

Evaluation of Physiological and Phytochemical Traits in Different *Trigonella foenum-graecum* Genotypes

ABSTRACT

Nowadays, medicinal plants are great alternatives to pharmaceuticals because they have fewer side effects. Fenugreek seeds and leaves are valued for their high flavonoids, alkaloids, saponins, and sterols content. The aim of the study is to assess the morphological, physiological, and biochemical traits of seven different fenugreek genotypes viz., Hisar Sonali, Rajendra Kranti, Gujarat Methi-1, Pusa Early Bunching, RMT-1, CO-1, and Pant Ragini at the pot house of department of Botany with complete randomised design. Hisar Sonali was the late-maturing genotype, taking an average of 150.18 days to reach maturity. Pant Ragini weighed the most in terms of both fresh and dried root weights (2.85 g and 0.11 g, respectively). Significant amounts of leaf chlorophyll content were found in all genotypes, with Pusa Early Bunching exhibiting the highest relative dry weight (0.23 g). Biochemical studies showed that Hisar Sonali had the highest total protein content (10.98 mg/g) and CO-1 had the lowest (5.23 mg/g). In addition to the numerous pharmacological and therapeutic uses of fenugreek, this review highlights the significance of its bioactive components, which hold great promise for enhancing human health and promoting sustainable farming methods.

INTRODUCTION

With their many biological and pharmacological properties, natural products are an excellent source for scientists looking for compounds to treat diseases and other health problems (Bouhenni *et al.*, 2021). Now days, medicinal plants are great alternatives to pharmaceuticals because they have fewer side effects (Li *et al.*, 2020). Medicinal and aromatic plants are typically used as medications to prevent, treat, and maintain ailments in both traditional and modern medicine (Temel *et al.* 2018). *Trigonella foenum-graecum* is an annual flowering plant that is primarily grown in India, the Mediterranean region, and Central Asia (Singh *et al.*, 2015). *Trigonella* is an important member of the legume genus with 135 species that are found all over the world and also referred to as "Greek hay" or "methi" in Hindi, is a member of the Fabaceae family (Pal & Mukherjee, 2020). The fenugreek plant has a finger-like root and a long, pink, cylindrical stem ranging from 30 to 60 cm. Its complex leaves are pinnate, with triangular stipules, long stalks and golden-yellow solid, smooth seeds with a life span of 4–7 months (Akan *et al.*, 2020). Fenugreek seeds contain 58% carbs, 23–26% proteins, 0.9% fats, and 25% fiber (Bakhtiar *et al.*, 2024). Additionally, according to Wani and Kumar (2018), fenugreek leaves provide 6%, 4.4%, and 1.1% of carbohydrates, proteins, and fiber, respectively (Ahmad *et al.*, 2016). Plant quality can either be negatively impacted (in terms of protein and diosgenin contents) or positively

impacted (in terms of crude oil, protein, and ash contents) by the timing of planting (Gökçe 2015; Anitha *et al.*, 2016). These characteristics are brought about by fenugreek's high concentration of bioactive phytochemicals, such as flavonoids, alkaloids, amino acids, vitamins, saponins, and fibers (Ouzir *et al.*, 2016; Bitarafan *et al.*, 2019). Bioactive compounds found in fenugreek had numerous health benefits, including better digestion, decreased blood glucose in diabetics, and preservation of plasma cholesterol levels, have been linked to the traditional usage of fenugreek seeds (Salarbashi *et al.*, 2019; Khan *et al.*, 2022). Fenugreek seeds also contain flavonoids, which are potent antioxidants. Antioxidants properties, fenugreek seeds may help sustain a robust immune system and reduce the frequency of chronic illnesses (Akbari *et al.*, 2019). Additionally, it is utilized in medicinal treatments for antidiabetic, hypocholesterolemic, immunological, antioxidant, and anticarcinogenic properties (Wani and Kumar 2018). Fenugreek leaves and stems are used medicinally to heal wounds, anemia, stomach ulcers, and antipyretics (Hasanuzzaman *et al.*, 2020) in addition to being used as a seasoning in food (Nannar *et al.*, 2023). It is well known that the phytonutrients have anti-inflammatory, antidiabetic, antifungal, antioxidant, antiviral, anticancer, hepatoprotective, and hypocholesterolaemia properties (Mohammedi *et al.*, 2020, Kulkarni *et al.*, 2020, Goyal *et al.*, 2016, Ghosal and Singh 2022). According to recent finding the present investigation was carried out to investigate the phytochemical profile of different fenugreek genotype.

MATERIAL AND METHODS

MATERIAL

Plant material and study location

In the current study, seven different fenugreek genotypes—Hisar Sonali, Rajendra Kranti, Gujrat Methi-1, Pusa Early Bunching, RMT-1, Co-1, and Pant Ragini—were used to assess their phytochemical and bioactive compounds using three replications in a pothouse using a completely randomized design at the Department of Botany's research area in Rohtak (HRY) during the Rabi season.

METHODS

Raising of crop and Experimental design: Fenugreek seeds were surface sterilized using 1% (w/v) mercuric chloride (HgCl₂) prior to their sowing. Five seeds were sown in pots containing 10 kg of farm soil. The experiment was conducted under controlled environmental conditions with Completely Randomized Design (CRD) to minimize variability and enhance statistical reliability.

OBSERVATION RECORDED:

The observation of various morpho-physiological and biochemical parameters was recorded. The data was recorded on three plants per genotype.

Number of days to maturity: To calculate the number of days to maturity, the number of days in each experimental plot between the date of planting and the day the plant reached full maturity was recorded.

Plant height at maturity (cm): From ground level to the tip of the final pod on the main stem, the average height of the related plants is expressed in cm.

Root Fresh and Dry weight (g): We measured the root's fresh and dried weights. After gathering ten mature roots of roughly comparable size, DDW (Double Distilled Water) was used to clean them. To absorb the extra water, tissue paper was utilized. Both fresh and dried weights were measured following room drying and drying, respectively.

Relative dry weight (g): The dry weight divided by the fresh weight is known as the relative dry weight, and it is frequently given as a percentage. The equation is as follows:

$$RDW = DW / FW \times 100.$$

Chlorophyll content (mg/g FW): The method developed by Hiscox and Israelstam (1979) was applied to extract chlorophyll. 100 mg of fresh leaf material was kept in 10 mL of dimethyl sulfoxide (DMSO) at 65 °C in the dark for 2 hours. The absorbance was measured at many wavelengths (480, 645, 520, and 663 nm) in a final volume of 10 mL. Chlorophyll-a (Chl a), -b (Chl b), and total chlorophyll (Chl a + b) concentrations were determined using the methods provided by Arnon (1949) and expressed as mg g⁻¹ FW.

Reducing Sugar Content (mg/g DW): Reducing sugar was extracted in 10 ml of 80% alcohol by boiling 0.1g of dry powdered material for 30 minutes at 80 to 90 °C degrees, centrifuging it at 5,000gm for 10 minutes, and then applying Nelson-Somogyi's method (Sadasivam and Manickam, 1992). The standard curve of a glucose solution (0 to 500 µg) was used to measure the amount of reducing sugar.

Non-reducing sugar (mg/g DW): For non-reducing sugar, the Malhotra and Sarkar (1979) method was applied, which included the same procedures as for reducing sugar with the added step of neutralizing the samples using 1ml of 1N NaOH.

Total protein content (g/100g of FW): Protein evaluation was done using the Lowry *et al.* [1951] technique. 1 N sodium hydroxide was used to crush the fenugreek seedlings for five minutes at 100 °C. For ten minutes, the alkaline copper reagent was combined at room temperature. Absorbance at 650 nm was taken 30 minutes after the Folin-Ciocalteu reagent was combined. The lysozyme standard curve was used to measure the quantity of protein.

Total Proline content (mg/100g): The method developed by Bates *et al.* (1973) was used to measure proline levels. After being incubated for an hour at 100°C, fenugreek seedlings were extracted using toluene, 3% sulphosalicylic acid, acid-ninhydrin, and acetic acid. A standard curve was used to calculate

and represent the amount of proline as $\mu\text{mol g}^{-1}\text{FW}$. The chromophore's absorbance at 520 nm was determined.

Phytochemical tests: Phytochemical tests were done for flavonoids, terpenoids, and steroids, (Diwakar *et al.*, 2016).

Test for Flavonoids: A few drops of sodium hydroxide solution were added to the extracts. The presence of flavonoids was detected by the formation of a bright yellow hue that turned colorless when diluted acid was added.

Test for Terpenoids: Two ml of chloroform were combined with the extract, and three ml of strong H_2SO_4 were carefully added to create a layer. The contact area developed a reddish-brown color, indicating the presence of terpenoids.

Test for Tannins: Each extract was boiled in a small volume with five ml of 45% (V/V) ethanol for five minutes. After cooling, the mixture undergoes filtering. Distilled water was used to dilute 1 ml of filtrate, and then 2 drops of 10% ferric chloride were added. A brief greenish-to-black colour indicated the presence of tannins.

Test for Steroids: After adding 3–4 drops of acetic anhydride to the test solution, it was heated, and cooled, and then 3 drops of sulphuric acid were added. At the intersection of the two layers, a brown ring emerges. When steroids are present, the top layer becomes green.

RESULTS:

The study assessed the morphological, physiological, and biochemical characteristics of different biotypes in terms of days to maturity, root fresh weight, root dry weight, reducing sugar, relative dry weight, leaf chlorophyll content, non-reducing sugar, total protein content, total proline content, flavonoids, terpenoids, tannins, and steroids. The data presented in the Table and graphs shows the mean values with standard errors (S.E.) for each parameter across biotypes: Coimbatore-1, Gujarat Methi-1, Hisar Sonali, Pusa Early Bunching, Pant Ragini, Rajendra Kranti, and Rajasthan Methi-1.

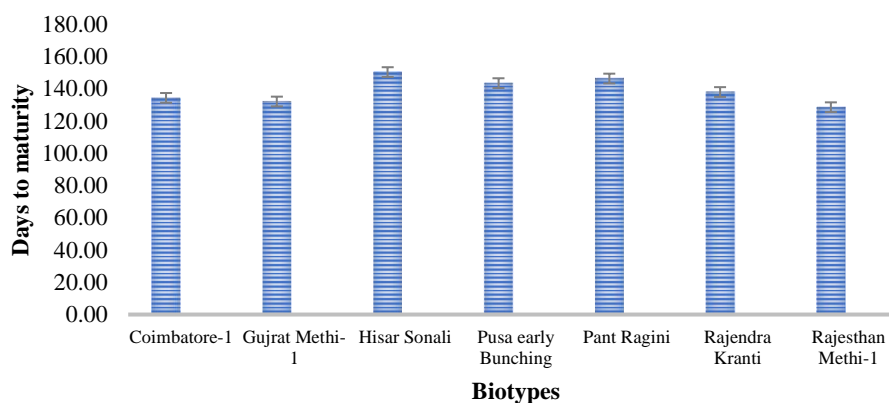


Fig-1. Number of days to maturity of different biotypes of *Trigonella foenum graecum* L.

Days to maturity: The number of days to maturity was significantly different across biotypes. Hisar Sonali showed the longest time to maturity (150.18 ± 1.094 days), indicating a slow maturity response. In contrast, Rajasthan Methi-1 reached maturity (128.42 ± 2.337 days) the fastest, indicating a faster growing stage. This variation may be associated with genetic or environmental adaptation differences among biotypes. The significant difference (S.D.) of 7.15 days confirms a statistically significant difference in maturity time, which is relevant for selecting biotypes with specific growing responses.

Plant Height at maturity (cm): The plant height at maturity differed significantly among biotypes. Pant Ragini displayed the highest plant height at maturity (53.65 ± 0.630 cm), while Rajasthan Methi-1 displayed the lowest plant height at maturity (46.11 ± 0.265 cm). Other biotypes, including Coimbatore-1 (47.86 ± 0.574 cm) and Gujarat Methi-1 (47.65 ± 0.916 cm), had moderate plant height. The critical difference (C.D.) value of 2.34 confirms the significant difference in plant height at maturity among biotypes.

Table 1: Morphological parameters of different biotypes of *Trigonella foenum graecum* L.

BIOTYPES	Plant Height (cm)		RFW (g)		RDW (g)	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
Coimbatore-1	47.86	± 0.574	1.92	± 0.030	0.07	± 0.001
Gujrat Methi-1	47.65	± 0.916	1.46	± 0.036	0.08	± 0.002
Hisar Sonali	50.25	± 0.785	2.15	± 0.026	0.05	± 0.015
Pusa early Bunching	52.18	± 0.598	2.68	± 0.066	0.10	± 0.021
Pant Ragini	53.65	± 0.630	2.85	± 0.042	0.11	± 0.007
Rajendra Kranti	48.49	± 1.213	2.08	± 0.012	0.09	± 0.011
Rajasthan Methi-1	46.11	± 0.265	1.25	± 0.030	0.06	± 0.030
C.D. (5%)	2.34		0.12		0.11	
SE(m)	0.76		0.04		0.06	
SE(d)	1.08		0.05		0.25	

RFW- Root fresh weight, RDW- Root dry weight, C.D.- Critical difference

Root Fresh and Dry Weight (g)

Significant variation in fresh weight of roots was observed among biotypes. Pant Ragini showed the highest average root fresh weight (2.85 ± 0.042 g), followed by Pusa Early Bunching (2.68 ± 0.066 g) and Hisar Sonali (2.15 ± 0.026 g). Rajasthan Methi-1 recorded the lowest fresh leaf weight of (1.25 ± 0.030 g). The significant difference (C.D.) value for fresh root weight was 0.12 g, indicating a statistically significant difference between biotypes. A

similar trend was observed in root dry weight, with both Pant Ragini and Pusa Early Bunching recording the highest values (0.11 ± 0.000 g for Pusa Early Bunching and 0.11 ± 0.000 g for Pant Ragini). Hisar Sonali also displayed a relatively high root dry weight (0.09 ± 0.000 g), while Rajasthan Methi-1 had the lowest dry leaf weight of (0.05 ± 0.000 g).

Relative Dry Weight (RDW; g)

The relative dry weight (RDW) differed significantly among biotypes. Pusa Early Bunching displayed the highest RDW (0.23 ± 0.012 g), while Pant Ragini displayed the lowest RDW (0.16 ± 0.075 g). Other biotypes, including Coimbatore-1 (0.20 ± 0.006 g), Rajendra Kranti (0.20 ± 0.006 g) and Rajasthan Methi-1 (0.20 ± 0.021 g), had same RDW levels. The critical difference (C.D.) value of 0.10 confirms the significant difference in RDW among biotypes.

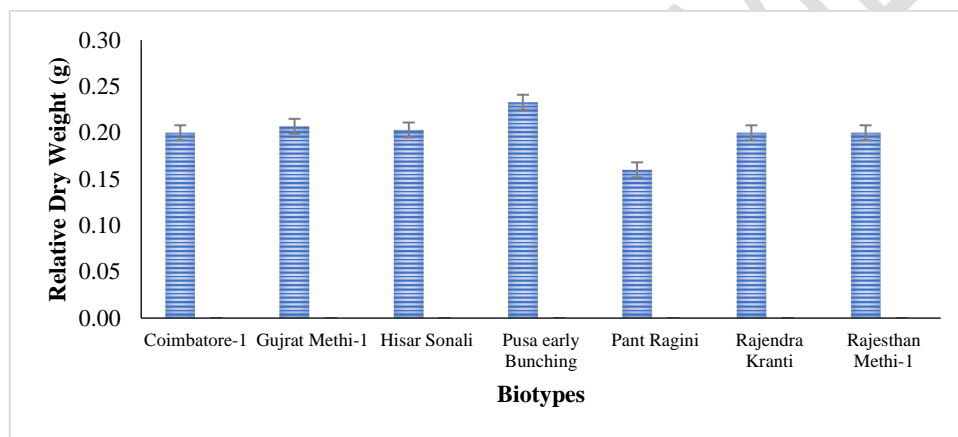


Fig-2 Relative dry weight (RDW; g) of different biotypes of *Trigonella foenum-graecum* L.

Leaf Chlorophyll content (mg/g FW): Chlorophyll levels were also variable, with Pant Ragini showing the highest concentration (4.90 ± 0.009 mg/g), followed by Pusa Early Bunching (4.54 ± 0.045 mg/g). Rajendra Kranti recorded medium chlorophyll levels (4.25 ± 0.059 mg/g), while Gujrat Methi-1 showed the lowest chlorophyll content (3.14 ± 0.009 mg/g). The CD value of 0.10 mg/g indicates that these differences are statistically significant, indicating diversity in biochemical traits among biotypes.

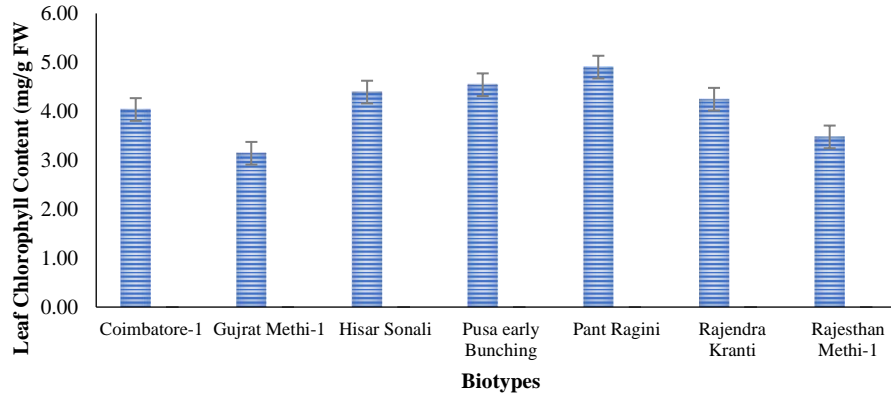


Fig-3 Leaf chlorophyll content of different biotypes of *Trigonella foenum-graecum* L.

Reducing sugar (mg/g DW): Reducing sugar value varied across biotypes, with Hisar Sonali exhibiting the highest average reducing sugar level (8.34 ± 0.012 mg/g of DW), followed by Rajasthan Methi-1 (7.56 ± 0.006 mg/g of DW) and Gujarat Methi-1 (6.67 ± 0.047 mg/g of DW). Coimbatore-1 had the lowest reducing sugar value (3.45 ± 0.033 mg/g of DW), indicating significant differences in the regulation of growth and development among biotypes. The significant difference (C.D.) of 0.17 mg/g of DW highlights the statistically significant variation observed in reducing sugar content.

Table 2: Estimation of phytochemical compounds in different biotypes of *Trigonella foenum-graecum* L.

BIOTYPES	Reducing sugar		Non-reducing sugar		Total Protein content	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
Coimbatore-1	3.45	± 0.033	17.35	± 0.217	5.23	± 0.042
Gujrat Methi-1	6.67	± 0.047	20.68	± 0.131	9.54	± 0.199
Hisar Sonali	8.34	± 0.012	21.75	± 0.276	10.98	± 0.015
Pusa early Bunching	5.18	± 0.104	20.25	± 0.318	8.56	± 0.068
Pant Ragini	5.34	± 0.006	19.98	± 0.395	7.23	± 0.098
Rajendra Kranti	4.45	± 0.083	18.55	± 0.327	6.54	± 0.110
Rajasthan Methi-1	7.56	± 0.006	21.45	± 0.357	10.55	± 0.078
C.D. (5%)	0.17		0.92		0.32	
SE(m)	0.06		0.30		0.10	
SE(d)	0.08		0.43		0.15	

Non-reducing sugar (mg/g DW): Non-reducing sugar value varied across biotypes, with Hisar Sonali exhibiting the highest average non-reducing sugar level (21.75 ± 0.276 mg/g DW), followed by Rajasthan Methi-1 (21.45 ± 0.357 mg/g DW) and Gujarat Methi-1 (20.68 ± 0.131 mg/g DW). Coimbatore-1 had the lowest reducing sugar value (17.35 ± 0.217 mg/g DW),

indicating significant differences in the regulation of growth and development among biotypes. The significant difference (C.D.) of 0.92 mg/g DW highlights the statistically significant variation observed in non-reducing sugar content.

Table 3: Estimation of phytochemical compounds in different biotypes of *Trigonella foenum-graecum* L.

BIOTYPES	Total Proline content		Flavonoid content		Terpenoids	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
Coimbatore-1	192.26	±3.401	18.78	±0.099	0.12	±0.000
Gujrat Methi-1	258.77	±6.329	20.98	±0.295	0.17	±0.000
Hisar Sonali	276.14	±6.120	21.75	±0.455	0.19	±0.000
Pusa early Bunching	250.95	±3.919	20.55	±0.083	0.16	±0.003
Pant Ragini	245.17	±0.260	19.54	±0.071	0.15	±0.003
Rajendra Kranti	234.75	±2.932	19.43	±0.253	0.14	±0.000
Rajasthan Methi-1	269.89	±3.935	21.25	±0.057	0.18	±0.006
C.D. (5%)	13.13		0.72		0.01	
SE(m)	4.29		0.23		0.00	
SE(d)	6.06		0.33		0.00	

Table 3: Analysis of bioactive compounds from different biotypes of *Trigonella foenum-graecum* L.

Total Proline content (mg/100g): Significant variation was also observed in total proline content, with Hisar Sonali (276.14 ± 6.120 mg/100g) and Rajasthan Methi-1 (269.89 ± 3.935 mg/100g) showing the highest total proline contents. Gujarat Methi-1 (258.77 ± 6.329 mg/100g) and Pusa Early Bunching (250.95 ± 3.919 mg/100g) also had high total proline content. In contrast, Coimbatore-1 had the lowest carbohydrate content at (192.26 ± 3.401 mg/100g). The C.D. value for total proline content (13.13 mg/100g) validates the statistical relevance of these observed differences.

Flavonoid (mg QCE/g of DWE): Using the AlCl₃ reagent and catechin as standard, the total flavonoids in leaves are in the range from (18.78 ± 0.099 – 21.75 ± 0.455 mg QCE/g of DWE) in Table 3. The highest value for the flavonoid content was (21.75 ± 0.455 mg QCE/g of DWE) in Hisar Sonali and the lowest was (18.78 ± 0.099 mg QCE/g of DWE) in Coimbatore-1. The following decreasing order of flavonoid content in leaves of different biotypes: Hisar Sonali > Rajasthan Methi-1 > Gujrat Methi-1 > Pusa Early Bunching > Pant Ragini > Rajendra Kranti > Coimbatore-1 was measured. Flavonoids are not easily detectable therefore extract using

AlCl₃ complexing reagent. The critical difference (C.D.) value of 0.72 mg QCE/g of DWE confirms the significant difference in flavonoids among biotypes.

Table 4: Estimation of bioactive compounds in different biotypes of *Trigonella foenum-graecum* L.

BIOTYPES	Tannin		Steroids	
	Mean	S.E.	Mean	S.E.
Coimbatore-1	0.49	±0.006	1.66	±0.006
Gujrat Methi-1	0.76	±0.015	3.64	±0.015
Hisar Sonali	0.86	±0.012	3.78	±0.068
Pusa early Bunching	0.61	±0.012	2.45	±0.054
Pant Ragini	0.55	±0.009	2.34	±0.009
Rajendra Kranti	0.52	±0.000	2.23	±0.026
Rajasthan Methi-1	0.77	±0.009	3.56	±0.050
C.D. (5%)	0.03		0.12	
SE(m)	0.01		0.04	
SE(d)	0.01		0.06	

Terpenoids (ug/ml): In terms of terpenoid content, Hisar Sonali displayed the highest concentration (0.19 ± 0.000 ug/ml), followed by Rajasthan Methi-1 (0.18 ± 0.006 ug/ml), suggesting the potential of these biotypes as a source of bioactive compounds. On the other hand, Coimbatore-1 showed the lowest terpenoid levels (0.12 ± 0.000 ug/ml). With a CD of 0.01ug/ml, these differences in terpenoid content are statistically significant, indicating substantial variability in secondary metabolite production among biotypes.

Tannin (mg TA/g of DWE): In the current study, there was a significant difference among the biotypes for tannin content in leaves which ranged from 0.49 ± 0.006 - 0.86 ± 0.012 (mg TA/g of DWE) with significant difference (C.D.) 0.03 mg TA/g of DWE. The maximum tannin content was recorded in the leaves of biotype Hisar Sonali (0.86 ± 0.012 mg TA/g of DWE) followed by Rajasthan Methi-1 (0.77 ± 0.009 mg TA/g of DWE), Gujrat Methi-1 (0.76 ± 0.015 mg TA/g of DWE), Pusa Early Bunching (0.61 ± 0.012 mg TA/g of DWE), Pant Ragini (0.55 ± 0.009 mg TA/g of DWE) > Rajendra Kranti (0.52 ± 0.000 mg TA/g of DWE) > Coimbatore-1 (0.49 ± 0.006 mg TA/g of DWE).

Steroids (g/100g DW): Steroid levels were also variable, with Hisar Sonali showing the highest concentration (3.78 ± 0.068 g/100g DW), followed by Gujrat Methi-1 (3.64 ± 0.015 g/100g DW). Pusa Early Bunching recorded medium steroid levels (2.45 ± 0.054 g/100g DW), while Coimbatore-1 showed the lowest steroid content (1.66 ± 0.006 g/100g DW). The CD

value of 0.12 g/100g DW indicates that these differences are statistically significant, indicating diversity in biochemical traits among biotypes.

DISCUSSION

This study gives the values of morpho-physiological parameters no. of days to maturity, fresh and dry root weight, relative dry weight, and leaf chlorophyll content. Analysis of the result showed that the Hisar Sonali is superior in all the biotypes in several days to maturity. In plant height, root fresh and dry weight Pant Ragini is uppermost in all the biotypes. The most crucial factor for a crop is its yield. According to Chauhan *et al.* (2017), the characteristics that contribute to yield vary among cereals, pulses, and seed spices. According to Senkal *et al.*, (2018), Panwar *et al.*, (2018), Gurbuz *et al.*, (2000), and Jain *et al.*, (2013) the height of fenugreek plants ranges from 32.42 to 46.58 cm, 19.22 to 64.66 cm, 68.57 to 91.33 cm, and 43.83 to 68.47 cm, respectively. Genetic traits of genotypes and environmental adaptability may cause varying reactions to plant height. Jhajhra *et al.*, (2017) and Shakthi *et al.*, (2020) reported similar outcomes with fenugreek. Bhutia *et al.*, (2017) found that fenugreek plants took the longest to attain maturity, at 133.00 days. Different fenugreek varieties' varying plant heights may be caused by variations in their genetic makeup and how they react to environmental factors. As the days go by, photosynthesis becomes more efficient in producing the sugar required for plant growth, increasing the amount of carbohydrates available for plant growth and development. The outcomes supported the findings of Duwal *et al.*, (2019) in Coriander and Latye *et al.*, (2016), and Aggarwal *et al.*, (2013) in fenugreek. The fresh weight of seedlings of root and shoot were recorded (0.834 and 0.119), But the dry weight of seedlings was found approximately eight times less (0.110 and 0.010) than the fresh weight of seedlings. Because sugars are used as sources of energy, carbon, and regulatory activities, sugars affect every stage of the plant life cycle, interact with other signaling molecules like phytohormones, and control plant growth and development (Jeandet *et al.*, 2022). Plants sown on October 15th had the highest total chlorophyll content (4.61 mg), followed by plants sown on November 1st (4.36 mg). The plot sown on December 15th had the lowest total chlorophyll content (3.83 mg) (Anitha *et al.*, 2016).

Chlorophyll production was more negatively impacted by the greater salt content. By causing damage to the enzymes that produce chlorophyll or destabilizing the chlorophyll protein complex through chlorophyll oxidation, salinity can produce such an effect (Wang *et al.*, 2019). Fenugreek's protein composition varied from 8.95 to 12.90%, according to various research. It was found that plants sown on October 15th had the highest protein content (12.90%), followed

by plants sown on November 1st (10.44%). The plots sowed on December 15th had the lowest protein content in seeds (9.55%) (Anitha *et al.*, 2016). The study found that floral parts had a higher content of terpenoids than leaves, which might be utilized as a substitute source to create a variety of products useful for human health (Das *et al.*, 2022). Flavonoids along with various natural antioxidants are comparatively less harmful than synthetic antioxidants, which have adverse consequences. This is why it is so important to use natural flavonoids in various products, such as foods, medications, and cosmetics (Rodríguez De Luna *et al.*, 2020). According to Ghevariya *et al.*, (2023), the seeds of *Trigonella foenum-graecum* L. showed the maximum tannin concentration (0.92 ± 0.02 mg TA/g of DWE), while the stem had the lowest tannin content (0.6 ± 0.08 mg TA/g of DWE). Microgreens (19.07 ± 0.20 mg QCE/g of DWE) had the highest TFC, while stems (17.13 ± 0.58 mg QCE/g of DWE) had the lowest. Moreover, a tiny variation of 0.13 mg QCE/g of DWE exists in the TFC content of leaves and seeds.

Conclusion: This study highlights significant variations among tested fenugreek genotypes in physiological, and biochemical traits. Hisar Sonali exhibited the longest maturation period, while Pant Ragini had the highest root weights. Pusa Early Bunching showed the highest relative dry weight, and Hisar Sonali recorded the highest protein content. Present study indicates the genotype Pusa and Hisar Sonali was most promising for phytochemical component. It can be utilized form further metabolic profiling as well as recommended for cultivation and consumption.

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REFERENCES

1. Bouhenni, H., Doukani, K., Hanganu, D., Olah, N. K., Şekeroğlu, N., Gezici, S., ... & Niculae, M. (2021). Comparative analysis on bioactive compounds and antioxidant activity of Algerian fenugreek (*Trigonella foenum-graecum* L.) and Syrian cumin (*Cuminum cyminum* L.) seeds. *Herba Polonica*, 67(1), 18-34.
2. Li, Y., Kong, D., Fu, Y., Sussman, M. R., & Wu, H. (2020). The effect of developmental and environmental factors on secondary metabolites in medicinal plants. *Plant Physiology and Biochemistry*, 148, 80-89. <https://doi.org/10.1016/j.plaphy.2020.01.006>
3. Kooti, W., Moradi, M., Ali-Akbari, S., Sharafi-Ahvazi, N., Asadi-Samani, M., & Ashtary-Larky, D. (2015). Therapeutic and pharmacological potential of *Foeniculum vulgare* Mill: A review. *Journal of Herbmed Pharmacology*, 4(1), 1-9.
4. Temel, M., Tınmaz, A. B., Öztürk, M., & Gündüz, O. (2018). Production and trade of medicinal and aromatic plants in the world and Turkey. *KSU Journal of Agriculture and Natural Sciences*, 21(Special Issue), 198-214.
5. Singh, K. P., Singh, B., Tomar, B. S., & Naidu, A. K. (2015). Trait variation in fenugreek. *Journal of Agronomy*, 5(3), 45-56.
6. Akan, H., Ekici, M., & Aytac, Z. (2020). The synopsis of the genus *Trigonella* L. (Fabaceae) in Turkey. *Turkish Journal of Botany*, 44(6), 670-693.
7. Maphosa, Y., & Jideani, V. A. (2017). The role of legumes in human nutrition. *Functional Food—Improve Health Through Adequate Food*, 1, 13.
8. Anonymous. (2015). *Indian Horticulture Database, Ministry of Agriculture, Government of India*.
9. Wani, S. A., & Kumar, P. (2018). Fenugreek: A review on its nutraceutical properties and utilization in various food products. *Journal of the Saudi Society of Agricultural Sciences*, 17(2), 97-106.

10. Ahmad, A., Alghamdi, S. S., Mahmood, K., & Afzal, M. (2016). Fenugreek: A multipurpose crop—Potentialities and improvements. *Saudi Journal of Biological Sciences*, 23(2), 300-310.
11. Bienkowski, T., Zuk-Golaszewska, K., Kaliniewicz, J., & Golaszewski, J. (2017). Content of biogenic elements and fatty acid composition of fenugreek seeds cultivated under different conditions. *Chilean Journal of Agricultural Research*, 77(2), 134-141.
12. Van Zelm, E., Zhang, Y., & Testerink, C. (2020). Salt tolerance mechanisms of plants. *Annual Review of Plant Biology*, 71, 403-433.
13. Gökçe, Z. (2015). Effect of sowing date on the yield and quality of fenugreek (*Trigonella foenum-graecum* L.) under the conditions of Kahramanmaraş. (Master's thesis). Kahramanmaraş Sütçü İmam University, Graduate School of Natural and Applied Sciences, Field Crops Department.
14. Anitha, B., Lakshmi Narayana Reddy, M., Dorajee Rao, A. V. D., Kiran Patro, T. S. K. K., & Salomi Suneetha, D. R. (2016). Effect of sowing date on yield and quality of fenugreek. *Plant Archives*, 16(1), 479-484.
15. Ouzir, M., El Bairi, K., & Amzazi, S. (2016). Toxicological properties of fenugreek (*Trigonella foenum-graecum* L.). *Food and Chemical Toxicology*, 96, 145-154.
16. Bitarafan, Z., Asghari, H. R., Hasanloo, T., Gholami, A., Moradi, F., Khakimov, B., Liu, F., & Andreasen, C. (2019). The effect of charcoal on medicinal compounds of seeds of fenugreek (*Trigonella foenum-graecum* L.) exposed to drought stress. *Industrial Crops and Products*, 131, 323-329.
17. Salarbashi, D., Bazeli, J., & Fahmideh-Rad, E. (2019). Fenugreek seed gum: Biological properties, chemical modifications, and structural analysis—A review. *International Journal of Biological Macromolecules*, 138, 386-393.
18. Khan, M. K. I., Ghauri, Y. M., Alvi, T., Amin, U., Khan, M. I., Nazir, A., Saeed, F., Aadil, R. M., Nadeem, M. T., Babu, I., *et al.* (2022). Microwave-assisted drying and extraction technique; kinetic modeling, energy consumption, and influence on antioxidant compounds of fenugreek leaves. *Food Science and Technology*, 42, e56020. <https://doi.org/10.1590/fst.56020>

19. Akbari, S., Abdurahman, N. H., Yunus, R. M., Alara, O. R., & Abayomi, O. O. (2019). Extraction, characterization, and antioxidant activity of fenugreek (*Trigonella foenum-graecum*) seed oil. *Materials Science for Energy Technologies*, 2(2), 349-355.
20. Micky, B. M., Abbas, M. A., & Sameh, N. M. (2019). Morpho-physiological status of fenugreek seedlings under NaCl stress. *Journal of King Saud University-Science*, 31(4), 1276-1282.
21. Hasanuzzaman, M., Araújo, S., & Gill, S. S. (2020). *The plant family Fabaceae*. Springer Singapore.
22. Petropoulos, G. A. (Ed.). (2002). *Fenugreek: The genus Trigonella*. CRC Press.
23. Aljuhaimi, F., Simsek, S., Ozcan, M. M., Ghafoor, K., & Babiker, E. (2017). Effect of location on chemical properties, amino acid, and fatty acid compositions of fenugreek (*Trigonella foenum-graecum*) seed and oils. *Journal of Food Processing and Preservation*, e13569.
24. Nannar, A. R., Raosaheb, S. S., & Salunkhe, K. S. (2023). Review on weight loss management by herbal therapy. *Systematic Reviews in Pharmacy*, 14(5), 28-34.
25. Wani, S. A., Bishnoi, S., & Kumar, P. (2016). Ultrasound and microwave-assisted extraction of diosgenin from fenugreek seed and fenugreek-supplemented cookies. *Journal of Food Measurement and Characterization*, 10(3), 527-532. <https://doi.org/10.1007/s11694-016-9331-2>
26. Niknam, R., Kiani, H., Mousavi, Z. E., & Mousavi, M. (2021). Extraction, detection, and characterization of various chemical components of *Trigonella foenum-graecum* L. (*fenugreek*) known as a valuable seed in agriculture. In *Fenugreek* (pp. 189-217). Springer.
27. Riaz, S., Hafeez, M. A., & Maan, A. A. (2020). The fenugreek seed: Therapeutic properties and applications. *Science of Spices and Culinary Herbs-Latest Laboratory, Pre-Clinical, and Clinical Studies*, 2, 65-91.
28. Tavangar, A., Karami, L., Hedayat, M., & Gholamreza, A. B. D. I. (2021). Effect of salinity and drought stress on morphological and biochemical properties of two Iranian fenugreek (*Trigonella foenum-graecum*) populations. *Notulae Botanicae Horti*

29. Mohamadi, N., Pournamdari, M., Sharififar, F., & Ansari, M. (2020). Simultaneous spectrophotometric determination of trigonelline, diosgenin, and nicotinic acid in dosage forms prepared from fenugreek seed extract. *Iranian Journal of Pharmaceutical Research: IJPR*, 19(2), 153.
30. Kulkarni, A. T., Agarkar, B. S., Sawate, A. R., & Pawar, P. P. (2020). Determination of cooking quality of the composite flour noodles incorporated with chia seeds powder. *Journal of Pharmacognosy and Phytochemistry*, 9(5), 76–78.
31. Goyal, S., Gupta, N., & Chatterjee, S. (2016). Investigating the therapeutic potential of *Trigonella foenum-graecum* L. as our defense mechanism against several human diseases. *Journal of Toxicology*, 2016, 1250387. <https://doi.org/10.1155/2016/1250387>
32. Ghoshal, G., & Singh, M. (2022). Characterization of silver nanoparticles synthesized using fenugreek leaf extract and its antibacterial activity. *Materials Science for Energy Technologies*, 5, 22–29. <https://doi.org/10.1016/j.mset.2021.10.001>
33. Hiscox, J. D., & Israelstam, G. F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany*, 57(12), 1332–1334.
34. Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, 24(1), 1.
35. Sadasivam, S., & Manickam, A. (1992). *Biochemical methods* (p. 187). Wiley Eastern Limited.
36. Malhotra, S. S., & Sarkar, S. K. (1979). Effects of sulfur dioxide on sugar and free amino acid content of pine seedlings. *Physiologia Plantarum*, 47, 223–228.
37. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193, 265–275.
38. Bates, L. S., Waldren, R. P., & Teare, I. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39(1), 205–207. <https://doi.org/10.1007/BF00018060>
39. Diwakar, R., Shaikh, I., Dawda, H., & Mukundan, U. (2016). Phytochemical evaluation and quantitative estimation of corosolic and ursolic acid from *Psidium guajava*. *World Journal of Pharmaceutical Science and Technology*, 5(3), 1318–1327.
40. Chauhan, J., Singhal, R. K., Kakralya, B. L., Kumar, S., & Sodani, R. (2017). Evaluation of yield and yield attributes of fenugreek (*Trigonella foenum-graecum*) genotypes under drought conditions. *International Journal of Pure and Applied Biosciences*, 5(3), 477–484. <https://doi.org/10.18782/2320-7051.4019>

41. Senkal, C. B., Cesur, C., Uskutoğlu, T., Dogan, H., & Kose, F. (2018). Determination of yield performance of fenugreek (*Trigonella foenum-graecum* L.) and coriander (*Coriandrum sativum* L.) plants grown in Yozgat ecological conditions. In *Proceedings of the 3rd International Bozok Symposium: Regional Development and Socio-Cultural Structure* (pp. 33). Yozgat, May 3–5, 2018.
42. Panwar, A., Sharma, Y. K., Meena, R. S., Solanki, R. K., Aishwath, O. P., Singh, R., & Choudhary, S. (2018). Genetic variability, association studies, and genetic divergence in Indian fenugreek (*Trigonella foenum-graecum* L.) varieties. *Legume Research*, 41(6), 816–821.
43. Gürbüz, B., Gümüşçü, A., & İpek, A. (2000). The effects of spring and winter sowings on yield and yield components of some fenugreek (*Trigonella foenum-graecum* L.) lines. *Journal of Field Crops Central Research Institute*, 9(1–2), 99–106.
44. Jain, A., Singh, B., Solanki, R. K., Saxena, S. N., & Kakani, R. K. (2013). Genetic variability and character association in fenugreek (*Trigonella foenum-graecum* L.). *International Journal of Seed Spices*, 3(2), 22–28.
45. Jhajhra, M. R., Rana, D. K., & Ola, A. L. (2017). Evaluation of fenugreek (*Trigonella foenum-graecum* L.) varieties under sub-tropical conditions of Garhwal Himalayas. *Chemical Science Review Letters*, 6(22), 684–689.
46. Shakthi, P. N., Meena, K. C., Naruka, I. S., Halder, A., & Soni, N. (2020). Performance of fenugreek (*Trigonella foenum-graecum* L.) genotypes for yield and yield contributing traits. *International Journal of Seed Spices*, 10(1), 11–15.
47. Bhutia, K. C., Bhutia, S. O., Chatterjee, R., & Chattopadhyay, N. (2017). Growth, phenology, and yield of fenugreek (*Trigonella foenum-graecum* L.) as influenced by date of sowing. *International Journal of Current Microbiology and Applied Sciences*, 6(10), 1810–1817.
48. Duwal, A., Nepal, A., Luitel, S., Acharya, S., Pathak, R., Poudel, P. R., *et al.* (2019). Evaluation of coriander (*Coriandrum sativum* L.) varieties for growth and yield parameters. *Nepalese Journal of Agricultural Sciences*, 18, 36–46.
49. Latye, P. T., Bharad, S. G., Kale, V. S., Nandeshwar, V. N., & Kholia, A. (2016). Varietal performance of fenugreek under Akola conditions. *International Journal of Minor Fruits, Medicinal and Aromatic Plants*, 2(1), 32–34.
50. Aggarwal, B. B., Gupta, S. C., & Sung, B. (2013). Curcumin: An orally bioavailable blocker of TNF and other pro-inflammatory biomarkers. *British Journal of Pharmacology*, 169(8), 1672–1692.

51. Jeandet, P., Formela-Luboińska, M., Labudda, M., & Morkunas, I. (2022). The role of sugars in plant responses to stress and their regulatory function during development. *International Journal of Molecular Sciences*, 23(9). <https://doi.org/10.3390/IJMS23095161>
52. Wang, Y. H., Zhang, G., Chen, Y., Gao, J., Sun, Y. R., Sun, M. F., & Chen, J. P. (2019). Exogenous application of gibberellic acid and ascorbic acid improved the tolerance of okra seedlings to NaCl stress. *Acta Physiologiae Plantarum*, 41(6), 93.
53. Das, S., Goswami, M., Yadav, R. N. S., & Bandyopadhyay, T. (2022). Quantitative estimation of terpenoid content in some tea cultivars of northeast India and their in vitro cell cultures using an optimized spectrophotometric method. *Journal of Advanced Scientific Research*, 13(03), 112–117.
54. Rodríguez De Luna, S. L., Ramírez-Garza, R. E., & Serna Saldívar, S. O. (2020). Environmentally friendly methods for flavonoid extraction from plant material: Impact of their operating conditions on yield and antioxidant properties. *Scientific World Journal*, 2020. <https://doi.org/10.1155/2020/6792069>
55. Ghevariya, H. H., Vatukiya, V. N., Mistry, N. H., & Jain, N. K. (2023). Comparative evaluation of bioactive compounds and antioxidant properties of fenugreek (*Trigonella foenum-graecum* L.) seed, stem, leaf, and microgreens.
56. Bakhtiar, Z., Hassandokht, M., Naghavi, M. R., & Mirjalili, M. H. (2024). Variability in proximate composition, phytochemical traits and antioxidant properties of Iranian agro-ecotypic populations of fenugreek (*Trigonella foenum-graecum* L.). *Scientific Reports*, 14(1), 87.
57. Pal, D., & Mukherjee, S. (2020). Fenugreek (*Trigonella foenum*) seeds in health and nutrition. In *Nuts and seeds in health and disease prevention* (pp. 161-170). Academic Press.