

Comparative evaluation of seed chlorophyll content, colour, and yield traits in Fenugreek (*Trigonella foenum-graecum* L.)

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Summary

Fenugreek has grown globally for thousands of years and is used in diverse ways, including as a food flavoring agent, essential oil production, and traditional medicinal practices. Ongoing research continues to explore new applications and potential uses for fenugreek by-products. The study included ten newly developed green-seeded fenugreek genotypes, which were evaluated alongside five released conventional yellow-seeded varieties for comparative analysis. The average seed yield ranged from 1,317 kg/ha (IC-0633368) to 2,260 kg/ha (IC-0633369). Among the yellow-seeded conventional fenugreek varieties, AFg-4 (2,120 kg/ha) and AFg-5 (2,108 kg/ha) consistently recorded higher yields across all seasons, suggesting superior genetic potential. Mean seed chlorophyll values ranged from 0.424 mg/g (AFg-4) to 0.678 mg/g (IC-0633362). Notably, green-seeded genotypes such as IC-0633362, IC-0633369, and IC-0633370 recorded higher mean chlorophyll contents (above 0.65 mg/g) with a dark olive-green shade. The higher value of chlorophyll percentage is related to the dark colour of the seed. The genotype IC-0633362, which has the maximum amount of chlorophyll content, is darkest in green colour with the shade name Dark olive green. The presence of the high amount of chlorophyll in green-seeded fenugreek represents a promising functional marker for use in breeding programs targeting improved seed quality for medicinal values and attractiveness. Specifically, it holds significant potential for enhancing the nutraceutical and medicinal value of fenugreek, particularly under diverse agro-climatic conditions.

JAE 2025, Vol 20

Received: 15 April 2025

Accepted: 30 April 2025

Published: 07 June 2025

<https://doi.org/10.58628/JAE-2520-103>

Associated Editor: Dr. MK Berwal

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Keywords: Fenugreek, seed chlorophyll, seed colour, PCA, green seeded

Introduction

Fenugreek (*Trigonella foenum-graecum*) is a potential seed spice crop recognized as one of the earliest known medicinal plants. The genus name *Trigonella*, meaning 'little triangle' in Latin, refers to the distinctive triangular shape of its yellowish-white flowers. Valued since ancient times, fenugreek is notable for its remarkable medicinal and nutritional properties (Flammang et al. 2004). It is widely cultivated as a spice crop globally, and the species name *foenum-graecum*, meaning "Greek hay" suggests its historical use as a forage crop (Acharya et al. 2017). It has been recognized as a medicinal herb since ancient times (Slinkard 2006). Fenugreek is one of all the oldest medicinal plants originating in central Asia ~4000 B.C. (Altuntas et al. 2005). In Ebers Papyrus (one of the oldest maintained medicinal documents), earlier in 1500 BC in Egypt mentioned its description and benefits (Betty 2008). Fenugreek is commercially important as a spice crop, valued for its diverse uses. It is extensively grown throughout the country, especially in the winter season, for its seeds, tender shoots, and fresh leaves (Malhotra 2011 and Patel et al. 2017; Singh et al. 2021). Primarily, fenugreek comprises choline, linoleic acid, linolenic acid, and stearic acid. Differential antioxidant potential of fenugreek was studied (Choudhary et al. 2017). The quantity of each of these chemical constituents varies based on the species or

cultivars, along with cultivation conditions such as soil type, weather, irrigation, pruning, and other horticultural practices (Malhotra 2011). Cultivated globally for millennia, it has been utilized worldwide for food flavoring, essential oil applications, and traditional medicines (Kaur 2021; Sarwar et al. 2020). Plant breeding has provided a sort of fenugreek varieties and studied genetic variability (Panwar et al. 2018, Meena et al. 2021, Maloo et al. 2020), omics (Kachhwaha et al. 2024), protein structure (Choudhary et al. 2016), weed management (Godara et al. 2015) and productivity (Godara et al. 2018), yield and economics (Godara et al. 2016). The requirement for fenugreek varieties with higher productivity, increased vigour, and elevated quantity of essential biochemical product, principally with higher diosgenin content, has driven additional breeding efforts during this crop (Petropoulos 2002). Fenugreek is a potential source of medicinal compounds and chlorophyll content (Singh et al. 2025a), but very limited variability is available in this crop for various traits. Hence, a comparative evaluation of identified green-seeded fenugreek genotypes along with the yellow-seeded fenugreek varieties was carried out to study the variation in morphometric, yield, seed chlorophyll and seed colour was carried out at ICAR-NRC on Seed Spices, Ajmer Rajasthan during the rabi season of 2020-21, 2021-22, 2022-23 and 2023-24.

Materials and Methods

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Plant material, experimental site, and experimental design

Initially, the Fenugreek plants were identified and selected from the field for green seed color from the Agro climatic Zone-IIA of Rajasthan. Collected plants were maintained and multiplied at ICAR-NRCSS, Ajmer field, and the multiplied 10 germplasm lines were registered at the ICAR-National Bureau of Plant Genetic Resources for the Indigenous Collection (IC) number. All ten green seed colored fenugreek genotypes viz., IC-0633362, IC-0633363, IC-0633364, IC-0633365, IC-0633366, IC-0633367, IC-0633368, IC-0633369, IC-0633370, and IC-0633371 were evaluated for morphometric, seed chlorophyll content and seed colour along with yellow seeded conventional fenugreek varieties as checks (AFg-1, AFg-2, AFg-3, AFg-4 and AFg-5). The experimental materials were planted in the field under RBD, replicated thrice with a plot size of 3x3 m² with a spacing of 30x10 cm during the rabi season of 2020-21, 2021-22, 2022-23, and 2023-24 at ICAR-NRCSS, Ajmer field. The region falls under agro climatic zone IIIa, "Semi-Arid Eastern Plain Zone" of Rajasthan. The field had a levelled topography and sandy loam texture. The climate of this zone is typically semi-arid and sub-tropical, characterized by mild winters and moderate summers associated with relatively high humidity during July to September. The mean annual rainfall is 550 mm, mostly received from the South-West monsoon during the last week of June to September.

Observation of agronomic traits

The agronomic data recorded in the study included plant height, number of primary branches per plant⁻¹, number of pods plant⁻¹, seed yield ha⁻¹, and test weight were measured by randomly selecting five plants from each plot (Tables 1 and 2).

Seed chlorophyll content and colour analysis

Chlorophyll contents of green and yellow seeded fenugreek were determined following the spectrophotometric assay described by Mazza and Oomah (1994). Ground seed samples (1 g) were homogenised with acetone in a pestle and mortar and extracted in a 50 ml centrifuge tube with 18 ml of acetone: 1 ml 0.1 N NHOH solution for 30 min and centrifuged at 5000 rpm. Supernatant was transferred to another test tube and read on a spectrophotometer at 700, 663, 645, and 626 nm. The 'colordesigner.io' tool was employed to identify and nomenclature the color shade (Table 3).

Statistical analysis

Statistical analysis was performed for all the traits observed, wherein mean values from four crop seasons, rabi 2020-21, 2021-22, 2022-23, and 2023-24, with three replications. The descriptive statistics were performed for all observed traits using OPSTAT and the MS Office Excel programme using the data analysis tool.

Results and Discussion

The present investigation evaluated ten green-seeded and five conventional fenugreek genotypes (*Trigonella foenum-graecum* L.) across four consecutive rabi seasons (2020–21 to 2023–24) for morphometric, seed yield, seed chlorophyll content, and seed colour traits. The analysis revealed significant differences among

genotypes and across years, indicating the presence of considerable genetic variability, which is a prerequisite for effective selection in crop improvement programs. The overall mean plant height was highest in AFg-3 (78.5 cm), followed by AFg-5 (76.7 cm) and AFg-4 (75.1 cm). The minimum mean height was recorded in IC-0633362 (62.0 cm). These findings align with previous studies of Sharma et al. (2016) wherein they reported significant genotypic variability for plant height in fenugreek, suggesting that taller genotypes may possess greater biomass accumulation capacity. The number of primary branches per plant ranged from 4.9 (IC-0633362) to 5.8 (AFg-2). Year-wise, 2022–23 recorded the highest average number of branches, reflecting favorable environmental conditions during that season. AFg-2, AFg-3, and AFg-5 exhibited superior branching ability, enhancing their photosynthetic and reproductive potential. Such variation is supported by Meena et al. (2021), who highlighted branching as a key trait influencing seed yield in fenugreek. Further, the number of pods per plant ranged from 45.6 (IC-0633362) to 60.4 (AFg-2) based on mean values. Genotypes AFg-2, AFg-3, and AFg-5 consistently outperformed others across all years, indicating higher sink strength and reproductive efficiency. These results are in agreement with Meena et al. (2011), who emphasized the significance of pod number as a yield-contributing trait in fenugreek.

Pod length exhibited minor fluctuations across genotypes and years, with mean values ranging from 11.9 cm (IC-0633364) to 12.8 cm (AFg-1 and AFg-5). Despite environmental variations, the trait remained relatively stable, indicating low environmental sensitivity. This trait, though less variable, contributes to seed development and correlates with seed number per pod. Choudhary et al. (2022) also reported limited variation in pod length among fenugreek accessions under multilocation testing. The number of seeds per pod ranged from 17.4 (IC-0633368) to 18.5 (AFg-5) across the genotypes. The conventional varieties, such as AFg-4 and AFg-5, consistently exhibited higher seed count across years, which directly contributes to seed yield. These findings are in line with Singh et al. (2013), who noted that seed number per pod is a stable and moderately heritable trait in fenugreek, making it ideal for selection. The seed yield of fenugreek genotypes exhibited notable variation across four consecutive rabi seasons (2020–21 to 2023–24), indicating the influence of both genotypic potential and environmental conditions. Mean seed yield ranged from 1,317 kg/ha (IC-0633368) to 2,260 kg/ha (IC-0633369). Among the conventional varieties, AFg-4 (2,120 kg/ha) and AFg-5 (2,108 kg/ha) consistently recorded higher yields across all seasons, suggesting superior genetic potential. In contrast, lower yields were observed in IC-0633367 (1,734 kg/ha) and IC-0633362 (1,848 kg/ha). These findings align with earlier studies by Choudhary et al. (2022) and Meena et al. (2021), which highlighted significant genotypic variability for seed yield in fenugreek under diverse agro-climatic conditions. Notably, the CV values across years ranged from 1.50 to 2.80, indicating reasonable experimental precision.

Test weight (1000-seed weight), an indicator of seed size and quality, showed considerable variation among genotypes and across years. Mean test weight ranged from 11.9 g (IC-0633363 and IC-0633364) to 14.7 g (AFg-2). The conventional varieties, such as AFg-2, AFg-3, and AFg-4, recorded consistently higher test weights, suggesting better seed filling and maturation. IC-0633365 and IC-0633367 also demonstrated relatively high-test weights among the green-seeded genotypes. This variability is consistent with findings by Shakthi et al. (2020), who reported that test weight is influenced by both genetic makeup and environmental conditions during seed development.

Chlorophyll-rich food is associated with anticancer properties, brain health, neuroprotective, endocrine disruptor effects, anti-obesity effects with ample antioxidant properties as well as a unique structure of it assists in scavenging harmful free radicals. Seed chlorophyll content is not only a unique botanical trait but also offers added medicinal value. Chlorophyll exhibits antioxidant, anti-inflammatory, and wound-healing activities, contributing to detoxification and cellular protection in the human body (Anu Udaya et al. 2019; Ferruzzi & Blakeslee 2007; Martins et al. 2023). These properties may enhance the already known therapeutic profile of fenugreek, which includes antidiabetic, hypocholesterolemic, and gastroprotective effects (Basch et al. 2003). Mean seed chlorophyll values ranged from 0.424 mg/g (AFg-4) to 0.678 mg/g (IC-0633362). Notably, green-seeded genotypes such as IC-0633362, IC-0633369, and IC-0633370 recorded higher mean chlorophyll contents (above 0.65 mg/g). Such variation in seed chlorophyll content was also reported by Singh et al. (2025a). In another study Singh et al. (2025b) reported that higher amount of chlorophyll content is directly related to higher amount of 4-Hydroxyisoleucine (4-OHIlle) content and lower amount of total soluble sugars which is very good dietary source for the persons struggling with diabetes and obesity. The results of the study reveal that green-seeded fenugreek, with its elevated chlorophyll content, presents a promising functional food ingredient and nutraceutical for managing metabolic disorders and enhancing overall health. The seed color shade and shade name identified which revealed that the green seeded fenugreek genotypes are having three different shades of green color (Dark olive green, Dark slate gray and Dim gray) whereas all five yellow seeded conventional fenugreek genotypes have with saddle brown shade of yellow color with different shade codes (Table 3). The Color shade of fenugreek seeds, as categorized visually, corresponded to the chlorophyll content data. Genotypes with higher chlorophyll (IC-0633362, IC-0633369, IC-0633370) exhibited a dark olive-green shade, while yellow-seeded conventional fenugreek genotypes with lower chlorophyll content were grouped under saddle brown (Table 3 and Plate 1). The maximum amount of chlorophyll content was recorded in genotype IC-0633362 for all four years during the study (Figure 2). The presence of chlorophyll in seeds of green-seeded fenugreek serves as a potential functional marker trait in

breeding programs aimed at enhancing the nutraceutical and medicinal value of the crop.

The principal component analysis (PCA) performed on green-seeded genotypes vs. conventional fenugreek varieties revealed substantial variation among the accessions for the studied morphological and yield-related traits. The biplot (Figure 1) explains a cumulative variance of 67.7% across the first two principal components, with PC1 (Dim1) accounting for 51.3% and PC2 (Dim2) contributing 16.4%. The genotypes clustered on the right side of the biplot, particularly AFg-1, AFg-2, AFg-4, and AFg-5, exhibited strong positive associations with high-yielding traits such as seed yield hectare⁻¹ (SYHA), test weight (TW), number of seeds per plant (NSP), and plant height (PH). Choudhary et al. (2022) reported such variation for yield and other contributing traits in fenugreek, and these traits can also be studied for a hybridization program in fenugreek. Further, the green-seeded genotypes IC-0633362 and IC-0633371 were separated from the main high-yielding cluster in the PCA biplot. Preliminary biochemical analysis indicates that these two accessions exhibited significantly higher total chlorophyll content in seed compared to most other genotypes in the study. While high-yielding accessions are critical for immediate genetic gain, physiologically superior but newly identified genotypes like IC-0633362 and IC-0633371 should not be overlooked. Their high total chlorophyll content in seed offers a valuable trait for breeding programs aiming to combine yield with nutraceutical significance. These identified accessions can be strategically used as donors in hybridization programs to improve the trait of interest, especially to cater to the needs of the pharmaceutical and nutraceutical industry. This strategy aligns with the suggestions of Singh et al. (2013), who recommended including genetically diverse and low-performing accessions in breeding programs to exploit heterosis and adaptability.

In the present study, the primary focus was on seed chlorophyll content, a novel and previously underexplored trait in fenugreek (*Trigonella foenum-graecum* L.). The presence of chlorophyll in seeds contributes not only to their distinctive green coloration but also holds promising implications for enhancing the crop's nutraceutical and medicinal value. This unique trait may increase the visual and functional appeal of fenugreek in health-conscious markets, thereby offering opportunities to improve its commercial potential and support higher income for farmers. While green-seeded genotypes demonstrated slightly lower average yields compared to conventional yellow-seeded varieties, this yield penalty can potentially be mitigated through the adoption of optimized agronomic practices, including precision nutrient management, improved sowing techniques, and genotype-specific cultivation strategies. With further genetic refinement and agronomic optimization, green-seeded fenugreek genotypes could emerge as a high-value crop segment that balances both quality and productivity, particularly in niche markets focused on medicinal and functional foods.

Table 1. Performance of green-seeded vs. conventional fenugreek genotypes growth related traits during 2020-21 to 2023-24

Genotypes	Plant height (cm)					No of Primary branches/plant					No. of Pods/plant					Pod length (cm)				
	2020-21	2021-22	2022-23	2023-24	Mean	2020-21	2021-22	2022-23	2023-24	Mean	2020-21	2021-22	2022-23	2023-24	Mean	2020-21	2021-22	2022-23	2023-24	Mean
IC-0633362	61.8	72.7	68.8	44.6	62.0	4.8	4.8	4.9	5.1	4.9	45.7	40.5	52.1	44.0	45.6	12.1	14.1	12.3	11.6	12.5
IC-0633363	72.9	81.5	68.4	51.0	68.5	5.3	4.5	5.2	5.8	5.2	40.6	42.3	60.7	40.7	46.1	11.9	13.5	12.4	11.6	12.4
IC-0633364	65.9	72.7	65.9	56.4	65.2	5.8	3.9	5.4	5.9	5.3	51.1	39.5	56.1	46.3	48.3	11.2	12.9	11.5	12.1	11.9
IC-0633365	64.0	76.2	64.9	54.8	65.0	5.0	4.8	4.7	5.6	5.0	49.0	37.6	56.0	44.4	46.8	11.2	13.5	11.2	11.9	11.9
IC-0633366	62.5	75.6	61.9	52.9	63.2	5.7	4.8	5.2	5.7	5.4	69.0	38.3	72.5	42.5	55.6	11.9	13.3	12.4	11.7	12.3
IC-0633367	71.3	77.3	73.5	62.3	71.1	5.6	4.9	5.2	5.5	5.3	51.2	43.4	53.5	43.1	47.8	11.5	13.8	12.4	11.7	12.3
IC-0633368	70.0	69.6	68.1	66.5	68.6	5.8	3.7	5.9	5.7	5.3	56.4	37.9	63.9	55.1	53.3	12.0	14.4	12.6	11.7	12.7
IC-0633369	66.8	72.1	65.1	58.9	65.7	5.3	4.1	5.4	5.3	5.0	50.3	40.5	58.6	53.9	50.8	12.1	13.0	11.0	12.5	12.2
IC-0633370	75.6	71.4	78.1	54.1	69.8	5.2	4.4	4.7	5.3	4.9	45.9	40.1	56.0	47.7	47.4	12.4	14.1	12.0	11.9	12.6
IC-0633371	73.4	74.7	74.9	51.8	68.7	5.6	3.8	5.1	5.8	5.1	41.2	35.8	54.3	42.3	43.4	13.5	14.1	12.3	12.2	13.0
AFg-1	70.3	73.6	71.5	79.5	73.7	5.1	4.3	5.7	5.9	5.3	63.4	39.1	71.5	58.7	58.2	11.9	13.5	12.2	11.8	12.3
AFg-2	75.1	79.1	86.8	82.7	80.9	5.7	4.4	5.5	5.5	5.3	69.0	37.0	85.2	50.5	60.4	12.5	13.3	13.2	12.1	12.8
AFg-3	80.3	84.9	82.5	75.5	80.8	5.0	5.2	5.6	5.7	5.4	45.6	45.5	71.7	40.9	51.0	11.9	13.9	12.8	12.5	12.8
AFg-4	80.2	75.3	84.0	78.6	79.5	5.1	4.4	5.7	5.7	5.2	59.0	41.9	85.4	46.0	58.1	12.9	13.7	11.8	11.8	12.5
AFg-5	75.8	76.0	79.7	75.3	76.7	5.3	4.0	5.6	5.5	5.1	59.0	38.7	79.5	45.8	55.8	13.3	13.3	12.5	12.2	12.8
Mean	71.10	75.5	72.9	63.0	70.6	5.4	4.4	5.3	5.6	5.2	53.1	39.9	65.1	46.8	45.6	12.1	13.6	12.2	12.0	12.5
CD (0.05%)	3.18	3.17	3.14	2.99	1.75	0.23	0.22	0.25	0.20	0.13	2.06	1.88	3.29	1.88	1.13	0.55	0.56	0.49	0.46	0.32
SE(d)	1.54	1.54	1.53	1.45	0.85	0.11	0.19	0.15	0.10	0.06	1.00	0.27	1.60	0.92	0.55	0.27	0.27	0.24	0.22	0.15
SE(m)	1.10	1.09	1.08	1.03	0.60	0.08	0.14	0.10	0.07	0.04	0.71	0.19	1.13	0.65	0.39	0.19	0.19	0.17	0.16	0.11
CV (%)	2.66	2.50	2.56	2.82	1.47	2.52	3.16	2.63	2.09	1.47	2.31	2.43	3.00	2.39	1.31	2.71	2.43	2.37	2.28	1.50

Table 2: Performance of green-seeded vs. conventional fenugreek genotypes for seed yield traits during 2020-21 to 2023-24

Genotypes	No. of seeds/pod					Seed yield (kg/ha)					Test weight (g)				
	2020-21	2021-22	2022-23	2023-24	Mean	2020-21	2021-22	2022-23	2023-24	Mean	2020-21	2021-22	2022-23	2023-24	Mean
IC-0633362	17.3	17.9	17.6	18.7	17.9	1,473	2,414	2,189	1,316	1,848	12.1	15.8	11.9	12.0	13.0
IC-0633363	17.1	16.8	17.2	18.7	17.5	1,554	2,691	2,296	1,223	1,941	12.2	11.9	12.9	11.9	12.2
IC-0633364	17.9	16.5	18.1	18.5	17.7	1,640	2,643	2,881	1,289	2,113	12.2	15.2	14.0	11.9	13.3
IC-0633365	17.6	17.6	17.9	18.8	18.0	1,651	2,753	2,855	1,343	2,151	12.4	14.9	12.1	12.0	12.8
IC-0633366	17.9	16.5	18.0	18.1	17.6	1,395	2,542	1,979	1,344	1,815	12.3	13.9	10.9	11.7	12.2
IC-0633367	18.1	17.9	18.2	18.2	18.1	980	2,457	2,050	1,449	1,734	12.1	14.9	12.8	11.6	12.8
IC-0633368	16.9	16.9	17.7	18.1	17.4	1,199	2,515	2,290	1,221	1,806	12.3	15.1	13.2	12.0	13.1
IC-0633369	17.2	17.9	18.2	18.1	17.8	1,618	2,871	2,967	1,583	2,260	12.6	14.4	13.3	11.8	13.0
IC-0633370	18.0	18.1	18.3	19.1	18.4	914	2,073	2,893	1,389	1,817	12.5	13.6	13.2	11.9	12.8
IC-0633371	18.0	17.3	18.5	18.5	18.1	1,528	2,411	2,320	963	1,806	12.5	14.6	11.0	11.5	12.4
AFg-1	17.6	17.7	18.7	17.8	18.0	1,353	2,617	3,094	1,338	2,100	13.0	14.8	13.5	12.5	13.5
AFg-2	17.0	17.5	18.7	18.3	17.9	1,348	2,574	2,770	1,417	2,027	11.6	13.7	14.4	11.7	12.9
AFg-3	17.8	18.2	17.9	18.8	18.2	1,456	1,152	2,677	1,448	1,683	12.2	13.5	16.8	14.8	14.3
AFg-4	17.6	18.1	18.6	18.9	18.3	1,496	2,582	2,895	1,508	2,120	12.5	13.4	16.6	13.7	14.0
AFg-5	17.1	17.7	20.3	18.7	18.5	1,535	2,342	3,047	1,506	2,108	11.5	16.1	16.5	13.0	14.3
Mean	17.5	17.5	18.3	18.5	17.9	1,409	2,442	2,614	1,356	1,955	12.3	14.4	13.5	12.3	13.1
CD (0.05%)	0.74	0.68	0.95		0.38	0.30	106.3	100.0	63.70	48.20	0.60	0.70	0.50	0.60	0.30
SE(d)	0.36	0.23	0.46	0.30	0.19	0.20	51.6	48.6	31.00	23.40	0.30	0.30	0.20	0.30	0.10
SE(m)	0.25	0.33	0.33	0.42	0.13	0.10	36.5	34.3	21.90	16.50	0.20	0.20	0.20	0.20	0.10
CV (%)	2.50	2.30	3.10	2.78	1.27	2.40	2.6	2.3	2.80	1.50	2.80	2.80	2.40	2.80	1.30

Table 3: Performance of green-seeded vs. conventional fenugreek genotypes for seed chlorophyll content, colour and shade names during 2020-21 to 2023-24

Genotypes	Total Chlorophyll (mg/100 g seed)					Colour analysis	
	2020-21	2021-22	2022-23	2023-24	Mean	Shade code	Color shade name
IC-0633362	0.690	0.740	0.450	0.830	0.678	#564724	Dark olive green
IC-0633363	0.570	0.420	0.360	0.630	0.495	#493e20	Dark olive green
IC-0633364	0.660	0.480	0.330	0.470	0.485	#524522	Dark olive green
IC-0633365	0.660	0.640	0.380	0.570	0.562	#473c20	Dark slate gray
IC-0633366	0.400	0.510	0.350	0.660	0.480	#4d3f20	Dim gray
IC-0633367	0.570	0.480	0.350	0.430	0.458	#584824	Dark olive green
IC-0633368	0.610	0.720	0.330	0.580	0.560	#584724	Dark olive green
IC-0633369	0.600	0.560	0.350	0.500	0.503	#524423	Dark olive green
IC-0633370	0.590	0.390	0.310	0.530	0.455	#4c4020	Dark olive green
IC-0633371	0.530	0.380	0.400	0.620	0.482	#463e1e	Dark olive green
AFg-1	0.070	0.090	0.080	0.020	0.065	#765125	Saddle brown
AFg-2	0.040	0.040	0.050	0.040	0.043	#6f4f29	Saddle brown
AFg-3	0.070	0.070	0.070	0.020	0.057	#765227	Saddle brown
AFg-4	0.100	0.120	0.040	0.040	0.075	#745029	Saddle brown
AFg-5	0.050	0.060	0.050	0.020	0.045	#74522b	Saddle brown
Mean	0.414	0.380	0.260	0.397	0.363		
CD (0.05%)	0.018	0.018	0.012	0.023	0.008		
SE(d)	0.009	0.009	0.006	0.011	0.004		
SE(m)	0.006	0.006	0.004	0.008	0.003		
CV (%)	2.626	2.863	2.792	3.446	1.382		

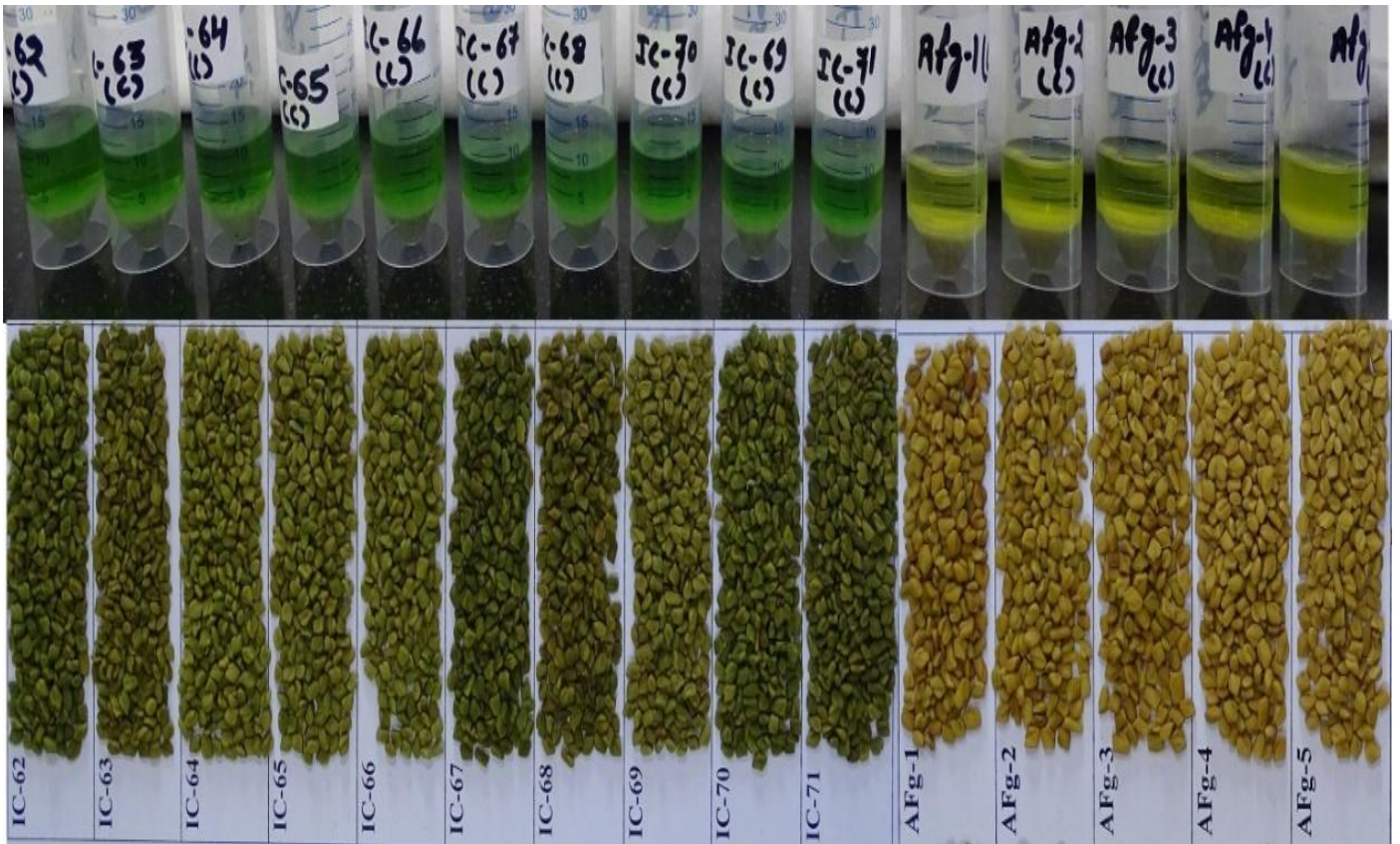


Plate 1. Observed colour variation in seeds and extract of green-seeded fenugreek genotypes and conventional fenugreek varieties

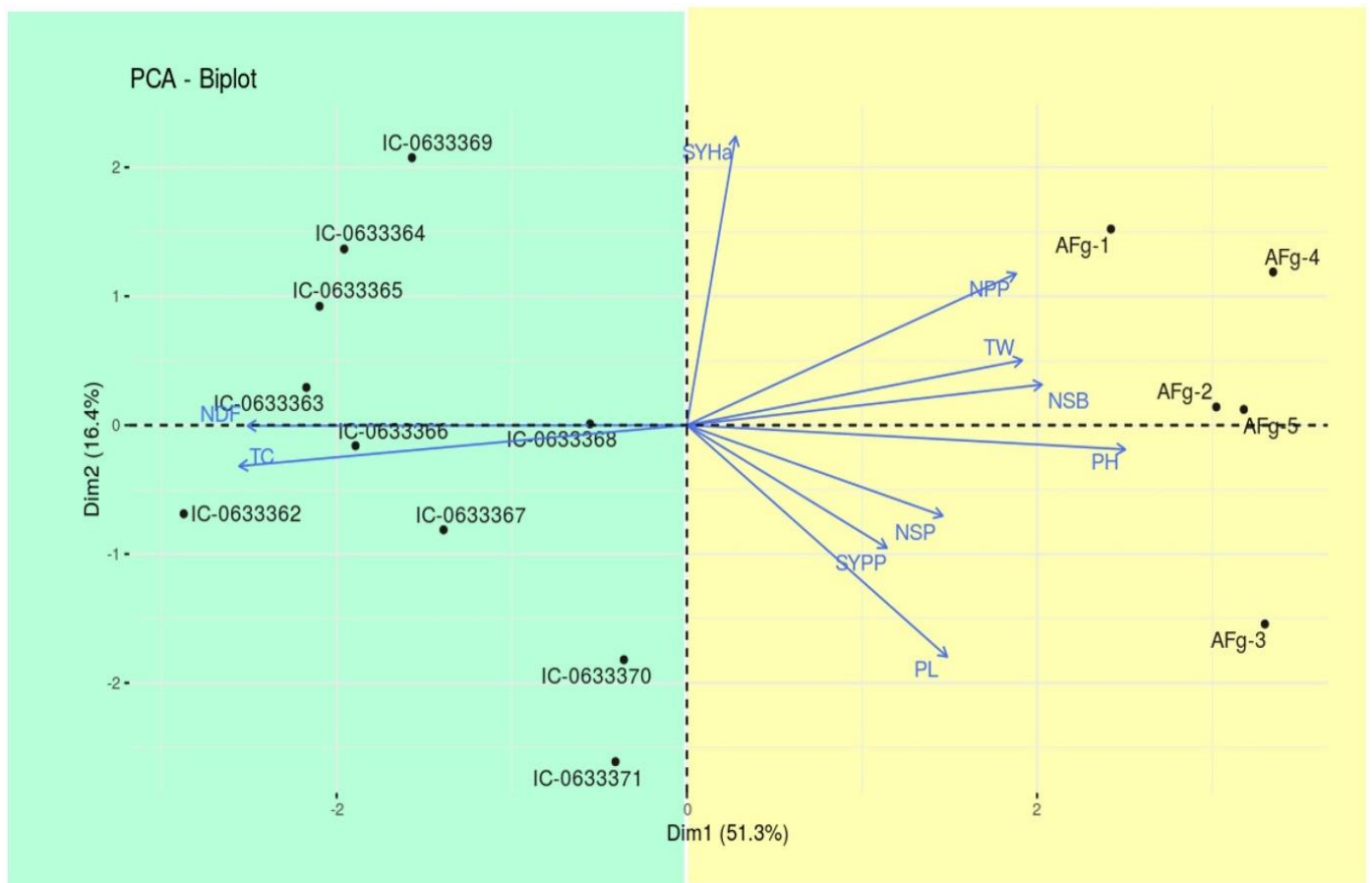


Figure 1. PCA bi-plot for the first and second principal components based on the morphometric, seed yield, seed chlorophyll content of green-seeded vs. conventional fenugreek genotypes

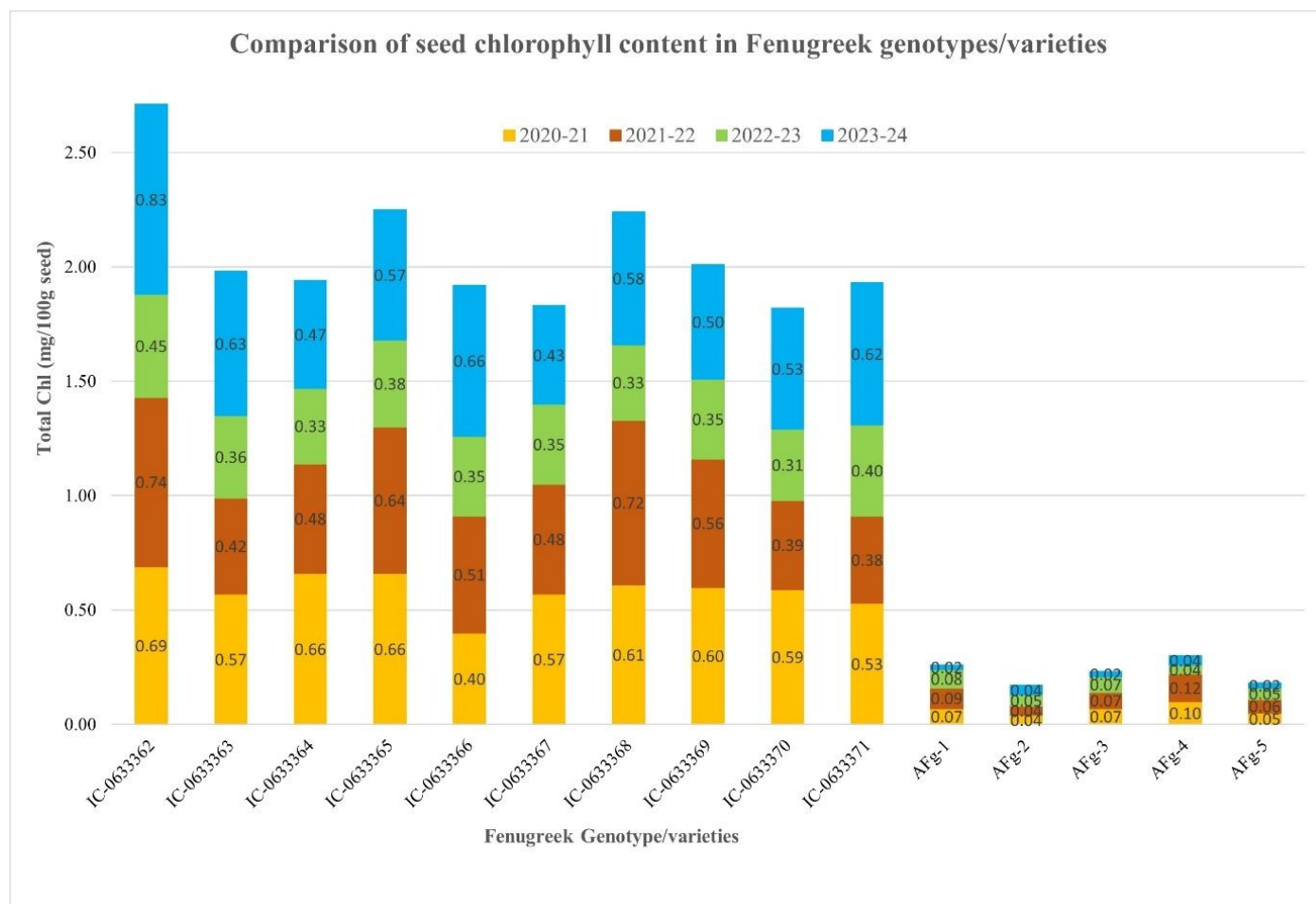


Figure 2. Chlorophyll content (%) in green-seeded fenugreek genotypes and conventional fenugreek varieties

Conclusion

The present study demonstrated significant genetic variability between green-seeded and conventional, yellow-seeded fenugreek (*Trigonella foenum-graecum* L.) genotypes, particularly in chlorophyll content, seed color, and key morphometric traits. The green-seeded genotypes, especially IC-0633362, exhibited superior chlorophyll content (>0.65 mg/g) and a distinctive dark olive-green seed color, distinguishing them from check varieties, which showed lower chlorophyll levels (0.423 to 0.075 mg/g) and Saddle Brown coloration. These findings highlight the potential of green-seeded fenugreek genotypes as valuable genetic resources for breeding programs aimed at enhancing seed quality, with particular relevance to nutraceutical and medicinal applications. The stability of these traits across four consecutive seasons further reinforces their adaptability and suitability for cultivation under diverse agro-climatic conditions. Although green-seeded genotypes showed moderately lower yields than yellow-seeded varieties, this limitation can be addressed through improved agronomic interventions. Future research should focus on elucidating the underlying genetic mechanisms and evaluating their biochemical and pharmacological benefits to fully harness their potential in crop improvement.

Acknowledgement

The authors gratefully acknowledge the Director, ICAR-NRCCS Ajmer, for providing research facilities and support to conduct the study.

Declaration of interests

The authors do not have any conflict of interest, and the research was conducted in the absence of any commercial relationship with anyone.

Data Sharing

All relevant data are within the manuscript and are available from the first author.

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Preferred citation: Singh R, Meena RS, Choudhary S, Meena NK, Meena RD, Verma AK, Ravi Y & Lal S. 2025. Comparative evaluation of seed chlorophyll content, colour, and yield traits in Fenugreek (*Trigonella foenum-graecum* L.). *Journal of Agriculture and Ecology*, 20: 18-26; <https://doi.org/10.58628/JAE-2520-103>