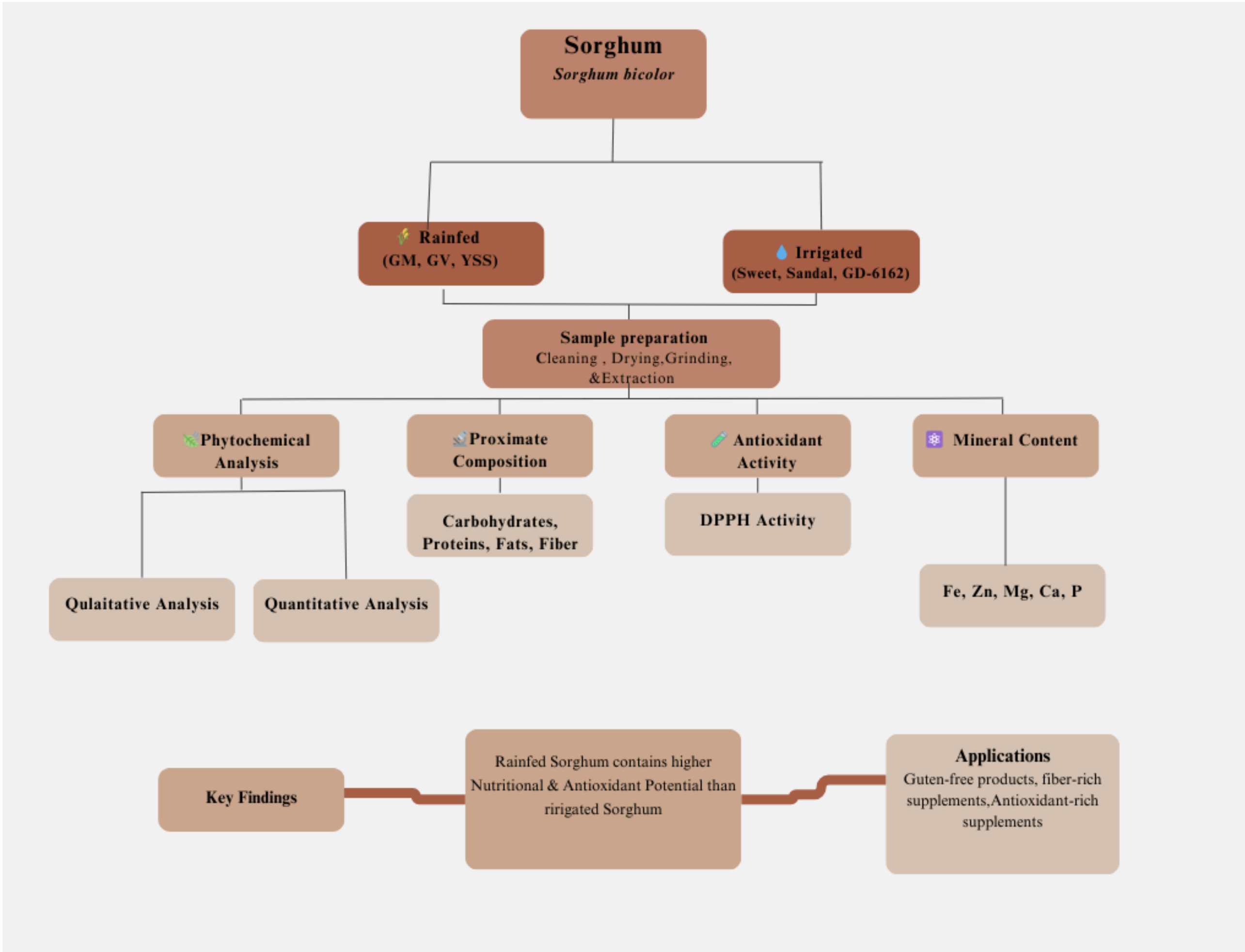
NUTRITIONAL, PHYTOCHEMICAL, AND ANTIOXIDANT POTENTIAL OF RAINFED AND IRRIGATED SORGHUM VARIETIES

Erum Khalid[1], Feroza Hamid Wattoo[1], Muhammad Javaid Asad[1], Saima Amin[1]

[1] UIBB, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi

ABSTRACT

One of the most popular cereal foods in the world, sorghum is thought to contain minerals and bioactive substances. This study assessed the mineral content, proximate composition, and antioxidant potential of rainfed (GM, GV, and YSS) and irrigated (Sweet, Sandal, and GD-6162) sorghum varieties. Variations in its phytochemical content, antioxidant qualities, and agronomic traits may result from its broad adaptive range. The purpose of the study was to evaluate the nutritional and phytochemical variations among these sorghum varieties cultivars under various growing circumstances. DPPH scavenging assay was used to examine antioxidant activities. The study also evaluated the mineral content, proximate composition, and phytochemical constituents, pointing out notable distinctions between irrigated and rainfed cultivars. According to the findings, rainfed sorghum varieties cultivars have greater nutritional and antioxidant potential than irrigated ones, which qualifies them for use in functional food applications and dietary enhancement initiatives.



Graphical Abstract

INTRODUCTION

Cereal crops, which come from the Gramineae family of monocot grasses, are vital food sources. They contribute significantly to the world's food energy and include wheat, maize, barley, rice, oats, and sorghum. Among cereals, sorghum, a gluten-free cereal is a rich source of bioactive polyphenols and dietary antioxidants. One of the most important crops in the world, Sorghum (Sorghum bicolor L.) Moench is a member of the Poaceae family and grows in arid and semi-arid tropical regions. It is known to be a great source of nutrition, offering minerals, proteins, starch, vitamin E bioactive compounds, phytosterols, and policosanols, along with a high fiber content. (Punia et al., 2021)

Sorghum is the fifth most important cereal in total world production. Cereals, which are grown on almost 60% of the world's farmland, are high in vitamins, minerals, proteins, fats, and carbohydrates. Consuming whole grains is associated with a lower risk of diet-related illnesses like inflammatory disorders, colon cancer, and coronary heart disease. Climate, soil conditions, and genetic factors all affect the nutritional makeup of cereals. Human nutrition may be impacted by poor crop quality caused by nutrient deficiencies in the soil as reported by Anglani (1998). Pakistan struggles to maintain cereal production due to its primarily arid climate and little rainfall, so managing the soil and crops is essential to addressing nutritional deficiencies. Pakistan's agricultural system uses both artificial and rainfed irrigation. Because of its semi-arid to humid climate, the Potohar region relies on monsoon rainfall to keep crops growing. From seeding to wax maturity, water availability is essential, and climate changes like too much or too little rainfall affect crop yields. Because minerals are available in arid soils, rainfed irrigation produces crops that are rich in nutrients; however, yields are typically lower than in irrigated agriculture. Although artificial irrigation guarantees a regulated water supply, it may eventually cause the depletion of soil minerals. Since rainfall variability continues to affect crop yields and quality, effective water management is crucial to maintaining cereal production and enhancing food security.

Table 1: Sowing and harvesting timelines of sorghum in rainfed and artificially irrigated areas of Pakistan

S.No	Cereal Name	Botanical Name	Nature of Irrigation	Sowing Time	Harvesting Time
1	Sorghum	*Sorghum bicolor*	Rainfed & Irrigated	March - June (Spring) / July - August (Monsoon)	September - December (Varies by region)

MATERIAL AND METHODS

Certified seed samples grown by artificial irrigation system

Certified samples grown by artificial irrigation system on plain lands were collected from;

a) NARC (National Agriculture Research Centre, Islamabad and Lahore)

b) NIFA (National Institute of Food and Agriculture)

c) Mandi Ahmed Abad, District Okara

Seed samples grown by Rainfed irrigation system

These certified sorghum seed samples were then cultivated on pure arid land environment of 'Koont Research Farm Chakwal' of Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi and fed with natural rainfed irrigation along with other required fertilizers as per crop cultivation protocol of the arid area.

Sample Preparation

By using West-Point dry miller, the grains of sorghum varieties were grinded to very fine powder after dried it in oven. Methanol extracts were prepared by dissolving 3 g of sample powder in 18ml of methanol. Electric shaker was used to mix the mixture for about 72 hours after that filtered it by using whattman filter paper and allow the filtrate to evaporate at room temperature in order to have concentrated extracts. For stock solution preparation, took the extract in the ratio of 1:10, means 1 g of extract dissolved in 100 ml of stock solution (methanol).

Proximate analysis

Proximate analysis is the determination of moisture content, ash content, crude fat, crude fibers, crude proteins and carbohydrate content of different samples. The proximate analysis of irrigated and rain-fed selected sorghum varieties were calculated according to the standard principle of (AOAC, 2005).

Screening of phytochemicals

The bioactive compounds that can be derived from plants such as flavonoids, phenol, alkaloids, carotenoids, tannin, saponin, coumarin, quinones, and antioxidants compounds, etc. There are about 4,000 phytochemicals known to be contained in plants that can be used to reduce, prevent, or minimize medical conditions such as cancer, strokes, and different metabolic syndromes. (Lamichhane et al., 2021).

Qualitative screening of phytochemicals

Qualitative Screening of Phytochemicals like alkaloids, flavonoids, terpenoids, saponins, phenol, coumarin, protein, quinones, carbohydrates and tannins was performed by using the standard procedure described in (Shaikh and Patil, 2020) Sorghum varieties were subjected to qualitative screening and different color confirmation indicate the presence of different phytochemicals.

Quantitative screening of phytochemicals

The total phenolic content in sorghum varities were determined by Folin-Ciocalteu method as described by (Daniel et al., 2021). The total flavonoid contents in sorghum varities were determined using the method was described earlier (Zainol et al., 2020). By using the Folin-Ciocalteu method, which was described by Saeed et al. (2012) and Ugwu and Anosike (2020), the total tannin content of sorghum varities was determined.

Estimation of minerals

The mineral composition of sorghum varities was determined by using Inductively Coupled Plasma-Mass Spectrometry technique (ICP-MS) (Topdas and Sengül, 2021).

Acid Digestion

The acid digestion of sorghum varities grains were accomplished for minerals estimation. For this purpose, took 1.00 g very fine powdered of sample in a conical flask and then added 10 ml nitric acid in. Placed it for 24 hours and poured 4.00 ml of perchloric acid in each flask containing sample and mixed well. Kept the digested sample on digester apparatus at about temperature 200 ^{o}C until the mixture turned transparent and only 2-3 ml left. After cooling the volume of the mixture was made up to 100ml by adding distilled water. Finally, the sample was ready to run on ICP-MS and Flame photometer for minerals estimation.

Estimation of vitamins

High Performance Liquid Chromatography was used for the determination of vitamins (Takeuchi et al., 1984; Surlehan et al., 2019).

Preparation of Extract by Using Soxhlet Extraction Procedure

The soxhlet extraction procedure was used for the estimation of vitamin E, fat soluble vitamin. For this purpose, took 1.00g of finely powdered of sorghum varities sample, wrapped it in filter paper. In Soxhlet extractor round bottom flask poured 250 ml of *n*-hexane. Kept the sample in the thimble at 90 ^{o}C up to started to boil. To avoid the dissipation of vitamins, the temperature was reduced to 60 ^{o}C. The complete process was allowed to run for about 4 hours, then cool down the extract at room temperature.

Preparation of Sample

For the collection of vitamin E, the extracted solvent from soxhlet extraction was allowed to evaporate. For this purpose, a rotary evaporator was used at about 60^{o}C and concentrated vitamin E in ethanol and filtered it. The sample was ready to run on HPLC for estimation of minerals, before injection onto the HPLC column the sample extract was filtered through a filter paper of 0.45 micrometers.

Preparation of Standard Solution

The standard stock solution of vitamin E were prepared to a concentration of 5 mg/ml in *n*-hexane and concentrated it in ethanol then filtered it. The prepared standard solution kept in dark at -20 ^{o}C and can stored it for at least 3 months.

High Performance Liquid Chromatography

The HPLC was equipped with fluorescence detector, the excitation wavelength of detector was set at 290 nm while the emission wavelength was 330 nm. Firstly, HPLC column was properly washed with the help of mobile phase having 94.6 % hexane, 3.6 % ethyl acetate and 1.8% acetic acid before starting the analysis. The flow rate was 1.0ml/min and temperature of the column oven was maintained at 25 ^{o}C. 0.02 ml accurately weighted oil sample was dissolved in 1.00 ml HPLC graded *n*-hexane and by using 1.00 ml syringe sample was injected into 1.5 ml vial. Vitamin E concentration of sorghum varities was evaluated according to the retention time and quantified by used standard calibration curved of HPLC for tocopherol.

Antioxidant scavenging activity

The following antioxidant scavenging activity were performed by several assays and absorbance was checked by UV-Mass spectrometry.

I. 1-diphenyl-2-picrylhydrazyl (DPPH) Radical-Scavenging Activity

1-diphenyl-2-picrylhydrazyl (DPPH) Radical-Scavenging Activity

Using the approach outlined by Ali et al. (2020) the antioxidant activity of sorghum varities extracts against DPPH was assessed. It was created to dilute DPPH to 0.0004 M in methanol. Each sample was extracted with 1 mL of methanol at various concentrations (25 g/mL, 50 g/mL, and 125 g/mL), then 2 mL of methanolic DPPH dilution was added. Kept the tubes holding the combination at room temperature and in the dark for 20 minutes before measuring the absorbance at 517 nm (Model, Smart Spec 3000). The DPPH was diluted in methanol to create the blank. The following formula was used to calculate the percentage of antioxidant scavenging activity for sorghum varities extracts:

$$\text{Scavenging }\% = \frac{\mathrm{CA}-\mathrm{SA}}{\mathrm{SA}}\ 100$$

Where, CA=Control absorbance SA= Sample absorbance

Statistical analysis

All the statistical analysis of sorghum varities were done by using SPSS and result will reported as mean ± SD (standard deviation). IC_{50} value will be calculated by linear regression analysis.

RESULTS AND DISCUSSION

Proximate Analysis

Table 2 Shows the proximate composition of irrigated sorghum varities varieties named sweet, sandal, and GD-6162 and rainfed GM, GV, and YSS. Sorghum contains a rich amount of protein and carbohydrates.

Table 2: Shows the proximate composition of irrigated and rainfed sorghum varieties.

S. NO	Cereal varieties	Moisture	Ash	Fiber	Fat	Protein	CHO
1	**Irrigated Sorghum varieties**						
1.1	Sweet	11.38±1.2	1.02±0.5	2.5±1.1	6.07±0.2	7.8±0.5	70.5±2.1
1.2	Sandal	13.1±0.05	0.98±1.0	2.1±0.6	6.04±0.5	8.5±0.5	69.4± 1.0
1.3	GD-6162	12.05±0.7	0.99±1.1	3.0±1.0	5.96±1.0	8.3±.02	69.0±0.5
2	**Rainfed Sorghum varieties**						
2.1	GM	1.38±1.2	4.02±0.5	2.17±0.4	0.58±0.6	10.5±0.4	81.4±0.9
2.2	GV	1.1±0.05	3.98±1.0	8.81±0.8	0.78±0.6	7.0±0.1	78.4±0.5
2.3	YSS	1.05±0.7	5.99±1.1	1.57±0.9	0.62±0.6	11.4±0.7	79.4±0.1

Phytochemical Analysis

Qualitative phytochemical analysis of cereals

Phytochemicals qualitative screening of was performed to identify secondary metabolites present in sorghum by using methanolic extracts. When these compounds with methanolic extract were allowed to react with different solution shows different color or precipitates. These screening confirmed the presence of coumarins, terpenoids, flavonoids, phenols, alkaloids and carbohydrates results are shown in table 4. Phenolic compounds found in sorghum grain could be utilized to create functional tea beverages (Xiaong et al., 2019). All the phytochemicals have their specific and functional role of cataracts in pateints with diabetics (Okwu and Omodamiro, 2005).

Table 3: Qualitative Phytochemical Analysis of Sorghum irrigated and rainfed varieties

Phytochemicals	**Rain fed sorghum varities**			**Irrigated sorghum varities**		
	GM	**GV**	**YSS**	**Sweet**	**Sandal**	**GD-6162**
Coumarins	-	-	+	-	-	-
Proteins	-	-	+	+	+	+
Tannins	-	-	+	+	+	+
Terpenoids	+	+	-	+	+	+
Cabohydrates	-	-	-	-	-	-
Quinones	+	+	+	+	+	+
Saponins	-	-	-	+	+	+
Alkaloids	-	-	+	+	+	+
Flavonoiods	-	-	-	-	-	-
Phenolics	+	+	+	-	-	+
Glycosides	+	+	-	-	+	-

+ Present, - Absent

Table 5, shows the phytochemical analysis of three sorghum varieties under irrigated and rain fed conditions. Variety GD-6162, YSS contained highest as compared to other varieties. Our results are closely related to (Plessis and Johanna, 2014 and Davi et al., 2011

Table 4: Quantitative Phytochemical analysis of Sorghum

S. NO	Cereal varieties	Phenol (mg/g)	Alkaloids (mg/g)	Flavonoid(mg/g)
1	**Irrigated Sorghum**			
1.1	Sweet	35.0±1.5	2.97±1.5	1.6±0.5
1.2	Sandal	30.67±2.1	5.12±.05	0.5±0.1
1.3	GD-6162	40±0.5	4.56±1.1	1.8±0.5
2	**Rainfed Sorghum**			
2.1	GM	39.45±0.5	5.10±0.4	1.01±1.4
2.2	GV	25.58±0.1	2.5±1.05	2.5±1.55
2.3	YSS	41.22±0.2	1.5±2.01	1.5±2.1

Minerals

Minerals are micronutrients required by the body in small amount, unlike macro nutrients they are not directly involved in energy generation but perform various important functions in the body i.e; co-factor, signal transduction, transport mechanism. Cereals contained different minerals. According to Bock. 2000 cereals contained 1.5 - 2.5% mineral. Current study- samples analysed for four minerals Ca, Zn, Mg and Cu. The results are presented in Table 5 and among the four minerals Mg is found in highest amount ranged 4.35- 9.38 ppm followed by Ca 0.17-1.48 ppm.

Mineral Analysis Through ICP

Table 5: Minerals content (ppm) in rainfed and irrigated Cereals

S.NO	Cereal	Ca	Mg	Cu	Zn
1	**Irrigated Sorghum varieties**				
1.1	Sweet	1.12	4.35	0.03	0.13
1.2	Sandal	1.09	9.27	0.05	0.27
1.3	GD-6162	0.64	7.22	0.01	0.01
2	**Rainfed sorghum varieties**				

2.1	GM	0.00	14.16	0.17	0.40
2.2	GV	0.00	11.89	0.05	0.34
2.3	YSS	0.00	19.18	0.06	0.40

Mineral Analysis Through Flame photometer

Table 6: Minerals (Sodium and Potassium) composition of sorghum varieties

S. No	Varieties	Sodium (mg/100g)	Potassium (mg/100g)
1	**Irrigated Sorghum varieties**		
1.1	Sweet	2.0±0.5	21±0.4
1.2	Sandal	2.1±0.1	35±0.2
1.3	GD-6162	3.3±0.4	61±0.7
2	**Rainfed Sorghum varities**		
2.1	GM	7.6±0.6	100±0.3
2.2	GV	5.8±0.4	83±0.5
2.3	YSS	6.0±0.5	83±0.9

Vitamin E

Vitamin E content of different cereals was determined by HPLC, the results are given in Table *7*. our results are comparable with that of documented by other researchers. Herting and Drury (1969) documented that whole grain contain 1.15mg/100g of vitamin E.

Table 7: Vitamin E content in rainfed and irrigated sorghum varieties

S.No	Cereal	Vit. E(mg/100g)
1	**Sorghum varieties**	
1.1	Sweet	0.001
1.2	Sandal	0.0045
1.3	GD-6162	0.1
2	**Rainfed Sorghum varieties**	
2.1	GM	0.3
2.2	GV	0.5
2.3	YSS	0.05

Antioxidant Activity

DPPH scavenging activity of the methanolic extract of rainfed and irrigated sorghum varities varieties was performed and the results are shown in **Error! Reference source not found.**, along w ith IC_{50} values. The highest % SCV (lowest IC_{50}) was recorded for Sweet sorghum varities and GV as compared to other two varieties of irrigated and rainfed sorghum varities respectively. DPPH scavenging assay of the sorghum varities varieties is shown Table 8. All the varieties showed lower IC_{50} values then standard. The lowest IC_{50} value showed by GV rainfed sorghum variety thus having maximum activity. The highest % SCV (lowest IC_{50}) was recorded for Sweet sorghum as an irrigated variety of sorghum as compared to other two varieties.

Table 8: Shows the DPPH assay of Sorghum irrigated and rainfed varieties

S. NO	Cereal varieties	Con. (µg/ml) 25	50	75	100	125	IC_{50}
1	**Irrigated Sorghum varities**						
1.1	Sweet	46±2.0	70±1.5	77.25±	80±2.08	90.2.03	**11.2**
1.2	Sandal	23.76±0.5	48.9±0.5	62.33±0.5	80.07±0.6	88.58±.5	**58.41**

1..3	GD-6162	48.12±0.76	59.3±1.0	66.78±1.0	70.93±1.2	85.65±1.2	**28.55**
2	**Rainfed sorghum varities**						
2.1	GM	56.0±0.8	66.1±0.5	72.33±0.5	78.70±0.6	96.94±0.6	**4.267**
2.2	GV	43.16±0.5	74±0.75	81.55±0.5	90.5±0.33	94.41±0.5	**9.81**
2.3	YSS	37.5±1.5	43.5±0.4	48.34±0.4	58.67±0.4	67.5±0.5	**72.04**
3	Ascorbic Acid	44.86±1.0	48.44±.50	62.46±.55	68.57±.58	82.43±.51	**51.49**

CONCLUSION

The overall study show that sorghum are not only good sources of macronutrients (carbohydrates, proteins and fats) but also exhibit a measurable number of micronutrients i.e; vitamins and minerals as well as considerable concentration of phytochemicals, antioxidants. Besides varieties cultivated under irrigated conditions, the varieties sown under **rainfed conditions** show high potent of the mentioned nutrients in almost all parameters.

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