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Salinity Stress Alerts Genome Stability and Genotoxicity of *Ocimum basilicum* Cultivars

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Abstract

Salinity is an important abiotic stress that greatly influences growth, secondary product content and genotoxicity in plants. *Ocimum basilicum* L. (family Lamiaceae) produces a volatile oil, which is used in many pharmaceutical industries, but the oil biosynthesis is affected by salt stress. The aim of this study was to evaluate the effect of salinity stress on genome stability and genotoxicity of three basil cultivars (Gigante, Gralissimum and Verde) using comet assays to study the genotoxic impact of salinity stress (0, 50, 100 and 200 mM NaCl) and a semi-quantitative real time polymerase chain reaction to study terpene gene expression. Both analyses revealed considerable genetic effects of salinity stress on the *O. basilicum* genome, detected by a regular increase in DNA damage and by diversity in the transcript levels of terpene biosynthesis and inhibitor genes. Our findings confirmed that basil plants were affected by NaCl salinity stress and that exposure to 200 mM NaCl resulted in significant DNA damage in the form of tail moment, DNA tail percentage and tail length. The accumulation of linalool synthase enzyme (LS) and hexokinase synthase (HK) gene transcripts was greatly increased in response to salinity, whereas FPPS, GPPS and DXR gene transcription was suppressed in all three basil cultivars.

Keywords: *Ocimum basilicum*; Salt stress; Genotoxicity; Basil cultivars; Genome

Introduction

Salinity is an environmental stress that affects plant genome persistence, as well as causing substantial damage to crop production, with significant economic and environmental impact in the affected areas. Globally, the effects of soil salinity on the agricultural sector productivity are greater than those of other factors, as increasing soil salinity ratios are converting reclaimed land into acreages unsuitable for cultivation (Saira *et al.* 2014). Salinity is one of the most important abiotic stresses that affect plant DNA, and plant genotoxicity induced by numerous stress agents is now under intensive investigation by many researchers. The comet assay is now recognised as a promising method for measuring the DNA damage and repair capacity at the single-cell level (Tomas *et al.* 2000). The accuracy, simplicity and need for only a single cell to obtain reliable results had led to an increased use of the comet assay in plant research that now extends beyond model plants like *Arabidopsis thaliana, Allium cepa, Vicia faba* or *Nicotiana tabacum* to a broad range of important crop species (Gichner *et al.* 2009; Ventura *et al.* 2013). Previous studies have employed comet assays for the evaluation of genotoxicity caused by chemicals, radiation, phytochemicals, pesticides, contaminated complex matrices, heavy metals and nanoparticles (Ghosh *et al.* 2015).

Medicinal and aromatic plants have many health benefits, which are found in many important plant families. One of the best known families is the Lamiaceae, as almost all members of this family possess volatile oil trichomes with high terpene contents. In this family, basil is a globally popular member that is widely used for many purposes, including insomnia treatments, pharmaceuticals, flavouring, aromatherapy, cosmetics and perfumes (Sonwa 2000; Labra *et al.* 2004). Basil is an annual herb, with a height between 20 to 60 cm and pink and white flowers. The plant is widely cultivated in the Mediterranean basin, Asia, Europe and in many other countries of the world (Omidbaig 2005). Recent studies have explored the importance of essential oils from basil as drug components for leukemia treatments and antibacterial and antifungal products (Moteki *et al.* 2002). However, the effects of salinity on essential oil production in basil have not been sufficiently investigated.

In the Kingdom of Saudi Arabia (KSA), some areas use saline water for irrigation. In addition, many reclaimed land areas are developing acute soil salinity problems. These factors have encouraged the introduction of new cultivars that are tolerant of soil salinity. Basil plants are economically important in the KSA and worldwide due to their large quantities of several essential oil components,

including terpenoids and phenylpropane derivatives, which have been characterised as the basic volatile components in basil oil (Hassanpouraghdam *et al.* 2010). Terpenoids are one of the most diverse classes of plant secondary metabolites, and they participate in many biological processes, including growth, development, photosynthesis and respiration (Gershenzon and Kreis 1999; Rodriguez and Boronat 2002).

Terpenoids also accumulate in plants exposed to environmental stresses, including salinity. Tissue culture technique has been frequently used as a tool to identify the cellular mechanisms that impart salt tolerance and to select for NaCl-tolerant plants. Plant tissue culture also avoids the need to cultivate whole plants, and the growth conditions are easily controlled in plant cell cultures (Davenport *et al.* 2003; Gu *et al.* 2004). The use of tissue cultures also simplifies genome analysis, such as by comet assays, for investigation of terpene biosynthesis at the transcriptional level and its behaviour under salinity stress (Ashour *et al.* 2010). For example, analysis of expression of the linalool synthase enzyme (LS) has confirmed that the accumulation of linalool in *Lavendula angustifolia* correlates closely with the transcript levels of LS gene (Lane *et al.* 2010). Several enzymes, including hexokinase synthase (HK), a glycolytic enzyme responsible for the ATP-dependent conversion of hexoses to hexose 6-phosphates (Jyan *et al.* 1997), and 1-deoxy-D-xylulose-5-phosphate synthase (DXS), a principal enzyme of the methylerythritol phosphate pathway, are known to be primarily regulated at the transcription level (Kai *et al.* 2011).

A number of genes have known involvement in essential oil synthesis. One example is the gene coding for farnesyl diphosphate synthase (FPPS), an important enzyme in the isoprenoid biosynthesis reaction that supplies sesquiterpene precursors for the synthesis of numerous essential metabolites, including ubiquinones, dolichols, sterols and carotenoids, as well as providing substrates for geranyl geranylation and farnesylation of proteins (Szkopińska and Plochocka 2005). Another is the geranyl diphosphate synthase (GPPS) gene that codes for a key enzyme in monoterpene biosynthesis and has a plastid localisation (Tholl *et al.* 2004). The aim of the present study was to use comet assays to evaluate the transcriptional level of terpene-related genes in tissue cultures of three basil cultivars following exposure to various levels of salinity. The other aim was to review the latest data on the use of this technique as an ideal approach for investigating the genotoxic effects of salinity stress on essential oil production in basil (*Ocimum basilicum* L.) plants.

Materials and Methods

The current study was carried out in the Plant Tissue Culture and Molecular Biology Laboratory, Biotechnology department, College of Science, Taif University, Taif, Saudi Arabia, from May 2019 to January 2020.

Plant materials and *in vitro* salinity assay

Seeds of three basil cultivars (*Ocimum basilicum* L. cvs. Gigante, Gralissimum and Verde) were purchased from Alhomaide Company, Taif, Saudi Arabia, and sterilised by washing for 30 sec with 70% ethanol containing a few drops of Tween20, washing three times with sterilized distilled water and then immersing in 5% commercial Clorox solution (1% sodium hypochlorite) for 5 min in a laminar air flow hood, followed by five washes with sterile distilled water. The sterilized seeds were inoculated aseptically into jars (3 seeds per jar) containing MS nutrient medium (Murashige and Skoog 1962) supplemented with 3% sucrose and solidified with (0.7%) agar. (Prior to inoculation, the pH was adjusted to 5.8, and 30 mL of medium was placed into each culture tube and sterilised by autoclaving at 121°C for 20 mins) The seed cultures were maintained in the dark at 25±2°C for 10 days. After germination, the seedlings were transferred to continuous cool white fluorescent light with a 16 h photoperiod at 2,000-Lux intensity. The germinated explants were then sub-cultured for 14 days under aseptic conditions in the basal MS nutrient medium with added 3% sucrose, 0.7% agar, and different levels of NaCl (0, 50, 100, and 200 mM) under the same culture conditions as above. After 14 days of salt stress, the genotoxic effect was determined using the techniques described below. Thirty-day-old seedlings of the three tested cultivars cultured on MS medium are shown in Fig. 1.

Isolation of nuclei

Individual leaf explants were removed from the seedlings and maintained in a petri dish on ice in Sörensen buffer [50 mM sodium phosphate, pH 6.8, 0.1 mM ethylenediamine tetraacetic acid (EDTA) and 0.5% dimethyl sulfoxide (DMSO)]. The leaf tissue explant was gently sliced with a razor blade and the obtained material was repeatedly immersed in the cold Sörensen buffer. The suspension including released nuclei was filtered through 30 µm disposable filter (Partec, Műnster, Germany) to exclude most of the cell debris and then centrifuged at 550 × g for 5 min at 4ºC.

Genotoxicity assay

Leaves were placed in a small Petri dish with 200 mL of cold 400 mM Tris-HCl buffer, pH 7.5 (on ice). Using a razor blade, the leaves were gently sliced into a "fringe" to liberate nuclei into the buffer, as viewed under yellow light. This method of nucleus isolation was confirmed as optimal for obtaining low values of DNA damage in the control cells. Slides were coated with 1% normal melting point (NMP) agarose, dried, wrapped with a mixture of 55 µL of nuclear suspension and 55 µL of LMP agarose (low melting point (LMP) (1% prepared with phosphate-buffered saline) at 40°C, and cover slipped. The slide was placed on ice for at least 5 min, and then the coverslip was removed. A 110 µL

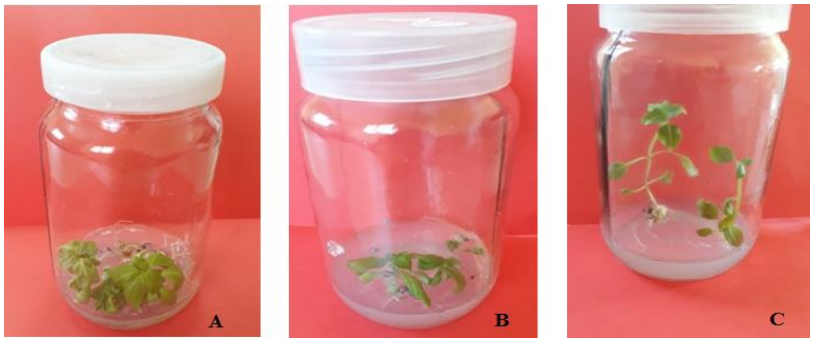

Fig. 1: *In vitro* culture of three basil cultivars on free hormone MS medium after 30 days. (A) *O. basilicum* cv. Gigante, (B) *O basilicum* cv. Gralissimum and (C) *O basilicum* cv. Verde

Table 1: Primers used for PCR simplification

Gene	*Forward primer 5′– 3′*	*Reverse primer 5′– 3′*	*Genes of terpene biosynthesis pathway*
Actin	GTTCTCAGTGGTGGCTCAACTATGT	GAGGAGCAACCACCTTAATCTTCAT	House keeping
FPPS	GGCACTAGAACTTTCAAACGAA	CTTGCTCTCGTACTCCATAAATG	Farnesyl pyrophosphate synthase
DXR	GTTGCGGTAAGAAATGAGTCAT	GCAACCTACTATCCCTGTAACTA	Deoxy-D-xylulose 5- phosphate reductoisom
HK	GATATTGTGGGAGAATTGACCAG	CATTTGTTCCAGTACCCAGTATC	Hexokinase
GPPS	AGTATTGGCAGGAGATCTTCTAC	GTAGTACTCATCTGCATGGTTTC	Geranyl diphosphate synthase
LS	CTTTCGACTTCTCAGACAACAAG	CAGCCTCTTCAAGTACTCTATCT	Linalool synthase

volume of LMP agarose (0.5%) was then carefully placed on the slide and the coverslip was placed again. After 5 min on ice, the coverslip was removed slowly. Single Cell Gel Electrophoresis (SCGE) slides with plant or cell nuclei were exposed to the mutagen solutions for 2 h at 26°C, followed by washing three times for 5 min in cold distilled water. The slides with plant cell nuclei were placed in a horizontal gel electrophoresis tank containing freshly prepared cold electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH>13) and incubated for 15 min. The electrophoresis was then run at 16 V, 300 mA for 30 min 16 V, 300 mA at 4°C. These electrophoresis conditions were previously confirmed as ideal as they resulted in only low levels of DNA damage in control cells and gave linear responses for comets after chemical mutagen treatment of these cultivars in pilot studies. The gels were then neutralised by washing three times in 400 mM Tris-HCl, pH 7.5, stained with ethidium bromide (20 µg/mL) for 5min, immersed in ice-cold distilled water and immediately analysed. For each slide, 50 randomLy selected cells were examined with a fluorescence microscope equipped with a 546 nm excitation filter, a 590 nm barrier filter and a computerised image analysis system (Komet Version 3.1 Kinetic Imaging, Liverpool, UK). The Tail moment (TM) and DNA (TD, %) served as the parameters of DNA damage (Jolanta *et al.* 2006).

RNA extraction and gene expression

RNA extraction: Total RNA was extracted from 0.5 g samples of basil plant tissues (MacRae 2007) by adding 500 µL of Trizol reagent, grinding the sample thoroughly, adding 100 µL chloroform, shaking well, and then centrifuging for 5 min at 13000 g. The upper layer was carefully removed, 250 µL isopropanol was added, and the mixture was shaken lightly to promote the formation of RNA strands. The tube was placed in a freezer at -2°C for 30 min until the RNA had precipitated. The tube was centrifuged for 5 min at 13000 g, the supernatant was removed, and the pellet was suspended in 500 µl diethylpyrocarbonate (DEPC)-treated water: ethanol (25%:75%) and centrifuged for 5 min. The supernatant was removed, the pellet was allowed to dry, and was then dissolved in 50 µL DEPC water in a 55–60°C water bath for 15 min. The quality of the isolated RNA was verified by agarose gel electrophoresis (1% agarose in 1X TBE buffer). The extracted RNA was checked for purity on the 1% agarose gel by visualisation with a UV transilluminator (Biometra UV star 15).

First strand cDNA synthesis reaction: The cDNAs were synthesised from 2 µL of total RNA in a final reaction volume of 20 µL using a Revert First Strand cDNA synthesis kit (Thermoscientific, Lithuania) according to the manufacturer's instructions. The cDNA protocol included the following steps: Oligo $(dT)_{18}$ primer (about 1 µL) was added to 2 µL RNA, 4µl 5X reaction buffer, 1 µL RiboLock RNase inhibitor (20 µg/µL), 2 µL 10 mM dNTP Mix, 1 µL Revert Aid RT (200 µg/µL), 9 µL nuclease-free water, and the final volume was adjusted to 20 µL by addition of DEPC-treated water, mixed, and incubated for 60 min at 42°C. The reaction was terminated by heating at 70°C for 5 min and then chilling on ice for at least 3–5 mins.

Polymerase chain reaction: Reverse transcription was performed with High Capacity Access RT-PCR System (Promega) using aliquots of total RNA extracted following

the manufacturer's instructions. The sequences of the selected primers for the secondary product genes (LS, HK, FPPS, GPPS and DXR) were obtained from previous studies (Table 1). The Master PCR reaction mix contained 2 µL cDNA, 0.6 µL primer, 4 µL PCR Master mix, in a volume adjusted to 20 µL by adding 12.8 µL distilled water; a negative control was also prepared. Semi-quantitative RT-PCR reactions were performed using a PXE 0.5 thermocycler (Thermo Scientific) with the following cycling program: Stage 1, 94ºC: 2–4 min; Stage 2 (40 cycles), 94ºC: 30 S; 61.1ºC: 1 min; 68ºC: 2 min; Stage 3: 68ºC: 7 min; Stage 4: hold at 4ºC. The sqRT-PCR products were visualised by conventional agarose gel electrophoresis. The generated bands were quantified using GelPro32 (version 4.03).

Agarose gel electrophoresis: The PCR products were examined by electrophoresis, as described previously, using 1.5% agarose gel at 100 V for 90 min. The samples were detected using UV trans-elements and imaged. A DNA ladder with a molecular size range of 100–1500 bp was used to determine the size of the reaction products. The results were analysed using a GEL pro computer program (Version32).

Statistical analysis

Each data element reflects the mean of three biological samples, with three replicates for each plant sample. Statistical analysis for all experiments was performed using Graph Pad Prism 8 (Graph Pad Software, La Jolla, CA, USA). One-way analysis of variance (ANOVA) was used to analyse the data. Values of $p < 0.05$ were considered statistically significant. The results were expressed as mean ± standard deviation (SD).

Results

DNA fragmentation dependent test (Comet assay)

Nuclear DNA damage was assessed in each basil cultivar cultured on MS medium supplemented with 0.0, 50, 100 or 200 mM NaCl. The important comet assay parameters measured in this study to evaluate DNA damage were Tail moment, DNA tail (%) and tail length. Fig. 2 shows greater dose-scored DNA damage in *O. basilicum* Gigante plants exposed to 50mM NaCl than in untreated control plants. Exposure to 200 mM NaCl resulted in DNA damage scores of 2.05% for DNA tail (%); this was a highly significant increment compared with the untreated plants (Fig. 2B). The score was 1.39%, again a significant increment over the control, when plants were treated with 100 mM NaCl. The levels of 100 and 200 mM NaCl gave highly significant damage, as determined by tail moment measurements of 2.1 and 4.4 units, respectively (Fig. 2C). Damage expressed by tail length in DNA was relatively higher and reached 1.13, 1.52 and 2.16 µm in *O. basilicum* Gigante plants cultured on MS medium with 50, 100 and 200 mM NaCl, respectively, whereas the control plants showed no damage based on the tail length in DNA (Fig. 2D). Similar results were recorded for the Gralissimum and Verde varieties using similar comet assay parameters and scoring against control plants Damage appeared to increase with increases in salt stress above 50 mM NaCl (Fig. 2, 3, 4B, C and D) in all samples, as evident by significant increases in DNA tail (%), tail moment and tail length.

Gene expression analysis

Exposure to NaCl stress affected the expression of FPPS, DXR, HK, GPPS and LS genes in *in vitro* cultures of all three basil cultivars and confirmed the correlation between NaCl stress of and changes in transcription of terpene biosynthesis genes in the three cultivars. The genes responsible for production of the expected precursors in the pathway were also monitored by semi-quantitative PCR using five primers for genes in the terpene biosynthesis pathway and recording the changes occurring in the basil cultivars treated with various levels of NaCl (Fig. 2, 3, 4F–G). The actin gene was used as a housekeeping gene. All three cultivars showed accumulation of LS and HK synthase transcripts in response to salinity stress when compared with the sesquiterpene synthase enzymes FPPS, 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) and GPPS.

The expression profiles of FPPS, GPPS and DXR indicated a significantly lower induction of transcript accumulation by salinity in the Gigante variety (Fig. 2). The expression patterns indicated a rapid response and sharp increase in the LS transcript amount (2.79) in the Gigante plants cultured on MS medium supplemented with 200 mM NaCl compared to control plants (0.06). Higher LS expression (1.84 and 1.54) was also observed in plants exposed to salinity stress (50 mM and 100 mM NaCl, respectively). The accumulation of the HK transcript also showed a significant increase to 3.17 in plants exposed to 200 mM NaCl versus the control plants (1.57). Notably, the HK transcript amounts were similar to the control levels (1.6) in plants exposed to 100 mM NaCl. The HK transcripts in plants cultured on 50 mM NaCl showed no clear trend and no significant difference in amount (2.1) compared to the unstressed control plants (Fig. 2).

The effect of different levels of salinity stress on the transcription of selected genes involved in terpene biosynthesis transcription was also examined in the Gralissimum variety (Fig. 3). The HK transcript content was similar to control levels in plants exposed to 50 mM NaCl (1.4), whereas plants cultured on 200 mM NaCl had HK levels of 2.8. The amount of LS transcript showed a gradual decrease in response to increases in salinity stress (Fig. 3).

The FPPS, DXR and GPPS transcript contents were lowest in the Verde variety and were minimal under salinity conditions. *O. basilicum* Verde plants treated with 0.0, 50,

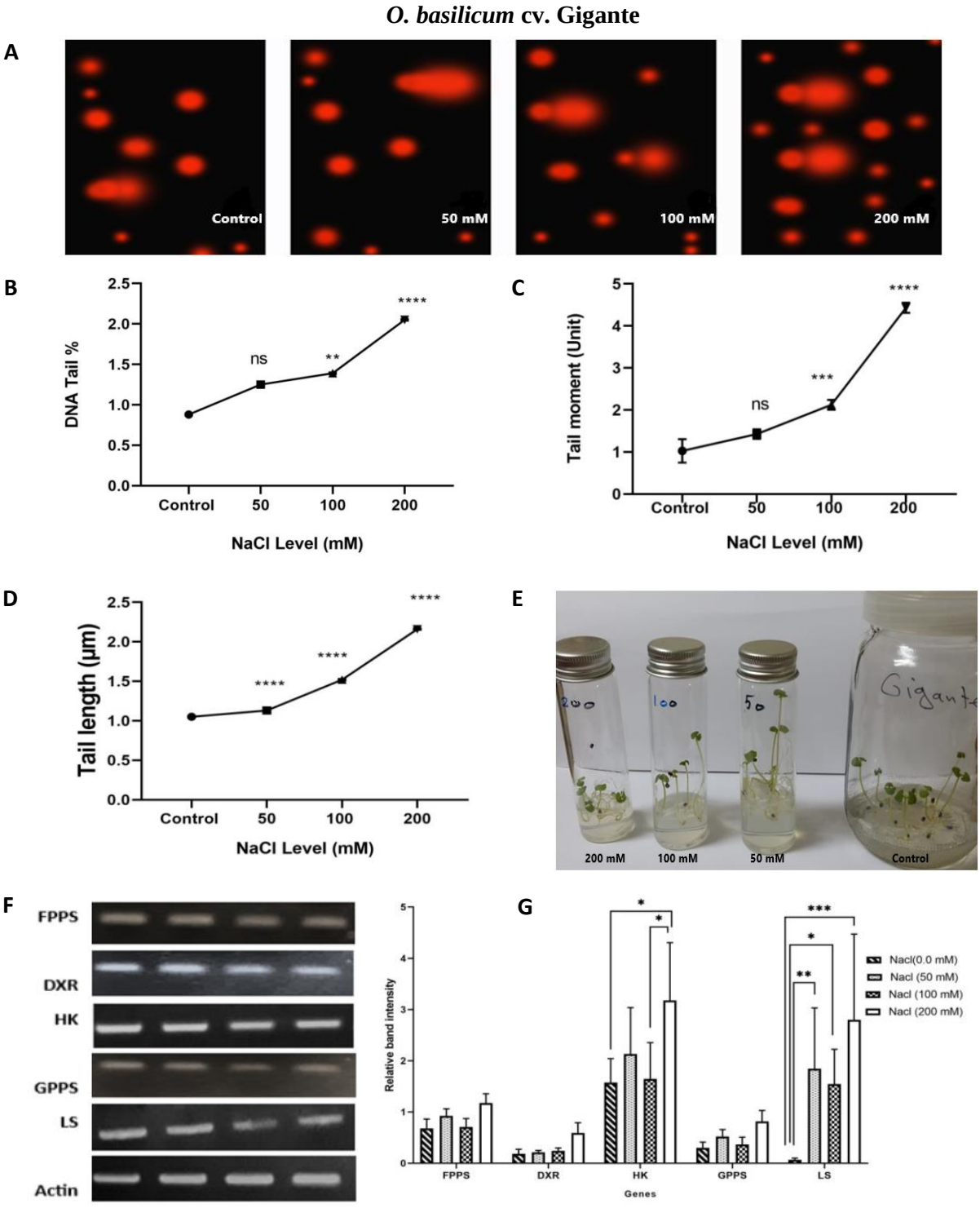

Fig. 2: Genotoxicity of salinity at four levels (0.0, 50, 100 and 200 mM NaCl) on Gigante cultivar of *O. basilicum* grown on MS medium. (A) Comet assay: Photomicrographs of ethedium bromide -stained DNA from protoplasts of *O. basilicum* Gigante exposed to four levels of NaCl stress, (B) DNA Tail (%), (c) Tail moment (Unit), (D) Tail length (µm), (E) *In vitro* shoot cultures of *O. basilicum* Gigante exposed to MS medium supplemented with (0.0, 50, 100 and 200 mM NaCl), (F) Agarose gel electrophoresis stained by ethidium bromide results showing the expression of FPPS, DXR, HK, GPPS and LS genes amplified in *O. basilicum* Gigante plant cultures exposed to salinity stress determined by semi-quantitative-PCR. Lane1: Control, lane 2: 50 mM, lane 3: 100 mM or, lane 4: 200 mM Nacl (G) Terpenes biosynthesis genes and their inhibitors / actin expression ratio

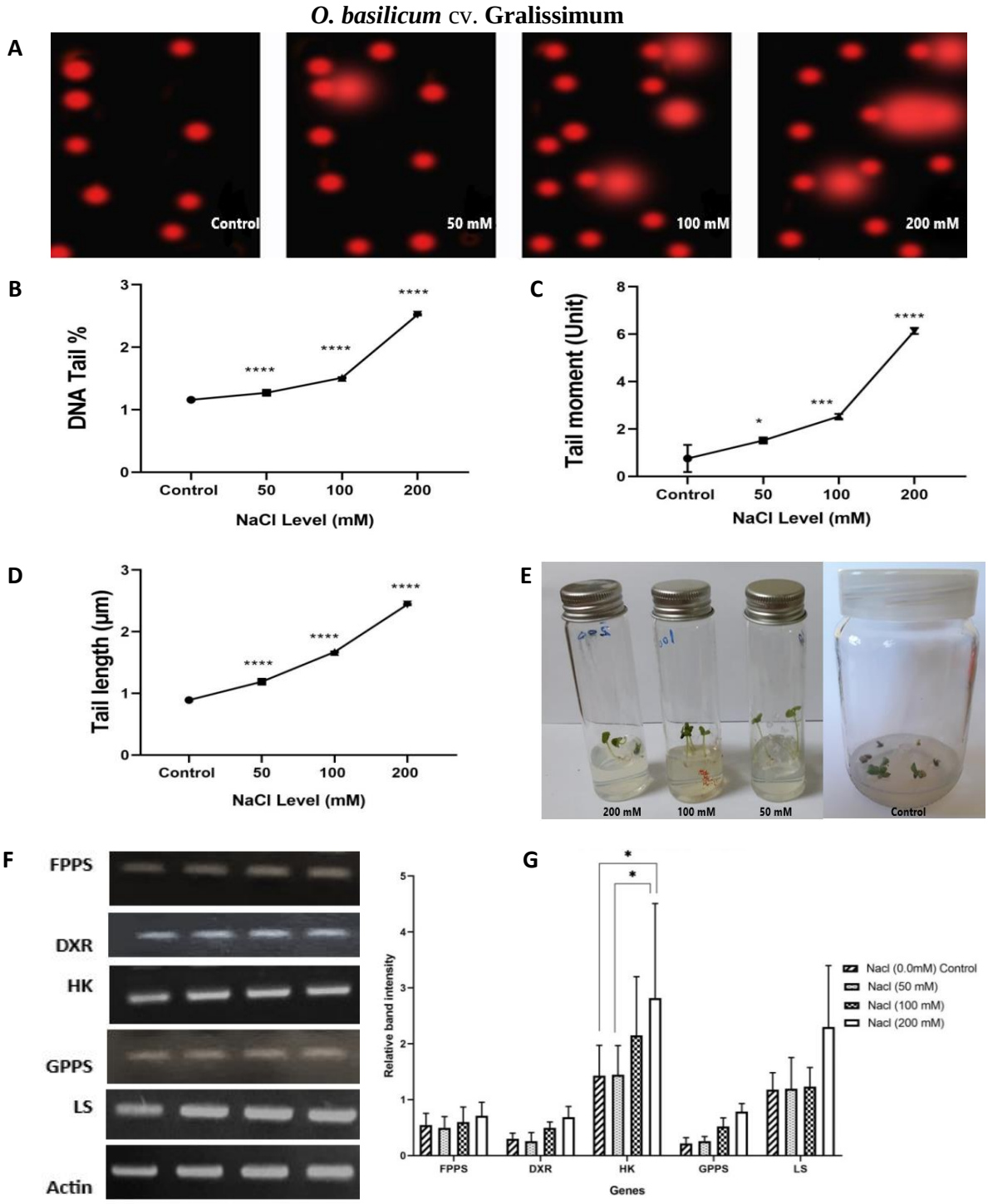

Fig. 3: Genotoxicity of salinity at four levels (0.0, 50, 100 and 200 mM NaCl) on Gralissimum cultivar of *O. basilicum* grown on MS medium. (A) Comet assay: Photomicrographs of ethedium bromide -stained DNA from protoplasts of *O. basilicum* Gralissimum exposed to four levels of NaCl stress, (B) DNA Tail %, (c) Tail moment (Unit), (D) Tail length (µm), (E) *In vitro* shoot cultures of *O. basilicum* Gralissimum exposed to MS medium supplemented with (0.0, 50, 100 and 200 mM NaCl), (F) Agarose gel electrophoresis stained by ethidium bromide results showing the expression of FPPS, DXR, HK, GPPS and LS genes amplified in *O. basilicum* Gralissimum plant cultures exposed to salinity stress determined by semi-quantitative-PCR. Lane1: Control, lane 2: 50 mM, lane 3: 100 mM or, lane 4: 200 mM Nacl (G) Terpenes biosynthesis genes and their inhibitors/ Actin expression ratio

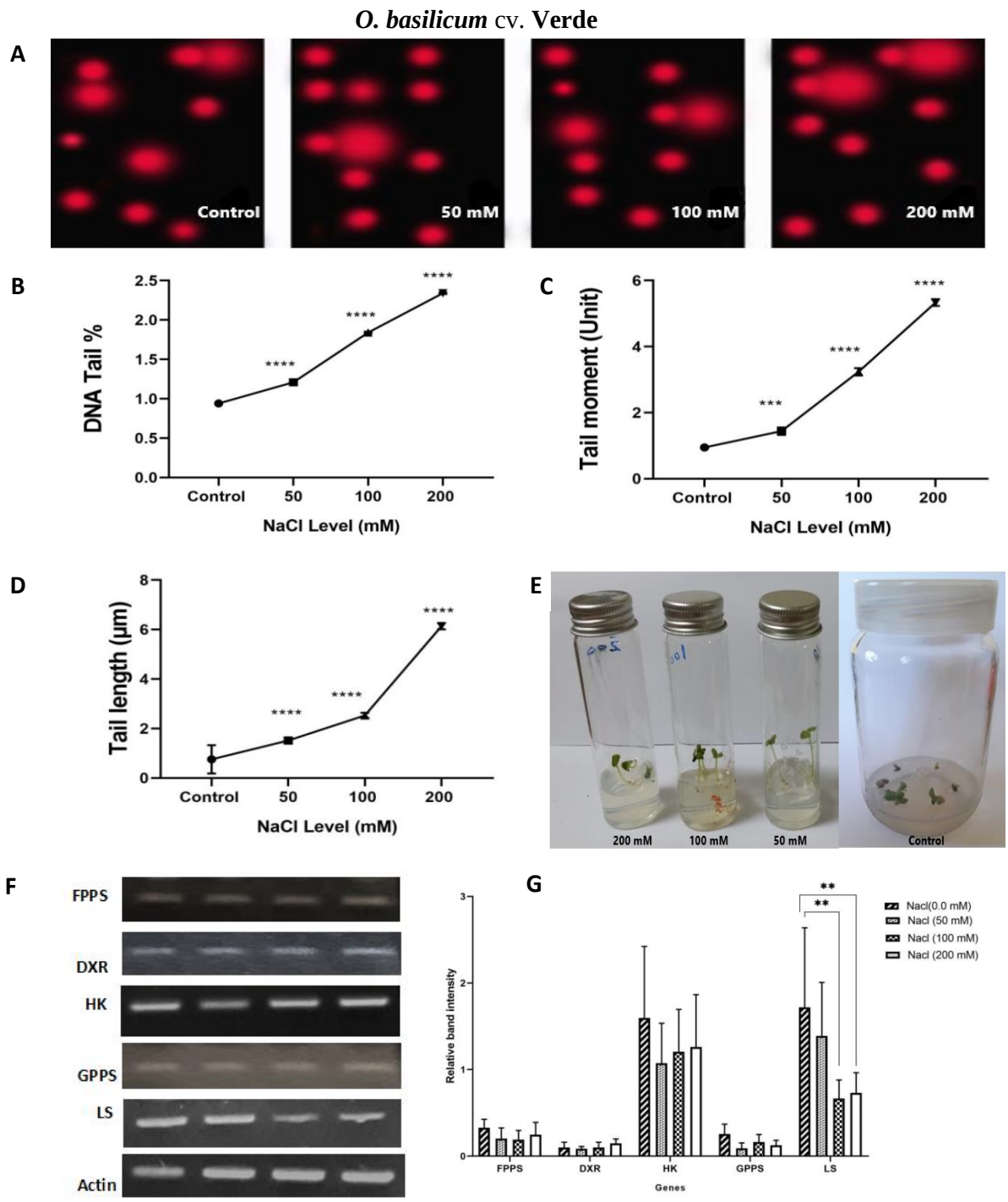

Fig. 4: Genotoxicity of salinity at four levels (0.0, 50, 100 and 200 mM NaCl) on Verde cultivar of *O. basilicum* grown on MS medium. (A) Comet assay: Photomicrographs of ethedium bromide -stained DNA from protoplasts of *O. basilicum* Verde exposed to four levels of NaCl stress, (B) DNA Tail %, (c) Tail moment (Unit), (D) Tail length (µm), (E) *In vitro* shoot cultures of *O. basilicum* Verde exposed to MS medium supplemented with (0.0, 50, 100 and 200 mM NaCl), (F) Agarose gel electrophoresis stained by ethidium bromide results showing the expression of FPPS, DXR, HK, GPPS and LS genes amplified in *O. basilicum* Verde plant cultures exposed to salinity stress determined by semi-quantitative-PCR. Lane1: Control, lane 2: 50 mM, lane 3: 100 mM or, lane 4: 200 mM Nacl (G) Terpenes biosynthesis genes and their inhibitors / Actin expression ratio

100 and 200 mM NaCl showed high amounts and specific activity of LS, the key enzyme in the linalool synthase pathway (Fig. 4), and the LS transcript levels were higher (1.7) in the control plants than in the plants exposed to salinity stress of 100 and 200 mM NaCl. A relative band intensity of 1.3 was recorded when Verde plants were cultured in 50 mM NaCl, but this value was not significantly different from the value obtained for the untreated control. Therefore, NaCl stress had an effect on the expression of genes involved in hexokinase biosynthesis the Verde plants; however, this increase in transcription in response to the different salinity treatments was not statistically significant. The expression patterns for the FPPS, DXR and GPPS genes under salinity stress are shown in Fig. 4 and indicated a dramatic decrease in transcription in response to salinity.

Discussion

The most accurate parameters reflecting salinity-induced DNA damage were the DNA tail percentage, which was expressed as the total intensity of the tailing, and the total intensity of the comet, which does not depend on the length of the tail. Boyko *et al.* (2010) and Nikolova *et al.* (2013) explained that DNA damage caused by treating plants with NaCl is considered a genotoxic effect that leads to altered transgene ratios and somatic changes in recombination rates, due primarily to exposure to Cl^- ions. Previous studies have highlighted the increases in oxidative stress as a cause of Al genotoxicity. Investigation of the mechanisms of Al genotoxicity by comet assays revealed a role for cell wall-bound NADHPX in the Al-mediated oxidative burst (Achary *et al.* 2012). The mechanics of signal transmission involved Ca^{2+} ions (Achary *et al.* 2013) and MAP kinases (Panda and Achary 2014), leading to Al-caused cell death and DNA damage. Monteiro *et al.* (2012) clarified that Cd toxicity induces DNA repair mechanisms and these create adducts of Cd-DNA that result in protein cross-links and long DNA fragments and/or it triggers a failure of the DNA repair mechanisms.

Two studies on *A. cepa* investigated the role of oxidative stress role in Pb-induced genotoxicity and found that the cell cycle has an important role in DNA damage (Jiang *et al.* 2014; Kaur *et al.* 2014). It was showed that the effects of NaCl on genome stability were due to Cl^- ions, whereas Na^+ ions had no effect on the recombination rates (RR), as media supplemented with Cl^- ions but not Na^+ showed the same increase in the frequency of genomic rearrangements (Smoleń *et al.* 2020). The mechanism involved in this Cl^- ion effect on genome equilibrium is still not specified. The toxic levels of Na^+ lead to marked defects in ion homeostasis in the plant cytoplasm that ultimately result in a K^+ deficiency (Hasegawa *et al.* 2000), but the genotoxicity of NaCl stress depends on Cl^-.

The imposition of NaCl salinity stress on the three basil cultivars caused adverse effects that were mainly observed in the content of plant secondary products. In fact, abiotic stresses like NaCl salt stress slow down plant growth, a response that is considered evidence of plant adaptation and vitality under salinity pressure (Sabir *et al.* 2012). Our findings illustrated that exposure to salt stress triggered a high accumulation of terpenoids, in spite of the cell damage and restrictions in plant growth, by maintenance of clear sources of metabolite precursors. Coordination of the terpenoid pathway-specific genes was the major reason for terpenoid accumulation. Sangwan *et al.* (2011) mentioned that the phytochemicals synthesised and included in glandular trichomes are very important for the plant defence in cases of exposure to biotic and abiotic stresses.

The transcription activity of FPPS, DXR and GPPS genes, as well as of LS (which is expressed strongly in *O. basilicum* Gigante and Verde when cultured on media supplemented with 50, 100 and 200 mM NaCl), was enhanced, as shown by semi-quantitative RT-PCR analysis (Table 1). Our data agreed with those of Lane *et al.* (2010), who reported that the major component of *Lavandula angustifolia* essential oil is linalool and that LS expression is responsible for the accumulation of linalool in lavender flowers. This is not unexpected, as a terpenoid synthase enzyme can convert a single molecule into various products (Toll *et al.* 2005; Degenhardt *et al.* 2009).

The HK gene is the second enzyme pathway gene that was highly expressed in the Gigante and Gralissimum cultivars in response to different salinity levels. As reported by Graham *et al.* (1994), HK is not only valuable for expression of sugar-inducible and sugar-repressible genes in higher plants but it has also been proposed to serve as a sensor for sugar suppression of genes involved in the glyoxylate cycle in higher plants. HK is a known enzyme of glycolysis that accelerates the ATP-dependent conversion of hexoses to hexose-6-phosphates, and it has been proposed as a glucose sensor in higher and lower eukaryotes (Jyan *et al.* 1997). In the current study, the band intensity of the RNA transcripts of selected genes (FPPS, DXR and GPPS) appeared to be lower when compared to the LS and HK gene transcripts in all three cultivars at all levels of salinity. Xiao *et al.* (2015) explained that, among the MEP pathway genes identified in *Salvia miltiorrhiza*, DXS was the principal rate-limiting reaction in the pathway. Kai *et al.* (2011) concluded that expression of SmDXS2 was related to the accumulation of phenanthrene secondary products like tanshinones.

FPPS is considered a key enzyme in isoprenoid biosynthesis reactions as it provides the sesquiterpene precursors for different essential metabolites, including ubiquinones, dolichols, sterols and carotenoids, as well as substrates for geranyl geranylation and farnesylation of proteins. It catalyses sequential head-to-tail condensation of two molecules; dimethylallyl diphosphate and isopentenyl diphosphate. FPPS is usually a homodimer of subunits, and the FPPS-encoding genes in *Arabidopsis thaliana* are controlled at both the expression and transcription levels (Szkopińska and Plochocka 2005). One possibility is that a mitochondrial isoform is transcribed and translated into a

protein or peptide without a signal sequence (Cunillera *et al.* 1997).

GPPS is a key enzyme in monoterpene biosynthesis and is localised in plastids (Tholl *et al.* 2004), the site of synthesis of most monoterpenes from dimethylallyl diphosphate and isopentenyl diphosphate. The heterodimeric form of GPPS consists of a non-catalytic small subunit (GPPS-SSU) that interacts with the large GPPS catalytic subunit and determines the product specificity (Michael *et al.* 2013).

Conclusion

The comet assay was an appropriate method for determining the DNA damage promoted by exposure of basil cultivars to known doses of a genotoxin, which was NaCl (200 mM) in the present study. Semi-quantitative RT-PCR of the LS and HK transcripts confirmed that these genes maintained comparatively stable expression under salinity stress in cell cultures from all three basil cultivars. The results highlight the potential of post-transcriptional regulation of LS and HK, which are abundantly expressed in plant cells under salinity stress.

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Conflict of interest

I declare no conflict of interest of any sort

Data Availability

All data relevant to this research are available with the author

Ethics Approval

No applicable

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