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Correlation and principal component analysis of morphophysiological and biochemical traits in sorghum under drought stress

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Abstract

Sorghum (*Sorghum bicolor* (L.) Moench) is a crucial global crop cultivated across diverse climatic conditions. This study investigates the correlation and principal component analysis (PCA) of morpho-physiological and biochemical traits in sorghum genotypes under both irrigated and rainfed conditions. Conducted at the University of Agricultural Sciences, Dharwad, the experiment involved twenty rabi sorghum genotypes planted in medium black soil, with two moisture levels: rainfed and irrigated. Correlation analysis revealed significant associations between morpho-physiological parameters and grain yield. Under drought stress, leaf chlorophyll content (SPAD) and relative water content (RWC) exhibited strong positive correlations with yield ($r = 0.684$ and 0.789 , respectively), indicating their critical role in enhancing yield potential under water scarcity. Plant height, leaf area, and dry weight also showed positive correlations with yield, emphasizing their importance in productivity under stressed conditions. Conversely, biochemical parameters such as proline and wax content demonstrated variable correlations with yield depending on moisture availability; proline and wax content had negative correlations under irrigated conditions but positive correlations under stress, suggesting their role in drought tolerance. PCA identified the first three principal components (PC1, PC2, and PC3) accounting for significant variance in trait data. PC1, explaining 47.6% of the variance under irrigated and 56.5% under rainfed conditions, was positively associated with traits like plant height, chlorophyll content, and yield parameters, reflecting genotypic responses to productivity and drought. PC2 was linked to chlorophyll content and proline, highlighting its relevance to drought tolerance mechanisms. Overall, the study underscores the complex interplay between physiological and biochemical traits in determining sorghum's adaptability to different moisture conditions, providing insights for breeding programs aimed at improving drought resilience and yield stability.

Keywords: Sorghum, rainfed, irrigated, correlation and principal component analysis

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is a vital global crop with significant agricultural and economic importance. Cultivated during both the kharif (rainy) and rabi (post-rainy) seasons, sorghum serves distinct purposes based on the season of cultivation. The rabi sorghum is predominantly used for human consumption, while kharif sorghum is primarily utilized for animal feed, starch production, and the alcohol industry. In India, where only 5% of sorghum cultivation is irrigated, the crop is primarily grown in Maharashtra and Karnataka, which together account for over 48% of the country's sorghum acreage. The rabi sorghum, especially in Peninsular India, is crucial as a post-rainy cereal crop, largely grown under rainfed conditions in the semi-arid regions of the Deccan Plateau, encompassing Maharashtra, Karnataka, and Andhra Pradesh. However, productivity in these areas is increasingly threatened by erratic and insufficient rainfall, leading to moisture stress during critical growth stages and resulting in significant yield losses (Kumar *et al.*, 2022) [8]. Consequently, maintaining adequate soil moisture is essential for successful sorghum production in arid regions. Drought stress, despite sorghum's inherent resilience to water scarcity, presents considerable challenges.

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Under drought conditions, sorghum exhibits marked physiological and biochemical impacts that adversely affect growth and yield. Key effects include reduced photosynthesis due to decreased stomatal conductance and lower chlorophyll content, leading to diminished biomass accumulation (Chaves *et al.*, 2003) ^[1]. Water use efficiency varies among sorghum varieties, with some maintaining high efficiency while others experience reduced performance, impacting overall grain yield and quality (Chaves *et al.*, 2003) ^[1].

Correlation coefficient analysis plays a crucial role in elucidating the relationships between various traits under drought stress. By assessing correlations between traits such as leaf chlorophyll content, leaf water potential, and grain yield, researchers can better understand how these traits interact and contribute to drought resilience. Previous studies have demonstrated that physiological parameters and water use efficiency often exhibit positive correlations with drought tolerance, indicating that these traits are beneficial for improving drought resistance (Turner and Begg, 2001) ^[12]. Conversely, traits such as days to flowering, biochemical parameters, and yield may show negative correlations under severe drought stress, highlighting trade-offs between stress resistance and yield potential (Kumar *et al.*, 2014) ^[9]. Analyzing these correlations is critical for identifying key traits that can be targeted in breeding programs to enhance drought tolerance in sorghum and other crops.

Materials and Methods

Experimental Site

The field experiment was conducted in plot No. 126 of E-block, Main Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad. The MARS is situated at 15°12' N latitude and 76°34' E longitude with an altitude of 678 meters above the mean sea level (MSL).

Experimental Setup

An experiment was conducted in medium black soil classified as vertic inceptisols, with a depth of 2-3 meters. Twenty rabi sorghum genotypes (Table 1) were sourced from the AICRP sorghum, Dharwad, India. The first season's sowing took place on October 24, 2021, followed by the second season's sowing on November 11, 2022. High-quality seeds were utilized for row sowing. Each plot consisted of 6 rows with a 45 cm gap between them, and the spacing between individual plants within a row was set at 15 centimeters. The experiment encompassed two moisture levels: rainfed and irrigated. In the rainfed condition, no irrigation was provided after sowing, whereas in the irrigated condition, two additional irrigations were conducted: the first one 35 days after sowing, and the second one 65 days after sowing.

Correlation Analysis and Principal Component Analysis Using R Software

Data for this study were collected from sorghum plants subjected to drought stress, encompassing various physiological and agronomic traits, including leaf chlorophyll content, leaf water potential, grain yield, days to flowering, and biochemical parameters. To perform correlation analysis, data were first imported into R software using the `read.csv()` function. The `cor()` function was utilized to compute the correlation matrix, which was subsequently visualized using the `corrplot` package to illustrate the strength and direction of relationships between traits. This approach enabled the identification of significant

correlations and provided insights into trait interdependencies under drought conditions (Wei & Simko, 2017) ^[14].

Principal Component Analysis (PCA) was carried out to reduce data dimensionality and elucidate the principal components that account for the most variance in the dataset. Data were standardized with the `scale()` function to ensure comparability across traits. PCA was conducted using the `prcomp()` function, and the results were summarized to assess the proportion of variance explained by each component. Visualization of PCA results was achieved through biplots and scree plots, using the `ggbiplot` and `ggplot2` packages to facilitate interpretation of the principal components' contributions (Jolliffe, 2002; Wickham, 2016) ^[7, 15].

Table 1: List of sorghum genotypes

Names of sorghum genotypes	
1	SVD-1272R
2	SVD-1358R
3	SVD-1528R
4	SVD-1403R
5	SPV-486
6	SPV-2217
7	CSV-216R
8	CSV-29R
9	ICSR-15001
10	Basavana pada
11	Tandur L
12	Phule Anuradha
13	Chitapur - L
14	DKS- 35
15	M-35-1
16	M 148-138
17	Basavan moti
18	Phule Vasudha
19	BJV-44
20	ICSR- 13025

Results

Correlation Coefficients between Morpho-Physiological Parameters and Grain Yield

This study evaluated the correlation between various morpho-physiological parameters and grain yield, revealing strong associations that underscore their significance in determining crop productivity. Table 2 presents the correlation coefficients between these parameters and grain yield. Notably, the SPAD chlorophyll reading exhibited a highly significant positive correlation ($r = 0.684$) with grain yield under stress conditions, suggesting that higher chlorophyll levels are linked to increased yield potential. Furthermore, the relative water content (RWC) displayed a significant positive correlation ($r = 0.789$) with yield under stress, indicating that better water retention contributes to higher yields.

Plant height also showed a positive correlation ($r = 0.401$), suggesting that taller plants generally yield more grain. Similarly, leaf area demonstrated a positive correlation ($r = 0.365$) with yield, indicating that a larger leaf surface area may enhance productivity. Additionally, dry weight had a significant positive correlation ($r = 0.763$) with yield under stress, highlighting that greater plant biomass is associated with higher grain yields (Table 2). It is noteworthy that each of these parameters exhibited significant inter-correlations, reflecting their interdependence. Under irrigated conditions, all morpho-physiological parameters maintained positive correlations with yield.

Table 2: Correlation coefficients of morpho-physiological parameters with yield

Correlation matrix based on irrigated data							
	Plant height	Leaf area	SPAD	Total chl content	RWC	Dry weight	Grain yield
Plant height	1	0.312	0.275	0.318	0.56*	0.732***	0.299
Leaf area		1	0.378	0.479*	0.664**	0.51*	0.328
SPAD			1	0.698***	0.272	0.626**	0.382
Total chl content				1	0.524*	0.741***	0.557*
RWC					1	0.648**	0.477*
Dry weight						1	0.64**
Grain yield							1
Correlation matrix based on rainfed data							
	Plant height	Leaf area	SPAD	Total chl content	RWC	Dry weight	Grain yield
Plant height	1	0.359	0.471*	0.453*	0.603**	0.71***	0.401
Leaf area		1	0.396	0.416	0.698***	0.567**	0.365
SPAD			1	0.867***	0.658**	0.87***	0.684***
Total chl content				1	0.785***	0.885***	0.787***
RWC					1	0.857***	0.789***
Dry weight						1	0.763***
Grain yield							1

*** Correlation is significant at 0.001 level (two tailed)

** Correlation is significant at 0.01 level (two tailed)

* Correlation is significant at 0.05 level (two tailed)

Correlation Coefficients between Biochemical Parameters and Grain Yield

In evaluating the performance of genotypes under both irrigated and rainfed conditions, distinct correlations were observed between certain biochemical parameters and grain yield. Specifically, proline and wax content exhibited variable relationships with yield depending on the environmental context. Under irrigated conditions, both proline ($r = -0.437$) and wax ($r = -0.441$) content were negatively correlated with grain yield (Table 3). Conversely, under stressed conditions, both proline ($r = 0.395$) and wax ($r = 0.326$) content showed positive correlations with yield. These findings suggest that the relationship between these biochemical parameters and grain yield is contingent upon the prevailing environmental conditions.

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was performed on thirteen variables, revealing that the first three principal components (PCs) accounted for the majority of the total variability. These three PCs, with eigenvalues greater than 1, contributed 70.6% and 78.8% to the total variation among sorghum genotypes

under irrigated and rainfed conditions, respectively (Table 4). From these, the two primary components, PC1 and PC2, were selected for further analysis due to their eigenvalues exceeding one, explaining 60% of the total variance for irrigated data and 69% for rainfed data.

Under irrigation, PC1 explained 47.59% of the total variance, showing positive correlations with plant height (PH), chlorophyll content (SPAD), total chlorophyll (TC), relative water content (RWC), membrane stability index (MSI), panicle weight (PW), grain yield per plant (GY/P), harvest index (HI), leaf area (LA), and grain yield per hectare (GY/ha). PC2, contributing 12.07% of the total variation, exhibited strong positive correlations with SPAD, thousand grain weight (TW), GY/P, HI, GY/ha, and proline (Table 4). In rainfed conditions, PC1 explained 56.4% of the total variance and was positively correlated with PH, SPAD, TC, RWC, MSI, PW, TW, GY/P, HI, LA, GY/ha, and proline. Similarly, PC2 accounted for 12.6% of the total variation, showing strong positive correlations with TC, TW, GY/P, HI, GY/ha, and proline (Table 5). Notably, under rainfed conditions, there was a significant positive correlation among GY/P, PW, GY/ha, SPAD, and proline, indicating a close association in genotype ranking.

Table 3: Correlation coefficients of biochemical parameters with yield

Correlation matrix based on irrigated data				
	Proline	Wax	Dry weight	Grain yield
Proline	1	-0.14	-0.522*	-0.437
Wax		1	-0.024	-0.441
Dry weight			1	0.64**
Grain yield				1
Correlation matrix based on rainfed data				
	Proline	Wax	Dry weight	Grain yield
Proline	1	0.586**	0.55*	0.395
Wax		1	0.642**	0.326
Dry weight			1	0.763***
Grain yield				1

*** Correlation is significant at 0.001 level (two tailed)

** Correlation is significant at 0.01 level (two tailed)

* Correlation is significant at 0.05 level (two tailed)

Table 4: Principle component analysis of various morpho-physiological and yield traits recorded in sorghum under irrigated conditions

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13
Eigenvalue	6.188	1.569	1.383	0.971	0.825	0.613	0.559	0.294	0.254	0.166	0.105	0.072	0
Percentage of variance	47.599	12.07	10.641	7.469	6.348	4.716	4.3	2.262	1.955	1.279	0.81	0.55	0
Cumulative percentage of variance	47.599	59.67	70.311	77.779	84.127	88.843	93.143	95.406	97.36	98.639	99.45	100	100
Factor loadings by various traits													
PH	0.244	-0.288	0.117	-0.293	0.497	-0.482	0.117	-0.155	0.273	-0.087	-0.395	-0.04	0
SPAD	0.267	0.054	-0.178	0.619	0.141	-0.017	-0.047	-0.457	0.436	-0.024	0.273	0.117	0
TC	0.339	-0.054	-0.131	0.277	-0.086	0.349	0.137	-0.131	-0.367	-0.281	-0.636	0.078	0
RWC	0.28	-0.079	0.471	-0.138	-0.001	0.011	0.27	-0.471	-0.465	0.182	0.355	0.061	0
MSI	0.269	-0.394	-0.232	0.056	0.175	0.03	-0.474	0.186	-0.23	0.574	0.006	0.206	0
Days.50..F	-0.224	-0.238	0.319	0.113	-0.591	-0.247	-0.36	-0.334	0.13	0.12	-0.309	-0.007	0
PW	0.344	-0.065	-0.226	0.125	-0.27	-0.295	0.12	0.123	-0.105	0.08	0.096	-0.771	0
TW	-0.015	0.586	0.293	0.303	0.3	-0.346	-0.364	0.089	-0.325	-0.005	-0.133	-0.08	0
GY/P	0.325	0.029	-0.125	-0.042	-0.362	-0.491	0.037	0.258	-0.096	-0.328	0.179	0.535	0
HI	0.313	0.38	-0.054	-0.333	-0.144	0.134	-0.14	-0.114	0.196	0.161	-0.096	0.007	-0.707
LA	0.236	-0.001	0.529	0.286	-0.084	0.125	0.321	0.495	0.32	0.299	-0.118	0.08	0
GY/ha	0.313	0.38	-0.054	-0.333	-0.144	0.134	-0.14	-0.114	0.196	0.161	-0.096	0.007	0.707
Proline	-0.278	0.238	-0.348	0.086	-0.058	-0.29	0.498	-0.147	-0.096	0.529	-0.228	0.196	0

Table 5: Principle component analysis of various morpho-physiological and yield traits recorded in sorghum under rainfed conditions

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13
Eigenvalue	7.339	1.647	1.283	0.801	0.653	0.473	0.251	0.189	0.144	0.092	0.081	0.04	0.005
Percentage of variance	56.455	12.669	9.871	6.16	5.026	3.642	1.93	1.456	1.111	0.705	0.622	0.311	0.042
Cumulative percentage of variance	56.455	69.124	78.995	85.155	90.181	93.823	95.752	97.209	98.32	99.025	99.647	99.958	100
Factor loadings by various traits													
PH	0.202	-0.534	0.203	-0.175	0.248	-0.296	0.308	-0.464	0.194	-0.02	-0.095	0.237	0.2
SPAD	0.332	-0.005	0.117	0.198	-0.194	-0.092	0.095	0.421	0.677	-0.148	-0.329	-0.12	-0.046
TC	0.35	0.005	0.019	0.183	0.028	0.084	-0.31	0.137	-0.115	-0.403	0.201	0.71	0.011
RWC	0.311	-0.218	-0.243	-0.262	0.032	-0.031	-0.364	-0.052	-0.244	-0.486	-0.235	-0.475	0.118
MSI	0.271	-0.242	0.093	0.572	0.11	-0.128	0.237	0.233	-0.546	0.215	-0.175	-0.129	-0.045
Days.50% F	-0.201	-0.13	-0.493	0.431	-0.363	-0.439	-0.255	-0.314	0.126	0.046	-0.04	0.077	-0.031
PW	0.352	-0.029	0.019	0.175	-0.125	0.128	-0.076	-0.085	0.155	0.203	0.64	-0.294	0.488
TW	0.227	0.405	-0.059	-0.305	-0.256	-0.622	0.354	0.117	-0.212	-0.108	0.194	0.03	0.021
GY/P	0.355	0.043	-0.117	0.056	0.129	0.105	0.104	-0.32	0.111	-0.025	0.27	-0.158	-0.777
HI	0.166	0.501	-0.407	0.208	0.329	0.216	0.291	-0.276	0.058	-0.076	-0.295	0.059	0.312
LA	0.19	-0.312	-0.46	-0.291	-0.447	0.394	0.288	0.13	-0.081	0.236	-0.087	0.214	-0.011
GY/ha	0.316	0.121	-0.121	-0.246	0.294	-0.217	-0.461	0.11	0.063	0.635	-0.173	0.121	-0.032
Proline	0.224	0.249	0.474	0.022	-0.513	0.155	-0.156	-0.452	-0.151	0.115	-0.332	0.025	0.002

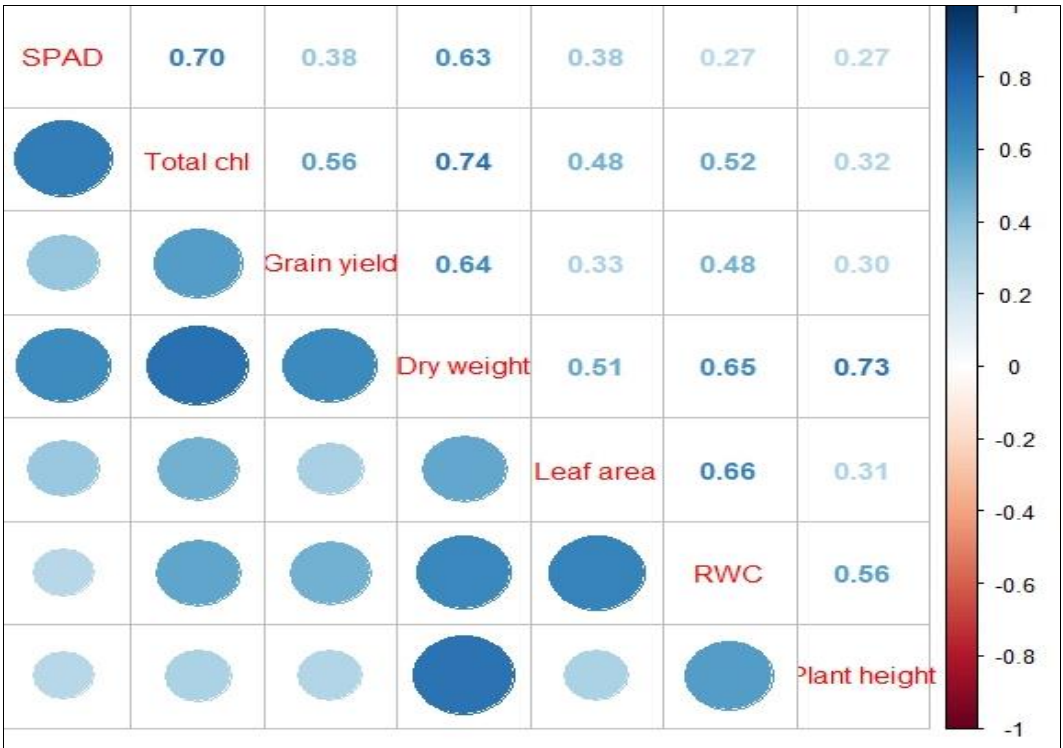
The PCA results highlighted that the variation among germplasm accessions could not be solely attributed to a few characteristics, suggesting the involvement of multiple traits in explaining the overall variance among the accessions. Specifically, in rainfed conditions, the highest contributions to the total variability under PC1 were from total leaf chlorophyll content (0.35), grain yield per plot (0.355), and panicle weight (0.352). These were followed by SPAD chlorophyll meter reading (0.33), leaf relative water content (0.312), and grain yield per hectare (0.316), as detailed in Table 5.

Discussion

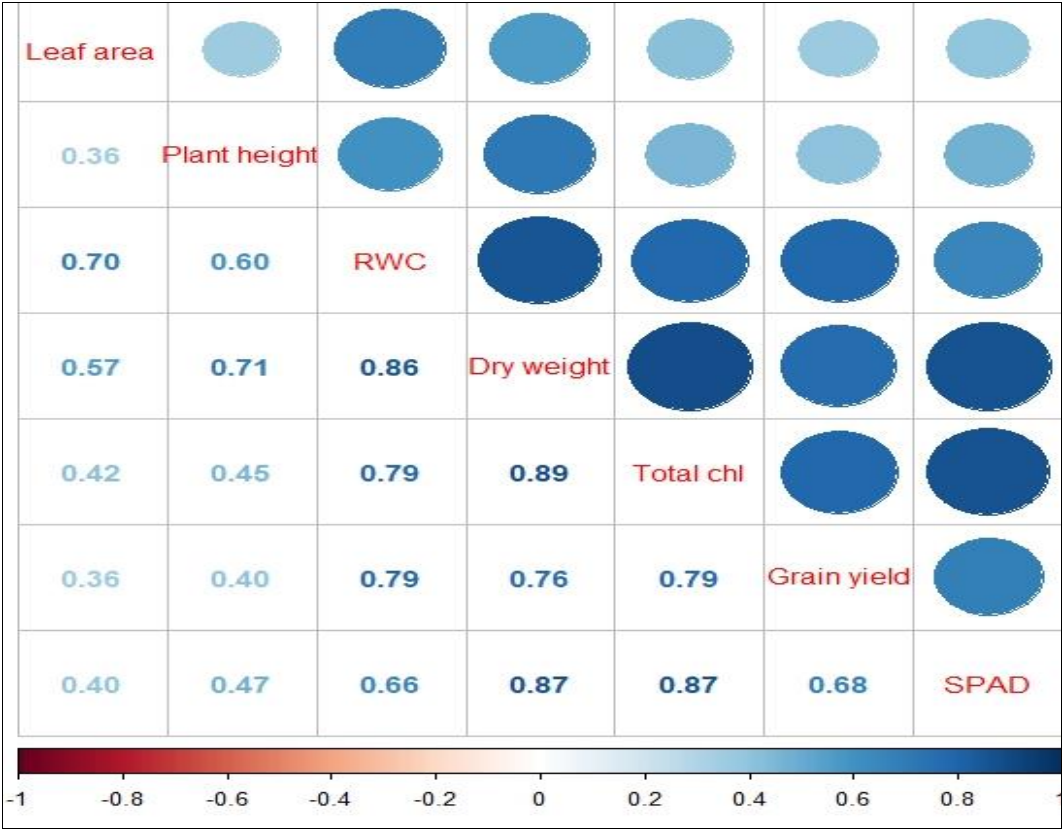
Correlation studies are crucial for understanding the relationships between variables by quantifying their associations. In this study, various morpho-physiological parameters showed strong correlations with sorghum genotype grain yield, as demonstrated in Figure 1. The SPAD chlorophyll reading exhibited a significant positive correlation of 0.684 with grain yield under stressful conditions (Fig. 1b) and 0.382 under irrigated conditions, indicating that higher chlorophyll levels are linked to increased yield potential. Similarly, relative water content (RWC) had a positive correlation of 0.789 under stress

(Fig. 1a) and 0.477 under irrigation, suggesting better water retention contributes to higher yields. Leaf area also showed significant positive correlations with yield under both rainfed (0.365) and irrigated (0.328) conditions, highlighting its role in productivity. These findings align with Ravali *et al.* (2021) ^[11], who emphasized the importance of these parameters in rainfed environments where water is limited. The stronger correlations observed under rainfed conditions suggest that traits like plant height, leaf area, and dry weight are more critical for yield when water availability is restricted. This supports Dhutmal *et al.*'s (2015) ^[4] findings on the positive impact of chlorophyll content on grain yield.

The correlation analysis of biochemical parameters, such as proline and wax content, revealed distinct associations with grain yield. In irrigated conditions, proline ($r = -0.437$) and wax ($r = -0.441$) content exhibited negative correlations with yield (Fig. 2), indicating that higher levels of these parameters may reflect reduced metabolic efficiency when water is not limiting. Under stressed conditions, however, both proline ($r = 0.395$) and wax ($r = 0.326$) content showed positive correlations with yield, suggesting that these biochemical adaptations enhance water stress tolerance.

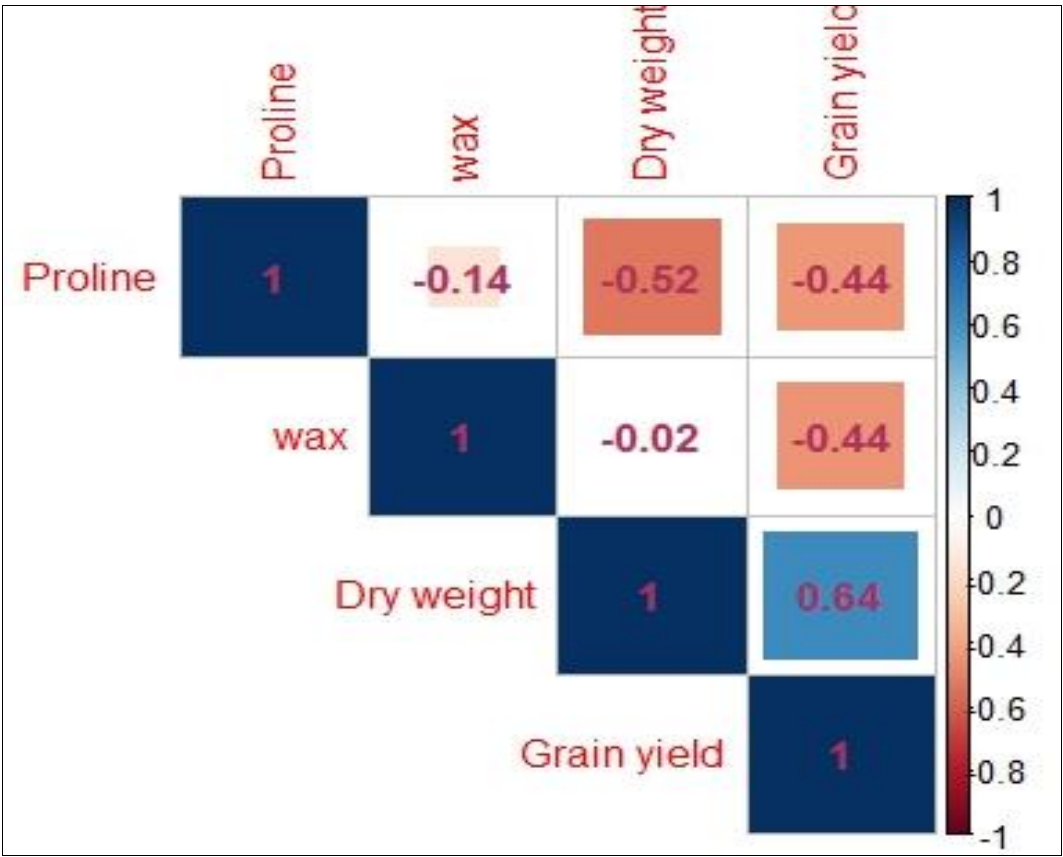


a) Correlation coefficient plot based on performance of sorghum genotypes under irrigated condition

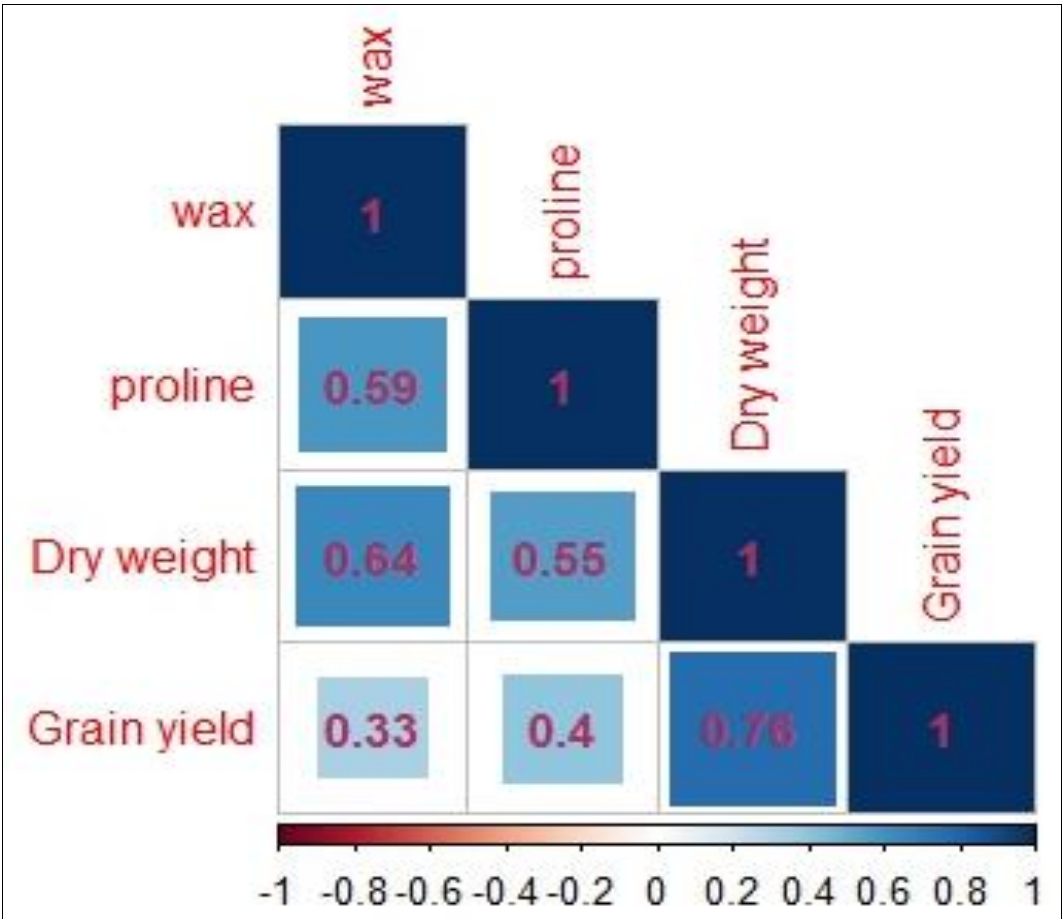


b) Correlation coefficient plot based on performance of sorghum genotypes under rainfed condition

Fig 1: Correlation coefficient of morpho-physiological parameters with yield under irrigated (a) and rainfed (b) conditions



a) Correlation coefficient plot based on performance of sorghum genotypes under irrigated condition

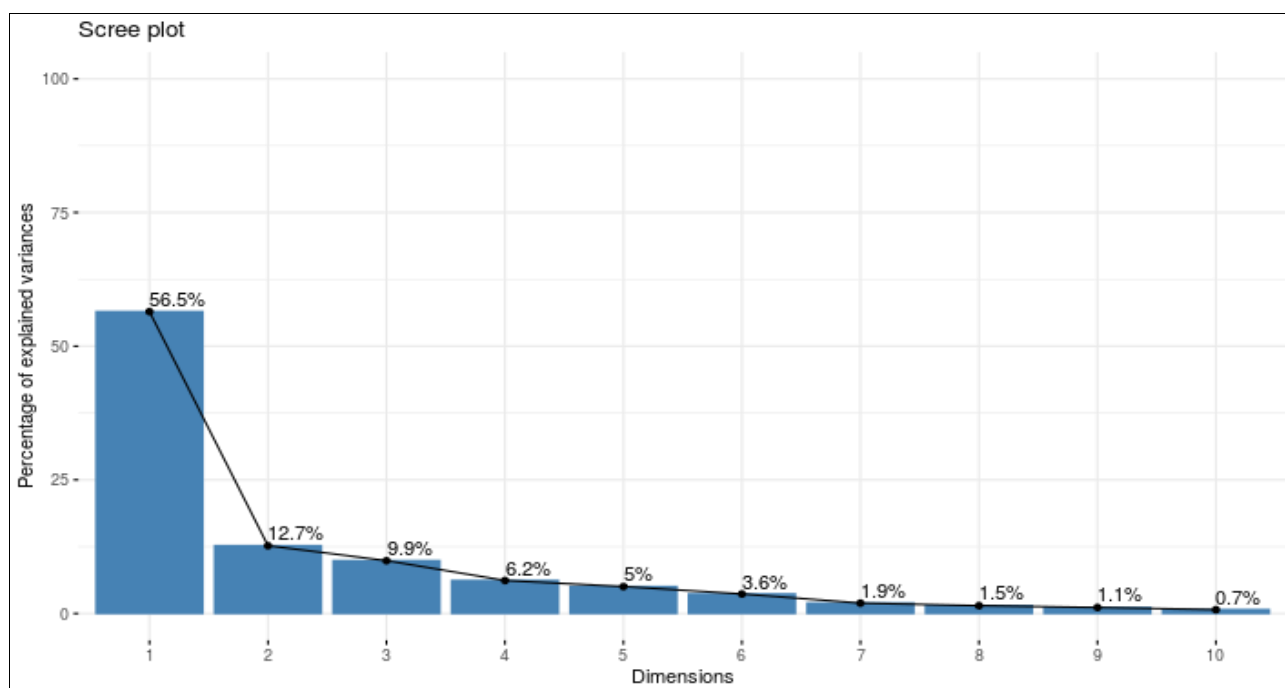


d) Correlation coefficient plot based on performance of sorghum genotypes under rainfed condition

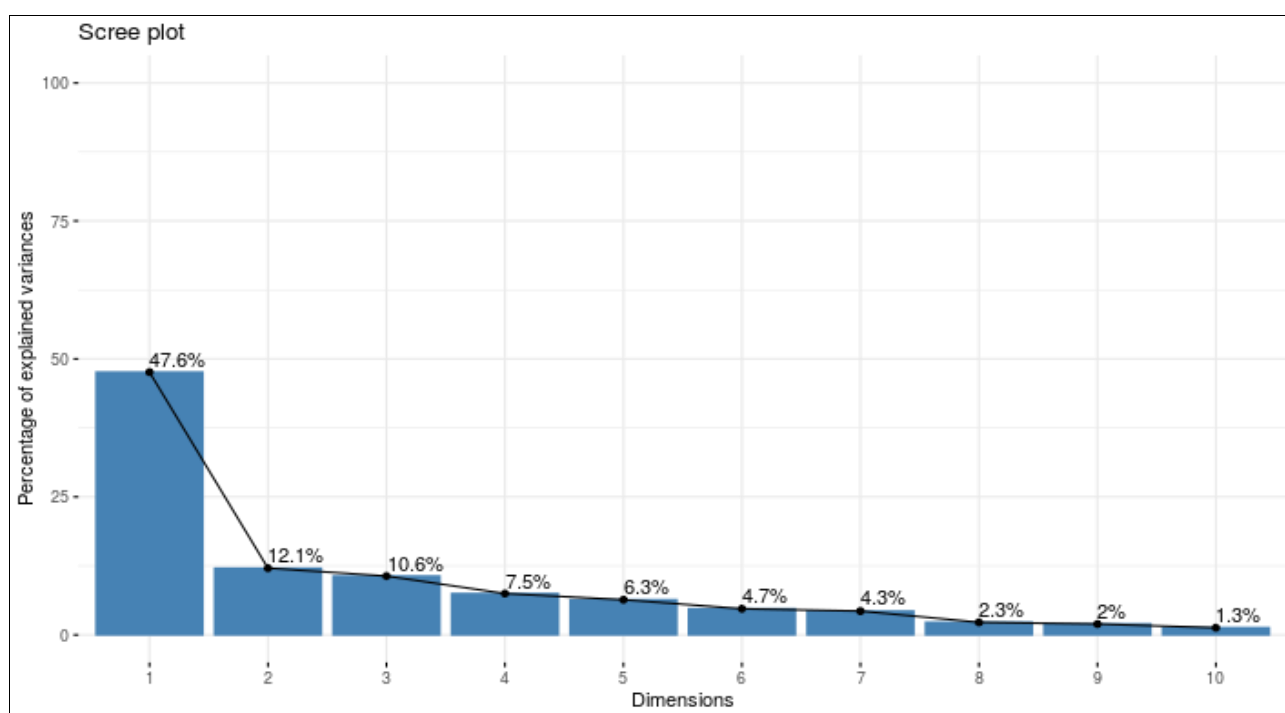
Fig 2: Correlation coefficient of biochemical parameters with yield under irrigated (a) and rainfed (b) condition

Principal Component Analysis (PCA) provided further insights into the variability among sorghum genotypes. The first three principal components accounted for the majority of the total variation, with PC1 and PC2 explaining 60% of the variance under irrigated conditions and 69% under rainfed conditions (Fig. 3). PC1 was positively correlated with traits such as plant height, chlorophyll content, and yield-related parameters (GY/P, GY/ha) under rainfed conditions, encapsulating genotypic responses related to productivity. PC2's correlation with chlorophyll content and proline suggests its link to drought tolerance mechanisms. The PCA results highlighted the complexity of trait variations among germplasm accessions,

emphasizing the importance of genotype-by-environment interactions. Traits like total leaf chlorophyll content, grain yield per plot, and panicle weight drove variance under PC1 in rainfed conditions, underscoring their role in drought response (Fig. 4). The combined influence of SPAD chlorophyll readings, leaf relative water content, and grain yield per hectare on overall variability further illustrates the need to consider multiple traits for assessing genotype adaptability to water stress and irrigated conditions (Fig. 5). The positive correlations observed in rainfed conditions among grain yield per plant (GY/P), panicle weight (PW), grain yield per hectare (GY/ha), SPAD, and proline indicate a close association in genotype ranking (Fig. 4).



a) Scree plot of principal components based on performance of sorghum genotypes under rainfed condition



b) Scree plot of principal components based on performance of sorghum genotypes under irrigated condition

Fig 3: Scree plot for percentage of variance and principal components based on morpho-physiological and yield traits recorded in sorghum under rainfed (a) and irrigated (b) conditions

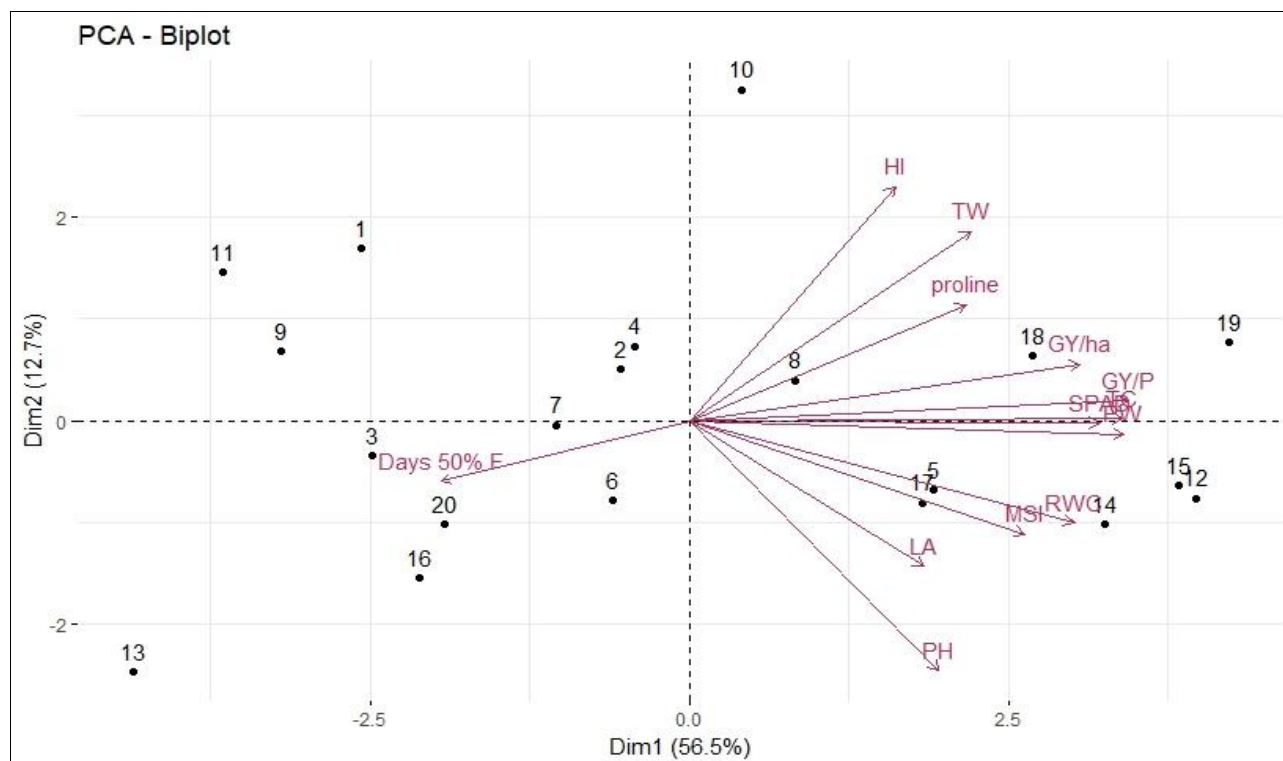


Fig 4: Biplot between PC 1 and PC 2 showing contribution of various traits in sorghum genotypes under rainfed condition

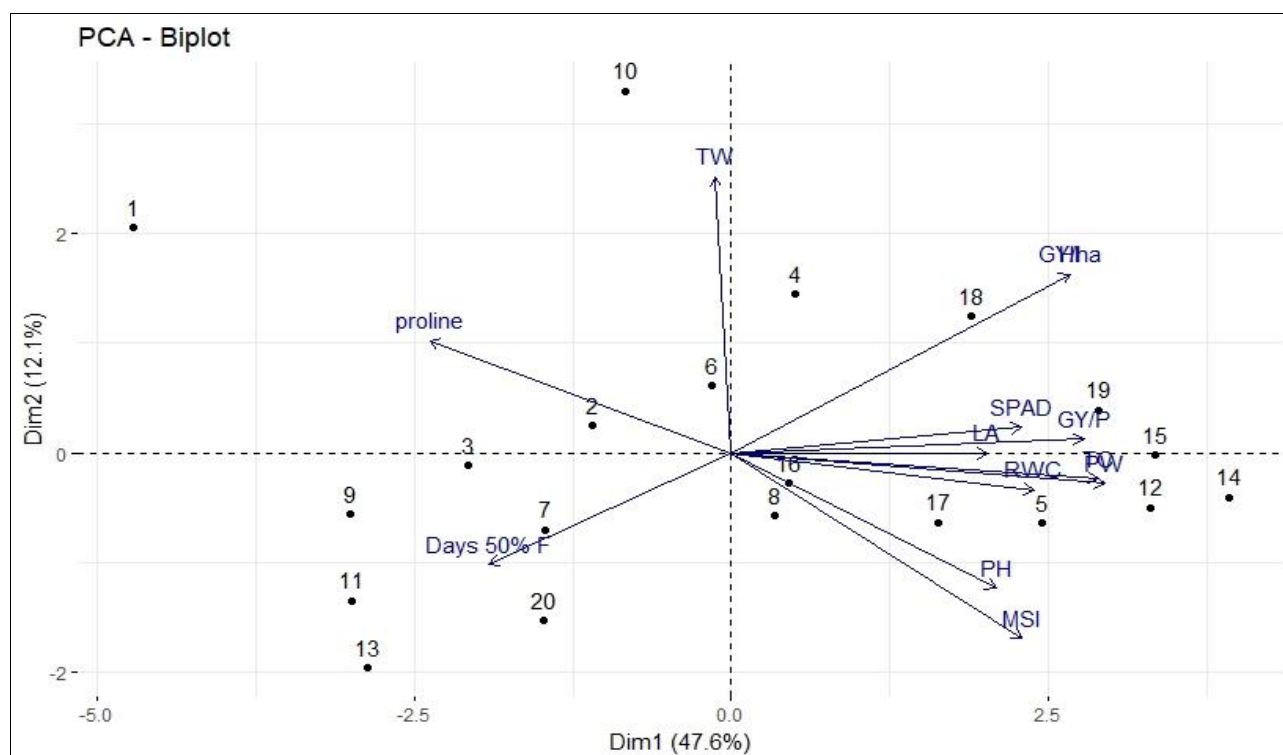


Fig 5: Biplot between PC 1 and PC 2 showing contribution of various traits in sorghum genotypes under irrigated condition

Conclusion

This study highlights the critical role of morpho-physiological and biochemical parameters in determining grain yield in sorghum genotypes under varying environmental conditions. Notably, SPAD chlorophyll reading and relative water content exhibited strong positive correlations with yield under stress, emphasizing their importance in water-limited environments. Additionally, leaf area showed significant correlations with yield in both rainfed and irrigated conditions, indicating its contribution to productivity. The distinct negative correlations of

proline and wax content with yield under irrigation and positive correlations under stress suggest their roles in stress adaptation. Principal Component Analysis further revealed the complexity of genotype-by-environment interactions, with key traits driving variability in different conditions. These findings underscore the need to consider multiple traits for optimizing sorghum yield, particularly in drought-prone areas, and highlight the potential for targeted breeding strategies to enhance crop performance under varying water availability.

Author contribution

Navyashree R, Mummigatti UV, Nethra P, Basavaraj B, and Hanamaratti NG played crucial roles in designing and implementing the research, analyzing results, and drafting the manuscript. Additionally, their collaborative efforts were crucial in interpreting data and refining the study's conclusions.

Conflict of interest

The authors declares that there is no conflict of interest

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