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Responses of the germination and phytochemical content of *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe. (Anatolian black pine) seeds to salt stress

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Abstract: The germination stage, which is known to be the most sensitive period of plant development, is very sensitive to salt stress. To determine how salt stress affected the germination behaviour of *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe, the seeds were germinated at gradually increasing salt concentrations. The detrimental effect of the salt stress increased parallelly to the salt concentration. The morphological and physiological changes were analysed in each application to detect the effect of the increasing salt concentrations on the germination stage, and the results were evaluated. The root and stem length, germination percentage, seed germination vigour index and seed mass decreased with the increasing concentration. The amount of volatile and phenolic compounds increased up to 100 mM of salt concentration, while similar parameters decreased at other salt concentrations. The gradual destruction of the habitats of these plants, which create sustainable living conditions with their ability to adapt to the ever-changing climatic conditions, and the increasing ecological negativities make it challenging for the quality of life and viability of these plants. The study was aimed at defining the responses to salt stress by examining the changes in the germination behaviour and phytochemical content, and the amount of Anatolian black pine seeds with an increasing soil salinity.

Keywords: germination; *Pinus*; phenolic compounds; salinity; volatile compounds

Today, the results of the anthropogenic impact on the forest ecosystem can be seen as the main factor in global climate change. The desire to benefit from plant-based products, particularly the demand for nutrition and shelter as the global population grows, has led to the establishment of new agricultural fields. The change of forestland to other types of land cover, the decreasing reduction of these areas (forests) has increased the acceleration of global climate change. This process has hindered living organisms from continuing their vital functions, causing in a slew of harmful implications including drought, salinity, flooding, exotic species invasion, and fire (Flowers et al. 2015).

Global climate change brings with it soil salinity with increasingly barren lands appearing (about 40% of the terrestrial area) (Huang et al. 2016). The excessive uptake of Na and Cl ions from the soil causes fatal biochemical processes in plants. Soil salinity, on the other hand, produces physiological drought by lowering the osmotic potential of soil solutions, and preventing the plant from taking up water from the soil. The cell water potential, cell turgor, and water uptake by the roots are all reduced when the plant is blocked from taking up water from the soil (Khan, Bano 2018). While the plant expends excessive energy to achieve an osmotic balance, the root development and growth cease. Since the high osmotic pressure of salinity causes disruptions in the uptake of some ions, such as chloride, sodium, and boron, the nutrient/toxicity balance is disturbed (Kalaji et al. 2018). Especially due to the suppression of cell division, the growth of young tissues decreases significantly and the rate of photosynthesis is decelerated (Da Silva et al. 2008). Soil salinity negatively affects all the biological periods of the plant, and its most dramatic impact is seen during germination (Croser et al. 2001). Because seed germination is the most delicate and crucial stage of plant development (Finch-Savage, Leubner-Metzger 2006), all the ecological conditions must be favourable to the germination process in order for it to succeed. Environmental stressors, such as insufficient/excessive temperature and humidity, drought, and salinity, have a negative impact on the seed germination and seedling development (Bewley et al. 2014). Similarly, when the germination responses of 15 different *Pinus nigra* Arnolds were examined under water stress conditions, it was found that water stress decreased the germination rate, germination percentage and germination value (Topacoglu et al. 2016). Plant life begins with seed germination and a key role in the successful germination requires plant survival and maintenance (Shu et al. 2017), monitoring and improvement of the germination processes under various stresses. Due to the utility of its wood for commercial uses, the Anatolian black pine is one of the most prevalent forest tree species used in Turkey (Gemici et al. 2019). The Anatolian black pine is primarily found at elevations of 400–1 800 m a.s.l. in the Central Black Sea, Western Black Sea, Marmara, Aegean, Mediterranean, and Central Anatolia Regions (Gülsoy, Cinar 2019). The Anatolian black pine is widely used for the rehabilitation of semi-arid and arid forest areas (Guner et al. 2022). In previous studies, a significant effect of drought on the growth of *Pinus* species grown in the Mediterranean climate was observed (Martín-Benito et al. 2008). It is clear that changing climatic conditions will cause soil salinity problems due to drought.

Salinity has a deleterious impact on the plant's morphological and anatomical features, as well as its physiological activities, causing considerable reductions in the biomass. Moreover, this also has a major impact on the secondary metabolite profile of the Anatolian black pine (*Pinus nigra* subsp. *pallasiana*), a valuable medicinal plant. While the plant's secondary metabolite develops its own defence system, its extract is widely used in the treatment of different ailments, including wound healing, inflammatory diseases, and skin damage. This feature is due to its high polyphenol content (Mármol et al. 2019). The fact that phytochemicals (phenols and flavonoids) belonging to many *Pinus* species have antioxidant activity, antimicrobial potential, and even cytotoxic activity against cancer cell lines could make them the pharmaceutical industry's key supply (Sharma et al. 2015). However, the increasing negative environmental impacts and changing limits of the habitats of such important medicinal plants cause a decrease in the quality and quantity of the phytochemicals produced. As a consequence, it is critical to identify the adaptations that will allow the plant to thrive in arid environments, particularly those with high salinity. Changes in the germination and phytochemical content of *Pinus nigra* subsp. *pallasiana* under salinity conditions should be defined for this aim. Climate change is one of the major elements responding to soil salinisation, land degradation, and desertification. Increasing the soil salinisation will cause a loss of biological material for the entire world (Rogel et al. 2000). Because seeds are a source of biologically active components, we wanted to figure out how Anatolian black pine seeds responded to salinity stress during the global climate change process, and how the germination parameters and the phytochemical content of the seeds in this process change. For action plans of future climate change scenarios, it is important to determine the germination responses of Anatolian black pine seeds under increasing salinity stress and to determine the measures to be taken to improve their germination and seedling development.

MATERIAL AND METHODS

Seed germination

Seeds were collected from a *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe (Anatolian black pine) natural stand at an altitude of 1 330–1 350 m a.s.l. in the Konya/Beyşehir Kurucuova location. To avoid any interference by toxins from fungus and bacteria, healthy and well-formed seeds taken from cones were sterilised with 15% sodium hypochlorite for 10 min and were cleaned of any woody parts. Afterwards, the seeds were thoroughly rinsed with distilled water. The following concentrations were used for experiments: 0.0 mM (control), 50 mM, 100 mM, 150 mM, and 200 mM (10 ml) salt (sodium chloride; NaCl). For each application, 30 seeds were placed in a 9 cm Petri dish covered with two layers of Whatman No. 1 filter paper. Each application was repeated three times, and the seeds were incubated at 20 ± 3 °C, with 16 : 8 (L : D) and 70% relative humidity conditions in a plant growth chamber. For 30 days, the data required for the seed germination were measured every 2 days. The seeds were considered germinated with the emergence of the radicle (≥ 2 mm) (Raccuia et al. 2004). If the shoot growth occurred without any root extrusion, a seed was deemed to have aberrant germination. The equations below were used to assess the impact of the salt stress on the seed germination and phytochemical content at various concentrations [0.0 mM (control), 50 mM, 100 mM, 150 mM, and 200 mM].

The equations below were used to define the germination index (*GI*) (Tiquia 2010) and germination vigour index (*GVI*) (Vashisth, Nagarajan, 2010).

The seed germination approach was used to analyse the phytotoxicity bioanalysis of the salt stress (Zucconi et al. 1981). The means of the germination were positive if the root was 5 mm in length. The germination index (*GI*) is the measure of the relative seed germination and the root elongation, relative seed germination, and relative root growth were calculated according to Equations (1–4).

The percentage increase in the seed mass (*WP*) was calculated by the following formula [Equation (5)] after determining the initial weight (*WD*) of the seeds and the 20-day germination weight (*WI*) of the same seeds (Xiang et al. 2019). A precision balance scale was used for weighing the seeds before and after germination (0.0001 g).

$$WP = \frac{WI - WD}{WD} \times 100 \tag{5}$$

where:

WP – seed mass increase (%);

WI – 20-day germination weight;

WD – initial weight.

Phytochemical content

Volatile analyses. Gas chromatography mass spectrometry (GC-MS) was employed to determine the volatile content of the seeds for each treatment (Li et al. 2021a). The essential oil components were examined using a polydimethylsiloxane (PDMS) coated fibre (100 m) and a manual solid-phase microextraction (SPME) holder (Supelco Inc., USA). Before the headspace collection, the fibre was exposed to the GC (gas chromatography) inlet for 3 min for thermal desorption at 250 °C. 1 g of the dry sample was placed in a 10 mL glass bottle, sealed with a rubber septum, and stored for 24 h in a drying cabinet at 25 °C. By manually penetrating the septum at 25 °C, the SPME fibre was exposed to each sample for 10 min (0.25 cm depth). For the analysis, it was withdrawn from the bottle and injected into the GC-MS injection port for 3 min. The gas chromatograph was connected to a mass spectrometer with an electron impact ion source for the GC-MS

$$\text{Relative seed germination (\%)} = \frac{\text{seeds germination in treatment}}{\text{seeds germination in control}} \times 100 \tag{1}$$

$$\text{Relative root growth (\%)} = \frac{\text{mean root length in treatment}}{\text{mean root length in control}} \times 100 \tag{2}$$

$$GI = \frac{\text{relative seed germination (\%)} \times \text{relative root growth (\%)}}{100} \tag{3}$$

where:

GI – germination index

$$GVI = \frac{\text{seedlings length}\left[\text{root + shoot }(\text{cm})\right] \times \text{germination percentage}(\%)}{100} \tag{4}$$

where:

GVI – germination vigour index

study. For the separations, a BP-5 Shimadzu fused silica capillary column (30 m with a 0.32 mm i.d., film thickness 0.25 µm) was used (Shimadzu, Japan). Helium, with a flow rate of 1.4 mL·min^{-1}, was used as the carrier gas (23 psi). The detector and injection port temperatures were 260 °C, split, 1 min (50 mL·min^{-1}). The column initial temperature was 35 °C. It was then raised to 140 °C with a rate of 5 °C·min^{-1}, and finally raised to 250 °C with a rate of 10 °C·min^{-1} and was held for 2 min. The active compounds from the SPME sampling were identified on the basis of the relative retention index (ESO 97, Database of Essential Oils, BACIS) and by using a mass spectrum database search (Varian NIST MS database and IMS Terpene Library). The quantification of the components was performed on the basis of their total ion current (TIC) GC peak areas on the column.

Phenolic compounds analyses. The phenolic compositions of the seeds of each application were determined by high-performance liquid chromatography (HPLC) (Kadri et al., 2015). The phenolic content and quantity in 0.02 g dried leaf samples were determined using a pump (LC10ADvp; Shimadzu, Japan), autosampler (SIL-10ADvp; Shimadzu, Japan), and column oven (CTO-10Avp; Shimadzu, Japan). Methanol and acetic acid (3%) were employed as the mobile phase with an Agilent eclipse XDB-C18 (Waldbronn, Germany) (2 504.60 mm, 5 m) column. The solvent flow rate was 0.9 mL·min^{-1} and the injection volume was 20 µL. The column temperature was adjusted to 30 °C. All the peaks' spectrum data were processed at a wavelength of λ_{max} = 200–400 nm and the chromatograms were acquired at different wavelengths based on the absorption maxima of the analysed compounds. The peak identification was performed by comparison of the retention times and diode array spectral characteristics with the standards and the library spectra. The absorbance observed in the chromatograms was used to quantify the phenolic compounds in comparison to the external standards. This procedure allowed us to identify ten compounds: caffeic, catechin, gallic acid, ferulic acid, (–)-epicatechin, taxifolin, rutin, p-hydroxybenzoic acid, resveratrol, and (+)-secoisolariciresinol.

Statistical analyses

The analytical data were generated from at least three independent experiments, processed using SPSS software (Version 21, 2020), and presented as the mean standard deviation (SD). The experimental data were analysed using an analysis of variance (ANOVA). An analysis of variance was used to evaluate the significant differences between the experiments followed by Duncan's multiple range test, and the significant differences between the means from the triplicate analyses were determined at $P \leq 0.05$.

RESULTS AND DISCUSSION

The increase in the number of summer forest fires and the rising salinity in the Mediterranean Region, which is located in an arid and semi-arid climate zone, are two of the most prominent causes contributing to the rapid degradation of ecosystem balance (Gupta, Huang 2014). Soil salinity, which is caused by the excessive presence of essential plant nutrients, such as sodium chloride (NaCl), magnesium chloride, and calcium chloride in the environment (≥ 50 mM), has a detrimental effect on the germination process, the first stage of the plant life cycle. As a result, both the growth and development processes of the plants, as well as their natural spread process, are negatively affected (Chen et al. 2019). Among the most frequently used quantitative parameters of forest tree seeds, various germination data, such as the germination capacity and germination energy, are used (Tomášková et al. 2014), and the germination success of plants can be determined in their ecological environment. For this reason, new data on future plant germination and propagation modelling describing the germination success of *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe (Anatolian black pine) seeds, as well as changes in the amount and quality of their phytochemical contents in increasing soil salinity caused by increasing drought and forest fires around the world, are presented.

Germination experiment

Anatolian black pine seedlings germinated at increasing salt concentrations were impacted by the salt stress in comparison to the control seeds (Figure 1).

The germination behaviour of Anatolian black pine seeds differed against the gradually increasing salinity levels ($P \leq 0.05$). At the 50 mM, 100 mM, 150 mM, and 200 mM salt concentrations, the root length was reduced by 24%, 62%, 71%, and 86%, respectively, compared to the control. The shoot length was reduced by 32%, 71%, and 77%,

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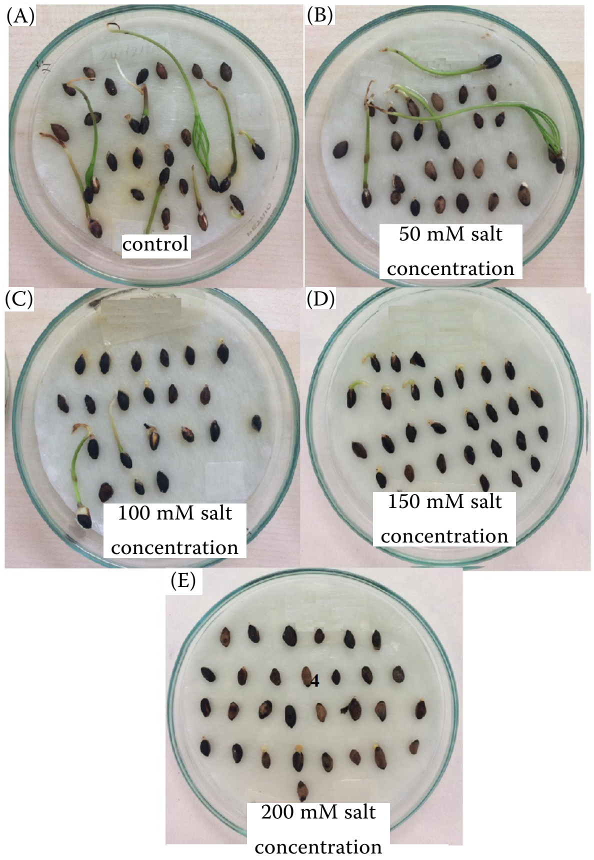

Figure 1. Anatolian black pine seeds in petri dishes with increased salt stress: (A) control; (B) 50 mM; (C) 100 mM; (D) 150 mM; (E) 200 mM salt concentrations

in parallel with the increasing salt concentrations of 50 mM, 100 mM and 150 mM, respectively, compared to the control. The shoot could not be detected at 200 mM absolute concentration. The most deleterious effects of the salt stress were identified at the concentration of 100 mM. In comparison to the 50 mM salt concentration, the root length was reduced by 60% and the stem length by 55% at this concentration. This effect was similar to the relative root growth rate. Root growth rates of 76% at 50 mM, 38% at 100 mM, 29% at 150 mM, and 14% at 200 mM were observed ($P \leq 0.05$). This effect on the root and shoot lengths, which were adversely affected by the increasing salt concentrations, resulted in a similar decrease in the germination percentage (decreased by 19%, 45%, 76%, and 91%, in line with increasing salt concentrations of 50 mM, 100 mM, 150 mM, and 200 mM, respectively, compared to control). This parameter, in particular, decreased by 58% at the 100 mM salt concentration (as compared to the 50 mM salt concentration), leading to the conclusion that this was the most effective salt concentration (Figure 2). Calcium ions, one of the essential nutrients of the plant, inhibit the flow of extracellular sodium ions from occurring in a saline environment, keeping the intracellular sodium and potassium balance in check (Anil et al. 2008). As the salinity

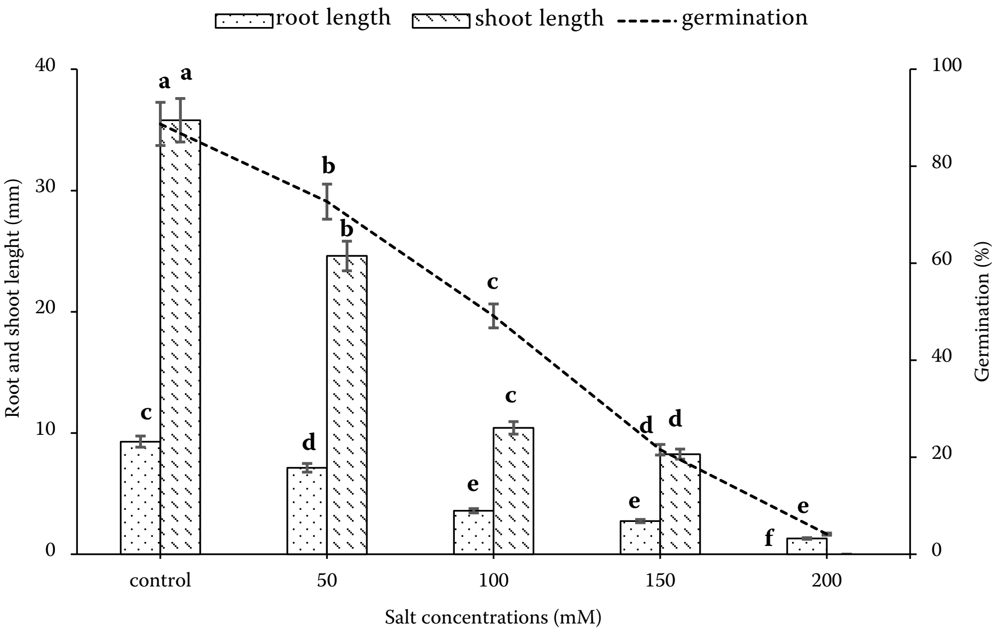

Figure 2. Anatolian black pine seeds which germinated at increasing salt concentrations germination data; bars indicate standard errors of the means (± SE); different letters over identical bars indicate significant differences (Duncan post-hoc test; $P \leq 0.05$)

rises, calcium ions are unable to maintain a metabolic balance, the cell is vulnerable to the harmful effects of sodium ions. The selective membrane permeability is negatively affected as a result of the accumulation of Na$^+$, which increases dramatically in the tissues owing to the soil salinity, creating an ionic imbalance, which causes significant physiological disorders (Flowers et al. 2015). Particularly, the membrane system, which is severely damaged with excessive salinity (Cui et al. 2018), had a negative impact on the water uptake capability of the seeds and suppressed the examined germination parameters. In fact, it was observed that at the highest salt concentration (200 mM), the radicular activity decreased by 86%, and there was no plumular activity. Salinity triggers both hypertonic and hyperosmotic stressors, which causes the plant's germination process to stop before it ever starts (Mahajan, Tuteja 2005). In addition, the seed germination is severely suppressed by the changes in the osmotic potential associated by salt (Pujol et al. 2000). The failure of the seeds to germinate, especially in saline soils, is directly proportional to the inability of the seeds to absorb water from the soil, overcome the high external potential (ψ_0) caused by the salinity, and maintain the water potential gradient (ψ_w) (Nedjimi, Guit 2021).

Multiple physiological and biochemical activities occur simultaneously during germination, the initial and most essential step of the vital process. Any negation during in this time has the potential to disrupt the entire vital process of plants (Zhang, Dai 2019). It was determined that the rate of suppression of the water potential and protein content in seeds germinated under salt stress (Wu et al. 2019) was similar to the rate of the seed germination vigour (*GVI*), defined as the seed vigour. Within this regard, when compared to control, at increasing salt concentrations (except 200 mM), the seed germination vigour index (*GVI*) and seed mass (*WP*) decreased by 43%, 86%, 95%, and 40%, 58%, 83%, respectively (Figure 3). Compared to the 50 mM salt concentration, the *GVI* decreased by 95% and the *WP* decreased by 32% at the 100 mM salt concentration. Whereas when compared to the 100 mM salt concentration, the *GVI* decreased by 10% while *WP* decreased by 31% at the 150 mM salt concentration. Data on the *GVI* are an important factor in determining the germination success of the plant (Rajjou et al. 2012). At the same time, the seedling growth and development success is related to the *GVI* rather than the seed germination rate (Chen et al. 2021). Since salinity causes excessive accumulation of reactive oxygen species (ROS), which is defined as secondary stress in plant cells (Zhang et al. 2015), proteins, lipids, and nucleic acids are peroxidised during germination (Farissi et al. 2014), so anomalies arise in the germination processes. It is noticeable that while the *WP* decreased as the salt concentration increased, the *GVI* did not decrease at the same rate as recorded in our study. This situation can be defined as an effort to alleviate the negative effect of salt stress by activating some mechanisms, such as the osmotic adaptation,

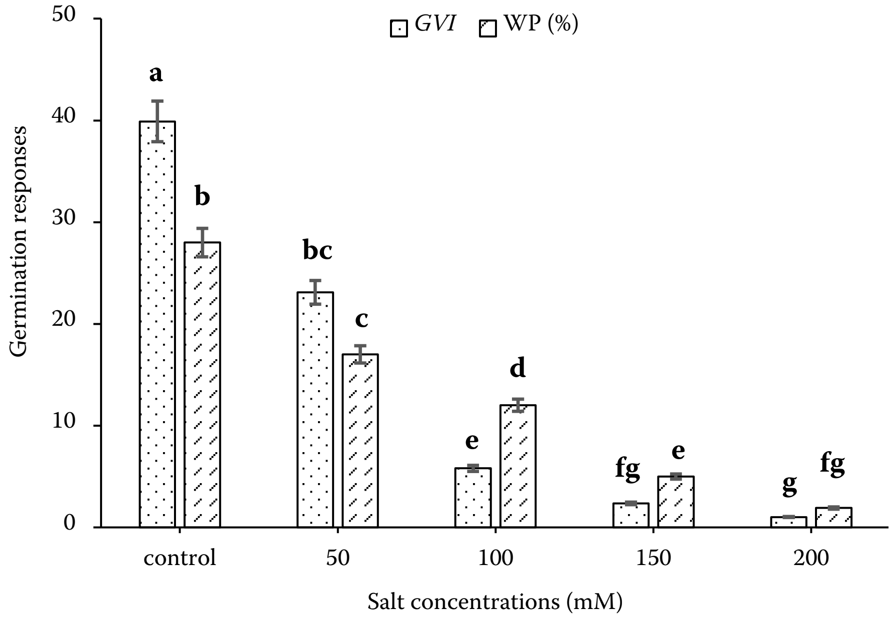

Figure 3. Anatolian black pine seed germination behaviour under increased salt stress; bars indicate standard errors of the means (± SE); different letters over identical bars indicate significant differences (Duncan post-hoc test; $P \leq 0.05$)

GVI – germination vigor index; *WP* – seed mass antioxidant mechanism, and biosynthesis of polyamines, which balance the mineral ion uptake mechanism due to the reduction of the ROS caused by salt stress during seed germination. Similarly, increased salt stress decreased the seed vigour more than the germination index in a study comparing the salinity tolerance levels of ornamental tree seeds, such as *Pinus taeda*, *Pinus elliottii*, *Lagerstroemia indica* and *Fraxinus chinensis*. It has been stated that seeds can germinate, but their survival is not possible, especially in high salt stress (200 mM) (Zhang, Yu 2019).

Physiological experiments

Volatile compounds. The phytochemical content and amount of Anatolian black pine seeds germinated at four different salt concentrations were defined. In comparison to the control, the tested parameters had varied responses at each salt concentration (Figure 4). 73 components were detected in the Anatolian black pine seeds exposed to salt stress, accounting for 96% of the total volatile compounds. It was determined that these compounds consist of six groups as monoterpenes, sesquiterpenes, aldehydes, alkanes, aromatic hydrocarbons and ketones. When compared to the control, the quantity of the components belonging to these groups altered depending on the salt concentrations ($P \leq 0.05$). At each salt concentration, including the control, the quantity of volatile compounds was concatenated as monoterpenes > ketones > aromatic hydrocarbons > aldehydes > sesquiterpenes > alkanes, where α-thujene, α-pinene, β-pinene, δ-3-carene, and limonene belonging to the monoterpenes, were determined as the most dominant compounds. In comparison to the control, these compounds affected the total amount of the monoterpenes by responding differently to the varying salt concentrations. The total monoterpene content increased by 17%, 45%, 7%, and 8% at the 50 mM, 100 mM, 150 mM, and 200 mM salt concentrations, respectively, compared to the control. The increase in the monoterpene content of the seeds is in parallel with the

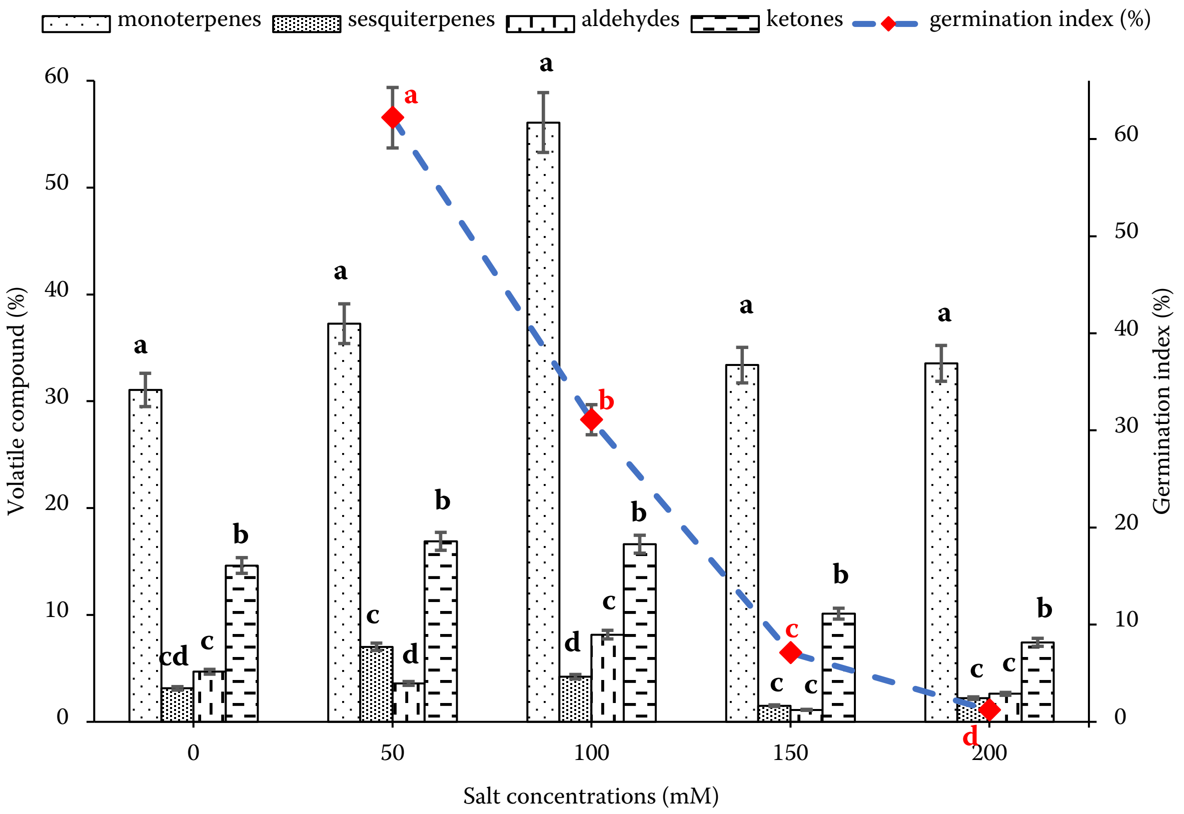

Figure 4. The association between volatile compounds and the germination of Anatolian black pine seeds at increasing salt concentrations; bars indicate standard errors of the means (± SE); different letters over identical bars indicate significant differences (Duncan post-hoc test; $P \leq 0.05$)

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long-term storage, ageing and prolongation of the viability of the *Pinus* sp. seeds. However, in studies of germination of similar seeds under different stress conditions, a similar inference could not be established between the increased amount of internal monoterpene and the germination (Tammela et al. 2003). The concentration of salt that increased the amount and content of the monoterpenes the greatest was found to be 100 mM. This ratio decreased with an increase in the salt concentration. The germination index began to decline at a salt concentration of 100 mM, where the monoterpenes were the highest, whereas the same parameters increased at the 50 mM salt concentration, where the germination index was the highest. However, with an increase in the salt concentration, this changed (150 mM and 200 mM), as the salt concentration increased, the amount and content of monoterpenes decreased in synch with the germination index. The germination index continued to decline at 150 mM and higher salt concentrations (7.1% and 1.2% at the 150 mM and 200 mM salt concentration, respectively), whereas the number of monoterpenes remained relatively constant (33.39% and 33.56% at the 150 mM and 200 mM salt concentration, respectively). α-pinene (16.56%) and limonene (23.61%) showed the highest value at the 100 mM salt concentration. Although α-pinene and limonene, which have greater lipophilic qualities than other monoterpenes, have a very great influence on the plant's oxidative metabolism, they have the highest value at the 100 mM salt concentration, reducing the seed germination and primary root growth (Abrahim et al. 2000). Monoterpenes, on the other hand, inhibit the germination, root elongation, and shoot elongation in pine seedlings by reducing the chlorophyll concentration in the cotyledon leaves and, thus, decrease the respiration capacity of the growing seeds (Halarewicz et al. 2021).

Phenolic compounds. Salt stress, up to a certain concentration, activates some defence mechanisms in the plant and reduces the oxidative damage, particularly thanks to the excessive increase in the

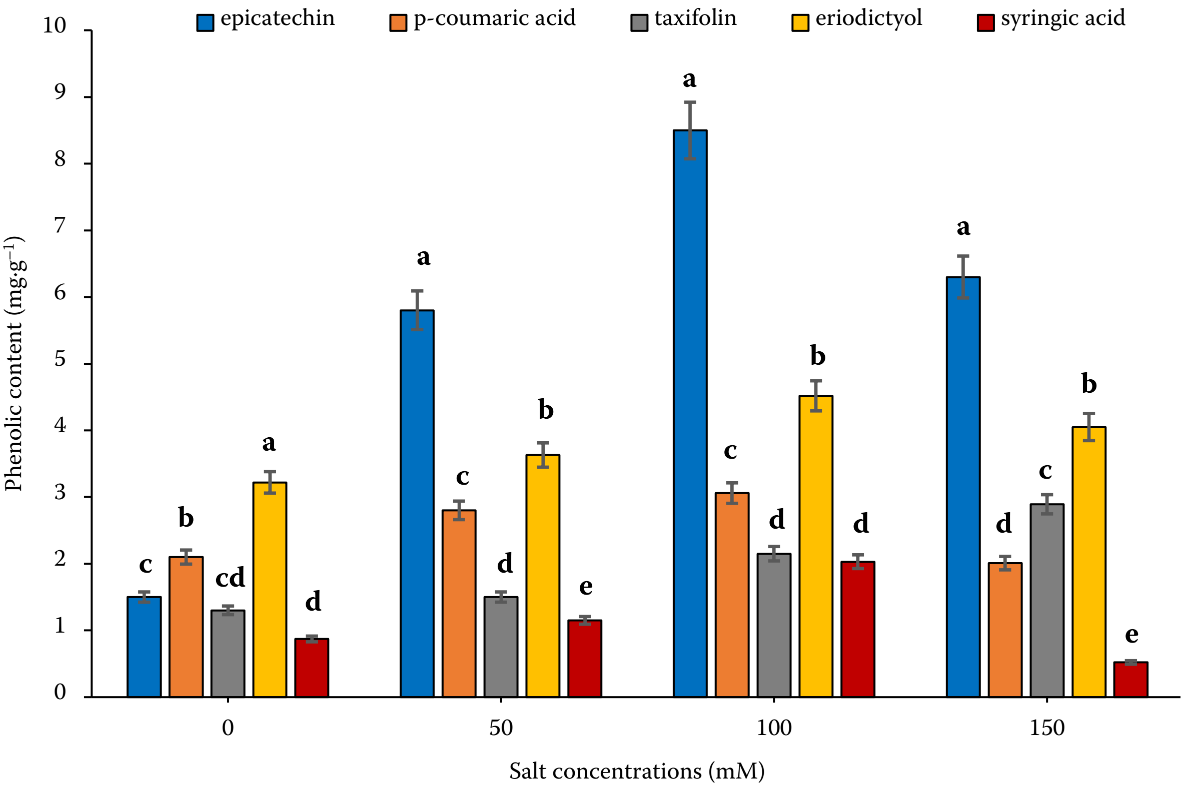

Figure 5. The effect of increasing salinity levels on the phenolic content of Anatolian black pine seeds; bars indicate standard errors of the means (± SE); different letters over identical bars indicate significant differences (Duncan post-hoc test; $P \leq 0.05$)

phenolic content (Tanase et al. 2019). The positive response to the stress was valid up to a salt concentration of 100 mM. The amount of phenolic compounds found in Anatolian black pine seeds grown under salt stress can be summarised as epicatechin > eriodictyol > p-coumaric acid > taxifolin > syringic acid (at the salt concentrations). As the salt concentrations increased, the amount of phenolic substance also changed, compared to the control ($P \leq 0.05$). For example, while epicatechin had the lowest rate in the control (1.5%), its rate increased as the salt stress increased (16.5% at 100 mM), where this dramatic increase was quite remarkable (Figure 5). While the stated ratio was expected to increase in parallel with the increase in the salt stress, it decreased with the opposite effect (6.3% at 150 mM). As a result, when compared to other applications and controls, the other phenolic compounds, particularly epicatechin, had the highest value at the 100 mM salt stress, while these compounds gradually decreased at the 150 mM and 200 mM salt concentrations, where it is actually undetectable at the 200 mM salt concentration. The increase in the salt concentration resulted in the accumulation of phenolic components, which allowed the stress maleffect to be recovered. As the salinity increased, this effect changed in the opposite direction and the negative effect of the salt stress could not be tolerated by the plant and it started to damage the plant by showing an inhibitory effect on the germination at high salt concentrations, such as 150 mM and 200 mM. The increase in the accumulation of small molecular weight phenolic compounds with the antioxidant activity is defined by their role in reducing the accumulation of the stress-induced ROS (Muscolo et al. 2001; Mittler et al. 2004). The accumulation of these compounds, described as organic osmolytes in a stress environment, aids in tolerating or improving the stress response (Cai et al. 2021). However, as the stress factor increases, this beneficial effect is reversed. Although there was a positive association between the increase in the salt concentration and the amount of phenolic components, a negative association was found between the germination statistics, which were examined, and the amount of phenolic components (valid up to a 100 mM salt concentration). At salt concentrations of 150 mM and 200 mM, this association terminated with a gradual decline in the amount of phenolic chemicals and germination parameters. Since the increase in the stress causes a decrease in the accumulation of the stress-protective compounds (Lattanzio et al. 2009), it causes the growth and development of the plant to decrease, and even the mechanisms that initiate the plant's death process to set in (Naikoo et al. 2019). In *Nitraria sibirica* plants grown at increasing salt concentrations, a 100 mmol·L^{-1} NaCl concentration positively affected the formation of compounds forming the precursor of various secondary metabolites, such as amino acids (proline, aspartic acid, methionine, etc.), organic acids (oxaloacetic acid, fumaric acid, nicotinic acid, etc.) and polyhydric alcohols (inositol, ribitol, etc.), whereas it was found that a 400 mmol·L^{-1} salt concentration negatively affected various amino acid metabolism rates, the tricarboxylic acid cycle, photosynthetic carbon fixation and sulfur metabolism and other metabolic pathways (Li et al. 2021b).

CONCLUSION

Soil salinity, which occurs as a result of the increasing average temperatures and falling annual precipitation with climate change, causes the vital processes of plants to end before they begin. The ability of pine plants, which play an important role in the forest ecosystem and human health, to adapt to salt stress caused by changing climatic conditions is critical for their survival and sustainable existence. Within the scope of the obtained results, the suitability of the germination, afforestation and rehabilitation work can be decided by taking the soil salinity in the forestry area into account with regards to the creation and rehabilitation work to be carried out in the next century. Therefore, the current and future soil salinity rates should be determined and these rates should be taken into account in afforestation studies with *Pinus nigra* Arn. subsp. *pallasiana* (Kuzu.) Holmboe. Moreover, it is predicted that the successful conclusion of forest afforestation programmes will be possible by understanding the responses of woody species to salinity stress encountered during the germination period and taking the necessary precautions to overcome the negative effects.

REFERENCES

Abrahim D., Braguini W.L., Kelmer-Bracht A.M., Ishii-Iwamoto E.L. (2000): Effects of four monoterpenes on germination, primary root growth, and mitochondrial respiration of maize. Journal of Chemical Ecology, 26: 611–624.

Anil V.S., Rajkumar P., Kumar P., Mathew M.K. (2008): A plant Ca^{2+} pump, ACA2, relieves salt hypersensitivity in yeast. Modulation of cytosolic calcium signature and activation of adaptive Na^+ homeostasis. Journal of Biological Chemistry, 283: 3497–3506.

Bewley J.D., Bradford K., Hilhorst H., Nonogaki H. (2014): Seeds: Physiology of Development, Germination and Dormancy. New York, Springer: 392.

Cai Z., Liu X., Chen H., Yang R., Chen J., Zou L., Wang C., Chen J., Tan M., Mei Y., Wei L. (2021): Variations in morphology, physiology, and multiple bioactive constituents of Lonicerae Japonicae Flos under salt stress. Scientific Reports, 11: 3939.

Chen K., Song M., Guo Y., Liu L., Xue H., Dai H., Zhang Z. (2019): MdMYB46 could enhance salt and osmotic stress tolerance in apple by directly activating stress-responsive signals. Plant Biotechnology Journal, 17: 2341–2355.

Chen X., Zhang R., Xing Y., Jiang B., Li B., Xu X., Zhou Y. (2021): The efficacy of different seed priming agents for promoting sorghum germination under salt stress. PloS ONE, 16: e0245505.

Croser C., Renault S., Franklin J., Zwiazek J. (2001): The effect of salinity on the emergence and seedling growth of *Picea mariana*, *Picea glauca*, and *Pinus banksiana.* Environmental Pollution, 115: 9–16.

Cui F., Sui N., Duan G., Liu Y., Han Y., Liu S., Wan S., Li G. (2018): Identification of metabolites and transcripts involved in salt stress and recovery in peanut. Frontiers in Plant Science, 9: 217.

Da Silva E.C., Nogueira R.J.M.C., De Araújo F.P., De Melo N.F., De Azevedo Neto A.D. (2008): Physiological responses to salt stress in young umbu plants. Environmental and Experimental Botany, 63: 147–157.

Farissi M., Aziz F., Bouizgaren A., Ghoulam C. (2014): Legume -rhizobia symbiosis under saline conditions: Agro-physiological and biochemical aspects of tolerance. International Journal of Innovation Science and Research, 11: 96–104. (in French)

Finch-Savage W.E., Leubner-Metzger G. (2006): Seed dormancy and the control of germination. New Phytologist, 171: 501–523.

Flowers T.J., Munns R., Colmer T.D. (2015): Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. Annals of Botany, 115: 419–431.

Gemici E., Yucedag C., Ozel H.B., Imren E. (2019): Predicting cone production in clonal seed orchard of Anatolian black pine with artificial neural network. Applied Ecology and Environmental Research, 17: 2267–2273.

Gülsoy S., Cinar T. (2019): The relationships between environmental factors and site index of Anatolian black pine (*Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe) stands in Demirci (Manisa) district, Turkey. Applied Ecology and Environmental Research, 17: 1235–1246.

Güner Ş.T., Arslan M., Özkan K., Çömez A., Kataras R., Celik N. (2022): İç Anadolu Bölgesindeki karaçam ağaçlandırmalarının odunsu tür ve taksonomik çeşitliliği. Ormancılık Araştırma Dergisi, 9: 1–11. (in Turkish)

Gupta B., Huang B. (2014): Mechanism of salinity tolerance in plants: Physiological, biochemical and molecular characterization. International Journal of Genomics, 2014: 701596.

Halarewicz A., Szumny A., Bączek P. (2021): Effect of *Prunus serotina* Ehrh. volatile compounds on germination and seedling growth of *Pinus sylvestris* L. Forests, 12: 846.

Huang J., Ji M., Xie Y., Wang S., He Y., Ran J. (2016): Global semi-arid climate change over last 60 years. Climate Dynamics, 46: 1131–1150.

Kalaji H.M., Račková L., Paganová V., Swoczyna T., Rusinowski S., Sitko K. (2018): Can chlorophyll- a fluorescence parameters be used as bio-indicators to distinguish between drought and salinity stress in *Tilia cordata* Mill? Environmental and Experimental Botany, 152: 149–157.

Kadri N., Khettal B., Aid Y., Kherfellah S., Sobhi W., Barragan-Montero V. (2015): Some physicochemical characteristics of *Pinus* (*Pinus halepensis* Mill., *Pinus pinea* L., *Pinus pinaster* and *Pinus canariensis*) seeds from North Algeria, their lipid profiles and volatile contents. Food Chemistry, 188: 184–192.

Khan N., Bano A. (2018): Effects of exogenously applied salicylic acid and putrescine alone and in combination with rhizobacteria on the phytoremediation of heavy metals and chickpea growth in sandy soil. International Journal of Phytoremediation, 16: 405–414.

Lattanzio V., Cardinali A., Ruta C., Morone Fortunato I., Lattanzio V.M.T., Linsalata V., Cicco N. (2009): Relationship of secondary metabolism to growth in oregano (*Origanum vulgare* L.) shoot cultures under nutritional stress. Environmental and Experimental Botany, 65: 54–62.

Li H., Tang X., Yang X., Zhang H. (2021a): Comprehensive transcriptome and metabolome profiling reveal metabolic mechanisms of *Nitraria sibirica* Pall. to salt stress. Scientific Reports, 11: 12878.

Li Z.J., Yan X.N., Zhang J., Wang S.Y., Cong R.Z., Wei R., Yu H.Y., Wang J.H., Si C.L. (2021b): Isolation and structural characterization of the chemical constituents of *Pinus pumila* seeds. Chemistry of Natural Compounds, 57: 985–987.

Mahajan S., Tuteja N. (2005): Cold, salinity and drought stresses: An overview. Archives of Biochemistry and Biophysics, 444: 139–158.

Mármol I., Quero J., Jiménez-Moreno N., Rodríguez-Yoldi M.J., Ancín-Azpilicueta C. (2019): A systematic review of the potential uses of pine bark in food industry and health care. Trends in Food Science & Technology, 88: 558–566.

Martín-Benito D., Cherubini P., Del Río M., Cañellas I. (2008): Growth response to climate and drought in *Pinus nigra* Arn. trees of different crown classes. Trees, 22: 363–373.

Mittler R., Vanderauwera S., Gollery M., Van Breusegem F. (2004): Reactive oxygen gene network of plants. Trends in Plant Science, 9: 490–498.

Muscolo A., Panuccio M.R., Sidari M. (2001): The effect of phenols on respiratory enzymes in seed germination. Plant Growth Regulation, 35: 31–35.

Naikoo M.I., Dar M.I., Raghib F., Jaleel H., Ahmad B., Raina A., Khan F.A., Naushin, F. (2019): Role and regulation of plants phenolics in abiotic stress tolerance: An overview. Plant Signaling Molecules, 2019: 157–168.

Nedjimi B., Guit B. (2021): Salinity and temperature influencing seed germination of Mediterranean Aleppo pine (*Pinus halepensis* Mill.): An ecological adaptation to saline environments. Baltic Forestry, 27: 247–252.

Pujol J.A., Calvo J.F., Ramirez-Diaz L. (2000): Recovery of germination from different osmotic conditions by four halophytes from southeastern Spain. Annals of Botany, 85: 279–286.

Raccuia S.A., Cavallaro V., Melilli M.G. (2004): Intraspecific variability in *Cynara cardunculus* L. var. *sylvestris* Lam. Sicilian Populations: Seed germination under salt and moisture stresses. Journal of Arid Environments, 56: 107–116.

Rajjou L., Duval M., Gallardo K., Catusse J., Bally J., Job C., Job D. (2012): Seed germination and vigor. Annual Review of Plant Biology, 63: 507–533.

Rogel J.A., Ariza F.A., Silla R.O. (2000): Soil salinity and moisture gradients and plant zonation in Mediterranean salt marshes of Southeast Spain. Wetlands, 20: 357–372.

Sharma A., Goyal R., Sharma L. (2015): Potential biological efficacy of *Pinus* plant species against oxidative, inflammatory and microbial disorders. BMC Complementary and Alternative Medicine, 16: 35.

Shu K., Qi Y., Chen F., Meng Y., Luo X., Shuai H., Zhou W., Ding J., Du J., Liu J., Yang F., Wang Q., Liu W., Yong T., Wang X., Feng Y., Yang W. (2017): Salt stress represses soybean seed germination by negatively regulating GA biosynthesis while positively mediating ABA biosynthesis. Frontiers in Plant Science, 8: 1372.

Tammela P., Nygren M., Laakso I., Hopia A., Vuorela H., Hiltunen R. (2003): Volatile compound analysis of ageing *Pinus Sylvestris* L. (Scots pine) seeds. Flavour and Fragrance Journal, 18: 290–295.

Tanase C., Bujor O.C., Popa V.I. (2019): Phenolic natural compounds and their influence on physiological processes in plants. In: Watson R.S. (ed.): Polyphenols in Plants: Isolation, Purification and Extract Preparation. London, Academic Press: 45–58.

Tiquia S.M. (2010): Reduction of compost phytotoxicity during the process of decomposition. Chemosphere, 79: 506–512.

Tomášková I., Vítámvás J., Korecký J. (2014): Testing of germination of spruce, pine and larch seed after 10 years from collection. Journal of Forest Science, 60: 540–543.

Topacoglu O., Sevık H., Akkuzu E. (2016): Effects of water stress on germination of *Pinus nigra* Arnold. seeds. Pakistan Journal of Botany, 48: 447–453.

Vashisth A., Nagarajan S. (2010): Effect on germination and early growth characteristics in sunflower (*Helianthus annuus*) seeds exposed to static magnetic field. Journal of Plant Physiology, 167: 149–156.

Xiang X., Zhang Z., Wu G. (2019): Effects of seed storage conditions on seed water uptake, germination and vigour in *Pinus dabeshanensis*, an endangered pine endemic to China. Seed Science and Technology, 47: 229–235.

Wu L., Huo W., Yao D., Li M. (2019): Effects of solid matrix priming (SMP) and salt stress on broccoli and cauliflower seed germination and early seedling growth. Scientia Horticulturae, 255: 161–168.

Zhang Q., Dai W. (2019): Plant response to salinity stress. In: Dai W. (ed.): Stress Physiology of Woody Plants. Boca Raton, CRC Press: 155–173.

Zhang Z., Yu F. (2019): Effects of salt stress on seed germination of four ornamental non-halophyte species. International Journal of Agriculture and Biology, 21: 47–53.

Zhang X.H., Zhou D., Cui J.J., Ma H.L., Lang D.Y., Wu X.L., Wang Z.S., Qui H.Y., Li M. (2015): Effect of silicon on seed germination and the physiological characteristics of *Glycyrrhiza uralensis* under different levels of salinity. The Journal of Horticultural Science and Biotechnology. 90: 439–443.

Zucconi F., Forte M., Monac A., De Beritodi M. (1981): Biological evaluation of compost maturity. BioCycle, 22: 27–29.

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