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Research Article

Elicitation effect of hydrogen peroxide to enhance tolerance of *Taxodium distichum* (L.) Rich. seedlings irrigated with saline water

Samah M. EL-SAYED[1], Amr S. MOHAMED[2], Azza A.M. MAZHAR[1], Yongdui CHEN[3]*

[1]*Ornamental Plants and Woody Trees Department, Agricultural and Biological Research Institute, National Research Centre, Giza, Egypt; ensamah_83@hotmail.com; azza856@yahoo.com*
[2]*Botanical Gardens Research Department, Horticulture Research Institute of Agricultural Research Center, Giza, Egypt; amr_amz_9@yahoo.com*
[3]*Yunnan Academy of Agricultural Sciences, Biotechnology and Germplasm Resources Institute, Yunnan Provincial Key Laboratory of Agricultural Biotechnology, Key Laboratory of South Crop Gene Resources and Germplasm Innovation, Ministry of Agricultural and Rural Affairs, Kunming, Yunnan 650205, China; yongduichen@126.com (*corresponding author)*

Abstract

Because of the scarcity of fresh water and its limited availability for human use, some places have resorted to utilizing alternative water sources, such as saline water, which negatively impacts plant growth and development. Hydrogen peroxide (H_2O_2) signalling molecule, aids in plant defence as a signal in the production of systemic acquired resistance and modulating ROS detoxification and regulating multiple stress-responsive pathways and gene expression. Our study suggests that using H_2O_2 as a stimulating substance to boost the production of antioxidant compounds in plants resulting in enhanced growth. During the 2022 and 2023 seasons, we used H_2O_2 as a foliar application at concentrations (0, 20, 40, 60, and 80 mM) on *Taxodium distichum* seedlings receiving saline water at concentrations (0, 3000, 5000, and 7000 ppm). The results indicated that applying H_2O_2 at 20 mM enhanced most of growth attributes and lowered the production of proline, lipid peroxidation (MDA), and improved the accumulation of chlorophyll a and b which suggests that plants Irrigated with this concentration were able to handle all of the salt levels. Compared to other treatments, applying a H_2O_2 at 40 mM treatment resulted in the highest plant Salt Resistance Index (SRI%) and root growth parameters. Carotenoids, flavonoids, phenols, and sugars in the plant reached peak production when treated with a 60 mM of H_2O_2, regardless of salinity concentrations. The activity of peroxidase (POD) as an antioxidant isoenzyme increased as the salinity and H_2O_2 concentrations increased. The treatment with salinity at 7000 ppm+ H_2O_2 at 60 or 80 mM showed the maximum number of bands and the greatest intensity of POD.

Keywords: abiotic stress; antioxidant enzyme; bioactive components; hydrogen peroxide; peroxidase; salinity; salt resistance index; signalling molecule; systemic acquired resistance; *Taxodium distichum*

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Introduction

Taxodium distichum (L.) Rich, widely recognized as bald cypress, is a species of tree in the Cupressaceae family. People often cultivate it for its pyramidal crown structure and use it as an ornamental tree. The unique, strengthened trunk of a mature species gives the tree a noticeable aspect (Arnold, 2002). Taxodium trees reach a maximum height of 20-27 meters and have a trunk diameter of 1-2 meters. The economic significance of *T. distichum* wood stems from its strength, softness, durability, and wide range of applications in various industrial wood products. *T. distichum* wood is highly resistant to deformation, rot, and white ants because it contains cypressene, an organic compound believed to have natural wood preservation properties (Sternberg and Wilson, 2004). Additionally, *T. distichum* trees are highly effective in preventing soil erosion due to their extensive root system growth, which spreads and stabilizes the soil (Koonce *et al*., 2020).

Our modern society is facing a multitude of environmental challenges. Soil salinity is a significant global concern due to its detrimental impact on agricultural yield. In certain areas, as the amount of high-quality water decreases, it is common practice to redirect it for human consumption. Urban landscapes frequently receive water from other sources, including municipal reclaimed water (Mahgoub *et al*., 2010). People widely recognize that using low-quality or saline water for irrigation significantly contributes to the formation of salt in soil (Sahab *et al*., 2021). Soil salinization affects almost 50% of irrigated croplands globally, leading to decreased plant growth, development, and survival (Guo *et al*., 2022; El-Ramady *et al*., 2024). The presence of sodium chloride (NaCl) in soil and irrigation water significantly hinders plant growth. The presence of salt stress affects a range of physiological and biochemical processes (Parida and Das, 2005; Tattini *et al*., 2006; Stepien and Johnson, 2009). The presence of high salinity levels led to a decrease in the amount of water in the plants and also caused toxicity due to excessive ions, resulting in an imbalance of ions. Aside from the osmotic and toxic consequences, salt stress also causes oxidative stress in plants. These processes collectively contribute to the detrimental effects of salinity (Hernández *et al*., 2001; Barba-Espín *et al*., 2011; Acosta-Motos *et al*., 2014).

Hydrogen peroxide (H_2O_2) is a reactive oxygen species (ROS) that is not a radical. It is an important metabolite in the reduction-oxidation process (REDOX). When present in high concentrations, it can cause oxidative damage to biomolecules and lead to cell death. Nevertheless, when present in small amounts, H_2O_2 acts as a signalling molecule and shares similarities with phytohormones (Cerný *et al*., 2018). Researchers have studied H_2O_2 for its ability to boost the production of bioactive compounds in plants, acting as a signalling molecule during defence responses triggered by the plant in various stressful situations. Hydrogen peroxide easily spreads through membranes and has a relatively extended period of time in which it breaks down, making it significant in the transmission of stress signals (Belchí-Navarro *et al*., 2019). Stress inducers provoke oxidative stress by stimulating oxidative bursts and the consequent accumulation of reactive oxygen species (ROS) (Considine and Foyer, 2021). Reactive oxygen species (ROS), especially hydrogen peroxide (H_2O_2), on the other hand, act as messengers that manage cell growth and development, photosynthesis, stomatal movement, and how plants react to stress (Liu *et al*., 2020; Asgher *et al*., 2021; Considine and Foyer, 2021). Several studies have shown that hydrogen peroxide (H_2O_2) improves the phenylpropanoid pathway. This makes the plant to produce more phenolic compounds and be more resistant to changes in its environment (Liu *et al*., 2019; Wang *et al*., 2022). The objective of this study was to evaluate the effects of varying rates of H_2O_2 on the growth of *T. distichum* seedlings and their ability to withstand saline water irrigation to identify the optimal rate of H_2O_2 that would boost seedling growth and promote their tolerance to saline water.

Materials and Methods

Study site and seasons

The experiment was conducted in the experimental farm of the Horticulture Research Institute (HRI), Agricultural Research Center (ARC), Giza, Egypt. over the course of the 2022 and 2023 seasons. Each season lasted for seven months, beginning in March and ending in September. The chemical estimates were conducted at the National Research Centre (NRC), Egypt.

Setup of experiment

Seedlings of *Taxodium distichum* (L.) Rich. were acquired from the HRI, ARC, with a height range of 20-23 cm. We planted the seedlings in plastic pots measuring 30 cm in diameter. The pots were filled with a soil mixture containing clay and sand in a 1:1 ratio. Table 1 provides the physical and chemical characteristics of the soil. The method described by Jackson (1973).

Table 1. Physical and chemical traits for soil mixture used in the experiment

Soil sample	Coarse sand%		Fine sand%		Silt%	Clay%			
Sandy clay	41.40		42.70		11.40	4.50			
	pH (1:2.5)	EC ($dS.m^{-1}$)	Anion (meq/l)			Cation (meq/l)			
	7.67	3.37	HCO_3^-	Cl	SO_4^-	Ca^{++}	Mg^{++}	Na^+	K^+
			0.61	29.2	2.19	9.00	4.90	17.50	0.60

We treated the seedlings with a preventative spraying with H_2O_2 at doses selected for the experiment (0, 20, 40, 60, 80 mM). Three weeks after the seedlings were transplanted; two sprays were conducted following the initiation of irrigation with saline water. The second spray was one month after the first spray, and the third spray occurred one month after the second spray.

The process of irrigating with saline water initiated at concentrations of 0 (distilled water), 3000, 5000, and 7000 ppm, four weeks after the seedlings were transplanted. During this process, the pots were closely checked to ensure that the field capacity in each pot remained at 60% by weight. The saline solution was prepared by dissolving 3, 5, and 7 g of NaCl in one litre of distilled water to get the desired concentration.

Recorded data

Growth attributes

Plant height (cm), stem diameter (cm), No. of branches/ plant, root length (cm), fresh and dry weights for leaves, stems and roots (g. $plant^{-1}$).

Salt Resistance Index (SRI %)

This equation is considered a true indicator of the plant's ability to tolerate salinity, which was mentioned by Wu and Huff (1983)

SRI (%) = (average root length of the salt – stressed plant/ average root length of the control plant) × 100.

Biochemical estimates

Photosynthetic pigments (mg. g^{-1} F.W.)

A known weight (0.1 g) of fresh leaves was crushed with 10 ml of 80% acetone (v/v). The supernatant was then used to measure the content of chlorophyll a, b, and carotenoids using a spectrophotometer at wavelengths of 663, 647, and 470 nm (Lichtenthaler and Wellburn, 1983). The carotenoids: total chlorophyll ratio was calculated by dividing the value of carotenoids on the summation of chl. a and b.

Enzymatic and non-enzymatic antioxidant

Total phenols content (mg. g^{-1} F.W.)

The total phenols were estimated using the method published by Singleton and Rossi (1965). The ethanol extract of fresh leaves was treated with Folin-Ciocalteu reagent and sodium carbonate (14%). The optical density was measured at wavelength 765 nm and calculated using the gallic acid standard curve.

Total flavonoids content

Chang *et al*. (2002) employed the aluminium chloride colorimetric method to determine the total flavonoid concentration of the ethanol extract of fresh leaves tissue.

Peroxidase isoenzyme patterns

Peroxidase isozymes (E.C. 1.11.1.7) The pure protein extract from 100 mg fresh leaf was separated on polyacrylamide gel using an electrophoresis device. After staining (benzidine and H_2O_2), isozyme patterns were compared on the bases of the number, density and electrophoretic mobility (Rf) values of peroxidase (Barceló *et al*., 1987).

Osmolytes

Total sugars content

Total sugars content was determined according to Dubois *et al*. (1956) by using sulphuric- phenol colorimetric method, The sample absorbance was measured at a wavelength of 490 nm using spectrophotometer.

Proline content

Proline content was determined using the method reported by Bates *et al*. (1973). Fresh leaves (0.2 g) were homogenized in 10 mL of 3% aqueous sulfosalicylic acid for 72 hours. After infiltration, 2 ml of acid-ninhydrin reagent was added to 2 ml of extract, which was then extracted using water bath that was boiling for an hour. The chromophore was separated with 4 mL of toluene, and its absorbance was measured at the wavelength of 520 nm using spectrophotometer.

Lipid peroxidation

Lipid peroxidation (μM. g^{-1} F.W.) was determined according to Rao and Sresty (2000). The thiobarbituric acid assay (TBA test) was used to measure lipid peroxidation by measuring malondialdehyde (MDA) content. Fresh leaf samples (0.5 g) were extracted with 5% trichloroacetic acid and centrifuged at 4000× g for 10 min. Two milliliters of the extract was mixed with 2 mL of 0.6% thiobarbituric acid (TBA) solution. The final volume was heated in a water bath (95 °C) for 30 min., after cooling the absorbance of the developed color was measured at 532, 600 and 450 nm. MDA content was determined using the following equation: $6.45 \times (A532 - A600) - 0.56 \times A450$.

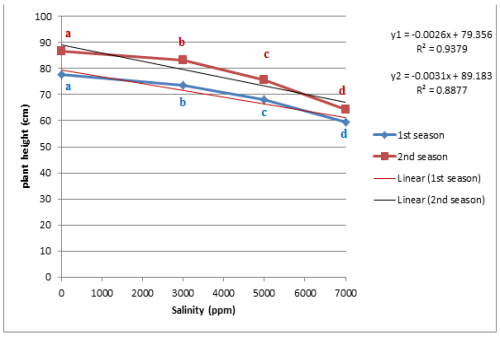
Statistical analysis and experiment layout

The experiment was designed as a factorial experiment in a complete block design consisting of 20 treatments (4 salinity × 5 H_2O_2), each treatment replicated three times. The average of the obtained data was subjected to statistical analysis using least significant differences (L.S.D.) at 5% level according to the method described by Snedecor and Cochran (1980), and the treatments' means were compared for significance by Duncan's new multiple range test (DMRT) where $p \leq 0.05$ (Duncan, 1955). All the statistical analyses were performed using CoStat (CoHort software, Monterey, CA, USA) V6.4 (2005).

Results

Growth attributes

According to the data attached in Figure 1, it was clear that irrigating *T. distichum* seedlings with saline water at different concentrations (3000, 5000, and 7000 ppm) led to a gradual decrease parallel to the increase in salinity concentration in the values of all estimated phenotypic traits as compared to control plants (irrigated with distilled water) which showed highly significant ($p \leq 0.05$). The values of the traits obtained from the untreated plants were as follows for each of plant height 77.85 and 86.55 cm, stem diameter 1.05 and 1.06 cm, No. of branches/ plant 10.13 and 12.73, root length 61.31 and 60.94 cm, leaves fresh weight 52.68 and 52.40 g/ plant, leaves dry weight 15.12 and 16.47 g/ plant, stem fresh weight 62.11 and 67.42 g/ plant, stem dry weight 23.75 and 25.77 g/ plant, root fresh weight 47.14 and 49.07 g/ plant and root dry weight 19.63 and 20.43 g/ plant, respectively, in 2022 and 2023 seasons.



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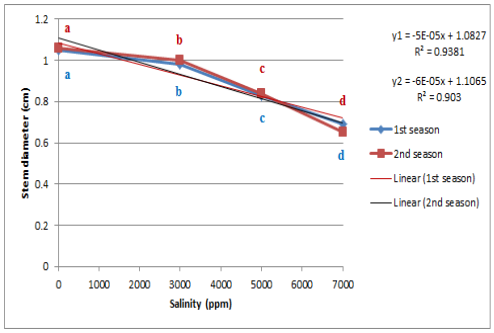

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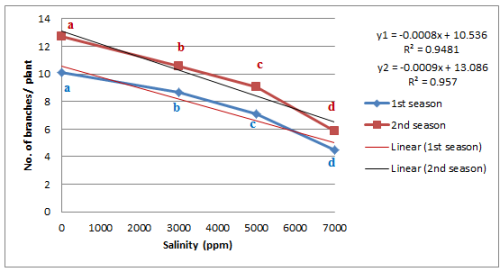

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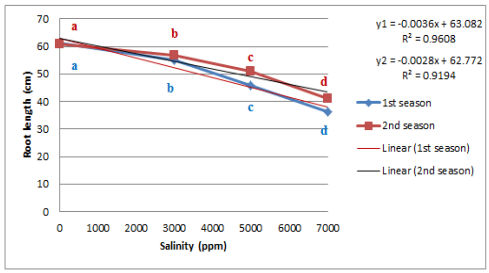

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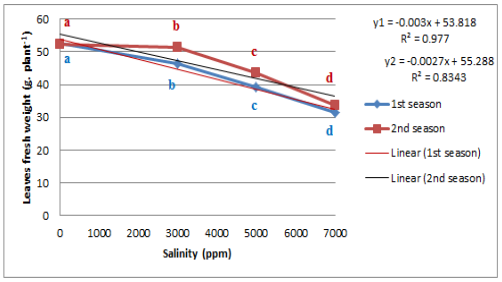

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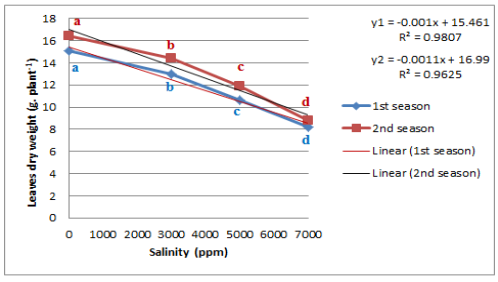

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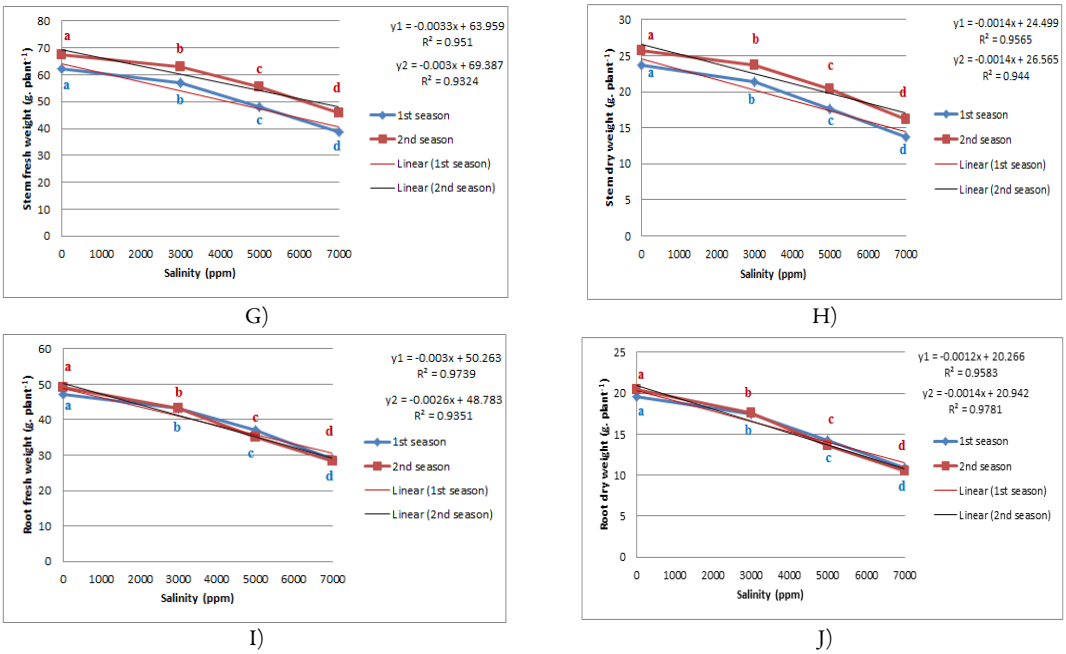
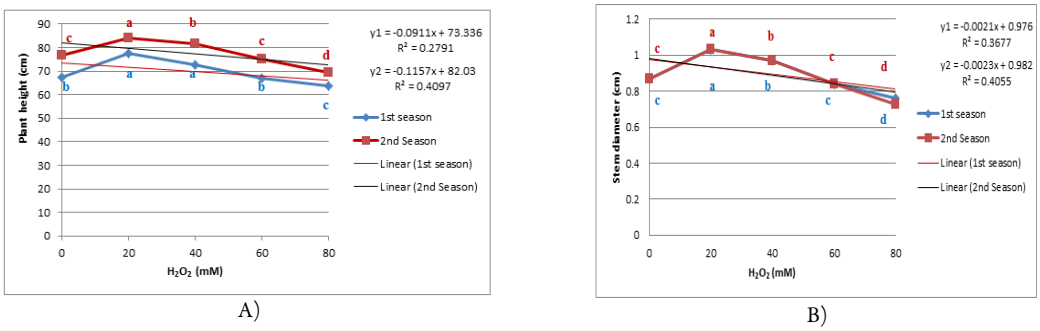


Figure 1. Linear regression of growth characters of *T. distichum* seedlings affected by irrigation with saline water during 2022 and 2023 seasons

Where A) Plant height; B) Stem Diameter; C) No. of Branches/plant; D) Root length; E) Leaves fresh weight; F) Leaves dry weight; G) Stem fresh weight; H) Stem dry weight; I) Root fresh weight; J) Root dry weight.

Attached letters of the express significant differences, and if different values take the same letter, this means that there is no significance between them according to the Duncan test, $p \leq 0.05$); R1: correlation coefficient in the 1st season, R2: correlation coefficient in the 2nd season ; y1: regression equation in the 1st season; y2: regression equation in the 2nd season

Linear regression in Figure 2 showed that treating plants with 20 mM H_2O_2 resulted in the most significant rise ($p \leq 0.05$) in some morphological traits. The largest percentage increment in plant height was 14.67-9.86, No. of branches/ plant 44.57-38.06, leaves fresh weight 40.66-39.40, leaves dry weight 50.98-49.60, stem fresh weight 35.62-25.19, stem dry weight 42.76-31.89%, respectively, in 2022 and 2023 seasons, in addition to stem diameter which gave the same value percentage increment 18.39% in both seasons. While the highest substantial increase in root characteristics was seen when seedlings were exposed to a concentration of 40 mM, where the highest percentage increment has been reached for root length 14.84-9.01, root fresh weight 8.53-13.00 and root dry weight 15.45-13.63%, respectively, in both seasons.



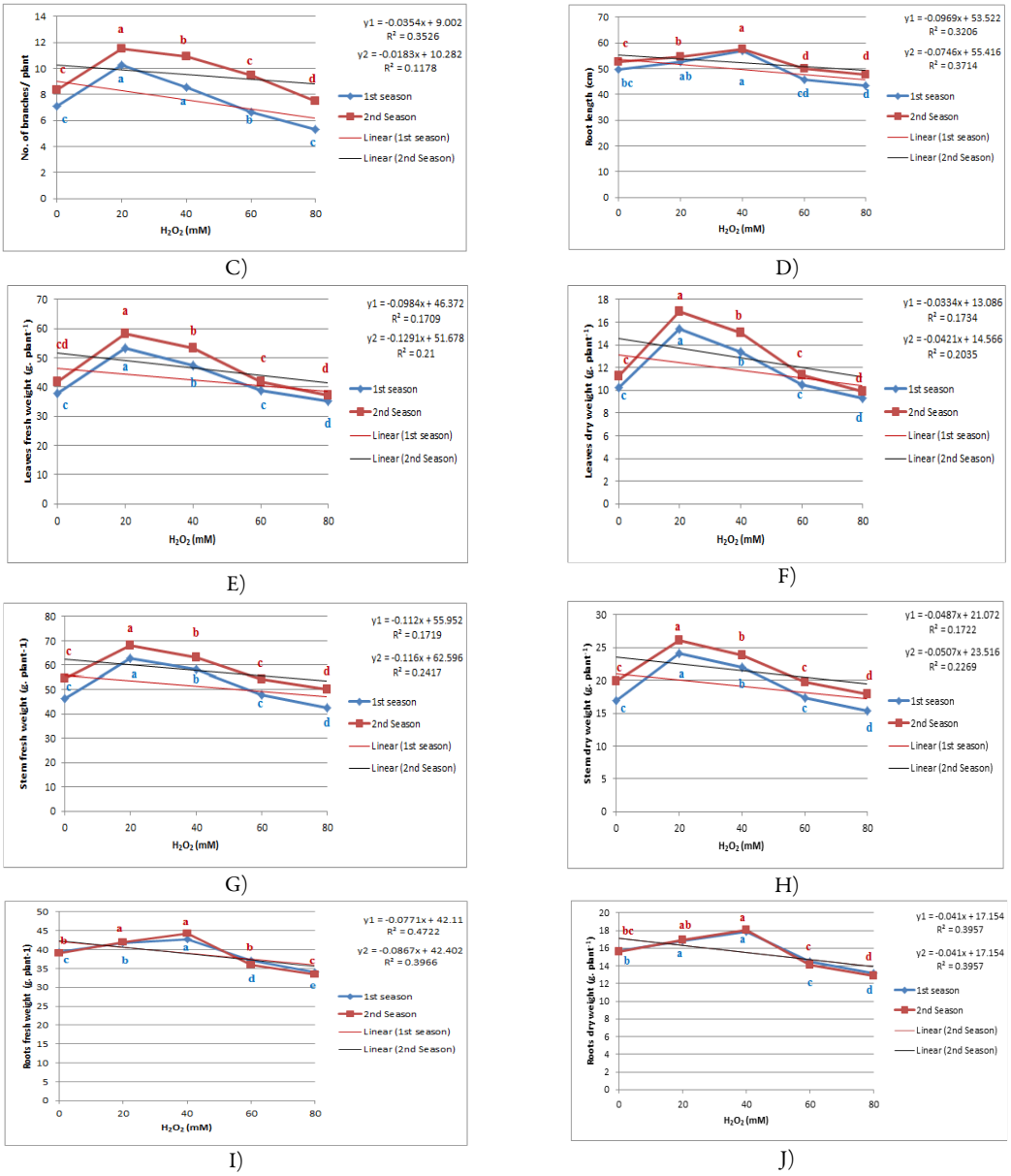


Figure 2. Linear Regression of effect of H_2O_2 growth characters of *T. distichum* seedlings during 2022 and 2023 seasons

Where A) Plant height; B) Stem Diameter; C) No. of Branches/plant; D) Root length; E) Leaves fresh weight; F) Leaves dry weight; G) Stem fresh weight; H) Stem dry weight; I) Root fresh weight; J) Root dry weight.

Attached letters of the express significant differences, and if different values take the same letter, this means that there is no significance between them according to the Duncan test, $p \leq 0.05$); R1: correlation coefficient in the 1st season, R2: correlation coefficient in the 2nd season; y1: regression equation in the 1st season; y2: regression equation in the 2nd season

The application of H_2O_2 on *T. distichum* seedlings which irrigated with saline water showed significant differences in the values of the obtained results Tables 2, 3 and 4. H_2O_2 treatment at a concentration of 20 mM improved growth traits in salinity-affected seedlings, including plant height, stem diameter, number of branches/plant, and fresh and dry weight of leaves and stems, followed by concentration of 40 mM resulted in similar improvements for both seasons as compared with other treatments of H_2O_2 under the same salinity

concentrations. In both seasons, the concentration of 40 mM had a direct effect on the recorded values of root characteristics.

Table 2. Effect of interaction between salinity and H_2O_2 on plant height, stem diameter, No. of branches/ plant and root length of *T. distichum* during 2022 and 2023 seasons

Salinity (ppm)	H_2O_2 (mM)	Plant height (cm)		Stem diameter (cm)		No. of branches/ plant		Root length (cm)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
0	0	75.23	87.33	1.04	1.07	10.00	11.00	62.17	61.00
	20	89.50	92.27	1.22	1.19	13.33	15.00	65.67	64.67
	40	82.33	90.67	1.17	1.15	11.67	14.67	67.73	65.50
	60	73.67	85.00	0.97	1.00	8.67	12.33	56.33	57.67
	80	68.50	77.50	0.85	0.87	7.00	10.67	54.67	55.87
3000	0	71.67	82.17	0.95	0.96	7.67	9.33	55.00	57.33
	20	80.67	90.00	1.13	1.18	11.33	12.33	59.83	58.33
	40	77.17	88.67	1.09	1.13	10.33	12.00	65.00	61.67
	60	70.33	80.87	0.92	0.93	7.33	11.00	50.17	53.90
	80	67.67	74.67	0.81	0.81	6.67	8.00	45.27	52.30
5000	0	67.82	75.23	0.82	0.84	7.00	7.67	44.50	51.47
	20	75.00	85.83	1.00	1.03	10.00	11.67	47.67	52.67
	40	70.00	80.33	0.88	0.89	7.33	10.33	53.50	55.10
	60	65.83	71.33	0.79	0.77	6.33	8.67	42.78	49.33
	80	61.50	65.47	0.68	0.66	4.67	7.00	41.00	46.33
7000	0	55.47	61.67	0.66	0.61	3.67	5.33	36.50	41.17
	20	64.67	68.50	0.75	0.72	6.33	7.00	37.83	42.20
	40	61.87	66.67	0.72	0.70	5.00	6.67	41.33	47.67
	60	58.50	63.33	0.67	0.64	4.33	6.00	33.27	38.67
	80	56.67	60.50	0.63	0.59	3.00	4.33	32.40	35.77
LSD 0.05		**5.86**	**6.08**	**0.09**	**0.08**	**2.13**	**2.21**	**6.17**	**6.81**

Table 3. Effect of interaction between salinity and H_2O_2 on fresh and dry weights of leaves and stems of *T. distichum* during 2022 and 2023 seasons

Salinity (ppm)	H_2O_2 (mM)	Leaves F.W. (g/ plant)		Leaves D.W. (g/ plant)		Stems F.W. (g/ plant)		Stems D.W. (g/ plant)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
0	0	51.00	55.79	14.43	15.78	60.45	66.50	22.97	25.27
	20	61.15	66.08	18.35	19.82	69.75	74.52	27.28	29.15
	40	55.24	61.82	16.09	18.00	68.00	70.24	26.36	27.23
	60	49.66	52.96	13.94	14.86	57.73	63.68	21.76	24.00
	80	46.36	50.36	12.77	13.87	54.61	62.17	20.37	23.19
3000	0	40.29	44.82	10.88	12.10	48.82	58.72	17.78	21.38
	20	58.57	62.49	17.28	18.43	68.37	71.77	26.68	28.01
	40	51.60	61.11	14.92	17.67	65.89	67.41	25.32	25.91
	60	42.02	47.58	11.44	12.96	53.42	61.61	19.78	22.81
	80	39.75	41.40	10.58	11.03	47.58	55.49	17.23	20.09
5000	0	31.87	35.97	8.32	9.39	39.67	50.18	14.09	17.82
	20	51.09	57.64	14.64	16.51	61.62	67.02	23.48	25.54
	40	47.84	52.00	13.28	14.43	57.39	62.91	21.44	23.50
	60	36.72	40.17	9.73	10.65	44.91	52.20	16.13	18.75
	80	28.25	32.28	7.29	8.33	36.88	45.28	12.98	15.94
7000	0	27.96	30.83	7.16	7.89	36.11	42.47	12.58	14.80
	20	41.73	47.13	11.33	12.80	51.22	59.45	18.82	21.85
	40	34.44	38.52	9.03	10.10	41.25	51.76	14.74	18.49
	60	27.37	26.62	6.96	6.77	34.38	39.12	11.88	13.52
	80	25.80	24.74	6.50	6.23	31.46	36.63	10.75	12.52
LSD 0.05		**6.55**	**6.03**	**1.96**	**2.20**	**6.40**	**6.83**	**2.96**	**3.43**

Table 4. Effect of interaction between salinity and H_2O_2 on fresh and dry weights of roots and salt resistant index (SRI %) of *T. distichum* during 2022 and 2023 seasons

Salinity (ppm)	H_2O_2 (mM)	Root F.W. (g/ plant)		Root D.W. (g/ plant)		SRI (%)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season
0	0	48.72	51.06	20.32	21.30	100.00	100.00
	20	50.22	51.37	21.13	21.61	105.63	106.02
	40	50.38	52.63	21.41	22.37	108.94	107.34
	60	44.49	47.03	18.3	19.35	90.61	94.54
	80	41.90	43.27	16.98	17.54	87.94	91.59
3000	0	43.64	43.98	17.80	17.94	88.47	93.98
	20	44.13	46.17	18.07	18.90	96.24	95.62
	40	47.65	49.66	19.72	20.56	104.55	101.10
	60	41.20	39.40	16.43	15.72	80.70	88.36
	80	39.77	37.12	15.59	14.55	72.82	85.74
5000	0	36.47	34.46	14.02	13.24	71.58	84.38
	20	41.00	37.35	16.24	14.80	76.68	86.34
	40	41.58	40.58	16.69	16.30	86.05	90.33
	60	35.39	33.29	13.33	12.54	68.96	80.87
	80	30.11	30.27	11.19	11.25	65.95	75.95
7000	0	28.80	26.82	10.65	9.92	58.71	67.49
	20	31.80	33.10	11.77	12.39	60.85	69.18
	40	31.45	33.75	13.52	12.81	66.48	78.15
	60	27.42	24.41	10.02	8.92	53.51	63.39
	80	24.32	22.91	8.78	8.27	52.55	58.64
LSD 0.05		**4.94**	**4.64**	**2.93**	**2.36**	**7.28**	**6.17**

The values of SRI (%) showed in Figure 3A cleared that the highest significant SRI ($p \leq 0.05$) was obtained from control plants giving values 98.62 and 99.90%, respectively, in 2022 and 2023 seasons, and then decreased by increasing salinity concentration.

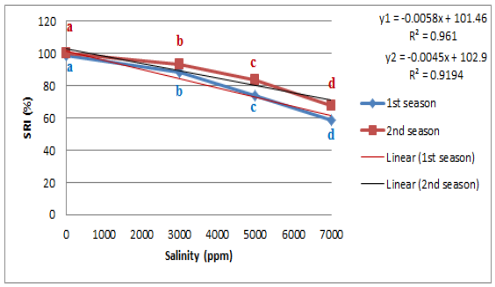
Concerning the effect of H_2O_2 treatment which appeared in Figure 4A illustrated that the seedling received H_2O_2 at concentration 40 mM showed highest significant ($p \leq 0.05$) giving values 91.51 and 94.23% respectively, in both seasons, as compared to the rest treatments.

Regarding the effect of interaction treatment between salinity and H_2O_2, the data presented in Table 4 clarified that the SRI increased by 108.94 and 107.34% with treatment 0 ppm salinity + 40 mM H_2O_2, respectively, in the first and second season as compared to control treatment (0 ppm salinity + 0 mM H_2O_2). Away from the control treatment (0 ppm salinity) and under the actual treatment of salinity (3000, 5000 and 7000 ppm), it was found that the highest value of SRI (104.55 and 101.1%) obtained from seedlings treated with salinity 3000 ppm+ 40 mM H_2O_2, respectively, in the first and second season as compared to other treatment under actual different salinity.

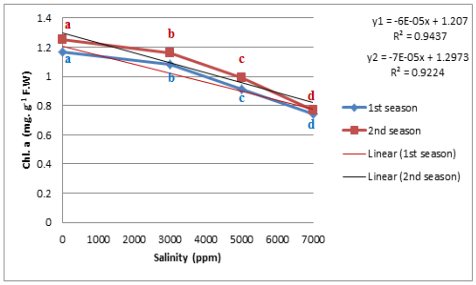
Biochemical estimates

Photosynthetic pigments content

The content of chlorophyll a and b negatively affected by raising salinity treatment as showed in Figure 3B, 3C, the highest values of chlorophyll a (1.17 and 1.25 mg. g-1 F.W., respectively, during 2022 and 2023 seasons) and chlorophyll b (the same value 0.44 for both seasons) were obtained from control treatment and then decreased gradually by increasing salinity level. On contrast, it was found that the carotenoid content and carotenoids: total chlorophyll ratio values increased progressively with increasing salinity concentration (Figure 3 D and E). Where the highest increment percentage of carotenoids 26.19 and 23.86% was appeared at concentration 7000 ppm, likewise carotenoids: total chlorophyll ratio which increased by 109.26 and 115.09% as compared to control plants during 2022 and 2023 seasons.



A)



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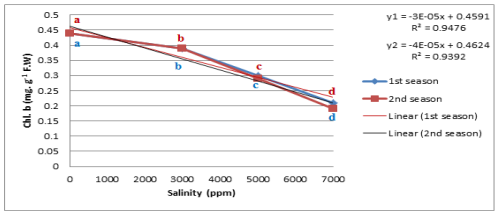
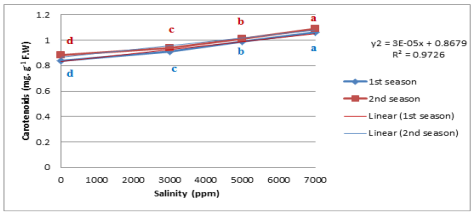


C)



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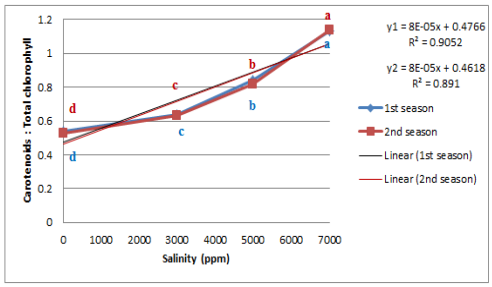
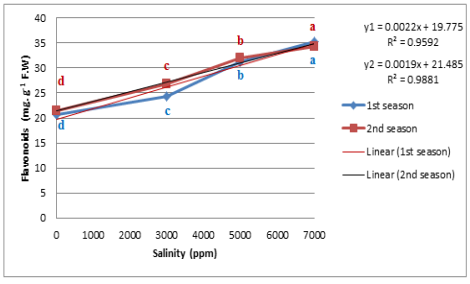


E)



F)

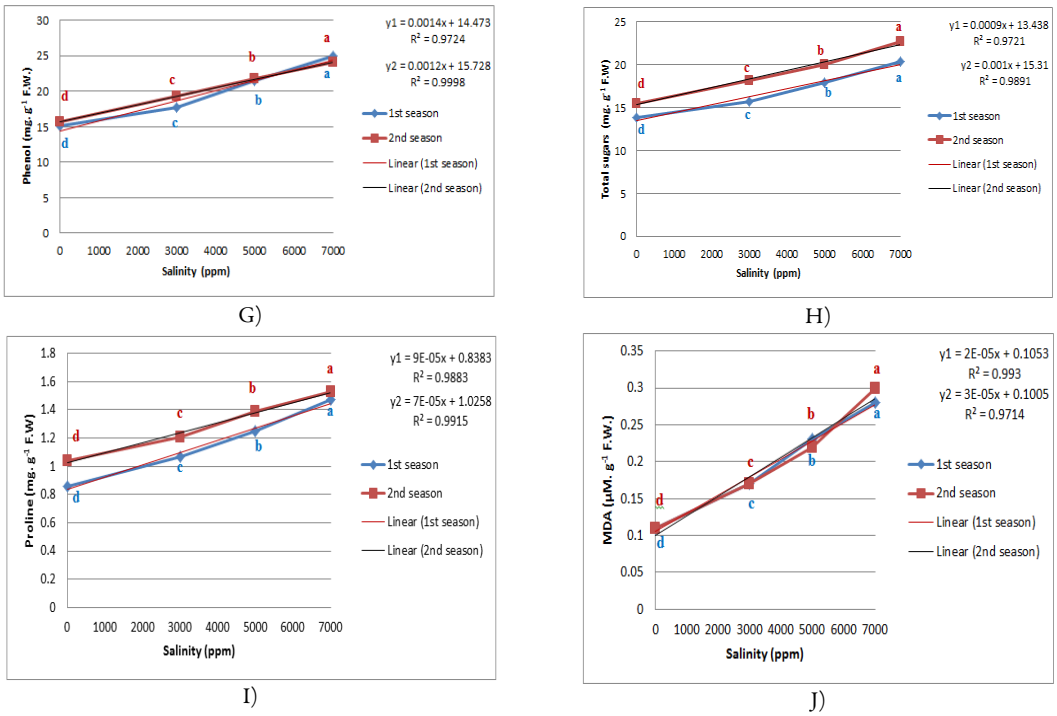
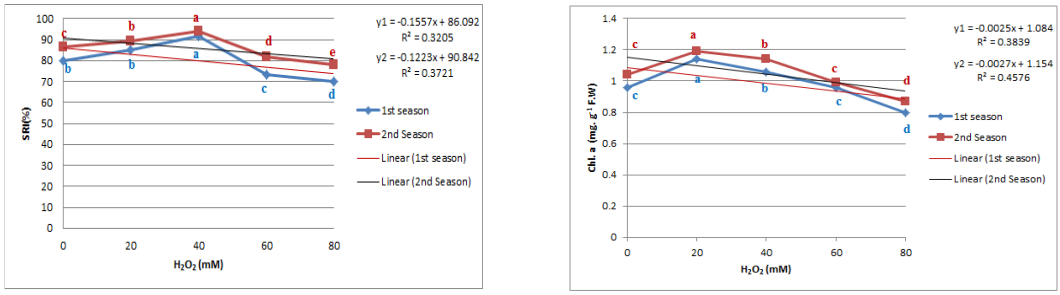


Figure 3. Linear regression of salt resistant index (SRI%) and chemical compositions of T. distichum seedlings affected by irrigation with saline water during 2022 and 2023 seasons

Where A) SRI; B) Chl. a; C) Chl. b; D) carotenoids; E) carotenoids: total Chlorophyll; F) Flavonoids; G) Phenol; H) Total sugar; I) Proline; J) MDA

Attached letters of the express significant differences, and if different values take the same letter, this means that there is no significance between them according to the Duncan test, $p \leq 0.05$); R1: correlation coefficient in the 1st season, R2: correlation coefficient in the 2nd season ; y1: regression equation in the 1st season; y2: regression equation in the 2nd season

The obtained results from Figure (4 B, C, D and E) indicated that H_2O_2 had significant effect ($p \leq 0.05$) on photosynthetic pigments content. *T. distichum* seedlings treated with H_2O_2 at 20 mM gave the highest content of chlorophyll a with values 1.14 and 1.19 and chlorophyll b with values 0.42 and 0.43 mg. g-1 F.W., respectively, in both seasons, as compared to control. The highest content of carotenoids was obtained from treatment 60mM giving values 1.02 and 1.04 mg. g-1 F.W., respectively, in both seasons, as compared to control. Moreover, the highest ratio of carotenoids: total chlorophyll with the same value in both seasons (0.93) was obtained from plants treated with 80 mM.



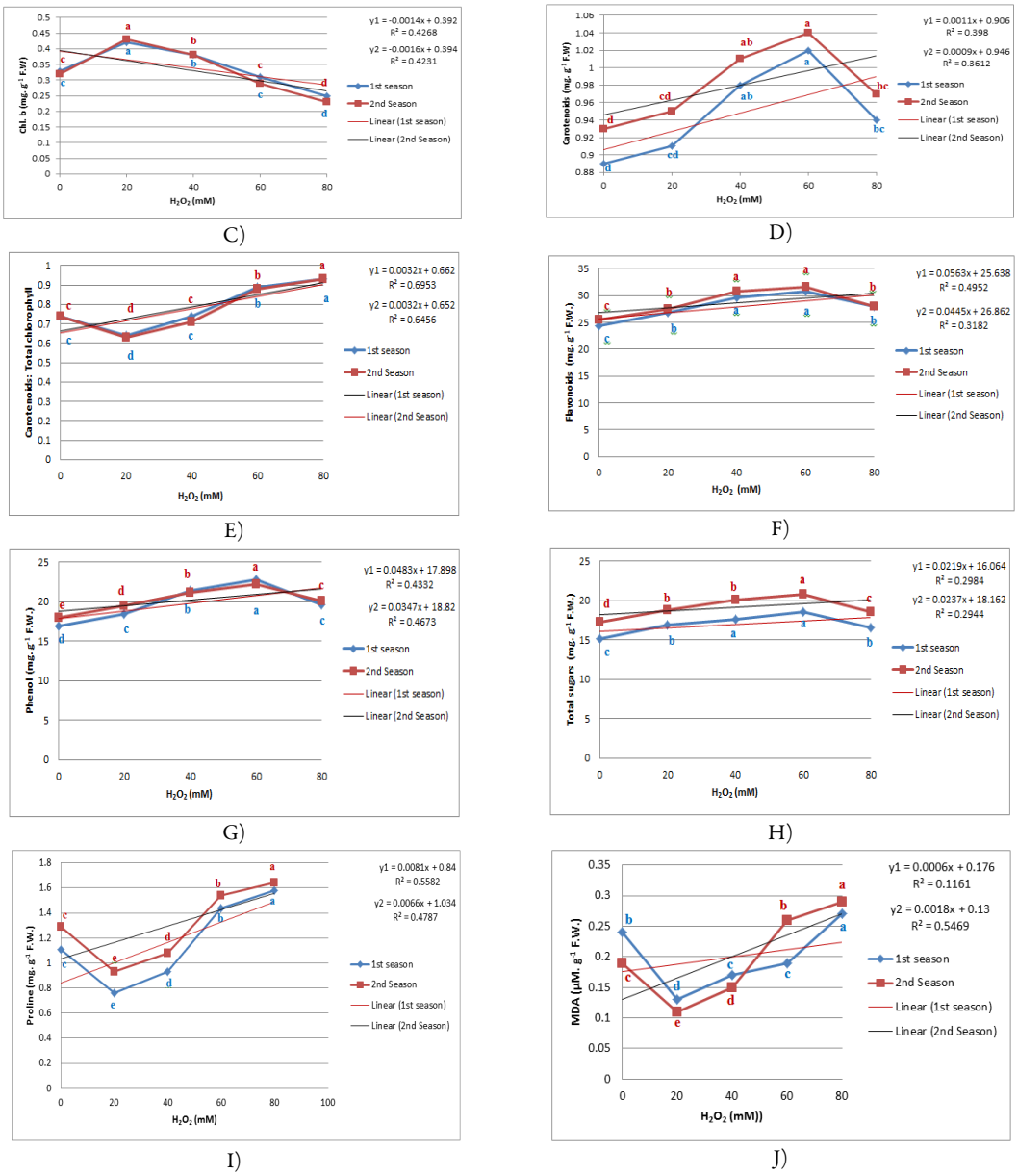


Figure 4. Linear regression of effect of H_2O_2 on salt resistant index (SRI%) and chemical compositions of *T. distichum* seedlings during 2022 and 2023 seasons

Where A) SRI; B) Chl. a; C) Chl. b; D) carotenoids; E) carotenoids: total Chlorophyll; F) Flavonoids; G) Phenol; H) Total sugar; I) Proline; J) MDA

Attached letters of the express significant differences, and if different values take the same letter, this means that there is no significance between them according to the Duncan test, $p \leq 0.05$); R1: correlation coefficient in the 1st season, R2: correlation coefficient in the 2nd season ; y1: regression equation in the 1st season; y2: regression equation in the 2nd season

The interaction factors had a clear effect on the plant content of photosynthetic pigments and carotenoids: total chlorophyll as shown in Table 5. The highest content of chlorophyll a (1.35 and 1.39 mg. g-1 F.W., respectively, during 2022 and 2023 seasons) and chlorophyll b (0.52 and 0.54 mg. g-1 F.W., respectively, during 2022 and 2023 seasons) were obtained from treating the plants with salinity at 0 ppm + H_2O_2 at 20 mM. While the treatment of salinity at 7000 ppm + H_2O_2 at 60 mM showed a clear increase in the

plant's carotenoid content with values 1.14 and 1.17 mg. g-1 F.W., also the carotenoids: total chlorophyll was affected by the same treatment giving values 1.23 and 1.26, respectively, in both seasons.

Table 5. Effect of interaction between salinity and H_2O_2 on photosynthetic pigments content (Chl. a, b and carotenoid) and carotenoids: chlorophyll of *T. distichum* during 2022 and 2023 seasons

Salinity (ppm)	H_2O_2 (mM)	Chl. a (mg. g^{-1} F.W.)		Chl. b (mg. g^{-1} F.W.)		Carotenoids (mg. g^{-1} F.W.)		Carotenoids: chlorophyll	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
0	0	1.18	1.25	0.45	0.47	0.80	0.83	0.49	0.48
	20	1.35	1.39	0.52	0.54	0.82	0.86	0.44	0.45
	40	1.29	1.34	0.50	0.50	0.85	0.91	0.48	0.49
	60	1.11	1.19	0.41	0.41	0.88	0.92	0.58	0.58
	80	0.90	1.06	0.31	0.30	0.84	0.87	0.69	0.64
3000	0	1.07	1.15	0.38	0.37	0.85	0.89	0.59	0.59
	20	1.24	1.31	0.48	0.51	0.90	0.93	0.52	0.51
	40	1.22	1.27	0.47	0.48	0.95	0.96	0.56	0.55
	60	1.01	1.13	0.35	0.34	0.96	0.98	0.71	0.67
	80	0.86	0.92	0.28	0.25	0.91	0.95	0.80	0.81
5000	0	0.87	0.97	0.29	0.27	0.92	0.96	0.79	0.78
	20	1.15	1.21	0.42	0.44	0.96	0.98	0.61	0.59
	40	0.96	1.12	0.31	0.33	1.01	1.03	0.80	0.71
	60	0.83	0.88	0.25	0.23	1.10	1.10	1.02	0.99
	80	0.75	0.79	0.22	0.20	0.97	1.00	1.00	1.01
7000	0	0.71	0.77	0.19	0.17	0.99	1.03	1.10	1.10
	20	0.81	0.85	0.24	0.23	1.02	1.04	0.97	0.98
	40	0.77	0.82	0.22	0.21	1.11	1.13	1.13	1.10
	60	0.72	0.74	0.21	0.19	1.14	1.17	1.23	1.26
	80	0.68	0.69	0.17	0.16	1.04	1.06	1.23	1.25
LSD 0.05		**0.09**	**0.10**	**0.07**	**0.07**	**0.10**	**0.09**	**0.09**	**0.10**

Enzymatic and non-enzymatic antioxidants

Increasing salinity level had a promoting effect ($p \leq 0.05$) on the content of determined non-enzymatic antioxidants including phenols, flavonoids as shown in the Figure (3 F, G). The highest content of flavonoids with values 35.27 and 34.36 and phenols with values 24.96 and 24.07 mg. g^{-1} F.W., respectively, in 2022 and 2023 seasons as compared with control treatment.

The data shown in Figure (4 F, G) manifested that seedling exposed to treatment 60 mM of H_2O_2 resulted in the most significant rise ($p \leq 0.05$) in non-enzymatic antioxidants content giving values 30.77 and 31.55 mg. g^{-1} F.W., respectively, in both seasons for flavonoids and 22.78 and 22.18 mg. g^{-1} F.W., respectively, in both seasons for phenols.

Regarding the effect of interaction treatments between salinity and H_2O_2, the data presented in Table 6 demonstrated that treating the seedlings with salinity at 7000 ppm + H_2O_2 at 60 mM gave the highest significant content of flavonoids (34.78 and 35.98 mg. g^{-1} F.W., respectively, in both seasons) and phenols content (28.71 and 25.39 mg. g^{-1} F.W., respectively, in both seasons) as compared to control treatment.

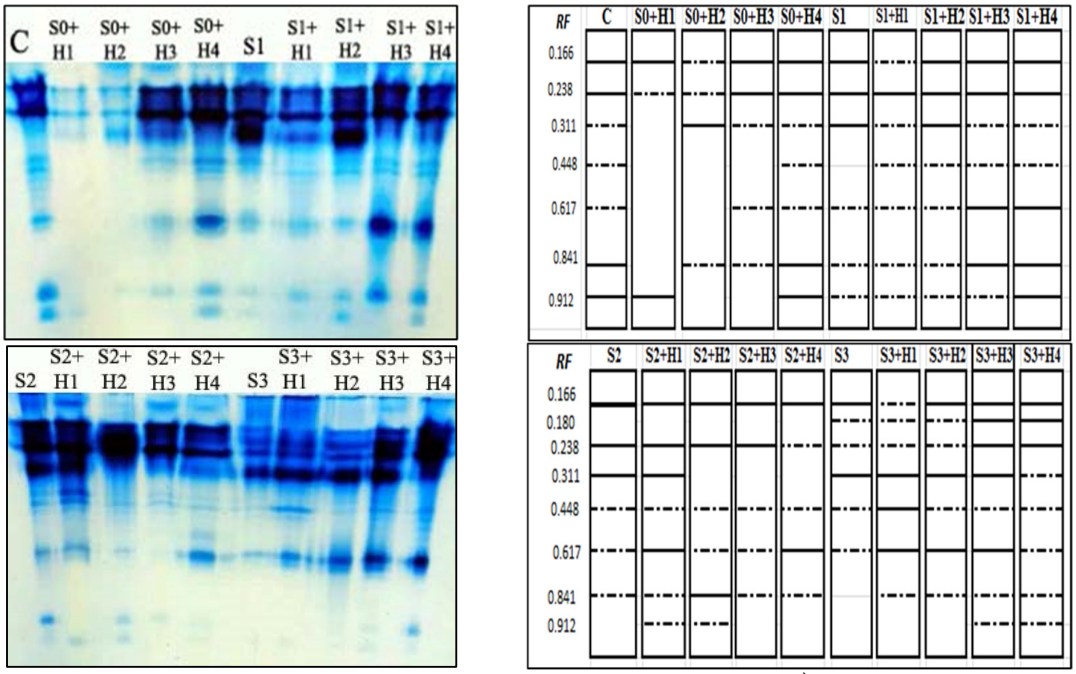
The data shown in Figure (5 A, B) demonstrates that the native PAGE of peroxidase isozymes (POD) yielded a total of 8 isozymes within the Rf range of 0.166 to 0.912. Variances in isozyme patterns were correlated with varying amounts of NaCl and H_2O_2. Plants subjected to high salinity levels of 3000 and 5000 ppm+ 0 mM H_2O_2 exhibited significantly an increase in POD activity, which then decreased at 7000+ 0 mM H_2O_2 compared to the control group. Plants treated with 3000 ppm NaCl+ 0 mM H_2O_2 showed 3 moderately dense and 3 faint bands, while plants treated with NaCl at 5000 ppm+ 0 mM H_2O_2 displayed one highly intense band at Rf (0.166), 3 moderate bands, and two faint bands. However, plants treated with NaCl at 7000 ppm+ 0 mM H_2O_2 experienced a significant decrease in peroxidase activity, with two moderate bands and 4 faint bands recorded.

Table 6. Effect of interaction between salinity and H_2O_2 on the content of total flavonoids, phenol, sugars and proline of *T. distichum* during 2022 and 2023 seasons

Salinity (ppm)	H_2O_2 (mM)	Total flavonoids (mg. g^{-1} F.W.)		Total phenols (mg. g^{-1} F.W.)		Total sugars (mg. g^{-1} F.W.)		Proline (mg. g^{-1} F.W.)		MDA (µM. g^{-1} F.W.)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
0	0	18.20	18.73	13.42	14.11	12.77	14.26	0.66	1.01	0.12	0.10
	20	18.55	19.47	13.67	14.62	13.37	14.97	0.49	0.62	0.06	0.04
	40	23.02	24.28	16.16	17.39	14.51	16.32	0.54	0.74	0.07	0.07
	60	23.52	24.53	16.21	17.80	14.80	16.61	1.16	1.35	0.11	0.15
	80	20.19	20.17	15.83	14.76	13.66	15.50	1.44	1.50	0.17	0.20
3000	0	20.34	21.82	15.90	16.25	14.23	15.84	0.92	1.10	0.24	0.13
	20	23.65	25.18	16.33	19.28	15.67	17.80	0.62	0.82	0.09	0.07
	40	24.98	30.67	19.76	20.35	16.27	19.63	1.00	1.12	0.16	0.14
	60	27.91	30.88	20.08	20.68	17.10	19.86	1.36	1.48	0.13	0.23
	80	24.60	25.67	16.85	19.75	15.16	17.46	1.47	1.55	0.25	0.26
5000	0	24.73	28.49	17.65	20.06	15.88	18.52	1.21	1.43	0.28	0.20
	20	30.47	31.36	20.12	20.92	17.40	19.12	0.84	1.06	0.17	0.12
	40	34.24	33.41	23.20	21.79	18.58	20.86	1.02	1.19	0.19	0.16
	60	34.85	34.80	26.12	24.84	20.37	21.72	1.54	1.57	0.21	0.29
	80	32.15	31.52	20.47	21.22	17.10	20.09	1.65	1.70	0.31	0.33
7000	0	33.96	33.07	20.68	21.46	17.69	20.38	1.63	1.60	0.33	0.32
	20	34.38	33.63	23.79	23.41	21.06	23.19	1.08	1.22	0.20	0.19
	40	36.44	34.87	26.43	25.27	21.24	23.76	1.15	1.27	0.24	0.24
	60	36.78	35.98	28.71	25.39	21.79	24.85	1.70	1.75	0.29	0.37
	80	34.79	34.26	25.21	24.80	20.11	21.44	1.77	1.82	0.35	0.38
LSD 0.05		**2.53**	**2.19**	**1.74**	**1.68**	**1.70**	**1.82**	**0.08**	**0.07**	**0.06**	**0.06**

The impact of applying H_2O_2 externally, without any salinity treatment, on the peroxidase activity expression is illustrated in Figures (5 A, B). The concentration of H_2O_2 had a gradual effect on the increase rate. The activity of POD was enhanced when seedlings treated with 20 mM H_2O_2, resulting in the production of two moderate bands and one faint band. Subsequently, at 40 mM H_2O_2, the enzyme activity increased at a faster rate, yielding one moderate band and three faint bands. Similarly, seedlings treated with 60 mM H_2O_2 exhibited two intermediate bands and three faint bands. The highest rate of enzyme activity was observed when H_2O_2 concentration reached 80 mM, with four intermediate bands and three faint bands. This rate surpassed both the control and other H_2O_2 treatments.

The experiment showed that H_2O_2 has a beneficial effect in mitigating the harmful effects of salinity. It was observed that the enzymatic expression of peroxidase activity increased gradually as the concentration of H_2O_2 increased (20, 40, 60, and 80 mM) under NaCl concentrations of 3000 and 5000 ppm. The plants treated with a sodium chloride (NaCl) concentration of 7000 ppm showed the highest number of bands, a novel band was formed with a retention factor (Rf) of 180 when exposed to H_2O_2 at concentrations of 60 or 80 mM. This band was not detected in any of the other experimental conditions.



A) B)

Figure 5. (A) isozyme banding patterns of peroxidase, (B) diagrammatic representation of the same isozymes

Where, Rf (retention factor), C: 0ppm NaCl+ 0mM H_2O_2; S0H1: 0ppm NaCl+ 20mM H_2O_2; S0H2: 0ppm NaCl+ 20mM H_2O_2; S0H3: 0ppm NaCl+ 40mM H_2O_2; S0H3: 0ppm NaCl+ 60mM H_2O_2; S0H4: 0ppm NaCl+ 80mM H_2O_2; S1: 3000ppm NaCl+ 0mM H_2O_2; S1H1: 3000ppm NaCl+ 20mM H_2O_2; S1H2: 3000ppm NaCl+ 40mM H_2O_2; S1H3: 3000ppm NaCl+ 60mM H_2O_2; S1H4: 3000ppm NaCl+ 80mM H_2O_2; S2: 5000ppm NaCl+ 0mM H_2O_2; S2H1: 5000ppm NaCl+ 20mM H_2O_2; S2H2: 5000ppm NaCl+ 40mM H_2O_2; S2H3: 5000ppm NaCl+ 60mM H_2O_2; S2H4: 5000ppm NaCl+ 80mM H_2O_2; S3: 7000ppm NaCl+ 0mM H_2O_2; S3H1: 7000ppm NaCl+ 20mM H_2O_2; S3H2: 7000ppm NaCl+ 40mM H_2O_2; S3H3: 7000ppm NaCl+ 60mM H_2O_2; S3H4: 7000ppm NaCl+ 80mM H_2O_2.

Osmolytes content

The data presented in Figure (3 H, I) showed that the salinity treatments showed a significant effect ($p \leq 0.05$) on osmolytes content in terms of total sugars and proline content. The seedlings irrigated with saline water at 7000 ppm gave the highest content for both total sugars by 47.47 and 46.30%, and proline content by 70.93 and 47.12% respectively, in both seasons as compared to control plants.

It was observed that H_2O_2 had a significant effect ($p \leq 0.05$) on osmolytes at different levels as shown in Figure (4 H, I), where the content of total sugars increased at the rate of 60 mM giving values 18.52 and 20.76 mg. g^{-1} F.W., respectively, in both seasons, whilst proline content increased at the rate 80 mM giving values 1.58 and 1.64 mg. g^{-1} F.W., respectively, in both seasons.

Osmolytes content were raised by the interaction between salinity and H_2O_2 (Table 6), where irrigating seedlings with saline water at 7000 ppm and exposed to H_2O_2 at 60 mM increased the content of total sugars producing values 21.79 and 24.85 mg. g^{-1} F.W., respectively, in both seasons. While the production and accumulation of proline content was increased by treating with salinity at 7000 ppm and exposure to H_2O_2 at 80 mM giving values of 1.77 and 1.82 mg. g^{-1} F.W., respectively, in both seasons.

Lipid peroxidation (MDA)

The data presented in Figure 3J showed a significant rising ($p \leq 0.05$) in MDA content parallel to increasing salinity concentration. The seedlings irrigated with saline water at 7000 ppm gave the highest

content with values of 0.28 and 0.30 μM. g^{-1} F.W., respectively, in 2022 and 2023 seasons as compared to control plants.

It was noticed that H_2O_2 had a significant effect ($p \leq 0.05$) on MDA content at different levels as shown in Figure 4J, where the highest content of MDA (0.27 and 0.29 μM. g^{-1} F.W., respectively, in both seasons) was produced in seedlings treated with H_2O_2 at rate 80mM.

Regarding to the interaction (Table 6), it was found that the highest content of MDA was produced in seedlings irrigated with saline water at concentration of 7000 ppm and treated with H_2O_2 at rate of 80 mM giving values 0.35 and 0.38 μM. g^{-1} F.W., respectively, in both seasons.

Correlation analysis

The obtained results from correlation analysis (Tables 7, 8, 9, 10) revealed that there is a positive correlation between all growth parameters and chlorophyll a and b, but the negative correlation was found between growth parameters and the other chemical component including the content of carotenoids, flavonoids, phenols, total sugars, proline and MDA under salinity stress conditions. Likewise, under H_2O_2 treatments, a remarkable positive correlation was noticed between growth parameters and chlorophyll a and b, but it was low positive correlation between growth parameters and total sugars content. Also, a negative correlation was found between growth parameters and the rest of chemical components during 2022 and 2023 seasons.

Table 7. Correlation analysis of vegetative growth parameters and biochemical compositions of *T. distichum* seedlings irrigated with saline water during 2022 season

	Plant height	Stem diameter	No. of branches	Root length	Leaves F.W.	Leaves D.W.	Stems F.W.	Stems D.W.	Roots F.W.	Roots D.W.	SRI	Chl.a	Ch.b	Carotenoids	Flavonoids	Phenols	Total sugars	Proline	MDA
Plant height	1																		
Stem diameter	0.994	1																	
No. of branches	0.999	0.992	1																
Root length	0.996	0.998	0.995	1															
Leaves F.W.	0.993	0.994	0.994	0.998	1														
Leaves D.W.	0.991	0.993	0.992	0.998	0.999	1													
Stems F.W.	0.996	0.999	0.995	0.999	0.997	0.996	1												
Stems D.W.	0.996	0.998	0.995	0.999	0.998	0.997	0.999	1											
Roots F.W.	0.999	0.997	0.997	0.997	0.994	0.992	0.998	0.998	1										
Roots D.W.	0.995	0.998	0.995	0.999	0.998	0.997	0.999	0.999	0.997	1									
SRI	0.995	0.998	0.995	1	0.998	0.998	0.999	0.999	0.997	0.999	1								
Chl.a	0.995	0.999	0.994	0.999	0.996	0.994	0.999	0.999	0.998	0.999	0.999	1							
Ch.b	0.995	0.999	0.994	0.999	0.996	0.995	0.999	0.999	0.998	0.999	0.999	0.999	1						
Carotenoids	-0.986	-0.990	-0.988	-0.996	-0.998	-0.999	-0.993	-0.995	-0.987	-0.995	-0.996	-0.992	-0.993	1					
Flavonoids	-0.978	-0.992	-0.977	-0.992	-0.992	-0.993	-0.991	-0.992	-0.984	-0.992	-0.992	-0.991	-0.992	0.995	1				
Phenols	-0.991	-0.996	-0.992	-0.999	-0.999	-0.999	-0.998	-0.998	-0.994	-0.998	-0.999	-0.997	-0.997	0.998	0.995	1			
Total sugars	-0.995	-0.995	-0.996	-0.999	-0.999	-0.999	-0.998	-0.998	-0.995	-0.998	-0.999	-0.996	-0.997	0.997	0.990	0.999	1		
Proline	-0.989	-0.987	-0.992	-0.994	-0.998	-0.998	-0.992	-0.993	-0.988	-0.994	-0.994	-0.989	-0.991	0.998	0.987	0.996	0.997	1	
MDA	-0.979	-0.984	-0.982	-0.991	-0.996	-0.997	-0.988	-0.990	-0.981	-0.991	-0.991	-0.986	-0.987	0.999	0.992	0.995	0.994	0.997	1

Table 8. Correlation analysis of vegetative growth parameters and biochemical compositions of *T. distichum* seedlings irrigated with saline water during 2023 season

	Plant height	Stem diameter	No. of branches	Root length	Leaves F.W.	Leaves D.W.	Stems F.W.	Stem D.W.	Roots F.W.	Roots D.W.	SRI	Chl.a	Ch.b	Carotenoids	Flavonoids	Phenols	Sugars	Proline	MDA
Plant height	1																		
Stem diameter	0.998	1																	
No. of branches	0.983	0.981	1																
Root length	0.997	0.995	0.993	1															
Leaves F.W.	0.995	0.994	0.962	0.987	1														
Leaves D.W.	0.988	0.992	0.993	0.994	0.974	1													
Stems F.W.	0.996	0.998	0.990	0.998	0.987	0.997	1												
Stem D.W.	0.994	0.996	0.991	0.996	0.983	0.999	0.999	1											
Roots F.W.	0.978	0.985	0.985	0.984	0.963	0.997	0.991	0.994	1										
Roots D.W.	0.974	0.982	0.983	0.980	0.958	0.995	0.989	0.992	0.999	1									
SRI	0.997	0.995	0.993	1	0.987	0.994	0.998	0.996	0.984	0.980	1								
Chl.a	0.997	0.999	0.988	0.998	0.990	0.996	0.999	0.999	0.989	0.986	0.998	1							
Ch.b	0.992	0.997	0.985	0.993	0.984	0.997	0.998	0.999	0.995	0.993	0.993	0.998	1						
Carotenoids	-0.984	-0.988	-0.993	-0.991	-0.968	-0.999	-0.995	-0.997	-0.998	-0.997	-0.991	-0.993	-0.996	1					
Flavonoids	-0.923	-0.937	-0.955	-0.938	-0.897	-0.969	-0.951	-0.959	-0.982	-0.986	-0.938	-0.946	-0.960	0.975	1				
Phenols	-0.942	-0.950	-0.976	-0.958	-0.914	-0.981	-0.965	-0.971	-0.987	-0.990	-0.958	-0.960	-0.970	0.986	0.995	1			
Sugars	-0.970	-0.974	-0.994	-0.982	-0.947	-0.994	-0.985	-0.989	-0.993	-0.993	-0.982	-0.982	-0.985	0.996	0.981	0.994	1		
Proline	-0.960	-0.969	-0.980	-0.970	-0.939	-0.990	-0.979	-0.984	-0.996	-0.998	-0.970	-0.976	-0.984	0.994	0.993	0.996	0.994	1	
MDA	-0.983	-0.984	-0.998	-0.992	-0.963	-0.997	-0.993	-0.994	-0.993	-0.991	-0.992	-0.990	-0.990	0.997	0.968	0.984	0.997	0.989	1

Table 9. Correlation analysis of vegetative growth parameters and biochemical compositions of *T. distichum* seedlings treated with H_2O_2 during 2022 season

	Plant height	Stem diameter	No. of branches	Root length	Leaves F.W.	Leaves D.W.	Stems F.W.	Stem D.W.	Roots F.W.	Roots D.W.	SRI	Chl.a	Ch.b	Carotenoids	Flavonoids	Phenols	Sugars	Proline	MDA
Plant height	1																		
Stem diameter	0.989	1																	
No. of branches	0.997	0.993	1																
Root length	0.997	0.993	1	1															
Leaves F.W.	0.991	0.968	0.977	0.977	1														
Leaves D.W.	0.991	0.967	0.977	0.977	0.999	1													
Stems F.W.	0.987	0.974	0.973	0.973	0.996	0.994	1												
Stem D.W.	0.988	0.974	0.973	0.973	0.996	0.996	0.999	1											
Roots F.W.	0.876	0.938	0.891	0.891	0.838	0.833	0.871	0.868	1										
Roots D.W.	0.841	0.909	0.852	0.852	0.812	0.806	0.852	0.848	0.993	1									
SRI	0.810	0.876	0.814	0.814	0.792	0.786	0.836	0.832	0.969	0.989	1								
Chl.a	0.966	0.981	0.975	0.975	0.936	0.932	0.943	0.941	0.923	0.883	0.827	1							
Ch.b	0.979	0.996	0.989	0.989	0.949	0.947	0.955	0.954	0.942	0.907	0.863	0.991	1						
Carotenoids	-0.181	-0.185	-0.223	-0.223	-0.110	-0.126	-0.067	-0.077	-0.139	-0.097	-0.102	-0.088	-0.190	1					
Flavonoids	-0.057	-0.086	-0.115	-0.115	0.037	0.023	0.073	0.065	-0.097	-0.050	-0.036	-0.024	-0.111	0.967	1				
Phenols	-0.108	-0.123	-0.158	-0.158	-0.026	-0.042	0.014	0.005	-0.103	-0.059	-0.057	-0.041	-0.137	0.993	0.991	1			
Sugars	0.151	0.113	0.100	0.100	0.231	0.218	0.257	0.249	0.053	0.074	0.056	0.199	0.099	0.924	0.960	0.952	1		
Proline	-0.938	-0.969	-0.955	-0.955	-0.896	-0.897	-0.903	-0.904	-0.949	-0.921	-0.895	-0.930	-0.969	0.382	0.301	0.331	0.122	1	
MDA	-0.927	-0.907	-0.915	-0.915	-0.932	-0.927	-0.934	-0.932	-0.786	-0.749	-0.694	-0.943	-0.907	-0.162	-0.254	-0.223	-0.478	0.779	1

Table 10. Correlation analysis of vegetative growth parameters and biochemical compositions of *T. distichum* seedlings treated with H_2O_2 during 2023 season

	Plant height	Stem diameter	No. of branches	Root length	Leaves F.W.	Leaves D.W.	Stems F.W.	Stem D.W.	Roots F.W.	Roots D.W.	SRI	Chl.a	Ch.b	Carotenoids	Flavonoids	Phenols	Sugars	Proline	MDA
Plant height	1																		
Stem diameter	0.999	1																	
No. of branches	0.937	0.937	1																
Root length	0.889	0.879	0.789	1															
Leaves F.W.	0.963	0.967	0.956	0.830	1														
Leaves D.W.	0.958	0.963	0.954	0.816	0.999	1													
Stems F.W.	0.969	0.974	0.953	0.819	0.998	0.998	1												
Stem D.W.	0.964	0.969	0.953	0.810	0.999	0.999	0.999	1											
Roots F.W.	0.930	0.923	0.833	0.994	0.880	0.868	0.872	0.865	1										
Roots D.W.	0.926	0.918	0.828	0.995	0.875	0.863	0.867	0.859	0.999	1									
SRI	0.889	0.879	0.790	1	0.830	0.816	0.819	0.810	0.994	0.995	1								
Chl.a	0.998	0.997	0.917	0.903	0.951	0.945	0.958	0.952	0.941	0.938	0.903	1							
Ch.b	0.995	0.997	0.920	0.865	0.971	0.968	0.979	0.975	0.913	0.908	0.865	0.993	1						
Carotenoids	-0.092	-0.105	0.203	-0.041	-0.065	-0.075	-0.095	-0.091	-0.079	-0.078	-0.042	-0.124	-0.172	1					
Flavonoids	0.042	0.030	0.339	0.095	0.089	0.079	0.056	0.060	0.062	0.063	0.095	0.009	-0.035	0.985	1				
Phenols	-0.062	-0.070	0.267	-0.065	0.018	0.013	-0.013	-0.006	-0.087	-0.088	-0.066	-0.103	-0.128	0.976	0.980	1			
Sugars	0.115	0.106	0.424	0.099	0.174	0.167	0.145	0.150	0.083	0.082	0.099	0.076	0.045	0.965	0.989	0.983	1		
Proline	-0.968	-0.971	-0.852	-0.892	-0.949	-0.947	-0.955	-0.951	-0.933	-0.930	-0.892	-0.974	-0.982	0.299	0.154	0.255	0.089	1	
MDA	-0.965	-0.967	-0.830	-0.888	-0.930	-0.926	-0.938	-0.933	-0.929	-0.926	-0.888	-0.974	-0.979	0.328	0.190	0.296	0.127	0.997	1

Discussion

The results obtained of the data recorded in the attached figures showed that salinity had a clear negative effect on all the mentioned vegetative traits, and these results were consistent with the results of some studies conducted by Salem *et al*. (2014), Alharbi and Alaklabi (2022), Akram *et al*. (2024) on *Carthamus tinctorius*, wheat plant, *Azadirachta indica*, respectively. Jabeen and Ahmed (2009), Misra and Srivastava (2000) have explained this clear decline in growth that occurs to the plants when irrigated with saline water, as it may be due to repeated irrigation with saline water which causes a significant accumulation of toxic ions in the soil, leading to an increase in the osmotic pressure of the soil, leading to a decrease the ability of the roots to draw water from the soil. This, in turn, leads to a decrease in the water content and the inability of the roots to compensate for the lost water. Consequently, the cells lose their swelling, halt their expansion and extension, and ultimately result in a decrease in plant growth, as demonstrated by the results of this study. In addition, the anatomical study conducted by Kilic and Kahraman (2016) on barley plants explained the decrease in the biomass of the leaves, as he stated that exposing the plants to grow in a salty environment led to a decrease in the number of stomata on the lower surface by 57% and on the upper surface by 45%, and consequently the shrinkage of the leaves. Moreover, salinity slows down shoot growth by limiting leaf initiation, elongation, and internode growth. It also speeds up leaf breakdown, which causes gymnosperms to shed their leaves early (Kozlowski, 1997).

The attached data show that as salt levels rise, the SRI (%) value decreases until the salinity reaches its peak concentration (7000 ppm). According to the equation used to create the SRI (%), which suggests a loss in root growth, a drop in mass, and an inability to endure salt, this is comparable with that reported by Mohammed *et al*. (2019) on the *Paspalum vaginatum* plant.

In the current investigation, the chlorophyll a and b content were decreased reversely by increasing salt level which is in line with the results of studies conducted by Akram *et al*. (2024), Alharbi and Alaklabi (2022). Various studies have attributed the cause of breaking and collapsing of chloroplasts, which causes the reduction in Chl a, b content; or accelerating the degradation of chlorophyll enzyme due to increasing salinity concentration (Pompelli *et al*., 2022). The obtained results showed that the carotenoids value increased in direct proportion to the increase in salinity concentration, and this result was consistent with the findings of Leiva-Ampuero *et al*. (2020). Carotenoids are lipid-soluble pigments found in the membranes of chloroplasts and chromoplasts. These pigments are important structural components of the aerial part, acting as auxiliary light-collecting pigments and photoprotectors of the photosynthetic apparatus against excessive light (Frank and Cogdell, 1996; Havaux, 1998). Variations in carotenoids content in response to salinity in some plants may be related to the plant's ability to cope with stress and oxidative damage. Salinity damages the photosynthetic machinery (Demmig-Adams and Adams, 1992), and as a result, reactive oxygen species (ROS) are produced as a stress byproduct. Consequently, plants can counteract the damage by synthesizing antioxidant molecules such as carotenoids, which serve as a non-enzymatic method for ROS detoxification (Havaux and Niyogi, 1999; Abogadallah, 2010). This inverse relationship, caused by a decrease in chlorophyll content and an increase in carotenoids content, explains why the value of the ratio between carotenoids and chlorophyll increases directly with salinity, indicating the plant's ability to resist oxidative stress.

Some researchers demonstrated that elevating salinity levels increased phenolic compounds content as a response to oxidative stress caused by ROS generation in antagonistic environmental conditions (Ksouri *et al*., 2008). These chemicals increase plant defence against harmful effects (Sonar *et al*., 2011). This could be attributed to the increase in enzymes activity occurring under salinity conditions, resulting in the synthesis of phenol and flavonoid molecules (Haghighi *et al*., 2012; Zhou *et al*., 2014; Tian *et al*., 2015; Noctor *et al*., 2015). The negative impact of salt on seedling growth parameters is caused by the decreased activity of antioxidant enzymes such as peroxidase (POD) when NaCl reaches toxic levels in the environment, triggering reactive

oxygen species (ROS), in agreement with Dash and Panda (2001). The effect of salt on seedling growth stages was dependent on the concentration of the salt treatment.

Osmolytes play an important role in maintaining the water level inside plant cells and swelling them. Among these osmolytes are total sugars and proline, whose concentration increases with salinity stress in an attempt by the plant to maintain growth as revealed in the current study. Sugars and other carbs build during salt stress. These sugars play an important function in salt stress mitigation through osmoprotection and ROS scavenging (Parida *et al*., 2004). In addition to their role in osmoregulation, soluble sugars provide energy and protect the photosynthetic system through Na+ sequencing compounds (Rahneshan *et al*., 2018; Karimi *et al*., 2018); the results obtained by Behzadi Rad *et al*. (2021) are consistent with the results of our current study regarding total sugars. The obtained results during this study showed that proline content increased by increasing salt stress level which in accordance with the results obtained by Pingle *et al*. (2022) and Miranda *et al*. (2014). When exposed to salt stress, many plants demonstrate proline accumulation, a basic defense mechanism to maintain osmotic pressure in a cell where proline amino acid is thought to be a source of energy, carbon, and nitrogen for plant tissue regeneration during salt stress, proline enhances salinity resistance through cell osmotic adjustment, membrane stabilization, and detoxification of damaged ions in plants exposed to salt stress (Hare *et al*., 1999; Slama *et al*., 2007). Additional role of proline; it is considered a signal to manufacture chemical molecules that are responsible for salt tolerance (Paliwal *et al*., 2017).

It was found through our study that the seedlings not exposed to salinity stress showed the lowest content of lipid peroxidation (MDA), while its content gradually increased with increasing salinity concentration, and the highest content appeared at the concentration 7000 ppm, as previously mentioned in the results; this is because MDA is believed to originate under stress conditions and has a high capability of reacting with multiple biomolecules such as proteins or DNA that leads to the formation of adducts. Excessive MDA production has been associated with different stress conditions (Ayala *et al*., 2014). Moreover, under physiological or low lipid peroxidation rates, the cells stimulate their maintenance and survival through constitutive antioxidants defence systems or signalling pathways activation that up-regulates antioxidants proteins resulting in an adaptive stress response (Lukatkin *et al*., 2020). Under medium or high lipid peroxidation rates, which occur during high stress conditions, the extent of oxidative damage overwhelms repair capacity, and the cells induce apoptosis or necrosis-programmed cell death (Ayala *et al*., 2014). Havugimana *et al*. (2023) mentioned similar results on different amaranth cultivars.

H_2O_2 is an important signalling molecule because it has the potential to regulate essential plant processes (Zlotek *et al*., 2014; Liu *et al*., 2019). Our current study found that using H_2O_2 at low concentrations (20 and 40 mM) improved all vegetative traits of *T. distichum* seedlings, increased the plant's ability to resist salinity (SRI%), and reduced the plant's induction to produce proline and MDA, on the other side, it increased the production of photosynthetic pigments (chlorophyll a and b), while antioxidant compounds (carotenoids, flavonoids, and phenols) decreased at a concentration of 60 mM under salinity stress condition. The findings of this investigation were consistent with those of earlier studies conducted and approved by Wang *et al*. (2023), Ferriz-Martinez *et al*. (2023), Barbosa *et al*. (2023) and Delis-Hechavarria *et al*. (2021).

Wang *et al*. (2023) reported that the level of chlorophyll a and b in lettuce plants increase, along with improving gas exchange, net photosynthetic, stomatal conductance, and water use efficiency. Asgher *et al*. (2021) and Nazir *et al*. (2019) attributed this to the ability of H_2O_2 to stimulate the activity of Rubisco, the Calvin cycle, and sugar metabolism, thus increasing the efficiency of photosynthesis, which results in improving plant growth. The carotenoids are small molecule antioxidants widely exist in many plants (Kim *et al*., 2016). Our results showed that H_2O_2 elicitation significantly increased the carotenoids in *T. distichum* seedlings.

Plants rely heavily on phenolic chemicals, such as flavonoids and phenolic acids (Wang *et al*., 2020; Rashmi and Negi, 2020). These compounds have been demonstrated to have high antioxidant activity due to their chemical structure, which consists of many hydroxyl groups attached to the benzene ring (Wang *et al*.,

2020; Daryanavard *et al*., 2023). Our investigation found that when H_2O_2 was utilized, these phenolic compounds rose, which may explain why *T. distichum* seedlings were able to tolerate stress caused by irrigation with saline water at various concentrations. This study supports the findings of prior investigations by Nurnaeimah *et al*. (2020) and Valizade *et al*. (2022). Many studies have demonstrated that the accumulation of phenolic compounds is vital in protecting plants from oxidative damage because H_2O_2 signals trigger genes responsible for phenylpropanoids biosynthesis and thus increasing phenolic compounds production and accumulation (Sun *et al*., 2021).

According to Brunetti *et al*. (2013), phenylpropanoid metabolism is strongly related to carbohydrate metabolism via shikimate pathway, with phenylpropanoid production requiring a wide range of carbon sources. This explains the findings of our current study, which showed a strong correlation where a rise in phenolic compounds corresponds to an increase in carbohydrates, which improves carbon supply when *T. distichum* seedlings are treated with H_2O_2. Hydrogen peroxide, which is abundant in apoplasts near the cell membrane, acts as a powerful signalling molecule in a variety of physiological processes and may engage in signal transmission due to its role in redox signalling. H_2O_2, generated by NADPH oxidase, which is encoded by RBOH genes, serves as a signal under abiotic stressors, enhancing plant stress resistance. The antioxidant enzyme system and non-enzymatic scavengers maintain the balance of reactive oxygen species (ROS) and control metabolic activity within cells. H_2O_2 especially generated by NADPH oxidase expressed by the RBOH gene, functions in reaction. We found that using H_2O_2 at lower concentrations was effective; this stimulating effect of H_2O_2 reveals that it can avoid or mitigate the negative effects of stress on plant growth, where POD plays a key role in the generation of the H_2O_2 signal and the redox steady state (Kapoor *et al*., 2015; Mohamed *et al*., 2023)

Conclusions

Salinity is one of the environmental challenges facing the world and affects the growth and productivity of plants, which leads to a decrease in the benefit that humans get from plants. The current study found that exposing *T. distichum* seedlings to irrigation with salt water at different concentrations (3000, 5000, 7000 ppm) led to a decrease in the values of all morphological traits and photosynthetic pigments, which reflects the toxic effects of salinity on the plant. On the contrary, it was found that the increase in the values of antioxidant compounds, whether enzymatic or non-enzymatic, an increase in oxidation indicators such as lipid peroxide, and an increase in osmolytes such as proline and sugars is an attempt by the plant to maintain its balance under salinity stress. When plants affected by salinity were treated with H_2O_2 as an elicitation substance at different concentrations, especially the concentration of 20 mM, which showed the best result for all the mentioned traits, this demonstrates the ability of H_2O_2 to prepare the plant for early defence without affecting growth traits.

Authors' Contributions

Conceptualization, S.M.E, A.S.M and A.A.M.M; Data curation, S.M.E, Y.C. and A.S.M; Formal analysis, A.S.M; and S.M.E. Funding acquisition, Y.C.; Methodology, S.M.E, and A.S.M. Re-sources, S.M.E, and A.S.M; Supervision, S.M.E, Y.C. and A.A.M.M Writing – original draft, S.M.E, Y.C. and A.A.M.M; Writing – review & editing, S.M.E, Y.C.; A.S.M and A.A.M.M. All authors have read and approved the final manuscript

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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