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Enhancement of Salinity Stress Tolerance in Cumin (*Cuminum cyminum* L.) Using Seed Priming with Amla Extract and NaCl.

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ABSTRACT

Salinity is one of the most crucial variables that limits crop productivity and quality. They influence various physiological processes, including seed germination and subsequent plant growth. The negative effects of salinity stress on germination could be minimized through different seed priming treatments. In this regard, this study was conducted to investigate the priming of cumin seed with both Amla extract and salt solution (NaCl) as an interesting strategy to improve salt tolerance and its impact on cumin (*Cuminum cyminum* L) seed germination and growth. Our data showed that seed priming before planting in Amla extract and salt solution improved growth parameters such as cumin length, number of branches, fresh weight, seed weight per plant, and number of seeds per umbel. The present study also showed a decrease in growth parameters under severe stress conditions (50 mM NaCl), while plants pre-soaked with both Amla extract and salt solution reduced this effect. Pre-soaking in Amla extract and salt solution reduced the inhibitory effect of salt stress on photosynthetic pigments and significantly increased the content of essential oils, total phenolic, flavonoids, amino acid content, proline, and antioxidant activity under the influence of severe salt stress. The findings of this research revealed that treatment of cumin plants with both Amla extract and NaCl strongly stimulated the expression of the SA, PRO, and POX genes.

Keywords: Amla, Cumin, gene expression, Priming, Salinity.

INTRODUCTION

Cumin (*Cuminum cyminum* L) is one of the most significant medicinal plants of the Apiaceae family. The ancient Egyptians knew cumin, which was widely grown on the banks of the Nile, and it is believed that Upper Egypt is the origin of it (Hansen, 2022). Because of the great importance of this plant, it has been cultivated by farmers due to the low water requirement and short growing season. Although the cumin plant is moderately salt resistant during its adult growth and reproductive phases, plants are extremely sensitive to salinity during seed germination and seedling growth (Hassanzadeh *et al.,* 2013). Salinity is a prevalent issue in Egypt's irrigated regions, with limited rainfall potentially arising as a significant barrier to seed germination (Kaya *et al.,* 2003).

One of the most significant factors limiting crop productivity and species dispersion is salt stress; it may influence various physiological processes, including seed germination and plant development. Developing new cultivars of commercially significant crops that are more tolerant of a wide variety of environmental changes is therefore important. (Lei Ma *et al.,* 2017). To increase plant resistance to salt stress, a variety of approaches can be used, including acclimation (Santangeli *et al.,* 2019) and seed priming (Schwachtje *et al.,* 2019). Seed priming was defined as pre-sowing procedures that involve soaking the seed in a priming agent, serving as an elicitor for some physiological changes, for a certain period. This permits the seed to absorb water and progress to the initial stage of germination (McDonald 1999). Using a minor stress-like signal, a chemical priming agent can induce abiotic stress in seedlings. This cue allows the development of a "priming memory," which is comparable to an acclimation response. As a result, this memory increases the plant's tolerance when it is subsequently exposed to various abiotic stress (Giordano *et al*., 2020). Priming agents, such as seaweed extract and hormones or polyamines (PAs) have been shown to enhance wheat seedling performance (Afzal *et al.*, 2006 and Ali *et al*., 2022) and validamycin A in rice (Abdelgawad *et al.*, 2014). Such memory works at the phenotypic level (Hilker *et al.*, 2015) and considers changes in gene expression, metabolism, and epigenetic modifications but not DNA sequence changes. It has been established that seed priming increases germination and emergence in seeds of numerous crops (Murungu *et al*., 2003; Demir *et al.*, 2006). Additionally, it has been

shown that these methods promote seed germination in a variety of stressful conditions, including salinity, drought, and temperature (Fujikura *et al.*, 1993; Ashraf and Foolad, 2005; Demir *et al.*, 2006).

Amla (*Phyllanthus emblica)*, commonly known as Indian gooseberry, belongs to *Euphorbiacea*e family, and is native to subtropical and tropical countries. It contains phytochemicals such as terpenoids, tannins, alkaloids, and flavonoids, which have several properties such as antioxidant, antigenotoxic, anticancer, antitumor, antimicrobial, anti-inflammatory, and anti-carcinogenic effects. It is commonly used to treat various ailments such as asthma, cough, diabetes, jaundice, diarrhea, vomiting, and kidney and urinary bladder disturbances (Sriwatcharaku, 2020). Previous reports revealed that some bioactive secondary metabolites were isolated and identified from different parts of the plant, as well as glycosides, phenolics, terpenoids, flavonoids, tannins, as well as alkaloids, and carbohydrates (Mohamed *et al.*, 2022).

The purpose of this study is to determine whether priming cumin (*Cuminum cyminum*) seeds with Amla extract and salt solution (NaCl) improves growth characteristics and yield under salinity stress. In addition, studied the impact of salinity stress and priming on gene expression and metabolite content in cumin.

MATERIALS AND METHODS

Cumin seeds were obtained from the Medicinal and Aromatic Plant Research Department's Experimental Farm in El-Kanater El-Khairia, Agricultural Research Center, Egypt, and sown in November for two seasons (2020–2021 and 2021–2022). Amla fruit extract (5%) was prepared by soaking 5g of dry fruits in water at 50°C for 20 minutes. After filtering, the extract was kept at 4°C.

The experiment design:

The experiment was performed in a greenhouse at the Horticulture Research Institute, ARC, Egypt, in a completely random design and included nine treatments; each treatment was replicated three times, and each replicate contained nine pots. Seeds were grown in pots (35 cm in diameter and 40 cm in depth) with 7 kg of soil. The characteristics of the soil were: sandy loam in texture, sand 84.2%, silt 12.9%, clay 2.9%, pH 7.7, EC 0.5 dSm, and organic matter 1.2%. The treatments were assigned to the pots as the following:

Control and salt treatments:

1. Seeds were soaked in distilled water for 12 hours before planting, and after planting, the soil was watered each week to its maximum capacity.
2. After two weeks of seedlings, they were irrigated weekly with 25 mM NaCl to the field's capacity.
3. Seedlings are irrigated weekly with 50 mM NaCl to attain the field's capacity.

II. Amla presoaking and salt treatments:

1. Seeds were soaked in a 5% Amla dry fruit extract for 12 hours before planting, and water to the field's capacity was used to irrigate them once a week.
2. After two weeks of sowing seedlings, they were irrigated weekly with 25 mM NaCl to the field's capacity.
3. Seedlings are irrigated weekly with 50 mM NaCl to the field's capacity.

III. **NaCl presoaking and salt treatments:**

1. Seeds are soaked in 40 mM NaCl for 12 hours before planting, and after planting, the seedling was irrigated weekly with water to its maximum capacity
2. After two weeks of sowing seedlings, they were irrigated weekly with 25mM NaCl to the field's capacity.
3. Seedlings are irrigated weekly with 50 mM NaCl to the field's capacity.

Fertilization:

Ammonium sulphate (20.6% N), calcium superphosphate (15.5% P2O5), and potassium sulphate (48% K2O) were added as the chemical fertilizers (NPK) at the prescribed level in three doses. During soil preparation, all of the phosphorus was supplied. After 30 and 60 days from seeding, NPK dosages were applied in two equal doses. At the fruiting stage, 15 weeks following treatment, seeds were collected and weighed. Plant length (cm), number of branches per plant, number of seeds per umbel, fresh weight per plant (g) and weight of seed per plant were measured.

Biochemical Analysis:

The contents of chlorophyll a, b and carotenoids were determined spectrophotometrically according to Mitic *et al*. (2013). The essential oil (EO) was determined according to the method of (British Pharmacopeia, 1963). The total phenolic content of the seed was determined by using the Foline Ciocalteu reagent (Merck) according to the procedure described by Dewanto *et al.* (2002). According to Chang *et al.* (2002), total flavonoid in seed were measured as mg of quercetin per gram of dry weight. The amount of free amino acids was calculated in seed according to the method described by (Jayaraman, 1985) as g/100 g of dried weight. Proline was determined in terms of seed calorimetrically according to Troll and Lindsley (1995). The reducing sugar content

(RSC) was estimated using the 3,5-dinitrosalicylicacid (DNSA) method. The measurement was performed according to the procedure described by Krivorotova and Sereikaite (2014).

Antioxidant activities (%DPPH):

According to Chen *et al*., (2008) protocols, the antioxidant activity was assessed using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging technique. To represent the percentage of DPPH that was scavenged, the antioxidant activity was reported as a percentage decline in absorbance when compared to the control. The percentage of DPPH, which was scavenged (% DPPHsc), was calculated using % DPPHsc: %DPPHsc = ($A_{control}$ - A_{sample}) ×100/$A_{control}$

Where $A_{control}$ was the absorbance of solution without extract and A_{sample} was the absorbance with different dilutions of extract, Ascorbic acid was used as a standard.

Amla extract analysis:

Phytohormones were determined in Amla extract as Gibberellic acid (GA3), abscisic acid (ABA) and indole acetic acid (IAA) used high-performance liquid chromatography (HPLC) for their operations (Hewlett Packard, HP1050) according to Horemans, *et al.* (1984). The total alkaloids were determined as recommended by Shamsa *et al*. (2008). The Cam and Hisil (2010) method was used to measure the content of tannins.

RNA Extraction and Gene Expression Analysis:

The leaves were used to extract the total RNA of untreated and treated cumin plants using the RNeasy Plant Mini kit from Qiagen (Hilden, Germany) with the DNase processing step as directed by the manufacturer. RNA quantity and quality were assessed by agarose gel electrophoresis and spectrophotometry (NanoDrop 2000; Thermo Scientific). First-strand cDNA was synthesized with 1 µg of total RNA using Super Script III RNase H Reverse Transcriptase (Promega), according to the standard protocol from the manufacturer. The expression pattern of genes was analyzed by semi-quantitative RT-PCR using gene-specific primers (Table 1). The RT-PCR was carried out using three specific primers of three genes, salicylic acid (SA), proline (Pro), and peroxidase (POX) genes, (Table 1). The following PCR cycle was used to amplify gene-specific products from cDNA: initial denaturation at 94°C for 3 minutes, denature (94°C) for 30 seconds, anneal (54°-60°C) for 30 seconds, extend (72°C) for 2 minutes each for 35 cycles, and final extension 72°C for 8 minutes. The PCR results from each sample were examined on 2% agarose gels following RT-PCR.

Statistical analysis:

The acquired data were statistically examined using Snedecor and Cochran (1969). One-way analysis of variance. Using the SPSS program version 16, means were compared using LSD at 5%.

Table 1. Primers sequence used for PCR-amplification.

Primer	Sequence 5'-3'	Tm	Primer	Sequence 5'-3'	Tm
SA F	5' -TTCTTCCACTTCGTCGGGTG- 3'	54°C	Pro R	5' -GAGGCAGGATATGTGTGCAG- 3'	56°C
SA R	5' -TGGACGCTAAGTTGTGTCTCT- 3'	54°C	POX F	5'-AGGGTGATACGATCAGCTCTT-3'	60°C
Pro F	5' -TGGGGACGTGATGCCTATTG- 3'	56°C	POX R	5'-AGCAGGATGCGAACAAACAAA-3'	60°C

RESULTS

Amla extract analysis:

The chemical properties of the extract from the Amla dry fruit **(***Phyllanthus emblica)* have been analyzed, and their values are given in Table (2).

Table 2. The Components of Amla fruit aqueous extract.

chemical properties	GA3 (mg/100g dry weight)	IAA (mg/100g dry weight)	ABA (mg/100g dry weight)	Total phenols mg/g	Total flavonoid mg/g	Total alkaloids mg/g	Total tannins mg/g
Amla extract	0.964	0.211	0.063	43.96	30.17	4.11	3.02

Cumin yield parameters:

At the fruiting stage, 15 weeks following treatment, cumin seeds were collected and weighed. Plant length (cm), number of branches per plant, number of seeds per umbel, fresh weight per plant (g), and weight of seed per plant were measured via the two seasons Table (3).

Table (3) shows that under salt stress conditions, plant length, number of branches per plant, and number of seeds per umbel, all decrease significantly. The reduction in these parameters was greater at 50 mM

NaCl, while the highest values of these parameters were attained for plants treated with Amla extract, followed by plants treated with NaCl solution under normal conditions. Under salt stress conditions (25 mM and 50 mM NaCl), there was a significant increase in plant length, number of branches per plant, number of seeds per umbel, fresh weight per plant (g), and weight of seed per plant in cumin plants priming in both Amla extract and NaCl solution compared to control plants. Therefore, soaking seeds in Amla extract and salt solution (40 mM NaCl) is effective in reducing the inhibitory effect of salt stress.

As shown in Table 3, there were significant decreases in the fresh weight of the plant and the weight of seeds / plant with an increase in the salt concentration. The application of 50 mM NaCl caused the highest reduction compared with the control. However, pretreatment of the seeds caused a significant increase in the fresh weight of the plant and the weight of the seeds of the plant in comparison with the control.

Table 3. Effect of Salinity on growth parameters and fresh plant weight and seed weight per plant of cumin plants priming in Amla extract and NaCl solution.

Treatment		Length of plant/ cm		No. branches /plant		No. seeds /umbel		Fresh weight/plant g		Weight of seed /plant	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
0 mM NaCl	Control	15.33	16.86	5.66	5.12	6.66	7.48	2.99	3.12	0.17	0.16
	Amla (Soak)	22.00	22.33	8.33	7.63	14.33	13.84	5.63	6.22	0.64	0.62
	NaCl (40mM) Soak	18.00	18.66	6.86	6.23	11.66	12.00	4.28	4.65	0.33	0.38
25 mM NaCl	Control	13.66	14.37	3.66	4.00	6.00	5.75	2.06	2.18	0.14	0.13
	Amla (Soak)	20.33	20.66	5.66	5.30	12.08	11.85	5.17	5.33	0.50	0.46
	NaCl (40mM) Soak	18.33	16.75	3.66	4.67	10.66	11.00	3.69	3.51	0.24	0.29
50 mM NaCl	Control	11.33	12.34	3.33	3.73	5.00	4.60	1.7	1.876	0.08	0.09
	Amla (Soak)	18.33	19.28	5.00	5.44	10	9.50	3.30	3.85	0.20	0.25
	NaCl (40mM) Soak	18.66	17.50	4.33	5.00	8.33	8.50	2.88	3.22	0.20	0.20
LSD		2. 01	2.28	1.10	0.9	2.08	1.92	0.88	0.72	0.062	0.066

Essential oil content:

The percentage of essential oil increased significantly at all salinity levels in both treated and untreated plants via two seasons. However, soaking in Amla extract and salt solution increased essential oil content in plants irrigated with 25 and 50 mM NaCl Table (4).

Table 4. The effect of Salinity on essential oil percentage and photosynthetic pigments of cumin plants priming in Amla extract and NaCl solution.

Treatment		Essential oil %		Chlorophyll A mg/g	Chlorophyll B mg/g	Carotenoids mg/g
		1st season	2nd season			
0 mM NaCl	Control	4.8	4.2	0.56	0.21	0.41
	Amla (Soak)	6.7	6.8	0.74	0.36	0.43
	NaCl (40mM) Soak	6.2	6.1	0.72	0.40	0.45
25 mM NaCl	Control	6.3	6.2	0.49	0.18	0.33
	Amla (Soak)	7.1	7.0	0.61	0.23	0.39
	NaCl (40mM) Soak	6.5	6.5	0.56	0.30	0.41
50 mM NaCl	Control	6.6	6.7	0.41	0.16	0.31
	Amla (Soak)	7.2	7.3	0.53	0.26	0.44
	NaCl (40mM) Soak	7.4	7.6	0.48	0.23	0.31
LSD		0.11	0.097	0.037	0.022	0.019

Biochemical Analysis:

Results in Table (4) indicated that there was an inverse relationship between salt concentration and chlorophyll A, B, and carotenoid content compared with the control. However, soaking seeds in Amla extract and salt solution without salt treatment resulted in a significant increase in chlorophyll A, B, and carotenoid content when compared to control plants. The pretreatment reduces the salinity stress effect in salt-treated plants by increasing chlorophyll A, B, and carotenoid content.

Total phenolic, flavonoid, amino acid, and proline content:

Plants pre-soaked with Amla extract and NaCl solution had fairly high total phenolic and flavonoid content (Fig.1a,b). At the same time, much higher contents of total phenols and flavonoids were observed in plants treated with Amla extract and irrigated with 50 mM NaCl. Fig.1 (c, d) shows that salt stress had a significant effect on total free amino acid and proline content in plants when compared to the control. Data indicated that the treatment of seeds with Amla extract and the salt solution had the highest content of amino acids and proline. In comparison to controls, plants treated with Amla extract followed by salt solution accumulated more total free amino acids and proline.

Antioxidant activity and reducing sugar content:

The results revealed an increase in the antioxidant activity of the DPPH radical in 50 mM NaCl as compared with the control. DPPH inhibition was most enhanced in 25 mM NaCl salt-stressed plants soaked in salt solution (40 mM NaCl) (Fig. 1e). Pre-soaking of seeds stimulated stress tolerance by further increasing antioxidant activity. There were no significant differences between plants treated with salt solutions and Amla extract.

The accumulation of reducing sugars tended to increase with increasing salinity. The increase in reducing sugar content in plants pretreated with salt solution resulted in a significantly increased reducing sugar at 25 mM NaCl and 50 mM NaCl treatment with Amla extract and salt solution as compared with controls (Fig. 1f).

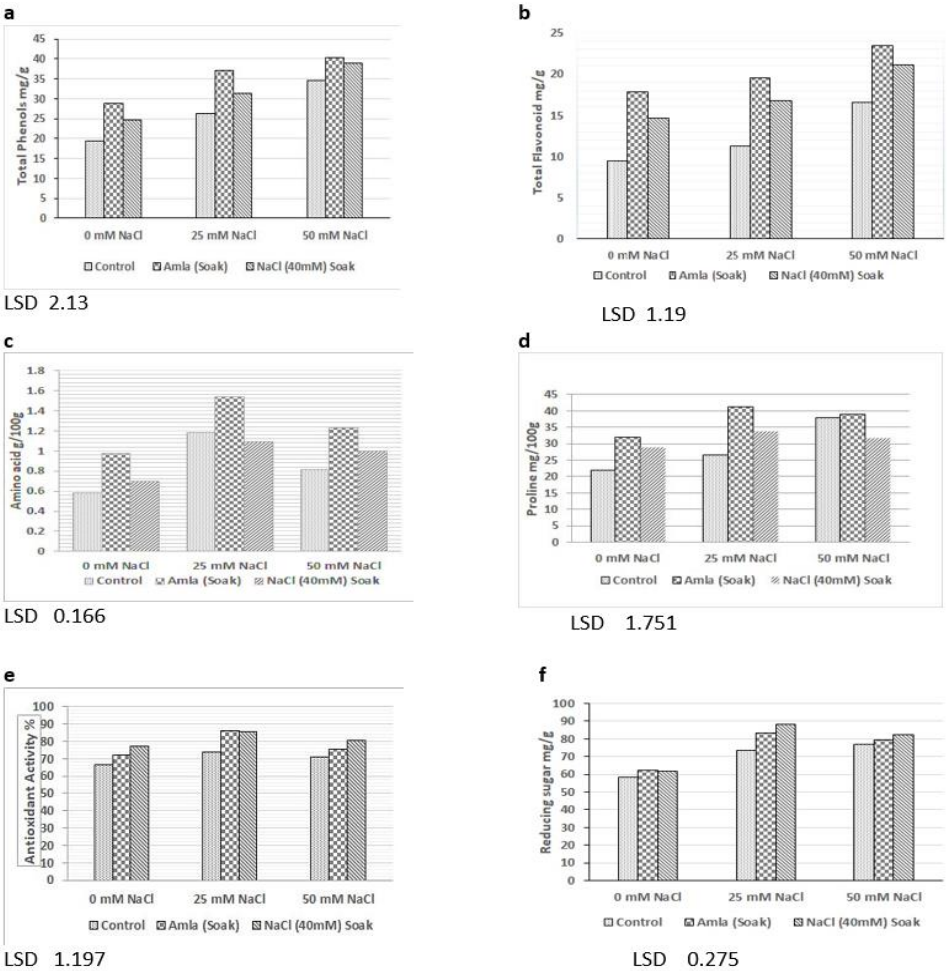

Fig. 1. Biochemical analysis of cumin plants priming in Amla extract and salt solution (40mMNaCL) under normal and Salinity conditions. a. Total phenolic content, b. Total flavonoid. c. Total free amino acids, d. Proline content, e. Antioxidant activity, and f. Reducing sugar content (dw).

Expression analysis of SA, Pro, and POX:

The expression patterns of three genes coding for salicylic acid (SA), proline (PRO), and peroxidase (POX) were tested. Results showed that untreated (control) and treated plants with both Amla extract and NaCl strongly induced the expression of SA, PRO, and POX genes. The expression of the salicylic acid (SA) encoding gene was reduced in the moderately stressed condition (25 mM NaCl) of treated plants with both Amla and NaCl, while significantly increasing under severe stress conditions (50 mM NaCl) and in Amla-treated control plants (Fig. 2). The proline (pro) transcripts showed a similar pattern of expression in both treated and untreated plants under salt stress conditions (Fig. 2), compared to the control, leading to the upregulation of the (Pro) gene in cumin plants. As shown in (Fig. 2), POX transcriptase was up-regulated in both untreated plants (control) and NaCl-treated plants in the absence of stress conditions expressed by two isoforms of the peroxidase enzyme pattern, while down-regulation occurred under severe salt conditions (50mM NaCl) in both control and NaCl-treated plants (Fig. 2).

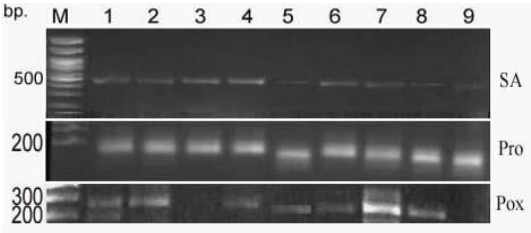

Fig. 2. Expression analysis of salicylic acid (SA), proline (pro), and peroxidase (POX) genes induced by seed priming of cumin and salt stresses: Lane M: 100 bp. Ladder, lane 1: control, Lane 2: Treated cumin with 25 mM NaCl, Lane 3: Treated cumin with 50 mM NaCl, Lane4: Seed primed with Amla extract 5%, Lane5: Seed primed with Amla extract and treated with 25 mM NaCl, Lane6: Seed primed with Amla extract and treated with 50 mM NaCl, Lane 7: Seed primed with NaCl solution, Lane 8: NaCl-primed seeds were treated with 25 mM NaCl. Lane 9: NaCl solution priming and treatment with 50 mM NaCl.

DISCUSSION

Throughout seed germination and seedling development, cumin plants are extremely sensitive to salt; however, during adult growth and reproductive periods, they are salt-resistant (Hassanzadeh *et al.,* 2013). Poor seed germination and seedling establishment are the results of salt in the soil. It is a major problem that hinders the growth and development of crop plants and reduces agricultural output. Different seed priming approaches can minimize the harmful and depressive impacts of salt stress on germination, according to earlier studies (Afzal *et al.,* 2005; Basra *et al.,* 2006). Additionally, it has been proven that using this method enhances plant performance. Priming the seeds of various crops, especially those of vegetables and small-seeded grasses, has been effectively shown to promote germination and emergence (Murungu *et al.*, 2003; Demir *et al*., 2006). According to our research, priming seeds in salt solution and Amla extract before planting enhanced growth characteristics such as cumin length, branch count, fresh weight, the weight of seed per plant, and the number of seeds per umbel. In this regard, a seed soaked in Amla extract (5%) showed a good response in growth factors. In comparison to the control, presoaking seeds in Amla extract increased fresh weight, the weight of seed per plant, and number of seeds per umbel of the cumin plant. The increased seedling growth might be due to the presence of some growth promoting substances. Natural plant extracts (PEs) are frequently abundant in bioactive substances (e.g., phenolics, flavonoids, carotenoids, etc.), making them efficient at controlling redox metabolism and consequently enhancing plant growth and productivity. (Drobek *et al*., 2019). Our findings agreed with past research on wheat (Ali *et al*., 2022), *Zea mays* (Al-Shakankery *et al*., 2014), as well as *Vigna sinensis* (Sivasankari *et al*., 2006).

Cumin's growth characteristics decreased when exposed to extreme salt conditions (50 mM NaCl), however this effect was mitigated when plants were presoaked in salt solution and Amla extract. This result is similar with those of Hassanzadeh *et al*., (2013) and Mahmood *et al.* (2014), who reported that priming cumin seed decreased sensitivity to salinity stress and that as salinity increased, root and shoot length decreased. Sedghi *et al.* (2010) observed that priming with NaCl and GA3 improves germination indices and seedling growth of pot marigold (*Calendula officinalis*) and sweet fennel (*Foeniculum vulgare*) under salinity stress conditions. Roodbari *et al*. (2013), reported that salt stress decreased the biomass of primary shoots, roots, germination percentage, and other physiological factors in the stage germination of cumin. PEs can help reduce salinity since they are rich in important phytochemicals such as vitamins, carotenoids, amino acids, mineral nutrients, plant hormones, phenolics, and antioxidants (Latef *et al*., 2017). This was consistent with our findings

that presoaking plants with Amla extract reduced the effect of salinity stress, which could be because of bioactive compounds in Amla aqueous extract, as shown in (Table 2). Biostimulants can improve the production of important secondary metabolites. The positive effect of GA3 and IAA on growth, seeds, and fruit production these compounds have been shown in several reports to be efficient against salinity (Drobek *et al.*, 2019; Zulfiqar *et al.*, 2020). Amla (*Emblica officinalis*) extract is a powerful antioxidant due to its high ascorbic acid content (ranging from 1,100 to 1,700 mg/100 g of fruit) (Tamer *et al.,* 2022). Furthermore Mahmood *et al*., (2014) reported that salt pretreatment increases germination percentage and speed, also can cause rising in dry matter and the growth rate of the plant. Salinity priming as well increased the ability to absorb K+, Mg2+ and Ca2+ in cumin and decreased Na^+ uptake.

Data in this study revealed a significantly increased percentage of essential oil at all salinity levels in both treated and untreated plants. Presoaking in Amla extract and salt solution increased essential oil content in plants irrigated with 25 and 50 mM NaCl (Table 4). *Coriandrum sativum* (Harrathi *et al.,* 2011), and *Nigella sativa* (Bourgou *et al.,* 2010) have all shown an increase in EO as salinity increases. The stimulation of EO production under salinity conditions may result from a greater density of oil glands and a rise in the number of glands formed (Harrathi *et al*., 2011).

The reduction in chlorophyll is a marker of cellular stress and can provide evidence of the severity of stress in plants (Harrathi *et al.,* 2011). The present study revealed that pretreatment with Amla extract and salt solution could minimize the negative effects of salt stress on photosynthetic pigments. Shobbar *et al*., (2012) findings for rice are consistent with the results that were obtained. Salinity can decrease photosynthetic activity by influencing non-stomatal characteristics, including smaller leaves, the oxidation of green pigment, or a decrease in the activity of photosynthetic enzymes. Salinity affects photosynthesis by harming the thylakoid membrane, which contains pigments for photosynthetic activity (Shaheen *et al*., 2012). The most prevalent secondary metabolite in plants, phenolic compounds function as antioxidants to scavenge the excessive ROS produced by the majority of stimuli. The phenolic chemical family member flavonoids also have antioxidant qualities (Tohidi *et al*., 2017). As a result of salt stress, our results showed a considerable increase in total phenolic and flavonoid components. The substantial correlation between polyphenols and resistance to abiotic stress is highly predictive and may serve as a marker for cellular redox state restoration (Sharma *et al*., 2019). Similar to what we found, Hichema *et al.,* (2009) discovered that salt stress had a substantial impact on the total phenolic and flavonoid components in two cultivars of maize (*Zea mays* L.). Similar findings were made in tomato and durum wheat grains by Martinez *et al*., (2016), Kumar *et al.* (2017), and Boukid *et al.*, (2019). Proline and free amino acid buildup in plant tissues may play a role in the osmotic modification of the plant and act as a protective agent for enzymes and membranes. Proline may be crucial as an osmoregulator, in the construction of macromolecules and the protection of enzymes, as well as a source of energy and nitrogen that can be used when exposed to Salinity (Tounektia *et al.*, 2011). This excess of amino acids at high salinity could be explained by enhanced protein degradation. There are numerous explanations that could account for this conflict. The most well-known ones are variations in the physiological stage between studies, stress severity, stress duration, and genotype or species (Ebrahim *et al*., 2020). According to Kumar *et al.,* (2017) the results in wheat are consistent. According to Ebrahim *et al.,* (2020), salt stress led to a significant accumulation of proline; a larger buildup of proline was observed in the leaves.

In addition to non-enzymatic antioxidative systems, enzymatic antioxidative systems are used to combat the damaging effects of reactive oxygen species (Khan *et al*., 2013). Salt stress had a big impact on the DPPH system's measurements of antioxidant activity. The beneficial effect of priming seeds in Amla extract and salt solution on the tolerance of cumin under salinity stress can be explained by the enhancement of phenolic content in treated plants. However, abiotic and biotic stresses can cause phenolic accumulation in plants (Dunja *et al.*, 2021). An increase in reducing sugar accumulation at higher NaCl defines its role as an osmo protectant in salinity-stressed cumin compared to controls; So, Na+ ion toxicity may directly limit starch production or cause the starch to degrade, leading to an increase in reducing sugar content (Kholová *et al*., 2009).

Abiotic stress tolerance is controlled by several genes and regulatory mechanisms at the molecular level. Transcriptional profiling is frequently utilized to determine the genes accountable for the stress response (Acosta- Motos *et al*., 2017). Numerous genes in plants are known to be induced by salt stress tolerance, and most of these genes are linked to salicylic acid (SA)-dependent activation. SA, an endogenous signaling molecule, controls plant responses and functions as a signal transducer (Gleadow *et al*., 2014). It guards against biotic and abiotic stresses and regulates activities including antioxidant defense, nitrogen metabolism, photosynthesis, water stress, and others to prevent the accumulation of toxins and cell death in plant cells (Gleadow *et al*., 2014). In this study, expression patterns of the SA-encoding gene were upregulated in

untreated (control) and treated cumin plants with both Amla extract and NaCl. The expression pattern of SA did not change under salt conditions except for a slight decrease in SA expression under moderate salt stress (in treated plants with both Amla extract and NaCl (Fig. 2). This reflects high endogenous levels of SA in cumin plants and rarely shows an increase in response to treatment. This is consistent with the (Nadarajah *et al*., 2021) finding that SA may not play a dominant role in regulation compared to other phytohormones like JA (Khan *et al*., 2012). Additionally, this shows that some of the oxidative stress generated after NaCl exposure is independent of the presence of SA (Nadarajah *et al.*, 2021). Given the variation in SA levels between plant species and the impact of the environment on endogenous SA levels, the capacity of this molecule to control the complete process of defense and resistance is still not fully understood (Verma and Kanwa, 2020). Previous studies showed that SA, like in plant-pathogen interactions, maybe a signaling molecule working in tandem with ROS to create a feedback amplification cycle under abiotic stress (Shim *et a*l., 2003; Yuan and Lin, 2008). The effects of the initial levels of ROS are thus amplified by the endogenous SA present. Endogenous SA, for instance, guards maize against cadmium stress (Metwally *et al.,* 2005) and shields rice plants from oxidative damage and biotic and abiotic challenges (Yang *et al*., 2004). Second, high SA accumulation can cause a programmed cell death pathway, which results in a hypersensitive response to stresses (Yuan and Lin, 2008). Proline is a powerful amino acid whose osmoprotective role has received substantial research. In this study, the expression of PRO was upregulated in untreated and treated cumin plants. In addition, we could not find any significant differences in proline expression between control and treated cumin plants with respect to their response to external stimuli. This reflects the high level of proline content in cumin plants. This result is consistent with the finding of (Özge and Selin, 2013) the level of proline in soybean mutant plants was not significantly different from that in control plants thus salt tolerance is independent of proline accumulation. Torabi and Niknam (2011) reported that accumulation of proline content depends on plant species and may vary from species to species. This finding agrees with Hassanzadeh *et al.* (2013), who reported that cumin is relatively salt resistant during adult growth and reproductive stages. Peroxides are antioxidant enzymes that play key roles in controlling cellular H_2O_2 levels, which are involved in many plant evolutionary responses. POXs are encoded by many genes. (Gaspar *et al*., 1991) suggest that several PODs act differently or cooperatively in physiological interactions regarding H_2O_2 metabolism. This may be one of the reasons why such large numbers of POD paralogs are expressed in a single plant species. As these enzymes exist in several isoforms with different substrate specificities, they have been involved in a wide range of physiological processes, including stress tolerance, defense against plant pathogenic attacks, and growth regulation (Foyer and Noctor, 2005). The expression of peroxidase genes in response to stressful environmental factors has been well documented in angiosperms recently (Marjamaa *et al*., 2006). In this regard, the current study found that the POX enzyme was upregulated in both untreated (Control) and NaCl-treated plants in the absence of stress conditions, as expressed by two peroxidase isotype isoforms, while it was downregulated in both control and NaCl-treated plants under extreme salt conditions (50 mM NaCl). Elevated salinity appears to be toxic to cumin plants, which is evidenced by the apparent decrease in growth parameters and by the down-regulation of the peroxidase gene. It was also observed that treating cumin plants with Amla extract reduced the salt stress effect in the plants, which was expressed through the upregulation of the expression of the peroxidase gene under severe salt stress conditions (50 mM NaCl). In this study, the results showed that the treatment of cumin plants with both Amla extract and NaCl, strongly induced the expression of the SA, PRO, and POX genes. Where the conditions of salt stress did not statistically affect the levels of both SA and PRO, while the increase in the expression of the POX gene indicates its involvement in adaptive regulation during salinity, and agrees with previous studies (Khan *et al*., 2012 and Sallam *et al.*, 2019).

CONCLUSION

The present study found that priming seeds in Amla extract and salt solution before planting improved growth parameters. Furthermore, it reduced the inhibitory effect of salt stress on photosynthetic pigments, and significantly increased the content of essential oils, phenolic compounds, flavonoids, amino acid content, proline, and antioxidant activity under the influence of severe salt stress. The results of this study also showed that treatment of cumin plants with both Amla extract and NaCl strongly stimulated the expression of the SA, PRO, and POX genes.

Conflict of interest:
The authors have no conflicts of interest to declare that are relevant to the content of this article.

Contribution of authors:

The corresponding author was involved in project conceptualization, execution, sample collection, writing and editing of the manuscript. Hala F. Mohammed was involved in analyzation, execution, sample collection, and writing of the manuscript. All the authors read and approved the manuscript.

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تعزيز تحمل الإجهاد الملحي في الكمون (*Cuminum cyminum* L) باستخدام نقع البذور فى مستخلص الأملا وكلوريد الصوديوم.

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تعتبر الملوحة من أهم المتغيرات التي تحد من إنتاجية المحاصيل و جودتها. فهي تؤثر على العمليات الفسيولوجية المختلفة ، بما في ذلك إنبات البذور ونمو النبات. يمكن التقليل من الآثار السلبية لإجهاد الملوحة على الإنبات من خلال معالجات تحضير البذور المختلفة. في هذا الصدد ، أجريت هذه الدراسة للتحقق من تحضير بذور الكمون بكل من مستخلص أملا ومحلول الملح (NaCl) كإستراتيجية مثيرة للاهتمام لتحسين تحمل الملح وتأثيره على إنبات بذور الكمون (*Cuminum cyminum* L) ونموها. أظهرت النتائج أن تحضير البذور قبل الزراعة في مستخلص أملا ومحلول الملح أدى إلى تحسين قياسات النمو مثل طول الكمون وعدد الأفرع والوزن الطازج ووزن البذور لكل نبات وعدد البذور لكل نورة. أظهرت الدراسة الحالية أيضًا انخفاضًا في متغيرات النمو في ظل ظروف الإجهاد الشديد (50 ملي مول كلوريد الصوديوم) ، بينما قللت النباتات المنقوعة بكل من مستخلص الأملا ومحلول الملح من هذا التأثير. قلل النقع في مستخلص املا ومحلول الملح من التأثير المثبط لإجهاد الملح على محتوى الكلورفيل وزاد محتوى الزيوت الطيارة والفينول الكلي والفلافونويد ومحتوى الأحماض الأمينية والبرولين ومضادات الأكسدة تحت تأثير إجهاد الملح الشديد. كشفت نتائج هذا البحث أن معالجة نباتات الكمون بكل من مستخلص الاملا و NaCl حفز بقوة التعبير عن جينات SA و PRO و POX.

الكلمات المفتاحية: أملا ، كمون ، تعبير جيني ، ملوحة