03, was utilized on a computer to quantify the created bands.

Statistics

The results' standard deviation and mean are displayed (SD). For each plant sample and four biological samples, each data point shows the average over five replicates. The software program GraphPad Prism 8 (GraphPad Software, La Jolla, California, USA) was used for all statistical calculations. To evaluate the data, analyzing variance in one way (ANOVA) with Duncan's test was employed to compare means. Major variations were detected when the p-values were less than 0.05.

Results

Characteristics of C. limon tissue cultures' growth

The salt tolerance of *C. limon* was examined by placing it under specific salinity conditions (0, 50, 100, and 200 mM of NaCl). According to our findings, *C. limon* plants exposed to 100 and 200 mM NaCl, respectively decreased plant length by 6.33 and 4.83 cm (*Figures 1, 2*), whereas, plants subjected to MS medium with 0 and 50 mM supplements of NaCl valued 8.63 and 7.4 cm. The number of leaves was also decreased with values 6.33 and 5 when plants exposed to 100 and 200 mM NaCl, while it was 10 and 8 in plants treated with 0 and 50 mM NaCl, respectively (*Figure 3*).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_18671883

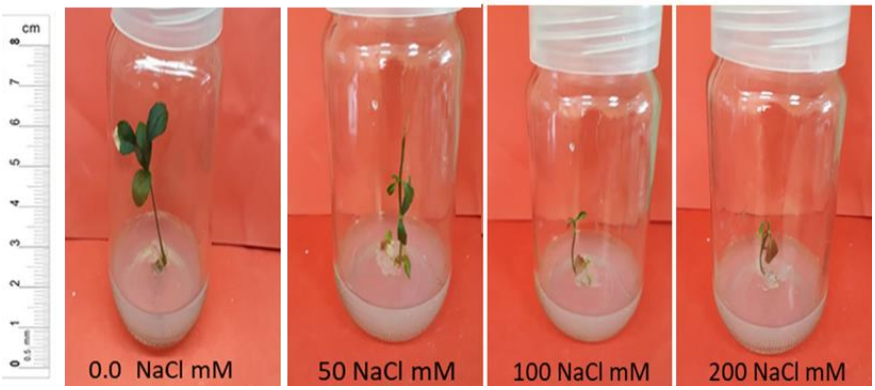

Figure 1. *After 14 days, the growth characteristics of C. limon plants grown on MS media supplemented with 0, 50, 100, and 200 mM of NaCl were assessed*

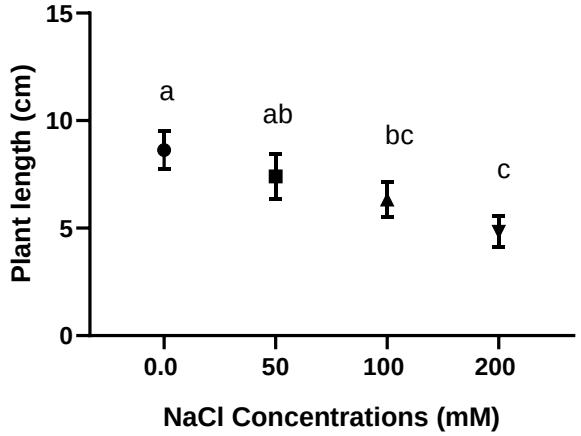

Figure 2. *C. limon plants were measured for plant length (cm) after being exposed to various doses of NaCl salt stress for an additional 15 days while being developed in full strength MS culture media. Bars with identical letter annotations are not substantially differ ($p < 0.05$, based on Duncan test)*

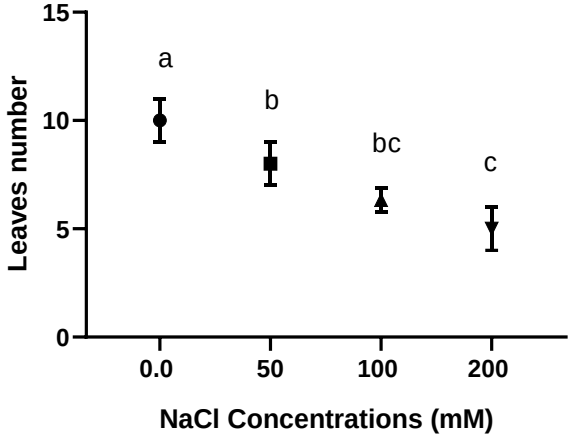

Figure 3. *The number of leaves on C. limon plants after 10 days of growth in full strength MS culture media under different degrees of NaCl salinity stress. Bars tagged with the same letter don't have notable variations ($p < 0.05$, according to the Duncan test)*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_18671883

Comet assay

Each *C. limon* plant cultivated in M.S media with 0.0, 50, 100, and 200 mM NaCl added was evaluated for DNA damage to the nucleus. *Figures 4 and 5* show that, in all samples, damage appeared to grow from 200 mM NaCl, which resulted in a notable rise in the tail moment, DNA tail%, tail length, DNA head%, and tail moment, respectively. *C. limon* plants underwent dose 100 mM NaCl scored DNA damage at a tail moment of 2.21 units in comparison to untreated plants (Control) as appeared in *Figure 5D*. DNA damage was reflected in the tail at 200 mM NaCl 15.62% (*Figure 5B*), which is a highly significant increase when compared to control plants. When comparing plants treated with 100 mM NaCl to untreated ones, the level of damage acquired 11.41% with a noticeable rise. When the tail length reached 10.65 µm, the levels of 100 mM NaCl caused a significant amount of damage (*Figure 5C*). When *C. limon* plants were cultivated on 100, 200, and 50 mM NaCl in M.S. medium, accordingly, damage reflected by head DNA% reached 94.84, 88.57, and 84.33% levels of damage, which is considered to be relatively significant (*Figure 5A*). Regarding DNA tail length and other criteria, the control treatment showed no damage. Prior measurements of the comet test parameters revealed non-significant damage in tail lengths of 6.57 and 8.06 µm. When 50 and 200 mM of NaCl were added to MS medium, the greatest decrease in all damage metrics as measured by control plants.

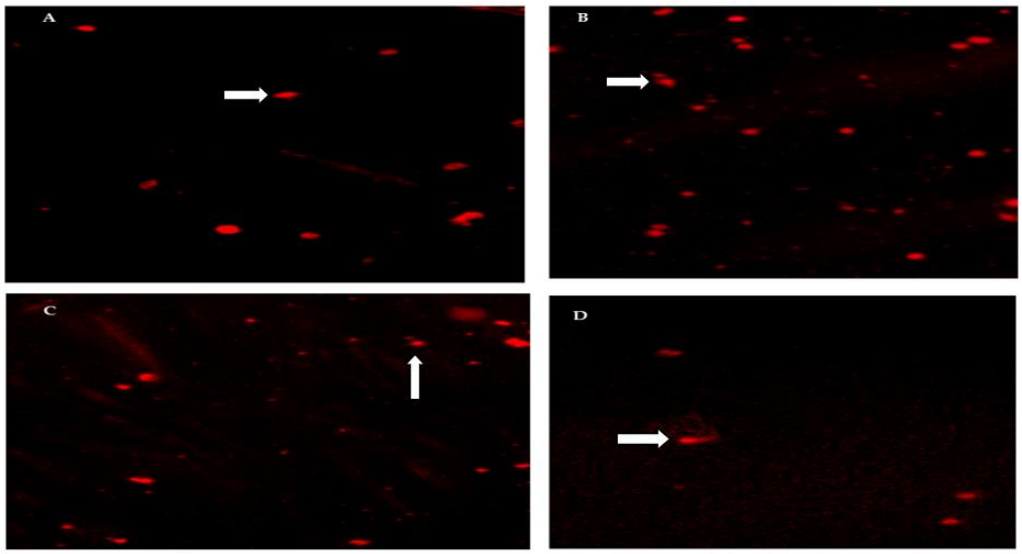

Figure 4. *DNA damage of C. limon seedling leaf nuclei cultivated on MS media at various salinity variant levels. (A) 0.0 mM NaCl, (B) 50 mM NaCl, (C) 100 mM NaCl, and (D) 200 mM NaCl. Arrows indicate to the changes in DNA damage among salinity levels*

Gene expression

Changes for terpene synthetic transcription are linked due to the plant's response to increased abiotic stress caused by NaCl, as shown by the impact of salinity on in vitro cultivation of four different *C. limon* treatments motivated *RtME*, *RtIDH*, *RtFPPS*, *RtLS*, *PAL* and *RtDXR* genes. Therefore, it was prudent to monitor the expected precursor number for the pathway.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_18671883

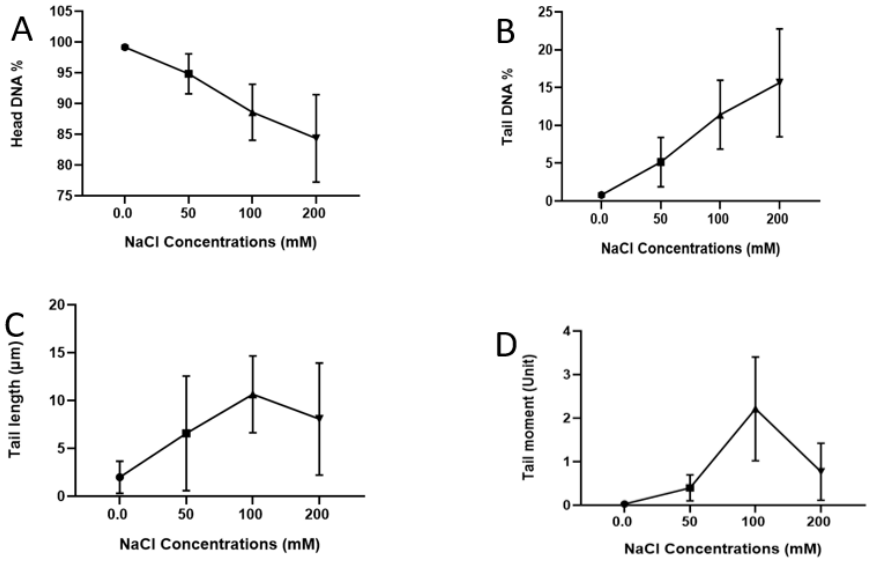

Figure 5. *DNA toxicity dose exposure curve for nuclei obtained from C. limon culture cells on MS medium at various NaCl concentrations. (A) Head DNA%, (B) Tail DNA%, (C) Tail moment (Unit), and (D) Tail Length (µm)*

Comparing *C. limon* plants that have received varied concentrations of housekeeping Actin gene and of NaCl to regulate plants, as well as any recorded changes related to these comparisons, is done using six primers of terpene biosynthesis pathway in semi-quantitative PCR (*Figure 6*). According to the expression profiles of *RtME* and *RtDXR*, the findings demonstrated that *C. limon* plants significantly minimized the alternative collection of transcripts produced by salt. In *C. limon* plants those were cultured on MS medium containing 100 mM NaCl, the expression patterns showed an immediate reaction and sharp increase in *RtIDH* transcript amount, which reached 1.91 as opposed to 1.13 in plants exposed to 50 mM NaCl. When *C. limon* plants were grown in a 200 mM NaCl medium, the transcript quantity of *RtIDH* decreased to 0.58. In contrast, plants grown in a zero mM NaCl media scored 1.01. On comparison to control, plants with a salinity problem those cultured on MS media at 50, 100, and 200 mM NaCl displayed enhanced *PAL* a description of 1.72, 2.08, and 2.1. Additionally, there was not a noticeable change in the results between the transcripted plants grown on media containing 100 and 200 mM NaCl. The plants received 100 mM NaCl got a considerably greater number of transcripts (2.21), when the *RtLS* increase of gene transcripts was observed in salt circumstances, in contrast to the untreated plants, which scored about 1.9, and compared with treated plants with 50 mM NaCl transcript level (2.003). It was clear that plants grown on media containing 100 mM NaCl had *RtFPPS* transcript levels of 2.02 that were comparable to plants grown on media without NaCl which transcript levels of 1.67. Whereas, plants supplied with 200 mM NaCl had transcript levels reduced to 1.8 that were comparable to plants grown on media with 100 mM NaCl, the same gene scored its lowest transcription 1.34 when *C. limon* cultures exposed to MS medium supplemented with 50 mM NaCl. After repairing *RtDXR* transcripts linked to plants grown in MS media at 0.0 and 200 mM NaCl, plants did not exhibit any clear targets; both transcripts were 0.9, and the difference between them was not statistically significant. While, the largest expression was 1.23 at the level of 50 mM NaCl and, the lowest expression 0.60 appeared when plants exposed to NaCl 100 mM. The findings revealed that plants grown on

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_18671883

medium with 100 mM NaCl scored 0.3 whereas those grown on medium containing 50 mM NaCl had *RtME* transcript 0.15 if compared with the level of NaCl 0.0 mM expressed 0.18, but the level of 200 mM NaCl scored the low expression of *RtME* gene 0.05. There was a not significant increase between the transcript amount 0.34 cultured on 100 mM NaCl when compared with the transcript amount 0.15 scored when culture plant medium supplemented to 50 mM NaCl, but there was a significant decrease between the transcript number of plants cultured on 200 mM NaCl and plants cultured on 100 mM NaCl.

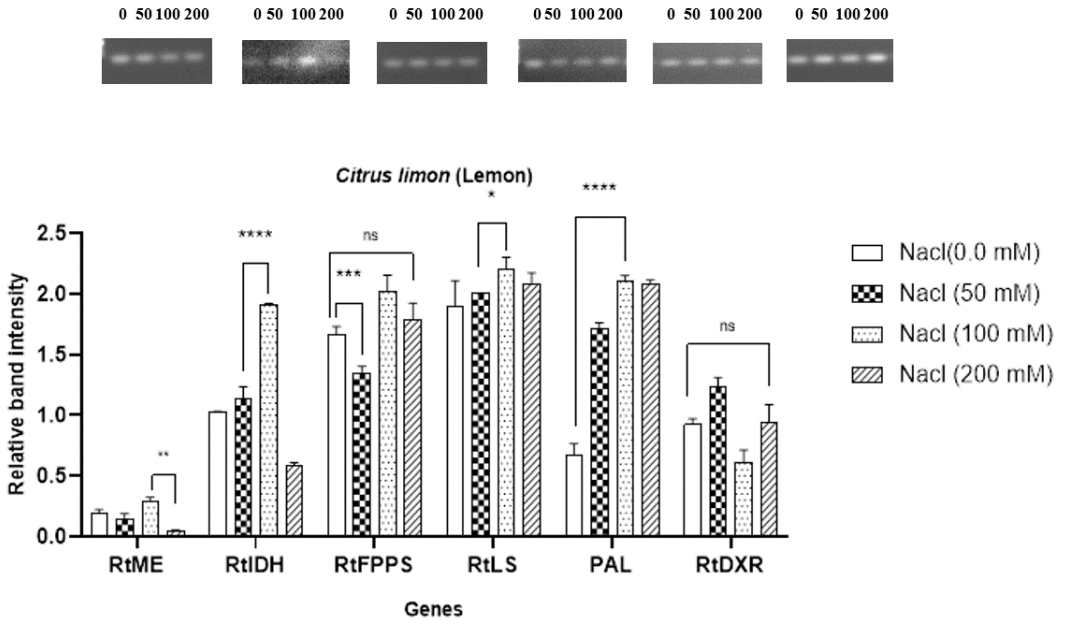

Figure 6. *RT-PCR analysis of C. limon cell cultures revealed transcription of RtME, RtIDH, RtFPPS, RtLS, PAL, and RtDXR was shown to increase when exposed to salt stress. The findings are presented as mean ± SD (ns, not significant, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001). Changes in terpene biosynthetic exposure to NaCl at levels of (0.0, 50, 100, and 200) are related to transcription. As an internal standard for normalization, actin was used*

The amount of *RtME* transcript gradually decreased under salt stress conditions. The amount of *RtDXR* in salinity conditions, transcript quality was at its lowest point, in cultured plants given 0, 50, 100, and 200 mM NaCl treatments, significant quantities and specific activity of *RtLS*, a crucial enzyme in the route of linalool synthesis, were discovered (*Figure 6*). In contrast to plants under salt stress at 200 and 100 mM NaCl, data proved the *RtLS* transcript levels in control plants increased significantly by 1.9. Compared to the treatments previously mentioned, in relation to band intensity rose when *C. limon* plants were cultured in 50 mM NaCl 2.0, but it was not statistically significant. The findings unmistakably shown that the involved gene in Phenylalanine Ammonia-lyase Biosynthesis *PAL* in *C. limon* plants was impacted. Nevertheless, this transcription increase was not induced by NaCl salinity at the various salinity concentrations, substantial. The genes' patterns of expression *RtFPPS*, *RtIDH*, and *PAL* were identified transcription was shown to increase when exposed to salt stress in response to salt exposure, as shown in (*Figure 6*).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_18671883

Discussion

Salt stress clearly inhibits poplar development, according to certain results (Wang et al., 2008). Three genera of sweet basil showed growth decline, which may be related to a reduction in water intake from salt growing media (Saira et al., 2014). Many plants showed reduced development caused by salinity as lucerne and canola (Rogers et al., 2003; Zadeh and Naeini, 2007). Additionally, restriction of growth in various medicinal plants in saline conditions further supports our findings (Zia and Khan, 2002). In the current study, the development of *C. limon* plants was reduced, which may have been caused by the culture medium's low water content, which increased osmotic pressure; this typically happens in situations of salt stress (Munns et al., 2006). Therefore, the growth of *C. limon* plants is impacted by various amounts of NaCl salt stress. Data cleared, the development of lemon plants was impacted by varying levels of NaCl salt stress, and non-saline lemon plants grew more than treated plants. In salt-affected plant cultures, plant development is mostly suppressed for two reasons. Firstly, salinity in plant cultivation firstly reduces the capacity of the plants to absorb from the media, which results in slower growth. Salt stress essentially causes this osmotic action. Second, the accumulation of salts in photosynthetic leaves can harm leaf cells, which can impair photosynthesis and plant growth (Munns et al., 2006). A decrease in water intake from saline growth media may be the cause of the growth depression in three salt levels of cultivars (Jamil et al., 2006), this result was agreed with current study. The results are also consistent with Keshavarzi (2011). Additionally, it was shown that plant growth is impeded by salinity stress because of the imbalance, ion toxicity, and low water potential that these factors excrete (Greenway and Munns, 1980). Current research shows that the 200 mM/L stress remedy with NaCl decreased the number of leaves. By obtaining a sizable amount of NaCl from the trees, the leaves served as a mechanism for salt removal (Chen et al., 1997). In their leaves, a number of halophytes show the presence of modified trichomes, which are salt secretory structures with epidermal origins (Shabala et al., 2014).

The percentage of DNA in the tail, which is independent of tail length and represents the comet's overall intensity, is the metric that most correctly represents DNA damage. NaCl is a genotoxic stimulus that alters recombination rates in a transgenic manner by causing somatic and rational alterations. It is stated how adding NaCl to plants can harm their DNA. These modifications were initially caused by contact with Cl ions (Boyko et al., 2010; Nikolova et al., 2013). An earlier investigation revealed that oxidative stress increases the genotoxicity of the element Al. Using the comet test, the mechanisms of Al genotoxicity were investigated, highlighting the function of bound cell walls anchored NADHPX in Al genotoxicity brought on by oxidative bursts (Achary et al., 2012). Moreover, the process by which Ca^{2+} sends messages (Achary et al., 2013) and the MAP Kinases (Panda and Achary, 2014) as a result of Al, DNA damage and cell death. As stated by Monteiro et al. (2012), these evident results are due to the creation of longer DNA fragments and protein cross-links because of high concentrations of Cd-DNA, as well as the incapacity of DNA repair systems. Lead induces genotoxicity, which plays a critical role in oxidative stress and DNA damage, both of which have been investigated in two studies on *Allium cepa* (Jiang et al., 2014; Kaur et al., 2014). Boyko et al. (2021) established that the chloride ion was responsible for sodium chloride's effect on genome stability. Rearrangements in the genome occur frequently and increase when chloride ions were added to the medium, whereas recombination rates (RR) were unaffected by sodium ions. How precisely the Cl^- ion harm influences the delicate complement of DNA is still a mystery. In plants, Na^+ poisoning results in a startling loss in ion homeostasis. In line

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_18671883

with Hasegawa et al. (2000), K^+ deficiency is brought on by salt stress, and genotoxicity requires Cl^-. NaCl salt was applied to four treatments of *C. limon* plants, with the main impact on the quantity of secondary plant products. In fact, delayed growth of plants under abiotic stresses like NaCl stress is seen to demonstrate their capacity for adaptation and continued health (Sabir et al., 2012). The present research showed that under salt stress, terpenoid synthesis increased significantly despite cell damage restricting growth and the availability of definite sources for precursors to metabolites. The cooperation of pathway- genes unique was primarily responsible for terpenoid accumulation (Sangwan et al., 2011) concluding that, phytochemicals produced by glandular cells are necessary for the plant to defend itself from abiotic and biotic stressors.

The transcription activity for *RtME, RtIDH*, *RtFPPS*, *RtLS*, *PAL*, and *RtDXR* was contrary to that of the secondary metabolites' synthase enzyme, substantially found in *C. limon* when cultivated on the medium supplemented with 200, 100, and 50 mM NaCl. Multiple isoforms of the protein family known as malic enzyme *RtME* can be found in various eukaryotic cell compartments. A crucial enzyme controlling the metabolism of malic acid is the malic enzyme *RtME*. It can work with reversible malate oxidative decarboxylation is catalyzed by the coenzyme, generate pyruvic acid and CO_2, and assist in the synthesis of NAD(P)H. Plant MEs are classified as either NAD^+ dependent malic enzymes (NAD-ME; EC 1.1.1.38 and EC 1.1.1.39) or $NADP^+$ dependent malic enzymes (NADP-ME; EC 1.1.1.40) depending on the cofactors (Liu et al., 2011). Furthermore, NADP serves as a crucial reducing agent by supplying NADPH for the synthesis of protective compounds including lignin and flavonoids. Additionally, it is a component necessary for the metabolism of reactive oxygen species (ROS). NAD-ME, on the other hand, predominantly functions as an oxidant during catabolism, which uses oxidation to produce energy (ATP) (Chen et al., 2019). Under 100 mM salinity stress, *RtME* in *C. limon* leaves considerably rises, and it is discovered that the de novo synthesis of NADP-ME is improved. Anions like malic acid and Cl build in cells during salt stress to counteract the excess Na^+ in plant. Currently, it is expected that the cells' ME produced will act a role in the metabolism of malic acid (Shao et al., 2011). Isocitrate dehydrogenase, NAD-dependent a Krebs cycle enzyme found in mitochondria is called *RtIDH*. Although it may be involved in glutamate synthesis, it is thought that the main role of *IDH* is as a Krebs cycle enzyme. The availability of NaCl to *C. limon* plants affects the transcript level of the gene coding for *IDH* (Lancien et al., 2000; Abiko et al., 2005). However, the gene encoding *FPPS* was up-regulated by a 100 mM NaCl supply, its transcript level only increased by 2.017 compared to 50 mM NaCl-treated *C. limon* plants, which only recorded a 1.347 increase. As a crucial enzyme in the isoprenoid biosynthesis process, farnesyl diphosphate synthase *FPPS* produces sesquiterpene precursors for a variety of essential metabolites, such as sterols, dolichols, ubiquinones, and carotenoids, as well as substrates for the geranyl geranylation and farnesylation of proteins. It causes the two molecules of dimethylallyl diphosphate and isopentenyl diphosphate to sequentially condense from head to tail. *FPPS* enzyme is the homodimer of subunits (Szkopinska and Plochocka, 2005). According to reports, the *Arabidopsis thaliana* encoding gene *FPPS* is under transcriptional and expression regulation. Without the signal sequence, it is still feasible to transcribe and translate a protein or peptide into its mitochondrial-targeted isoform (Cunillera et al., 1997). The 100 mM NaCl boosted the activity of linalool *RtLS* in *C. limon*. The present data supported (Lane et al., 2010) in which linalool was shown to be the main ingredient in *Lavandula angustifolia* essential oil, and it was directly demonstrated that *RtLS* expression is fully tied to the linalool

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_18671883

content in lavender flowers. This is not remarkable because terpenoid synthases can consistently change a single molecule into a multitude of molecules (Tholl et al., 2005; Degenhardt et al., 2009). Results showed disagreement, heterogeneity in gene transcript levels, and genotype-specificity overall. In response to culture conditions, it is found that each genotype exhibits variable levels of their up- or down-regulation. In comparison to *C. limon* plant cultures exposed to free NaCl, additionally, the *PAL* gene's expression was raised by up to 2.1-fold. As the first stage in the phenylpropanoid pathway and a crucial transitional regulator between primary and secondary metabolism, cinnamic acid is created when *PAL* catalyzes the deamination of Phe (Raes et al., 2003). For functional investigation of the *PAL* gene family in plant growth, development, and responses to environmental challenges, furthermore, other studies have made use of genetic and molecular methods to mute or interfere with the *PAL* genes (Dixon and Paiva, 1995). In *C. limon* culture media supplemented with 50 mM NaCl, *DXR* expression level increased to 1.2, but lower expression levels were connected to the levels of 0.0 and 100 mM NaCl. *DXR*, also known as 1-deoxy-D-xylulose-5-phosphate reductoisomerase, is the second enzyme in the pathway, which uses the cofactors Mg^{+2} and NADPH to catalyze the reduction and isomerization of *DXP* to 2-methyl-D-erythritol-4-phosphate (MEP) (Oyakhirome et al., 2007). These results suggest that *DXR* is a legitimate target, and that apicomplexan's chemotherapy might benefit from using inhibitors of this enzyme.

Conclusions

In a separate experiment, the plant comet assay seemed to be a suitable technique for determining DNA damage brought on by subjecting plants to known genotoxic doses, especially high salt stress levels (100 and 200 mM NaCl). Semi-quantitative RT-PCR was used to demonstrate the relatively steady expression of *RtME* and *RtDXR* transcripts in *C. limon* plants under the pressure of saltiness. The results corroborated the idea that *RtIDH*, *RtFPPS*, *RtLS*, and *PAL* might be regulated post-transcriptionally, resulting in their abundance in plant cells under salinity stress.

Acknowledgements. The author would like to thank the Deanship of Scientific Research at Taif University for funding this work through the project number (202214), Taif University, Taif, Saudi Arabia.

REFERENCES

[1] Abad-García, B., Garmón-Lobato, S., Berrueta, L. A., Gallo, B., Vicente, F. (2012): On line characterization of 58 phenolic compounds in Citrus fruit juices from Spanish cultivars by high-performance liquid chromatography with photodiode-array detection coupled to electrospray ionization triple quadrupole mass spectrometry. – Talanta 99: 213-224.

[2] Abiko, T., Obara, M., Ushioda, A., Hayakawa, T., Hodges, M., Yamaya, T. (2005): Localization of NAD-isocitrate dehydrogenase and glutamate dehydrogenase in rice roots: candidates for providing carbon skeletons to NADH-glutamate synthase. – Plant Cell Physiol 46: 1724-1234.

[3] Achary, M., Parinandi, N. L., Panda, B. B. (2012): Aluminum induces oxidative burst, cell wall NADH peroxidase activity, and DNA damages in root cells of *Allium cepa* L. – Environ Mol Mutagen 53: 550-560.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_18671883

[4] Achary, V. M., Pariani, N. L., Panda, B. B. (2013): Calcium channel blockers protect against aluminium - induced DNA damages and block adaptive response to genotoxic stress in plantcells. – Mutat Res Genet Toxicol Environ Mutagen 751: 130-138.
[5] Angelis, K. J., Dusinská, M., Collins, A. R. (1999): Single cell gel electrophoresis: detection of DNA damage at different levels of sensitivity. – Electrophoresis 20: 2133-2138.
[6] Ashour, M., Wink, M., Gershenzon, J. (2010): Biochemistry of terpenoids: monoterpenes, sesquiterpenes and diterpenes. – Annu Plant Rev 40: 258-303.
[7] Azizi, A., Yan, F., Honermeier, B. (2009): Herbage yield, essential oil content and composition of three oregano (*Origanum vulgare* L.) populations as affected by soil moisture regimes and nitrogen supply. – Ind Crop Prod 29: 554-561.
[8] Azqueta, A., Meier, S., Priestley, C., Guzkow, K., Brunborg, G., Sallette, J., Soussaline, F., Collins, A. (2011a): The influence of scoring method on variability in results obtained with comet assay. – Mutagenesis 26(3): 393-399.
[9] Azqueta, A., Shaposhnikov, S., Collins, A. (2011b): DNA repair measured by comet assay. – In: Kruman, I. (ed.) DNA repair. Intech, Croatia, pp. 615-636.
[10] Bañuls, J., Serna, M. D., Legaz, M., Primo-Millo, E. (1997): Growth and gas exchange parameters of Citrus plants stressed with different salts. – J Plant Physiol 150: 194-199.
[11] Bhatia, H., Pal, S. Y., Manhas, R. K., Kumar, K. (2015): Traditional phytoremedies for the treatment of menstrual disorders in district Udhampur, J&K, India. – J Ethnopharmacol 160: 202-210.
[12] Boyko, A., Golubov, A., Bilichak, A., Kovalchuk, I. (2010): Chlorine ions but not sodium ions alter genome stability of *Arabidopsis thaliana*. – Plant Cell Physiol 51: 1066-1078.
[13] Boyko, A., Tsepkova, P., Aleshin, V., Artiukhov, A., Mkrtchyan, G., Ksenofontov, A., Baratova, L., Ryabov, S., Graf, A., Bunik, V. (2021): Severe spinal cord injury in rats induces chronic changes in the spinal cord and cerebral cortex metabolism, adjusted by thiamine that improves locomotor performance. – Front Mol Neurosci 14: 620593.
[14] Chen, Q., Wang, B., Ding, H., Zhang, J., Li, S. C. (2019): The role of NADP-malic enzyme in plants under stress. – Plant Sci 281: 206-212.
[15] Chen, S., Wang, S., Arie, A., Huttermann, A. (1997): Genotypic variation in drought tolerance of poplar in relation to abscisic acid. – Tree Physiol 17: 797-803.
[16] Collins, A., Azqueta, A., Brunborg, G., Gaivão, I., Giovannelli, L., Kruszewski, M., Smith, C. C., Stetina, R. (2008): The comet assay: topical issues. – Mutagenesis 23: 143-151.
[17] Collins, A., Moller, P., Gajski, G., Duthie, S. J. et al. (2023): Measuring DNA modifications with the comet assay: a compendium of protocols. – Nature Protocols 18: 929-989.
[18] Cunillera, N., Boronat, A., Ferrer, A. (1997): The *Arabidopsis thaliana* FPS1 gene generates a novel mRNA that encodes a mitochondrial farnesyl-diphosphate synthase isoform. – J Biol Chem 272: 15381-15388.
[19] Davenport, S. B., Gallego, S. M., Benavides, M. P., Tomaro, M. L. (2003): Behaviour of antioxidant defense system in the adaptive response to salt stress in *Helianthus annuus* L. cells. – Plant Growth Regul 40: 81-88.
[20] Degenhardt, J., Köllner, T. G., Gershenzon, J. (2009): Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. – Phytochemistry 70: 1621-1637.
[21] Dixon, R. A., Paiva, N. L. (1995): Stress-induced phenylpropanoid metabolism. – Plant Cell 7: 1085-1097.
[22] Doubnerová, V., Ryslavá, H. (2011): What can enzymes of C4 photosynthesis do for C3 plants under stress? – Plant Science 180(4): 575-583.
[23] Duran-Vila, N., Gogorcenna, Y., Ortega, V., Ortiz, J., Navarro, L. (1992): Morphogenesis and tissue culture of sweet orange (*Citrus sinensis* L.) Osb: Effect of temperature and photosynthetic radiations. – Plant Cell Tis Org Cult 29: 11-18.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_18671883

[24] Feng, F. C., Wang, Z. C., Li, R. F., Wu, Q., Gu, C., Xu, Y., Peng, W. P., Han, D., Zhou, X. M., Wu, J., He, H. L. (2019): Citrus alkaline extracts prevent fibroblast senescence to ameliorate pulmonary fibrosis via activation of COX-2. – Biomedicine & Pharmacotherapy 112: 108669.
[25] Gichner, T., Menke, M., Stavreva, D. A., Schubert, I. (2000): Malic hydrazide induces genotoxic effects but no DNA damage detectable by the comet assay in tobacco and field beans. – Mutagenesis 15(5): 385-389.
[26] Gichner, T., Znidar, I., Wagner, E., Plewa, M. (2009): The use of higher plants in the Comet Assay. – In: Dhawan, A., Anderson, D. (eds.) Isues in Toxicology 5: The Comet Assay in Toxicology. Royal Society of Chemistry, London, pp. 98-119.
[27] Goetz, P. (2014): *Citrus limon* (L.) Burm. f. (Rutacées). Citronnier. – Phytotherapie 12: 116-121.
[28] Gómez-Cadenas, A., Tadeo, F. R., Primo-Millo, E., Talon, M. (1998): Involvement of abscisic acid and ethylene in the response of citrus seedlings to salt shock. – Physiol Plant 103: 475-484.
[29] Greenway, H., Munns, R. (1980): Mechanism of salt tolerance in non-halophytes. – Ann Rev Plant Physiol 31: 149-190.
[30] Gu, R., Liu, Q., Pei, D., Jiang, X. (2004): Understanding saline and osmotic tolerance of *Populus euphratica* suspended cells. – Plant Cell Tissue Organ Cult 78: 261-265.
[31] Hasegawa, P. M., Bressan, R. A., Zhu, J. K., Bohnert, H. J. (2000): Plant cellular and molecular responses to high salinity. – Annu Rev Plant Physiol Plant Mol Biol 51: 463-499.
[32] Hassanein, A. M., Azooz, M. M. (2003): Propagation of *Citrus reticulata* via in vitro seed germination and shoot cuttings. – Biol Plant 47: 173-177.
[33] Jamil, M., Lee, D. B., Jung, K. Y., Lee, S. C., Rha, E. S. (2006): Effect of salt (NaCl) stress on germination and early seedling growth of four vegetables species. – J Central Europ Agri 7(2): 273-282.
[34] Jang, J. C., León, P., Zhou, L., Sheen, J. (1997): Hexokinase as a sugar sensor in higher plants. – The Plant Cell 9(1): 5-19.
[35] Jiang, Z., Qin, R., Zhang, H., Zou, J., Shi, Q., Wang, J. (2014): Determination of Pb genotoxic effects in *Allium cepa* root cells by fluorescent probe, microtubular immunofluorescence and comet assay. – Plant Soil 383: 357-372.
[36] Jomaa, H., Wiesner, J., Sanderbrand, S., Altincicek, B., Weidemeyer, C., Hintz, M., Türbachova, I., Eberl, M., Zeidler, J., Lichtenthaler, H. K., Soldati, D., Beck, E. (1999): Inhibitors of the nonmevalonate pathway of isoprenoid biosynthesis as antimalarial drugs. – Science 285(5433): 1573-1576.
[37] Juchimiuk, J., Gnys, A., Maluszynska, J. (2006): DNA damage induced by mutagens in plant and human cell nuclei in acellular comet assay. – Folia Histochemica Et Cytobiologica 44(1): 127-131.
[38] Kaur, G., Singh, H. P., Batish, D. R., Kohli, R. K. (2014): Pb-inhibited mitotic activity in onion roots involved DNA damage and disruption of oxidative metabolism. – Ecotoxicology 23: 1292-1304.
[39] Keshavarzi, M. H. (2011): Effect of salt stress on germination and early seedling growth of savory (*Satureja hortensis*). – Aust J Basic Appl Sci 5(2): 3274-3279.
[40] Lancien, M., Gadal, P., Hodges, M. (2000): Enzyme redundancy and the importance of 2-oxoglutarate in higher plant ammonium assimilation. – Plant Physiol 123: 817-824.
[41] Lane, A., Boecklemann, A., Woronuk, G., Sarker, L., Mahmoud, S. (2010): A genomics resource for investigating regulation of essential oil production in *Lavandula angustifolia*. – Planta 231(4): 835-845.
[42] Lemaitre, T., Hodges, M. (2006): Expression analysis of *Arabidopsis thaliana* NAD-dependent isocitrate dehydrogenase genes show the presence of a functional subunit that is mainly expressed in the pollen and absent from vegetative organs. – Plant Cell Physiol 47(5): 634-643.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_18671883

[43] Liepman, A. H., Olsen, L. J. (2004): Genomic analysis of aminotransferases in *Arabidopsis thaliana*. – Critical Reviews in Plant Sciences 23(1): 73-89.
[44] Liu, C., Liu, X. F., Tetsuo, T., Liu, C. K. (2011): Research progress of NADP-ME in higher plants. – Genomics Appl Biol 30: 1255-1260.
[45] MacRae, E. (2007): Extraction of plant RNA. – Methods Mol Biol 353: 15-24.
[46] Monteiro, C., Santos, C., Pinho, S., Oliveira, H., Pedrosa, T., Dias, C. (2012): Cadmium-induced cyto- and genotoxicity are organ-dependent in lettuce. – Chem Res Toxicol 25: 1423-1434.
[47] Munns, R., James, R. A., Läuchli, A. (2006): Approaches to increasing the salt tolerance of wheat and other cereals. – J Exp Bot 57: 1025-1043.
[48] Murashige, T., Skoog, F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. – Physiol Plant 15(3): 473-497.
[49] Nicot, N., Hausman, J-F., Hoffmann, L., Evers, D. (2005): Housekeeping gene selection for real-time RT-PCR normalization in potato during biotic and abiotic stress. – Journal of Experimental Botany 56(421): 2907-2914.
[50] Nikolova, M., Vitkova, A., Zayova, E., Petrova, M. (2013): Flavonoid profiles, polyphenolic content, and antiradical properties of cultivated plants of *Arnica montana* L. – AgroLife Scientific Journal 2(2): 20-24.
[51] Otang, W. M., Afolayan, A. J. (2016): Antimicrobial and antioxidant efficacy of *Citrus limon* L. peel extracts used for skin diseases by Xhosa tribe of Amathole District, Eastern Cape, South Africa. – South Afr J Bot 102: 46-49.
[52] Oyakhirome, S., Issifou, S., Pongratz, P., Barondi, F., Ramharter, M., Kun, J. F., Missinou, A. M., Lell, B., Kremsner, P. G. (2007): Randomized controlled trial of fosmidomycin-clindamycin versus sulfadoxine pyrimethamine in the treatment of *Plasmodium falciparum* malaria. – Antimicrob Agents Chemother 51(5): 1869-1871.
[53] Panda, B. B., Achary, V. M. (2014): Mitogen-activated protein kinase signal transduction and DNA repair network are involved in aluminium induced DNA damage and adaptive response in root cells of *Allium cepa* L. – Front Plant Sci 5: 256.
[54] Papp, N., Bartha, S., Boris, G., Balogh, L. (2011): Traditional uses of medicinal plants for respiratory diseases in Transylvania. – Nat Prod Commun 6(10): 1459-1460.
[55] Plant, J., Stephens, B. (2015): Effects of essential oils on transcriptional regulation in human cells. – Med Aromat Plants 4(3): 200.
[56] Raes, J., Rohde, A., Christensen, J. H., Van de Peer, Y., Boerjan, W. (2003): Genome-wide characterization of the lignification toolbox in Arabidopsis. – Plant Physiol 133: 1051-1071.
[57] Riaz, A., Khan, R. A., Mirza, T., Mustansir, T., Ahmed, M. (2014): In vitro/in vivo effect of *Citrus limon* (L. Burm. f.) juice on blood parameters, coagulation, and anticoagulation factors in rabbits. – Pak J Pharm Sci 27: 907-915.
[58] Rogers, M. E., Grieve, C. M., Shannon, M. C. (2003): Plant growth and ion relations in Lucerne (*Medicago sativa* L.) in response to the combined effects of NaCl and P. – Plant Soil Sci 253: 187-194.
[59] Roistacher, C. N., Navarro, L., Murashige, T. (1976): Recovery of citrus selections free of several viruses, exocortis viroid and spiroplasma citri by shoot-tip grafting in vitro. – Proc 7 Conf Int Organization Citrus Virol, IOCV, Riverside, pp. 186-193.
[60] Russo, M., Bonaccorsi, I., Costa, R., Trozzi, A., Dugo, P., Mondello, L. (2015): Reduced time HPLC analyses for fast quality control of citrus essential oils. – J Essent Oil Res 27: 307-315.
[61] Sabir, F., Sangwan, R. S., Kumar, R., Sangwan, N. S. (2012): Salt stress-induced responses in growth and metabolism in callus cultures and differentiating in vitro shoots of Indian ginseng (*Withania somnifera* Dunal). – J Plant Growth Regul 10: 344-356.
[62] Saira, K., Zafar, U. Z., Habib, R. A., Rehana, K. (2014): Physiological and biochemical basis of salt tolerance in *Ocimum basilicum* L. – Journal of Medicinal Plants Studies 2: 18-27.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_18671883

[63] Salwa, E. (2018): In vitro evaluation and selection for salinity tolerance in some Citrus rootstock seedlings. – Journal of Horticultural Science & Ornamental Plants 10(1): 17-27.
[64] Sangwan, N. S., Kumar, R., Srivastava, S., Kumar, A., Gupta, A., Sangwan, R. S. (2011): Recent developments on secondary metabolite biosynthesis in *Artemisia annua* L. – J Plant Biol 37: 1-24.
[65] Shabala, S., Bose, J., Hedrich, R. (2014): Salt bladders: Do they matter? – Trends Plant Sci 19: 687-691.
[66] Shao, H. B., Liu, Z. H., Zhang, Z. B., Chen, Q. J., Chu, L. Y., Brestic, M. (2011): Biological roles of crop NADP-malic enzymes and molecular mechanisms involved in abiotic stress. – Afr J Biotechnol 10: 4947-4953.
[67] Storey, R., Walker, R. R. (1999): Citrus and salinity. – Scientia Horticulturae 78: 39-81.
[68] Szkopinska, A., Plochocka, D. (2005): Farnesyl diphosphate synthase; regulation of product specificity. – Acta Biochimica Polonica 52(1): 45-55.
[69] Tholl, D., Chen, F., Petri, J., Gershenzon, J., Pichersky, E. (2005): Two sesquiterpene synthases are responsible for the complex mixture of sesquiterpenes emitted from Arabidopsis flowers. – Plant J 42(5): 757-771.
[70] Tholl, D., Kish, C. M., Orlova, I., Sherman, D., Gershenzon, J., Pichersky, E., Dudareva, N. (2004): Formation of monoterpenes in *Antirrhinum majus* and *Clarkia breweri* flowers involves heterodimeric geranyl diphosphate synthase. – Plant Cell 16: 977-992.
[71] Ventura, L., Giovannini, A., Savio, M., Donà, M., Macovei, A., Buttafava, A., Carbonera, D., Balestrazzi, A. (2013): Single cell gel electrophoresis (Comet) assay with plants: research on DNA repair and ecogenotoxicity testing. – Chemosphere 92: 1-9.
[72] Wang, R. G., Chen, S. L., Zhou, X. Y., Shen, X., Deng, L., Zhu, H., Shao, J., Shi, Y., Dai, S., Fritz, E., Hüttermann, A., Polle, A. (2008): Ionic homeostasis and reactive oxygen species control in leaves and xylem sap of two poplars subjected to NaCl stress. – Tree Physiol 28(6): 947-957.
[73] Zadeh, M. H., Naeini, M. B. (2007): Effects of salinity stress on morphology and yield of two cultivars of canola (*Brassica napus* L.). – J Agron 6(3): 409-414.
[74] Zerihun, A. D., Lukman, S. S., Soheil, S. M. (2011): Cloning and functional characterization of b-phellandrene synthase from *Lavandula angustifolia*. – Planta 233: 685-696.
[75] Zhao, W. N., McAlister-Henn, L. (1996): Expression and gene disruption analysis of the isocitrate dehydrogenase family in yeast. – Biochemistry 35: 7873-7878.
[76] Zhou, G.-L., Zhu, P. (2020): De novo transcriptome sequencing of *Rhododendron molle* and identification of genes involved in the biosynthesis of secondary metabolites. – BMC Plant Biology 20: 1-19.
[77] Zia, S., Khan, M. A. (2002): Comparative effect of NaCl and sea water on seed germination of *Limonium stockii*. – Pak J Bot 34: 345-350.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_18671883