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CHARACTERIZATION OF SWEET SORGHUM ACCESSIONS FOR GRAIN-RELATED TRAITS USING METABOLOMICS ANALYSIS

Muhammad Umar Hussain [1,2]*, Tayyaba Noor [2], Abdullah Ahmad [2], Muhammad Sohail Nasir [1] and Mumtaz Khan [1]

[1]Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad, Pakistan
[2]National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan

*Corresponding author: omee4777@gmail.com

ABSTRACT

Sorghum is the 4th largest cereal crop in the world with diverse flavonoid and phenolic compounds. It is resistant to biotic and abiotic stresses. Sweet sorghum is mostly grown for its grain as well as for the production of ethanol and biofuels. Sorghum is also the main food source with numerous health benefits. Plant growth and development are sustained by metabolites which include both primary and secondary metabolites. Sorghum varieties are characterized on the basis of morphological, molecular, and biochemical factors. Morphometric characterization is a simple and cost-effective method; however, it is greatly influenced by environmental factors. While metabolite profiling pinpoints the basis of plant morphological variations. Metabolomics provides a link between morphological and genetic factors. Moreover, it has applications in gene discovery via metabolomic quantitative traits loci (mQTL) identification. This study was aimed at characterizing three sweet sorghum accessions with characteristic seed colors. Phenotypic data related to grain-related traits was recorded. Flavonoid and phenolic contents of these accessions were also analyzed. Sorghum accession with dark red seed color contained higher phenolic contents as compared to the accession with white seed color. The study provides an interpretation of morphological variations in the grain-related traits of these accessions from the metabolomics perspective.

***Keywords*:** Morphometric Characterization, Metabolomic Quantitative Traits Loci, Total Flavonoids and Phenolic Contents, Accessions and Grain-Related Traits.

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1. INTRODUCTION

In Pakistan, sorghum is majorly produced in Punjab and Sindh (Iqbal et al. 2015). According to 2015, in Punjab, 47% of the land is under sorghum production and in Sindh 26% of the land is used for sorghum crop but 60% of total area for sorghum is irrigated and the remaining land is fed by rain (Iqbal et al. 2017). An average of 1.5-million-hectare land is occupied by sorghum crop in Pakistan (Zuo et al. 2019; Wang et al. 2023). From this land, a yield of 150 mT is obtained (USDA). In recent years, developments in Pakistan's environment in response to heat and drought as well as the suitability of sorghum to grow in arid regions have led to the re-imagining of sorghum as a fodder crop (Tonapi et al. 2020; Zhao et al. 2023). Apart from resistance or adaptation to extreme conditions, sorghum has phytochemicals that increases its market value and importance (Getachew et al. 2016; Chakravathi et al. 2017).

Phytochemicals are essential for the growth and development of plants (Awika and Rooney 2004; Tiwari et al. 2015; Dykes 2019). Recently 651 metabolites were identified in sorghum (Pavli et al. 2013; Zhou et al. 2020). Metabolomics is a study to determine and understand how the variations among phenotypes are affected by genetic diversity in plants (Harrigan et al. 2007; Zhang et al. 2012; Carreno-Quinterro et al. 2013; Hamany et al. 2020). Being able to grow in diverse environments is an important trait of Sorghum. Due to such traits, a large diversification is expected in metabolome of the sorghum (Ramalingam et al. 2021; Tondé et al. 2023).

The purpose of this study was to characterize sorghum accessions with contrasting seed colors, analyze total flavonoid content (TFC) and total phenolic content (TPC) levels in each accession and comprehensively compare them to interpret phenotypic differences from the viewpoint of metabolomics (Shi et al. 2022).

The current study analyzed differences in the phenolic and flavonoid profile of three sorghum accessions and evaluation of their morphological traits. Three sorghum accessions were planted in the field and their morphological evaluation was done.

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AGROBIOLOGICAL RECORDS
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2. MATERIALS AND METHODS

The study was performed in Somatic Cell Genetics Lab, Center of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad (UAF), Pakistan.

2.1. Plant Material

The plant material was comprised of three accessions of sweet sorghum. These accessions had contrasting colors (Fig. 1 and 2). The seeds of sweet sorghum accessions were collected from Agriculture Research Service (ARS), United States Department of Agriculture (USDA).

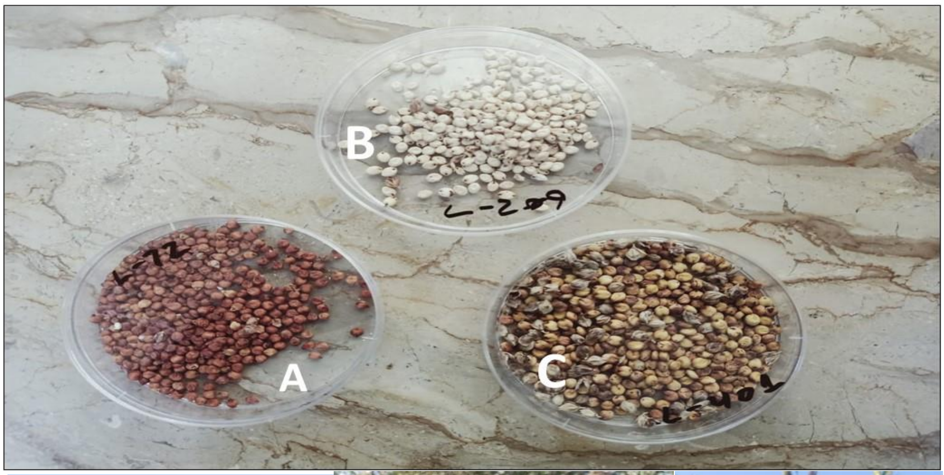

Fig. 1: Three sorghum accessions having contrasting color. A: IS 2306, B: ETS 3008 C: PB-53

Fig. 2: Accession PI454407 (EST 3008) shown on the left, PI 496316 (PB-53) shown in middle, PI570284 (IS 23064) shown in right.

2.2. Morphological Evaluation of Sorghum Accession

Sorghum accessions were planted in the field of University of Agriculture, Faisalabad, under Randomization Complete Block Design (RCBD). Faisalabad lies between 31°-26° N latitude and 73°-6° E longitude. Plant to plant and row to row distance was 25 cm and 75 cm respectively. For good plant stand, two seeds were sown in one hole. Dibbler was used during sowing of sorghum seeds. All agronomic and plant protection measures were carried out to get healthy crops. Traits were measured at maturation stage. Data of following traits were taken to study sorghum accessions; Plant height (cm), Stem width (cm), Plant moisture content (%), 100 grain weight (g), number of leaves, Leaf length (cm), Leaf width (cm), Sugar content or Brix values (ºBx) and Shell color (Fig. 3-13).

2.3. Metabolite Profiling

Different parameters were used in metabolite profiling i-e; TFC (Total flavonoid content) and TPC (Total Phenolic content). TFC and TPC were calculated according to the Julkenen-Titto method (Julkunen-Tiitto 1985). The extraction was done by using methanol because it is easily available and has good extraction power. Seeds for the metabolic studies were washed and dried for 1 day, under shade. Dry seeds were grinded in sterilized pestle and mortar by using liquid nitrogen. For the protection of metabolites from degeneracy in the seeds, chemicals weren't used during grinding. Powder seeds samples of 8g were taken in a falcon tube with methanol. The samples were labeled according to their accession number. The cap of falcon tube was removed for drying methanol from extract. After 14 days of incubation at room temperature all methanol evaporated. The samples were prepared from grinded seeds extract after drying all the methanol from these samples (Nielsen et al. 2016; Zhou et al. 2020). TFC and TPC were studied for three sorghum extracts. The parameters were checked in Biochemistry lab, University of Agriculture Faisalabad, Pakistan.

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2.4. Determination of Total Phenolic Content (TPC)

Phenolic content estimation was done through "Julkenen-Titto" method and by using methanolic extracts (Zhou et al. 2020). Folin-Ciocalteau's reagent was used during phenolic estimation. For determination of TPC. Seed samples were homogenized in 80% acetone. The samples were centrifuged for 10min at 12000rpm and supernatant was taken. After this, 1ml of Folin-Ciocalteau's reagent was added in supernatant. The volume was raised up to 10 ml. The 755 nm wavelength was used to read the absorbance (Fig. 14-15).

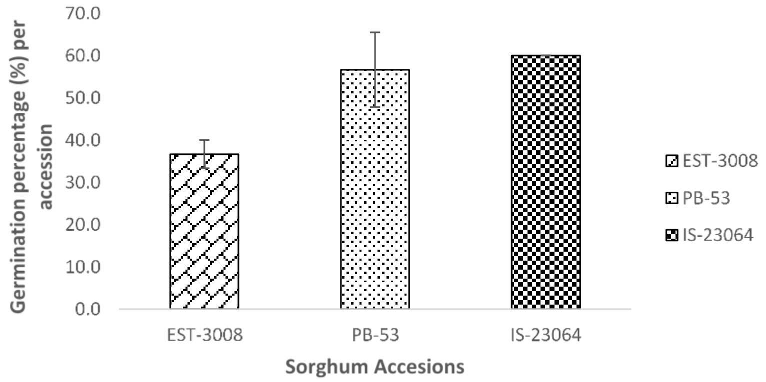

Fig. 3: Comparison of germination percentage in sorghum accessions.

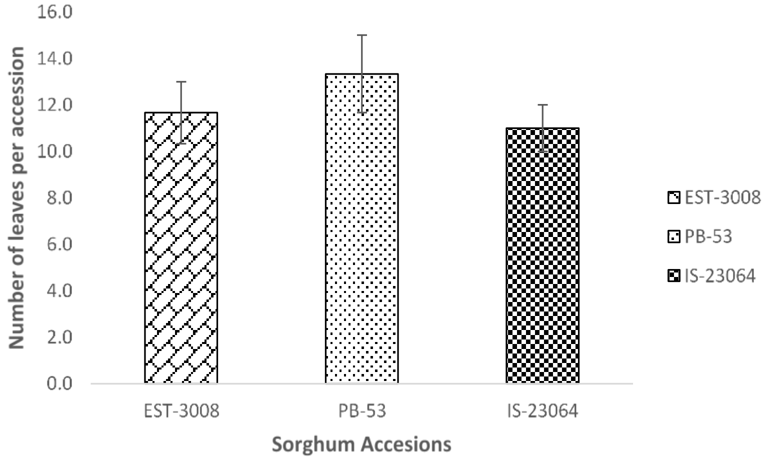

Fig. 4: Comparison of number of leaves in sorghum accessions.

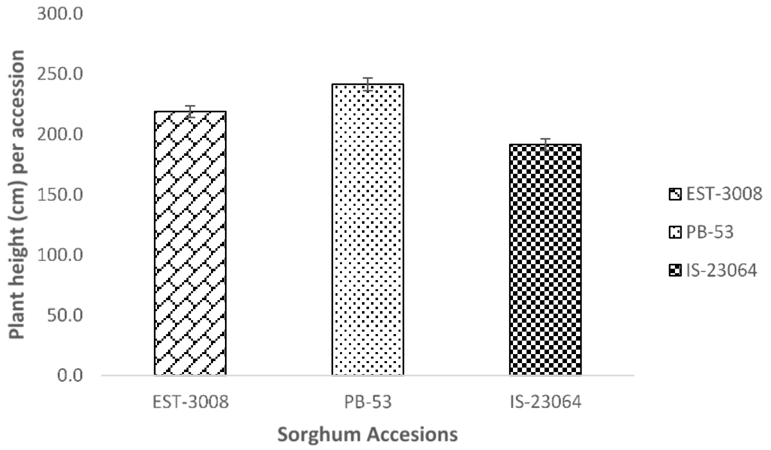

Fig. 5: Comparison of Plant height in sorghum accessions.

2.5. Determination of Total Flavonoid Content (TFC)

Flavonoid content estimation was done through "Julkenen-Titto" method and by using methanolic extracts (Zhou et al. 2020). Folin-Ciocalteau's reagent was used during phenolic estimation. For determination of TFC. Seed samples were placed in 10mL volumetric flask and volume was made up to 5mL by adding distilled water. 0.3mL of sodium nitrate was added and after 5min 3mL of Aluminum chloride was added. 2mL of 1mol per liter of sodium hydroxide was added after 6min. The volume was made up to 10mL by adding distilled water. The solution was shaken to mix

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it well and absorbance was checked at 510 nm (Fig. 16-17). In this method, Catechin was used as a standard (Fig. 16-17).

2.6. Determination of Association between Total Flavonoid Content and Total Phenolic Content with Seed and Plant Morphology

Kumar and Pandey 2013, illustrated that flavonoids have an anti-oxidant and growth regulative capability by affecting various developmental processes (Taylor and Grotewold 2005; Kumar and Pandey, 2013). For the determination of association between content of flavonoids and phenols present in sorghum accessions.

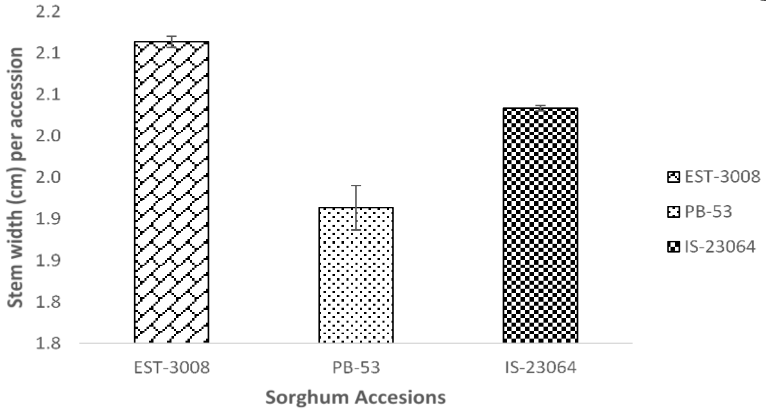

Fig. 6: Comparison of Stem width in sorghum accessions.

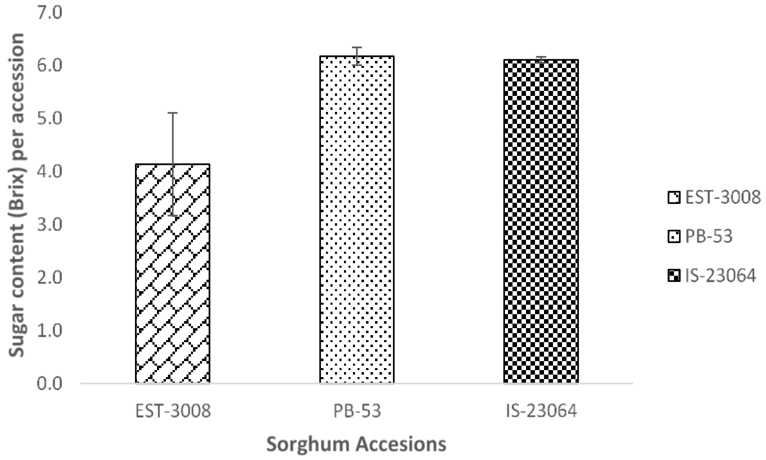

Fig. 7: Comparison of Sugar content (brix) in sorghum accessions.

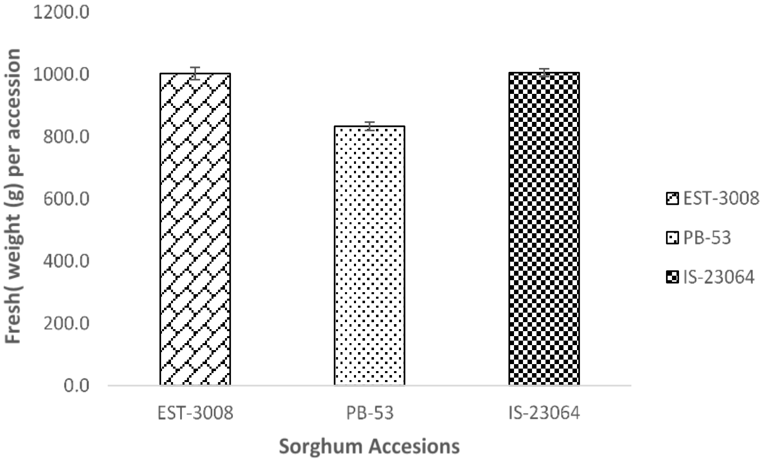

Fig. 8: Comparison of Plant fresh weight (g) in sorghum accessions.

Plant morphological characters were determined (Fig. 3-13). Morphological characters were linked with Total Flavonoid contents (TFC) and Total Phenolic content (TPC) of EST-3008, PB-53 and IS-23064 through graphical representation (Fig. 18-19). Association was confirmed through statistical analysis by observing correlation test (Table 2).

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3. RESULTS AND DISCUSSION

3.1. Sorghum Accessions used in Study

Accession PI 454407 (ETS 3008) was originated and collected from Ethopia under the project of 'Gebrekidan, Brehane Ethiopian Sorghum Improvement Project'. It is maintained at 'Plant Genetic resource Conservation Unit, Griifin, GA'. This accession is stored at 'National Laboratory for Genetic Resources Preservation, USA' (Source: USDA, GRIN-Global). Accession PI 496316 (PB-53) was originated in Burundi and collected from Burundi (IBPGR).

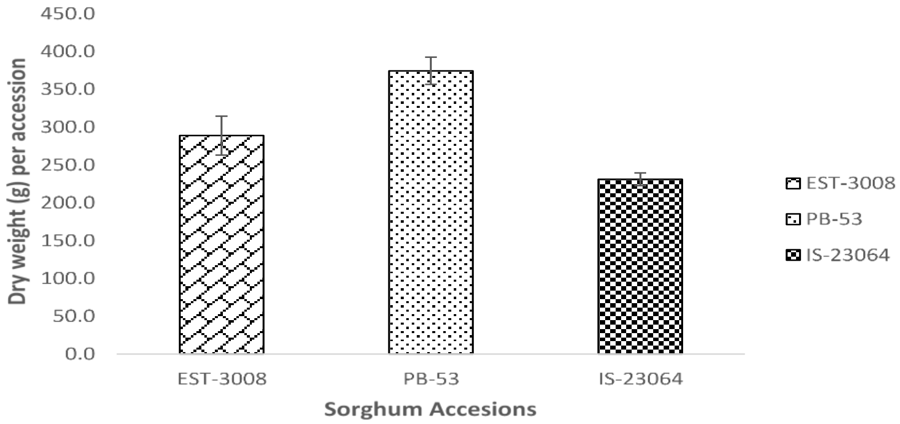

Fig. 9: Comparison of Plant dry weight (g) in sorghum accessions.

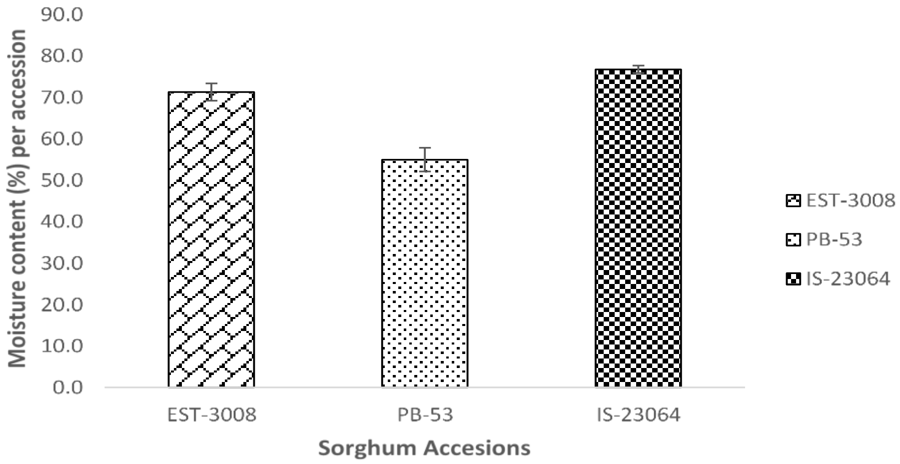

Fig. 10: Comparison of Moisture content (%) in sorghum accessions.

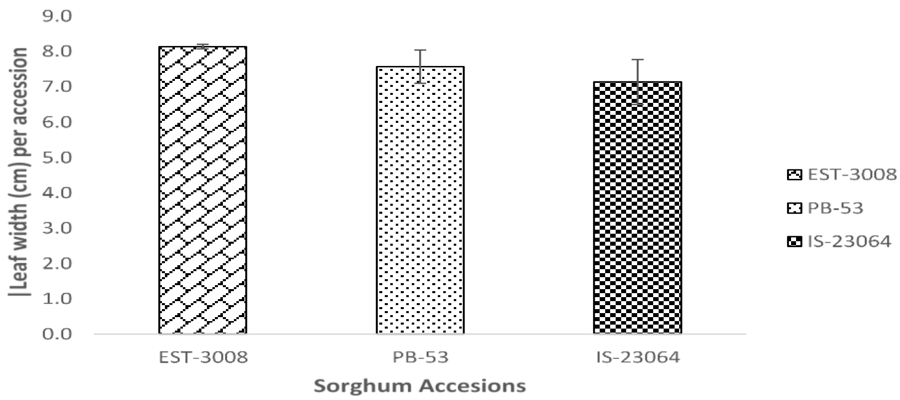

Fig. 11: Comparison of Leaf width (cm) in sorghum accessions.

It was maintained at 'Plant Genetic Resource Conservation Unit, Griffin, GA, US'. The PI was assigned to this group in 1985 (Source: USDA, GRIN-Global). Accession PI 570284 (IS 23064) was originated in Sudan and donated by Omer, Hilu Sorghum & Millet Research, Sudan on 10 June, 1993 and was maintained at 'Plant Genetic Resources Conservation Unit, Griffin, 1109 Experiment Street Griffin, Georgia in the form of seeds. (Source: USDA, GRIN-Global) (Fig. 1 and 2).

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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3.2. Morphological Evaluation of Sorghum Accessions

The sample accessions were analyzed for their morphological characters. The morphological characteristics from 3 accessions showed an average of 51.111±20.62% (EST-3008; 36.66±5.77%, PB-53; 56.66±15.27% and IS-23064; 60± 0%) germination. 12.0±23.07 (EST-3008;11.6±2.3, PB-53;13.3±2.8 and IS-23064; 11±1.7) number of leaves, 2.17±4.57cm (EST-3008; 218.66±8.5, PB-53;241.33±9.07 and IS-23064; 191.3±8.5) Plant height, 2.02±0.04476cm (EST-3008;2.11±0.11, PB-53;1.9±0.046 and IS-23064;2.0±0.005) average stem width, 5.4667±12.47 (EST-

Table 1: F-values from one-way ANOVA for germination percentage (GP), Number of leaves (NL), Plant height (PH), stem width (SW), sugar content (SC), fresh weight (FW), Dry weight (DW), Moisture content (MC), leaf width (LW), Leaf length (LL), 100 Grain weight (where, *P<0.05,**P<0.01)

Morphological characters	one-way ANOVA		
	DF	SS	MS
GP	8	955.56	477.778
NL	8	8.6667	4.33333
PH	8	3760.89	1880.44**
SW	8	0.0608	0.0304**
SC	8	8.0067	4.00333
FW	8	58381.6	29190.8**
DW	8	31208.7	15604.3*
MC	8	770.159	385.08**
LW	8	1.50889	0.75444
LL	8	93.669	46.8344*
GW	8	10.1681	5.08404**

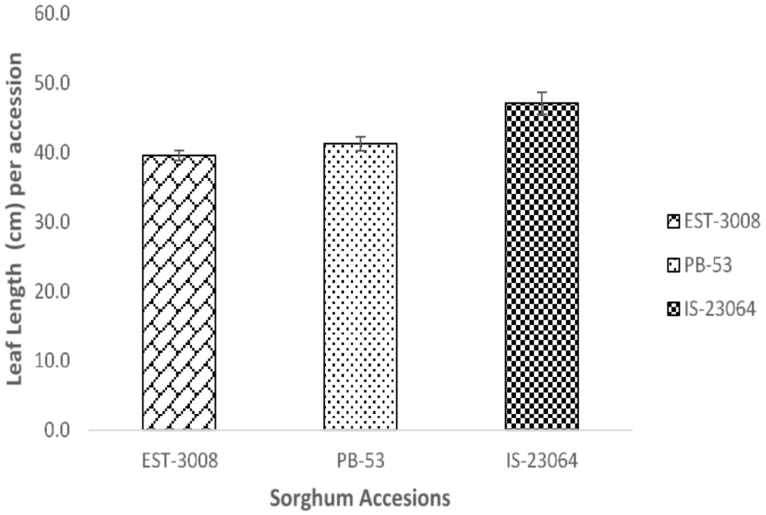

Fig. 12: Comparison of Leaf length (cm) in sorghum accessions.

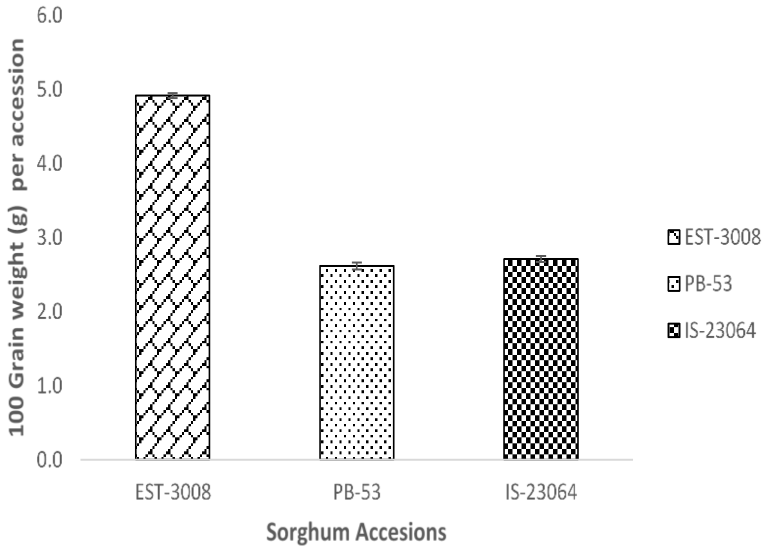

Fig. 13: Comparison of grain weight (100g) in sorghum accessions.

3008;4.1±1.677, PB-53;6.16±0.288 and IS-23064;6.1±0.1) average Sugar content (brix), 946.89±3.39 g (EST-3008;1002.3±34.15 , PB-53;66.33±269 and IS-23064;1005.33±22.14) fresh weight, 298±10.01g (EST-3008;288.6±44.79, PB-53;374.33±31.21and IS-23064;231±14.52) dry weight, 67.668±4.69% (EST-3008; 71.29±3.57, PB-53;54.97±4.94 and IS-23064;76.74±1.5) moisture content, 42.611±4.94cm (EST-3008;8.1±0.115, PB-53;7.56±0.814 and IS-23064;7.1±1.1) width of leaf, 7.611±12.26cm (EST-3008;39.53±1.26, PB-53;41.23±1.75 and IS-23064;47.06±2.75) length of leaf and 3.4144±2.29g (EST-3008;4.9±0.56, PB-53;2.6±0.83 and IS-23064;2.7±0.06) weight of 100 grains.

Among all accessions, germination was found to be highest in PB-53 and lowest in IS-23064 (Fig. 3). The number of leaves were found to be most in EST-3008 and PB-53 and least in IS-23064 (Fig. 4). The height of PB-53 was maximum and IS-23064 had minimum height (Fig. 5). Stem width was less in IS-23064 (Fig. 6). IS-23064 and PB-53 showed the highest brix amounts (Fig. 7). Moisture content was observed to be in high amounts in IS-23064 and least in PB-53 (Fig. 10).

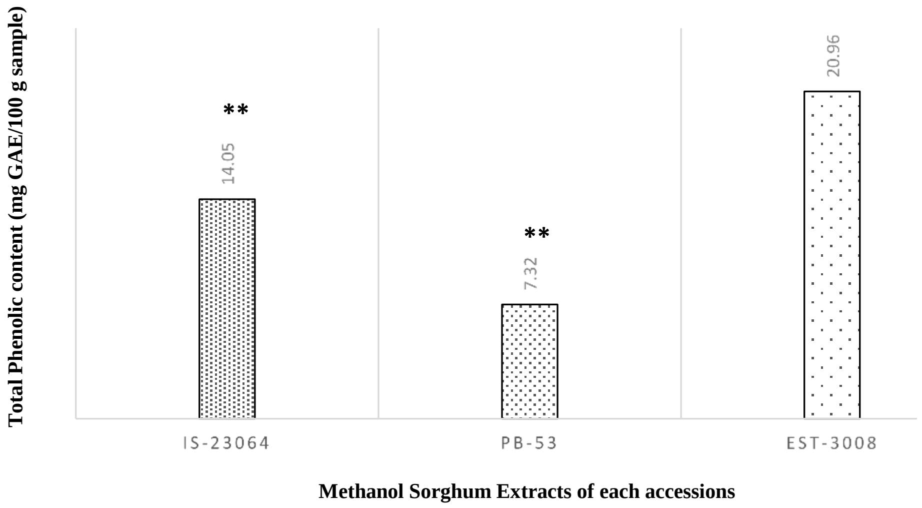

Fig. 14: Graphical representation of Total Phenolic content (mg GAE/100g sample) in methanolic sweet sorghum extracts.

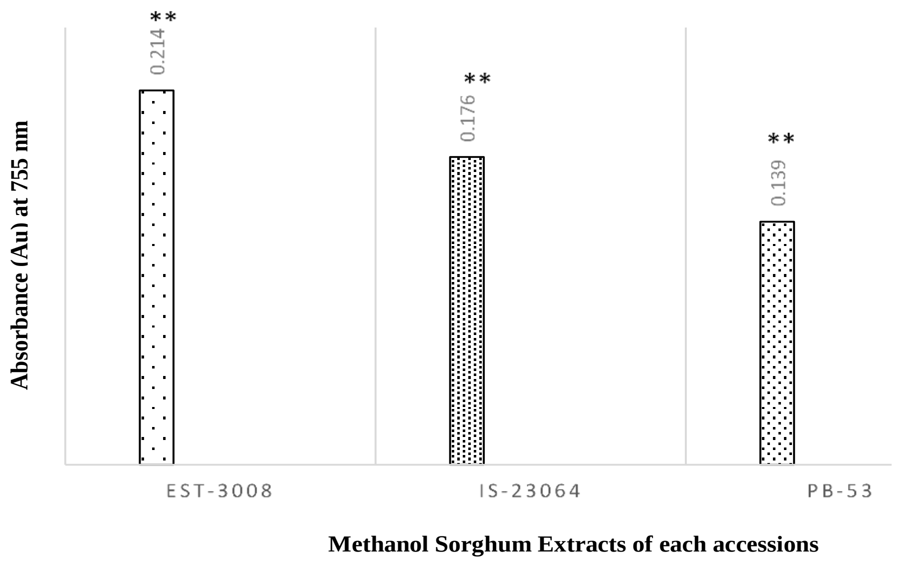

Fig. 15: Graphical representation of absorbance of EST-3008, IS-23064 and PB-53 in methanolic sweet sorghum extracts for TPC at 755nm.

Width of leaf was largest in EST-3008 (Fig. 11). Length of leaf was highest in IS-23064 (Fig. 12). Weight of grain was found to be highest in EST-3008 (Fig. 13).

Values found for each morphological character were significantly different (*P<0.05) except for means of germination, number of leaves, brix or sugar content and leaf width data (*P<0.05) (Table 1).

3.3. Determination of Total Phenolic content (TPC)

Jevcsak et al. 2017 observed 38.45 to 375.80mg GAE/g of TPC in their samples. Since the absorbance value determines the phenolic content present in a sample, the results indicate that EST-3008 had maximum amount of

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phenolic contents and highest absorbance (Fig. 14). Then Gallic acid and sample absorbance were compared. The GAE was used to describe results.

On other hand, phenolic content estimation was done through Folin-ciocalteu reagent. In this method methanol extracts were used. Gallic acid and tunic acid were used as markers for phenolic content estimation. In methanolic extracts, the maximum concentration of total phenols was found in the EST-3008 accession of sorghum to be 20.96mg GAE/g. TPC was 14.05, 7.32, and 20.96mg GAE/g for IS-23064, PB-53 and EST-3008, respectively in methanolic extracts (Fig. 14). PB-53 had the minimum amount of phenolic compounds and had white color seeds. On other hand, seeds with darker color have more phenolic compounds. Red color seeds of EST-3008 had maximum phenolic. As the color of seeds became darker its phenolic contents increased dramatically.

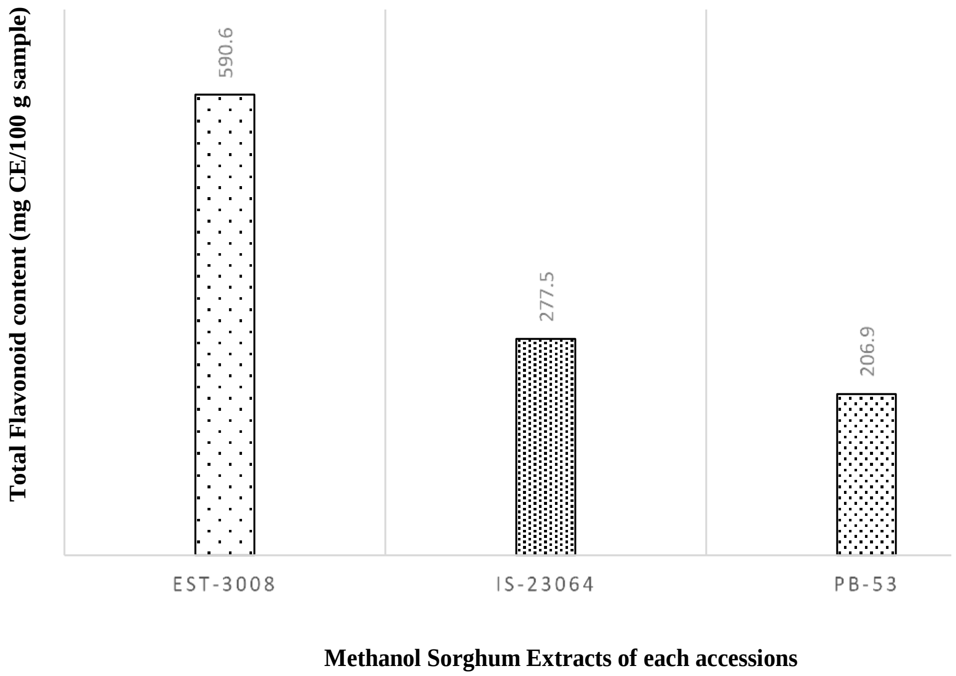

Fig. 16: Graphical representation of Total Flavonoid content (mg CE/g sample) in methanol sorghum extracts

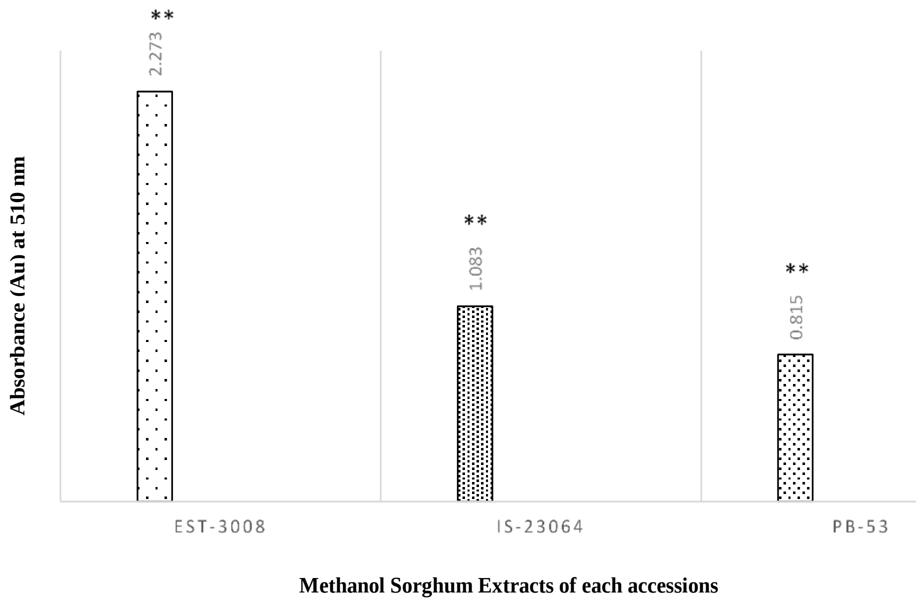

Fig. 17: Graphical representation of absorbance of EST-3008, IS-23064 and PB-53 in methanolic sweet sorghum extracts for TFC at 510nm.

3.4. Total Flavonoid Content (TFC)

The flavonoid extraction efficiency was high by using methanol extract. When catechin was used as a standard reference, the methanolic extraction process showed to be the most efficient for flavonoids, yielding the largest quantities. Flavonoid contents of the sample obtained are illustrated in Fig. 17. Average amount of 106.26 to 117.93 mg CE/100g TFC have been reported by Jevcsák et al. (2017).

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Table 2: Determination of association between total flavonoid content and total phenolic content with plant morphology through statistical method (correlation)

	BR	DW	FW	GER	GW	LL	LW	MOS	NOL	PH	SW	TFC
DW	-0.0019											
p-value	0.9960											
FW	-0.3951	-0.766*										
	0.2926	0.0160										
GER	0.7831 *	-0.1029	-0.2678									
	0.0126	0.7922	0.4859									
GW	-0.7557*	-0.1345	0.5088	-0.7901*								
	0.0185	0.7300	0.1619	0.0113								
LL	0.3667	-0.4752	0.2470	0.4836	-0.5925							
	0.3317	0.1961	0.5216	0.1872	0.0927							
LW	-0.2733	0.0830	0.1689	-0.2041	0.4978	-0.4963						
	0.4767	0.8319	0.6640	0.5984	0.1727	0.1741						
MOS	-0.1522	-0.9739*	0.8872*	-0.0373	0.3007	0.3752	0.0227					
	0.6958	0.0000	0.0014	0.9240	0.4317	0.3198	0.9537					
NOL	-0.1536	0.6725	-0.4484	-0.2799	-0.1314	-0.2157	-0.5099	-0.6399				
	0.6932	0.0472	0.2260	0.4657	0.7361	0.5773	0.1608	0.0635				
PH	0.1083	0.7519 *	-0.8000*	-0.0004	0.0167	-0.7354*	0.2397	-0.7802*	0.2852			
	0.7816	0.0195	0.0096	0.9991	0.9660	0.0240	0.5345	0.0131	0.4569			
SW	-0.6532*	-0.5234	0.8331 *	-0.6688	0.7900*	-0.0446	0.1292	0.6627 *	-0.1508	-0.5614		
	0.0564	0.1482	0.0053	0.0489	0.0113	0.9093	0.7404	0.0518	0.6985	0.1158		
TFC	-0.7454*	-0.2509	0.6028	-0.7641*	0.9900*	-0.4825	0.4386	0.4116	-0.1839	-0.1058	0.8590*	
	0.0212	0.5150	0.0857	0.0165	0.0000	0.1883	0.2376	0.2711	0.6358	0.7865	0.0030	
TPC	-0.5934	-0.6237	0.8634*	-0.5646	0.8353*	-0.1417	0.2650	0.7566*	-0.3650	-0.4771	0.9497*	0.8990*
	0.0921	0.0727	0.0027	0.1132	0.0051	0.7161	0.4908	0.0183	0.3341	0.1941	0.0001	0.0010

Correlation between total flavonoid content and total phenolic content with plant morphology through statistical method

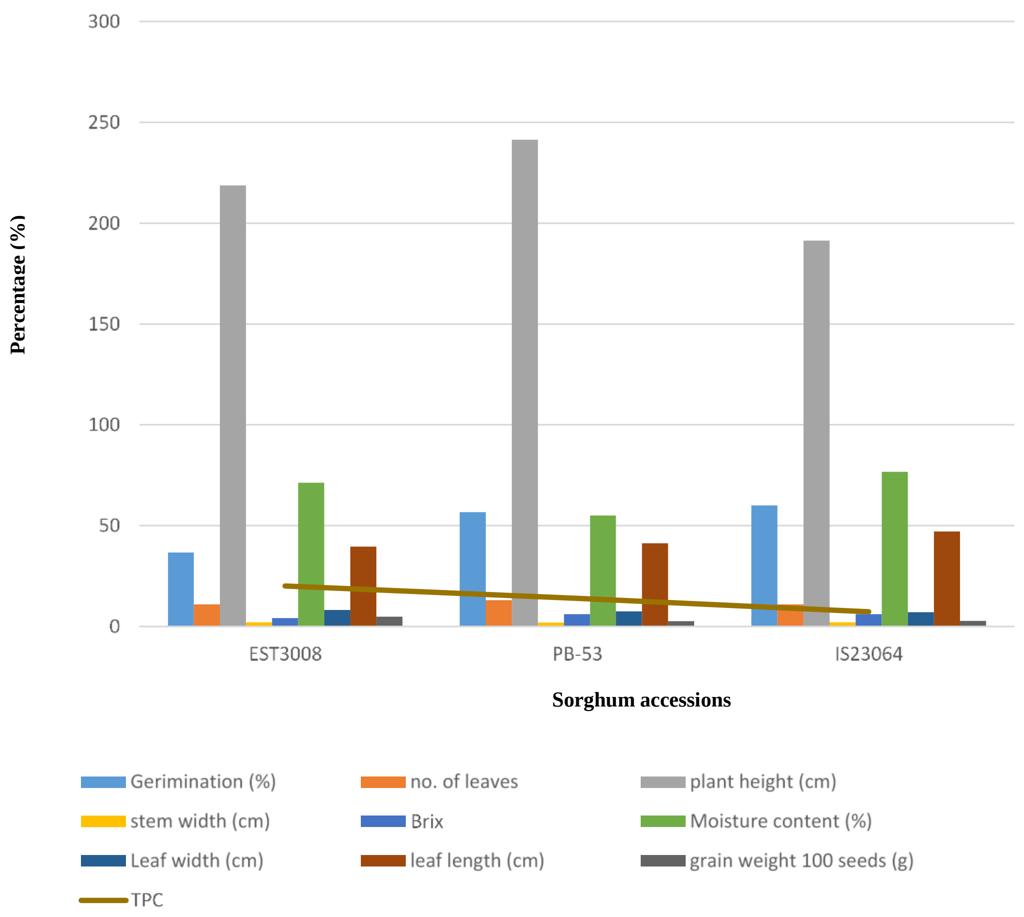

Fig. 18: EST-3003, PB-53 and IS-23064 accessions traits and their relation with Total Phenolic content (TPC).

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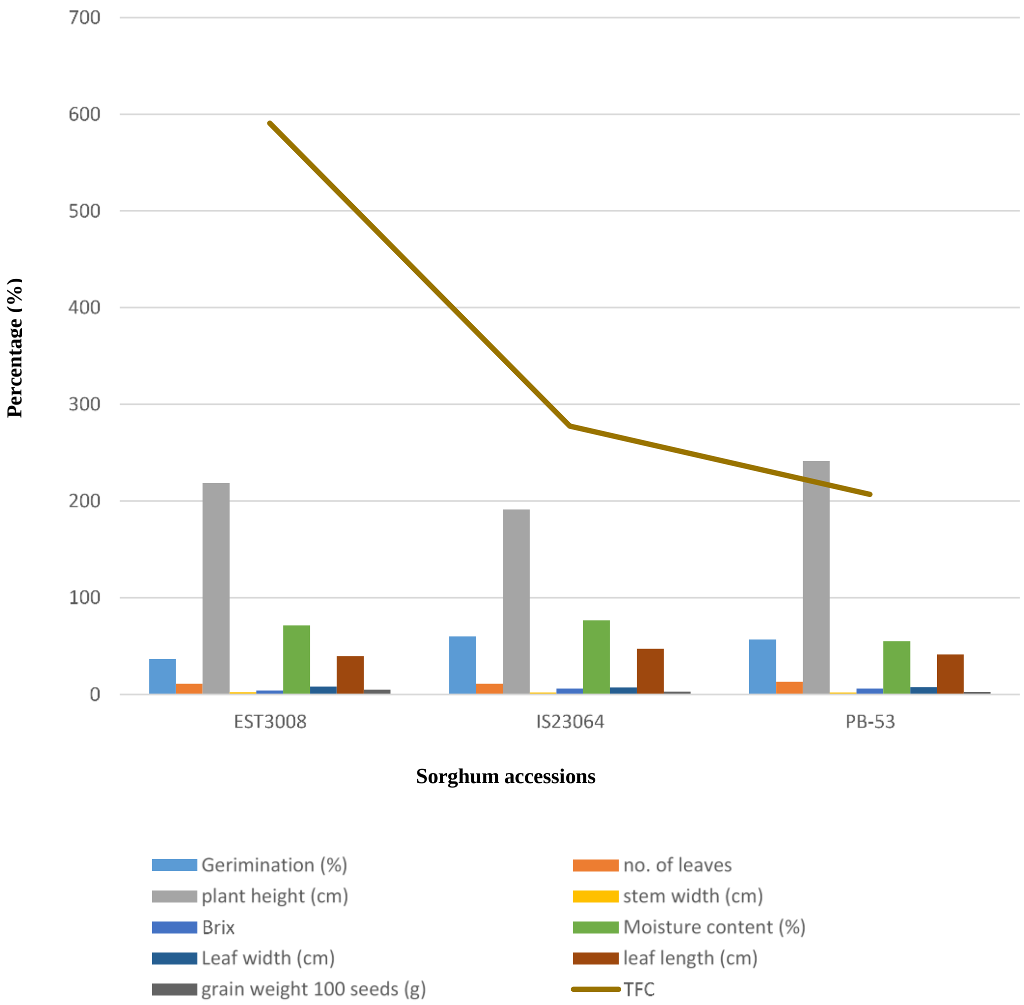

Fig. 19: EST-3003, PB-53 and IS-23064 traits and their association with Total Flavonoid content (TFC).

The absorbance of the sample also determines the level of or amount of total flavonoid content present in the sample. From the results, it is evident that EST-3008 has the highest amount of flavonoids since the absorbance is highest (Fig. 17). In order to determine the amount of flavonoids, the catechin standard curve was used as a reference and amounts of flavonoids in each sample were obtained (Fig. 16).

The flavonoid extraction efficiency was high with methanol. The EST-3008 sorghum accession had the highest concentration of flavonoids in methanolic extracts, although IS-23064 had lower flavonoids extracted as compared to EST-3008. The flavonoid concentration in the PB-53 accession is lower than in the other varieties. The Flavonoid contents of accessions were 590.6, 277.5 and 206.9 mg CE/g of sample for EST-3008, IS-23064 and PB-53 respectively (Fig. 16). The accessions with white color seed coat had minimum amount of flavonoids. On other hand, sorghum with red seed coat had highest flavonoid contents. For both TFC and TPC the color of seeds play an important role. Methanol is more successful for extracting TFC and TPC from the samples, which can be explained by the methanol's higher polarity.

3.5. Determination of Relationship between Total Flavonoid Content and Total Phenolic Content with Plant Morphology

Association of TPC and TFC with the morphological characteristics through graph (Fig. 18, 19) showed that with the decrease in TPC from EST-3008 to PB-53 and IS-23064, the decrease was also observed in leaf width, stem width, number of leaves and grain weight. Leaf length, Brix and germination showed inverse relation with TPC. As TPC decreased, the length of leaf, Brix and germination percentage was observed to increase. Plant height and moisture content showed non-symmetric behavior showing no relation with TPC (Figure 18). It can be observed that, with the decrease in TFC, Number of leaves, stem width, leaf width and grain weight also decreased. Brix and moisture content showed inverse relation with TFC, as TFC increased the value of brix and moisture content in samples also increased.

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Leaf length and plant height showed non symmetric behavior with TFC (Figure 19). Correlation (Table 2) of germination percent with morphological characters through statistical analysis showed that, strong positive relation ($P<0.05$) was found with brix values and grain weight. Dry weight of sorghum, fresh weight of sorghum, leaf length, leaf width, moisture content, number of leaves, plant height and stem width showed negative relation with percentage of germination in sample plants. Correlation of morphological characters with TPC and TFC showed that TFC had positive relation with fresh weight of the plant, percentage of germination, grain weight, leaf width, moisture content and stem width. On the other hand, TPC was observed to have strong positive relation with fresh weight of the plant, grain weight, leaf width, moisture content, stem width and TFC. Brix values, dry weight of the plants, germination percent, leaf length, number of leaves and plant height were found to have negative relation/effect by both TFC and TPC (Table 2).

4. Conclusion

Sorghum is a C_4 plant with a high level of photosynthesis and a fast rate of CO_2 modification, which allows it to thrive in arid or even saline or alkaline environments. Sorghum contains a lot of phenolic and flavonoid chemicals in them. Some phenolic chemicals are involved in the development of biotic and abiotic resistance to sorghum, while others perform multiple functions such as insecticide, antifungal, antiviral, and UV protection. This research presented the comparison of three sorghum accessions for their phenolic and flavonoid concentrations along with their morphological traits. Significant results were obtained which depicted the strong contribution of TPC and TFC on developmental characters of sorghum. So, the results could be used for further analysis of other metabolites having significant contribution towards agronomical important traits in sorghum.

Author's Contribution

MUH and TN: Conceptualization, Data collection, Drafting the manuscript; AA and MSN: writing, Review and editing manuscript; MK: Review, and Editing. All authors have reviewed the manuscript critically and approved the final draft for publication in Agrobiological Records.

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