Int.J.Curr.Microbiol.App.Sci (2024) *13*(07): 94-99

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 13 Number 7 (2024)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2024.1307.011

Studies on Biochemical Properties of Potential Sorghum Lines in Rabi Season for Micronutrient Densification

Shradha Aherkar[1]*, R. B. Ghorade[2], V. V. Kalpande[2], N. M. Konde[3], Priti Sonkamble[1], Arjun Bhuyar[2] and P. S. Kamble[2]

[1]*Department of Agricultural Botany, Dr. PDKV, Akola, Maharashtra, India*
[2]*Sorghum Research Station, Dr. PDKV, Akola, Maharashtra, India*
[3]*Department of Soil Science and Agricultural Chemistry, Dr. PDKV, Akola, Maharashtra, India*

**Corresponding author*

Keywords

Sorghum, malnutrition, Biofortification, Iron, Zinc, Protein, Carbohydrates, Proline

Article Info

Received: 25 May 2024
Accepted: 29 June 2024
Available Online: 10 July 2024

A B S T R A C T

Millets are the most important and nutrient rich food preferred by most of the people for their daily consumption. However, these millets if enriched with nutrients will eradicate the problem of hidden hunger i.e. malnutrition prevalent in the weaker sections of the society. Sorghum, the King of Millets which is rich in starch, protein, fibres, etc and have efficient amount of Zinc and Iron bioavailability. However, sorghum biofortification would provide a sustainable solution to iron and zinc densification in sorghum. The present study was undertaken to study the ten potential sorghum lines for their biochemical properties such as protein, carbohydrates and proline along with the micronutrients such as iron and zinc at Sorghum Research Station, Dr. PDKV, Akola during *rabi* season 2023-24. In the present investigation, it was observed that, highest and lowest protein content was recorded by the line CSV-29R (8.98%) and PKV Kranti (6.33%), respectively. The highest and lowest carbohydrate content was recorded in the line Parbhani Shakti (86.19 g/100g) and CSV-29R (49.23 g/100g), respectively. The highest and lowest proline content was recorded by the line CSV-22R (1 g/100g) and Parbhani Shakti (0.5 g/100g), respectively. From the correlation studies it was observed that, carbohydrates had significantly positive correlation with iron content (r=0.663*) whereas, had positive correlation with zinc content (r=0.286). On the other hand, negative correlation was observed between protein content and micronutrients.

Introduction

Millets are collective group of small seeded annual grasses that are grown as grain crops, especially in dry areas of temperate, sub-tropical and tropical regions. Millets are good sources of energy. They not only provide protein, fatty acids, minerals, vitamins, dietary fibre and polyphenols but also are a good source of antioxidants, such as phenolic acids and glycated flavonoids Amadou *et al.,* (2013). The King of Millets, Sorghum is the fifth most important cereal crop grown in Africa, Asia, and America and also one of the major staple crops cultivated in India. It comprises of 25 species of flowering plants belonging to the Family of Poacae (Graminae), (Price *et al.,* 2005) Genus: Sorghum and Species: bicolor. Sorghum grain is rich in starch, protein, micronutrients and crude fiber but low in fat (Chavan and Patil, 2010), making it a good staple. It is gluten-free and a safe food for celiac patients or people with known gluten allergies. The slow digestibility of sorghum starch makes it a food option for diabetics (Wong *et al.,* 2009).

Based on the WHO recommendation nutrient intake (RNI), sorghum is a good source of energy and eleven nutrients, of which nine are micronutrients that are critical to health and well-being. The bioavailability of Fe in sorghum is 5% and that of Zn is 20% depending upon the form in which sorghum is consumed (United Sorghum Checkoff Program, 2010). Biofortification of sorghum by increasing mineral micronutrients (especially iron and zinc) in the grains provides a sustainable solution to iron and zinc deficiency (Pfeiffer and McClafferty, 2007). Sorghum cultivars differ in Fe and Zn content in grain.

In view to the losses caused by deficiency of the micronutrients, the present study was undertaken with objective to study the biochemical properties of sorghum lines in response to micronutrients densification.

Biochemical analysis was done to understand the metabolic behaviour of the sorghum grains. It was further suggested that, the lines showing good potential can be used in breeding program through hybridization and selection.

Materials and Methods

Ten potential sorghum lines were selected for study in the *rabi* season. The selected lines comprised of promising varieties, derived lines and advanced breeding lines. It varied in seed size, seed shape, seed colour, seed luster, etc.

Specific features of the lines which were selected is mentioned below

AKSV-438

It is a derived line maintained at Dr. PDKV, Akola and registered by NBPGR (INGR23040). It has recorded to be high in zinc and iron content. It has white pearly grain appearance.

AKSV-440

It is the derived line maintained at Dr. PDKV Akola and registered by NBPGR (INGR23041). It has recorded to be high in zinc and iron content. It has pale type plant with reddish bold seeds.

AKSV-442

It is the derived line maintained at Dr. PDKV, Akola. It has high zinc and iron content. It has yellowish grains colour and elongated grain shape.

CSV-26R

It is a *rabi* sorghum variety superior for its nutritional and roti qualities. Due to its dual purpose use, it is highly acceptable.

CSV-22R

This is a *rabi* sorghum variety developed at MPKV, Rahuri. The variety has round bold grain.

M-35-1

It is a *rabi* sorghum variety selected from a local landrace. It produces high stable yields of grain and has recorded superior grain and fodder quality.

CSV-29R

It is a *rabi* sorghum variety developed through one back cross followed by Pedigree breeding. It is developed from the cross [(CSV216R x DSV5) x CSV216R].

PKV Kranti

It is a *rabi* sorghum variety which has been developed at Dr. PDKV, Akola. It is a pedigree selection derived from the cross SPV – 1201 X Ringani.

Parbhani Shakti

It is India's first biofortified sorghum variety with significantly higher iron and zinc content. It is developed by ICRISAT from the improved variety ICSR 14001 and was collaboratively released for cultivation by Vasantrao Naik Marathwada Krishi Vidyapeeth (VNMKV), Parbhani, Maharashtra and ICRISAT.

AKSV-439

It is the derived line maintained at Dr. PDKV, Akola. It has recorded to be high in zinc and iron content.

Biochemical Characters

The biochemical analysis was done to infer the amount of protein, carbohydrates as well as proline in the sorghum grain samples. The study was undertaken to understand the metabolic behaviour of selected sorghum lines.

Protein content (%)

Protein content in the grain sample was estimated by the Kjeldhal method Anantakrishnan *et al.,* (1952). For digestion of the sample, 0.5g powdered seed sample was weighed in digestion tube to that 1g of catalyst mixture and 10 ml concentrated H2SO4 was added and the tubes were placed in the block digester heated at 1000C until the digestion was completed and a transparent liquid was obtained. After that tubes were cooled by adding sufficient quantity of distilled water and volume made upto 50ml.

For sample distillation, 20ml boric acid solution was taken in a conical flask condenser outlet of distillation apparatus was dipped in the boric acid solution. 10ml of digested aliquot was taken in the distillation tube and in that 40% of 40ml NaOH was added.

The distillation was then carried out for 9 minutes. The color of boric acid in the conical flask changes to green from pink. This boric acid was then titrated with 0.1N H2SO4 drop by drop the boric acid changed the color to pink. The reading on the burette was recorded.

Carbohydrates content (g/100g)

Carbohydrates were estimated by utilizing Anthrone method of Carbohydrates estimation Dreywood (1946) Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green colored product with an absorption maximum at 630 nm. 100 mg of the sample was weighed into a boiling tube and hydrolyzed in boiling water bath for 3 hours with 5mL of 2.5 N-HCl and cooled to room temperature. The volume was made up to 100ml and centrifuged.

The supernatant was collected and 0.5 and 1ml aliquots were taken for analysis. The standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard ('0' serves as blank).

The volume was made upto 1 ml in all the tubes including the sample tubes by adding distilled water. 4ml of anthrone reagent was then added and cooled rapidly. The colour change was observed from green to dark green color at 630nm and observations recorded.

A standard graph was plotted by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph, the amount of carbohydrate present in the sample tube was calculated.

Proline content (g/100 g)

Proline will be estimated by the Proline assay protocol (Bates *et al.,* 1973). 0.5 g sample was homogenized in 10 ml of 3% sulphosalicylic acid. The homogenate was then filtrated through double layer filter paper. 2 ml filtrate was then taken in test tube to which 2 ml of acid ninhydrin reagent and 2 ml glacial acetic acid was added.

The test tube containing mixture was placed in water bath for 1 hour. The test tubes were then cooled by keeping in ice bath. The contents were further transferred to separating funnel and 4 ml toluene was added and mixed vigorously.

The coloured toluene fraction was separated and measured at 520 nm in spectrophotometer. The proline concentration was determined from a standard curve using D-Proline

Iron content (ppm) / Zinc content (ppm)

The content of iron as well as zinc in the grain samples was determined by using the di-acid digestion method and the iron and zinc content in the aliquot was determined by Atomic Absorption Spectrometer (ASS).

Firstly, seed sample of 1 gm was digested with 30ml of di-acid mixture (HNO3+HClO4) in 9:4 proportions in 100 ml of volumetric flask by placing it on a hot plate at moderate temperature. After 3-5 minutes the seed sample started to digest and emitted a dull red colored smoke. The digestion is done until 3-5 ml of colorless liquid was obtained in the flask.

After cooling the flask, 20 ml of deionized or glass-distilled water was added and the volume was made up with deionized water. Sample was then filtered through Whatman no 42 filter paper.

The aliquot of this solution was then used for determination of Iron content as well as zinc content with the help of Atomic Absorption Spectrophotometer (ASS).

Results and Discussion

Protein content (%)

Protein is an important constituent in the body needed for growth and cell repair. They are immensely important in seeds, as they make up 40% of the seeds weight and serve to store amino acids for the developing embryo. The highest protein content was recorded by the line CSV-29R (8.98%) followed by M-35-1 (8.46%) and CSV-22R (8.25%) and the lowest protein content was recorded in the genotype PKV Kranti (6.33%). (Table 1)

Carbohydrates (g/100g)

Carbohydrates are the sugar molecules and is one of the three main constituents found in food. The body breaks down carbohydrates into glucose which is the main source of energy for body's cells, tissues and organs.

The highest carbohydrate content was recorded in the line Parbhani Shakti (86.19g) followed by AKSV-440 (80.97g) whereas, lowest carbohydrate content was recorded by the line CSV-29R (49.23g). (Table 1)

Proline (g/100g)

Proline is an amino acid, which plays a beneficial role in plants which are exposed to various stress conditions. Stress leads to an overproduction of proline in plants which imparts stress tolerance by maintaining cell turgor or osmotic balance. The lowest proline content was recorded by the line Parbhani Shakti (0.5 g) followed by PKV Kranti (0.65g) whereas, highest proline content was recorded by the line CSV-22R (1g) followed by AKSV-442 (0.97g) (Table 1).

Iron content (ppm)

Iron is an essential micronutrient for all the living organisms which helps to boost the immune system. It plays a major role in metabolic processes of life such as DNA synthesis, respiration and photosynthesis. Maximum iron content was recorded by the sorghum line Parbhani Shakti (55.28 ppm) whereas, minimum iron content was recorded in the line CSV-26R (27.20 ppm). (Table 1)

Zinc content (ppm)

Zinc is an essential micronutrient for human metabolism which plays an important role in catalyzing more than 100 enzymes as well as facilitates protein folding and helps in regulation of gene expression. Maximum zinc content was recorded in the line AKSV-442 (25.79 ppm) and minimum zinc content was recorded by the line CSV-26R (17.61 ppm) (Table 1).

Correlation studies

The correlation analysis was done between the biochemical parameters and the micronutrients to study the significant influence of biochemical properties on the micronutrients. It was observed that carbohydrates had significantly positive correlation with iron content (r = 0.663*) but had non-significantly positive correlation with zinc content. Also, protein had negative correlation with iron (r = -0.381) and zinc content (r = -0.303) and iron and zinc were reported to have positive correlation (r = 0.329). Whereas, all the other biochemical parameters had non-significant association with the micronutrients (Table 2).

The geometric mean of protein, carbohydrate and proline was 7.56%, 66.50 g/100g and 0.69 g/100g respectively for all the selected sorghum lines. The protein content was observed in the range 6.33-8.98 %, carbohydrates in the range 49.23-86.19 g/100g and 0.5-1 g/100g was the range of proline content in the sorghum lines studied. For all the evaluated parameters F test was found to be significant. Similar results in this direction was also observed by Chavan *et al.,* (2020) where potential sorghum lines were studied for micronutrient enrichment in the *kharif* season. Also a study was reported by Kardes *et al.,* (2021) where they observed crude protein content of (>9.65%) and carbohydrates content of (>77.07%) which was in accordance with the present study.

Table.1 Protein, Carbohydrates, Proline and Grain yield per plant of the selected sorghum lines

Sr. No	Parents	Protein content (%)	Carbohydrates (g/100g)	Proline (g/100g)	Iron (ppm)	Zinc (ppm)
1	AKSV-438	6.85	59.667	0.69	38.30	18.87
2	AKSV-440	7.84	80.97	0.72	33.25	22.00
3	AKSV-442	6.91	80.67	0.97	38.80	25.79
4	CSV-26R	7.33	69.63	0.77	31.80	17.61
5	CSV-22R	8.25	60.03	1	28.90	17.69
6	M-35-1	8.46	66.24	0.71	38.50	18.13
7	CSV-29R	8.98	49.23	0.75	24.50	20.20
8	PKV Kranti	6.33	61.89	0.65	27.20	21.77
9	Parbhani Shakti	7.12	86.19	0.5	55.28	18.07
10	AKSV-439	6.89	68.94	0.82	48.30	19.71
	Parental Mean	7.56	66.50	0.69	36.48	19.98
	Range	6.33 - 8.98	49.23 - 86.19	0.5 - 1	24.5-55.28	17.613 - 25.787
	F test	S	S	S	S	S

Table.2 Correlation between the biochemical parameters and micronutrients

	Protein content (%)	Carbohydrates (g/100g)	Proline (g/100g)	Iron content (ppm)	Zinc content (ppm)
Protein content (%)	1	-0.384	0.191	-0.381	-0.303
Carbohydrates (g/100g)		1	-0.19	0.663*	0.286
Proline (g/100g)			1	-0.357	0.297
Iron content (ppm)				1	0.329
Zinc content (ppm)					1
*** - significance at 5%**					

The correlation studies stated that, carbohydrates had significantly positive correlation with iron content and had non-significant positive correlation with zinc content however, no study was found to support this finding but on the other hand, positive correlation between protein and iron and zinc content was reported in the findings of Badigannavar *et al.,* (2016) which contradicted the present findings of negative correlation between protein and iron content and zinc content.

The present study evaluated the potential sorghum lines and identified the lines high in iron and zinc content. It was also observed that, carbohydrates had significantly positive correlation with iron content and had positive correlation with the zinc content from which it can be inferred that carbohydrates and micronutrient studies can be carried out simultaneously. For all the evaluated parameters F test was found to be significant.

Acknowledgements

The authors are thankful to all the members who are part of the research work for their support and help and for providing necessary facilities to carry out this experimental work.

Author Contributions

Shradha Aherkar: Designed the model, analysed the data, computational framework original draft preparation, and wrote the manuscript. Dr. R. B. Ghorade: Conceived the original idea Designed the model and Project Administration. Dr. V. V. Kalpande: Supervision and

Investigation. Dr. N. M. Konde: Supervision and Methodology. Dr. Priti Sonkamble: Visualization and Supervision. Arjun Bhuyar: Reviewing and Editing, and Supervision. Dr. P. S Kamble: Supervision and provided Resources

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Amadou, I., Gounga, M. E. and Le, G. W., 2013. Millets, nutritional composition, some health benefits and processing. *Emir J Food Agric*, *25*(7), pp.501-508. https://doi.org/10.9755/ejfa.v25i7.12045.

Anantakrishnan, S. V. and Srinivasa Pai, K. V., 1952, October. The kjeldahl method of nitrogen determination. In *Proceedings of the Indian Academy of Sciences-Section A* (Vol. 36, pp. 299-305). Springer India. https://doi.org/10.1007/BF03172239

Badigannavar, A., Girish, G., Ramachandran, V. and Ganapathi, T. R., 2016. Genotypic variation for seed protein and mineral content among post-rainy season-grown sorghum genotypes. *The Crop Journal*, *4*(1), pp.61-67. https://doi.org/10.1016/j.cj.2015.07.002

Bates, L. S., Waldren, R. P. A. and Teare, I. D., 1973. Rapid determination of free proline for water-stress studies. *Plant and soil*, *39*, pp.205-207. http://dx.doi.org/10.1007/BF00018060

Chavan, S., Ghorade, R. B., Kalpande, V. V., Badigannavar, A., Kanwade, D. G. and Konde, N. M., (2020). Studies on Biochemical Properties of Potential Sorghum Lines for Micronutrient Enhancement. *Int J Curr Microbiol*. App.Sci (2020) Special Issue-11: 672-677

Chavan, U. D. and Patil, J. V., 2010. *Grain Sorghum Processing: Health, Ethnic, and Industrial Food Products from Grain Sorghum (sorghum Bicolour L. Moench)*. Ibdc Publishers.

Dreywood, R., 1946. Qualitative test for carbohydrate material. *Industrial & Engineering Chemistry Analytical Edition*, *18*(8), pp.499-499. https://doi.org/10.1021/i560156a015

Kardeş, Y. M., Kaplan, M., Kale, H., Yılmaz, M. F., Karaman, K., Temizgül, R. and Akar, T., 2021. Biochemical composition of selected lines from sorghum (*Sorghum bicolor* L.) landraces. *Planta*, *254*, pp.1-13.

Pfeiffer, W. H. and McClafferty, B., 2007. Harvest Plus: breeding crops for better nutrition. *Crop Science*, *47*, pp.S-88. https://doi.org/10.2135/cropsci2007.09.0020IPBS

Price, H. J., Dillon, S. L., Hodnett, G., Rooney, W. L., Ross, L. and Johnston, J. S., 2005. Genome evolution in the genus Sorghum (Poaceae). *Annals of Botany*, *95*(1), pp.219-227. https://doi.org/10.1093/aob/mci015

United Sorghum Checkoff Program 2010.

Wong, J. H., Lau, T., Cai, N., Singh, J., Pedersen, J. F., Vensel, W. H., Hurkman, W. J., Wilson, J. D., Lemaux, P. G. and Buchanan, B. B., 2009. Digestibility of protein and starch from sorghum (*Sorghum bicolor*) is linked to biochemical and structural features of grain endosperm. *Journal of cereal science*, *49*(1), pp.73-82.

How to cite this article:

Shradha Aherkar, R. B. Ghorade, V. V. Kalpande, N. M. Konde, Priti Sonkamble, Arjun Bhuyar and Kamble, P. S. 2024. Studies on Biochemical Properties of Potential Sorghum Lines in *Rabi* Season for Micronutrient Densification. *Int.J.Curr.Microbiol.App.Sci.* 13(7): 94-99. **doi:** https://doi.org/10.20546/ijcmas.2024.1307.011