



Physiological homeostasis for ecological success of *Typha* (*Typha domingensis* Pers.) populations in saline soils

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Abstract The natural capacity of plants to endure salt stress is largely regulated by multifaceted structural and physio-biochemical modulations. Salt toxicity endurance mechanism of six ecotypes of *Typha domingensis* Pers. was evaluated by analyzing photosynthesis, ionic homeostasis, and stomatal physiology under different levels of salinity (0, 100, 200 and 300 mM NaCl). *Typha* populations were collected across different areas of Punjab, an eastern province in Pakistan. All studied attributes among ecotypes presented differential changes as compared to control. Different salt treatments not only affected gas exchange attributes but also shown significant modifications in stomatal anatomical changes. As compared to control, net

photosynthetic rate, transpiration rate, total chlorophyll contents and carotenoids were increased by 111%, 64%, 103% and 171% respectively, in Sahianwala ecotype among all other ecotypes. Similarly, maximum water use efficiency (WUE), sub stomatal CO₂ concentration, sodium (Na⁺) and chloride (Cl⁻) contents were observed in Sahianwala (191%, 93%, 168%, 158%) and Knotti (162%, 75%, 146%, 182%) respectively, as compared to the others ecotypes. Adaxial and abaxial stomatal areas remained stable in Sahianwala and Knotti. The highest abaxial stomatal density was observed in Gatwala ecotype (42 mm²) and maximum adaxial stomatal density was recorded in Sahianwala ecotype (43 mm²) at 300 mM NaCl salinity. The current study showed that *Typha* ecotypes responded varyingly to salinity in terms of photosynthesis attributes to avoid damages due to salinity. Overall, differential photosynthetic activity, WUE, and changes in stomatal attributes of Sahianwala and Knotti ecotypes contributed more prominently in tolerating salinity stress. Therefore, *Typha*

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domingensis is a potential species to be used to rehabilitate salt affected lands for agriculture and aquatic habitat.

Keywords *Typha* populations · Stomatal regulation · Structural modifications · Transpiration rate · Water-use efficiency

Introduction

Soil salinity is one of the most injurious environmental constraints that rigorously impede plant growth, development, and productivity. Salt stress usually increase disorders in plants (Acosta-Motos et al. 2017). Among these salinity associated perturbations in plants, reduced leaf area, declined photosynthesis (Agarwal et al. 2013), stomatal closure and over-production of Reactive Oxygen Species (ROS) (Hussain et al. 2016) are well-known and cause alarming reduction in plant growth and productivity (Phang et al. 2008). Imbalances in ionic homeostasis coupled with osmotic stress due to Na^+ ions enhance toxicity (Horie et al. 2012). Besides, unequal ionic concentration in plant parts leads to irregularity in stomatal functions (Nilson and Assmann 2007). Such changes in stomatal functions directly influence crucial plant processes such as transpiration, gaseous exchange and photosynthesis. Salt tolerant plants mainly use three types of tolerance mechanisms. These include toxic ion exclusion, limitation/deposition of ions into specific plant cells/tissues as well as subcellular organelles and shoot ion-independent salinity tolerance (Munns and Tester 2008). Other physiological processes i.e. photosynthesis, transpiration, ionic homeostasis, antioxidant defense are also parallel contributors in these mechanisms for making plant tolerant to salt stress (Negrão et al. 2017). These facts suggest a strong and positive correlation between physiological and anatomical attributes in plants that collectively determine the survival of a plant species growing in saline soil.

Different plant populations such as grasses and macrophytes naturally show better tolerance to salinity stress (Hameed and Ashraf 2008). For instance, salt tolerant plants i.e. halophytes showed tolerance by adjusting their histo-physiological and biochemical attributes in their life cycle and such endurance to stress conditions is reflected in terms of growth and productivity (Khan and Gul 2002). *Typha domingensis* Pers. can inhabit variety of salt and metal affected lands e.g. salt range, wetlands, and coastal marshes. This plant is native to South America and found as invasive macrophyte in different regions of the world. *Typha* has shown high potential for phytoremediation (Oliveira et al. 2018) and its tolerance to deteriorated rooting medium is due to the evolution of specific histological traits (Wu 1981). Survival of *Typha* plants in

salinity affected rhizosphere would provide mechanistic insight into working relationship between anatomical and physiological characteristics. It also supports the notion that interaction between plants and soil is not limited to single feature. Although much work has been done on physiological aspects of salinity tolerance in plants yet other aspects including morphological changes in plant tissues with respect to salt stress needs extensive investigations (Noreen et al. 2019; Yoo et al. 2009; Zulfiqar et al. 2020a). Previously, *Typha* plants have been evaluated for their tolerance to heavy metals and other nutrient uptake studies (da Cunha Cruz et al. 2020; Oliveira et al. 2018).

It was hypothesized that histological modifications in stomatal characteristics were directly linked to physiological modulations. Therefore, primary objective of this study was to assess the changes in stomatal attributes and their link to different physiological attributes in *T. domingensis* Pers. affecting their distribution and tolerance to salinity. Intra-species differences among *Typha* populations inhabiting fresh water or saline rhizosphere were also focused to determine the most tolerant ecotype in relation to different sites. It was revealed that salinity levels significantly affected the stomatal density on epidermal surface and indicated correlated physiological responses across the *Typha domingensis* Pers. populations.

Materials and methods

Six *Typha domingensis* Pers. ecotypes were collected from different areas of Punjab province in Pakistan; viz. Sahianwala (saline waterlogged), Knotti (salt range), Jahlar (hyper saline), Gatwala (Fresh water), Treemu (fresh water), and Sheikhpura (industrially polluted) (Fig. 1, Table 1). These plants were individually grown in plastic pots of size 10 × 10 × 10 (L × W × H) and 500 g weight filled clayey-loam soils and sand in 1:1 ratio, of pH:6.8 and then filled with 10 L irrigation water in the Botanic Garden at University of Agriculture, Faisalabad under natural environmental conditions for a period of 2 months. Then the acclimatized plants were treated with salt treatment for 3 months (Naseer et al. 2017). The average temperatures of day and night were 38–41 °C and 24–26 °C, respectively and light period ranged from 13 to 16 h. The relative humidity alternate from 46.8 to 59.3% (Zulfiqar et al. 2020b).

Establishment of saline levels

The levels of salt used in this experiment were 0, 100, 200, and 300 mM NaCl with half-strength Hoagland's nutrient solution (Hoagland and Arnon 1950) applied as rooting

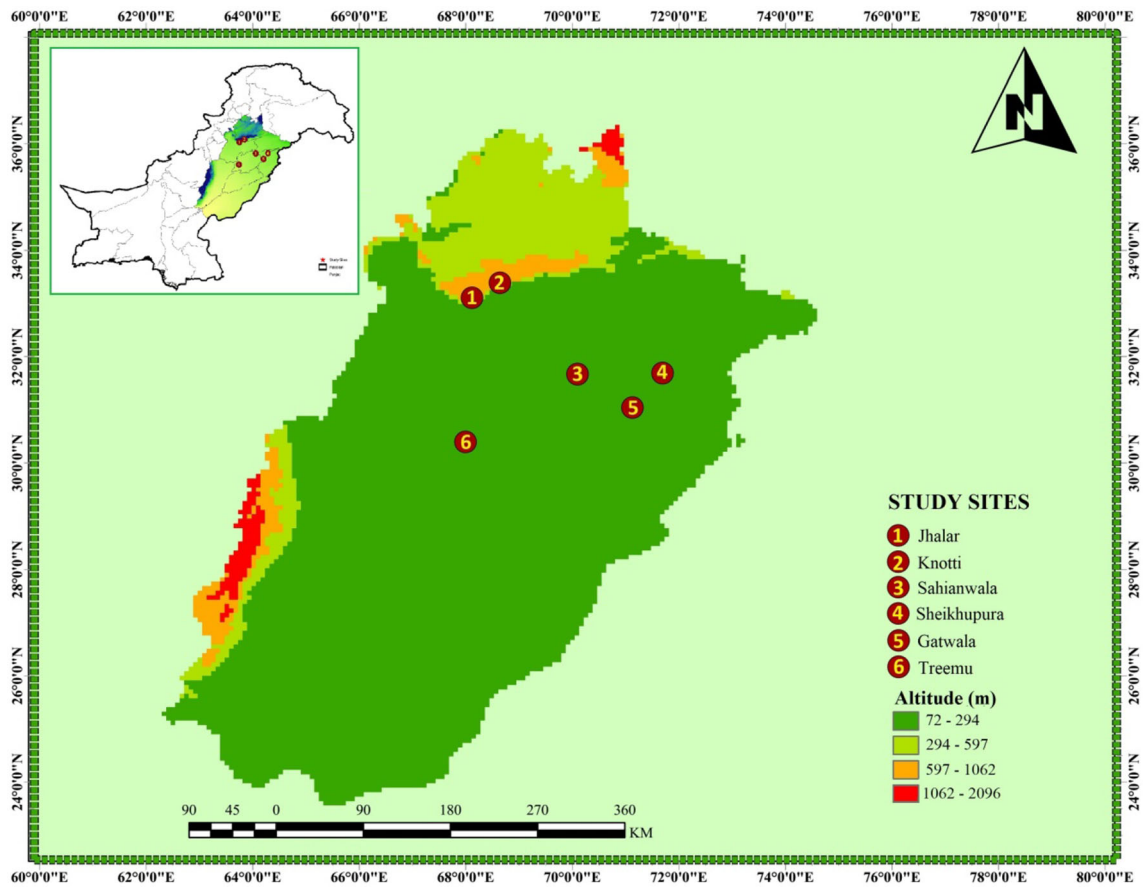


Fig. 1 The map of the Punjab, Pakistan showing six different collection sites for *Typha domingensis* Pers. ecotypes

Table 1 Data about *Typha* ecotypes, their habitats and collection sites

Ecotype name	Accession number	Date of collection	Place name	Collection area	Geographic coordinates of location	No. of plants	Stage of sample
Jahlar	17001	12-04-2017	Jahlar lake, hypersaline, Punjab, Pakistan	Lake	32° 56' N, 72° 41' E	15	Vegetative
Sheikhupura	17002	22-04-2017	Sheikhupura road, industrial polluted area, Punjab, Pakistan	Road side	31° 72' N, 73° 91' E	15	Vegetative
Sahianwala	17003	02-05-2017	Sahianwala area, water-logged wetlands Punjab, Pakistan	Urban	31° 43' N, 73° 63' E	15	Vegetative
Gatwala	17004	10-05-2017	Gatwala fresh water lake Punjab, Pakistan	Canal bank	31° 71' N, 73° 12' E	15	Vegetative
Treemu	17005	14-05-2017	Treemu head, fresh water,Punjab, Pakistan	River	31° 08' N, 72° 08' E	15	Vegetative
Knotti	17006	20-05-2017	Knotti garden,salt range, Punjab, Pakistan	Wetland	32° 42' N, 72° 14' E	15	Vegetative

medium to all the *Typha* (*Typha domingensis* Pers.) ecotypes. Salt treatment was given once in week along with all media. After 15 days of treatment tissues were harvested for further measurements.

Plant pigments

Chlorophyll a, b, and carotenoids were determined according to the method of Arnon (1949), and Davies (1976). The chlorophyll a, b, and carotenoids were calculated using following formula:

$$\text{Chl a (mg/g f.wt.)} = [12.7(\text{OD } 663) - 2.69(\text{OD } 645) \times V/1000 \times W]$$

$$\text{Chl b (mg/g f.wt.)} = [22.9(\text{OD } 645) - 4.68(\text{OD } 663) \times V/1000 \times W]$$

$$\text{Carotenoids (mg/g f.wt.)} = \text{Acar/Em} \times 100$$

Determination of photosynthetic activity

Net CO₂ assimilation rate (P_N), transpiration rate (E), and stomatal conductance (g_s) were measured on one fully expanded flag leaf from each plant using an open system *LCA-4 ADC* portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). The data were recorded for different parameters with molar flow of air per unit leaf area 403.3 mmol m⁻² s⁻¹, atmospheric pressure 99.9 kPa, maximum light intensity (PPFD) at leaf surface 1,711 μmol m⁻² s⁻¹, leaf temperature ranging from 28.4 to 32.4 °C, ambient temperature from 22.4 to 27.9 °C, and ambient CO₂ concentration of 352 μmol mol⁻¹ (Aqeel et al. 2021; Noreen et al. 2018).

Digestion of plant material

For the estimation of nutrients firstly plant material was digested according to the prescribed methodology (Wolf 1982; Ali 2018). The dried, well ground plant material (0.5 g in each tube) was placed in digestion tubes and 5 mL of concentrated H₂SO₄ was added to each tube. All tubes were incubated overnight at room temperature. Then 0.5 mL of H₂O₂ (35% A. R. Grade extra pure) was poured down the sides of the digestion tube, ported the tubes in a digestion block and heated at 350 °C until fumes were produced. The heating was continued for another 30 min. The digestion tubes were removed from the block and cooled. 0.5 mL of H₂O₂ was slowly added to each tube and tubes were placed back into the digestion block. The above step was repeated until the cooled digested material was colorless. The volume of the extract was brought up to

50 mL. The extract was filtered and used for determining ionic contents.

Estimation of ionic contents

Na⁺ and K⁺ were determined with a flame photometer (Jenway, PFP-7, Burlington, NJ, USA). A graded series of standards (ranging from 5 to 25 mg L⁻¹) of Na⁺ and K⁺ were prepared and standard curves were plotted. The values from flame photometer were compared with standard curves and total quantities were computed. (Horneck and Hanson 1998; Jiang et al. 2019; Ditta et al. 2018; Jackson 1962). P content was measured by following the protocol of Jackson (1962) by using Spectrophotometer (Hitachi-220, Tokyo, Japan). Magnesium (Mg²⁺) and calcium (Ca²⁺) contents in shoot were determined by EDTA titration according to the method of Tucker and his co-worker (Tucker and Kurtz 1961; Yang et al. 2020). Cl⁻ contents of leaf were determined by following the method described by Tavakkoli et al. (2010). Shoot samples (100 mg each) were ground and heated in 10 ml of H₂O at 80 °C until the volume became half. The volume was brought to 10 mL again by adding distilled water. Cl⁻ content was determined with a chloride meter (Model 926, Sherwood Scientific Ltd., Cambridge, UK).

Anatomical attributes

For stomatal studies, 1 cm piece from the leaf used for gas-exchange measurements was taken and fixed in FAA (formalin 5%, acetic acid 10%, ethanol 50%, and water 35%) solution for 48 h. The material was then transferred to acetic-alcohol (v/v, acetic acid 25%, and ethanol 75%) solution for long-term storage and till for further analysis (Johansen 1940; de Andrade Silva et al. 2020). The paradermic piece of leaf from adaxial and abaxial sides of leaf were taken using steel blades and further sections were stained with 0.1% safranin according to (Kraus and Arduin 1997; de Andrade Silva et al. 2020). Stomatal density, area, and orientation were studied by scratching the leaf surface. The basal portion of the leaf (1 cm long) was selected for stomatal studies. Measurements and photographs were taken with the help of a camera equipped light microscope (Nikon 104, Japan).

Statistical design

The experiment was planned in a Completely Randomized Design (CRD) with two factors (plant species and salinity) and three replications. The data was subjected to statistical analysis using Minitab statistical software (Version: Minitab 19.1.1) for analysis of variance (ANOVA) and LSD for comparison of mean values (Steel et al. 1997; Kim

2014). Graphs were prepared using Origin Pro 9.1 (OriginLab Corporation 2014), and statistical program “R (v 4.0.1)” was used for estimating Pearson’s correlation coefficient and for Principal component analysis among various measured variables (R Development Core Team 2017).

Results

Physiological parameters

Plant chlorophyll-a content reduced significantly in Gatwala ecotype in response to increase in salt levels. A profound decline was recorded in *Typha* collected from Sheikhpura, Sahianwala, Treemu, and Knotti only at 300 mM NaCl. In comparison, a minor reduction in Chl. a

content was observed in Jahlar ecotype in response to increased salinity in growth medium (Fig. 2). A prominent and significant reduction was recorded in Chl. b content in ecotypes from Sheikhpura, Gatwala and Treemu in response to progressive increase in salt levels. Chl. b value remained stable up to 200 mM salinity in the Jahlar and Knotti ecotypes and showed significant reduction at 300 mM salt level. In the Sahianwala ecotype, this parameter showed significant reduction at moderate levels of salt stress (100 and 200 mM NaCl). However, high dose of salt (300 mM NaCl) yielded non-significant variation. Total chlorophyll contents were significantly reduced in Jahlar, Sheikhpura, Gatwala, and Treemu region *Typha* plants. However, ecotypes from Knotti and Sahianwala showed non-significant changes up to 200 mM salt level, but a significant reduction was recorded at the 300 mM salinity. Carotenoids invariably increased in *Typha*

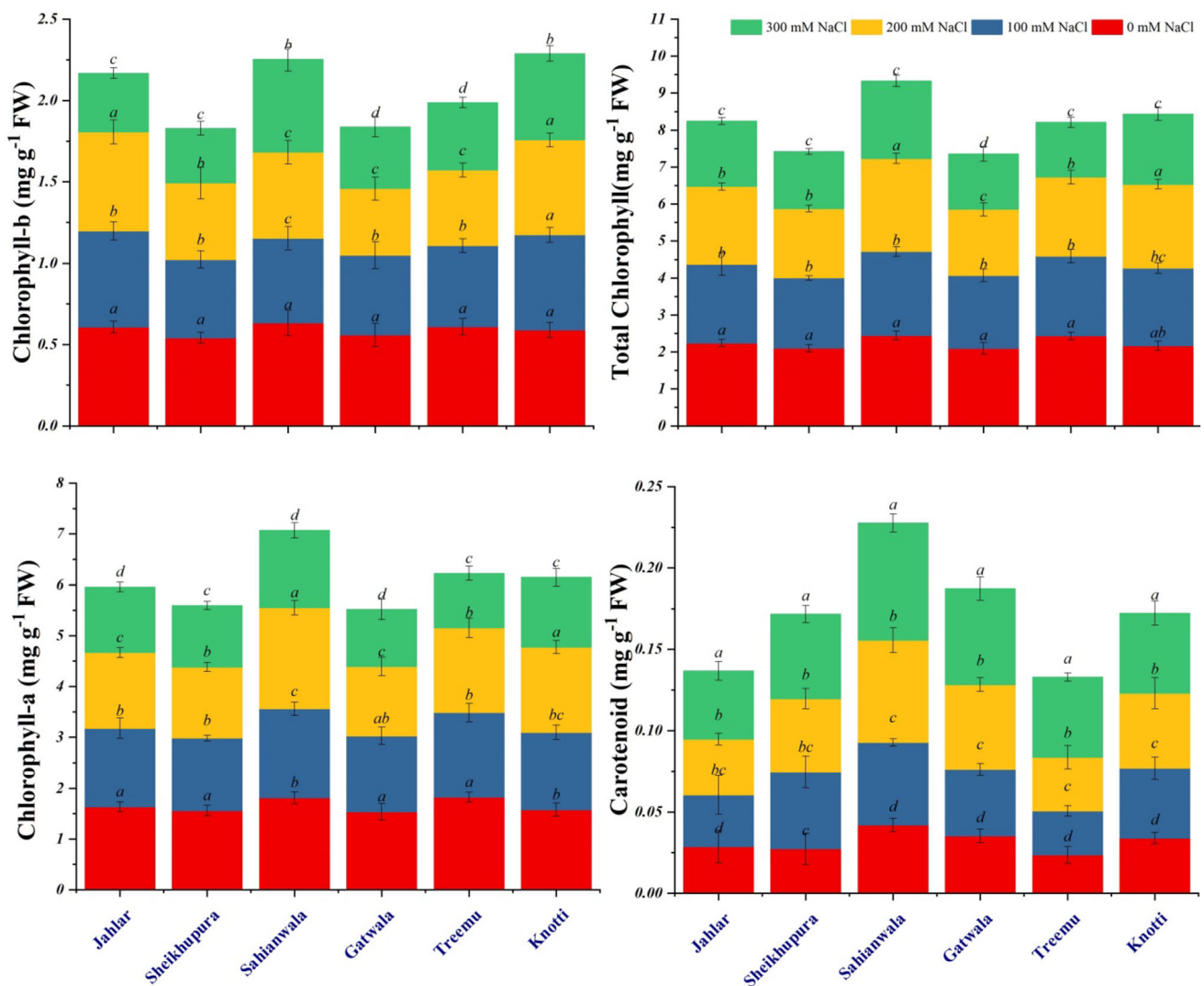


Fig. 2 Plant pigments of six ecotypes of *Typha domingensis* Pers. under different levels of salt stress. After 15 days of treatment, tissues were harvested for pigment measurements.

ecotypes in response to salt stress, particularly at the highest salt level (Fig. 2). Carotenoid content however, was significantly higher in the Sahianwala ecotype as compared to all other ecotypes. In short, Sahianwala ecotype was tolerant in term of chlorophyll a, b, and carotenoids content as least reduction was observed in these pigments as compared to control. On the other hand, *Typha* ecotypes from Sheikhpura and Gatwala were sensitive that showed greater reduction in chlorophyll a, b and total chlorophyll as compared to control.

Photosynthetic parameters

Transpiration rate (E) was decreased in all populations of *Typha domingensis* Pers. under salinity stress as compared to that of control. Sheikhpura and Gatwala populations showed lowest transpiration rate. Reducing transpiration is a good indicator of stress tolerance. P_N (net CO_2 assimilation) was also recorded to reduce under salt stress in all populations except Sahianwala, a population from high saline habitat, which showed marked increase in net CO_2 assimilation in response to gradual increase of salt stress levels. Substomatal CO_2 concentration was less affected due to salinity stress. This may be correlated to an increase in stomatal density which reduces loss of CO_2 under saline conditions. Sahianwala populations showed marked enhancement in sub-stomatal CO_2 concentration along with increased salinity levels (Fig. 3). Various levels of salinity influenced stomatal conductance (g_s) adversely. Populations from fresh water and industrially polluted areas; Gatwala, Treemu and Sheikhpura showed more sensitivity as compared to Sahianwala, Knotti and Jahlar populations from highly saline habitat. WUE was progressively increased under higher salinity levels in all populations which depict their salt tolerance mechanism. Sahianwala and Knotti populations from high saline soils showed more WUE as compared to Jahlar population from saline-waterlogged habitat, Gatwala, Treemu and Sheikhpura populations from fresh water to industrially polluted habitats. *Typha* ecotypes from Sahianwala and Knotti showed better adaptation as compared to other ecotypes. Tolerance behavior was recorded in Sahianwala ecotype that showed the highest net photosynthetic rate, high WUE and least effected stomatal conductance and sub-stomatal CO_2 activities. The Sheikhpura ecotype was sensitive in term of decrease photosynthetic rate, transpiration rate, sub-stomatal CO_2 concentration and stomatal conductance as compared to control.

Ionic contents

Shoot sodium (Na^+) content in all ecotypes of *Typha* showed progressive and significant increase along with

increased salt levels of growth medium. Ecotype from Gatwala accumulated highest shoot Na^+ content as compared to all other ecotypes. On the other hand, ecotype from Knotti revealed least increase in this parameter in response to salt stress as compared to control. There was a profound and significant reduction in shoot K^+ in all ecotypes with increase in salt conc. of external growth medium. The greatest reduction in shoot K^+ was observed in Sheikhpura ecotype at 300 mM NaCl as compared to other ecotypes (Fig. 4). Shoot P value was gradually and prominently reduced in the Sheikhpura, Gatwala and Treemu ecotypes in response to increase salinity levels of growth medium. Ecotypes from Jahlar, Sahianwala and Knotti showed non-significant differences among P content variations. Shoot Cl^- content progressively and significantly increased in all *Typha domingensis* ecotypes as compared to control in response to salt stress. Highest increase was observed at 300 mM NaCl. Data relevant to shoot Mg^{2+} content was gradually and significantly decrease in all ecotypes except Knotti site *Typha* ecotype. Shoot Ca^{2+} value showed a significant decrease in ecotypes from Jahlar, Sheikhpura, Gatwala and Treemu. However, in ecotypes from Sahianwala and Knotti, the pattern of variation of Ca^{2+} was stable and non-significant changes were observed under salinity stress (Fig. 4). Overall, Sahianwala ecotype showed high Na^+ and Cl^- contents and least reduction in Ca^{2+} , Mg^{2+} and P. Tolerant behavior was observed in Sahianwala ecotype as compared to all other *Typha* (*Typha domingensis* Pers.) ecotypes. The sensitive ecotype was Sheikhpura that showed reduced values of Na^+ , K^+ , Ca^{2+} , Mg^{2+} and P.

Stomatal area and density

Stomatal area refers to the area occupied by stomatal pore as per unit surface of leaf (abaxial and adaxial) and density is the count of number of stomata on either abaxial or adaxial leaf surface (Figs. 5 and 6). Both these characteristics have inverse relation to each area, as increase density will provide less area to stomata. Under salinity stress Treemu and Gatwala populations showed reduced stomatal area of abaxial and adaxial leaf surfaces (Fig. 7). However, Sahianwala, Knotti and Jahlar populations showed non-significant variations in adaxial and abaxial stomatal area. Adaxial stomatal area of Jahlar population was increased under different salinity levels. Salinity induced decrease in stomatal area, transpiration rate E , which ultimately enhance WUE, is an effective strategy under stress condition. Net CO_2 assimilation increased with decreasing stomatal area. Stomatal density however, showed non-significant

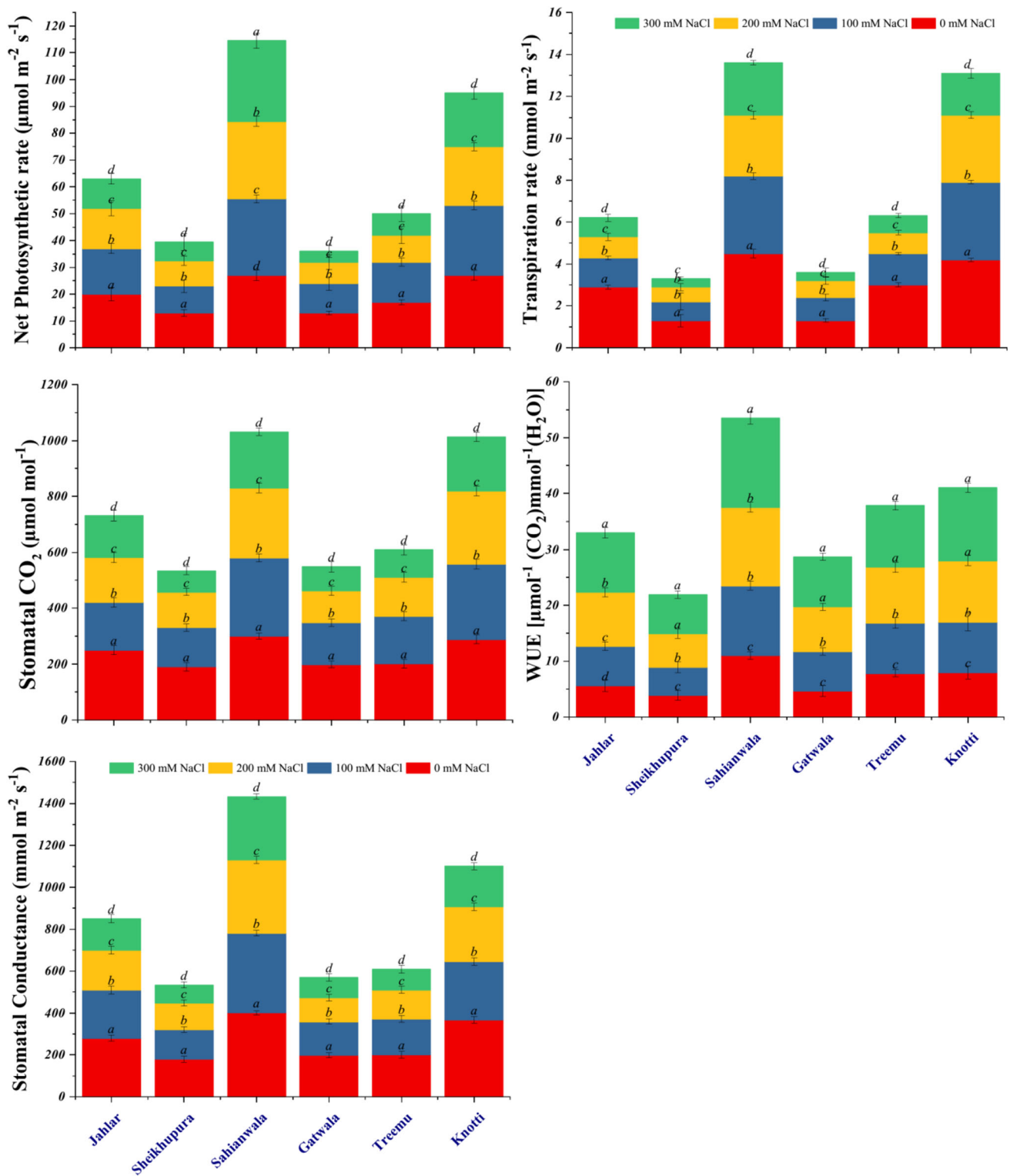


Fig. 3 Gas exchange characteristics of six ecotypes of *Typha domingensis* Pers. under different levels of salt stress

pattern of variation among all populations, overall enhanced abaxial stomatal density was observed.

In Jahlar population decrease in adaxial stomatal density was recorded under increase salt stress, but in other

populations this parameter increased progressively under salt stress. Lowest stomatal density was recorded in Treemu population which showed increase in adaxial stomatal density, but abaxial side showed increase in

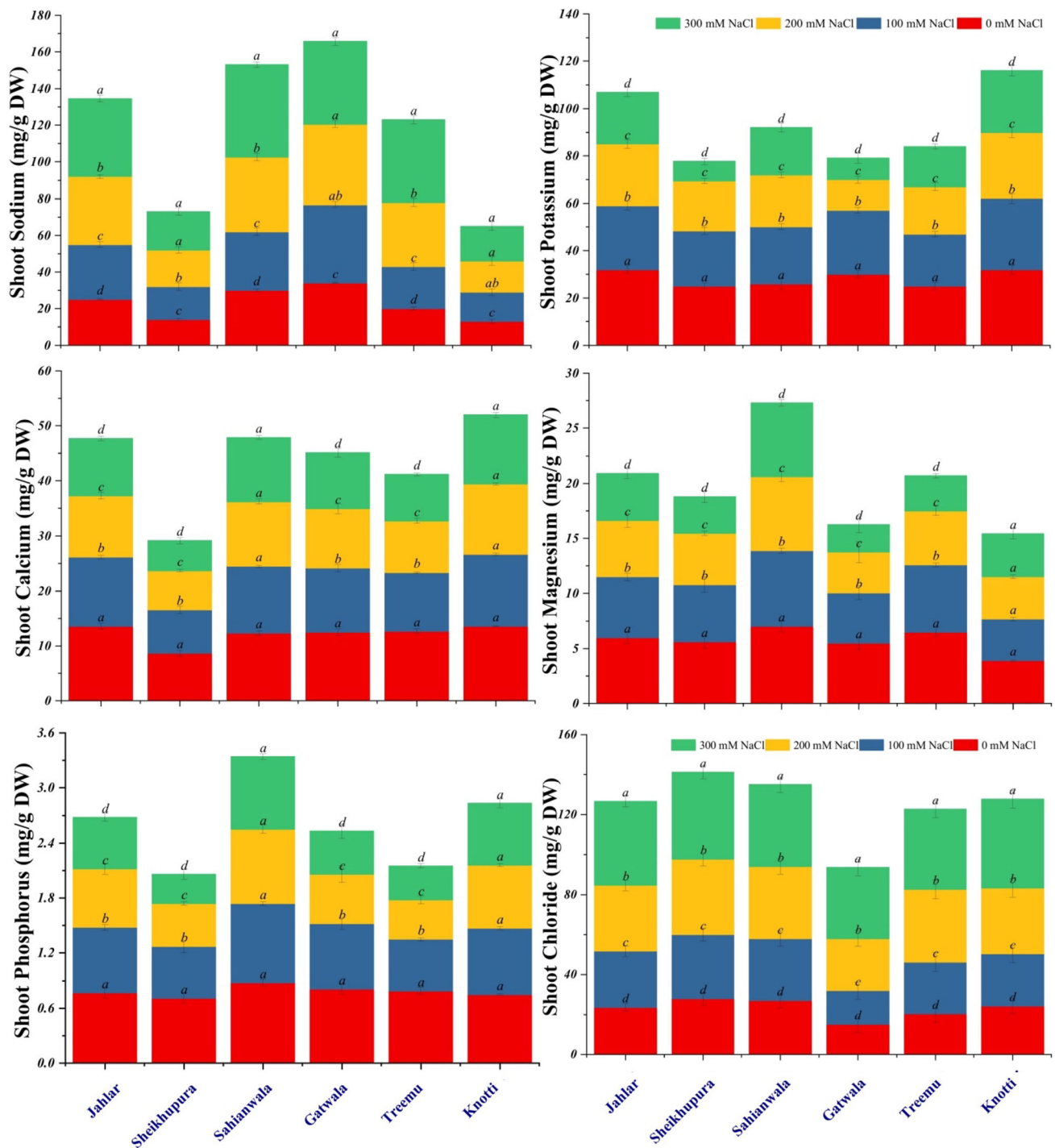


Fig. 4 Shoot ionic contents of six ecotypes of *Typha domingensis* Pers. under different levels of salt stress

stomatal density only at 100 mM NaCl, thereafter gradual decrease was recorded. Increased stomatal density provide better stomatal conductance which could be an effective precautionary measure under water limiting conditions (Figs. 5, 6, and 7). The high abaxial and adaxial stomatal density, and adaxial stomatal area was recorded in

Sahianwala ecotype. Sahianwala ecotype also showed least reduction in abaxial stomatal area as compared to other *Typha domingensis* Pers. ecotypes. So, Sahianwala ecotype showed tolerance in comparison with other ecotypes.

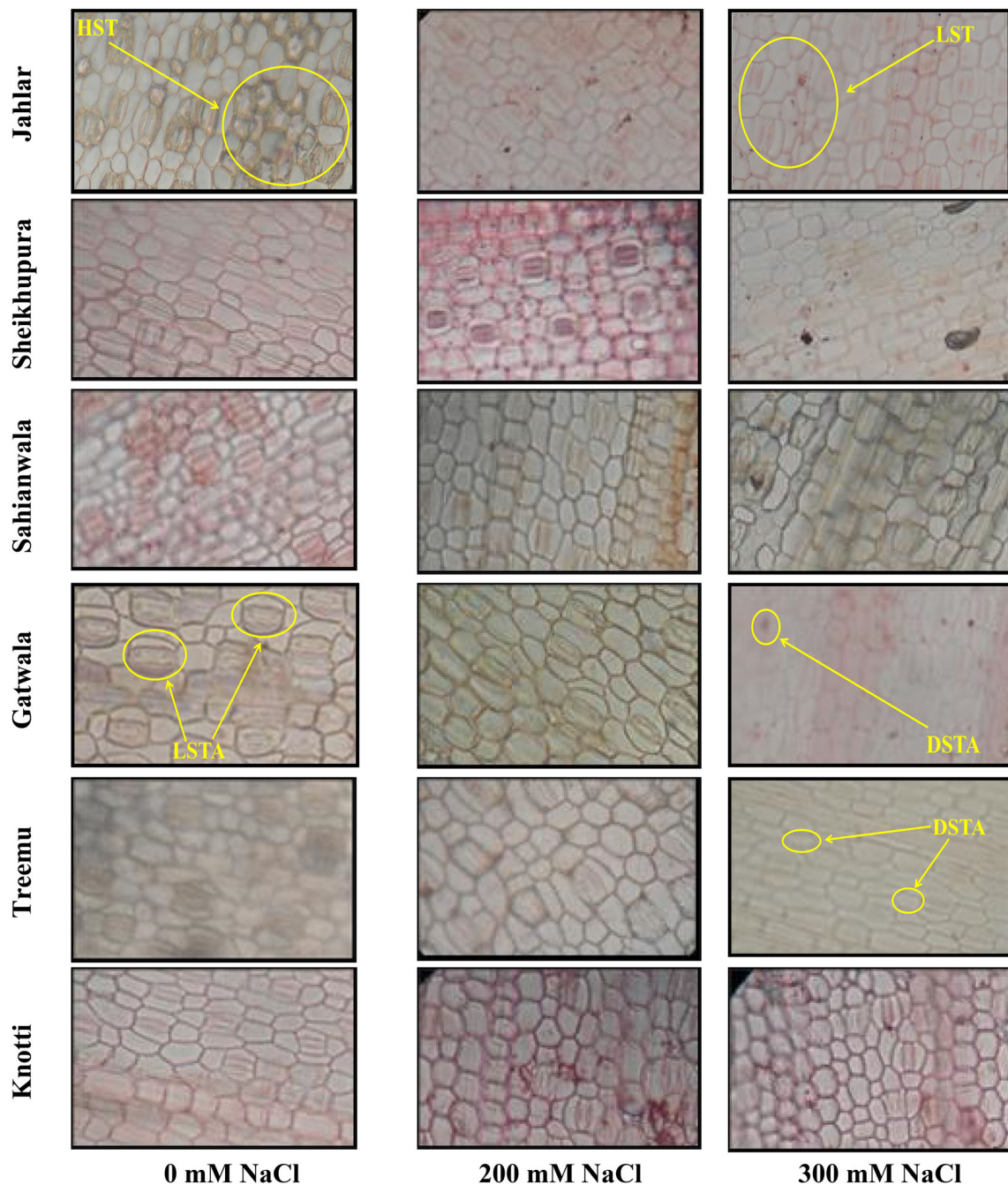


Fig. 5 Adaxial epidermis of six ecotypes of *Typha domingensis* Pers. under different levels of salt stress. *HST* high stomatal density, *LST* low stomatal density, *LSTA* large stomatal area, *DSTA* decrease stomatal area

PCA and Pearson correlation

For assessment of inter-relationship among various attributes of *Typha* ecotypes and salinity levels, the principal component analysis and Pearson correlation was executed (Figs. 8 and 9). Pearson’s correlation showed a significant positive correlation of salinity levels with transpiration rate, WUE, sub-stomatal CO₂ concentration, Shoot P, Ca²⁺ and stomatal modifications. Adaxial and abaxial stomatal

area is highly correlated but negatively correlated with stomatal density. Shoot Na⁺ and Cl⁻ is negatively correlated with K⁺. Furthermore, a significant negative correlation is evident for Cl⁻ concentrations with Chl a, b and total chlorophyll. This correlation represented a close connection and relationship between salinity and plant attributes (Fig. 8).

On a factor plane in PCA, the directions of the cases with the Bi-plot were imaged. A clear separation of *Typha*

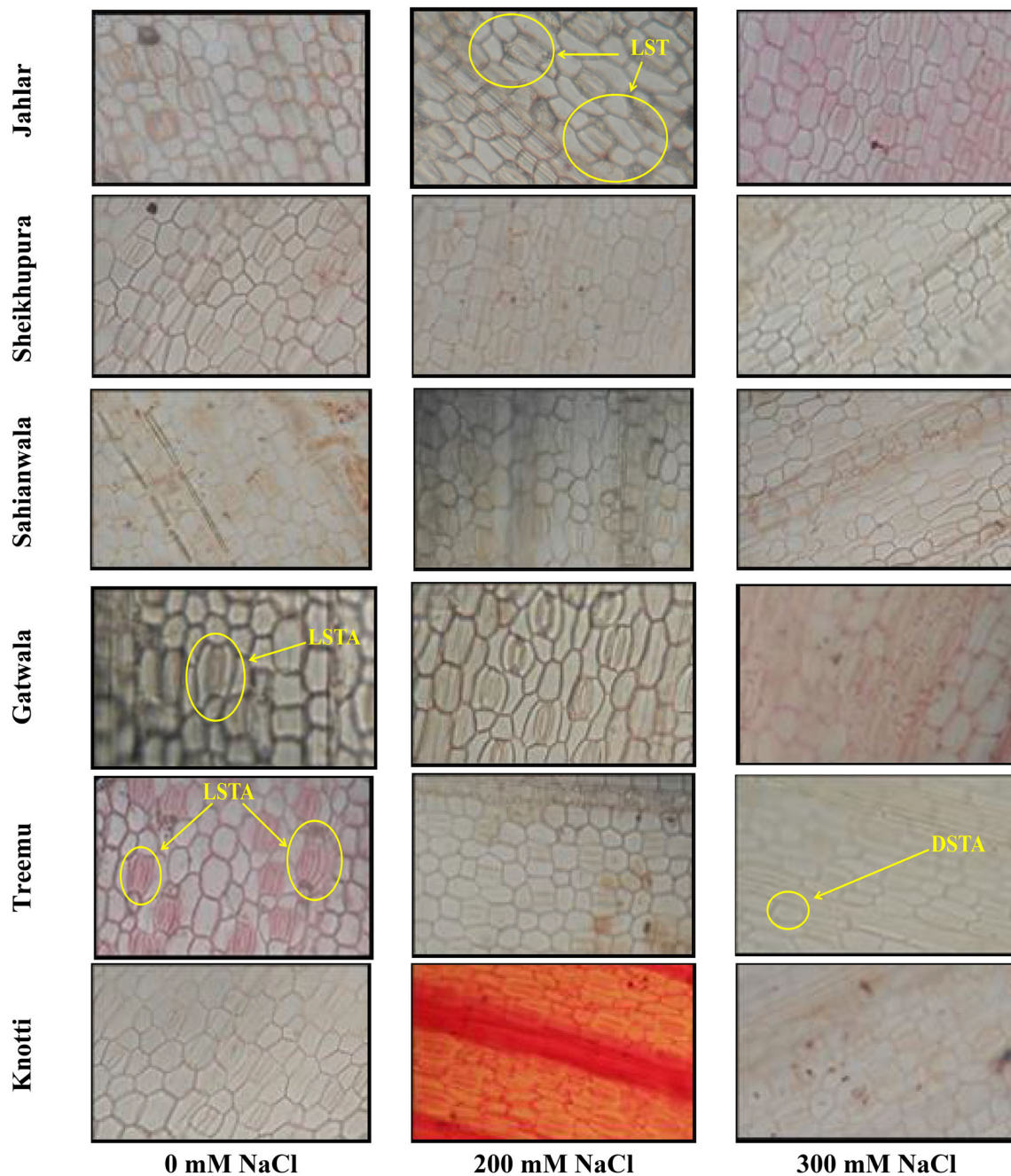


Fig. 6 Abaxial epidermis of six ecotypes of *Typha domingensis* Pers. under different levels of salt stress. *LST* low stomatal density, *LSTA* large stomatal area, *DSTA* decrease stomatal area. All images were taken with light microscope.

(*Typha domingensis* Pers.) ecotypes attributes in response to different salinity levels were shown by PC1. Of the total variation, contribution of PC1 was 32.7% as compared to 21.5% contribution by PC2. We detected a clear separation among different attributes studied in *Typha* ecotypes. Although we observed a strong overlap yet there is a clear bias between the ecotypes. The maximum variation in PC1 was elaborated by g_s , P_n , adaxial stomatal area, abaxial and adaxial stomatal density, Na^+ and Cl^- (Fig. 9).

Discussion

Naturally growing monocot species are best adapted to grow in habitats facing environmental stresses such as salinity. Such successful adaptation depicts evolution in their genetic makeup and certain structural as well as functional modifications of certain tissues and organs (Hameed et al. 2010). Major processes like photosynthesis, respiration and transpiration show functional modifications

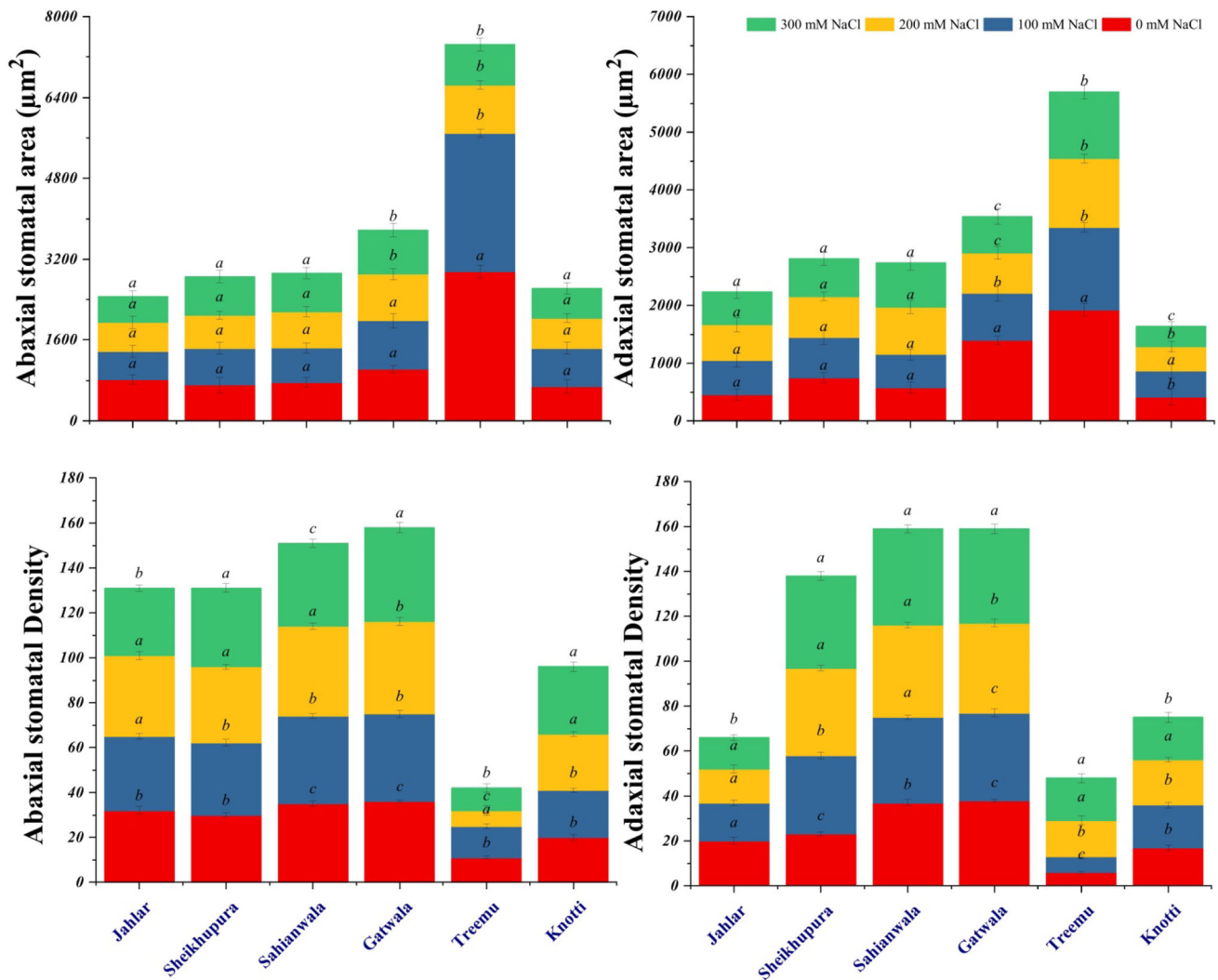


Fig. 7 Stomatal area and density of six ecotypes of *Typha domingensis* Pers. under different levels of salinity stress. These results are average of data obtained in three replicates

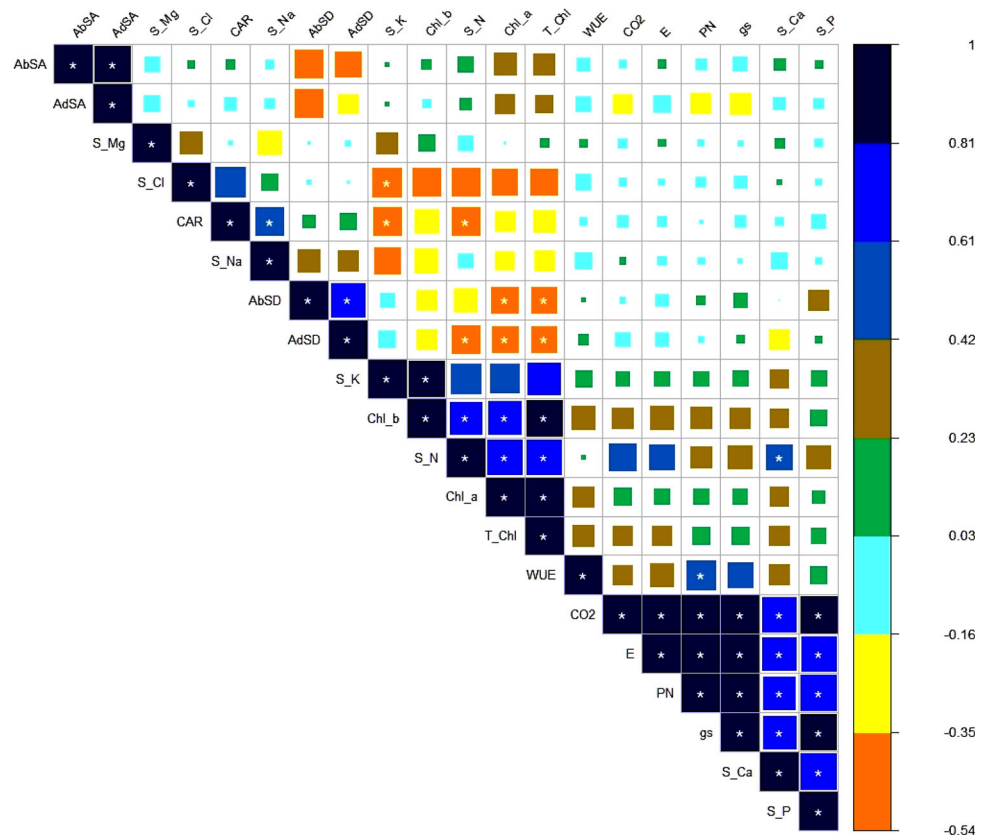
that make plants able to compete stress. Stomata are the vital organs to regulate these processes by maintaining water and CO₂ supply under limiting environmental conditions (Naz et al. 2010; Tufail et al. 2020; Yang et al. 2004). In present study, all ecotypes of *Typha domingensis* Pers. exhibited declined transpiration rate (*E*) and stomatal conductance (*g_s*) with increasing salinity levels. This is depiction of the fact that plants decrease transpiration rate and stomatal conductance as an effective strategy to prevent water loss and dehydration for maintaining normal functioning of photosynthetic machinery under saline conditions (Naz et al. 2010; Robinson et al. 1998; Tufail et al. 2020).

In ecotype Sahianwala, larger adaxial and abaxial stomatal area along with increased stomatal density under salt stress contributed in maximum CO₂ supply for photosynthesis. This attribute correlated with increased sub-

stomatal CO₂ in the same ecotype and emerged as an evidence supporting process of photosynthesis. Besides, certain invaginations of epidermal tissues in the Sahianwala ecotype also elaborated a direct relationship between increased stomatal area and corresponding stomatal conductance to prevent transpirational losses. Our stance is supported by the mechanistic explanation presented by Naz and co-workers (Naz et al. 2010). According to them, in *Aeluropus lagopoides* controlled transpiration rate and high water-use efficiency were major players in salinity tolerance that was tightly controlled by fewer and smaller stomata on adaxial and abaxial leaf surfaces as well as stomatal encryption due to epidermal invaginations (Naz et al. 2010).

Furthermore, Jahlar ecotype showed an increase in adaxial stomatal area. Increased stomatal area and the size of stomatal pore may be decreased by epidermal

Fig. 8 Pearson Correlation between different attributes in *Typha* (*Typha domingensis* Pers.) reveal relationship to support ecological survival of different ecotypes subjected to multiple salinity regimes. AbSA: Abaxial Stomatal Area; AbSD: Abaxial Stomatal Density; AdSA: Adaxial Stomatal Area; AdSD: Adaxial Stomatal Density; CAR: Carotenoid; Chl_a: Chlorophyll-a; Chl_b: Chlorophyll-b; CO₂: Internal Carbon dioxide; E: Transpiration rate; gs: Stomatal Conductance; PN: Net Photosynthetic Rate; S_Ca: Shoot Calcium; S_Cl: Shoot Chloride; S_K: Shoot Potassium; S_Mg: Shoot Magnesium; S_N: Shoot Nitrogen; S_Na: Shoot Sodium; S_P: Shoot Phosphorus; T_Ch: Total chlorophyll; WUE: Water Use Efficiency



appendages that act as limiting factors to reduce transpiration. However, CO₂ could diffuse through discrete stomatal pore (Parkhurst 1994). This is a beneficial aspect of architectural adjustments for survival. Such managed size, presence and number of stomata are critical traits not only for regulation of stomatal function but also for photosynthetic attributes. Contrary to these findings in ecotypes i.e. Sahianwala and Jahlar, freshwater ecotypes i.e. Gatwala and Treemu displayed marked diminution of abaxial and adaxial stomatal area due to salinity which leads to dropped transpiration rate under saline conditions and marked their sensitivity to salinity. Sheikhpura ecotype from industrially polluted sites presented unaffected stomatal area under salinity.

Under salinity, transpiration rate is reduced to minimize water loss and increase WUE in many macrophytes (Ashraf 2004). Decreased stomatal area contributes to maintain maximum water use efficiency thus enhancing photosynthesis (Franks and Farquhar 2007). Increased stomatal density on both abaxial and adaxial surfaces of leaf was an adaptive response to salinity in all ecotypes. Increased stomatal density enhanced number of stomata. Consequently, it reduces the diameter of stomatal pore to tackle stressed conditions that damage the plant and avoid direct contact with external harsh conditions (Jordan et al. 2008). High WUE-water use efficiency is the major determinant

among other salinity tolerance indicators observed in all ecotypes.

Higher WUE was correlated to increased stomatal density that projected greater number of sunken stomata to prevent water loss and ensuring maximum water availability for photosynthetic activity under osmotic stress due to higher salinity treatments. Sunken stomata are complementary support in survival process that prevent their direct contact with environmental harshness (Jordan et al. 2008). The considerable WUE and other photosynthetic attributes in Sahianwala and Knotti ecotypes along with enhanced stomatal attributes are adaptive components of salinity tolerance. Unaffected or least affected stomatal behavior point out successful adaptation of these ecotypes related to the modified photosynthetic parameters. This can be due to the effectiveness of stomatal closure instead of stomatal density and area (Parkhurst 1994). The salinity affected chlorophyll contents but managed photosynthetic activity in Sahianwala and Knotti ecotypes is sufficient proof of supplementary aid to avoid much metabolic loss. Similarly, ionic homeostasis in terms of different concentrations of various ions was among the prominent supporters to the survival along with WUE, g_s and stomatal density. The same had been recorded by Zhu and co-workers (Zhu et al. 2016).

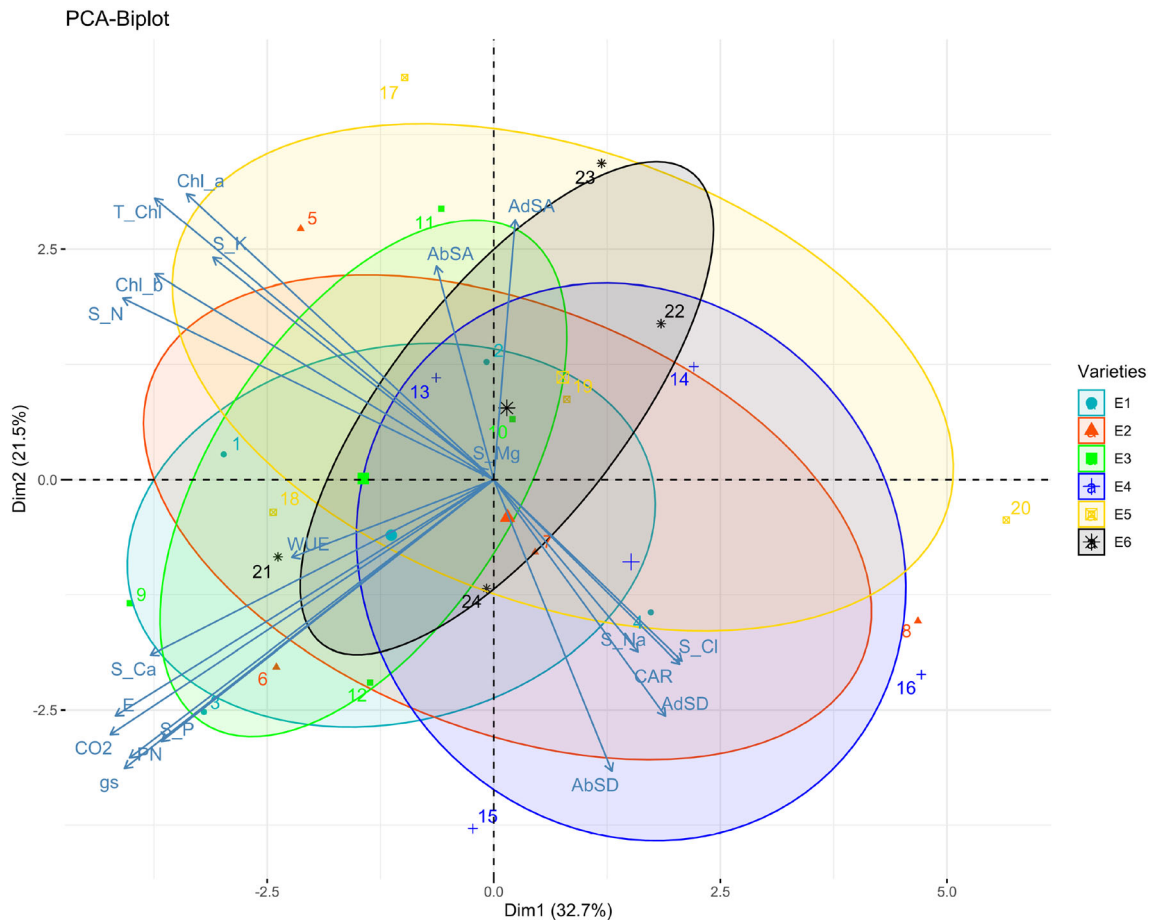


Fig. 9 Principal component analysis showing relationship between different attributes and *Typha* ecotypes under salinity stress

Different ions significantly contributed for tolerance and this is exhibited by the correlation between ionic homeostasis and stomatal functions. Maintenance of appropriate balance between ions and their optimal concentration in plants is a key player in salinity tolerance (Cuin et al. 2009). Hence, plant’s capacity to transport K^+ to the shoot and its retention in mesophyll in exchange with Na^+ would be a chief participant for maintenance of optimal K^+/Na^+ ratio in leaf that influence salinity tolerance in plants. It is on record that salinity tolerant plants maintain a balance between ionic concentrations (Shabala et al. 2013). Our findings are consistent with this fact. Overall, osmotic stress due to high salt content in root zone decreased stomatal conductance (Munns 2002) and this constriction may govern for many weeks, until explicit ion toxicity in leaves begins to play a main role. Noteworthy correlations were recorded between salinity tolerance and stomatal conductance as well as stomatal density. Ecotypes with high stomatal conductance and stomatal density have ability to show better tolerance against salt stress.

Decrease in stomatal area and vice versa for stomatal density plays crucial role in stress tolerance as decreased

area provide minimum water loss and increased density gives better stomatal conductance as well as diffusion of CO_2 for photosynthesis. These adaptations in macrophytes like *T. domingensis* allow this species to survive in areas facing lots of environmental stresses like salinity, drought, heavy metals, light and temperature that induces modifications in gas exchange characteristics to regulate photosynthesis and transpiration (Naz et al. 2010; Noman et al. 2017, 2012; Aqeel et al. 2021; Khalid et al. 2021).

Conclusion

In summary, the competitive superiority and survival of *T. domingensis* i.e. Sahianwala and Knotti ecotypes was due to its better capacity in terms of anatomical and physiological modulations. The considerable WUE, other photosynthetic pigments and attributes in Sahianwala and Knotti ecotypes along with enhanced stomatal attributes are adaptive components of salinity tolerance. Stomatal behavior in connection with physiological modulations points out successful adaptation of these ecotypes. Such

anatomical and physiological indices as observed in these ecotypes are best indicators of survival. The histo-physiological and functional relationship contributed in enhanced capability of Sahianwala and Knotti ecotypes in stress tolerance and survival, which deserves further study at molecular level.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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