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ORIGINAL ARTICLE

POTASSIUM AND CALCIUM ENHANCE THE ADAPTATION OF INDIAN ALMOND SEEDLINGS TO HEAT STRESS

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Abstract: In light of global warming, adapting plants to a new area may reduce the damage of climatic changes. Indian almond seedlings were planted in pots subjected to calcium and potassium (250 and 500 mg l^{-1}) to reduce the negative impact of high field temperature. Compared with the control, all treatments improved the height plant, branch numbers, leaf numbers, and leaf area. The treatments increased total chlorophyll and carotenoid. However, the treatments modulated proline, total soluble carbohydrate, phenolic, and ascorbic acid contents. Heat stress increased abscisic acid content and electrolyte leakage percentage, whereas calcium and potassium treatments decreased abscisic acid and electrolyte leakage. The treatments modulated protein profile in the number of proteins separated into bands on the gel. The thickness and density of these bands indicate differences in the molecular weights of proteins by treatments. The seedlings responded to heat stress during the second week of July, and the untreated plants were more responsive to the temperature rise than the treated plants. The adaptability of the Indian almond during the summer in semi-tropical regions to high temperatures by using calcium and potassium to reduce heat stress damage on the plant.

***Key words*:** Adaption, Calcium, Potassium chloride, Heat stress, Electrolyte leakage, Indian almond.

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1. Introduction

Plant adaptation to critical environmental conditions depends on the maximum temperature and duration of exposure, the type of plant and other environmental factors [Henry (2020)]. Botanists interested in temperature stress seek to discover the plant responses to heat tolerance and explore how plants manage in high-temperature environments [Raza *et al*. (2019)].

The temperature is constantly increasing in Iraq and the world, where it is expected that the temperature will increase by 5°C in the year 2100 and that the transfer of temperature to and from the plant depends on the difference between the temperatures of the plant and the environment in which, it is developing when plants are exposed to high field temperatures leads to drought and deterioration of growth the plant [Guillot *et al*. (2019)]. The stress resulting from high temperature causes a reduction in photosynthesis and most of the physiological activities in the plant [Al-Khafajy *et al*. (2020)] and effect on cell membranes and proteins [Raza *et al*. (2019)].

The availability of excellent and vigorous seedlings is one of the most important means of spreading and developing multipurpose plants, including Indian almonds [Phulwaria *et al*. (2012)]. The Indian almond (*Pithecellobium dulce* (Roxb.)) is one of the perennial trees grown as an ornamental plant in gardens and parks. It is also used for fixing dunes and planting forests. Mature trees are often used for decoration for the beauty of their appearance, as they have straight trunks topped with horizontal branches and dark green leaves. The flowers are oval, grow at the branches' end and are greenish-white [Rojas-Sandoval and Meléndez- Ackerman (2013)].

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The Indian almond requires adequate care, especially during the summer period. It is a deciduous plant that needs low winter and moderate temperatures for the spring and summer [Akubude *et al*. (2018)]. Adapting plants to external conditions provides additional factors that improve plant tolerance to unfavorable environmental conditions and enhance biochemical processes [Shareef *et al*. (2020)].

The plant management process requires the development of mechanisms and technologies based on bearing environmental stress and limiting its adverse effects on agriculture and production [Henry (2020)]. Therefore, it is necessary to use some mechanisms that increase the heat tolerance of the Indian almond plant, especially in the Middle East, which is witnessing increases in temperature during spring and summer. Some compatible osmolytes, such as calcium and potassium exogenous applications, are among these mechanisms or technologies.

Calcium directs plant reactions to different ecological anxieties, including heat stress [Jasim *et al*. (2016), Lateef *et al*. (2021)]. Heat stress increases calcium particles in the cell, diminishing hotness injury and empowering plant cells to endure better [Naeem *et al*. (2013)]. Heat tolerance in many plants improved using exogenous calcium, which may be related to increased activities of antioxidant enzymes and reduced lipid peroxidation in cell membranes [Bahamonde *et al*. (2018)]. Calcium use preserves chlorophyll under heat stress, possibly by reducing photo-oxidation or maintaining the integrity of cell membranes [Jiang and Huang (2001)].

Potassium is an element that modulates the osmotic pressure in cells of the plant (osmoticum) to relieve various stresses [Shahid *et al*. (2019), AL-Taey and Burhan (2021)]. Potassium-induced regulation of plant water relations reduces heat sensitivity, while potassium availability under stress enhances photosystem II quantitative yield, chlorophyll content and enzymatic activities. Similarly, the potassium availability with heat stress conditions improves plants' ability to fix carbon (carboxylation) by regulating the synthesis of RuBisCO and sucrose synthase [Zahoor *et al*. (2017)]. Homeostasis with K enhances enzyme synthesis, relieves oxidative stress, and improves signal transduction during stress [Souto *et al*. (2018)]. Furthermore, the plant can retain chlorophyll and sucrose translocation for a relatively long time in the presence of potassium than without [Chrysargyris *et al*. (2017), Shareef (2019)].

A change in the extreme temperatures in recent years exceeded 50°C, which negatively affected the growth of plants and led to their death, especially in the seedling stage. Note that the importance of the Indian almond plant lies in the diversity of its uses for medicinal purposes and as an ornamental plant, in addition to its use as windbreaks and the possibility of obtaining seeds that are used as a primary method for its propagation, which helps to spread it and obtain a profitable economic return as a result of using the currently imported seeds for breeding in local nurseries [Kaneria *et al*. (2018)]. The experiment aims to reduce the adverse consequence of heat stress by treating the seedlings with different calcium chloride and potassium chloride concentrations.

2. Materials and Methods

Seeds of Indian almonds (*Pithecellobium dulce*) were obtained from Aljouri Agricultural materials/ (UAE). The seeds were grown in 20 × 25 cm pots in a local nursery for four months. On September 1, 2020, fifty-four seedlings were transferred to plant at the wooden canopy at (30°33'47.3"N 47°44'38.4"E). Then, large plastic pots (30 × 32 cm) were prepared, washed well with water and sterilized with formalin, and then filled with sterilized growth medium by autoclaving at a rate of one plant per pot and in soil consisting of (1:1) Bet moss: soil, the electrical conductivity (EC) of soil was 2 dSm^{-1} and to the water of irrigation is EC 1.2 dS m^{-1}. The process of servicing the plants was carried out symmetrically from fertilization and irrigation, and the plants were approximately 30-40 cm in height. All the experimental plants were fertilized with the compound fertilizer NPK (20-20-20) from 1 February 2021 once per month at rate1g pot^{-1}. The pots were taken from the wooden canopy and put in the field on February 10, 2021. On 9 March 2021, the treatments were conducted monthly and over four months as a foliar spray until 9 May 2021. Tween 20 was added at 1 ml l^{-1} as a diffuser to reduce the surface tension of the leaves. It was sprayed in the early morning using polyethylene bags to prevent contamination from the effect of the treatments. The following nine treatments were applied: 1) Control (spray withdistilled water), 2) KCl 250 mg l^{-1}, 3) KCl 500 mg l^{-1}, 4) $CaCl_2$ 250 mg l^{-1}, 5) $CaCl_2$ 500 mg l^{-1}. On day 60th of treatment, the leaves were collected for morphological and biochemical

assay from every treatment. In July, the minimum temperature was 28°C, the maximum was 46°C, the relative humidity was 27% and the light intensity was 1750 μmol m^{-2} s^{-1}.

2.1 Growth parameters

Measure the height of the plants from the soil surface of the pot to the end of the growing top using a tape measure. The number of lateral branches of each plant was calculated and the means were recorded. The number of leaves on the main stem and the lateral branches was calculated and the average was taken and multiplied by the number of branches to get the leaves number. The leaf area was measured using the ImageJ program according to Easlon and Bloom (2014) and after taking ten leaves for each replicate, they were placed in a scanner. The readings representing the leaf area of the plant were taken according to the following equation:

The plant leaf area (cm^2) = Leaf area (cm^2) × No. of leaves.

2.2 Assessment of total chlorophyll

The substance of pigments was taken out from the leaves as per the method depicted by Lichtenthaler and Wellburn (1983). Preliminary 0.2 g of the sample leaf were crushed in 10 ml acetone (80%) and centrifuged at 2000 rpm for five min. The 645, 663 and 534 nm absorbance was utilized to pick the chlorophyll.

2.3 Soluble carbohydrate estimation

The Phenol-sulphuric acid technique assessed the carbohydrate, followed by Yemm and Willis (1954). 100 mg fresh leaf sample was homogenized in 5 mL of 2.5 N HCl and put in a boiling water bath for 3 h. The cooled rough homogenate was balanced and centrifuged at 10000 rpm for 10 minutes. To 100 μl of supernatant, 100 μl phenol [5% (v/v)] and 500 μl of sulfuric acid [96% (v/v)] was mixed. The absorbance was estimated at 490 nm. Glucose going from 0-500 μg mL^{-1} was utilized as a standard to calculate the soluble carbohydrate of the examples.

2.4 Proline estimation

The proline content was assessed by Bates *et al*. (1973). 0.5 g of the test was homogenized with 5 mL of 3% sulfosalicylic acid. The example was separated, and 3 mL of this separate was mixed with ninhydrin reagent and glacial acetic acid, 3 mL each. This combination was warmed in a bubbling water bath for an hour and cooled. A chromophore was shaped by adding 4 mL toluene to this cooling solution. By 520 nm, absorbance utilizing the UV-VIS spectrophotometer proline was measured. Proline going from0-10 μgmL^{-1} was used as standard and a diagram was plotted from which the proline content was assessed.

2.5 Total Phenolic assay

The phenols extract was estimated utilizing the Folin-Ciocalteu strategy, as Waterman and Mole (1994) indicated. 25 μL of the concentrate (500 μg mL^{-1}) was utilized, to which 25 μL of (1:1) Folin-Ciocalteu reagent and 100 μL of 7.5% sodium bicarbonate solution were the arrangement and hatched at room temperature for 2 hours in dim circumstances. 765 nm using a UV-VIS spectrophotometer to record the absorbance. Gallic acid going about 0-100 μg mL^{-1} was utilized as a standard to calculate the phenol content of the examples.

2.6 Ascorbic acid content

Ascorbic acid (ASA) by Luwe *et al*. (1993) was measured. Leaf tests (0.6 g) were homogenized with 12 ml of 5% trichloroacetic acid. The concentrate was blended in 2 ml of 2% dinitrophenylhydrazine (pH 5) trailed using 10% thiourea as a drop (in 70% ethanol). For 15 min in a water bath, the blend was bubbled, then cooling the combination at room temperature, 5 ml of 80% (v/v) H_2SO_4 was mixed into the mixture at 0°C. 265 nm was used to record the absorbance. The ASA content is determined as a standard bend plotted with available content.

2.7 Abscisic acid analysis

One gram of new leaf tissue was homogenized in 70 % methanol at 4°C; it was mixed for the time being. Under a vacuum, the concentrate was separated and dissipated with Whatman channel paper (No. 1). The pH of the aqueous stage was changed following 8, utilizing a 0.2 mol phosphate buffer. The aqueous phase was divided twice using methanol. A rotary evaporator eliminates the methanol stage. pH edited to 2.5, utilizing 1 N HCl through the aqueous stage. The concentrate injection specified abscisic acid into a turned around stage HPLC, C12 column in an isocratic elution mode using a convenient stage including $(CH_3)_2CO$: H_2O (26:74) with 30 mmol phosphoric acids according to Tang *et al.* (2011). 1 N sodium hydroxide using to keep up pH at 4. The transition rate was 0.6 ml min^{-1}, and the elution of abscisic acid was seen at 270 nm at 25ºC.

Fig. 1: (a) Show exposure of Indian almond seedlings to thermal stress, (b) Show the recovery of Indian almond seedlings from thermal stress

2.8 Electrolyte leakage

Leaf segments one gram utilized with 12 ml separated water was saved at 27 ºC overnight alongside vibration. The first conductivity (C1) was determined after getting a thermometer test of 27ºC along with a conductivity meter. The models get autoclaved for 15 min and cooled to 26ºC; by then, the second conductivity (C2) will be determined. The film unfaltering quality rundown was once assessed to determine the estimation of C1 and C2 and imparted among the ratio [Shanahan *et al*. (1990)].

2.9 Minerals concentration

Fresh leaf samples were dried at 75 °C until arriving at a consistent weight; Cresser and Parsons (1979) method was applied. The arrangement was straightforward and was used to finish the potassium transmission flame photometer. Calcium focus is controlled by atomic absorption spectrophotometer at 422.7 nm [Waling *et al*. (1989)].

2.10 Extraction of proteins and gel electrophoresis

Protein was separated (350 mg) in buffer [0.2 mol, tris-hydroxymethyl aminomethane (Tris) + 0.001 mol ethylenediamine tetraacetic acid + (Na_2+EDTA) + 12% glycerol + 0.01 M dithiothreitol (DTT) + 0.05 mmol phenylmethylsulfonyl fluoride (PMSF)] a mortar and pestle use to mixed. after, the samples put at 14,000 ×g for 20 min centrifuged; then the buffer comprised of 0.125 M Tris HCl (pH 6.8) + 4% SDS + 20%, glycerol + 10% b-mercaptoethanol + 0.01% bromophenol blue. The water bath at 90°C for 3 min was used to denaturize the protein tests. Electrophoresis of protein was acted in SDS polyacrylamide gel following the methodology Laemmli (1970). The groups of protein exhibited that investigated by ImageJ programming.

2.11 Response to heat stress

Percentage of plants responding to heat stress (%) = number of plants responding to stress / total number of plants.

2.12 Statistical analysis

The experiment was conducted as a randomized block complete design of nine treatments. Each one consisted of four replications. The information was exposed to the examination of difference (ANOVA) utilizing SPSS variation 20.0 (SPSS, Chicago, IL) and the means were isolated utilizing the Duncan test at the 0.05 level.

3. Results

3.1 Growth parameters and total chlorophyll

All growth parameters in Indian almond seedlings are subjected to potassium and calcium under high field temperature increased (Table 1). Application of K and Ca improved the height of the plant, branches number, leaves number, and leaf area related to the control. Application of KCl 250, KCl 500 and $CaCl_2$ 500 increased plant height compared with other treatments.

Treatments of KCl 250, KCl 500 and $CaCl_2$ 250 increased branch number and leaf numbers compared with control. However, $CaCl_2$ 250 treatment increased leaf area compared with other treatments. $CaCl_2$ 500 treatment increased total chlorophyll related to other treatments.

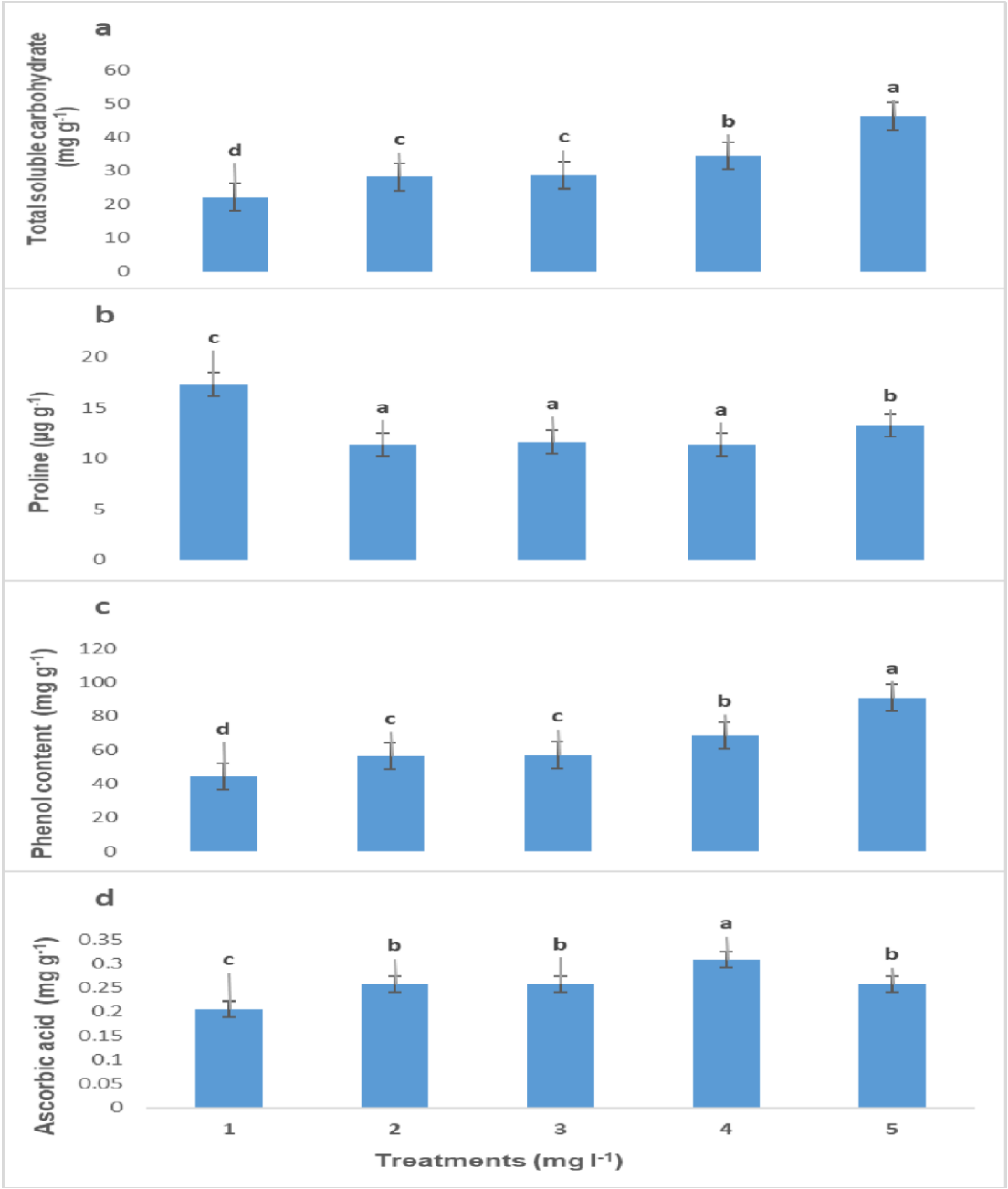

Fig. 2: Exogenous potassium and calcium on total soluble carbohydrate (a), proline content (b), phenol content (c), and ascorbic acid (d) content in Indian almond seedlings under high field temperature in July. Treatment numbers are described as 1) Control (spray with distilled water), 2) KCl 250, 3) KCl 500, 4) $CaCl_2$ 250, 5) $CaCl_2$ 500. By Duncan's multiple range test, values within the same column show significant differences at $P \leq 0.05$. Data are means of four replicates ±SD

3.2 Metabolic compounds

Proline content increased under heat stress, whereas total soluble carbohydrate, phenolic and ascorbic acid contents decreased (Fig. 2). All treatments modulated proline, total soluble carbohydrate, phenolic, and ascorbic acid contents. Moreover, $CaCl_2$ 500 treatments significantly increased total soluble carbohydrate and phenolic contents compared with other treatments. Applications of KCl 250, KCl 500 and $CaCl_2$ 250 decreased proline content significantly compared with other treatments. The application of $CaCl_2$ 250 increased ascorbic acid significantly related to other treatments.

Heat stress increased abscisic acid content and electrolyte leakage percentage, whereas potassium and calcium decreased (Fig. 3). The applications of KCl

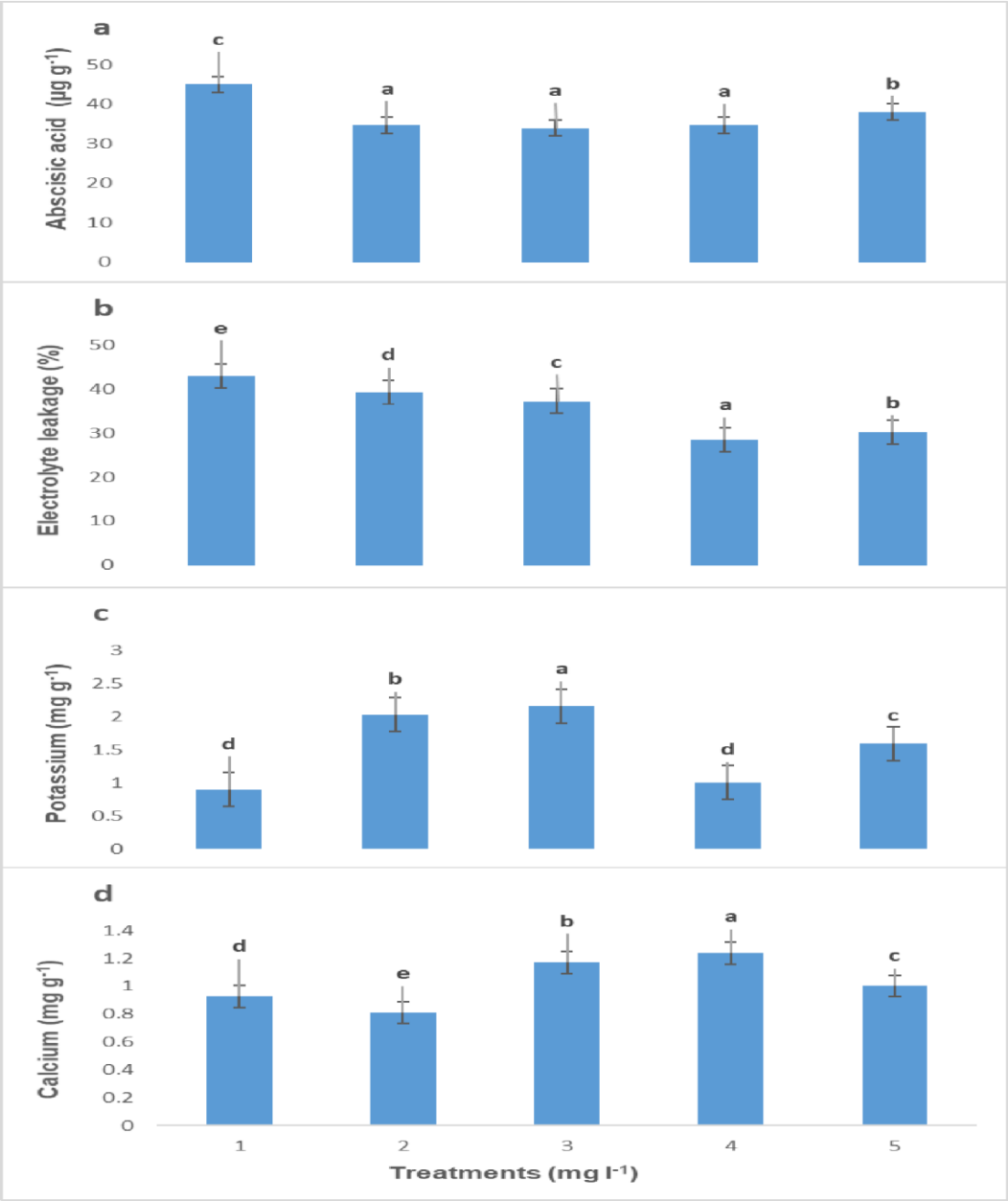

Fig. 3: Exogenous potassium and calcium on total abscisic acid content (a), Electrolyte leakage (%) (b), and Potassium content (c) in Indian almond seedlings under high field temperature in July. Treatment numbers are described as 1) Control (spray with distilled water), 2) KCl 250, 3) KCl 500, 4) $CaCl_2$ 250, 5) $CaCl_2$ 500. By Duncan's multiple range test, values within the same column show significant differences at $P \leq 0.05$. Data are means of four replicates ±SD

250, KCl 500, and $CaCl_2$ 250 decreased abscisic acid content. Moreover, $CaCl_2$ 250 decreased electrolyte leakage significantly compared with other treatments. KCl 500 application increased potassium content significantly related to other treatments. Whereas, $CaCl_2$ 250 application increased calcium content significantly related to other treatments.

3.3 Change in protein pattern

Fig. 4 illustrates the process of gel electrophoresis of the leaves of Indian almond seedlings due to spraying with potassium and calcium. Differences in the number of proteins separated into bands on the gel and the thickness and density of these bands indicate differences in the molecular weights of proteins. It is clear from

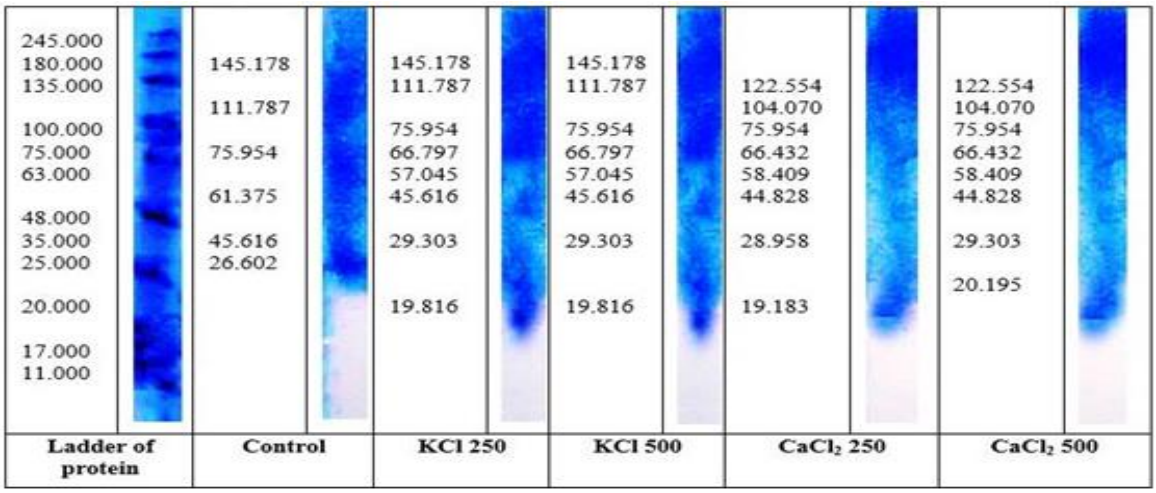

Fig. 4: Protein patterns analysis by One-D SDS-PAGE extracted from the leaf shows changes in molecular protein weights of Indian almond seedlings under high temperatures and treatments

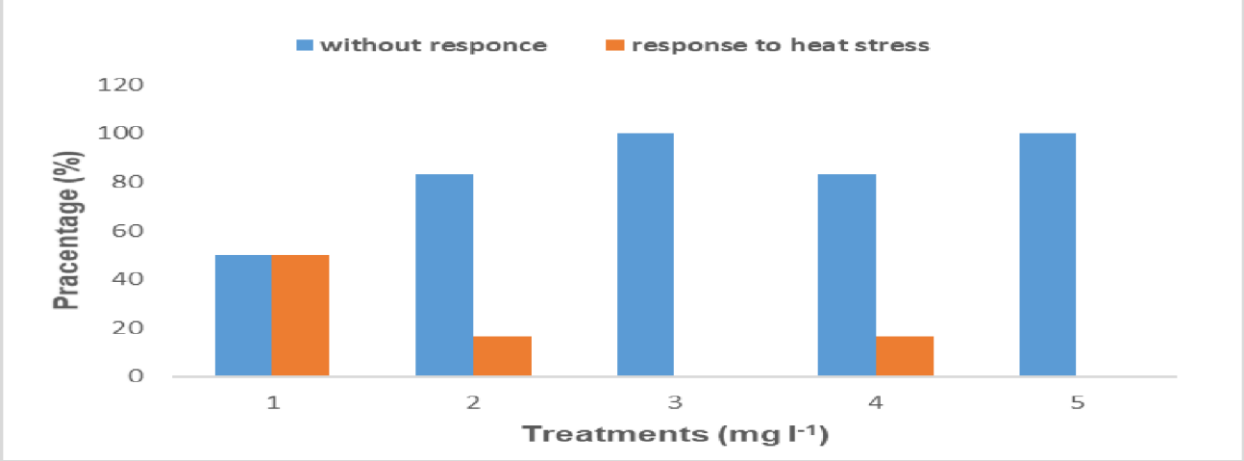

Fig. 5: Exogenous potassium and calcium response to heat stress in Indian almond seedlings under field high temperature in July. Treatment numbers are described as 1) Control (spray with distilled water), 2) KCl 250, 3) KCl 500, 4) $CaCl_2$ 250, 5) $CaCl_2$ 500

the figure that the control treatment gave eight protein bands with molecular weights that included (286.786, 145.178, 111.787, 75.954, 61.375, 45.616, 26.602, 19.816) kDa. $CaCl_2$ 250 and $CaCl_2$ 500 showed eight similar bands with molecular weights (122.554, 104.070, 75.954, 66.797, 58.409, 44.828, 28.958, 19.183) kDa and only two are different (29.303, 20.195) kDa in treatment of $CaCl_2$ 500. The treatment of KCl 250 and KCl 500 have eight similar bands with molecular weights (145.178, 111.787, 75.954, 66.797, 57.045, 45.616, 29.303, 19.816).

3.4 Response to Heat stress

Fig. 4 shows potassium and calcium's effect on the response rate to heat stress during the second week of July. The results of the experiment released that with an increase in the average temperature during the second week to 40.28°C (49/31°C), the seedlings responded to heat stress, and the control treatment was more responsive to the temperature rise than the other treatments; the response rate to heat stress in control reached 50%, followed by treatments of KCl 250 and $CaCl_2$ 250 with a response rate of 16.66%. In contrast, the other treatments did not respond to heat stress this week. The data is not shown; the high and low temperatures varied, and when the temperatures reached the average of 34°C (42/26°C) in mid-September, all seedlings recovered their growth.

Table 1: Exogenous potassium and calcium on heightof plant, number of branches, leaves number, leaf area, and chlorophyll in Indian almond seedlings under high field temperature.

Treatments ($mg\ l^{-1}$)	Plant height (cm)	Number of branches	Number of leaves (Leaf $plant^{-1}$)	Leaf area ($cm^2\ plant^{-1}$)	Total chlorophyll ($mg\ g^{-1}$)
Control	63.25±3.30 c	2.50±.57 b	167.50±35.64 b	168.50±35.85 d	6.04±0.09 c
KCl250	91.00±2.58 a	4.25±0.95a	319.75±71.39 a	385.93±86.17b	7.21±0.15 b
KCl500	89.75±4.78 a	4.50±0.57a	381.25±45.71 a	464.40±61.75b	7.25±0.11 b
$CaCl_2$ 250	81.75±7.50 b	5.00±0.00 a	381.25±22.50 a	903.18±53.30a	7.41±0.11 b
$CaCl_2$ 500	93.75±3.59 a	3.25±0.50 b	121.50±31.89 b	287.83±75.54c	7.79±0.27 a

Means of 4 replications ±SD. Using Duncan's multiple range test means different letters at $p \leq 0.05$.

4. Discussion

In light of global warming, planting new areas may reduce the damage of climatic changes. The distribution of plants on the planet is associated with high temperatures as a limiting factor to adaption. In this study, a tropical plant of Indian almond was grown in a subtropical environment. Subtropical regions suffer from high temperatures and little or no rain in the summer. Biochemical reactions within plants are sensitive to high-temperature stress, which vary with temperature, duration of exposure and plant type [Fragkostefanakis *et al*. (2015)]. High temperatures cause cell damage or plant death within minutes and moderate temperatures may cause cell injury or death with prolonged exposure [Nievola *et al.* (2017)]. High temperature impairs plant growth stages and physiological processes and significantly reduces the productivity of many plant species [Hatfield and Prueger (2015)]. High-temperature formats the harmful substances in plants due to disruption in the processes of photosynthesis and the ability to respiration in plants; subsequently, leaves turn yellow, inhibiting growth processes in plants and destroying chlorophyll [Jamloki *et al*. (2021)]. Water evaporates greatly from plants, causing dryness in plants (Fig. 1(a)). Higher summer temperatures cause a decrease in growth parameters, photosynthetic pigments and metabolic compounds, whereas proline and abscisic acid (ABA) increase (Figs. 2, 3). The high temperatures increase the activity of the enzyme chlorophyllase, destroy chlorophyll and increase the level of ABA, which accelerates the decomposition of chlorophyll [Jamloki *et al*. (2021)].

The impact of heat stress was reversed due to the foliar spray with potassium and calcium. The increase in growth parameters when treated with potassium may be due to its positive role in improving the overall physiological activities within the plant cells, the most important of which is improving the effectiveness of photosynthesis and the composition of cell organelles [Aly *et al*. (2015)], as well as improving hormonal balance by increasing growth-promoting hormones and reducing growth-inhibiting hormones [Hasanuzzaman *et al*. (2018)]. The increase in vegetative growth indicators, mainly when treated with potassium may be due to potassium's role in reducing environmental stress damage by reducing the rate of transpiration, which leads to reducing osmotic stress, reducing plant water loss, and prolonging plant survival live under heat stress. The increase in leaf area by potassium sprayed was similar to Al-Furtuse *et al*. (2019) on cowpea plants.

When treated with calcium, the increase in leaf area is one of the components of cellular membranes and its increased stability when exposed to external stresses-cells in carrier cells in phloem under stress conditions [White and Broadley (2003)]. The protective role of calcium is attributed to maintaining the structural integrity of the plasma membrane and thus controlling ion uptake [Jasim *et al.* (2016)]. However, the increased growth is due to cell expansion and photosynthesis activation by increasing membrane stability (Fig. 3(b)).

The increase in plant pigments when treated with potassium may be due to its effect on increasing the absorption of some elements, including iron and magnesium, which play an essential role in building chlorophyll, or it may be due to potassium's role it plays in increasing the activity of the ATPase enzyme in plasma membranes and vacuole membrane (Tonoplast) [Hasanuzzaman *et al.* (2018)].

The reduction in total soluble carbohydrates in the control treatment due to the impacts of high temperature and exposure of seedlings to thermal quiescence programming is represented by a decrease in the leaf

area and the total chlorophyll content (Table 1). The activity of the enzymes responsible for carbon dioxide reduction, especially the RuBSCO enzyme and RuBP Carboxylase [Kahrizi *et al*. (2012)]. When sprayed with calcium and potassium, the increase in the total soluble carbohydrates is due to these two compounds' roles in increasing the efficiency of the photosynthesis process and the leaf area exposed to light, which leads to an increase in carbohydrates leaves.

The proline accumulation under heat stress conditions gives the plant the energy needed for growth and endurance to stress [Liang *et al*. (2013)]. The proline accumulation under heat stress conditions gives the plant the energy needed for growth and endurance to stress [Liang *et al*. 2013)]. Proline acts in many physiological roles and functions under environmental stress conditions, where it performs the process of osmoregulation inside the plant cell, in particular between the vacuole and the cytoplasm, as the accumulation of proline inside the cytoplasm works to regulate the state of homeostasis and facilitate the process of water absorption by the roots. In addition, proline protects the organelles from harmful effects such as oxidizing factors and contributes to the destruction of free radicals [Kishor *et al*. (2015)]. The treatment with calcium and potassium led to a modification of the proline content in the leaves. It can be attributed to their role in relieving heat stress on plants.

Phenolic compounds are plants' most important secondary metabolites that act as natural antioxidants, free radical scavengers, inhibitors of free radical production and catalysts for antioxidant synthesis [Cosme *et al*. (2020)]. The high in total phenols by calcium is due to this mineral's critical role in reducing respiration. Increased phenols indicate plant adaptation to extreme environmental conditions [Cosme *et al*. (2020), Ismael *et al*. (2022)].

The reduction in plant growth at 49/31°C was associated with the emergence of stress damages that decreased membrane stability. The membrane damage is probably due to a loss of ability to rapidly and completely reorganize cell membranes. Previous studies have indicated membrane permeability as a valuable indicator of heat stress damage [ElBasyoni *et al*. (2017), Jia *et al*. (2020)]. Our findings show that heat stress decreases the membranes' stability when electrolyte leakage is used as the permeability index. A higher temperature tolerance was observed in plants grown than those not treated with calcium and potassium. The higher membrane stability index resulting from calcium and potassium is related to the antioxidant responses that protect the plant from oxidative damage and the higher ionic content and induced activities of antioxidant enzymes.

Potassium increases in the leaves when the plant is treated with potassium and calcium increases when the plant is treated with calcium (Fig. 3(c), (d)). The act of antioxidants thus reduces the oxidative process, increasing the activity of the plant's vital functions [Alhamrani *et al*. (2019)]. In addition to being a stimulant for synthesizing proteins and enzymes that accompany carbohydrate representation, photosynthesis and ionic balance, as well as being an osmotic regulator that participates in the processes of opening and closing stomata, etc. [Da Silva *et al*. (2021)].

Heat stress is the cause of an increase in ABA. ABA, known as one of the transduction signal components, leads to gene induction, forming proteins necessary to protect plants under stress conditions [Vishwakarma *et al*. (2017)]. Examination of protein patterns in control and treatments showed that the relative density in Indian almonds was more significantly related to the KCl 250, KCl 500 and $CaCl_2$ 250 treatments than the other treatments (Fig. 4). Plant development and variation in ecological circumstances are unequivocally affected by protein metabolism [Schlüter *et al*. (2013)]. Different examinations have demonstrated that the development of new proteins connects with changes in natural plant circumstances, for example, heat stress, prompting an expansion or diminishing in polypeptides. The results demonstrate that heat stress prompted the development of new proteins and the disappearance of others, accompanied by a distinctive appearance of stress proteins. The formation of new proteins affected by high temperature demonstrates that these proteins were shaped ahead of schedule by programming cell passing as we noticed shrinkage and stiffness in plants (Fig. 1(a)). All ecological burdens invigorate the creation of a gathering of proteins called heat-shock proteins (Hsps), or stress-incited proteins. The acceptance of the record of these proteins is a typical peculiarity in every living being. These proteins are assembled in plants into five classes as per their approximatemolecular weight: (1) Hsp100, (2) Hsp90, (3) Hsp70, (4) Hsp60 and (5) small heat shock

proteins (sHsps). This broadening of these proteins reflects variation in enduring heat stress. The heat shock protein gene is controlled by regulated proteins called heat stress transcription factors (HSFS). These proteins likewise coordinate in all periods of cyclic reactions to high temperature (operation, maintenance and recovery) (Fig. 1(b)) [Islam *et al*. (2018)].

The plant's response to the high temperature in the summer, which included all the treatments, indicates its behavior in the semi-tropical regions with high temperatures in the summer, as it is a deciduous plant in the winter. When the plants are exposed to temperatures higher than 45°C, the plants are exposed to thermal stress. The hardening and shedding of leaves under the influence of high temperature may be attributed to the high concentration of growth inhibitors such as abscisic acid, which leads to the production of ethylene and thus stiffness and defoliation [Islam *et al*. (2018)]. Applying treatments led to a delay in the plant's response to heat stress, due to the role of those compounds such as calcium and potassium in the plant's tolerance of environmental stresses, including heat stress, by reducing oxidative stress and creating a state of metabolic compounds. One of the treatments that delay affecting thermal stress is the treatment of calcium, which may be attributed to the physiological roles of calcium in protecting the plant from the harmful effects of high and low temperatures, reducing the damage of environmental stresses, reducing the harmful effect of ozone, the severity of freezing, drought stress, and its role in plant tolerance to drought from the method of closing the stomata during the day, which is essential to protect plants from high temperatures, and thus leads to a delay in plant aging [Khan *et al*. (2012)].

5. Conclusion

Growing plants in an environment different from their original environment must improve their tolerance to new environmental conditions. The adaptation of the Indian almond plants to high temperatures during the summer in semi-tropical regions is possible by using antioxidants to reduce heat stress damage on the plant. Using calcium or potassium chloride improved theplants' tolerance to heat stress.

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