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Impacts of Sodium Nitroprusside on Morphological, Biochemical, and Anatomical Features of *In vitro* Propagated *Khaya senegalensis* Desr A. Juss under Salt Stress

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Abstract

Salinity interferes with plant morphological and biochemical functions, which eventually results in serious losses. *In vitro* shooting and rooting abilities of *Khaya senegalensis* Desr A. Juss were examined under four levels of salinity stress (0, 1000, 3000, and 5000 ppm), and treated by sodium nitroprusside (SNP) at 0.0, 1.5, 3.0 and 6.0 mg l^{-1} to alleviate the adverse effect of salt stress. Using SNP in the culture media under salinity levels attained to 3000 ppm had a stimulation impact on *in vitro* grown and survived plants, shootlets number, length, and rooting percent. Increasing SNP level to 6 mg l^{-1} in the salinized culture media at all tested levels (0-5000 ppm) enhanced photosynthetic pigments content as well as the estimated minerals (N, P, K, Ca%, and K+/Na+ ratio), while had an inhibitory effect on Na and Cl%, total phenols and proline under the same salinity level. Leaf and stem transverse sections were showed that salt stress induced an adverse effect on their anatomical traits, however SNP at 6.0 mg l^{-1} mitigated the harmful effects of salinity stress on histological characteristics of leaf i.e. thickness of leaf midvein & lamina, xylem & phloem tissues, xylem vessels diameter, palisade and spongy tissue thickness, as well as epidermis, cortex, fibers, phloem and xylem tissues thickness for stem structure

Keywords: African mahogany; micropropagation; sodium nitroprusside; salinity; biochemistry

1. Introduction

African mahogany (*Khaya senegalensis* Desr A. Juss.) is known as a tree species of the genus Khaya, belonging to the Meliaceae family native to Africa. It is a woody and evergreen ornamental tree suitable for wood production in agroforestry systems or mixed species plantations. Thus, the production generates economic returns, due to its durable, hard timber, which is sought for construction, luxury furniture, interior finishes, and musical instruments with high commercial value, at the same time it brings food security and greater financial stability to the farmers [1]. *K. senegalensis* has medicinal and nutritional properties, used to treat various diseases and as an alternative in livestock feed [2]. It has been reported as anti-cancer, anti-lipidemic, antibacterial, antifungal, anti-plasmodial, anti-diabetic, antioxidant, antitrypanosomal, nematicidal, and has antiflarial activities [3]. Furthermore, the extracted *K. senegalensis* seeds oil possessed a high free fatty acid value that was used as a projected green energy biodiesel production [4].

Salinity is a substantial abiotic factor due to its significance in the multiple physiological processes of plant. Egypt has about 0.9 million hectares (~25%) of the total irrigated cultivable croplands suffering from salinization problems [5]. Thus, in growing media affected by salinity, more than one approach should be adopted to reduce the effects of salinity and improve plant growth and its productivity.

Sodium nitroprusside (SNP) is a nitric oxide (NO) donor due to its ability to provide an ongoing supply of NO, that considered a reactive nitrogen species and is essential for plant physiological functions as well as it has defensive responses

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against stress [6]. Nitric oxide is a multitasking signaling molecule and is involved in the stress-acclimation processes of plants [7]. Under abiotic stresses, SNP can enhance antioxidant activity, reduce ROS-induced cytotoxic activities such as electrolyte or ion leakage, DNA fragmentation, and inhibit cell death [8]. SNP can be used as a stimulator to promote multiple shoot production, plant development, photosynthesis, stomatal movement, and recovery of cell membrane [9, 10].Therefore, the objective of this investigation was to assess the effects of SNP complementation on the morphological, biochemical, and anatomical responses of stressed *in vitro K. senegalensis* plant.

2. Materials and Methods

2.1. Plant material

The present study was conducted at the Tissue Culture Technique Laboratory, Department of Ornamental Plants and Woody Trees, Central Laboratories, National Research Centre (NRC), Egypt, during the years 2023 and 2024. Seeds were harvested from *K. senegalensis* trees maintained at the National Research Centre farm for research and production in Al-Emam Malek village, Al-Nubaria region, Al-Behira Governorate, Egypt.

2.2. Procedure layout

2.2.1. Surface sterilization

Under aseptic conditions in a laminar air flow hood, healthy uniform un-coated seeds of K. senegalensis were disinfested by soaking in 70% (v/v) ethanol for 30 sec, followed by commercial sodium hypochlorite solution 15% then washed three times with sterile autoclaved distilled water. The sterilized seeds were left to germinate on MS basal medium + sucrose at 2.5% (w/v) + agar at 0.7% (w/v) which was adjusted to 5.7 ± 0.2 pH medium, and incubated in the dark until germination.

Culture medium and incubation condition

The epicotyls (~1 cm length) of four weeks old seedlings were excised and cultured for one month on a MS basal medium modified with De Fossard medium vitamins [11] (Nicotic acid 4.9 mg l^{-1} + Calcium pantothenate 0.88 mg l^{-1} + riboflavin 3.6 mg l^{-1} + Ascorbic acid 1.8 mg l^{-1} + choline chloride 1.4 mg l^{-1}+L-cystatin14.5 mg l^{-1}) and supplemented with 25 g l^{-1} sucrose, 7g l^{-1} agar for one month.

For *in vitro* shootlets proliferation, the nodal explants of regenerated shoots from previous step were cultured on MS medium modified with De Fossard medium vitamins which supplemented with 1 mg l^{-1} of BA + 0.05 mg l^{-1} of IBA at the presence of 2gl^{-1} activated charcoal as was described by Abdel-Magied [12]

For testing *in vitro* shooting and rooting abilities under salinity stress, *K. senegalensis* explants were cultured on media included sodium chloride at four levels (0, 1000, 3000, and 5000 ppm), and treated with four concentrations of sodium nitroprusside at 0.0, 1.5, 3.0 and 6.0 mgl^{-1} to mitigate the adverse effect of salt stress. Cultures were incubated at 25±2°C under photoperiod 16h of fluorescent light with 30µmol m^2sec^{-1}, on a growth chamber.

Data of *in vitro* micropropagation ability under salinity stress were recorded after three months which were represented in the survived explants (%), shootlets number/explant, shootlets length (mm), leaves number/shootlet as well as the *in vitro* root growth criteria (rooting percentage, roots number/shootlets, and root length mm).

2.2.2. Biochemical analysis

Photosynthetic pigments: chlorophylls a, b, and total carotenoids were determinate according to Saric [13].

Determination of some mineral elements: Nitrogen, calcium, sodium, and chloride% were determined according to Cottenie [14], phosphorus% was estimated according to Snell and Snell [15] and potassium% according to Chapman and Pratt [16].

Shootlets extraction: 5g of fresh shootlets were soaked in 50 ml of 80% ethanol, and were shaken for 48h at room temperature, and then filtered and extracted twice.

Total phenols content: The final extract was used for the assay of the total phenols using Folin–Ciocalteu's reagent, according to Singleton and Rossi [17].

Determination of proline content: Proline content was determined by Bates[18].

Microscopic measurements: *In vitro* samples of *K. senergalensis* leaves and stems were taken for anatomical study from the stem middle internode. Samples were killed and fixed in FAA (10 ml formalin, 5 ml glacial acetic acid, 50 ml ethyl alcohol (95%), and 35 ml distilled water) for at least 48h. Samples were dehydrated in n-butyl series and embedded in paraffin wax. 15µ thickness cross sections were cut, stained in crystal violet-erythrosine combination, and mounted in Canada balsam according to Nassar and El-Sahhar [19].

Statistical analysis: The treatments' means were compared for significance by Duncan's New Multiple Range test [20] at a 5% level of probability using the MSTAT Computer Program (MSTAT Development Team) [21]. Analysis of variance was estimated as two factorials in a completely randomized block design. The data were statistically analyzed according to Steel and Torrie (1980) [22].

3. Results and Dissection

3.1. In vitro growth capacity

Results in Table (1) and Fig.(1) illustrate that increasing salinity level in the culture media had an inhibition effect on multiple shoots of *K. seneglansis*, which caused the lowest one (56.25) at the highest used level (5000 ppm) in comparison with control. This inhibition effect of high salinity level was also noticed on the formed roots (50% rooting, 1.5 for root number, and 41.25 mm for root length).

Meanwhile, *in vitro* shoots could be grown and highly promoted when the explants were introduced in MS culture medium supplemented with sodium nitroprusside (SNP) and attained to the greatest survival (100%), shootlets number (1.72), shootlets length (78.72 mm), rooting percentage (89.44%), roots number (2.28), and length (72.08 mm) at the maximum used concentration (6 mg l^{-1}) of SNP. The *in vitro* growth capacity could be induced when SNP interacted with the saline condition at most used concentrations that attained to 3000 ppm, caused 100% survival, 1.89 shootlets number, 80.00 mm shootlets length, and 80 % rooting.

In plant tissue culture protocols, use of SNP results in an increment in shoot and root formation, enhancing micropropagation success [23]. In this study, *in vitro* plant growth was negatively impacted by salt stress on both its shooting and rooting abilities. Our findings are consistent with other investigations indicated that stressed *in vitro* plants experience reduced plant growth features [24]; however, SNP supplementation significantly increases the growth [25]. SNP is influential in the signal transmission of cytokinin, thereby regulates cell division, elongation, and boosts the micropropagation [26]. SNP can alleviate the unfavorable effects of stress [27]. This effect of SNP on *in vitro* grown explants may be attributed to the positive effect on the cell wall constituents that may cause wall relax, membrane fluidity to increase, and results in trigger cell, cell expansion, wall loosening, ultimately induces plant growth [28]. It was mentioned that nitric oxide changed the cell wall through a reaction with phospholipids, which eventually allowed plants to grow again in stressful conditions [29]. The stimulation effect of SNP explained that it derived NO has been proposed as a new phytohormone [30], and has been applied to the creation of different plant tissue culture procedures [31].

The micropropagated *K. senegalensis* plants were successfully acclimatized on sand and peat moss (1: 1 v/v) and survived with 83%.

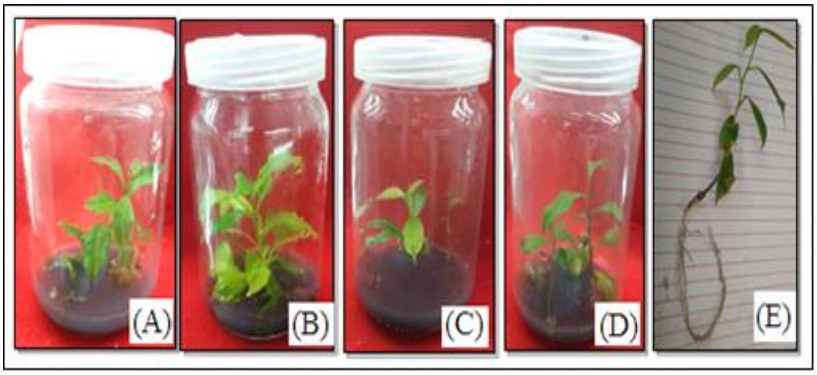


Fig.1 (A-E): In vitro shooting and rooting ability of Khaya senegalensis Desr A. Juss (a) Shootlets development that were cultured on MS+0ppm NaCl+0 mgl-1 SNP (The control) , (b) Shootlets treated with 6 mgl-1 SNP, (c) Shootlets grown under salinity conditions at 5000ppm NaCl, (d) Shootlets grown under 5000ppm NaCl+6 mgl-1 SNP, and (e) Rooted plantlet for acclimatization.

Table 1. *In vitro* growth capacity of *K. senegalensis* under various concentrations of salinity (NaCl) and sodium nitroprusside (SNP)

Treatment **A** NaCl (ppm)	Treatment **B**	Survival %	Number of shootlets	Length of shootlets	Number of leaves	Rooting %	Root number	Root length
0	0 mgl^{-1} SNP (The control**)**	100 a	1.00 e	56.67 de	7.67 ef	100.0 a	1.67 bc	51.33 def
	1.5 mgl^{-1} SNP	100a	1.53 bcd	67.77 bcd	12.33 ab	100.0 a	1.67 bc	60.00 cd
	3 mgl^{-1} SNP	100a	1.67 abc	76.67 abc	10.33 cd	100.0 a	2.00 bc	65.00 bc
	6 mgl^{-1} SNP	100a	1.78 ab	83.24 a	13.67 a	100.0 a	2.10 bc	90.00 a
1000	0 mgl^{-1} SNP	100a	1.33 cd	60.00 de	9.00 de	66.67bc	1.67 bc	40.00 fgh
	1.5 mgl^{-1} SNP	100a	1.33 cd	63.33 cde	11.00 bc	66.67bc	1.83 bc	33.33 h
	3 mgl^{-1} SNP	100a	1.56 a-d	80.00 ab	9.67 cd	100.0 a	2.33 bc	73.33 b
	6 mgl^{-1} SNP	100a	1.67 abc	88.30 a	12.00 b	100.0 a	2.67 ab	96.67 a
3000	0 mgl^{-1}SNP	100a	1.22 de	56.67 de	7.33 f	33.33 d	1.33 bc	38.33 fgh
	1.5 mgl^{-1} SNP	100a	1.56 a-d	62.78 cde	9.00 de	66.67 bc	1.33 bc	45.00 e-h
	3 mgl^{-1} SNP	100a	1.78 ab	58.89 de	9.56 cd	77.77 b	3.67 a	50.00 d-g
	6 mgl^{-1} SNP	100a	1.89 a	80.00 ab	11.00 bc	80.00 ab	2.33 bc	55.00 cde
5000	0 mgl^{-1}SNP	100a	1.00 e	51.67 e	7.00 f	33.33 d	1.00 c	36.67 gh
	1.5 mgl^{-1} SNP	100a	1.44 bcd	55.00 de	9.00 de	33.33 d	1.33 bc	40.00 fgh
	3 mgl^{-1} SNP	100a	1.50 bcd	55.00 de	9.00 de	55.56 c	1.67 bc	41.67 e-h
	6 mgl^{-1} SNP	100a	1.56 a-d	63.33 cde	9.67 cd	77.78 b	2.00 bc	46.67 e-h
Mean **A**	0 ppm NaCl	100a	1.49 a	71.08 a	11.00 a	100.0 a	1.86 a	66.58 a
	1000 ppm NaCl	100a	1.47 a	72.91 a	10.42 ab	83.33 b	2.12 a	60.83 b
	3000 ppm NaCl	100a	1.61 a	64.58 b	9.22 bc	64.44 c	2.17 a	47.08 c
	5000 ppm NaCl	100a	1.38 a	56.25 c	8.67c	50.00 d	1.50 b	41.25 d
Mean **B**	0 mgl^{-1} SNP	100a	1.14 c	56.25 c	7.750 c	58.33 b	1.42 b	41.58 c
	1.5 mgl^{-1} SNP	100a	1.46 b	62.22 bc	10.33 b	66.67 b	1.54 b	44.58 c
	3 mgl^{-1} SNP	100a	1.63 a	67.64 b	9.639 b	83.33 a	2.42 a	57.50 b
	6 mgl^{-1} SNP	100a	1.72 a	78.72 a	11.58 a	89.44 a	2.28 a	72.08 a

Different letters in columns show a significant difference between treatments based on Duncan's multiple range test

3.2. Photosynthetic pigments content

The determined pigments content in obtained plants were significantly influenced by salinity stress (Table 2). In comparison with the control treatment, increasing NaCl level gradually declined Chl. a, b, and carotenoids to the minimum contents (31.72, 14.54, and 22.83 mg100 g^{-1} F.W., consecutively) at 5000 ppm of NaCl. From the presented data in Table 2, it could be observed that increasing SNP concentration to 6 mg l^{-1} in the salinized culture media at all tested levels (0-5000ppm) acted as promoters to enhance photosynthetic pigments content. Noticeable increment of Chl. a, b, and carotenoid contents were recorded (64.58, 23.86, and 33.74 mg100 g^{-1} F.W., respectively) when the highest concentration of SNP (6 mg l^{-1}) was added in a non-salinized MS culture medium. The present findings go in line with Li [32] who noticed that the quantity of chlorophyll determines the photosynthetic ability, and salinity stress greatly reduces chlorophylls. Chlorophyll degradation can be caused by an increase in sodium ions, which leads to a decrease in potassium and magnesium ions, which are essential elements in chlorophyll biosynthesis, moreover, the accumulation of ROS in chloroplasts can lead to damage the photosynthetic system by degradation of the chloroplast membrane due to salinity stress [33]. SNP has a protective role on photosynthetic pigments by reducing in salt-induced ROS accumulation and protecting the membranes of the chlorophyll-containing cell organelles [34, 35].

Table 2. Photosynthetic pigments content (mg100 g^{-1} F.W.) of *K. senegalensis* under various concentrations of salinity (NaCl) and sodium nitroprusside (SNP)

Treatment **A** NaCl (ppm)	Treatment **B**	Chlorophyll - a	Chlorophyll – b	Carotenoids
0	0 mgl^{-1} SNP (The control**)**	51.54 e	17.81 d	34.45 bc
	1.5 mgl^{-1} SNP	60.82 c	23.02 b	39.88 a
	3 mgl^{-1} SNP	66.09 b	25.58 a	40.68 a
	6 mgl^{-1} SNP	79.54 a	27.41 a	40.68 a
1000	0 mgl^{-1} SNP	46.29 f	18.83 cd	30.14 d
	1.5 mgl^{-1} SNP	52.67 de	20.34 c	33.12 c
	3 mgl^{-1} SNP	55.33 d	20.98 bc	35.22 b
	6 mgl^{-1} SNP	67.29 b	26.39 a	30.03 d
3000	0 mgl^{-1} SNP	28.62 h	15.02 ef	25.02 f
	1.5 mgl^{-1} SNP	29.00 h	17.35 de	25.77 ef
	3 mgl^{-1} SNP	44.15 f	17.00 de	27.08 e
	6 mgl^{-1} SNP	61.21 c	22.62 b	34.76 bc
5000	0 mgl^{-1} SNP	20.29 j	9.35 g	17.43 g
	1.5 mgl^{-1} SNP	23.29 i	13.03 f	18.47 g
	3 mgl^{-1} SNP	33.00 g	16.78 de	25.95 ef
	6 mgl^{-1} SNP	50.30 e	19.00 cd	29.48 d
Mean **A**	0 ppm NaCl	64.50 a	23.45 a	38.92 a
	1000 ppm NaCl	55.40 b	21.64 b	32.13 b
	3000 ppm NaCl	40.75 c	18.00 c	28.16 c
	5000 ppm NaCl	31.72 d	14.54 d	22.83 d
Mean **B**	0 mgl^{-1} SNP	36.69 d	15.25 d	26.76 d
	1.5 mgl^{-1} SNP	41.44 c	18.43 c	29.31 c
	3 mgl^{-1} SNP	49.64 b	20.08 b	32.23 b
	6 mgl^{-1} SNP	64.58 a	23.86 a	33.74 a

Different letters in columns show a significant difference between treatments based on Duncan's multiple range test

3.3. Minerals concentration

Results in Table (3) revealed a significant decrease in the N, P, K, Ca%, and K^+/Na^+ ratio by increasing NaCl level in the culture media that attained the minimum rates (1.24, 0.21, 1.92, 0.43 %, and 2.05, in order) at highest salinity level (5000 ppm) comparing to the control. However, Na% and Cl% showed opposite trends whereas, the maximum values (1.02 and 4.84 %) were recorded on the same salinity level (5000 ppm). Furthermore, it has been noticed that the above-mentioned minerals (N, P, K, Ca%, and K^+/Na^+ ratio) are directly proportional to SNP level in the culture media. The the salinity causes limiting of P- movement and decreases its adsorption [40]. The positive impacts of SNP treatments on nutrient elements may be best results (2.07, 0.35, 3.45, 0.69%, and 6.30, respectively) were found with the highest tested level of SNP (6 mg l^{-1}), while caused the lowest Na% and Cl% (0.60 and 2.98%, respectively) as compared to the control.

The highest percent of Na and Cl (0.86 and 4.17 %) was obtained in untreated shootlets with SNP. The interaction between the salt stress and SNP showed the efficiency of SNP treatment to reduce the stress, where, using a high level of SNP (6 mg l^{-1}) showed a significant simulative effect on the estimated minerals (N, P, K, Ca%, and K^+/Na^+ ratio) at each salinity level (0.0, 1000, 3000, and 5000 ppm) which had an inhibitory effect on Na and Cl% under the same salinity conditions.

The significant decrease in the contents of N, P, K%, and K^+/Na^+ ratio under saline conditions (Table 3) might be due to decreasing of the nitrate reductase enzyme activity which affected protein synthesis and total nitrogen [36], furthermore, depolarization of the plasma membrane permeability and ionic homeostasis disturbances [37], which negatively affects plant growth and can lead to an imbalance of nutrients, increasing sodium and chlorine and reducing potassium and calcium in plant tissues [38, 39]. In addition, because of its vital role in mitigating high salt in plant tissues, through inhibited Na+ transfer to the shoot by increasing its accumulation in the roots and instead transferred more K+, which significantly induced the K^+/Na^+ ratio [39]. The regulating of K^+/Na^+ in cytoplasm cells relates to the boosted gene expression of vacuolar H+-ATPase, H^+-ATPase in the plasma membrane as well as H^+-PPase activities, thus the Na^+/H^+ antiporter assists compartmentation of Na^+ [41].

Table 3. Effect of various concentrations of salinity (NaCl) and sodium nitroprusside (SNP) on some minerals (%) of *K.senegalensis* shootlets

Treatment A NaCl (ppm)	Treatment **B**	N%	P%	K%	Ca%	Na%	Cl%	K^+/Na^+ ratio
0	0 mgl^{-1} SNP (The control)	1.42 d-g	0.26 ef	2.77 c-f	0.51 de	0.65 fg	3.10 g	4.25 def
	1.5 mgl^{-1} SNP	1.69 b-e	0.34 cd	3.06 bcd	0.58 c	0.61 g	2.74 g	5.00 de
	3 mgl^{-1} SNP	2.10 b	0.40 b	3.71 b	0.70 b	0.50 h	2.20 h	7.38 b
	6 mgl^{-1} SNP	2.86 a	0.49 a	4.54 a	0.92 a	0.46 h	2.00 h	9.98a
1000	0 mgl^{-1} SNP	1.22 e-h	0.22 fgh	2.14 fgh	0.48 def	0.69 fg	3.49 f	3.11 f-i
	1.5 mgl^{-1} SNP	1.31 d-h	0.23 fgh	2.39 d-g	0.50 def	0.63 fg	3.46 f	3.78 efg
	3 mgl^{-1} SNP	1.75 bcd	0.30 de	2.88 cde	0.54 cd	0.54h	3.10g	5.37 cd
	6 mgl^{-1} SNP	1.98 bc	0.38 bc	3.09 bc	0.66 b	0.49 h	2.92g	6.35 bc
3000	0 mgl^{-1}SNP	0.91 h	0.18 hi	1.60 hij	0.51 de	0.83 d	4.44c	1.93 ijk
	1.5 mgl^{-1} SNP	1.15 fgh	0.21 fgh	2.00 ghi	0.47 ef	0.77 de	4.03 d	2.61 ghi
	3 mgl^{-1} SNP	1.59 c-f	0.20 ghi	2.79 c-f	0.59 c	0.70 ef	3.64 ef	3.97 ef
	6 mgl^{-1} SNP	1.78 bcd	0.25 efg	3.35 bc	0.65 b	0.62 fg	3.05 g	5.39 cd
5000	0 mgl^{-1}SNP	0.87 h	0.15i	1.18 j	0.37 g	1.29 a	5.66 a	0.92 k
	1.5 mgl^{-1} SNP	1.04 gh	0.19 ghi	1.37 ij	0.39 g	1.03 b	5.02 b	1.33 jk
	3 mgl^{-1} SNP	1.39 d-g	0.23 fgh	2.29 efg	0.45 f	0.93 c	4.74bc	2.46 hij
	6 mgl^{-1} SNP	1.68 b-e	0.27 ef	2.83 cde	0.50 def	0.81d	3.94 de	3.48 fgh
Mean **A**	0 ppm NaCl	2.02a	0.37 a	3.52 a	0.68 a	0.56c	2.51 d	6.65 a
	1000 ppm NaCl	1.56b	0.28 b	2.63 b	0.55 b	0.59 c	3.24 c	4.65 b
	3000 ppm NaCl	1.36c	0.21 c	2.44 b	0.56 b	0.73 b	3.79 b	3.48 c
	5000 ppm NaCl	1.24d	0.21 c	1.92 c	0.43 c	1.02a	4.84 a	2.05 d
Mean B	0 mgl^{-1} SNP	1.11c	0.20 d	1. 92 c	0.47 c	0.86a	4.17 a	2.55 d
	1.5 mgl^{-1} SNP	1.30c	0.24 c	2.21 c	0.48 c	0.76b	3.82 b	3.18 c
	3 mgl^{-1} SNP	1.71b	0.28 b	2.92 b	0.57 b	0.67c	3.42 c	4.80 b
	6 mgl^{-1} SNP	2.07a	0.35 a	3.45 a	0.69 a	0.60d	2.98 d	6.30 a

Different letters in columns show significant differences between treatments based on Duncan's multiple range test

3.4. Total phenols and proline contents

Plants must produce suitable organic solutes such as phenols and proline in the cytosol to counteract the osmotic stress caused by salt stress. Results in Table (4) recorded the positive effect of salt concentration (NaCl) on the shootlets contents of both total phenols and proline. In comparison with the control, the highest salinity level (5000 ppm) augmented both of them to the highest values (34.83 and 34.11 µg/g, consecutively) which were decreased gradually by decreasing NaCl concentration.

The addition of SNP at various levels (0.0-6.0 mg l^{-1}) had a negative effect on total phenols and proline contents whereas, the highest value was obtained with untreated shootlets (control) then decreased by increasing SNP level, reached the lowest values (20.91 and 18.05 µg/g, respectively) at the highest SNP level (6.0 mg l^{-1}). The inhibitory impact of SNP treatment on total phenols and proline was also observed when combined with saline conditions. Using high levels of SNP declined their contents to the lowest value at all tested salinity levels (0.0-5000 ppm).

Concerning total phenols and proline contents respond to saline conditions (Table 4), similar results were reported in a variety of plant species when exposed to salinity [42, 43, and 44]. Under salinity conditions, since glutamate is a precursor for chlorophyll synthesis as well as proline, and chlorophyll content is suppressed under stress, nitrogen metabolism switches from glutamate to proline synthesis as chlorophyll content declines, causing an increment in proline [45]. A build-up of these osmolytes (total phenols and proline) may decrease oxidative respiration or cellular acidification brought on by stress, hence

supplying recovery energy *in vitro* cultures by encouraging cell growth and enhancing the antioxidative actions of explants [46]. In this respect, restructuring biochemical pathways by applying alleviators that reduce salinity externally may help plants adapt to the negative impacts of abiotic stressors [47]. Treatment of SNP related with significant reductions in phenols and proline, SNP as an antioxidant molecule interacts with ROS which reduces the oxidative stress of salinity thus enhancing plant tolerance [48]. SNP reduces the unfavorable impacts of salinity, declining the accumulation of some osmolytes such proline [49, 50].

Table 4. Effect of various concentrations of salinity (NaCl) and sodium nitroprusside (SNP) on total phenols and proline contents (µg/g) of *K. senegalensis* shootlets

Treatment **A** NaCl (ppm)	Treatment **B**	Total phenols (µg/g)	Proline (µg/g)
0	0 mgl^{-1} SNP (The control)	20.10 hi	17.33 gh
	1.5 mgl^{-1} SNP	18.76 i	15.40 i
	3 mgl^{-1} SNP	16.30 jk	13.62 j
	6 mgl^{-1} SNP	15.06 k	10.45 k
1000	0 mgl^{-1} SNP	22.39 g	18.33 fg
	1.5 mgl^{-1} SNP	21.08 gh	16.76 h
	3 mgl^{-1} SNP	19.00 i	15.00 ij
	6 mgl^{-1} SNP	16.85 j	13.94 j
3000	0 mgl^{-1} SNP	31.25 cd	29.85 c
	1.5 mgl^{-1} SNP	27.54 e	28.26 d
	3 mgl^{-1} SNP	25.00 f	23.26 e
	6 mgl^{-1} SNP	21.99 g	18.89 f
5000	0 mgl^{-1} SNP	40.00 a	41.36 a
	1.5 mgl^{-1} SNP	37.53 b	36.00 b
	3 mgl^{-1} SNP	32.02 c	30.17 c
	6 mgl^{-1} SNP	29.75 d	28.93 cd
Mean **A**	0 ppm NaCl	17.55 d	14.20 d
	1000 ppm NaCl	19.83 c	16.01 c
	3000 ppm NaCl	26.44 b	25.07 b
	5000 ppm NaCl	34.83 a	34.11 a
Mean **B**	0 mgl^{-1} SNP	28.44 a	26.72 a
	1.5 mgl^{-1} SNP	26.23 b	24.10 b
	3 mgl^{-1} SNP	23.08 c	20.51 c
	6 mgl^{-1} SNP	20.91 d	18.05 d

Different letters in columns show significant differences between treatments based on Duncan's multiple range test

3.5. Anatomical studies of leaf and stem

Anatomical observation of transverse sections of *K. senegalensis* plant leaf and stem showed that salt stress induced adverse effect on their anatomical traits. Light microscope observation of obtained leaves under various salinity levels (0, 1000, and 5000 ppm), and SNP at 6 mgl^{-1} was shown in Table (5) and Fig. (2). The stressed plant, especially under the maximum level of salt (5000 ppm), were exhibited a clear reduction in the dimensions of the main vascular bundle length and width by 25.10%, and 16.41%, respectively, as well as the thickness of xylem and phloem tissues by 33.10% and 42.48 %, respectively. Likewise, fibers, and palisade tissues thickness were reduced by 27.70%, and 12.99%, below to the control.

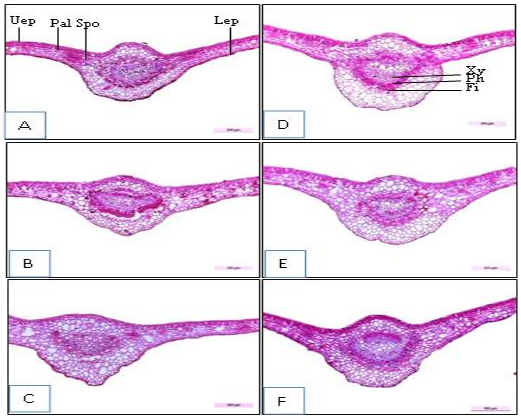
The harmful effects of salinity stress could be alleviated by using SNP. It was clear that added SNP (6 mg l^{-1}) either in non-salinized medium or under different salinity levels had a positive impact on leaf histological characteristics. Relative to the control, application of SNP at 6 mg l^{-1} increased the leaf midvein thickness, xylem, phloem tissue thickness, xylem vessels diameters, and fibers tissue thickness by 29.75, 12.05, 14.06, 95.14, and 48.66%, respectively. The increments in the thickness of leaf lamina, mesophyll and palisade tissues were 29.42, 35.21, 30.21 % as well as 41.45% for spongy tissue as compared with control.

Concerning the interaction between SNP and the salinity stress, the same trend was obtained where leaf midvein thickness showed an increment by 12.79 and 15.09 %, for plants grown under 1000 and 5000 ppm NaCl in addition with SNP (6 mg l^{-1}) as compared to the control. Furthermore, xylem vessel diameters increased by 35.78 and 65.05%, phloem thickness by 45.45

and 3.75%, as well as the palisade, and spongy tissues were increased by 35.09, and 4.17% for palisade tissue and, 74.02 and 40.31% for spongy tissue, respectively at SNP at 6 mg l^{-1} interacted the same studied saline levels (1000, and 5000 ppm NaCl, respectively) as compared to the control. Relative to the highest level of NaCl (5000 ppm), plants grown under 5000 ppm NaCl and received 6 mg l^{-1} SNP were recorded an increments in xylem, phloem, and leaf lamina by 26.42, 80.36, and 4.45%, respectively. Similarly, fibers tissue, palisade and spongy tissues were increased by the same trends. It was worth to mention that the application of SNP at 6 mg l^{-1} in the culture medium that was *K. senegalensis* plantlets grown under salinity stress caused a prominent increase in the most leaves anatomical features as compared with those grown on other media without SNP.

Table 5. Microscopical measurements of anatomical features in transverse sections of *K. senegalensis* leaves grown under various concentrations of salinity (NaCl) and sodium nitroprusside (SNP).

Anatomical characters (µ)	0 ppm NaCl (The control)	1000 ppm NaCl	5000 ppm NaCl	0 ppm NaCl + 6 mg l^{-1} SNP	1000 ppm NaCl + 6mg l^{-1} SNP	5000 ppm NaCl + 6 mg l^{-1} SNP
Leaf midvein thickness	445.44	418.56	521.72	577.96	502.41	512.66
Main vascular bundle length	228.12	215.74	170.88	262.78	238.10	281.76
Main vascular bundle width	325.77	325.48	272.32	363.06	323.90	316.03
Xylem tissue thickness	37.85	28.14	25.32	42.41	37.13	32.01
Xylem vessels diameter	7. 21	7.07	7.23	14.07	9.79	11.90
Phloem tissue thickness	19.21	15.90	11.05	21.91	27.94	19.93
Fibrous tissue thickness	21.27	23.91	15.83	31.62	29.83	33.01
Leaf lamina thickness	110.72	152.60	130.13	143.29	168.44	136.04
Upper epidermis thickness	7.71	15.10	9.75	9.75	15.06	8.91
Mesophyll tissue thickness	95.31	115.89	109.10	128.87	150.37	119.78
Palisade tissue thickness	30.72	34.47	26.73	40.00	41.50	32.00
Spongy tissue thickness	62.56	81.42	78.42	88.49	108.87	87.78
Lower epidermis thickness	7.46	9.16	12.59	12.00	9.05	8.16



Uep: Upper epidermis; Pal: Palisade tissue; Spo: Spongy tissue; Lep: Lower epidermis; Xy: Xylem; Ph: Phloem; Fi: Fibers

Fig. 2. Transverse sections of the middle leaf blade on the main stem of *K senegalensis* as affected by salt stress (zero, 1000, and 5000 ppm of NaCl) and treated with 6 mg l^{-1} SNP. A: the control plant (0 ppm), B: 1000 ppm of NaCl C. 5000 ppm of NaCl, D. 6 mg l^{-1} of SNP, E. 1000 ppm of NaCl + 6 mg l^{-1} of SNP, and F. 5000 ppm of NaCl + 6 mg l^{-1} of SNP.

As shown in Table (6) and Fig. (3), by examining stem transverse sections from control and treated plants; it was observed that increasing salinity level reduced most anatomical traits measurements of *K. senegalensis* stem. This reduction could be ascribed to the reduction of the stem diameter to 7.70% at 5000 ppm NaCl. In addition, the achieved reduction in

whole stem diameter was followed by a reduction in the thickness of epidermal tissue, fibrous tissue, phloem, and xylem tissues (vascular tissue) by 22.43, 26.60, 49.34 and 29.25%, respectively at 5000 ppm salinity stress below to the control plants. In contrast, cortex thickness was increased.

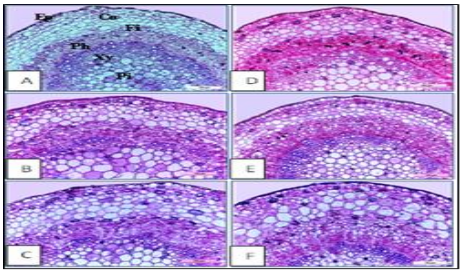
These results are in accordance with those obtained by El-Taher *et al.* (2021) [51] who found that increasing cortex thickness under salinity condition may conserve more water to overcome the adverse moisture condition and is considered as defensive strategy to reduce Na^+ toxicity [52]. From the tabulated data in Table (6), using SNP at 6 mg l^{-1} caused a notable increment in the most measurements of anatomical traits of *K. senegalensis* stems as follows; epidermis, cortex, fibers, phloem and xylem tissues thickness, the increased percentages in these tissues were 13.19, 7.12, 21.43, 6.46, and 17.06 %, respectively more than control.

Regarding the interaction between the salt stress and additive SNP to growing media produced an increment in the thickness of epidermis, and xylem tissues by 9.37 and 19.64 % for plants grown under 1000 ppm of NaCl, and treated with 6 mg $l^{-1}$of SNP, respectively, as compared to 1000 ppm of NaCl. Moreover, increasing the salinity level to 5000 ppm NaCl + 6 mg l^{-1} SNP recorded increments by 3.60, 7.48, 21.30, 51.62 and 23.33% in the thickness of epidermis, cortex, fibers, phloem and xylem tissues, respectively, over the plants grown under 5000 NaCl individually.

The reductions are common as adverse effects of salinity on leaf and stem anatomical measurements might due to damage and swelling of mesophyll chloroplasts which reduces net photosynthesis rate and some spongy growth which could reduce ROS [53]. Salinity conditions induced many changes in leaf anatomical structure as reduction in midvein, and palisade [54]. Also, salt stress caused changes in shape, size of chloroplasts and cell wall properties then leaf turgor and photosynthesis rate [55], and reduced grana numbers [56], these changes may be the reason for the reduction in the leaf and stem measurements. The harmful effects of salinity stress could be mitigated using SNP. From the above-mentioned results, it is clear that, 6 mg l^{-1} of SNP showed an increase in the most anatomical observations of *K. senegalensis* leaves and stems. These increments were associated with noticeable increases in some internal tissues. The epidermal layer in such treatment (6 mg l^{-1} of SNP) is thicker than the other plants in control or at salinity levels. The thick epidermal layer covering plant leaves plays a role in reducing water loss by transpiration. Other tissue increases might be occur as a result of the positive effects of SNP on increasing photosynthetic pigments through synthesis, regeneration, and/or preventing its degradation [57]. Nevertheless, stem and pith diameters were decreased due to SNP application under saline conditions, this reduction may be beneficial to plants in conserving energy for survival [58].

Table. 6. Microscopical measurements of anatomical traits in transverse sections of *K. senegalensis* stem grown under various concentrations of salinity (NaCl) and sodium nitroprusside (SNP).

Anatomical characters (µ)	0 ppm NaCl (The control)	1000 ppm NaCl	5000 ppm NaCl	0 ppm NaCl + 6 mg l^{-1} SNP	1000 ppm NaCl + 6mg l^{-1} SNP	5000 ppm NaCl +6 mg l^{1} SNP
Stem diameter	1352.70	1080.00	1248.57	1072.01	1000.00	1095.87
Epidermis thickness	9.63	7.90	7.47	10.90	8.64	7.74
Cortex thickness	128.15	130.23	162.42	137.28	123.72	174.57
Fibrous tissue thickness	38.50	36.36	28.26	46.75	36.00	34.28
Phloem tissue thickness	40.23	35.48	20.38	42.83	31.25	30.90
Xylem tissue thickness	62.50	54.54	44.22	73.16	65.25	54.54
Pith diameter	535.64	400.00	516.22	440.98	384.00	379.53



Ep: Epidermis; Co: Cortex; Fi: Fibers; Ph: Phloem; Xy: Xylem; Pi: Pith

Fig. 3 Transverse sections of the median portion of *K. senegalensis* stem as affected by salt stress (zero, 1000, and 5000 ppm) and treated with 6 mg l^{-1} of SNP, a: the control, B. 1000 ppm of NaCl, C: 5000 ppm of NaCl, D: 6 mg l^{-1} of SNP, E: 1000 ppm of NaCl +6 mg l^{-1} of SNP, F:5000 ppm of NaCl + 6 mg l^{-1} of SNP.

4. Conclusion

The present study concluded that different salt levels adversely affected the *in vitro* growth, biochemical, and anatomical characteristics of *K. senegalensis*, while SNP treatment has potential benefits in mitigating the negative impacts of salinity. The highest concentration of SNP (6mg l^{-1}) was effective for enhancing the in vitro-grown plant traits under saline conditions.

5. Conflicts of interest

There are no conflicts to declare

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