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Exploring Adaptive Strategies and Resilience to Drought Stress Across Diverse Populations of Sporobolus Ioclados in the Cholistan Desert

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Abstract

The present research was carried out at the Khawaja Fareed Government College, Rahim Yar Khan, Pakistan, to check the drought tolerance mechanisms of fifteen distinct ecotypes of *Sporobolus ioclados*. These ecotypes were cultivated using regular nursery techniques, and then exposed to four different degrees of drought in a carefully monitored experimental setting. The experiment's two-factorial arrangement of populations and drought treatments was performed three times to achieve statistical significance. *Sporobolus ioclados's* plant height, number of roots, flag leaf area, stomatal features, and proline concentration all varied significantly as a result of the plant's physiological and morphological responses to drought stress. A mixture of statistical methods, including ANOVA, PCA, HCA, and DFA, offered a thorough knowledge of the plant's adaptation processes and the level of ecotype diversity within the species. Drought stress caused significant changes in morphological and physiological features, and proline content was negatively correlated with photosynthetic activity, indicating that proline protects against drought. *Sporobolus ioclados* populations have varied adaptation strategies, and the species' potential for afforestation in dry environments was shown using PCA and DFA analysis. This work significantly contributes to climate change research and ecosystem conservation by revealing drought-tolerant plants for desert places.

Introduction

The perennial grass *Sporobolus ioclados* is well-known for its hardiness and adaptation to dry and semiarid climates. This species is crucial to the health of desert ecosystems because it helps keep sand dunes in place, feeds cattle, and increases biodiversity [1]. *Sporobolus ioclados* has a broad distribution in many arid environments worldwide. This species thrives in the Cholistan Desert, located in the Punjab area of Pakistan. *Sporobolus ioclados* can withstand the high-temperature swings and lack of precipitation typical of the Cholistan Desert thanks to its remarkable flexibility [2].

Given global warming and desertification, understanding drought tolerance in plants like *Sporobolus ioclados* is crucial. This data might be utilized to protect semi-arid and desert ecosystems and create drought-resistant crops [3]. *Sporobolus ioclados*, a perennial grass, is well-known for its exceptional adaptability across a wide range of habitat types, especially in dry and semi-arid regions. Scientists interested in how plants react to drought stress have taken notice of this species due to its ecological adaptability [4]. Due to its capacity to preserve soil and provide food for animals during times of great drought, the Cholistan Desert in Punjab, Pakistan is an important habitat for *S. ioclados* [5]. The widespread distribution of *S. ioclados* in the area attests to the importance of this species ecologically and to its potential effectiveness in addressing desertification issues.

The ecotype *Sporobolus ioclados* has been discovered to have had population fragmentation across its range. Habitat degradation originates from human actions such as overgrazing, as well as natural events such as protracted droughts [6]. The *S. ioclados'* range, habitat preferences, and population fragmentation causes must be understood to conserve the species and its environment. The knowledge can help conservationists cope with climate change and growing human activities.

Sporobolus ioclados has substantial genetic variability within and across groups. Variety makes the species more adaptable to new environment. In dry and semi-arid areas, *S. ioclados* populations exhibit substantial

genetic diversity to survive and adapt to drought ecosystem [7]. This research aims to understand drought tolerance by studying *S. ioclados* genetic diversity and population structure.

Researchers have used molecular markers, biochemical structural analysis, and DNA sequencing to study these communities' genetic variance. Genetic markers and DNA variations have helped researchers explore *S. ioclados'* drought resistance mechanism [8]. Genetic research has shown the variability of this species and helped us understand its drought-resistance functions.

Studying the response of *Sporobolus ioclados* to drought is vital for the conservation and restoration efforts in the Cholistan Desert since this species has significant value. Studying the mechanisms by which *S. ioclados* adapt to drought conditions might enhance the durability and effectiveness of restoration initiatives [9]. These findings are crucial for environmentalists striving to save the region's ecological variety and halt the process of desertification.

The fact that different populations of *S. ioclados* possess unique genetic characteristics makes propagation and replanting issues prevalent. The genetic variants may impact the germination efficiency of seeds, the strength of young plants, and their performance following transplantation [10]. In order to surmount these challenges, researchers need a comprehensive understanding of the genetic variability of *S. ioclados* and its impact on the process of propagation and establishment in restoration sites.

Ecotypes of *S. ioclados* with enhanced resistance to drought conditions could be used to improve ecosystem resiliency and longevity. Amidst anticipated conditions of heightened aridity and unpredictable climate, restoration practitioners may significantly improve the chances of survival and ecological effectiveness in replanted areas by choosing ecotypes with superior drought tolerance [11]. This technique leads to the creation of a more robust and diversified ecosystem, while also contributing to the broader conservation objectives in the Cholistan Desert.

Drought tolerance, along with other interrelated physiological and morphological features, is the focus of this study on several populations of *Sporobolus ioclados* native to the Cholistan Desert. Essential for both ecological comprehension and practical conservation measures, this effort seeks to decipher the nuances of how these features orchestrate the plant's resilience to dry circumstances [12].

Genetic diversity and local adaptations likely account for the observed disparities in drought tolerance across *Sporobolus ioclados* populations [8]. The plants of *Sporobolus ioclados* population drought tolerance is strongly influenced by changes in soil moisture and nutrient availability due to variances in habitat [13]. Cholistan Desert conservation and restoration programs might benefit greatly from learning more about drought tolerance and related qualities [14], which would help achieve larger ecological and conservation goals.

The anticipation is that the findings of this investigation could provide a robust scientific basis for forthcoming activities in the areas of conservation, restoration, and research pertaining to *Sporobolus ioclados* and other species indigenous to arid climates. The purpose of this study is to enhance our understanding of the processes that enable organisms to tolerate drought and the genetic basis for these mechanisms. This information is crucial for the development of resilient and sustainable ecosystems in the

Cholistan Desert. The importance of this research is hardly overstated, as climate change increases uncertainty and the need to protect and restore biodiversity becomes more pressing.

Materials and methods

• Plant material and growth conditions

Since 2019, the Khawaja Fareed Government College in Rahim Yar Khan, Pakistan, has hosted a collection of fifteen different ecotypes of *Sporobolus ioclados*. Each consists of a group of plants grown from seeds collected from the Cholistan desert (Southern Punjab, Pakistan, Table 1). The ecotypes included in this study were chosen based on their potential use in afforestation in drought conditions, representing diverse habitats in southern Punjab. Standard nursery practices were followed to establish this collection by using 300-cm^3 containers filled with a peat-vermiculite mixture (3:1 v: v). Based on this study, we assumed nursery cultivation and its environmental effects had no significant impact on subsequent growth.

Table 1
Environmental Features of Locations in and around the Cholistan Desert, Rahim Yar Khan, Pakistan

No.	Location Name	Coordinates (Approximate)	MASL (m)	Tmax (°C)	Tmin (°C)	P (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

Key: MASL: Meters Above Sea Level, Tmax: Average Temperature of the Warmest Month, Tmin: Average Temperature of the Coldest Month, P: Average Annual Rainfall

• Experimental setup and design

The seedlings were transplanted at the flowering nursery of Khawaja Fareed Government Post Graduate College, Rahim Yar Khan, which features a dry thermo-Mediterranean climate with hot, dry summers and warm winters, and an average annual rainfall of 459.4 mm. The experiment was designed in a completely randomized pattern with a two-factorial layout (populations and drought treatments) and repeated thrice. In February 2019, the plantation was set up. The soil was sub-soiled to a depth exceeding 20 cm using a ripper with a singular tine. Following this, soil clods were disintegrated using a spring harrow and cultu2re mulcher, ensuring a smooth planting surface. The drought levels maintained throughout the experiment were: T1-Control (100% field capacity), T2-75% field capacity, T3-50% field capacity, and T4-25% field capacity.

• Traits assessment

A combination of soft and hard traits [15], was measured to evaluate drought tolerance. Soft traits consisted of the anatomical-morphological features of *Sporobolus ioclados* such as the area of the flag leaf, total number of adventitious roots per plant, height of the plant, length and width of stomata, the thickness of the stem's sclerenchyma, and the cortex's thickness in the root. Physiological traits, considered as “hard” traits, encompassed attributes like the contents of proline in the plant.

Statistical Analysis

Several statistical analyses were performed in our extensive research of drought tolerance across distinct *Sporobolus ioclados* populations in order to figure out the complex correlations between morphological and physiological features under varying drought circumstances.

An Analysis of Variance (ANOVA) was crucial in our study as it provided insight into the varying reactions of *Sporobolus ioclados* populations to drought stress. The physical characteristics of plant height, number of roots, and flag leaf area showed significant changes across various drought treatments. PCA was used to elucidate the links and distinctions among the various *Sporobolus ioclados* ecotypes. The results were visually represented using a scatter plot, showcasing the findings in an aesthetically pleasing manner. This analysis effectively caught the fundamental distinctions and resemblances among the ecotypes. In order to categorize the variables based on their similarities and differences, we used Hierarchical Cluster Analysis (HCA). The dendrogram's visual representation of the grouping greatly facilitated the comprehension of the data.

The inclusion of Discriminant Function Analysis (DFA) in our statistical approach was crucial as it offered a quantitative assessment of the relationships between ecotypes and their associated characteristics. DFA coefficients reveal the relative importance of each parameter in ecotype divergence, allowing us to deduce how *Sporobolus ioclados* adjusts to drought. All analyses were rigorous, ensuring the accuracy of our findings and permitting meaningful interpretations and discussions.

Results

1. Variation in Morphological and Physiological Traits

In order to evaluate the drought tolerance of several *Sporobolus ioclados* populations, a comprehensive study was done to better understand the variations in morphological and physiological parameters across a range

of drought circumstances. The results of an ANOVA showed clearly how these groups reacted to drought stress.

Photosynthetic rate, stomatal conductance, and proline concentration varied greatly between drought stresses treatments. This was shown by p-values that were considerably lower than 0.05. Varying populations of *Sporobolus ioclados* exhibited distinct responses to drought stress, which were evident in the proline concentration, a key indicator of drought tolerance. The drought treatments had a significant influence on the physical characteristics of the plants, including their height, number of roots, and flag leaf area. This was confirmed by the ANOVA analysis, which revealed very low p-values. The findings demonstrate the response of *Sporobolus ioclados* to drought, as shown by alterations in its growth and structural attributes. These changes may impact its survival and adaptive capabilities. The treatments did not have a significant effect on stem cell width, stomatal height, and stomatal breadth ($p > 0.05$). The lack of a statistically significant disparity between the two groups might suggest that the attributes being examined are more resilient to drought, or that the range of drought conditions used in this study did not provoke a discernible reaction from the characteristics under investigation (Table 2).

Table 2
ANOVA of all measurements and comparing across different treatments

Measurement	F-statistic	P-value
Photosynthetic Rate	13.32	0.00000112
Stomatal conductance	24.13	0.000000374
Proline	23.75	0.000000479
Plant height	9.60	0.00003275
Number of Roots	30.66	0.00000000737
Flag Leaf Area	6.78	0.000557
Width of Stem Cortex Cells	1.73	0.172
Height of Stomata	0.35	0.786
Width of Stomata	0.63	0.598

Key: Hp (Plant height), FLA (Flag leaf area), TNR (Total number of roots), LS (Length of stomata) WC (width of stomata), RPS (Rate of photosynthesis), RSC (Rate of stomatal conductance), PRO (Proline)

2. Correlation among response variables

Photosynthetic Rate, Stomatal Conductance, and Proline

A striking positive correlation was observed between Photosynthetic Rate and Stomatal Conductance (0.881), indicating a synchronous increase in these variables under certain environmental conditions. Proline, on the other hand, demonstrated a strong negative correlation with both Photosynthetic Rate (-0.596) and Stomatal Conductance (-0.579), suggesting that higher proline content is associated with conditions that lead to a lower photosynthetic rate and stomatal conductance.

The graphical data shows that the variations were present at different nine sites in all physiological parameters and the values of some parameters were negative at some sites like proline (Fig. 1). The values of morphological parameters were also found negative at some study sites (Fig. 2).

3. Data clustering and classification

A dendrogram visually represents the clustering of several variables according to their similarities and dissimilarities, as determined by the results of a Hierarchical Cluster Analysis (HCA). Every vertical line in the dendrogram corresponds to an individual cluster, and the width of the connecting lines between clusters shows the extent of their dissimilarity. The capacity to classify analogous attributes into distinct categories is a potent methodology for investigating the adaptive mechanisms of *Sporobolus ioclados* populations in the Cholistan Desert, which are tailored to their distinctive environment.

Principal Component Analysis (PCA) Graphical representation of data points on a coordinate plane, where each point represents the values of two variables. This map efficiently represents the relationships and fluctuations among different ecotypes in a simplified style. The x-axis represents Principal Component 1 (PC1), which explains the bulk of the variation, while the y-axis represents Principal Component 2 (PC2), which explains the remaining variance (Fig. 3).
Each 'X' symbol on the diagram represents a singular observation, and its matching hue shows the ecotype for which that observation was recorded. Due to their unique color patterns, ecotypes such as DG, Yazman, Surian, and Nawab Wala may be readily contrasted in the PCA space. To exemplify the inherent diversity within this group, let us examine the DG ecotype, characterized by its data points that are widely scattered around the plot. The DG ecotype may exhibit a significant level of phenotypic flexibility or genetic variety due to the wide range of characteristic values it exhibits. The PCA analysis significantly enhances our comprehension of the adaptation strategies and genetic variability of *Sporobolus ioclados* by illustrating the interconnections among the many ecotypes of this species in a lower-dimensional space (Fig. 4).

Discriminant Function Analysis (DFA)

Through the use of DFA, we obtained insightful coefficients that elucidate the relationships between factors and *Sporobolus ioclados* ecotypes. The coefficients, shown in Table 3, indicate the size and direction of the observed associations.

Table 3
Discriminant Function Analysis (DFA) results depicting the coefficients associated with different measurements for various ecotypes

Site Name	Photosynthetic Rate	Stomatal Conductance	Proline	Width of Stem Cortex	Height of Stomata	Width of Stomata
ANG	-0.240464	-276.208616	-48.439837	-2.352337	-17.362142	-18.695058
B.cht.wala.	-0.605388	458.059811	16.030802	0.401216	-6.460517	-11.852102
CH 50	-3.366457	145.174185	36.950736	12.897991	-19.501406	-18.009499
CH.36	-2.156461	387.284746	26.205041	-8.324765	-25.713376	-27.47038
CIDS	1.045633	-617.248773	-41.894519	-7.950186	5.102907	15.010977
DF	4.058034	-721.348391	-61.994991	-12.983478	-0.237858	14.384357
DG	-1.206147	165.744850	23.295055	-12.856764	-12.332282	-5.104188
KKI	5.586354	-838.775721	-109.870953	-21.733043	26.395212	38.911124
Kandi	3.463396	-385.596510	-71.356097	2.825377	28.084056	16.869037
Marula	-0.036448	-266.566006	-6.778051	25.822424	10.753230	4.314866
Nawab Wala	-0.229273	931.422939	85.004641	-7.617506	-17.496045	-26.945728
R. wala	-1.982337	320.209986	-13.233441	-10.141978	23.071645	19.131551
Surian	-3.992324	645.818634	80.601484	1.771180	4.235657	3.048342
Tharula	-0.306549	-487.414326	-10.351668	24.788577	6.552256	4.095808
Yazman	-0.031570	539.443190	95.831798	15.453292	-5.091337	-7.689101

Key: Hp (Plant height), FLA (Flag leaf area), TNR (Total number of roots), LS (Length of stomata) WC (width of stomata), RPS (Rate of photosynthesis), RSC (Rate of stomatal conductance), PRO (Proline)
In the ANG and B.cht.wala ecotypes, the coefficients for Photosynthetic Rate and Stomatal Conductance are negative, whereas the coefficient for Flag Leaf Area is positive in both. This suggests a potential trade-off 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 ecotypes CIDS and DF exhibit contrasting coefficients for the same trait, with CIDS displaying a positive coefficient for Photosynthetic Rate, whereas DF has a significantly negative coefficient. This demonstrates the variable levels of photosynthetic efficiency across different ecotypes. The KKI and Kandi ecotypes have a significant association with larger leaf areas, which might contribute to their ability to tolerate drought. This is evident from the high positive coefficients observed for the Flag Leaf Area.

The presence of negative coefficients for Stomatal Conductance and Proline in the Marula ecotype, together with the positive coefficient for Proline in the Nawab Wala ecotype, indicates that these two ecotypes use distinct strategies when faced with drought stress. The R. wala and Surian ecotypes both have negative

coefficients for the Flag Leaf Area. However, these two groups show distinct relationships with Photosynthetic Rate and Stomatal Conductance. The variety of adaptive mechanisms seen suggests the intricate nature of these two ecotypes. The Tharula and Yazman ecotypes demonstrate different strategies for surviving drought. The Tharula ecotype does this by having a negative coefficient for Photosynthetic Rate and Stomatal Conductance, whereas the Yazman ecotype achieves this via a positive coefficient for Proline. DFA coefficients elucidate the intricate connections between different ecotypes of Sporobolus ioclatus and their observable morphological and physiological characteristics, hence uncovering viable strategies for adaptation in response to drought (Table 4).

Table 4
Discriminant Function Analysis (DFA) results depicting the coefficients associated with morphological measurements for various ecotypes

Site Name	Plant Height	Number of Roots	Flag Leaf Area
ANG	-0.068148	-0.032414	0.908086
B.cht.wala.	-0.491110	-0.009397	-0.274930
CH 50	0.710068	0.048403	-0.920534
CH.36	-0.132224	0.023626	0.490780
CIDS	0.482803	-0.066253	1.013689
DF	0.082313	-0.154431	1.909309
DG	0.051582	0.046770	0.711326
KKI	-0.344746	-0.227773	3.230797
Kandi	-0.825668	-0.132718	4.191518
Marula	0.287999	0.046649	-1.216802
Nawab Wala	-0.976993	0.144601	-0.642378
R. wala	-0.214151	0.066273	-2.121496
Surian	0.379409	0.121022	-1.862615
Tharula	0.804884	0.062588	-2.965737
Yazman	0.253981	0.063055	-2.451011

Key: Hp (Plant height), FLA (Flag leaf area), TNR (Total number of roots), LS (Length of stomata) WC (width of stomata), RPS (Rate of photosynthesis), RSC (Rate of stomatal conductance), PRO (Proline)

Discussion

An insightful analysis of drought tolerance mechanisms in different *Sporobolus ioclados* ecotypes is provided by a recent study undertaken by researchers at Khawaja Fareed Government College in Rahim Yar Khan, Pakistan. It can withstand a broad range of salinity levels. This plant's capacity to colonize new areas within the Cholistan Desert is evidence of its ecological versatility and adds depth to the already substantial

intraspecific variety that exists within the species. The plant's morphology [16] [17], physiology [18], and genetic variety [19], are only some of the biological aspects that exhibit this sort of variation. Across different populations of *S. ioclados*, there is a wide range of leaf size, shape, and color [16]. 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 in severely salinated settings [18]. Genetic studies conducted by [19], revealed a high degree of diversity among the species, which aided its ability to adjust to and persist in shifting coastal environments. The results obtained from a series of statistical analyses, including Analysis of Variance (ANOVA), Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), and Discriminant Function Analysis (DFA), offer a solid grasp of how these ecotypes respond to various degrees of drought stress.

Morphological and Physiological Adaptations

Sporobolus ioclados's sophisticated adaptation mechanisms are on display in the wide range of reported morphological and physiological changes in response to varying drought conditions. Results from an analysis of variance showed that drought has a significant effect on plant height, root number, and flag leaf area, all of which are crucial to the plant's adaptation to drought [15]. These results corroborate earlier studies [20], which highlighted the significance of morphological changes in plants experiencing drought.

Photosynthetic rate, stomatal conductance, and proline concentration were only a few of the physiological indicators that showed wide change across treatments. As has been documented for other plant species [21], the negative connection between proline content and both photosynthetic rate and stomatal conductance implies that proline accumulation may be a defensive strategy adopted by *Sporobolus ioclados* under drought stress.

Ecotypes Variation and Drought Tolerance

The genetic diversity and adaptation potential within *Sporobolus ioclados* populations were highlighted by the PCA and DFA analyses, which demonstrated considerable ecotype variation in response to drought. The DG ecotype's broad dispersion of data points in the PCA plot implies high phenotypic flexibility or genetic variety, which may be essential for its survival in changeable settings [22].

The DFA coefficients demonstrated possible trade-offs and adaptation methods by illuminating the connections between characteristics and ecotypes. For instance, the ANG and B.cht.wala ecotypes had negative coefficients for photosynthetic rate and stomatal conductance, indicating a trade-off between these features, which has been found in other drought-tolerant plant species [23]. However, the positive coefficient for flag leaf area in these ecotypes suggests that the plants are trying to grow their leaves in size, maybe to increase their ability to absorb light and produce more biomass while facing water scarcity.

Implications for Afforestation and Conservation

Implications for afforestation initiatives in dry areas, especially in Southern Punjab, Pakistan, are substantial as a consequence of this research. Selecting appropriate plant material for reforestation operations in drought-prone locations benefits greatly from the identification of drought-tolerant ecotypes and knowledge of their adaptation mechanisms [24].

Declarations

Ethical Acceptance and Participation

The institutional ethics council at the University of Jamshoro, Sindh, Pakistan gave its approval to 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.

Statement regarding plant guidelines

Research regarding this manuscript was conducted inside Institute of Plant Sciences, University of Sindh, Pakistan and no animal or plant was harmed and no work was performed on new germline or new species. Furthermore, the plants were treated after the approval and guidelines from the departmental permission and standard methods were used to analyze the plant materials.

Consent for Publication

Not Applicable

Permission to publish

All participants granted their approval for the textual information (the "Material") to be published in the aforementioned article and journal. For the publication of this manuscript, all authors have provided their written approval.

Competing Interest

All authors declare that there are no competing interests.

Voucher specimens

Plant specimens were identified by Dr. Iftikhar Ahmad associate professor of Botany, University of Sargodha, Sargodha, Pakistan and Dr. Mansoor Hameed, Professor at University of Agriculture Faisalabad. Voucher specimens are available at Institute of Plant Sciences, University of Sindh, Pakistan, P1828-P1840/R1002.

Authors' contributions

Abdul Rehman and Rabia Asma Memon collected the samples, designed the experiments, Mansoor Hameed and Nargis Naz analyzed the data. Shifa Shaffique and Anis Ali Shah worked on contents, Mansour K. Gatasheh and Toqeer Abbas reviewed the literature, designed the graph and tables; all authors 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 file.

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Figures

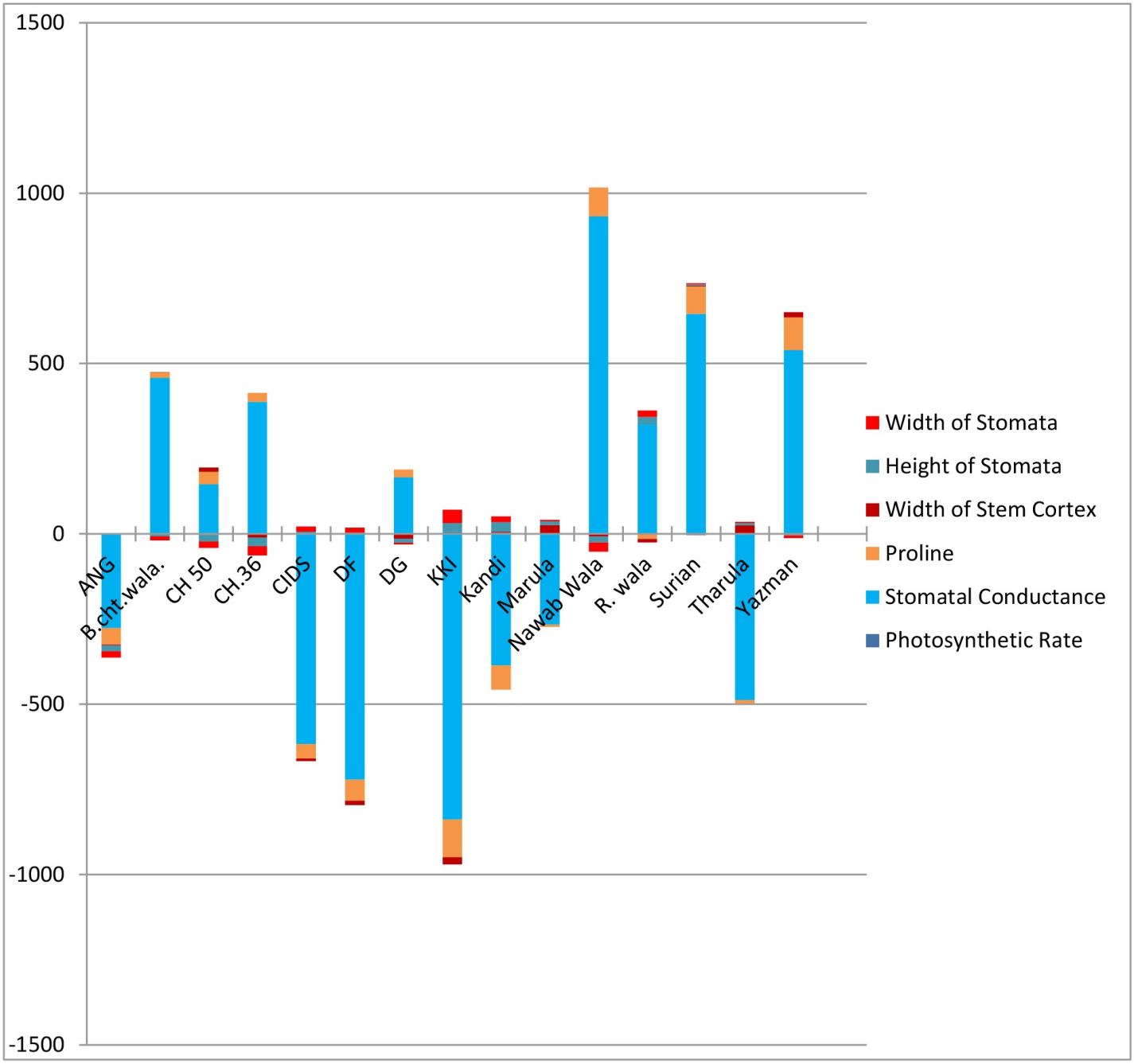

Figure 1

Physicochemical characteristics of *Sporobolus ioclados* in fifteen different study sites

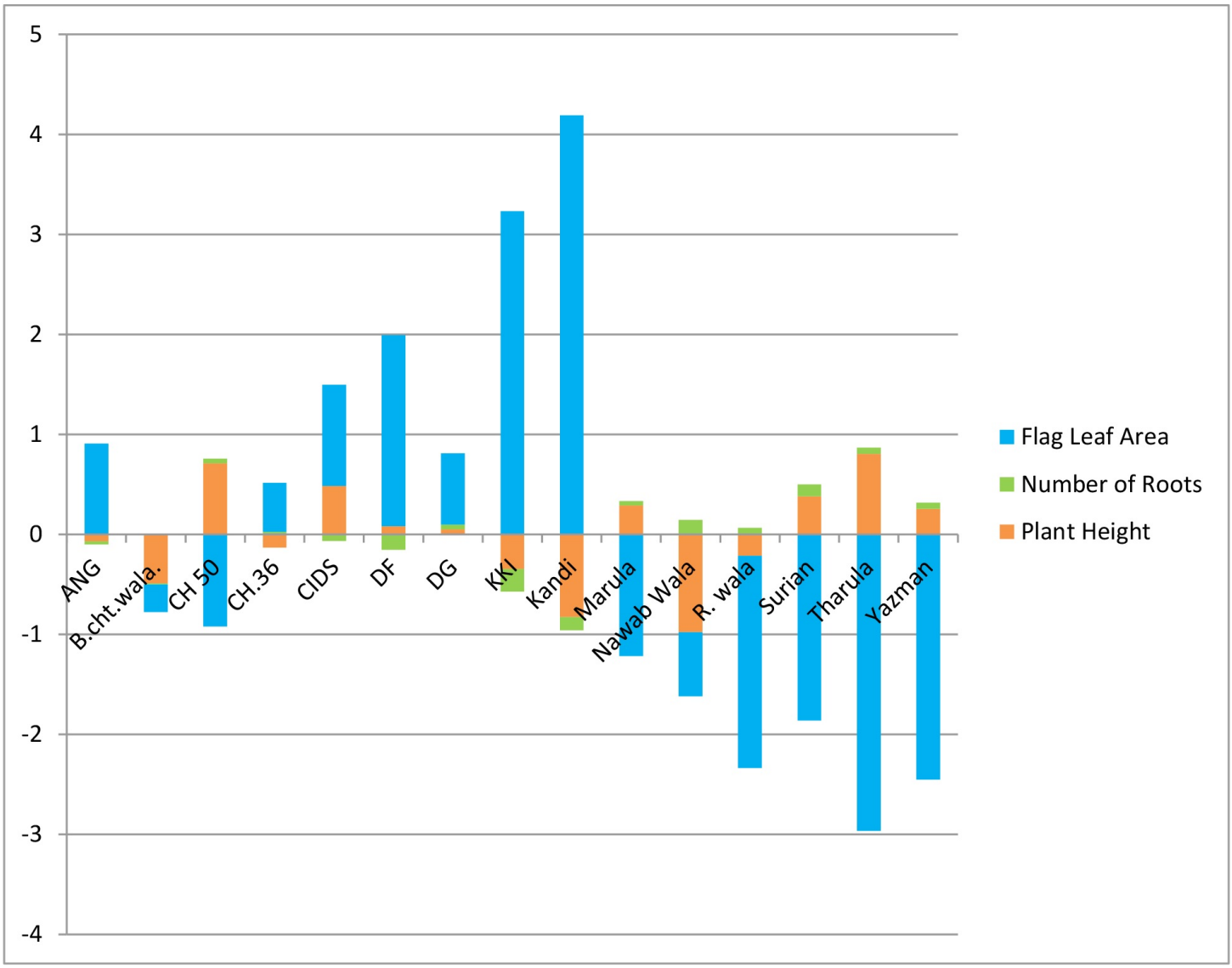

Figure 2

Morphological characteristics of *Sporobolus ioclados* in fifteen different study sites

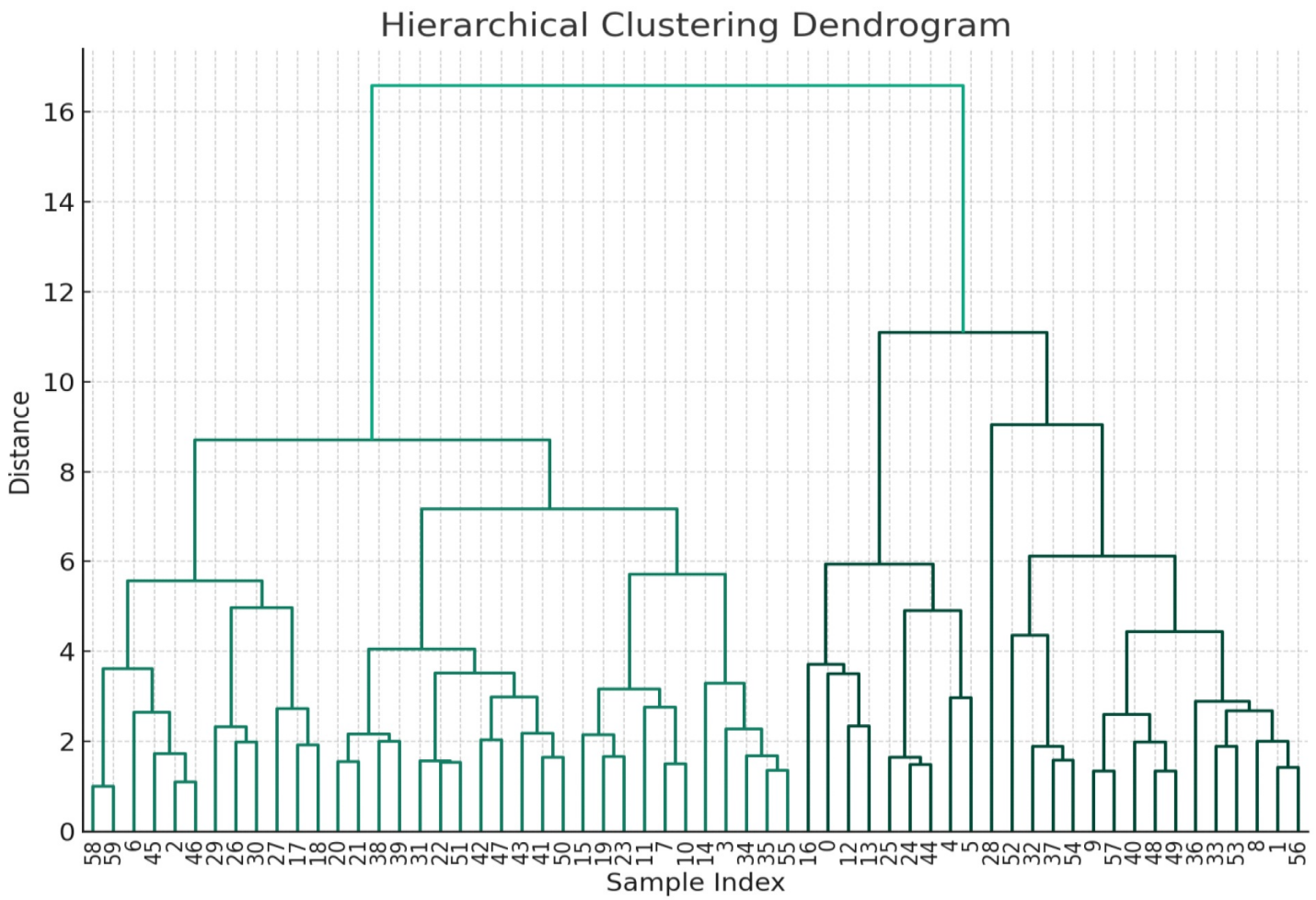

Figure 3

1Hierarchical Clustering Dendrogram for Dataset Analysis

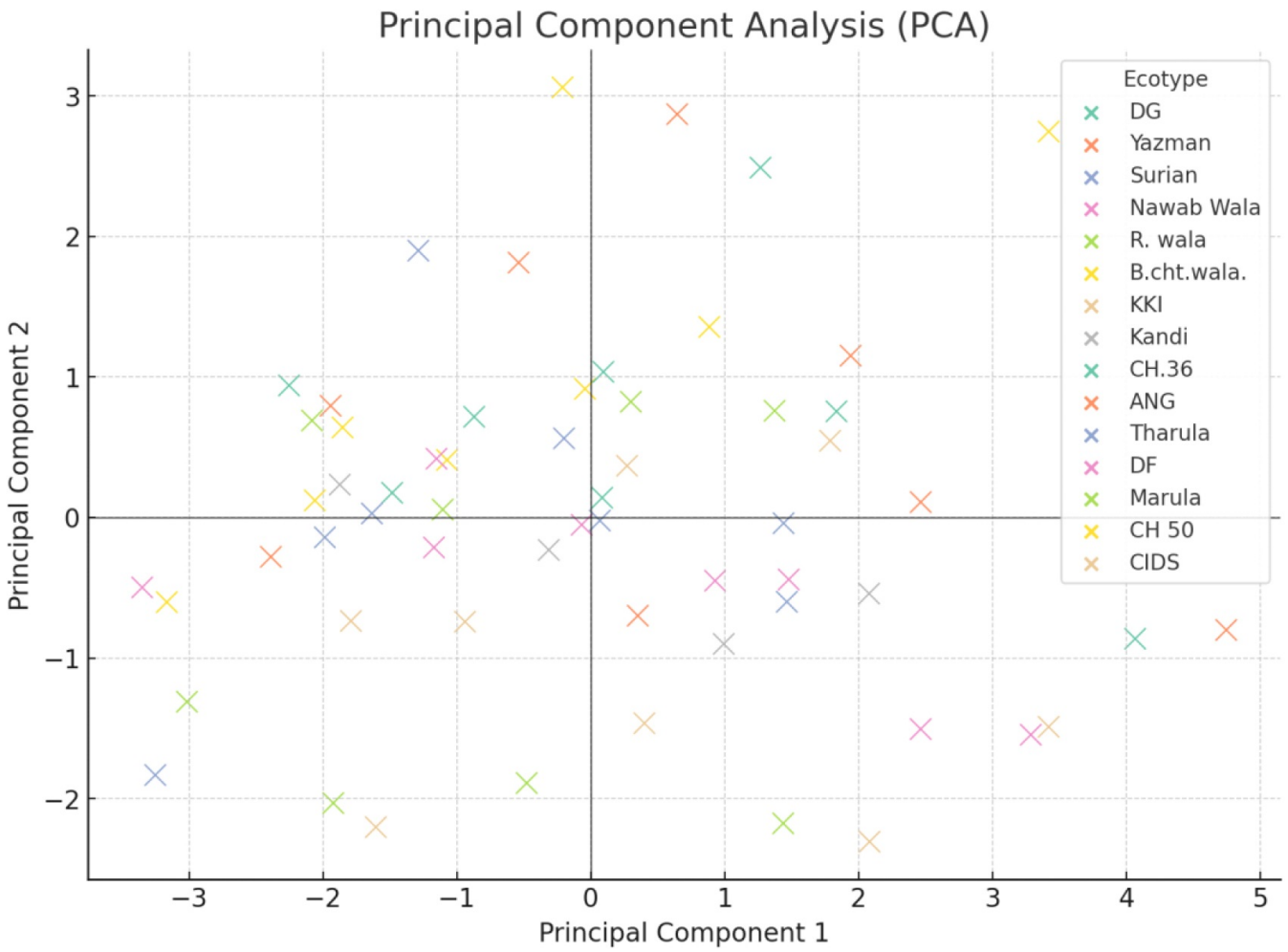

Figure 4

Principal Component Analysis (PCA) Scatter Plot of Different Ecotypes