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Evaluating genetic diversity using molecular markers, examining growth potential and morphological adaptation in *Vachellia nilotica* against abiotic stress

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Abstract

The current study was designed to assess the genetic diversity based on Inter Simple Sequence Repeats (ISSR) markers, analyzing the growth behavior and biochemical response of *Vachellia nilotica* against salt stress. For this purpose, a pot experiment was conducted at the Forest Nursery and Experimental Area at the Institute of Forest Sciences, The Islamia University of Bahawalpur. The disease free, homogenous and uniform sized seedlings of *Vachellia nilotica* were collected from the nursery and shifted into the pots. Seventeen plants of this species were taken and applied six different treatments such as T_1 (control), T_2 (10 dSm^{-1}), T_3 (15 dSm^{-1}), T_4 (20 dSm^{-1}), T_5 (25 dSm^{-1}), and T_6 (30 dSm^{-1}). Each treatment contains three replicates. The results show that plant height, plant diameter, shoot dry weight, and root dry weight were maximum in T_1 (Control) and minimum in T_6 (30 dSm^{-1}) respectively. Furthermore, the biochemical parameters such as SOD, POD, and CAT concentration were maximum (33.9 U mg^{-1} Protein, 15.36 U mg^{-1} Protein, 5.3 U mg^{-1} Protein) in T_6 (30 dSm^{-1}) and minimum (19.22 U mg^{-1} Protein, 11.21 U mg^{-1} Protein, 2.7 U mg^{-1} Protein) in T_1 (control). But in the case of photosynthetic parameters, such as chl a and chl b contents, were maximum (0.52 mg g^{-1}FW,0.32 mg g^{-1}FW,0.84 mg g^{-1}FW) in T_1 (control) and minimum (0.21 mg g^{-1}FW,0.16 mg g^{-1}FW,0.38 mg g^{-1}FW) in T_6 (30 dSm^{-1}). Seventeen *Vachellia nilotica* species were analyzed with seven ISSR markers to evaluate genetic relationships among the plants. The molecular characterization of all ISSR amplification profiles of the selected plants revealed polymorphic bands. The obtained findings will be helpful in the future to recover salt-affected soils in Pakistan.

1 Introduction

Soil salinization is a worldwide problem amplified by global warming, especially in arid and semiarid regions (Soares *et al*. 2021; Ullah *et al*. 2022). The negative effects of soil salinity, particularly in dry regions, include a decrease in fertility, damage to soil characteristics and the loss of plant diversity or even complete plant extinction (desert) (Naorem *et al*. 2023; Syed *et al*. 2021). The problem of salinity is widespread and is steadily expanding in the areas it impacts (Dagar and Minhas 2016; Ali *et al*. 2023). Salinity and sodicity impact over half of the arable land in some nations (Ahmed *et al*. 2023). Salinity is defined as the presence of an excess amount of soluble salts that reduces plant growth and germination due to excessive ion toxicity (Mehmood *et al*. 2021). Most neutral salts dissolve in water, such as Na^+, Ca^{2+} and Mg^{2+} chlorides and sulfites. To thrive in salty soils, plants typically experience restricted development. A soil is said to be sodic if its qualities are affected by an excessive amount of exchangeable sodium, which in turn prevents the germination of the

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majority of crops and plants (Zaman and Ahmad 2009). Soil characteristics and plant and growth are both negatively impacted by the high concentrations of soluble salts and SAR seen in saline-sodic soil (Bin Yousaf *et al*. 2022). Because plants are negatively affected by soils with high salt concentrations, agricultural productivity is greatly affected by soil salinity (Syed *et al*. 2022; Sumra *et al*., 2023). Approximately 20% of the world's irrigated areas are currently afflicted by salinity. Reservoirs lose some of their storage capacity when salt affects the soil (Mohanavelu *et al*. 2021).

One of the biggest problems with using land resources is soil salinization. Because salty soils change the soil environment and reduce plant growth, agricultural production is reduced (Dahlawi *et al*. 2018; Liu *et al*. 2018). Both the plants and the air absorb the water quickly, but the salts remain in the water when it evaporates. (Walker and Salt 2012), irrigated land is supplemented with one metric ton of salts per hectare. The main environmental concern in the Indus Basin is now the presence of salty soils. Land that has excessive amounts of soluble salts is characterized as saline, sodic, or saline-sodic, having a large amount of sodium as well as chloride. Approximately 85% of topsoil affected by salt is salty, 10% is sodic and 5% is saline-sodic on a global scale. 62% of subsurface soils are salty, 24% are sodic and 14% are saline-sodic (Negacz *et al*. 2022). The salinity of the soil solution is the quantity of dissolved salts in the soil. Soluble salts such as Na^+, Ca^{2+}, Cl^-, Mg^{2+}, K^+, SO_4 and HCO_3 are the most common in soils (Rengasamy *et al*. 2022).

Sodic soils refer to the soils having excessive amounts of sodium (Na^+) ions and are black color appearance (Choudhary and Kharche 2018). When clay particles expand and disperse in soil solutions, it can lead to soil structure collapse. This, in turn, can have negative effects like the formation of impermeable soil layers, root growth restriction, and increased erosion susceptibility (Li *et al*. 2016). Carbon dioxide concentrations affect alkalinity; these concentrations rise with root and microbiome respiration and can mitigate OH^- formation to some extent (Msimbira and Smith 2020; Afzal *et al*., 2020). The formation of the insoluble calcite occurs when carbonates precipitate out of soil solutions when there is an excess of calcium. When this happens, the soil pH usually stays below 8. The solubility of sodium carbonates, however, can cause pH values well above 8 in soil solutions with a high relative content of Na. Soil alkalinity, therefore, gives clues about soil sodicity (Johan *et al*. 2021).

Salinization is the most detrimental environmental stress among abiotic stresses since it impacts at least twenty percent of the arable land of the world. In an agricultural nation, the soil is the foundation of the economy (Hussain *et al*. 2018). The soil fertility of a certain area determines its cultivability. Soil salinity is a problem that reduces crop and fruit production (Chandio *et al*. 2017; Zafar *et al*. 2023). Some tree species can grow under salt-affected soils (Yousaf *et al*. 2021; Aminullah et al. 2023). Trees are seen as having greater potential than other vegetation types because of their longer growth periods, larger biomass production and higher tolerance index (Yousaf *et al*. 2021; Yasin *et al*., 2024). Research on the salt tolerance of various Acacia species has been documented (Abbas *et al*. 2022; Azhar *et al*. 2013). Typically, soil salinity has been the unit of analysis in these types of studies. There is a lack of research on the salt tolerance of acacias when it comes to irrigation with seawater dilutions or brackish water (Dhupper *et al*. 2013; Riaz *et al*. 2022).

The revised subfamily taxonomy of the Leguminosae, which incorporates the genera of the mimosoid clade in Caesalpinioideae, has changed *Acacia nilotica* to *Vachellia nilotica* (Sambou *et al*. 2010). The Leguminosae tree species *V. nilotic*a is significant from a social, economic and ecological perspective. Traditional veterinary medicine, agriculture, pastoral medicine, industrial medicine and food are just a few of the many uses for *V. nilotica* (Zhu 2001; Mehmood *et al*. 2021; Bin Yousaf *et al*. 2022). It is a versatile tree that serves multiple purposes in agroforestry systems around the globe, including providing green fertilizer, lumber, and fodder (Dagar and Minhas 2016). *Vachellia nilotica* improves soil fertility and crop yield, which is important for a sustainable future because it helps mitigate the effects of climate change, including flooding, high temperatures, wind and water erosion (Bargali and Bargali 2009). It is suggested that future experiments can be performed by screening salt-sensitive and salt-tolerant cultivars based on variations in salt sensitivity among different genotypes of the same species (Hussain *et al*. 2018; Mir *et al*., 2024).

Therefore, this study explored the potential of *Vachellia nilotica* against salt stress at the seedling stage in terms of morpho-physiological growth and ionic response. In this work, we assessed how *Vachellia nilotica* physiologically, biochemically, and growth responses were affected under water deficit conditions. Inter-simple sequence repeat (ISSR) markers are a valuable tool in molecular biology due to their ease of use and reproducibility. These markers require only a small quantity of DNA and do not necessitate prior knowledge of the DNA sequence. ISSR primers are designed based on simple sequence repeat (SSR) motifs, making them applicable to a wide range of plant species, provided that these motifs are present and appropriately distributed within their genomes. Techniques utilizing DNA markers facilitate the identification of genotypes at a molecular taxonomic level and enable the assessment of genetic diversity both within and

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between species. This study aims to assess the genetic diversity using molecular markers, examining growth potential and morphological adaptation in *Vachellia nilotica* against salt stress.

2. Material and Methods

Study site

The purpose of the experiment was to determine how *Vachellia nilotica* will respond to salt stress in terms of its growth capacity and morphological adaptability. The research trial was conducted at the Forest Nursery and Experimental Area, Institute of Forest Sciences, Baghdad-Ul-Jaded Campus, The Islamia University of Bahawalpur. Bahawalpur is the 11th most populous city in Pakistan and is known for its carpets, embroidery, and pottery. Bahawalpur, formerly part of the Rajputana State in United India, is recognized for landmarks such as Noor Mahal, Darbar Mahal, Sadiq Ghar Palace, and the historic Fort of Derawar situated in the Cholistan Desert. The city's metropolitan region experienced a population increase from 845,000 in 2019 to 895,000 in 2022. Bahawalpur is situated approximately 117 meters above sea level in a dry climate characterized by minimal rainfall.

Climatic conditions

In the summer, the Bahawalpur district experiences hot and dry weather, while in the winter, it is chilly and dry. The temperature ranges from 1°C at the lowest to 48°C at the highest. April to October is its long summer season, with May, June, and July being the three hottest months. The winter season, in contrast, starts in November and lasts until March. December, January, and February are the three coldest months. The temperature was between 20 and 25 degrees centigrade. Bahawalpur's current metro area population in 2023 is 920,000, a 2.79% rise from 2022. The Abbasi family of Nawabs ruled Bahawalpur, a former princely state, from its founding in 1748 until 1955. The Nawabs left behind a significant architectural heritage, and now, Bahawalpur is well-known for its Nawabi-era structures. District of Bahawalpur measures 24,830 km^2 and lies at 71.6198686 longitudes and 29.3666752 latitude. The area is situated at an elevation of 118 m above sea level. The Cholistan Desert, which stretches into the Thar Desert of India, covers almost two-thirds of the district's (16,000 km^2) total area.

Experiments layout

The current study was conducted to examine growth potential and morphological adaptation in *Vachellia nilotica* against salt stress. The disease free, uniform sized seedlings of *Vachellia nilotica* were taken from a forestry nursery for the experiment's purpose. Seventeen plants of targeted species were taken and six treatments such as T_1 (control), T_2 (10 dSm^{-1}), T_3 (15 dSm^{-1}) and T_4 (20 dSm^{-1}), T_5 (25 dSm^{-1}), T_6 (30 dSm^{-1}). Each treatment contains 3 replicates.

Data collections

At the time of harvesting, the following parameters were measured for the required data.

Growth parameters

Before harvesting, we used a measuring tape to record the plants' heights from the soil's surface to their highest point. The stem diameter, on the other hand, was measured at the collar both before and after harvesting using a digital caliper. To get a uniform weight, the samples were dried in an oven for four days at a temperature of 74 degrees Celsius using a DGH-9202 series thermal electric thermostat drying oven. After taking the samples out of the oven, we measured their dry weight in shoots and roots by using an electronic weighing scale (Electronic Scale JJ3000B.

Biochemical parameters

To extract and measure the amounts of soluble proteins as well as the activity of antioxidant enzymes (SOD, CAT and POD), the experimental procedure that was reported by (Alici and Arabaci 2016) Naqvi et al. (2011) was applied. This required using a mortar and pestle to combine 0.5 grams of leaves with 2 milliliters of phosphate buffer that was 50 millimeters in thickness. First, the extracts were filtered and then they were centrifuged at 8,000 revolutions per minute for ten minutes to measure the antioxidant activity of the ingredients. For analysis, the extracts were separated from residues in Eppendorf tubes. Because the extract is perishable, it was kept at -800 ºC until it was analyzed.

A segment of the new leaf (0.5g) was centrifuged in 50mMl^{-1} cooled buffer Phosphate 5ml (pH 7.8). To conduct a SOD assay at a temperature of 4 degrees Celsius, the homogenized sample was centrifuged at 15000 revolutions per minute for twenty minutes. In a glass beaker with a capacity of 25 milliliters, combine 2.725 milliliters of RS that have been made according to the normal methodology with 0.25 milliliters of hydrogen peroxide and 0.025 milliliters of ethylene oxide. Read all samples spectrophotometrically at 560nm. A fresh sample of leaf 0.5g was immersed in 5ml of extracting buffer (50mML phosphate pH 7.8). For 20 minutes, the homogenized sample was centrifugal at an enzyme assay using supernatant 15000 rpm at 4C°. Take 2.7ml PBS standard solution + 0.1ml EE + 0.1ml H_2O_2+ 0.1ml Guaiacol. Before performing spectrophotometry at 470 nm, after setting a timer for thirty to sixty seconds, carefully shake the sample four to five times. The equation that Casual and Maehly (1995) provided will serve as the base for the calculations of the activity of POD.

A fresh 0.5g leaf sample was homogenized in 5 ml of buffer extraction (50 ml, phosphate, pH 7.8). The prepared homogenous sample was centrifuged at 15,000 revolutions per minute for twenty minutes and the enzyme extract was used for the examination. The whole procedure was carried out at 0-4 C°. Take 2.8ml BPS standard solution +0.1ml of EE +0.1ml of H_2O_2 give constant shaking and take the measurement from the spectrophotometric at 240nm. A fresh 0.5g leaf sample was homogenized in 5 ml of buffer extraction (50 ml, phosphate, pH 7.8). The prepared homogenous sample was centrifuged at 15,000 revolutions per minute for twenty minutes and the enzyme extract was used for the examination. The whole procedure was carried out at 0-4 C°. Take 2.8ml BPS standard solution +0.1ml of EE +0.1ml of H_2O_2 give constant shaking and take the measurement from the spectrophotometric at 240nm**.**

After pulverizing 1g of frozen sample in a motor and pestle with 2ml of phosphate buffer (pH 6.4), we added 600 µl of thiobarbituric acid (0.5%) and 600 µl of trichloroacetic acid (20%), heated at 95°C for a minute, quickly cooled in an ice-bath for 5 minutes and centrifuged at 4000 rpm for 10 minutes. There was a recording of the absorbance at both 532 nm and 600 nm.

MDA= (532-600)/1.56*105

The Bradford method was used to count the amount of soluble protein (Bradford 1976). Two milliliters of Bradford reagent were added to a microcentrifuge tube containing fifty microliters of the sample. The only thing in the blank was Bradford reagent. The absorbance was measured at 595 nm in frequency. The amount of protein was measured using a standard curve generated with various BSA (bovine serum albumin) concentrations.

Photosynthetic pigments

The methods of Amon (1949) and Davies (1986) were used to quantify the amounts of carotenoid and chlorophyll. 0.5 grams of leaf discs were spun for five minutes over 14000 revolutions per minute after the chlorophyll was extracted from them using 10 milliliters of 80% acetone at a temperature of 10 degrees Celsius for an entire night. We evaluated the absorbance of the supernatant at three different wavelengths: 645, 652, 663 and 480 nm, with a spectrophotometer (PG, T60U). Calculating the levels of carotenoid along with chlorophyll required the use of the following formulas:

Chl_a (mg g^{-1} FW) = [12.7(OD663)-2.69(OD645)] × v/1000 × w

Chl_b (mg g^{-1} FW) = [22.9(OD645)-4.68(OD663)] × v/1000 × w

Carotenoids (mg g^{-1} FW) = A^{car}/E_{mx}^{100}

V= Volume of sample extract and W= Weight of the sample

A^{car} = (OD480) + 0.114(OD645); $Emax^{100}$ cm =2500

Molecular analysis

DNA was extracted from 18 selected plants by using the standard cetyltrimethyl ammonium bromide (CTAB) method (Doyle 1990). In the current study inter-simple sequence repeats (ISSR) primers were used for the detection of genetic diversity in *Vachellia nilotica*. Seven selected simple sequence repeat (ISSR) primers were initially screened to determine the suitability of each primer for the study. Primers were selected for further analysis based on their ability to detect distinct, clearly resolved and polymorphic amplified products within the varieties. PCR analysis was performed using specific ISSR primers (UBC-818, UBC-825, UBC-827, UBC-837, UBC-844, UBC-864, UBC-889) for all selected plants. The amplification products were separated on a 1% agarose gel in 1x TBE buffer, stained with ethidium bromide on a 100 bp ladder (Life Technologies-GIBCO BRL).

Statistical analysis

The collected data were organized and analyzed using Statistic 8.1 software. Differences among group means were evaluated by one-way analysis of variance (ANOVA), and statistical significance was considered at $P < 0.05$.

3. Results

Growth parameters

The results indicate that the maximum plant height (53 cm) was measured in T_1 (Control), while the minimum plant height (36.6 cm) was recorded in T_6 (30 dSm^{-1}). The findings showed that the minimum stem diameter (5 mm) was recorded in T_6 (30 dSm^{-1}), while the maximum stem diameter (12.3 mm) was reported in T_1 (Control). Maximum shoot dry weight (23 g) was measured in T_1 (Control) while the minimum shoot dry weight (10.6 g) was recorded in T_6 (30 dSm^{-1}). The maximum root dry weight (17 g) was measured in T_1 (Control), while the minimum root dry weight (8 g) was recorded in T_6 (30 dSm^{-1}). The maximum total biomass (40.33 g) was measured in T_1 (Control), while the minimum total biomass (20.66 g) was recorded in T_6 (30 dSm^{-1}). As we increased the stress level the plant height, stem diameter, shoot dry weight, root dry weight and total biomass were decreased, respectively.

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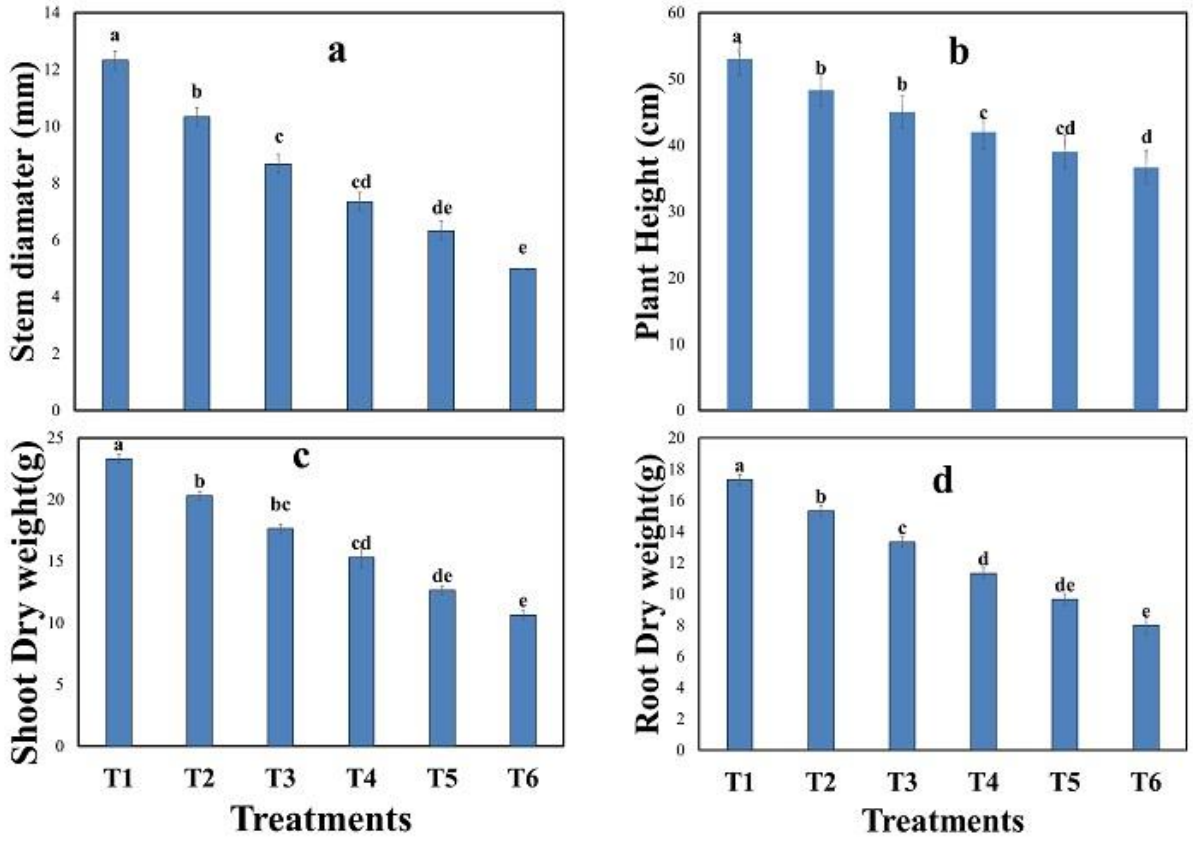


Fig. 1 Effect of different salinity levels on the growth parameters of *Vachellia nilotica*

Biochemical parameters

Salinity stress depicted a significant variation in peroxidase concentration. Our findings indicate that the maximum peroxidase concentration (15 U mg^{-1} Protein) was measured in T_6 (30 dSm^{-1}), while the minimum peroxidase concentration (11 U mg^{-1} Protein) was recorded in T1 (Control). Our findings indicate that the maximum superoxide dismutase concentration (33 U mg^{-1} Protein) was measured in T_6 (30 dSm^{-1}), while the minimum peroxidase concentration (19.22 U mg^{-1} Protein) was recorded in T_1 (Control). The maximum catalaze activity (5.3 U gm^{-1}protein) was measured in T_6 (30 dSm^{-1}), while minimum peroxidase (2.7 U gm^{-1}protein) was recorded in T_1 (Control).We observed that the maximum malondialdehyde content (1.45 mol g^{-1}FW) was measured in T_6 (30 dSm^{-1}), while the minimum malondialdehyde content (0.67 mol g^{-1}FW) was recorded in T_1 (Control). As we increased the stress level the peroxidase concentration, superoxide dismute concentration, catalyze activity, and malondialdehyde content were increased respectively.

Photosynthetic parameters

Different levels of salinity stress produce a significant variation in Chl a content. The results indicate that the maximum Chl a (0.5 mg g^{-1}FW) was measured in T_1 (Control), while minimum chlorophyll a (0.22 mg g^{-1}FW) was recorded in T_6 (30 dSm^{-1}). As we increase the stress level the Chl a were decrease respectively. The results indicate that the maximum chl b content (0.32 mg g^{-1} FW) was measured in T_1 (Control), while minimum chl b content (0.16 mg g^{-1}FW) was recorded in T_6 (30 dSm^{-1}). The maximum total chlorophyll content (0.8 mg g^{-1} FW) was measured in T_1 (Control), while the minimum total chlorophyll content (0.3 mg g^{-1} FW) was recorded in T_6 (30 dSm^{-1}). The results indicate that the maximum carotenoid content (0.39 mg g^{-1}FW) was measured in T_1 (Control), while the minimum carotenoid content (0.21 mg g^{-1}FW) was recorded in T_6 (30 dSm^{-1}). Our results indicate that maximum soluble protein content (12 mg g^{-1} FW) was measured in T_1 (Control), while the minimum soluble protein content (7.13 mg g^{-1} FW) was recorded in T_6 (30 dSm^{-1}). As we increased the stress level on Chl a content, Chl b content, total chlorophyll content,carotenoid content and soluble protein contents were decreased respectively

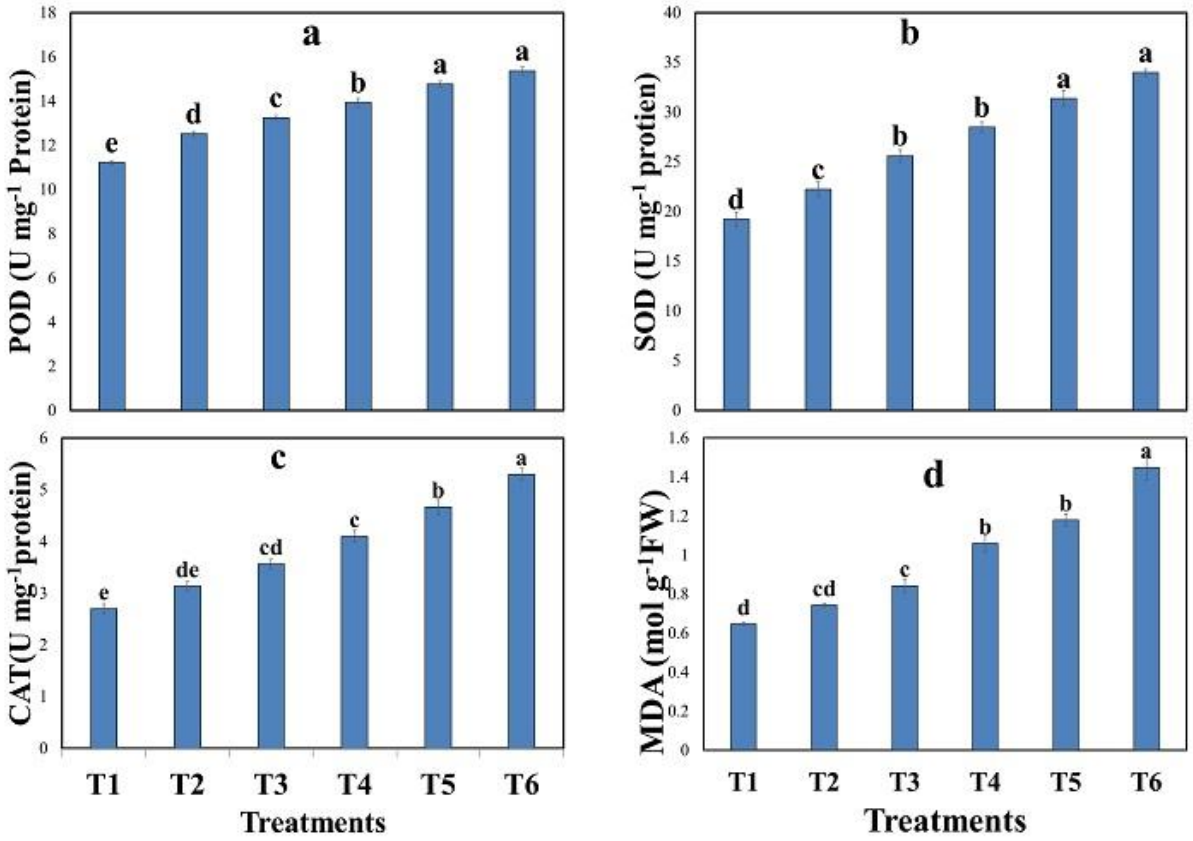


Fig. 2 Effect of different levels of salinity on the biochemical parameters of *Vachellia nilotica*

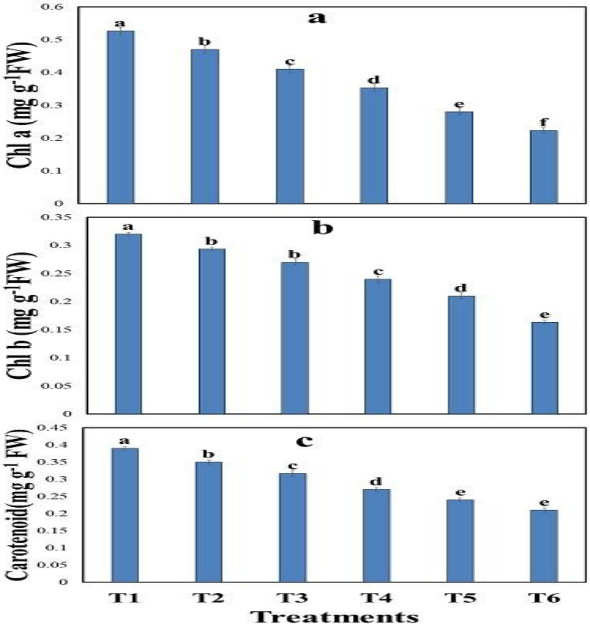


Fig. 3 Effect of different levels of salinity on the photosynthetic pigments of *Vachellia nilotica*

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Molecular Result

The present investigation was to evaluate the level of genetic diversity of the 18 species of *Vachellia nilotica*. The amplification profiles of the selected plants generated using the ISSR markers were showed good polymorphism among seventeen genotypes Fig. 4 a and b. Molecular scoring band of seven transcription factors in selected plants of *Vachellia nilotica* are shown in Table 1.

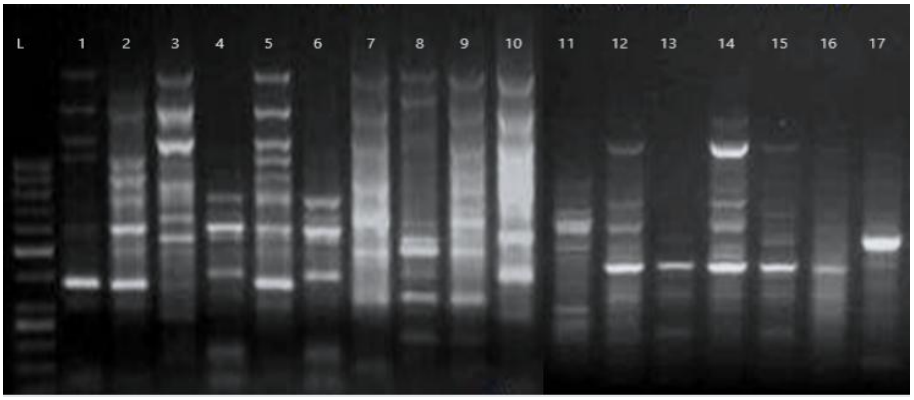


Fig. 4 a. DNA Amplification obtained with primer: ISSR (UBC-825)

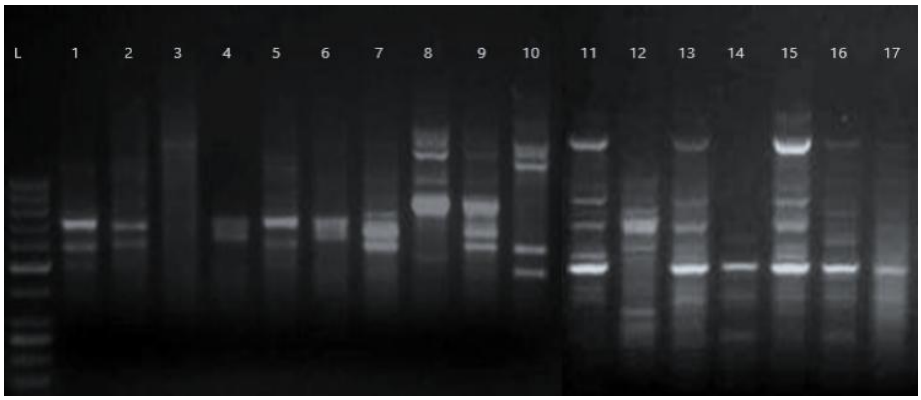


Fig. 4. b. DNA Amplification obtained with primer: ISSR (UBC-827)

Table 1 Molecular scoring band of seven transcription factors in selected plants of *Vachellia nilotica*

Plants	UBC-818	UBC-825	UBC-827	UBC-837	UBC-844	UBC-864	UBC-889
P1	✓	✓	✓	✓	✓	✓	✓
P2	✓	✓	✓	✓	✓	✓	✓
P3	✓	✓	✓	✓	✓	✓	✓
P4	✓	✓	✓	✓	✓	✓	✓
P5	✓	✓	✓	✓	✓	✓	✓
P6	✓	✓	✓	✓	✓	✓	✓
P7	✓	✓	✓	✓	✓	✓	✓
P8	✓	✓	✓	✓	✓	✓	✓
P9	✓	✓	✓	✓	✓	✓	✓
P10	✓	✓	✓	✓	✓	✓	✓
P11	✓	✓	✓	✓	✓	✓	✓
P12	✓	✓	✓	✓	✓	✓	✓
P13	✓	✓	✓	✓	✓	✓	✓
P14	✓	✓	✓	✓	✓	✓	✓
P15	✓	✓	✓	✓	✓	✓	✓
P16	✓	✓	✓	✓	✓	✓	✓
P17	✓	✓	✓	✓	✓	✓	✓

4. Discussion

The extent to which salinity is a problem is expanding at an alarming rate around the world (Dagar and Minhas 2016). Salinity and other abiotic pressures are reducing the world's productive area at a time when the global population is

rising, which is a problem for food security (Srivastava *et al*. 2019). One efficient, sustainable and cost-effective way to enhance the condition of soil that has been harmed by salt is to use green plants (Gangwar *et al*. 2020; Sabir *et al*., 2023). With a greater tolerance index, greater biomass production and longer growth periods, trees are seen as having greater potential among vegetation (Herr *et al*. 2019).

The effects of salt stress on the growth of *Vachellia nilotica* are substantial. Key growth indicators, including stem diameter, root dry weight, and plant height, all declined as salt stress concentration rose. As salt toxicities rose, *Vachellia nilotica* biomass dropped dramatically; this loss might be due to the root's restricted growth caused by less cell division. *Vachellia nilotica* growth may be suppressed by elevated salt concentrations in the soil. Lower rates of growth for roots and shoots are common indicators of this inhibition. A water deficit in the plant tissues results from salt stress, which also hinders the roots' ability to absorb water. This reduces the growth of the plant overall by affecting the elongation and expansion of its cells.

The results of previous studies have also revealed that a decline in plant growth under salt stress was closely linked with specific ion toxicity (Abrol *et al*. 1988; Rahman *et al*., 2025), nutritional imbalance (Karimi and Tari 2017) and osmotic stress (Liu et al. 2018). These problems result in damaging plant cells, tissue and organs, ultimately reducing the plant growth throughout the life cycle (Song and Wang 2015). *Vachellia nilotica* can tolerate a wide variety of soils, although it thrives in sites with good drainage. There is a 40% reduction in growth at an ECe of approximately 8 $dS.m^{-1}$ (Singh *et al*. 1991). Higher salt concentrations had a significant effect on the biochemical parameters of *Vachellia nilotica* as well. When the concentration of the biochemical parameters SOD, CAT, POD, and MDA increased with the rise in salt ratio, they demonstrated a positive response to the saline conditions. Even for plants that have evolved to thrive in dry environments, the availability of water is the single most important environmental factor regulating photosynthesis and growth. Less stomatal conductance for CO_2 fixation by mesophyll cells is the main effect of salt stress (Rajesh *et al*. 1998). A fair amount of research has focused on the physiological effects of salinity on mangrove photosynthesis, primarily on transpiration and stomatal conductance.

The efficiency of carboxylation, stomatal conductance and photosynthetic rate were all significantly reduced by salinity stress. Enhanced chlorophyll activity or alterations in the lipid-protein ratio of pigment-protein complexes may account for the decline in chlorophyll concentration observed at increasing salt levels (Lotfi *et al*. 2020). Halophytic plants can experience a drop in photosynthetic activity due to salinity stress (Angon *et al*. 2022; Khan *et al*., 2023). This can happen through various mechanisms, including partial stomatal closure, decreased carboxylation efficiency and CO_2 saturation photosynthesis and an inhibition of the light reaction mechanism (Gamage *et al*. 2018). The growth parameters vary significantly depending on the degree of salt stress (Ji *et al*. 2022; Rahman *et al*., 2025). The finding shows that plant growth parameters like (plant height, stem diameters, shoot dry weight, and root dry weight) were record in T_1 (Control) maximum (53cm,12.3mm, 27g, 23g, 24g, 17g, 40.33g), while the minimum record (36.6cm, 5mm, 18.6g, 10.6g, 16.6g, 8g 20.66g) in T_6 ($30dSm^{-1}$). As we increase the stress level, all the growth parameters decrease respectively. Different levels of salinity stress showed a significant variation in biochemical parameters like (POD, SOD, CAT, MDA). Salinity stress depicted a significance variation in peroxidase concentration(POD), superoxide dismutase concentration(SOD), catalase activity concentration(CAT) and malondialdehyde contents (MDA) were minimum in T_1(Control) 11Umg^{-1} Protein, 19.22 U mg^{-1} Protein, 2.7 Umg^{-1} Protein, 0.67$molg^{-1}$FW 0.2 mgg^{-1}FW, while the maximum values in T_6($30dSm^{-1}$) 15 U mg^{-1} Protein,33 Umg^{-1} Protein, 5.3 Umg^{-1} Protein, 0.74mgg^{-1}FW).There is a significant variation in photosynthetic parameters like chlorophyll a, chlorophyll b, total chlorophyll, carotenoid, and soluble protein under salinity stress.

The results indicate that the (chl a, chl b, and carotenoid content) were maximum measured in T_1(0.5 mg g^{-1}FW, 0.32 mg g^{-1} FW, 0.8 mg g^{-1} FW, 0.39 mg g^{-1}FW, 12 mg g^{-1} FW), while the minimum (chl a, chl b, total chl content, carotenoid content and soluble protein content) were measured in T6($30dSm^{-1}$). As we increased the stress level chl a, chl b, total chl content and soluble protein contents were decreased, respectively. The order of treatments was as follows: T_1; control (Non-saline), T_2; 10 dS m^{-1}, T_3; 15 dS m^{-1}, T_4; 20 dS m^{-1}, T_5; 25 dS m^{-1}, T_6; 30 dS m^{-1}. The study revealed that there exists a large amount of genetic diversity within and among selected species. Analysis of variation in ISSR fragments revealed a high level of variation among the plant species along with a high level of diversity. Similar observations were made by various authors in forest species (Casiva *et al*. 2002; Heider *et al*. 2007; Aparajita and Rout 2009).

5. Conclusion

The study revealed that salt stress negatively affects growth and photosynthetic parameters, while certain biochemical responses, like the activity of enzymes, are heightened. This research provides valuable insights into how

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Vachellia nilotica responds to salt stress and can inform future efforts to grow this species in marginal, saline soils. Additionally, the findings on genetic diversity are crucial for selecting plants with superior traits for such environments. It was revealed that a significant amount of genetic diversity existed among the 18 plant species. Therefore, understanding genetic variation between and within the genomic groups is very important for the evaluation of plants with superior traits. Overall, *Vachellia nilotica* can be successfully planted in highly saline soils, although its biomass and growth significantly decrease under higher salt stress conditions.

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