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Cold plasma technology as a pre-treatment for seed priming enhances germination and reduces salinity stress in *Prosopis Koelziana*

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The *Prosopis koelziana* genus, part of the Fabaceae family, plays a crucial role in the ecology and economy of arid regions. It is commonly used for restoring deserts, forests, and degraded soils that have low fertility and high salt concentrations. However, recent research has shown that the seedlings of *P. koelziana* are highly susceptible to salinity stress, despite their overall high tolerance. This study aimed to explore the use of cold plasma technology as a seed pretreatment to enhance germination rates and seedling tolerance to salt stress. In the study, the germination rates of *Prosopis koelziana* seeds improved significantly: from 44% to 21% at 100 mM and 200 mM salinity, respectively, to 100% and 68% after an 8-minute plasma treatment. This improvement is attributed to various physical and biochemical changes occurring within the seeds, such as increased permeability to water and gases. Furthermore, after the 8-minute plasma treatment, membrane lipid peroxidation—an indicator of oxidative stress—was reduced by 35% and 50% in plants subjected to 100 mM and 200 mM salt stress, respectively. Under both moderate and severe salt stress, the activities of the enzymes catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX) significantly increased in plasma-treated plants compared to those not treated with cold plasma. In contrast, the activity of polyphenol oxidase (PPO) decreased. These findings suggest that cold plasma priming is a promising method for combating salinity stress and highlight its potential in enhancing the survival and conservation of *Prosopis koelziana* in arid ecosystems.

Keywords *Prosopis Koelziana*, Soil salinity, Cold plasma, Germination, Resilient seedlings

Desertification has become a major environmental issue in many countries, including Iran. More than half of Iran's area is covered by arid and semi-arid deserts. The main reasons for the spread of desert areas are reduced precipitation and soil salinity. To prevent soil erosion and dust particle dispersion, many tactics have been proposed. Planting indigenous vegetation in arid and semi-arid areas is one of the most effective ways to prevent wind and water erosion. It is recommended to use native plant species in these regions as they are more resistant to severe environmental conditions[1].

Prosopis koelziana is a plant commonly found in the arid and semi-arid regions of Iran. It is a member of the Mimosaceae or Fabaceae family and can thrive in harsh environmental conditions. This plant is highly beneficial for restoring desiccated forests, rehabilitating degraded lands, and reclaiming highly saline and decertified terrains[2,3]. There are 44 distinct species of *Prosopis*, but the *koelziana* species is a standout and make a significant contribution to the ecology and economy of the Iranian desert. The plant has various industrial, medicinal, and afforestation applications, and its adaptability to harsh environmental conditions makes it ideal for these purposes[4]. Although mature *Prosopis koelziana* plants have impressive salt tolerance, the seedlings are sensitive to high salinity levels. Therefore, enhancing the salt resistance of the seedlings can greatly improve their proliferation. This, in turn, can help preserve biodiversity, improve soil quality, and enhance environmental stability in arid regions.

Soil salinity is the presence of dissolved salts in the soil, which usually happens due to water evaporation and the re-emergence of these salts on the soil surface[5]. Salinity can harm plants by directly reducing water uptake[5], stalling physiological processes[6], and degrading proteins and enzymes[5]. It can also indirectly affect them by making them more vulnerable to plant diseases[7], reducing crop output and quality[8], decreasing biodiversity[9],

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and disrupting soil ecosystems[8]. Seed priming is a valuable technique for mitigating the negative impacts effects of salinity on plants. This involves treating seeds before planting, resulting in more vigorous plants that can better withstand harsh environmental conditions. Studies have shown that seed priming is an effective method for reducing salinity damage and improving plant resistance to stress[10–12]. Several physical and chemical methods have been used for seed priming[13,14].

Plasma is a relatively new technology being used in agricultural crop production, and has already shown benefits for several plant species[11,15–17]. *The term "plasma" refers to the fourth state of matter, which comprises atoms, molecules, ionized gases, free electrons, and radicals. Plasma is widely used in various commercial sectors including microelectronic technology, medicine, power fusion, and food processing*[18–20]. *Plasma treatment is an efficient, low-cost, fast, and environment-friendly technology that can enhance seed quality, growth, and, ultimately, plant production*[18,20]. In agriculture, plasma treatment has been found to enhance seed germination, reduce plant diseases, promote plant growth, and increase tolerance to abiotic stresses[21–24].

In regions where the climate is arid, the soil is becoming increasingly salty, making it difficult for *Prosopis koelziana* seedlings to survive. Therefore, it is essential to find ways to improve seed germination rates and seedling establishment under these saline stress conditions. ،The present study examined the use of cold plasma technology to pretreat *Prosopis koelziana* seeds. This study used different treatment times, gases, and applied voltages to produce seedlings that can withstand and adapt to saline environments. This research aims to prevent the decline of this species and its vegetative communities and ultimately save *Prosopis koelziana* from the risk of extinction. The results of this study could be critical in achieving this goal.

Materials and methods

Source of plasma production

To create a plasma medium, several components were used, including a gas flowmeter that accurately measures the inlet gas flow to the DBD chamber, a high voltage pulsed.DC power supply that applies an electrical potential difference to the electrodes, a discharge chamber made up of two Teflon parts (including places to install electrodes and gas inlet and outlet), two flat disk electrodes made of Aluminum with round edges, and dielectric sheets with a thickness of 0.2 mm from Mica with a dielectric strength of 30 kV/mm. Both electrodes were covered by that dielectric, and the sample holder was installed on the dielectric of the bottom electrode (Fig. 1). That holder also playedthe role of spacer between those parallel electrodes. These components made it possible to adjust parameters such as gas flow rate, pulse height of applied voltage, and distance between electrodes.

Seed pretreatment with plasma

There are several species in the genus Prosopis, but Prosopis kuelziana is particularly important for its ecological, physiological, and desertification-related significance. To ensure accurate identification of this species, the herbarium number is required. The seeds used for this experiment were obtained from native *Prosopis koelziana* plants in Shahdad, located close to the Dasht-E-Lut desert in the Kerman province of Iran (Herbarium number: MIR-4752). This area is not protected, so seed collection is allowed. A small quantity of seeds from this plant was collected for research purposes. Healthy and uniform Prosopis seeds were selected for both control and plasma treatment. The seeds were treated with plasma for 6, 8, and 10 min, using a voltage of 10 V and air as input gas.

Seed germination and seedlings growth parameters

Seeds were treated with plasma for 0, 6, 8, and 10 min, then placed in petri dishes with 15 seeds each on filter paper soaked with distilled water or 100/200 mM NaCl solutions. The petri dishes were kept in a germinator at 25 °C with a 16/8 light/dark cycle for one week. Germination percentage was calculated after 7 days, with three petri dishes per treatment as replicates.

The percentage of seed germination was calculated based on the following formula:

$GR = (SG / ST) \times 100\%$.

In this formula.

GR = Germination rate.

SG = Number of germinated seeds.

ST = Total number of seeds.

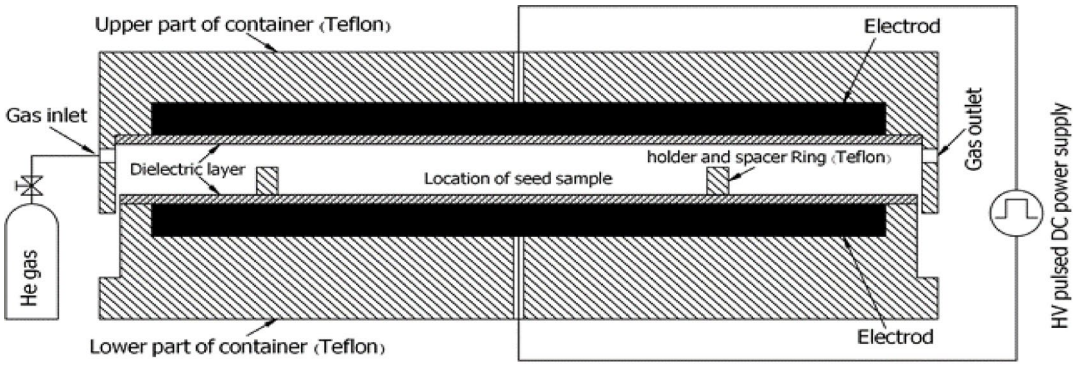
After 7 days, seedlings were harvested and the biomass of seedling measured in different treatment and recorded as g fresh weight per seedlings.

Planting a plant in pot

Pre-treated seeds were placed in pots with washed sand, while untreated seeds served as controls. Three replicates per treatment were used, with Hoagland nutrient solution (1/2 dilution) applied weekly. After 30 days, when 4–6 leaves per plant emerged, the plants were treated with 100 or 200 mM NaCl solutions for 14 days, with control plants watered with distilled water. To prevent high salt concentrations near the roots, pots were flushed twice a week. After two weeks, plant leaves were frozen in liquid nitrogen for further analysis.

Lipid peroxidation assay: lipid peroxidation assay

Malondialdehyde (MDA) content of leaf tissue was estimated by using Heath and Packer methods[25]. For MDA calculation, the value of non-specific absorption at 600 nm was subtracted from the 532 nm reading. The MDA content was calculated with an extinction coefficient of 155 $mM^{-1}Cm^{-1}$ and expressed as μmol MDA per g fresh weight.



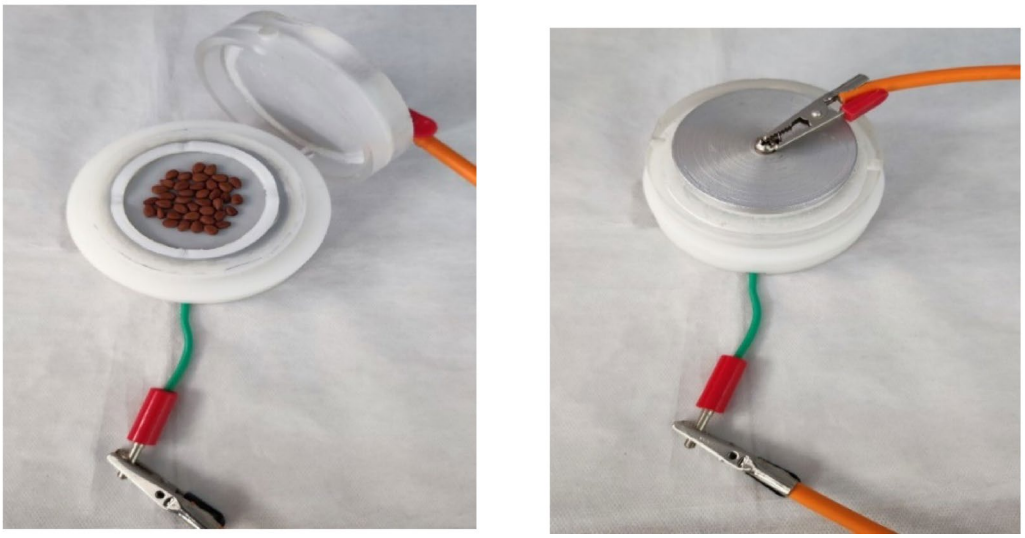


Fig. 1. A homemade DBD instrument which was used in this experiment.

Total soluble sugar determination: total soluble carbohydrates

The Fales method[26] was used to determine the total soluble carbohydrates present in the plant sample. To perform this method, 0.1 g of the sample was mixed with 2.5 mL of 80% ethanol and heated at 95 °C for an hour. The extracts were then filtered, and the remaining alcohol was evaporated. The residue was dissolved in 2.5 mL of distilled water. Next, 500 µL from each sample was taken and mixed with 5 mL of anthrone reagent. The tubes were then heated in a 90 °C water bath for 17 min. After cooling, the absorbance of the samples was measured at 625 nm. A standard curve was constructed using different concentrations of glucose.

Quantitative determination of proline content

The proline content was determined using the protocol developed by Bates[27]. First, 0.02 g of frozen plant tissue was homogenized in 10 mL of 3% sulfosalicylic acid. The resulting mixture was then centrifuged at 4000×g for 5 min. Next, 2 mL of the obtained supernatant was mixed with 2 mL of ninhydrin reagent and 2 mL of pure acetic acid. The mixture was then incubated at 100 °C for 60 min and rapidly cooled in an ice bath. After cooling, 4 mL of toluene was added and shaken. Finally, the proline content was determined by measuring the absorbance of the upper layer at 520 nm.

Quantification of photosynthetic pigments in leaves tissue

The Lichtenthaler[28] method was used to measure the levels of photosynthetic pigments in leaves. The process involved homogenizing 0.2 g of plant sample in 15 milliliters of 80% acetone. The resulting supernatant was then measured for its absorbance at 646.8 nm and 663.2 nm wavelengths to determine the levels of chlorophyll a, chlorophyll b, and total chlorophyll. Finally, the concentration of chlorophyll was expressed as mg g^{-1} FW and calculated using the following equations.

Chl a (µg/mL) = 12.25 A663.2–2.79 A646.8.
Chl b (µg/mL) = 21.21 A646.8–5.1 A663.2.
Chl t (Chl a + Chl b) = 7.15 A663.2 + 18.71 A646.8.

Enzyme extraction and activity determination

Leaf tissue (300 mg) was homogenized in 3 mL of a 50 mM potassium phosphate buffer. After centrifugation at 10,000×g for 20 min at 4˚C, the supernatant was collected and used for enzyme activity and protein content assays. Protein concentration was assessed using Bradford's method[29], with Bovine serum albumin serving as the reference standard.

Guaiacol peroxidase (GPX) (EC1.11.1.7)

The GPX activity was measured using the method described by Plewa[30]. To determine the enzyme activity, a reaction mixture was prepared that contained: 50 mM potassium phosphate (pH 7.0), 0.3% (v/v) H_2O_2, 1% (v/v) guaiacol, 100 µL enzyme extract. The activity of the enzyme was measured as the amount of enzyme that produced 1 µmol of tetra guaiacol per minute. This was considered as one unit (U) of enzyme activity. The enzyme activity was recorded as U per milligram of protein.

Ascorbate peroxidase (APX) (EC 1.11.1.11)

APX activity was measured according to Nakano and Asada[31]. 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 were mixed in the test tube and then 150 µL of enzyme extract was added to it. The absorbance was recorded at 290 nm ($\mathcal{E}$=2.8 $mM^{-1}cm^{-1}$). The enzyme activity was expressed in U per milligram protein.

Catalase activity (CAT) (EC 1.11.1.6)

CAT activity was measured according to the method of Dhindsa[32]. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 15 mM H_2O_2 and 100 µL of the enzyme extract. The decline in absorbance of the mixture was measured at 240 nm ($\mathcal{E}$=40 $mM^{-1}Cm^{-1}$). The enzyme activity was recorded in U per milligram protein (1 µM of H_2O_2 reduction min^{-1} mg^{-1} protein).

Polyphenol oxidase (PPO) activity assay (EC 1. 14. 18. 1)

The activity of polyphenol oxidase was determined using the method described by Nicoli[33]. The reaction solution consisted of 50 mM potassium phosphate buffer (pH = 7.0), 20 mM pyrogallol, and 100 µL enzyme extract. After a reaction time of 3 min, the absorbance of the solution was recorded at 420 nm. The activity was then calculated using an extinction coefficient of 6.2 mM^{-1} cm^{-1}.

Measurement of K^+ and Na^+ content in root and shoots of plants

To prepare the samples, 100 mg of dried material was digested in a solution of 67% (v/v) HNO_3. The samples were left overnight in 10 ml of HNO_3. The samples were heated for 45 min at 90 °C and then boiled at 150 °C for at least three hours until a clear solution was obtained. Digestion continued until the volume was reduced to about one ml. Finally, the extracts were filtered and diluted with distilled water. The ion contents were determined using an atomic absorption spectrometer (Spectra AA 220, Varian, Australia), and a standard curve was used to calculate each ion concentration[34].

Statistical analysis

A completely randomized factorial design with three replications was used. Data were analyzed using SPSS software, with ANOVA conducted at a 95% confidence level. Duncan's multiple range test was applied to identify significant differences among means.

Results

Germination percentage

According to the experiment, treating Prosopis seeds with cold plasma resulted in an increase in their germination percentage under normal conditions. The increase was approximately 25%, 56% and 45% for 6, 8, and 10 min treatments, respectively. Moreover, seed pretreatment also improved the germination percentage under salinity stress conditions. The 8-minute treatment was found to be the most effective, which resulted in a two-fold and three-fold increase in germination at 100 and 200 mM NaCl, respectively (Table 1).

Seedling growth

Data showed that pretreatment of seeds with cold plasma increased the fresh wight of seedling under control and salinity stress. The 8-min pretreatment had most effective (Table 2). As shown in Fig. 8, plasma had a promotive effect on both the shoot and root growth of the plant, especially under salinity stress. It appears from the image that the root mass of plants which underwent plasma priming is higher compared to the control group. In the plasma-primed plants, new leaves were observed even under salinity stress. There seems to be a notable difference in growth between the plasma pretreated and non-pretreated plants when exposed to salinity stress.

Peroxidation of membrane lipids

Lipid peroxidation in cell membranes serves as an indicator of oxidative stress and increased by approximately 2-fold and 3-fold in plants subjected to salinity stress of 100 mM and 200 mM, respectively. At 100 mM salinity, pretreating seeds with plasma for 6 and 8 min decreased lipid peroxidation by 22% and 37%, respectively. Under more severe salinity conditions of 200 mM, plasma treatment for 6 and 8 min reduced lipid peroxidation by 38%

	Control	Salt stress 100mM NaCl	Salt stress 200mM NaCl
Ct	64% (c)	44% (d)	21% (f)
P 6 min	80% (b)	67% (c)	35% (e)
P 8 min	100% (a)	100% (a)	68% (a)
P 10 min	93%(a)	62% (c)	32% (e)

Table 1. The effect of seeds priming with cold plasma at 6, 8 and 10 min on germination percentage of *Prosopis Koelziana* seeds in control and salinity stress conditions. Means were compared with duncan's test. $P < 0.05$ was considered as a significant difference. Different letters indicate significance, and similar letters' averages do not differ statistically.

	Control	Salt stress 100mM NaCl	Salt stress 200mM NaCl
Ct	1.15 g (c)	0.761 g (d)	0.62 g(e)
P 6 min	2.66 g(b)	2.1 g(c)	1.85 g(d)
P 8 min	3.66 g(a)	3.66 g(a)	2.91 g(a)
P 10 min	2.11 g(a)	1.21 g(c)	1.11 g(d)

Table 2. The effect of *Prosopis Koelziana* seeds priming with plasma for 6, 8 and 10 min on seedling fresh weight (gr) under control and salinity conditions. Means were compared with duncan's test. $P < 0.05$ was considered as a significant difference. Different letters indicate significance and averages with similar letters do not different statistically.

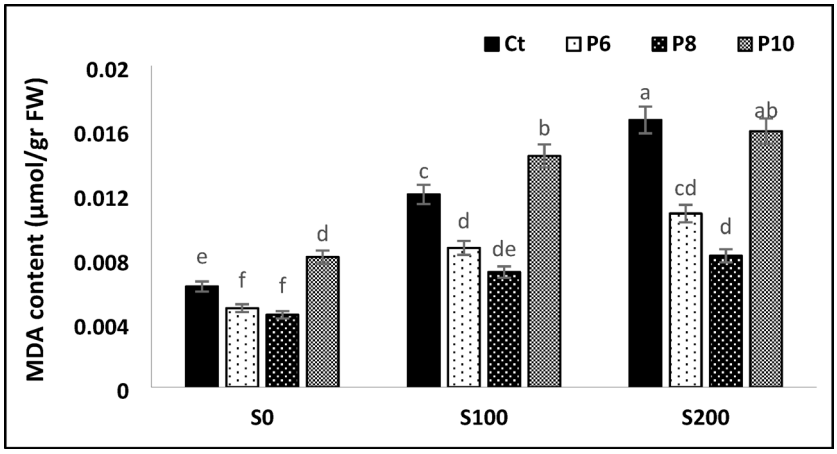


Fig. 2. Effect of cold plasma pre-treatment of seeds lasting for 6, 8, and 10 min on the malondialdehyde (MDA) content in the leaf tissue of *Prosopis koelziana* plants under both control and saline stress conditions. The means were subjected to statistical analysis using Duncan's multiple range test, and differences were considered significant at $P < 0.05$. Means denoted by different letters were found to represent significant differences, while those with identical letters were statistically similarity. (S0 = Normal condition, S100 = salinity 100mMNaCl, S200 = salinity 200mM NaCl, Ct = non pretreated plant, P6 = pretreated of seeds with plasma for 6 min, P8 = pretreated of seeds with plasma for 8 min, P10 = pretreated of seeds with plasma for 10 min).

and 50%, respectively. Conversely, the 10-minute plasma treatment increased peroxidation in both conditions, indicating it may not be the best option for this plant species (Fig. 2).

Soluble sugars content

In this study, it was found that mild salinity (100 mM) resulted in a 30% decrease in sugar content compared to the control seedlings. In contrast, seedlings subjected to severe salinity (200 mM) experienced an approximate 70% reduction in sugar content. Under salinity stress conditions of 100 mM and 200 mM, pretreatment of seeds for 6 and 8 min with plasma, increased the sugar content of seedlings by about 2 and 5 times, respectively, compared to untreated seeds. However, a 10-minute plasma exposure did not significantly influence sugar content, indicating that it had no effect under saline conditions (Fig. 3).

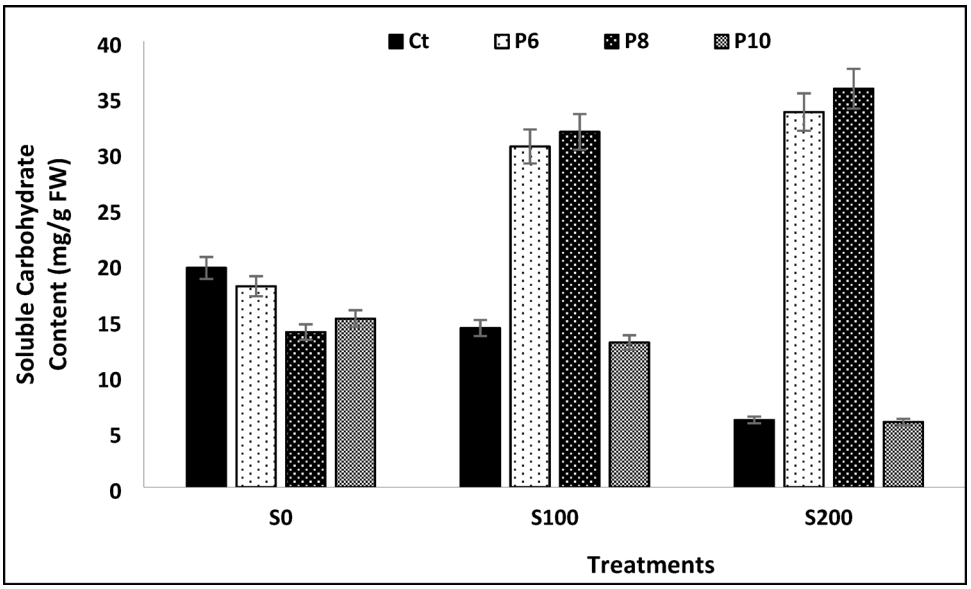


Fig. 3. Effect of cold plasma pre-treatment of seeds lasting for 6, 8, and 10 min on the soluble carbohydrates content in the leaf tissue of *Prosopis koelziana* plants under both control and saline stress conditions. The means were subjected to statistical analysis using Duncan's multiple range test, and differences were considered significant at $P < 0.05$. Means denoted by different letters were found to represent significant differences, while those with identical letters were statistically similarity. (S0 = Normal condition, S100 = salinity 100mMNaCl, S200 = salinity 200mM NaCl, Ct = non pretreated plant, P6 = pretreated of seeds with plasma for 6 min, P8 = pretreated of seeds with plasma for 8 min, P10 = pretreated of seeds with plasma for 10 min).

Proline content

Figure 4 Demonstrates that the proline content in *Prosopis koelziana* increased by 2-fold under moderate salinity stress and by 2.5-fold under severe salinity stress, indicating that higher salt concentrations lead to greater proline accumulation. Under moderate salinity conditions, pretreatment of seeds with cold plasma for 6 and 8 min resulted in a decrease in proline levels of approximately 20% and 40%, respectively. In the presence of 200 mM salinity stress, pretreating seeds with cold plasma for 6 and 8 min led to a reduction in proline content of about 35% and 50%, respectively (Fig. 4).

Quantification of photosynthetic pigments

Figure 5 shows that salinity stress significantly reduced chlorophyll a, b, and total chlorophyll in *Prosopis koelziana*. Pre-treatment with 6- and 8-minute plasma exposures significantly increased chlorophyll levels under both salinity conditions compared to untreated plants. However, the 10-minute plasma treatment reduced chlorophyll levels in both control and saline conditions.

Activity of antioxidant enzymes (Catalase, Guaiacol peroxidase, Ascorbate peroxidase, and Polyphenol oxidase)

Figure 6 shows that salinity stress, especially at 100 and 200 mM, significantly increased CAT and GPX activities in *Prosopis koelziana*. Pre-treatment with 6- and 8-minute plasma exposures improved these effects. The 10-minute plasma treatment enhanced GPX activity under high salinity but only increased CAT activity under non-stress conditions. APX activity also increased with salinity, and 8- and 10-minute plasma pre-treatments enhanced its activity. PPO activity rose under salinity stress, but 6- and 8-minute plasma treatments reduced it, while the 10-minute treatment increased PPO activity and plant stress (Fig. 6).

Sodium and potassium ion concentrations in root and leaf tissues of *Prosopis koelziana*

Figure 7 shows that salinity stress significantly increased sodium levels in both leaves and roots. Plasma pre-treatment did not affect sodium content in leaves but reduced it in roots, with the 8-minute plasma exposure showing the most significant effect. Potassium levels decreased only in plants exposed to 200 mM salinity, and plasma pre-treatment had little impact on this parameter.

Discussion

Salinity stress presents a significant challenge that greatly impairs seed germination and early plant growth. One of the primary effects of saline conditions is the restriction of water uptake. The high concentration of salt surrounding the seed prevents adequate hydration, which delays the onset of germination[35]. Furthermore, the excess sodium and chloride ions in plant tissues disrupt ionic balance, damage cell membranes, and hinder the functioning of essential enzymes necessary for early growth[36,37]. Due to low rainfall and increased soil salinity in the habitats of the *Prosopis* plant, its germination and seedling growth have decreased, putting the species at

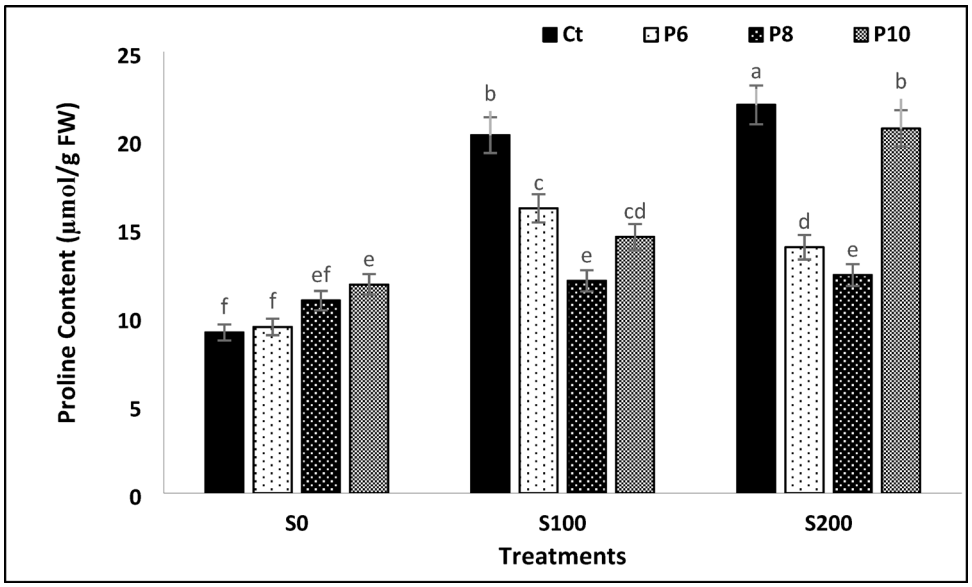


Fig. 4. Effect of cold plasma pre-treatment of seeds lasting for 6, 8, and 10 min on proline content in the leaf tissue of *Prosopis koelziana* plants under both control and saline stress conditions. The means were subjected to statistical analysis using Duncan's multiple range test, and differences were considered significant at $P < 0.05$. Means denoted by different letters were found to represent significant differences, while those with identical letters were statistically similarity. (S0 = Normal condition, S100 = salinity 100mMNaCl, S200 = salinity 200mM NaCl, Ct = non pretreated plant, P6 = pretreated of seeds with plasma for 6 min, P8 = pretreated of seeds with plasma for 8 min, P10 = pretreated of seeds with plasma for 10 min).

risk of extinction. The present study showed that the application of plasma through the DBD method induces morphological changes on the seed surface as well as physiological changes within the seed, leading to enhanced germination and growth under controlled conditions and in the presence of salt stress. The benefits of plasma treatments for seeds are believed to arise from changes to the surface properties of the seeds which causes more water absorption and the activation of specific metabolic pathways[17,23,38,39] However, excessive exposure to plasma can negatively affect germination by damaging the seed embryo or disrupting the seed's physiological mechanisms. Thus, the optimal duration for plasma treatment varies among different seed types and should be carefully tailored in each study. In this research, treatments lasting 6 and 8 min proved to be more effective than a 10-minute exposure. In fact, exposing seeds for 10 min resulted in damage compared to the shorter treatments. Therefore, 8 min was determined to be the optimal time for treating mesquite seeds. Research conducted by Los et al.[19] and Štěpánová et al.[40] has confirmed that plasma treatments effectively enhance the germination of wheat and pepper seeds. Similar results have been observed in other crops, such as barley and rice, where plasma treatment has improved seedling vigor and stress tolerance by promoting better water uptake and activating antioxidant responses[41,42]. The effect of plasma on hormone balance, particularly gibberellin, may contribute to the increased germination of seeds that have been pretreated with plasma. This approach offers a promising method for mitigating the adverse effects of salinity on seed germination and the early growth of seedlings[18,20],. For example, exposure to plasma has been shown to enhance gibberellin synthesis in wheat, resulting in more vigorous seedling growth even under stressful conditions[43]. Studies on other crops, such as rice and maize, indicate that plasma treatments can enhance hormonal balance and improve antioxidant responses, helping plants become more resilient to environmental stressors[44,45].

Excess salt disrupts metabolic processes, leading to reduced protein synthesis and negatively impacting the enzymes necessary for chlorophyll production. Furthermore, increased salinity levels promote the formation of reactive oxygen species (ROS), which can degrade chlorophyll and damage the photosynthetic machinery[46]. Salinity often leads to a decline in photosynthesis, which results in stunted plant growth. This occurs because salinity interferes with essential metabolic processes needed for energy production and development[47]. However, plant responses to salinity can vary significantly based on genetic traits, the severity of salinity, and environmental conditions[48]. The current study demonstrates that treating seeds with plasma can help preserve and even enhance chlorophyll levels in plants subjected to saline stress. This treatment may mitigate the effects of reactive oxygen species (ROS) and improve metabolic efficiency, presenting a promising strategy for maintaining photosynthetic performance under challenging conditions[18,44].

Salinity-induced oxidative stress often leads to increased lipid peroxidation, which occurs when membrane lipids are oxidized, ultimately compromising cell integrity and function. Elevated lipid peroxidation is a well-established indicator of increased oxidative stress under saline conditions[49,50]. This elevated lipid peroxidation occurring during salt stress is a direct result of the increased ROS production. This investigation highlights the benefits of plasma pretreatment in reducing lipid peroxidation in seedlings of *Prosopis koelziana*, suggesting enhanced resilience to oxidative stress. Seeds that have been pre-treated with plasma demonstrate increased

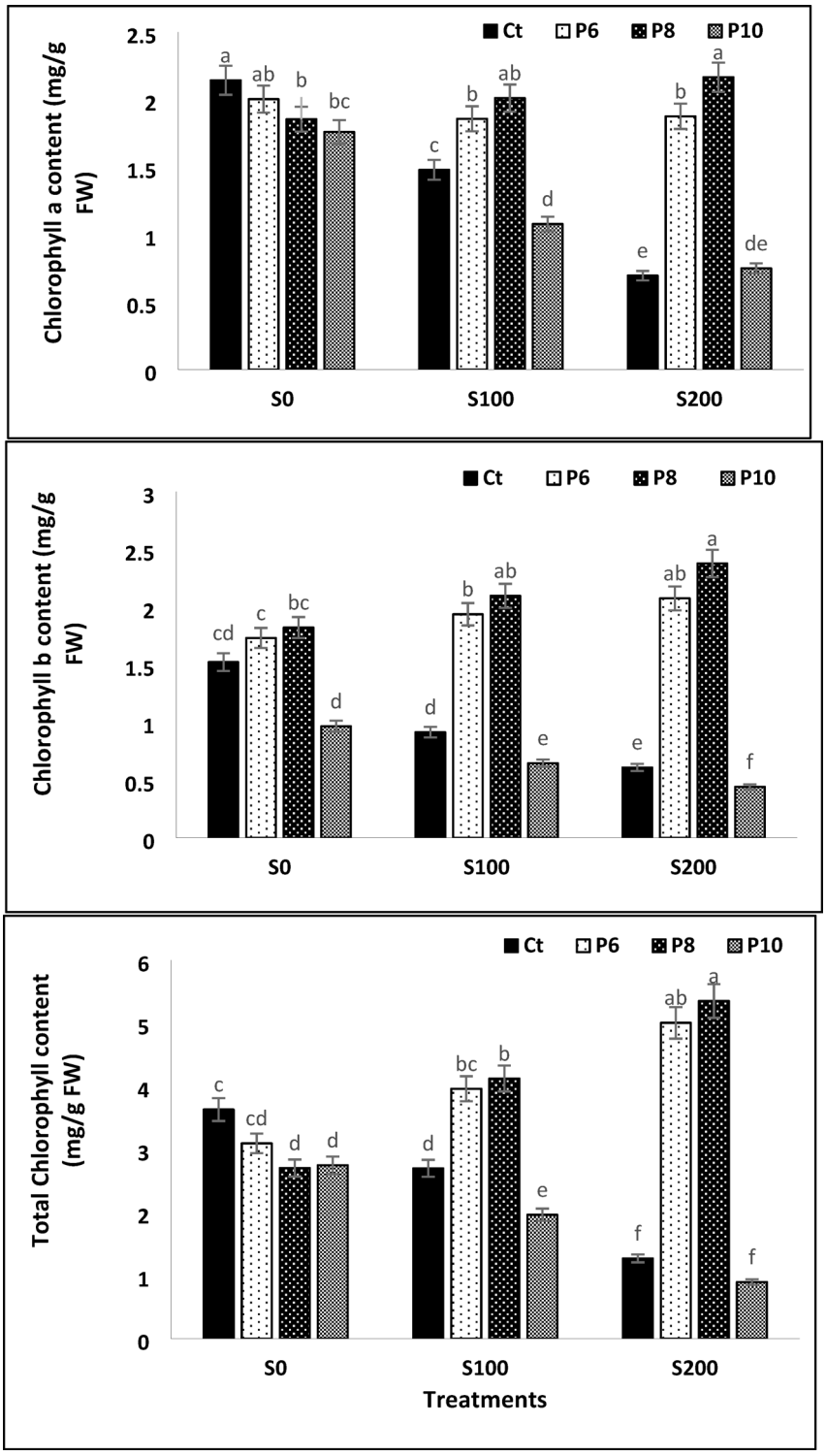


Fig. 5. Effect of cold plasma pre-treatment of seeds lasting for 6, 8, and 10 min on the photosynthetic pigments in the leaf tissue of *Prosopis koelziana* plants under both control and saline stress conditions. The means were subjected to statistical analysis using Duncan's multiple range test, and differences were considered significant at $P < 0.05$. Means denoted by different letters were found to represent significant differences, while those with identical letters were statistically similar. (S0 = Normal condition, S100 = salinity 100mM NaCl, S200 = salinity 200mM NaCl, Ct = non pretreated plant, P6 = pretreated of seeds with plasma for 6 min, P8 = pretreated of seeds with plasma for 8 min, P10 = pretreated of seeds with plasma for 10 min).

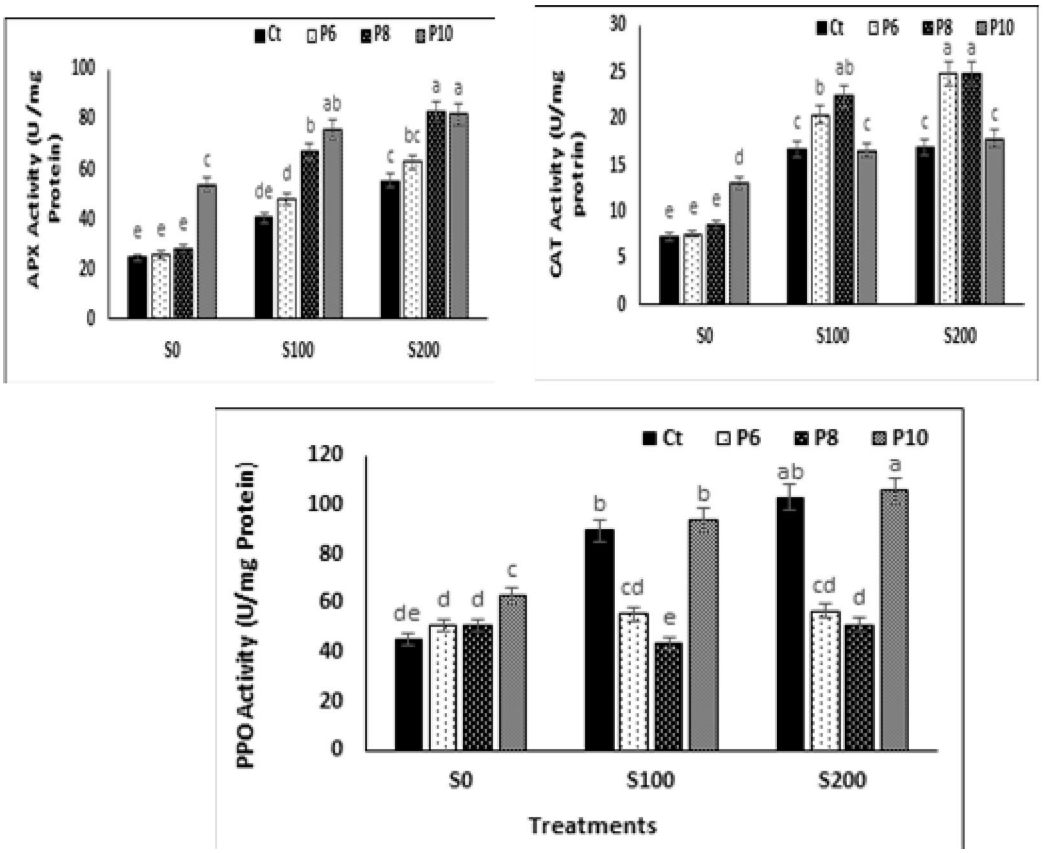


Fig. 6. Effect of cold plasma pre-treatment of seeds lasting for 6, 8, and 10 min on antioxidative enzymes activity in the leaf tissue of *Prosopis koelziana* plants under both control and saline stress conditions. The means were subjected to statistical analysis using Duncan's multiple range test, and differences were considered significant at $P < 0.05$. Means denoted by different letters were found to represent significant differences, while those with identical letters were statistically similarity. (S0 = Normal condition, S100 = salinity 100mMNaCl, S200 = salinity 200mM NaCl, Ct = non pretreated plant, P6 = pretreated of seeds with plasma for 6 min, P8 = pretreated of seeds with plasma for 8 min, P10 = pretreated of seeds with plasma for 10 min).

enzymatic activities, which help to mitigate the effects of oxidative stress, thereby preserving cellular integrity and functionality[51]. Similar effects of cold plasma on enhancing antioxidant potential have also been observed in other plants. For example, research on wheat and barley has shown that plasma-treated seeds exhibited increased activities of SOD and catalase, leading to reduced lipid peroxidation and improved growth under saline conditions[44,52]. While some enzymes exhibited increased activity during the 10-minute plasma treatment, other indicators—such as heightened lipid peroxidation, reduced levels of photosynthetic pigments, and lower sugar concentrations—suggest that this duration imposes extra stress on the plant. This stress likely exceeds the plant's capacity to handle it, ultimately resulting in damage.

Under saline conditions, plants often experience significant fluctuations in carbohydrate levels and activity, which act as compatible solutes. These changes can negatively impact photosynthesis, leading to reduced production of soluble sugars and impairing overall plant growth[53,54]. Interestingly, this study has shown that pretreating of *Prosopis* seeds with cold plasma can positively affect carbohydrate metabolism in plants experiencing salt stress, as reported in earlier research on other plants[44,55].

When plants experience stress, the levels of proline increase as a defensive response, helping to regulate osmotic balance. It also acts as a powerful antioxidant by neutralizing reactive oxygen species (ROS), thereby reducing oxidative damage. Proline accumulation helps preserve cellular integrity and maintain osmotic balance[56,57]. In this experiment, it was observed that plasma pretreatment reduced proline levels in stressed *Prosopis koelziana* plants. This suggests that cold plasma may activate alternative mechanisms for coping with stress. This effect could be attributed to the stimulation of the plant's immune system, which reduces the necessity for proline accumulation by enhancing other defensive responses, primarily through increased antioxidant activity. Research on wheat has indicated that plasma pretreatment under drought stress increases the activity of important antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT). This enhancement improves the plant's resistance to stress without leading to excessive proline synthesis[58,59]. This study suggests that plasma pretreatment may help plants neutralize reactive oxygen species (ROS) more effectively during saline stress, which could reduce the need for proline as a defensive mechanism. Additionally, cold plasma seems to regulate cellular osmotic balance by increasing soluble sugar levels, further decreasing the plant's reliance on

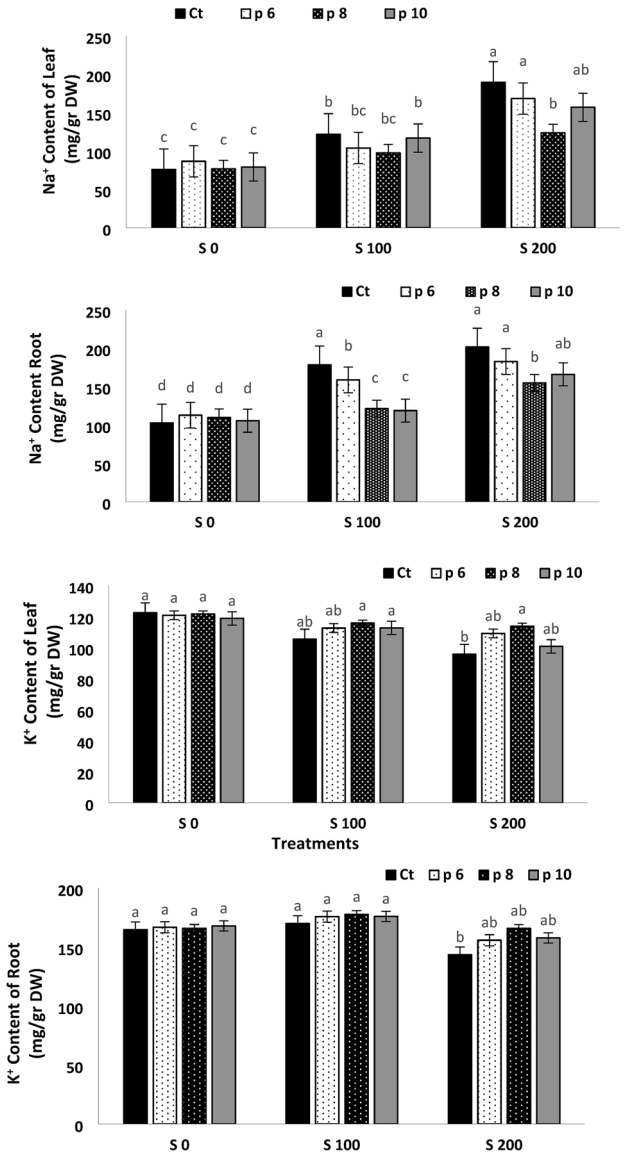


Fig. 7. Effect of cold plasma pre-treatment of seeds lasting for 6, 8, and 10 min on the Na and K content of root and shoot of *Prosopis koelziana* plants under both control and saline stress conditions. The means were subjected to statistical analysis using Duncan's multiple range test, and differences were considered significant at $P < 0.05$. Means denoted by different letters were found to represent significant differences, while those with identical letters were statistically similar. (S0 = Normal condition, S100 = salinity 100mM NaCl, S200 = salinity 200mM NaCl, Ct = non pretreated plant, P6 = pretreated of seeds with plasma for 6 min, P8 = pretreated of seeds with plasma for 8 min, P10 = pretreated of seeds with plasma for 10 min).

proline synthesis to maintain water equilibrium. Previous studies have also reported similar findings, noting that plasma-treated plants exhibited reduced proline accumulation along with higher sugar levels and improved growth metrics under salinity stress. These observations indicate a shift in osmotic adjustment strategies[44,55].

High salt levels disrupt the ionic balance within plant tissues, particularly by competing with essential ions such as potassium (K^+), which are necessary for the optimal functioning of many enzymes[53,60]. Therefore, maintaining ionic equilibrium in salt-stressed plants is crucial to ensuring normal cellular activity and growth. This study found that salt-stressed *Prosopis koelziana* plants accumulated significantly higher levels of sodium ions compared to the control group. However, pretreatment with cold plasma notably reduced Na^+ accumulation in the roots, indicating that plasma priming may enhance the plant's ability to regulate ion transport. This response seems to be linked to the function of ion antiporters, specifically K^+/H^+ and Na^+/H^+ exchangers, which rely on membrane H^+-ATPase pumps to maintain cellular ionic balance, as previously described by Li et al.[61]. Research on barley and rice has yielded similar findings, indicating that plasma pretreatment can reduce sodium uptake and improve ion balance during periods of salinity stress, which in turn promotes better growth performance[55]. Although the precise mechanism by which plasma priming affects H^+-ATPase activity is not yet

Fig. 8. Growth dynamics of *Prosopis koelziana*, which were exposed to an 8-minute cold plasma treatment using Helium gas in a greenhouse setting. The results showed that the plasma had a promotive effect on both the shoot and root growth of the plant, especially under salinity stress. The accompanying images depict one-month-old seedlings of *Prosopis koelziana*. It appears from the image that the (**A**) root mass of plants which underwent plasma priming is higher compared to the control group. (**B**) in the plasma-primed plants, new leaves were observed even under salinity stress. (**C**)There seems to be a notable difference in growth between the plasma pretreated and non-pretreated plants when exposed to salinity stress. (**D**) Positive effect of plasma pretreatment on the root and shoot growth of *prosopis* plants under salinity stress.

fully understood, recent studies suggest that plasma treatments may alter the electrochemical properties of cell membranes. This alteration could enhance ion exchange and stabilize the membranes[44,62].

Conclusion

This study shows that exposing seeds to cold plasma for 8 min can improve germination rates and enhance plants' resilience to salinity stress. This benefit comes from boosting the plant's immune system and reducing harmful reactive oxygen species (ROS), which helps support healthier growth. Cold plasma also increases the production of plant pigments, further strengthening their ability to resist stress. These findings are crucial for developing robust seedlings and improving salinity tolerance in *Prosopis koelziana*. By enhancing antioxidant responses and regulating ion transport, plasma treatments offer a promising way to increase plant health and productivity, especially under stress. This study serves as a foundation for understanding plasma technology's potential in agriculture. With continued research, it could become a vital tool for farmers to improve crop resilience and productivity in the face of climate change and soil salinity challenges.

Data availability

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request. All data generated or analyzed during this study are included in this published article.

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Author contributions

Z.M. Contribution to the experimental work- Collection, writing of the manuscript and analysis of data. F.N. Supervision- Performance the experiment- Data analyzes- Interpretation of data and writing of the manuscript. H.N. Plasma instrument provide and plasma analysis- Helped in the correction of the manuscript. All authors contribute in reading and approving the final manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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