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Exploring the adaptive mechanisms and strategies of various populations of *Sporobolus ioclados* in response to arid conditions in Cholistan desert

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Abstract

This study explored the drought resistance mechanisms of different populations of *Sporobolus ioclados* (Poaceae), locally known as "Sawri," "Drabhri" and "Dhrbholi" native to Africa and the Indian Subcontinent. These populations were grown in conventional nursery practices at Khawaja Fareed Government College in Rahim Yar Khan, Pakistan, and subsequently subjected to four distinct levels of drought within carefully monitored experimental settings. The experiment was conducted in a two-factorial design involving populations and drought treatments and was repeated three times. The physiological and morphological responses of *S. ioclados*, including plant height, number of roots, root length, flag leaf area, stomatal features, proline concentration and nitrogen content, displayed significant variability in response to the imposed drought stress. Drought resulted in increases in proline concentration and nitrogen content. The number of roots decreased, while the length and width of the stomata increased in various populations. A combination of advanced statistical techniques, such as ANOVA, PCA, HCA, and DFA, provided a comprehensive understanding of the mechanism of plant adaptation and the extent of population diversity within the species. The Yazman and Nwab Wala populations exhibited the highest rates of photosynthesis and stomatal conductance, while *S. ioclados* demonstrated notable drought tolerance at the T4 level of drought stress. A negative correlation was found between proline levels, nitrogen contents, and photosynthesis, suggesting that proline has a protective role in drought. The diverse adaptation strategies indicated by *S. ioclados* populations have revealed the potential of this species for afforestation and climate change mitigation in dry environments.

Keywords Cholistan desert, Drought adaptation, Plant physiology, Ecological features, Water scarcity, Proline contents, Nitrogen contents

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Introduction

The perennial grass *Sporobolus ioclados* is renowned for its resilience and ability to thrive in arid and semiarid climates. This particular species plays a crucial role in the ecological balance of desert environments by stabilizing sand dunes, serving as a source of nourishment for livestock, and promoting biodiversity [1]. The ecotype *S. ioclados* is found worldwide in a variety of dry environments. These plant species thrive particularly in the Cholistan desert of Pakistan Punjab Province. Because of its amazing adaptability *S. ioclados* is able to withstand high temperature variations and has limited rainfall characteristics in its environment [2, 3].

Understanding drought tolerance in plants such as *S. ioclados* is essential due to global warming and desertification. Various processes at different levels contribute to the ability of plants to respond to drought, resulting in improved root systems, leaf structure, osmotic adjustment, water content, and stomatal function [4]. Because of the exceptional ecological endurance of *S. ioclados*, scientists are particularly interested in how this species responds to drought conditions [5]. Owing to its ability to preserve soil and provide food for animals during times of great drought, *S. ioclados* is a very important species in the Cholistan desert in Punjab, Pakistan [6]. The ecological relevance of the species and its potential effectiveness in combating drought conditions are demonstrated by the widespread occurrence of *S. ioclados* in the area.

The Cholistan desert lies in the southern part of the province of Punjab, Pakistan, between latitudes 270 42˚ and 290 45˚ North and longitudes 690 52˚ and 750 24˚ East. This desert occupies an area of approximately 26,000 km^2 with a length of 480 km and a width of 32–192 km [7]. The climate in the Cholistan desert is arid, characterized by low and infrequent rainfall, elevated summer temperatures, low relative humidity and robust winds in summer. The mean winter temperature is 6.60˚C (December-January) and the mean summer temperature is 46.40˚C (June-July) with an absolute maximum of approximately 51˚C [8]. Despite the extreme climatic conditions, plant species are less dense. The sparse vegetation consists of xerophytic grasslands of *Eragrostis barreleri, Aristida adscensionis, Cenchrus ciliaris, Cenchrus biflorus, Cymbopogon jwarancusa, Cyperus conglomeratus, Ochthochloa compressa, Panicum antidotale, Panicum turgidum, Lasiurus scindicus, Aeluropus lagopoides and Sporobolus ioclados*. Grasses are the most common plants in the Cholistani rangelands, but perennial grasses are highly important [9]. *Sporobolus ioclados* (Poaceae), is a typical halophyte that is ponderously grazed by cattle and other livestock. *S. ioclados* is very well distributed in different climatic zones in South Asia including Pakistan's semi-deserts. It is one of the nine hard perennial grasses in the desert of Cholistan that invades and flourishes in highly alkaline sodic soils and has tremendous potential for competition [10].

The ecotype *Sporobolus ioclados* has been observed to exhibit population fragmentation throughout its distribution range. Habitat degradation is a consequence of human activities such as deforestation in addition to natural events such as protracted droughts [11]. The conservation of species and ecosystems depends on research on the distribution, ecology, and fragmentation factors of *S. ioclados*. Understanding its response to drought is key for conservation in the Cholistan desert. Research on how *S. ioclados* adapts to drought can improve restoration efforts [12]. These findings are crucial for environmentalists striving to save the ecological vegetation of the region and stop the process of desertification.

A research was carried out on eight plant species that have adapted to arid conditions, comprising a variety of grasses, shrubs, and forbs, which were subjected to three distinct levels of summer rainfall in a garden located in the Great Basin (Owens Valley, California). The findings revealed that two grass species (*Sporobolus airoides* and *Leymus triticoides*) and a salt-tolerant shrub species (*Atriplex confertifolia*) presented an increase in their photosynthetic rate and/or stomatal conductance when provided with increased water. There was a notable association between water availability and nutrient absorption in five of the eight species that were examined [13]. Research on the abiotic responses of four ecotypes in industrial drain and river sandy environments indicates that abiotic stress impacts the rate of photosynthesis [12]. Ecotypes of *S. ioclados* with increased drought resistance can increase ecosystem resilience and endurance [14, 15]. Restoration practices can improve survival and ecological effectiveness in replanted areas by selecting ecotypes with superior drought tolerance [16]. This technique led to the creation of a more robust and diversified ecosystem, while also contributing to broader conservation strategies in the Cholistan desert.

Plants exhibit various strategies to cope with drought conditions. The ability of a plant to accelerate the phases of its life cycle or flowering is known as drought escape. Drought avoidance, on the other hand, involves the ability of a plant to mitigate water loss and increase water uptake by undergoing morphological changes in the root system [17]. Physiological adjustments such as stomatal closure and decreased photosynthetic rates are key components of drought tolerance [18, 19]. This investigation explored the drought tolerance of indigenous *S. ioclados* species in the Cholistan desert, by examining their interconnected physiological and morphological features. A comprehensive understanding of ecology and the implementation of effective conservation strategies, aimed at revealing the interconnected mechanisms through which these features contribute to the ability of plants to withstand desert environments has been reported [20]. Because of variations in their habitat, the plants of the *S. ioclados* population are very susceptible to drought through changes in soil moisture and nutrient availability [21]. Programs aimed at conserving and restoring the Cholistan desert might stand to acquire a great deal of knowledge regarding drought resistance and related characteristics [9, 22], which would help to achieve ecological and conservation goals.

The present study aims to investigate the relative drought tolerance of selected populations of *S. ioclados* inhabiting different habitats of the Cholistan desert, to unearth the adaptive mechanism of drought tolerance by describing morphological and physiological parameters associated with drought tolerance, and to identify potential structural traits and physiological indicators that can be used for future studies. The study hypothesized that *S. ioclados* is resistant to drought and arid climates. This study aims to provide a scientific basis for the conservation, restoration, and research of *S. ioclados* and other arid climate species. In the context of increasing concerns about climate change, this study aims to improve the understanding of drought tolerance mechanisms for ecosystems that are resilient in the Cholistan desert.

Materials and methods

Plant material and growth conditions

The flora of the Cholistan desert consists of xerophytic species that have evolved to thrive in diverse and severe temperature, moisture, and soil conditions. The Cholistan desert is home to a distinctive array of biodiversity and geological characteristics. Since 2019, the Khawaja Fareed Government College in Rahim Yar Khan, Pakistan, has held a collection of *Sporobolus ioclados* from fifteen distinct localities (Fig. 1). Each ecotype consists of a group of plants grown from seeds collected from the Cholistan desert (Southern Punjab, Pakistan, Table 1). The *S. ioclados* populations included in this study were chosen on the basis of their potential use in afforestation under drought conditions, as they represent diverse habitats in southern Punjab. The collection was performed by adopting the standard nursery protocols, utilizing 300-cm^3 containers filled with a peat-vermiculite mixture at a 3:1 ratio (v:v).

Experimental setup and design

The seedlings were transplanted at the flowering nursery of the study site which boasts a dry thermo-Mediterranean climate characterized by hot, arid summers and mild winters, along with an average annual rainfall of 459.4 mm. The experiment was performed in a completely randomized design (CRD) with a two-factorial (populations and drought treatments) arrangement and three replications. The soil of the Cholistan desert is saline or saline sodic with pH ranging from 8.2 to 8.4 and from 8.8 to 9.6, respectively. Soil (unsterilized) was prepared by mixing peat, sand, litter and animal manure in equal quantities. The soil was weighed and 2 kg of soil were added to each pot. Tap water (unsterilized) was used for the drought treatments. The average day temperature during the experiment ranged from 40–48 °C. The drought levels maintained during the experiment were T1-Control (100% field capacity), T2-75% field capacity, T3-50% field capacity and T4-25% field capacity. The experiment was conducted in an open area, and the moisture content was maintained after samples were weighed regularly at 60 h intervals, and thereafter watered to obtain the required field capacity. No nutrients were added to the soil for experimentation, and the available nutrients in the peat, litter and animal manure were sufficient for the growth and development of *Sporobolus ioclados.* The soil moisture level was subsequently, determined by weighing the sample before and after drying it in an oven [23].

Trait assessment

A combination of soft and hard traits (morphological and physiological) [24], was measured to evaluate drought tolerance. Soft traits include the morphological features of *Sporobolus ioclados* such as plant height, the flag leaf area, number of roots, root length, and length and width of the stomata. Physiological traits include attributes such as proline content, the photosynthetic rate, stomatal conductance, and nitrogen content in the plant.

The flag leaf area was measured with a meter rod via the following formula:

- Leaf area = Maximum length x Maximum width × 0.68
- The plant fresh weight was measured instantly after the whole plant was uprooted on a portable digital balance. The plants were then held at 60 °C until a constant weight was achieved (after 6 days). The plants were then weighed on a portable digital balance.
- The total number of adventitious roots present on a single plant was counted.
- The length of the roots was measured via a scale from the root-shoot junction to the top of the roots.

Proline determination

The Lee technique was used to estimate the concentration of proline [25]. In 10 ml of 3% sulfo-salicylic acid, 0.5 g of fresh leaf tissue was pulverized and

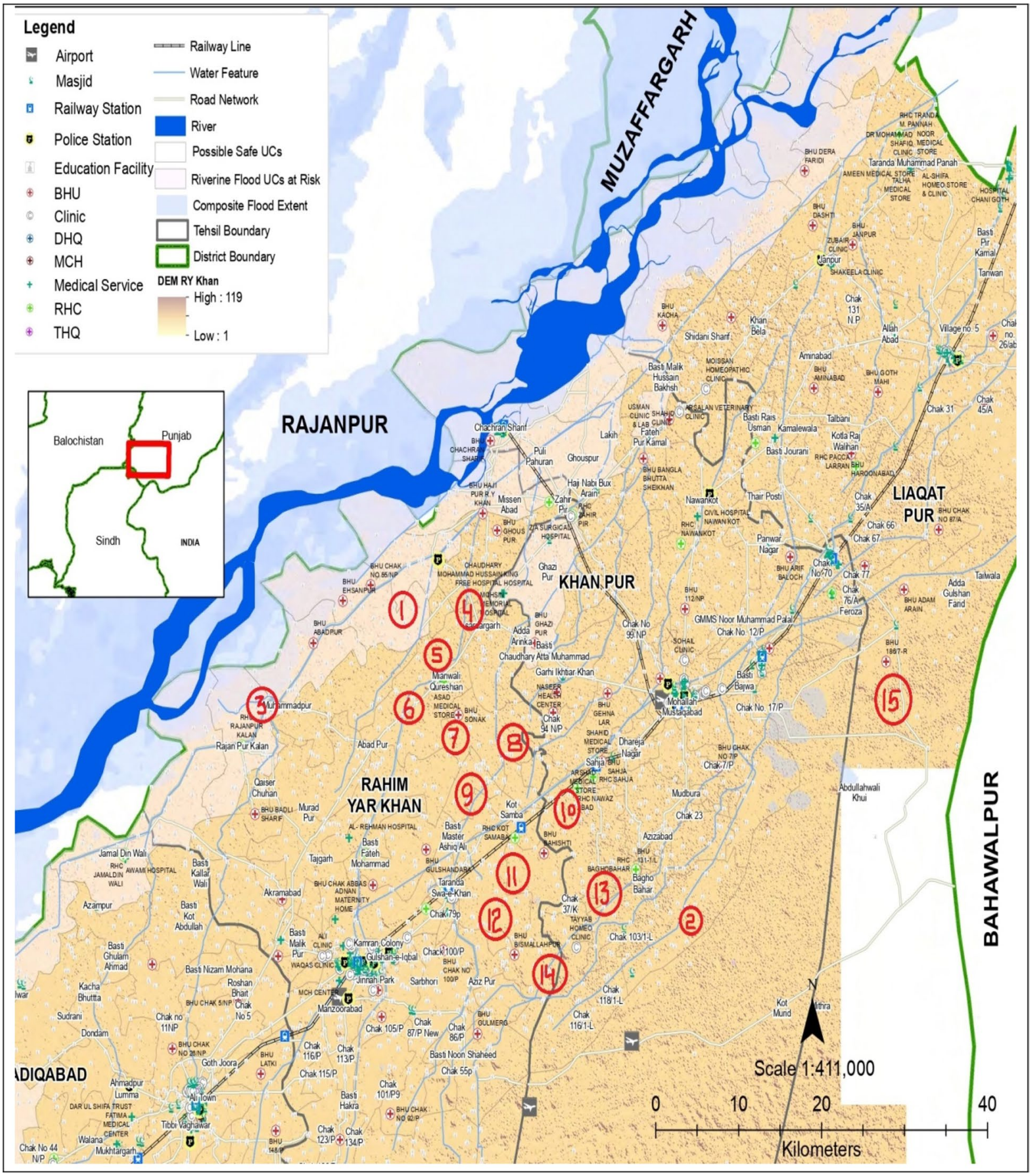

Fig. 1 Rahim Yar Khan, Punjab Cholistan desert Map showing fifteen different locations of *Sporobolus ioclados* collection sites. (1, Deen Garh; 2, Yazman; 3, Surian; 4, Nawab Wala; 5, Rahar Wala; 6, Bahoo Chahat Wala; 7, Toba Kaki; 8, Toba Kandi; 9, Chak.36; 10, Toba Angatra; 11, Toba Tharu Wala; 12, Derawar Fort; 13, Toba Maru Wala; 14, Chak. 50; 15, Cholistan Institute of Desert Studies)

homogenized. Whatman No.2 filter paper was used to filter the homogenate. In a test tube, two ml of the filtrate were combined with two ml of acid ninhydrin and two ml of glacial acetic acid for an hour at 100 °C. To cool the mixture and stop the reaction, an ice bath was used. Toluene (4 ml) was added to the reaction

Table 1 Environmental Features, locations, temperature, rainfall and coordinates in and around the Cholistan desert, Rahim Yar Khan, Pakistan

No.	Site Name	Coordinates (Approximate)	Meters above Sea Level (MSL) (m)	Average Temperature of the Warmest Month (Tmax) (°C)	Average Temperature of the Coldest Month (Tmin) (°C)	Average Annual Rainfall (AR) (mm)
1	Deen Garh	28°42′N, 70°12′E	130	42	3	100
2	Yazman	29°05′N, 71°44′E	125	42	4	120
3	Surian	28°38′N, 70°10′E	130	42	3	100
4	Nawab Wala	28°45′N, 70°15′E	130	42	3	100
5	Rahar Wala	28°40′N, 70°20′E	130	42	3	100
6	Bahoo Chahat Wala	28°42′N, 70°22′E	130	42	3	100
7	Toba Kaki	28°43′N, 70°25′E	130	42	3	100
8	Toba Kandi	28°40′N, 70°30′E	130	42	3	100
9	Chak. 36	28°35′N, 70°35′E	130	42	3	100
10	Toba Angatra	28°32′N, 70°40′E	130	42	3	100
11	Toba Tharu Wala	28°30′N, 70°45′E	130	42	3	100
12	Derawar Fort	28°46′N, 71°20′E	120	42	4	100
13	Toba Maru Wala	28°42′N, 70°50′E	130	42	3	100
14	Chak. 50	28°38′N, 70°55′E	130	42	3	100
15	Cholistan Institute of Desert Studies	29°24′N, 71°40′E	125	42	4	120

mixture. Using a vortex mixer, the reaction mixture was vigorously shaken for 1–2 min. The absorbance of the toluene containing chromophore was measured at 520 nm after it was isolated from the liquid phase and warmed at room temperature.

The amount of proline was estimated using the following formula on the basis of the fresh weight of the sample. "µmoles per gram tissue = [(µg proline/ml) x ml toluene)/115.5 µg/µmole] / [(g sample)/5]".

Photosynthetic parameters

The net CO_2 assimilation rate (A), transpiration rate (E), and rate of conductance of stomata (g) were investigated by using a portable infrared gas analyzer (open system LCA-4 ADC). To measure these parameters the fully expanded third leaf from the apex of the main tiller was chosen. The experiment was conducted from 9:00 a.m. until 11:00 a.m. with the following specifications:

403.3 mmol m-2 s-1 = molar air flow per unit leaf area; 9.9 K pa = atmospheric pressure; the pressure of water vapour entering the chamber varied between 6 and 8.9 mbar; photosynthetically active radiation (PAR) at the surface of the leaf peaked at = 1711 mmol m-2 s-1; the leaf temperature ranged from = 28.4 to 32.4°C; the CO_2 content in the surrounding air = 352.

Nitrogen estimation

After digestion, steam distillation was performed in the presence of NaOH to release free NH_4 from the solution. $(NH_4)\ SO_4 + 2NaOH$à$2NH_3 + Na_2SO_4 + 2H_2$.

Ammonium borate complex is formed when NH_3 accumulates in excess boric acid. $NH_3 + H_3BO_3$à$NH_4 + H_2\ BO_3 + 2H_2O$.

The nitrogen estimation procedure was conducted to fill the Kjeldahl apparatus with 10 ml of the filtrate. Fifteen ml of strong sodium hydroxide was mixed with the mixture. Finally, a 500 ml conical flask was used for distillation over steam into boric acid (10 ml).The procedure was continued for ten minutes.

The calculations were performed by the following method:

Weight of N in the plant sample=wg volume of 0.1 normal of H_2SO_4 =50 ml

For back titration, 0.1 normal of NaOH was utilized. = vml suppose consumption of 0.1 normal NaOH = 20 ml.

The volume of 0.1 N H_2SO_4 used in the reaction for production of NH_3 = 30 ml (volume of 0.1N NH_3 = 30 ml).

Since 100 ml of 1 N NH_3 contains = 17 g of NH_3 "OR"14 g of N 100 ml of 0.1 N of NH_3 contains = 1.7 g of NH_3 "OR" 1.4 g of N, the amount of N is = $30 \times 1.4 \div 1000 = 0.042$ g of N.

OR $0.042 \times 100\ 0.1 = 4.2\%$.

Statistical analysis

Analysis of variance (ANOVA) was used to understand how different *Sporobolus ioclados* populations reacted to drought stress. PCA was employed to reveal connections and differences among the populations, with the results displayed in a scatter plot for clear visualization. To categorize the morphological and physiological variables on the basis of their similarities and differences, we used hierarchical cluster analysis (HCA). The dendrogram's visual representation of the grouping greatly facilitated the representation of the data. The inclusion of discriminant function analysis (DFA) in our statistical approach was crucial for assessing relationships between ecotypes and their associated characteristics. The DFA coefficients reveal the relative importance of each parameter in terms of ecotype divergence, and provide the basis for how *S. ioclados* adjusts to drought. The graphs were designed via Microsoft Excel 2010 and XLSTAT.

Results

Determination of morphological characteristics

To assess the drought resistance of multiple *Sporobolus ioclados* populations, an extensive investigation was conducted to enhance comprehension of the morphological differences under various drought conditions. The results of analysis of variance (ANOVA) clearly revealed the responses of these populations to drought-induced stress.

The variations in adventitious roots, plant height, root length, flag leaf area, and stomatal width, significantly differed among the drought stress treatments, as indicated by p-values greater than 0.05. Conversely, the treatments did not have a notable effect on stem cell width, stomatal height, or stomatal width ($p > 0.05$). Shortage of moisture resulted in a significant decline in the number of roots in all the populations with increasing severity of drought. Approximately one-third of the populations responded with an increase in the initial level of water stress followed by decreasing trend whereas 20% reflected unusual behaviour but finished with a reduced height of the stomata. At the maximum level of water stress (25%), with a minimum decrease of 21.25% in the flag leaf area at the Deen Garh site was proved, the most resistant populations. Approximately 50% the populations revealed a smooth response of either an increase or decrease in stomatal size to various levels of drought (Table 2). In the present study, decreasing the water content of the soil in the pots resulted in an overall decrease in root length, and a maximum decrease of 52% compared with the respective control treatments was recorded in Bahoo Chahat Wala (Table 3A).

The variations in plant height and root length significantly differed among the drought stress treatments (Fig. 2A & B). The number of adventitious roots and flag leaf area also varied across the different study sites under the different treatment conditions. The flag leaf area was noted high at the Toba Kaki site at the highest level of drought (T4). Variations were recorded in growth of roots some selective populations at various levels of water availability (Fig. 3A, B, C & D). The Deen Garh site showed the greatest number of adventitious roots at 100% field capacity (T1) (Fig. 4A & B). The values of morphological parameters such as plant height and number of roots were also negative at the Toba Kaki and Toba Kandi sites, whereas positive associations were found at the Surian and Toba Tharu Wala sites. The Toba Tharu Wala site presented a strong negative correlation with the flag leaf area and the Toba Kandi site presented a high positive correlation with the flag leaf area (Fig. 5).

The results of the ANOVA revealed a significant decrease in the number of adventitious roots of the

Table 2 Analysis of variance (ANOVA) for different morphological and physiological parameters across different treatments of *Sporobolus ioclados*

Measurement	F-statistic	P-value
Photosynthetic Rate (PR)	13.32	0.00000112
Rate of Stomatal conductance (RSC)	24.13	0.000000374
Proline (PRO)	23.75	0.000000479
Plant height (HP)	9.60	0.00003275
Total Number of Roots (TNR)	30.66	0.00000000737
Flag Leaf Area (FLA)	6.78	0.000557
Width of Stem Cortex Cells (WSC)	1.73	0.172
Height of Stomata (HS)	0.35	0.786
Width of Stomata (WC)	0.63	0.598

Table 3 Analysis of variance (ANOVA) for root length, and number of adventitious roots of *Sporobolus ioclados* from fifteen different study sites at Cholistan desert

3A: Analysis of variance (ANOVA) for root length					
SOV	**df**	**SS**	**MS**	**F-ratio**	**SE**
Sites	14	2101.311	150.0937	21.56174**	0.761638
Treatment	3	4731.439	1577.146	226.5653**	0.393308
S x Tr	42	611.3111	14.55503	2.090906**	1.523276
Error	120	835.3333	6.961111		
Total	179	8279.394			
3B: Analysis of variance (ANOVA) for number of adventitious roots					
SOV	**df**	**SS**	**MS**	**F-ratio**	**SE**
Sites	14	2101.311	150.0937	21.56174**	0.761638
Treatment	3	4731.439	1577.146	226.5653**	0.393308
S x Tr	42	611.3111	14.55503	2.090906**	1.523276
Error	120	835.3333	6.961111		
Total	179	8279.394			

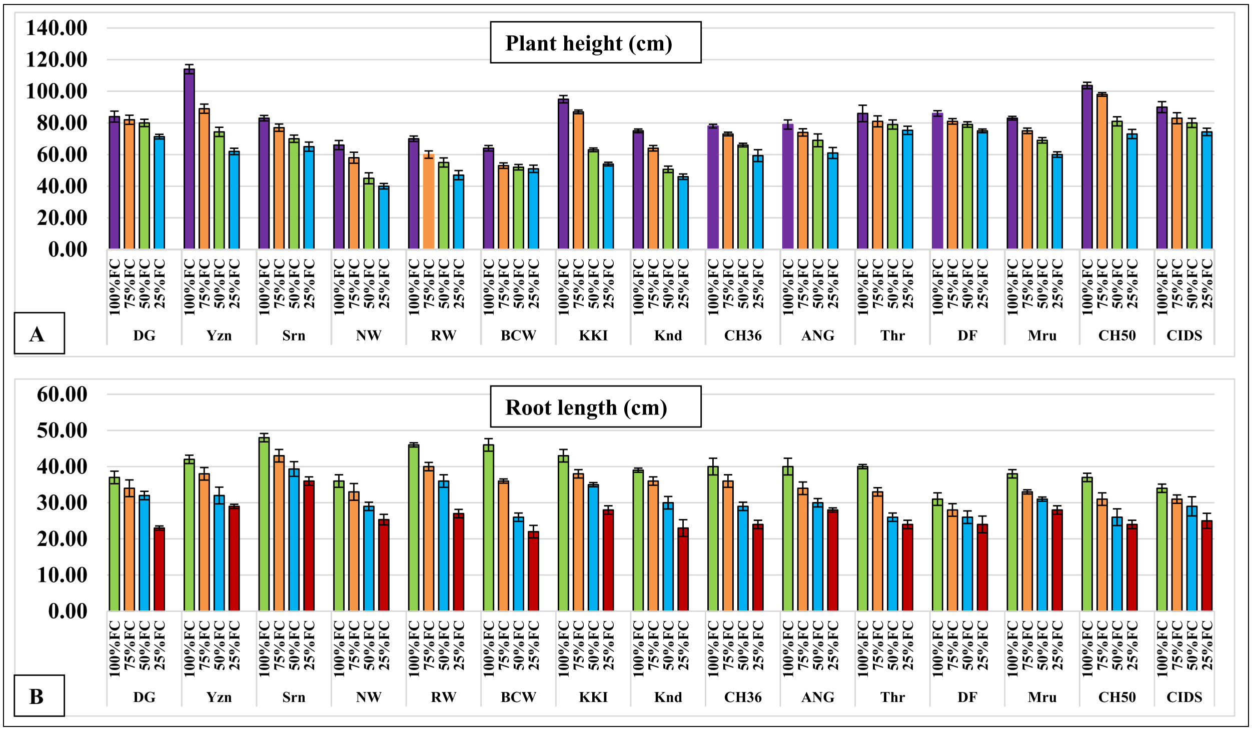

Fig. 2 **A-B** Effect of different stress levels on plant height (cm), and root length (cm), of fifteen populations of *Sporobolus ioclados* at 100, 75, 50 and 25% FC (Field capacity). (DG, Deen Garh; Yzn, Yazman; Srn, Surian; NW, Nawab Wala; RW, Rahar Wala; BCW, Bahoo Chahat Wala; KKI, Toba Kaki; Knd, Toba Kandi; CH36, Chak.36; ANG, Toba Angatra; Thr, Toba Tharu Wala; DF, Derawar Fort; Mru, Toba Maru Wala; CH50, Chak. 50; CIDS, Cholistan Institute of Desert Studies)

fifteen populations in the drought treatments (T1, T2, T3 and T4) and an interaction effect between population and treatment at p values < 0.05 and < 0.01. On the basis of the percentage decline in the number of roots the majority of the populations fell into three categories reflecting decreases b/w of 60% -70%, 70% -80%, and 80% -90% respectively at the highest level of drought (T4) compared with those in the control treatment (T1). Approximately 40% of the populations presented 70% -80% decreases in the number of roots. The maximum reduction was observed in 20% of the populations including the Rahar Wala and Toba Angatra sites. In contrast, the Yazman site revealed the lowest reduction of 50.89% at the highest level of water stress. An abrupt change in the number of roots ranging from 3.52% to 73.24% was noted at the Toba Maru Wala site (Table 3B).

Length of stomata (µm)

An inconsistent and disparate response of stomata was observed when exposed to drought. Depending upon the response to drought the under investigation populations can be categorized into four categories. About 26.67% populations at Bahoo Chaht Wala, Toba Kandi, Tobar Maru wala and Cholistan institute of desert studies (CIDS) responded with increase in height of stomata to increasing drought. They ranged between 32.68 µm in Bahoo Chahat Wala in control to 57.19 µm in Toba Kaki in T4. About 20% populations at Deen Garh, Nawab Wala and Toba Kandi showed decrease in the trait. The minimum height of stomata. i.e. 38.12 µm was recorded in Deen Garh and Nawab Wala. About one third of the populations responded with increase on initial level of water stress followed by declining trend whereas 20% reflected unusual behaviour but finished with reduced height of stomata (Fig. 6A).

Width of stomata (µm)

Width of stomata manifested a diverse response to drought stress. About 46.67% populations revealed smooth response of either increase or decrease in width of stomata to various levels of drought. The populations at Deen Garh and Nawab Wala reflected a decline in width of stomata. Nawab Wala with 65.36 µm showed minimum width of stomata in T4. The populations Bahoo Chaht Wala, Toba Kaki and Cholistan institute of desert studies (CIDS) showed increase in width of stomata to rising level of drought. In these populations width of stomata varies between 68.08 -119.82 µm. 33.33% populations revealed a smooth decrease in trait followed by an

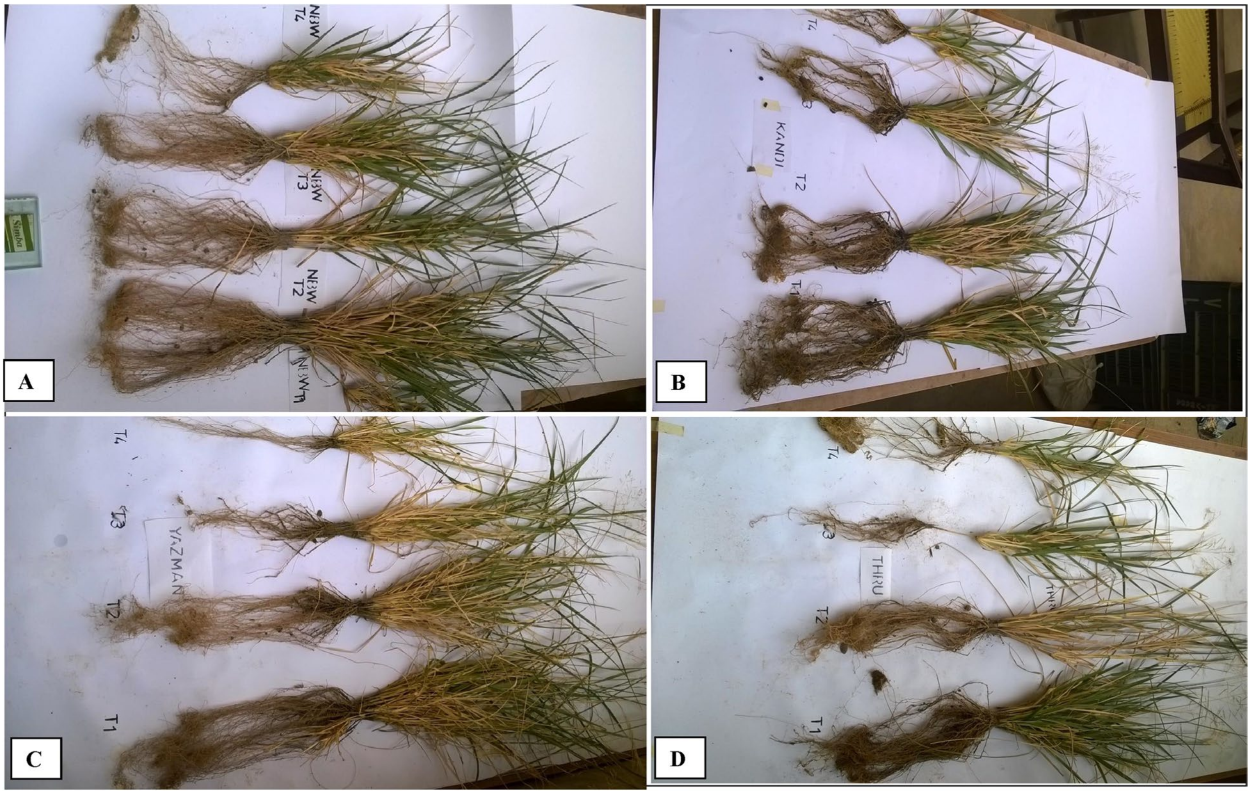

Fig. 3 **A-D** Comparative growth of roots some selective populations at various levels of water availability. Nawab Wala (**A**). Toba Kandi (**B**). Yazman (**C**), and Tob Tharu Wala (**D**)

increase in width of stomata at initial level of drought stress (Fig. 6B).

Determination of the photosynthetic rate, stomatal conductance, and proline content

The photosynthetic rate and stomatal conductance reached a maximum at the Yazman and Nawab Wala sites at 100% field capacity (T1), whereas the lowest values were recorded at the Cholistan institute of desert studies (CIDS) at 25% field capacity (T4) (Fig. 7A & B). A strong positive correlation was observed between the photosynthetic rate and stomatal conductance (0.881), indicating a synchronous increase in these variables under certain environmental conditions. On the other hand, proline was strongly negatively correlated with both the photosynthetic rate (-0.596) and stomatal conductance (-0.579), suggesting that a relatively high proline content was associated with conditions that led to a relatively low photosynthetic rate and stomatal conductance. The graphical data analysis revealed that there were significant variations in all physiological parameters across the fifteen different sites. It was noted that some parameters, such as proline levels, presented negative values at certain locations (Fig. 8).

Statistical analysis revealed significant results at $p < \underline{0}.01$ and $< \underline{0}.05$ for populations, treatments, and their interaction. The photosynthesis rate decreased with increasing drought. The Yazman and Nawab Wala sites presented the highest rates of photosynthesis at 9.860 and 9.946 μmol/m^2/s, whereas the Surian and Nawab Wala sites presented the lowest rates at 0.29 and 0.60 μmol/m^2/s under maximum drought (Table 4A).

Statistical analysis revealed significant results at $p < 0.01$ and < 0.05 for populations, treatments, and their interaction. All the populations presented decreased trait expression with increasing drought levels. In the control treatment, populations were ranked on the basis of stomatal conductance. Under extreme drought, some populations are more affected than others are. Yazman and Nawab Wala presented the greatest reduction in trait expression (Table 4B).

Proline is one of the most important osmolytes synthesized during stress conditions. The populations used in the current study expressed a significant variation between the stress treatments and among the populations. All the populations can be categorized into three groups on the basis of their differential proline synthesizing potential. The populations of

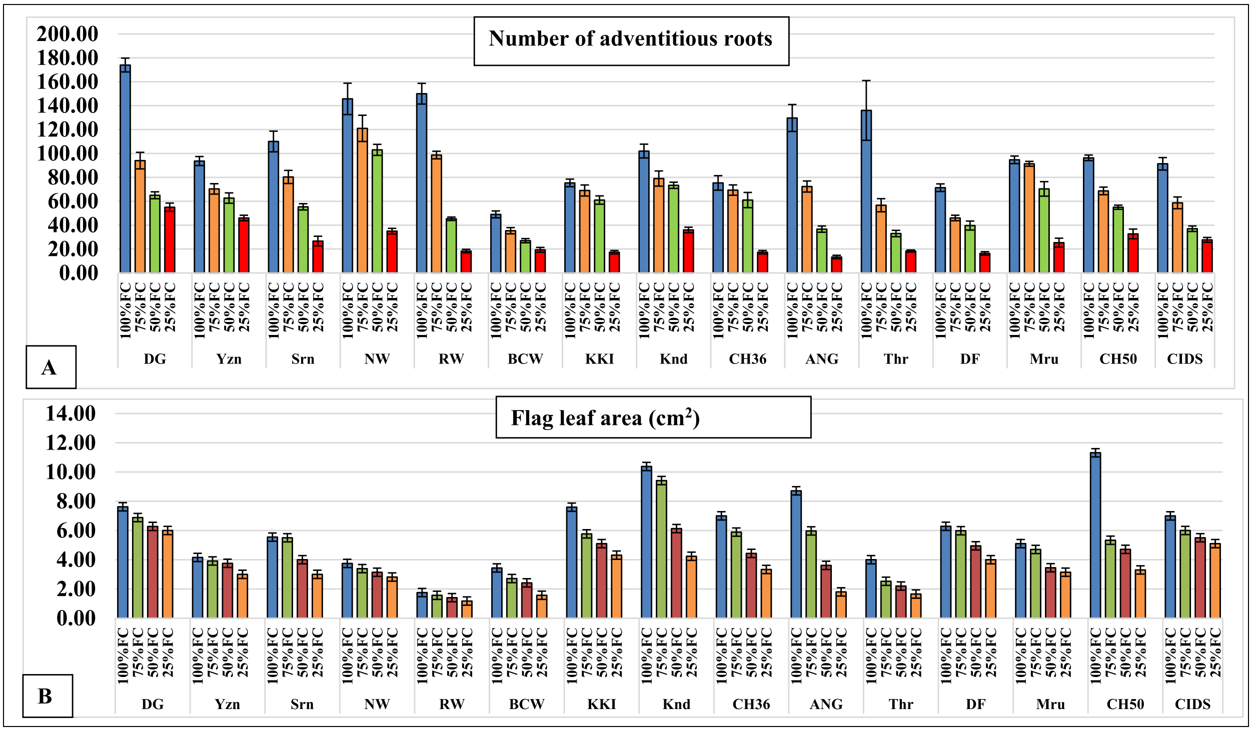

Fig. 4 **A-B** Effect of different stress levels on number of roots, and flag leaf area (cm.[2]), of fifteen populations of *Sporobolus ioclados* at 100, 75, 50 and 25% FC (Field capacity). (DG, Deen Garh; Yzn, Yazman; Srn, Surian; NW, Nawab Wala; RW, Rahar Wala; BCW, Bahoo Chahat Wala; KKI, Toba Kaki; Knd, Toba Kandi; CH36, Chak.36; ANG, Toba Angatra; Thr, Toba Tharu Wala; DF, Derawar Fort; Mru, Toba Maru Wala; CH50, Chak. 50; CIDS, Cholistan Institute of Desert Studies)

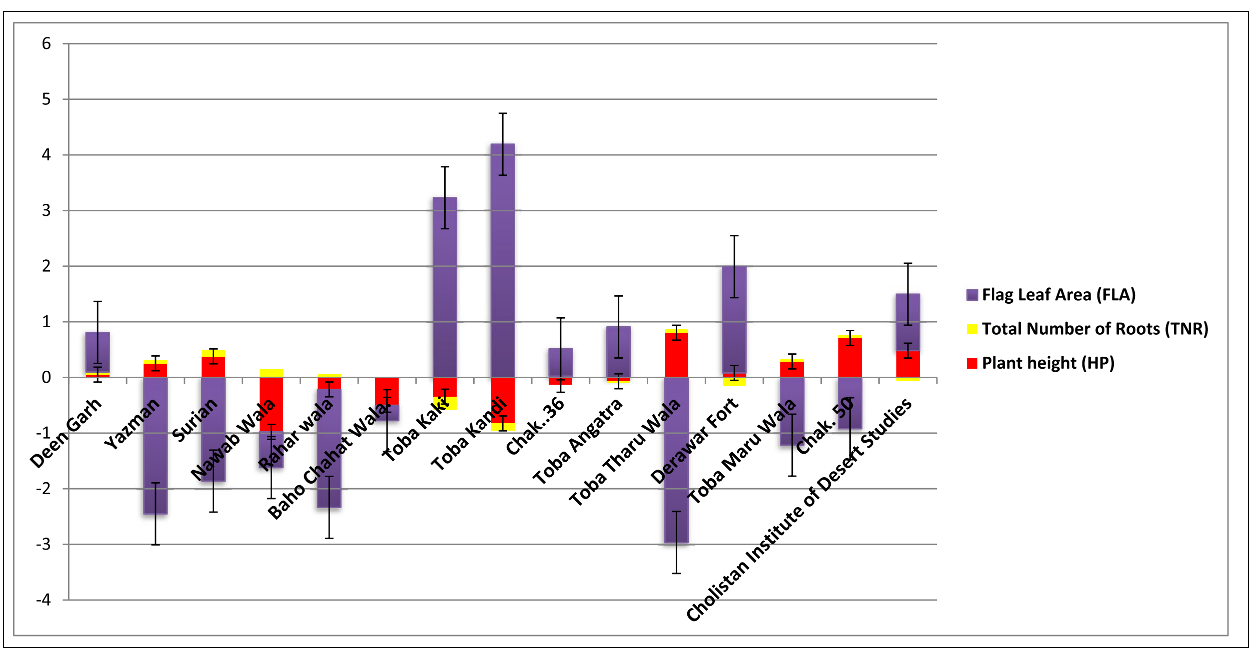

Fig. 5 Comparison of morphological characteristics of *Sporobolus ioclados* collected from the Cholistan desert at fifteen different study sites. (Flag leaf area, FLA; Total number of roots, TNR; Plant height, HP)

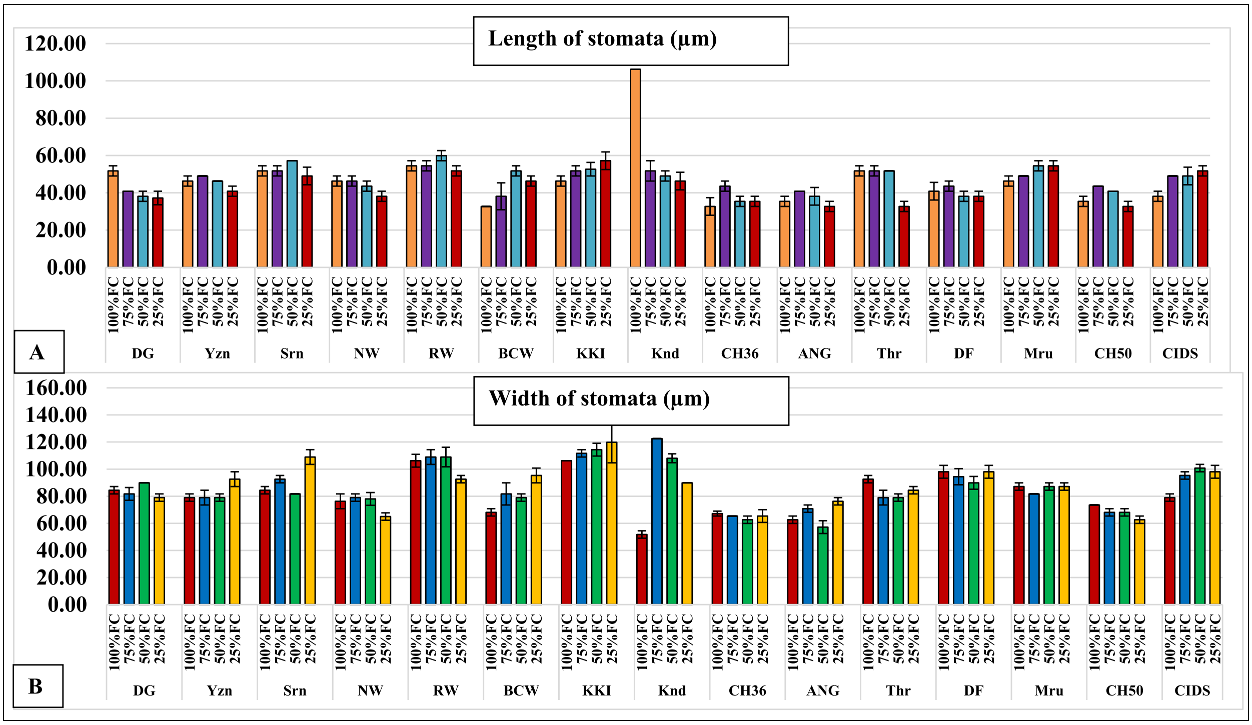

Fig. 6 **A-B** Effect of different stress levels on length of stomata (µm), and width of stomata (µm), of fifteen populations of *Sporobolus ioclados* at 100, 75, 50 and 25% FC (Field capacity). (DG, Deen Garh; Yzn, Yazman; Srn, Surian; NW, Nawab Wala; RW, Rahar Wala; BCW, Bahoo Chahat Wala; KKI, Toba Kaki; Knd, Toba Kandi; CH36, Chak.36; ANG, Toba Angatra; Thr, Toba Tharu Wala; DF, Derawar Fort; Mru, Toba Maru Wala; CH50, Chak. 50; CIDS, Cholistan Institute of Desert Studies)

Toba Kandi exhibited the lowest percentage increase in proline content i.e., 45.18% (0.239 µmol/g fresh weights in T1 v/s 0.347 µmole/g fresh weight in T4). The populations from the Deen Garh, Yazman and Surin sites showed maximum proline concentration as the water stress levels got elevated. The Yazman site showed a maximum increase in proline concentration of 299.37% (0.165 µmol/g fresh weight in T1 v/s 0.528 µmole/g fresh weights in T4). The performance of the remaining populations was between these two extremes (Fig. 9A).

Nitrogen estimation

All fifteen populations revealed an increase in nitrogen content with the increasing water stress. This increase was highly significant at different levels of drought within populations individually and among different populations. The range in nitrogen accumulation with increasing drought was observed at p values < 0.01 and < 0.05. At the maximum level of drought, the minimum increase in nitrogen content was noted in populations at Toba Tharu Wala, whereas the maximum rise in nitrogen content in the populations at Raher Wala was recorded (Fig. 9B).

Data clustering and classification

A dendrogram graphically illustrates the grouping of multiple physiological analyses based on their similarities and differences, as identified through hierarchical cluster analysis (HCA). Each vertical line in the dendrogram represents a specific cluster, and the thickness of the lines connecting clusters indicates the degree of dissimilarity between them. The ability to categorize similar traits into separate groups is a powerful approach for studying the adaptive strategies of *Sporobolus ioclados* populations in the Cholistan desert, which illustrates their unique habitat. The Derawar Fort populations which are shown in the dendrogram, are widely scattered around the plot compared to other populations.This map efficiently represents the similarities and dissimilarities among different ecotypes in a simplified style. The x-axis represents Principal Component 1 (PC1), which explains the bulk of the variation, whereas the y-axis represents Principal Component 2 (PC2), which explains the remaining variance (Fig. 10).

Each 'X' symbol on the diagram represents a singular observation, and its matching hue shows the ecotype for which that observation was recorded. Owing to their unique color patterns, *Sporobolus ioclados* populations at the Deen Garh, Yazman, Surian, and Nawab Wala sites

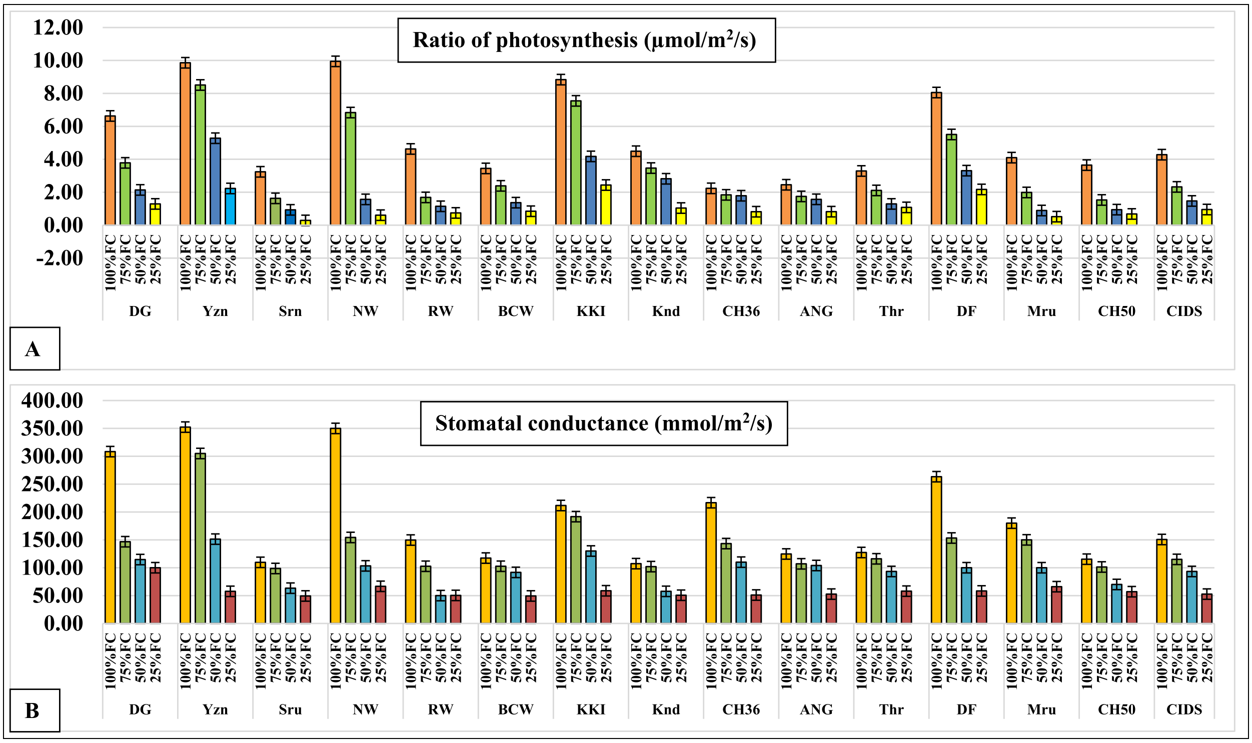

Fig. 7 **A-B** Effect of different stress levels on ratio of photosynthesis (µmol/m^2/s), and stomatal conductance (mmol/m.^{2}/s), of fifteen populations of *Sporobolus ioclados* at 100, 75, 50 and 25% FC (Field capacity). (DG, Deen Garh; Yzn, Yazman; Srn, Surian; NW, Nawab Wala; RW, Rahar Wala; BCW, Bahoo Chahat Wala; KKI, Toba Kaki; Knd, Toba Kandi; CH36, Chak.36; ANG, Toba Angatra; Thr, Toba Tharu Wala; DF, Derawar Fort; Mru, Toba Maru Wala; CH50, Chak. 50; CIDS, Cholistan Institute of Desert Studies)

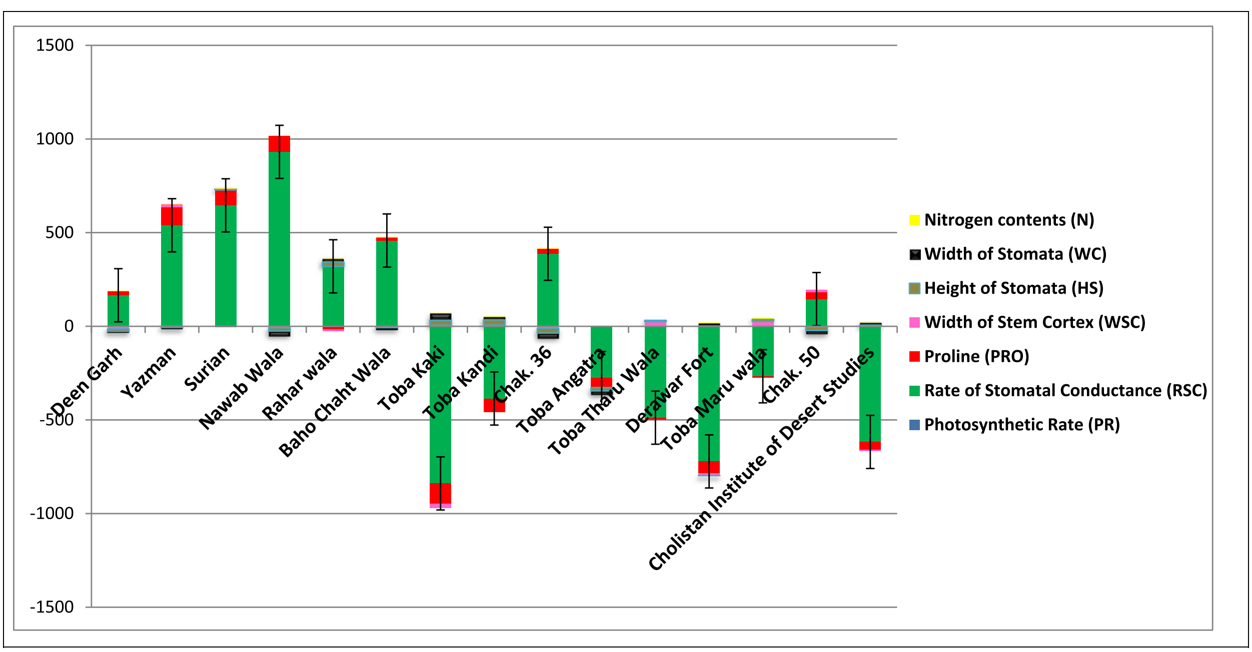

Fig. 8 Comparison of physiological characteristics of *Sporobolus ioclados* collected from Cholistan desert from fifteen different study sites. (Nitrogen contents, N; Width of stomata, WC; Height of stomata, HS; Width of Stem Cortex, WSC; Proline, PRO; Rate of stomatal conductance, RSC; Photosynthetic rate, PR)

Table 4 Analysis of variance (ANOVA) for photosynthetic rate, stomatal conductance and adventitious roots of *Sporobolus ioclados* from fifteen different study sites at Cholistan desert

4A: Analysis of variance (ANOVA) for photosynthetic rate					
SOV	**Df**	**SS**	**MS**	**F-ratio**	**SE**
Sites	14	458.6823	32.76302	8321.354**	0.018114
Treatment	3	448.7364	149.5788	37,990.94**	0.009354
S x Tr	42	169.9486	4.046395	1027.728**	0.036227
Error	120	0.472467	0.003937		
Total	179	1077.84			
4B: Analysis of variance (ANOVA) for stomatal conductance					
SOV	Df	SS	MS	F-ratio	SE
Sites	14	275,224	19,658.86	84.24422	4.409795
Treatment	3	448,456.5	149,485.5	640.5911	2.277209
S x Tr	42	206,986.6	4928.252	21.11907	8.819591
Error	120	28,002.67	233.3556		
Total	179	958,669.8			

may be readily contrasted in the PCA space. To exemplify the inherent diversity within this group, let us examine the Deen Garh population which is characterized by data points that are widely scattered around the plot. The Deen Garh populations may exhibit a significant level of phenotypic flexibility and adaptive capability due to the wide range of characteristic values. PCA (principal component analysis) significantly enhances our comprehension of the adaptation strategies of *S. ioclados* by revealing the interconnections among the many ecotypes of this species in a lower-dimensional space (Fig. 11).

Discriminant function analysis (DFA)

Through discriminant function analysis (DFA), we obtained insightful coefficients that elucidate the relationships between factors and *Sporobolus ioclados* ecotypes. The coefficients, shown in Table 5, indicate the size and direction of the observed associations.

The coefficients for the photosynthetic rate and stomatal conductance at the Toba Angatra and Bahoo chahat Wala sites are negative, whereas the coefficient for the flag leaf area is positive for both. These findings suggest a potential correlation between the efficiency of photosynthesis and the ability of stomata to regulate gas exchange, as well as the size of the flag leaf, under certain environmental conditions. The populations at the Cholistan Institute of Desert Studies and the Derawar Fort site presented contrasting coefficients for the same trait, with the Cholistan Institute of Desert Studies site displaying a

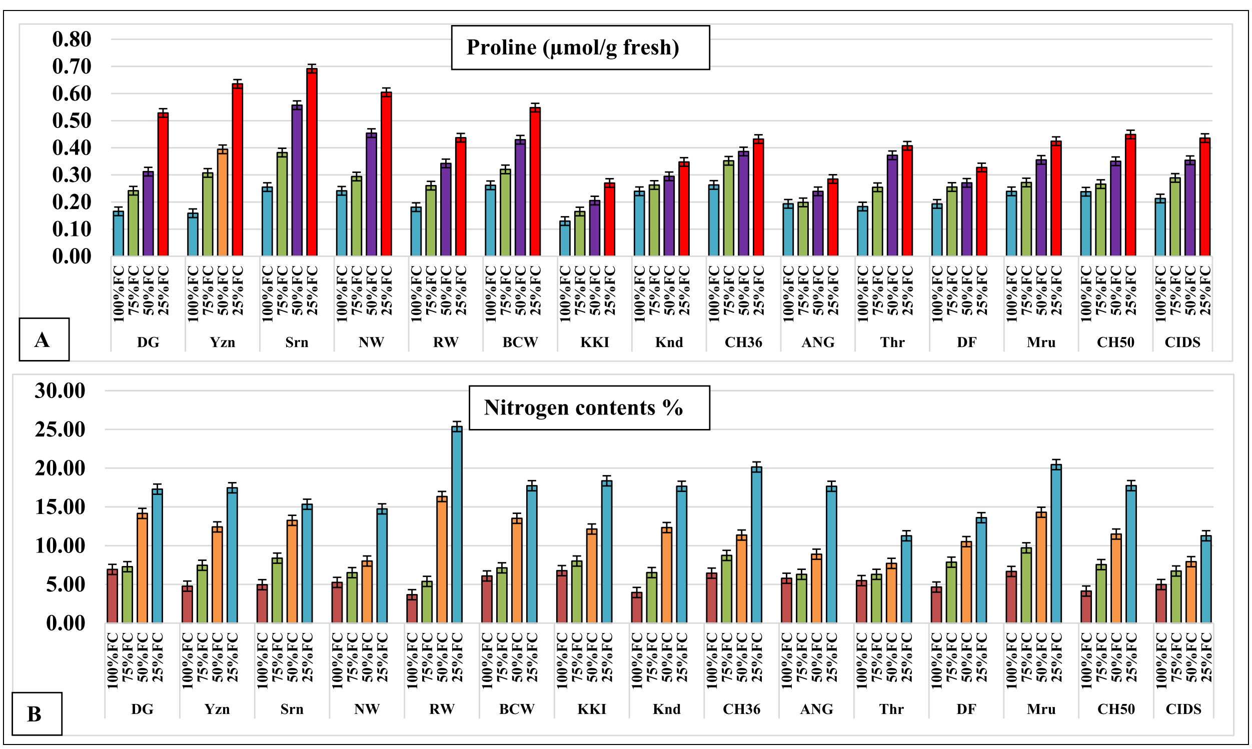

Fig. 9 A-B Effect of different stress levels on proline (µmol/g fresh), and nitrogen (N%), of fifteen populations of *Sporobolus ioclados* at 100, 75, 50 and 25% FC (Field capacity). (DG, Deen Garh; Yzn, Yazman; Srn, Surian; NW, Nawab Wala; RW, Rahar Wala; BCW, Bahoo Chahat Wala; KKI, Toba Kaki; Knd, Toba Kandi; CH36, Chak.36; ANG, Toba Angatra; Thr, Toba Tharu Wala; DF, Derawar Fort; Mru, Toba Maru Wala; CH50, Chak. 50; CIDS, Cholistan Institute of Desert Studies)

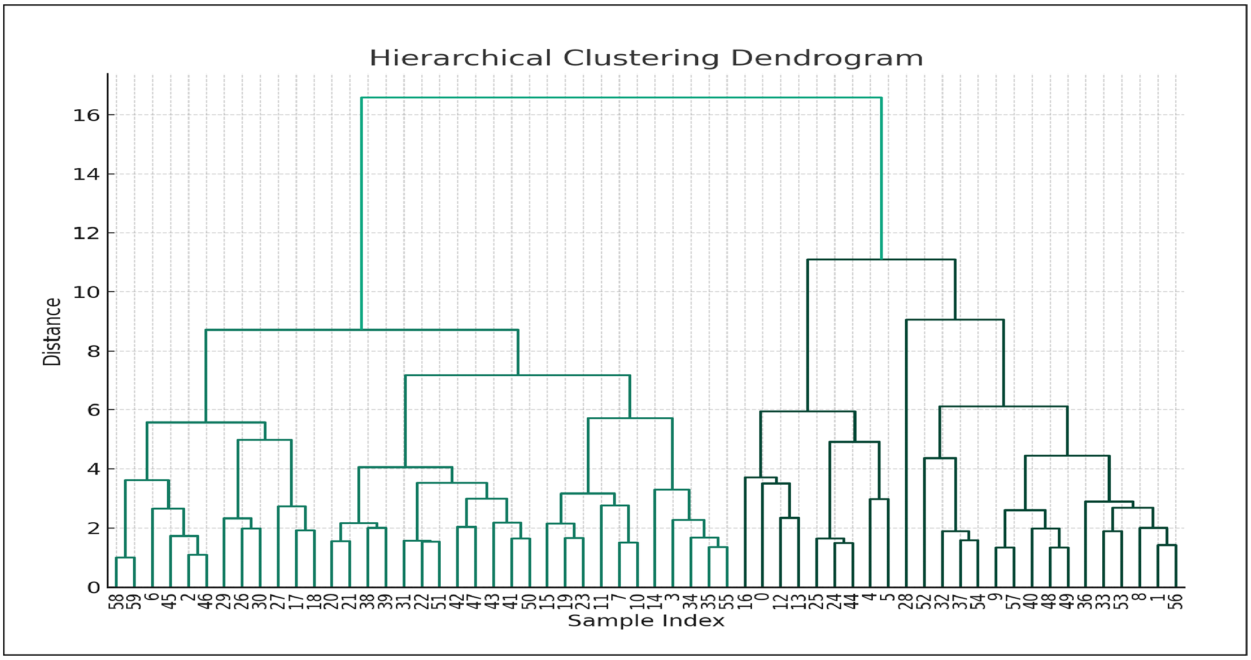

Fig. 10 Hierarchical Clustering Dendrogram (HCD) for data-set analysis of *Sporobolus ioclados* collected from the Cholistan desert at fifteen different study sites

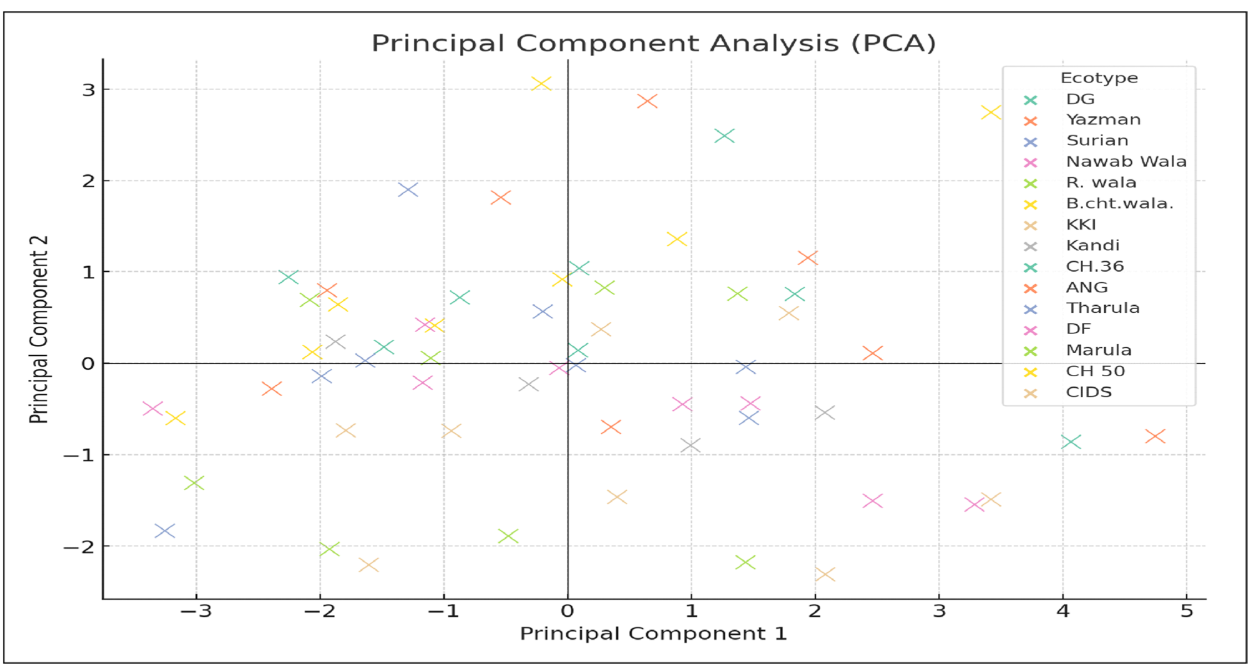

Fig. 11 Principal Component Analysis (PCA) scatter plot of different *Sporobolus ioclados* populations. (DG, Deen Garh; Yzn, Yazman; Srn, Surian; NW, Nawab Wala; RW, Rahar Wala; BCW, Bahoo Chahat Wala; KKI, Toba Kaki; Knd, Toba Kandi; CH36, Chak.36; ANG, Toba Angatra; Thr, Toba Tharu Wala; DF, Derawar Fort; Mru, Toba Maru Wala; CH50, Chak. 50; CIDS, Cholistan Institute of Desert Studies). (PC1, principal component along X axis; PC2, principal component along Y axis)

Table 5 Discriminant Function Analysis (DFA) results for *Sporobolus ioclados* depicting the coefficients associated with different measurements for various morphological and physiological parameters

Site Name	Photosynthetic Rate (PR)	Rate of Stomatal Conductance (RSC)	Proline (PRO)	Width of Stem Cortex (WSC)	Height of Stomata (HS)	Width of Stomata (WC)
Deen Garh	-1.206147	165.744850	23.295055	-12.85676	-12.332282	-5.104188
Yazman	-0.031570	539.443190	95.831798	15.453292	-5.091337	-7.689101
Surian	-3.992324	645.818634	80.601484	1.771180	4.235657	3.048342
Nawab Wala	-0.229273	931.422939	85.004641	-7.617506	-17.496045	-26.945728
Rahar wala	-1.982337	320.209986	-13.23344	-10.14197	23.071645	19.131551
Baho Chaht Wala	-0.605388	458.059811	16.030802	0.401216	-6.460517	-11.852102
Toba Kaki	5.586354	-838.775721	-109.8709	-21.73304	26.395212	38.911124
Toba Kandi	3.463396	-385.596510	-71.35609	2.825377	28.084056	16.869037
Chak. 36	-2.156461	387.284746	26.205041	-8.324765	-25.713376	-27.47038
Toba Angatra	-0.240464	-276.208616	-48.43983	-2.352337	-17.362142	-18.695058
Toba Tharu Wala	-0.306549	-487.414326	-10.35166	24.788577	6.552256	4.095808
Derawar Fort	4.058034	-721.348391	-61.99499	-12.98347	-0.237858	14.384357
Toba Maru wala	-0.036448	-266.566006	-6.778051	25.822424	10.753230	4.314866
Chak. 50	-3.366457	145.174185	36.950736	12.897991	-19.501406	-18.009499
Cholistan Institute of Desert Studies	1.045633	-617.248773	-41.89451	-7.950186	5.102907	15.010977

positive coefficient for the photosynthetic rate, whereas the Derawar Fort site presented a significantly negative coefficient. This finding highlights the variable levels of photosynthetic efficiency across different populations. The Toba Kaki and Toba Kandi were significantly associated with relatively large leaf areas, which might have contributed to their ability to tolerate drought. This is evident from the high positive coefficients observed for the flag leaf area.

The inclusion of negative coefficients for stomatal conductance and proline in the Toba Maru Wala populations, along with the positive coefficient for proline in the Nawab Wala populations, suggests that these two populations adapted different tactics in response to drought stress. Conversely, the populations at the Rahar Wala and Surian sites presented negative coefficients for flag leaf area. These two groups have distinct relationships with the photosynthetic rate and stomatal conductance. The variety of adaptive mechanisms observed suggests the related nature of these two ecotypes. The Toba Tharu Wala and Yazman site populations demonstrate different strategies for surviving drought stress. The Toba Tharu Wala populations presented a negative coefficient for the photosynthetic rate and stomatal conductance, whereas the Yazman populations presented a positive coefficient for proline. Discriminant function analysis (DFA) coefficients elucidate the intricate connections between different ecotypes of *Sporobolus ioclados* and their observable morphological and physiological characteristics, hence *revealing* viable strategies for adaptation in response to drought (Table 6).

Discussion

An insightful analysis of drought tolerance mechanisms in different *Sporobolus ioclados* populations was conducted in a recent study by researchers at Khawaja Fareed Government College in Rahim Yar Khan, Pakistan. This species demonstrates resilience across a wide spectrum of drought and salinity levels.

This ability of this plant to colonize new areas within the Cholistan desert is evidence of its ecological versatility and adds depth to the already substantial intraspecific diversity that exists within the species. The morphology [26, 27], physiology [28], and genetic variety [29], of plants are only some of the biological aspects that exhibit this type of variation. Across different populations of *Sporobolus ioclados*, there is a wide range of leaf sizes, shapes, and colours [26, 30]. Amazing physiological resistance to salt stress has been shown by this plant, with studies highlighting its capacity to maintain cellular homeostasis and photosynthetic efficiency even under severe drought and saline conditions [28]. Genetic studies conducted by Gairola et al. [29], revealed a high degree of diversity among the species, which aided their ability to adjust to and persist in shifting coastal environments. The results of a series of statistical analyses, including analysis of variance (ANOVA), principal component analysis (PCA), hierarchical cluster analysis (HCA), and

Table 6 Discriminant Function Analysis (DFA) results for *Sporobolus ioclados* depicting the coefficients associated with different measurements for various morphological parameters

Site Name	Plant height (HP)	Total Number of Roots (TNR)	Flag Leaf Area (FLA)
Deen Garh	0.051582	0.046770	0.711326
Yazman	0.253981	0.063055	-2.451011
Surian	0.379409	0.121022	-1.862615
Nawab Wala	-0.976993	0.144601	-0.642378
Rahar wala	-0.214151	0.066273	-2.121496
Baho Chahat Wala	-0.491110	-0.009397	-0.274930
Toba Kaki	-0.344746	-0.227773	3.230797
Toba Kandi	-0.825668	-0.132718	4.191518
Chak.36	-0.132224	0.023626	0.490780
Toba Angatra	-0.068148	-0.032414	0.908086
Toba Tharu Wala	0.804884	0.062588	-2.965737
Derawar Fort	0.082313	-0.154431	1.909309
Toba Maru Wala	0.287999	0.046649	-1.216802
Chak. 50	0.710068	0.048403	-0.920534
Cholistan Institute of Desert Studies	0.482803	-0.066253	1.013689

discriminant function analysis (DFA), revealed how these populations respond to various degrees of drought stress.

Morphological adaptations

The adaptation mechanisms of *Sporobolus ioclados* are evident in the diverse array of documented morphological and physiological adaptations in response to diverse drought conditions. Findings from a variance analysis revealed that drought has a substantial effect on plant height which decreased significantly with increasing moisture deficiency in all populations under study. Compared with the control population, the Toba Tharu Wala population showed a reduction of 12.40%. These results are comparable with the findings of Taleb et al. [31] and Alzoheiry et al. [32].

A shortage of moisture resulted in a significant decrease in the number of roots in all the populations with increasing severity of drought. The decrease in the number of roots ranged between 60 and 90%. Among all the populations 20% revealed a maximum reduction ranging between 80% and almost 90%. These populations include Tharu Wala and Toba Angatra sites. The reproduction of the root system requires metabolic expenditure which may constitute more than 50% of daily photosynthesis [33, 34]. A limited supply of water limits photosynthesis and meristematic activity resulting in a reduced number of roots. These results are comparable with the results of Kim et al. [35] in rice and Wasaya et al. [36]. The results are contradicting the findings of Quandahor et al. [37]. In present study decreasing the water content of the soil in the pots resulted in an overall decrease in root length. The results are comparable with the results of Slette et al. [38], and Li et al. [39].

Leaf area decreases in response to water stress to avoid the negative effects of drought. This decrease in the leaf area occurs because of the impediment in leaf expansion due to a reduction in the rate of cell division, which ultimately leads to a loss of turgor [40]. A significant decrease in flag leaf area was recorded in all the populations with increasing levels of drought stress, ranging from 0.85% to 79.34%. These results support earlier studies [41–43], which highlighted the significance of morphological changes in plants experiencing drought. The size of stomata (length and width of stomata) manifested a diverse response to drought stress. Approximately 50% of the populations revealed smooth response of either an increase or a decrease in stomatal size to various levels of drought. In 33.33% of the populations, there was no significant variation in the overall diameter of the stomata. Overall, an increase was recorded in the stomta size at most of the studied sites. These results are similar to the findings of Ouyang et al. [44] in rice, Dubey et al. [45] in cotton and Li et al. [46] in wheat.

Physiological adaptations

The photosynthetic rate, stomatal conductance, and proline concentration were the only physiological indicators that changed widely across the treatments. As documented for other plant species [43], the negative correlations between proline content, the photosynthetic rate and stomatal conductance demonstrate that proline accumulation may constitute a defensive strategy adopted by *Sporobolus ioclados* under arid environment. Our findings are consistent with those of prior studies demonstrating the impact of physiological changes in drought-stressed plants [47, 48]. The observed heterogeneity in drought tolerance among *S. ioclados* populations highlights the importance of genetic diversity in adjusting to environmental stresses [49–51].

Population variation and drought tolerance

Principal component analysis (PCA) explained the bulk of the variation in both the physiological and morphological characteristics of *Sporobolus ioclados* adopted under drought stress at Cholistan desert. PCA revealed that populations of *S. ioclados* exhibit a significant level of phenotypic flexibility or genetic variability due to the wide range of characteristic values at the study sites. Arid climates and drought can have many detrimental effects on plants, reducing transpiration rates, altering metabolism, or altering water and nutrient uptake [52]. The differences in the characteristic features of *S. ioclados* under different treatment conditions were also determined by hierarchical cluster analysis (HCA). The adaptive strategies of *S. ioclados* populations were underscored by PCA and DFA analyses, which revealed significant population diversity in their response to drought. The broad dispersion of data points in the PCA plot of the Deen Garh site population implies high phenotypic flexibility or genetic variety, which may be essential for its survival in changeable environments [53].

The DFA coefficients demonstrated possible relationships and adaptation methods by illuminating the connections between characteristics and ecotypes. For instance, in the Toba Angatra and Bahoo chahat Wala sites, populations presented negative coefficients for the photosynthetic rate and stomatal conductance, indicating a correlation between these features, which has been found in other drought-tolerant plant species [52, 54, 55]. However, the positive coefficient for flag leaf area in these ecotypes suggests that the plants are trying to grow their leaves in size, possibly to increase their ability to absorb light and produce more biomass while facing water scarcity.

Nitrogen contents

In the present investigation, a highly significant increase in nitrogen content was recorded with increasing levels of drought stress, which ranged from 5.79% to 592%. This is due to the utilization of nitrogen in the synthesis of amino acids, proteins and sugars involved in osmoprotection. These results are analogous to those of Ding et al. [56] and Iqbal et al. [57]. Drought stress restrains the growth of plants by altering their physiology and biochemistry [58]. The degree of drought tolerance depends on the existence of potential antioxidant system for scavenging reactive oxygen species (ROS) in plants [59] and certain osmolytes such as soluble sugars and proline for osmotic adjustment [60]. Nitrogen is an essential element of chlorophyll, plant proteins including enzymes, hormones and organic osmolytes. Thus it plays a significant role not only in the normal life of plants but also in their survival under drought stress. Our results are similar to a study conducted by Yousefzadeh-Najafabadi, and Ehsanzadeh (2021) [61] which revealed that drought reduced gas exchange, water relation, rooting characteristics, and shoot dry mass in the three genotypes to varying degrees, while increasing substomatal CO_2 concentration, proline concentration, and root/shoot dry mass.

Implications for afforestation and conservation

The findings of this research have significant implications for afforestation efforts in arid regions, particularly in southern Punjab, Pakistan. The success of reforestation projects in areas prone to drought heavily relies on the careful selection of plant species that are tolerant to water scarcity. By identifying ecotypes that can thrive under dry conditions and understanding their unique adaptation mechanisms, policymakers and practitioners can increase the effectiveness and sustainability of afforestation initiatives in these challenging environments. This knowledge can ultimately lead to the establishment of resilient forest ecosystems that can withstand the pressures of climate change and contribute to biodiversity conservation and ecosystem restoration efforts [62].

Conclusion

This study provides insights into the drought tolerance mechanisms and ecological versatility adopted by *Sporobolus ioclados* populations in the Cholistan desert. The considerable differences in morphological and physiological features among populations reveal their distinct adaptation mechanisms, which are critical to their survival in the Cholistan desert. The negative connection between proline levels and photosynthetic performance implies that proline plays a protective role in drought tolerance. The identification of drought-tolerant ecotypes and their unique adaptation strategies has significant implications for afforestation and conservation efforts in arid regions, particularly southern Punjab, Pakistan. These results greatly improve our understanding of drought tolerance in plants, with implications for climate change mitigation, agricultural research, and ecosystem conservation. Identifying drought-tolerant plants and understanding their adaptation processes allows us to create successful methods for recovering damaged ecosystems and fostering ecological resilience in the face of environmental instability. Further research can build upon these findings to explore the potential of *Sporobolus ioclados* in mitigating drought impacts and promoting ecosystem resilience.

Recommendations

Further studies might be conducted to investigate drought tolerance mechanisms in different plant species to identify common and distinct adaptation methods. Field studies are being conducted to confirm laboratory findings and investigate the effects of environmental variables on drought tolerance. Scientists can combine molecular and genetic approaches to better understand the underlying mechanisms of drought adaptation expand the study area to explore geographic variations in drought tolerance and adaptation strategies, and investigate the impact of climate change on drought tolerance and adaptation mechanisms in plant species.

Limitations of the study

This study examined only morphological and physiological characteristics, overlooking potential molecular and genetic mechanisms. Future research can integrate these aspects for a more comprehensive understanding. The research was limited to a specific region (southern Punjab, Pakistan). Expanding the research area can reveal more diverse adaptation strategies.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05666-5.

Supplementary Material 1.

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Authors' contributions
Abdul Rehman and Rabia Asma Memon collected the samples and designed the experiments; Mansoor Hameed and Nargis Naz analyzed the data; Anis Ali Shah worked on the contents; Ihab Mohamed Moussa and Eman A. Mahmoud helped in review and funding; Toqeer Abbas and Shifa Shaffique reviewed the literature; and designed the graph and tables. All authors have revised the manuscript.

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Availability of data and materials
The datasets or supplementary files analyzed during the study are available as supplementary files.

Declarations

Ethics approval and consent to participate
The institutional ethics council at the University of Jamshoro, Sindh, Pakistan approved each of the protocols used in this study. All of the experimental procedures used in this investigation complied with all applicable guidelines and laws. The authors state that the manuscript had never before been published.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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