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AN EVALUATION OF KEY NUTRITIONAL COMPONENTS OF VEGETABLE MESTA (*HIBISCUS CANNABINUS* L.): A STUDY ON VARIABILITY AND BIOCHEMICAL PROFILE

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ABSTRACT

An investigation was conducted on the genetic variability of 24 locally collected accessions of mesta (*Hibiscus cannabinus* L.) for several biochemical traits during summer season (2024). The experiment was laid out in Randomized Complete Block Design with two replications. The analysis of mesta leaves for several key nutrients was done in an order to develop its nutrient profile. A solid and wider range of variation among studied accessions was revealed by analysis of variance (ANOVA). Based on the *per se* performance, it was noticed that different genotypes had performed well in terms of different biochemical parameters. Substantial level of coefficient of variation at genotypic (GCV) and phenotypic (PCV) level was noted for all of the biochemical characters except ash and antioxidants, which had recorded medium level of GCV and PCV values. This signifies the true expression of traits with less or no influence of environment. The traits, total phenol content and calcium can easily transferred from parents to progeny because of their high heritability values. Additive gene action had its complete control on titratable acidity, fat content and crude fibre of mesta leaves, which can be fixed through appropriate breeding technique. Thus, our investigation had unpacked the nutritive potentiality of mesta crop. However, this basic information can be utilized to develop a mesta cultivar with a strong nutrient profile and can be harnessed at economical level.

Keywords: Mesta, variability, nutrient profile, biochemical traits

Introduction

Mesta is an underutilized leafy vegetable flourishes in the tropical India. It belongs to the malvaceae family ($2n=2x=36$) and closely related to cotton and okra (Shill, 2019). Mesta crop is originated in India but few literatures mentions that its center of origin is tropical Africa (Karmakar *et al.*, 2008). It is a bisexual, short day plant which, produces cream-colored flowers with dark red centers annually (Gray *et al.*, 2006). Vegetable mesta is undoubtedly a C_4 plant (Lam, 2000), quick developing with tall stature,

potentially requiring less inputs in raising it. Both simple and compound leaves are present on the same plant with green or red stems. Mesta is more adaptable to various environmental conditions, especially adapted to northern dry land areas of Karnataka and it can grow anywhere and still perform better than many commercial crops (Sen and Karmakar, 2014).

A developing country like India, with its growing population facing the problem of lack of supply of nutrient rich food. Rice and wheat are considered as staple foods in India but these grains do not provide

overall nutrition needed to human body. Leafy vegetables are important for a healthy and balanced diet, because they are excellent source of minerals, vitamins, antioxidants and low calories. Including greens in foods can improve taste, texture, color and provide many health benefits, whether eaten raw in salads or cooked. Nowadays people have become health conscious and searching nutrient rich food in some exotic leafy vegetables like spinach, kale, collard greens and so on. Now it is high time to focus on locally available vegetables packed with nutrients like mesta, which is majorly consumed by northern Karnataka people.

Mesta is considered to be the cheapest source of vitamins and minerals. It is rich in crude protein, which can replace soybean, sunflower and fish meal, as these well-known protein ingredients are currently expensive. Due to its diverse applications in different industries (fibre and paper), it is an important multifunctional crop. Mesta contains a great number of bioactive compounds which can be of great importance in human and animal health (Garjia 2016). Hence this is an essential food, fiber and medicinal plant. Tender foliage of mesta is utilized for preparation pundi bhaji or chutnies in a few parts of South India. Calyces of the blossoms are utilized in processed items like jelly, jam, pickles and acidic syrups with an appealing colour (Islam *et al.*, 2016). Since it is acidic in nature, the items prepared from mesta will be stored for 3-4 days. This plant is a great source of calcium and iron, dishes of this vegetable can be prepared more nutritious by including cereals (maize, jowar and rice), beans (greengram, chickpea and pigeonpea) and oilseeds (groundnut).

The present study is focused on collection, identification and exploitation of diversity in the locally available germplasms of mesta. It provides an easy path to a plant breeder to initiate the crop improvement program. The vegetable breeder has to study genetic variability in an order to improve quantitative and biochemical characters for successful selection. To achieve this, evaluation of 24 locally available mesta genotypes has been covered in our research study.

Material and Methods

Plant material and experimental design

The present study was conducted at University of Horticultural Sciences, Bagalkot (Karnataka) during summer season (2024). Experiment was conducted in Randomized Complete Block Design (Panse 1957), with 24 locally collected mesta genotypes replicated twice. The seeds were sown by hand in 2m x 2m plot at

a spacing of 30 cm X 10 cm. A fertilizer dose of 40:20:20 kg N: P₂O₅: K₂O/ha was applied at the time of sowing. All the cultural practices were carried out at time to time. Irrigation was provided by flooding at 3 days interval during initial stages of growth and 1 week interval during later stages of growth. Weeding operation was done at 20 DAS and 35 DAS.

Data collection

The data were collected from 3 randomly selected plants from each plot (genotype) at pre-bud stage (generally 45 DAS). Healthy leaves were separated from plants, oven dried and powdered. This sample was used in the estimation of ascorbic acid (AA), protein, fat, ash, crude fibre (CF), total phenols (TP), calcium (Ca) phosphorous (P), iron (Fe), antioxidant activity (AO) and titratable acidity (TA).

Estimation of ascorbic acid (mg/100g)

Ascorbic acid or vitamin C content was estimated by using 2,6-Dichlorophenol Indophenol visible titration method.

Estimation of protein (%)

Estimation of protein was done by micro kjeldhal method by following three steps (digestion, distillation and titration). The crude protein content was determined by using the formula given below.

$$\text{Crude protein content (\%)} = \text{Nitrogen(\%)} \times 6.25$$

Estimation of fat (%)

Fat estimation was done by using Soxhlet plus apparatus (Ojure and Quadri, 2012).

Estimation of ash (%)

Ash content was determined by burning dried leaf samples of mesta in muffle furnace with the help of crucibles. Ash percentage was calculated using following formula (Aziz *et al.*, 2019).

$$\text{Total ash(\%)} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Estimation of crude fibre (%)

Crude fibre of dried leaf sample was estimated by acid-alkali hydrolysis method by using Fibra plus-FES-6 instrument.

$$\text{Crude fibre (\%)} = (\text{Loss in weight on ignition} \div \text{Weight of the sample}) \times 100$$

Estimation of total phenols (mg GAE/100g)

Total phenolic content in the fresh leaf sample was estimated according to Folin-Cicoalteu method (Singleton and Rossi, 1965).

Estimation of Calcium (mg/100g)

Calcium content was estimated by “versenate method” using stand

Estimation of Phosphorous (mg/100g)

Phosphorous in the di-acid digested leaf sample was estimated by using vanadomolybdate reagent. Per cent phosphorous content was calculated by using below formula (Kaur *et al.*, 2017).

Estimation of Iron (mg/100g)

AAS (Atomic Absorption Spectrophotometry) method was used to estimate iron content in the leaf sample. Di-acid digested sample of dried mesta leaf was fed into the machine to obtain the results. It was expressed as milligram per 100 gram of leaf sample (Lindsay and Norvell, 1978).

Estimation of Anti-oxidant (FRAP method)

Anti-oxidant activity in mesta leaf sample was determined by FRAP (ferrous ion reducing antioxidant method). Absorbance was recorded at 593 nm using spectrophotometer. Antioxidant activity was expressed as mg AAE/100gm (AAE represents ascorbic acid equivalent).

Antioxidant activity (mg AAE/100g) = (OD value at 593nm x volume made up x 100)

Aliquot taken x Weight of sample (g) x 1000

Estimation of Titratable acidity (%)

Simple titration method was carried out with 0.1 N NaOH. The colour change from red to faint pink indicated the end point. The acidity was calculated and expressed in per cent by using the formula.

$$\text{Acidity (\%)} = \frac{(\text{Titrateable value} \times \text{Normality of NaOH}) \times \text{Volume made up} \times \text{Eq. weight of acid}}{(\text{Volume of the sample}) \times \text{weight of the sample taken} \times 1000}$$

Statistical analysis

The mean value of each observation was subjected for different biometric analysis with the help of the tools like Microsoft excel (Microsoft 365, United States of America). ANOVA was calculated to understand the significance of genotypes. Estimation of genetic parameters (σ^2_g and σ^2_p), Coefficients of variability (GCV: Genotypic coefficient of variation and PCV: Phenotypic coefficient of variation) was calculated by the method suggested by Burton and Devane (1953). Heritability in broad sense (Hanson *et al.*, 1956) and genetic advance over mean (Johanson *et al.*, 1955) were also estimated.

Results

Analysis of variance (ANOVA) and variability

Analysis of variance (ANOVA) for 11 biochemical parameters are presented in Table 1. It revealed that mean sum of squares due to treatments (genotypes) were remarkably significant for all the biochemical traits. Based on the *per se* performance of vegetable mesta accessions, the superiority of genotype was assessed (Table 2). Among 24 genotypes, ascorbic acid content was recorded maximum in genotype UHSB-M-8 (144.91 mg/100g) and minimum in UHSB-M-3 (16.92 mg/100g) with the overall mean of 76.70. Protein composition of mesta leaves varied from 2.75 % (UHSB-M-2) to 11.08 % (UHSB-M-12).

Table 1: Analysis of variance of twenty-four genotypes of mesta for several traits

Source of variations	Degrees of freedom	Ascorbic acid	Protein (%)	Fat (%)	Ash (%)	CF	TP	Ca	P	Fe	AO	TA
Replication	1	1.41	0.41	0.55	1.35	0.01	0.01	25.35	0.01	0.10	6.37	0.93
Treatment	23	1545.84**	7.63**	6.87**	9.94**	55.75**	17588.6**	2808.95**	0.01**	3.01**	22.85**	34.81**
Error	23	1.21	0.07	0.05	0.55	0.19	2.45	0.65	0.00	0.09	1.49	0.04

AA: Ascorbic acid (mg/100g), CF: Crude fibre (%), TP: Total phenols (mg GAE/100g), Ca: Calcium (mg/100g), P: Phosphorous (mg/100g), Fe: Iron (mg/100g), AO: Antioxidants (mg AAE/100g), TA: Titratable acidity (%) * and ** significant at 5% and 1% respectively

Fat content was almost negligible in mesta. It ranged from 0.56 % (UHSB-M-7) to 6.47 % (UHSB-M-17) with an average of 3.41. UHSB-M-1 had recoded highest ash content (18.81 %) whereas, UHSB-M-10 had recoded lowest value (10.43 %) in terms of ash. The cumulative value of crude fibre was 10.31 and it ranged from 2.34 % (UHSB-M-22) to 24.00 % (UHSB-M-5). Total phenols had highest mean value (280.40) than the other parameters. It had wide range of variation from 128.13 mg GAE/100g (UHSB-

M-19) to 571.57 mg GAE/100g (UHSB-M-14). A significant range of variation was observed for mineral nutrients of mesta like calcium, phosphorous and iron. The genotype UHSB-M-16 (135.06 mg/100g) had shown high calcium content in leaves whereas, UHSB-M-8 (13.71 mg/100g) was a genotype with poor calcium content. Along with this, phosphorous content of mesta leaves varied from 0.06 mg/100g (UHSB-M-23) to 0.39 mg/100g (UHSB-M-10). Similarly, genotype UHSB-M-24 was found to be the good

source of iron (7.68 mg/100g). On the other hand, UHSB-M-19 (3.23 mg/100g) genotype was poor in iron content with overall mean of 5.10. Antioxidant activity varied from 13.99 mg GAE/100g (UHSB-M-1) to 27.92 mg AAE/100g (UHSB-M-7) with cumulative value of 20.56. Highest titratable acidity was observed in genotype UHSB-M-1 (15.34 %) and lowest in UHSB-M-9 (2.13 %).

Estimation of variance and heritability components

Estimation of variability at genetic level along with heritability components are presented in Table 3. The results of the analysis showed higher (>20 %) genotypic coefficient of variance (GCV) and phenotypic coefficient of variance (PCV) values were observed for most of the biochemical traits. The highest GCV and PCV valued were noticed in the trait titratable acidity (59.58 % and 59.64 % respectively). However, other traits *viz.*, ascorbic acid, protein, fat, crude fibre, total phenols, calcium, phosphorous and iron had recoded higher coefficient of variations along with titratable acidity. On the contrary, medium range of GCV and PCV was recorded for ash (16.26 % and 17.19 % respectively) and antioxidants (15.89 % and 16.97 % respectively).

Assessment of gene action was carried out through the estimation of heritability (bs) and genetic advance over mean (GAM) and presented in the Table 3. All of the studied traits exhibited higher heritability coupled with higher GAM values. Among them, total

phenol content of mesta leaves showed significantly highest heritability (99.97 %) in broad sense followed by calcium (99.95 %). Additionally, remarkable GAM values were observed for the traits, titratable acidity (122.61 %), fat (110.74 %) and crude fiber (104.93 %) of mesta leaves.

Discussion

Variability studies in any crop is a foundational research activity of a plant breeder to take the initiative steps for the further improvement. Our comprehensive biochemical analysis of locally collected mesta genotypes have yielded numerous noteworthy results with substantial implications for the development of nutrient rich mesta cultivar. A solid basis for the selective breeding attempts is provided by ANOVA results, which showed significant variation in the nutrient profile of studied genotypes. A wide range of variation was recoded for all the traits, which builds a strong foundation for selective breeding. Tareq *et al.* (2021) obtained the greater difference in concentrations of different minerals, vitamins, moisture and ash in roselle (*Hibiscus sabdariffa*). This report quotes that the one possible reason for the variation of contents is the use of different land races/accessions in the experiment. Several reports suggests the role of agro-climatic conditions and soil type, was being involved in changing vitamin and mineral contents (Atta *et al.*, 2013 and Islam 2019).

Table 2: *Per se* performance of mesta genotypes for several biochemical parameters

Genotype	AA	Protein (%)	Fat (%)	Ash (%)	CF	TP	Ca	P	Fe	AO	TA
UHSB-M-1	49.94	5.65	5.08	18.81	12.30	209.75	102.32	0.26	3.81	13.99	15.34
UHSB-M-2	26.15	2.75	5.22	15.83	18.61	256.49	133.22	0.23	3.68	21.66	2.98
UHSB-M-3	16.92	10.39	3.75	13.31	11.09	229.14	58.39	0.29	4.44	21.63	3.38
UHSB-M-4	74.77	8.50	1.92	12.36	20.55	417.85	69.02	0.16	5.36	23.17	15.09
UHSB-M-5	39.63	8.73	1.51	14.04	24.00	297.13	121.07	0.20	5.51	20.73	14.74
UHSB-M-6	87.05	10.74	2.74	14.32	7.39	287.68	45.39	0.15	6.08	19.24	6.49
UHSB-M-7	102.58	9.70	0.56	12.43	6.36	429.11	94.27	0.19	4.38	27.92	12.21
UHSB-M-8	144.91	7.88	0.70	14.10	8.46	230.10	13.71	0.26	4.88	21.74	2.57
UHSB-M-9	68.38	9.45	4.56	11.95	13.39	284.93	61.76	0.07	4.56	23.52	2.13
UHSB-M-10	89.97	10.06	2.35	10.43	5.38	261.57	78.36	0.39	5.53	19.59	4.71
UHSB-M-11	107.68	10.68	3.57	13.85	9.55	251.85	34.26	0.24	3.46	17.01	8.04
UHSB-M-12	88.81	11.08	1.46	13.08	7.54	234.14	29.81	0.11	6.68	22.04	3.46
UHSB-M-13	109.61	8.69	5.68	14.04	9.42	433.26	32.02	0.14	5.60	26.57	3.17
UHSB-M-14	87.33	6.72	6.38	10.76	16.48	571.57	64.00	0.13	4.52	23.24	6.43
UHSB-M-15	74.78	8.89	2.67	10.50	7.66	234.67	44.44	0.17	6.49	20.32	7.89
UHSB-M-16	73.34	8.00	5.29	10.76	9.41	286.15	135.06	0.30	4.88	22.34	3.49
UHSB-M-17	91.37	7.69	6.47	12.42	14.60	235.84	55.56	0.21	3.50	16.53	2.70
UHSB-M-18	82.71	11.00	4.41	12.13	8.59	265.89	110.95	0.21	4.60	19.92	8.42
UHSB-M-19	74.76	10.69	1.41	10.96	5.22	128.13	75.91	0.15	3.23	21.65	11.01
UHSB-M-20	65.83	9.35	4.54	11.76	9.97	229.76	129.15	0.14	5.19	16.72	5.59
UHSB-M-21	57.85	10.20	3.46	17.18	5.32	269.67	119.82	0.09	4.55	15.92	4.68

UHSB-M-22	53.21	10.53	0.59	16.15	2.34	213.73	105.04	0.23	6.61	15.70	10.32
UHSB-M-23	80.10	10.34	2.66	12.53	6.23	228.93	102.71	0.06	7.34	19.81	5.36
UHSB-M-24	93.15	7.82	5.00	16.16	7.71	242.31	129.12	0.15	7.68	22.48	7.85
SEM	0.78	0.19	0.15	0.53	0.31	1.11	0.57	0.01	0.21	0.86	0.13
CD @ 5%	2.28	0.55	0.44	1.54	0.90	3.23	1.67	0.04	0.60	2.53	0.39

AA: Ascorbic acid (mg/100g), CF: Crude fibre (%), TP: Total phenols (mg GAE/100g), Ca: Calcium (mg/100g), P: Phosphorous (mg/100g), Fe: Iron (mg/100g), AO: Antioxidants (mg AAE/100g), TA: Titratable acidity (%)

Table 3: Estimates of genetic variability and heritability components for different characters in mesta

Traits	Mean	Range		Variances		Coefficient of variance		h ² bs (%)	GA	GAM (%)
		Min.	Max.	σ^2_g	σ^2_p	GCV (%)	PCV (%)			
Ascorbic acid (mg/100g)	76.70	16.92	144.91	772.32	773.53	36.23	36.26	99.84	57.20	74.58
Protein (%)	8.98	2.75	11.08	3.78	3.85	21.66	21.86	98.15	3.97	44.19
Fat (%)	3.41	0.56	6.47	3.41	3.46	54.12	54.49	98.66	3.78	110.74
Ash (%)	13.33	10.43	18.81	4.69	5.24	16.26	17.19	89.46	4.22	31.67
Crude fibre (%)	10.31	2.34	24.00	27.78	27.97	51.11	51.28	99.32	10.82	104.93
Total phenols (mg GAE/100g)	280.40	128.13	571.57	8793.08	8793.52	33.44	33.45	99.97	93.14	68.88
Calcium (mg/100g)	81.05	13.71	135.06	1404.15	1404.80	46.23	46.24	99.95	77.17	95.21
Phosphorous (mg/100g)	0.19	0.06	0.39	0.01	0.01	41.22	42.57	93.74	0.15	82.20
Iron(mg/100g)	5.10	3.23	7.68	1.46	1.55	23.70	24.38	94.51	2.42	47.47
Antioxidant (mg AAE/100g)	20.56	13.99	27.92	10.68	12.17	15.89	16.97	87.94	6.31	30.67
Titratable acidity (%)	7.00	2.13	15.34	17.39	17.42	59.58	59.64	99.79	8.58	122.61

σ^2_g : Genotypic variance, σ^2_p : Phenotypic variance, GCV: Genotypic coefficient of variation, PCV: Phenotypic coefficient of variation, h²bs: Heritability in broad sense, GA: Genetic advance, GAM: genetic advance over mean.

Among 24 genotypes, UHSB-M-8 contributed high magnitude of ascorbic acid but this genotype was a poor yielder due to less viable seeds which failed to germinate (Arivazhagan and Kandasamy 2019). Protein composition of UHSB-M-12 genotype was appreciable (Andini *et al.*, 2013 in amaranthus, Umakanta *et al.*, 2015). The line, UHSB-M-17 had fair amount of fat, as fat content was almost negligible in mesta. The accession UHSB-M-1 had recoded highest ash content and titratable acidity. Whereas, crude fibre was significantly high in UHSB-M-5 mesta line. These results are in close proximity with report of Alexopoulou *et al.* (2015), Umakanta *et al.* (2015), Ryu *et al.* (2017) and Tetteh *et al.* (2019). They confirmed that high palatability, crude proteins and fibre content in tender leaves of mesta was a good indication for human consumption and animal feed as well. Total phenols was recorded highest in UHSB-M-14. According to Park *et al.* (2020), high magnitude of individual phenolic compounds due to the level of kaempferitrin, a major compound found in kenaf (mesta). A significant range of variation was observed for mineral composition of mesta like calcium, phosphorous and iron. The genotype UHSB-M-16 had shown high calcium content in leaves. Along with this, UHSB-M-10 exhibited great phosphorous content. Similarly, genotype UHSB-M-24 was found to be the good source of iron. These results are in line with (Gharneh and Davodahosseini, 2015, Umakanta *et al.*, 2015) previous record of mineral content of leafy

vegetables. Additionally, antioxidant activity was observed high for UHSB-M-7 genotype of mesta (Pandjaitan *et al.*, 2005).

Ascorbic acid, protein, fat, crude fibre, total phenols, calcium, phosphorous and iron had recoded higher coefficient of variation (GCV and PCV) along with titratable acidity (highest). These traits are more likely to respond positively to selection. A critical role of environment on expression level of ash and antioxidant was due to their medium range of GCV and PCV values. Traits with higher levels of variances are considered perfect candidates for simple selection. The narrow difference was observed between GCV and PCV in mesta, which indicates the least effect of environment on the expression of the traits. This outcome was in agreement with Deshmukh and Pacharne (2022), Saifullah and Rabbani (2009), Abo-kaied and Abuozaid (2008).

The high heritability for all the important nutrients was one of the most encouraging results. Particularly, total phenol content and calcium. The high heritability of total phenols and calcium content is exciting, as it opens up all kind of possibilities for producing mesta cultivar with enhanced phenols and mineral content. Heritability coupled with genetic advance is one more important estimation for complete understanding of the gene action. The titratable acidity, fat content and crude fibre demonstrated highest GAM values. The traits showing high heritability and GAM are governed by additive gene action, which can be fixed through

suitable breeding activity. These results are in conformity with Panda *et al.* (2017) and Gerrano *et al.* (2015). The mesta genotypes (UHSB-M-1, UHSB-M-4 and UHSB-M-5) with enhanced acidity in leaves can be utilized in preparation of processed products. Similarly, strong genetic base for fibre content of mesta leaves could lead to the development of cultivars for optimized dietary fibre. This implies that these traits are determined by genetic factors rather than environmental conditions, making them perfect candidates for breeding initiatives focused on enhancing the nutritional profile of mesta.

Conclusion

The substantial variation in the nutrient content reported among genotypes show the untapped potential for nutrient augmentation in mesta. Not a single genotype was rich in all kind of nutrients but selective genotypes had high concentration of particular nutrient. Development of nutrient-dense mesta cultivar can be made possible by choosing the genotype with high heritability and genetic advance for major biochemical traits. To sum up, this investigation highlights the considerable possibility of developing mesta cultivars with a very strong nutrient profile. In order to find even more insights, future study should widen the genetic base by including more genotypes in the study and adopting better breeding program. Thus, our study provides an insight into the basic knowledge of biochemical composition of mesta, which can be utilized in selecting a target candidate for future crop improvement.

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