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Article

Fenugreek (*Trigonella foenum-graecum* L.) Cultivation and Genetic Diversity Assessment in Ethiopia

Abukiya Getu [1,*] and Biruk Hirko [1]

[1]Ethiopian Institute of Agricultural Research, Tepi Agricultural Research Centre. P.O.Box 34, Teppi, Ethiopia

* Correspondence e-mail: birukhirko@gmail.com
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Abstract: Fenugreek is mainly used as a spice, food, and medicinal plant in different parts of the world and Ethiopia. There is a suitable agroecology for the production of fenugreek in the country, however, the production volume and productivity are very low compared to its potential due to a lack of improved optional varieties and production packages. From the total production in the country, more than 98% of the production volume is only from Oromia and Amahara regions (58.88% and 39.92%), respectively. Studies on the assessment of genetic variability of fenugreek in Ethiopia revealed the crop was highly variable for several traits which indicates the possibilities for genetic improvement of the crop through selection and cross-breeding. In Previous times there are a few numbers of improved varieties that were developed under seed spices, however recently the research attention given to seed spices is better than the previous, and different varieties were released and recommended for specific and wider agroecology cultivation. In the country, more than nine fenugreek varieties (Chala, Hunda'ol, Ebisa, Bishoftu, Burka, Jamma, Wereillu, Teru, and Chefe) were released for yield, fixed and essential oil, and seed quality from regional and federal research institute. However, variety improvement in Ethiopia was only through the selection of superior yielding and quality landraces. Therefore, developing variety through hybridization and mutation breeding for yield, organoleptic and nutraceutical properties is vital for future breeding work to exploit the potential of the crop in the country.

Keywords: Hybridization; utilization; variability; variety.

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1. Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is native to the Mediterranean's eastern shores, the crop is widely grown in India, Egypt, Ethiopia, Morocco, and, on rare occasions, England [6]. The cultivation and production of fenugreek in Ethiopia started long years ago and the crop is currently produced in a different part of the country as a major cash crop mainly in highland areas similar to those of other highland legumes such as faba bean, field pea, lentils, chickpea, and grass pea, etc. Fenugreek is used for different purposes in Ethiopia, including: as a food condiment, its flour is used to flavor traditional bread and keeps the soft texture of "tef-injera", as a rotation crop to enhance soil structure and fertility [11].

Knowledge of the scope and pattern of variability of genetic resources present in a population of a given crop is essential for further improvement. Ethiopia is rich in genetic resources fenugreek, however, the crop is still being cultivated by traditional methods of farming without optional improved varieties and production packages to diverse agroecology. To increase its large-scale production across different ecology of the country and development of better varieties with economically important traits of interest, the study of morphological/molecular diversity on available materials is very essential. Therefore, to best use the available genetic resources, unraveling the information on the magnitude and nature of genetic diversity of the population and the inter-relationships among traits that would help in formulating an efficient scheme of selection based on multiples of traits is of utmost importance.

To determine the extent and pattern of genetic diversity for morpho-physiological traits and associations between the geographic origins of the germplasm and genetic diversity and to establish such fundamental genetic facts as heritability and covariance of traits is of interest for further improvement of the crop [16]. In the country different regional and federal research institutes involved in the improvement of fenugreek and the nutraceutical property of the crop were studied. Accordingly, different research was undertaken on: genetic diversity assessment on available genetic resources, variety improvement (more than nine varieties were developed for specific and wider cultivation across the country), improved production packages developed for agronomic practices, and survey and identification of major disease pest were undertaken.

Fenugreek is mostly grown in Ethiopia in areas where the climate is similar to that of highland pulse crop produced area. In the country fenugreek, planting time fluctuates depending on rainfall distribution, accordingly the crop is planted at the end of the main rain season because the crop grows mainly by using residual soil moisture and as it doesn't tolerate heavy rainfall throughout the growing stages. Fenugreek best performs in well-drained black soil mainly the alluvial soil type which doesn't have a water logging problem. In addition, productivity is affected by the soil's ability to provide adequate moisture throughout the growing season [17]. The climate in these areas is primarily subtropical, with a wet season followed by a dry season. According to Simon *et al* [23]., fenugreek grows best in temperatures ranging from 8 to 27 degrees Celsius, with annual precipitation ranging from 400 to 1500 millimeters, and on rich, well-drained soils with a pH of 5.3 to 8.2. Cold temperatures and moist soils impede and weaken growth. Fenugreek, as a leguminous plant, requires little to no nitrogen fertilizer and can enhance soils with nitrogen. The mid-to-high plateaus (1800-2300 m.a.s.l.) of Ethiopia's fenugreek-growing regions are characterized by a subtropical environment with rainy and dry seasons [3].

The cultivation and production of fenugreek in Ethiopia are nearly similar to those of other cool-season food legumes. Fenugreek stands as number one in generating cash among pulse crops [3]. In the country, fenugreek is cultivated in different agroecology and regions. Amhara and Oromia regions are the major producer and contribute 98.80% of production.

2. Materials and Methods

In this work, the cultivation and genetic diversity assessment were searched using the database of different journals, and books. The data were updated more from recent published materials using google scholar, pub med and Scopus search engine in May 2022, using "hybridization, variability, variety, fenugreek and genetic diversity" as keywords for this review.

The paper also presents an up-to-date overview of chemical constituents and use of fenugreek in Ethiopian and world.

3. Results and Discussion

Table 1. Production status of Fenugreek in 2021 (2013 E.C) across regions and in Ethiopia.

Region	Production area in (ha)	Production (quintal)	Yield (quintal/ha)
Amahara	15,014.21	202,611.20	13.49
Oromia	26,596.97	298,804.38	11.23
Ethiopia	**42,344.28**	**507,472.35**	**11.98**

Amahara and Oromia regions covered 98.26% (41,611.18 ha) of the total production area and produce 98.80% (501,415.58 quintal/ha) from the production volume in 2020/21 or 2013 E.C cropping seasons. Fenugreek is also grown in small amounts in other regions (Tigray, Benishangul Gumuz, and South Nation and Nationality and Peoples (SNNP)). The productivity is high in the Amhara region (13.49 quintal/ha), 12.6% larger than the national average yield (11.98 quintal/ha). More than 419,222 farmers are involved in the production of fenugreek across the country.

3.1. Utilization of Fenugreek

3.1.1. Fenugreek as Spice Crop

The oil derived from fenugreek seeds accounts for about 68 percent of the seed weight and has a fetid odor and an unpleasant flavor. The oil's unsaponifiable fraction is reported to include a lactation-stimulating component (3.9 %). Because of its distinctive smell, the oil is used as an insect repellent for cereals and clothing. Perfumes and cosmetics include traces of oil [8]. Fenugreek has been used to flavor or adds taste and aroma to a variety of dishes in India, Egypt, and North Africa. During non-fasting periods, the seeds needed to prepare Ethiopia's traditional sauce and in "wot," by roasting, crushing, and flouring. Fenugreek flour is also widely used to flavor "injera" (a thin pancake-like bread made of fenugreek).

3.1.2. Fenugreek as Food Crop

Supplementing wheat flour with a modest percentage of fenugreek flour has been shown to improve both the nutritional and organoleptic qualities of bread in Egypt [25], [9] and also enhanced physicochemical, nutritional, and rheological qualities [21]. In meals like soups, sauces, and ice cream, galactomannan (mucilage or gum) in fenugreek works as a thickening or stabilizer [20].

Fenugreek is consumed by nursing moms in Ethiopia, who ingest increased quantities of pulses to preserve breast milk production. Breakfast snacks include sprouted fenugreek seeds sweetened with sugar or honey. Fenugreek seeds are mixed into the flour, boiled, and sweetened with sugar or honey before being fed to babies aged 4-6 months. Because of religious, cultural, or economic causes, meatless meals are observed for extended periods in India, Ethiopia, and Turkey. Because of its high protein content, fenugreek aids in bridging the nutritional gap created by vegetarian diets [1].

3.1.3. Fenugreek as Medicinal Plant

Al-Habori and Raman [1], state that fenugreek has anti-diabetic, anti-fertility, anti-cancer, anti-microbial, anti-parasitic, and hypocholesterolemic properties, as well as a protective effect against ethanol toxicity [26]. Fenugreek includes three medicinally significant chemical constituents: steroidal sapogenins, galactomannans, and isoleucine. These properties have made fenugreek one of the most well-known "nutraceutical" or health-food items [24].

3.1.4. Genetic Diversity of fenugreek

The occurrence of differences among individuals or groups of individuals due to differences in their genetic composition and/or the environment in which they are grown leads to variation and/or genetic divergence. The difference in character expression of two individuals grown in an identical environment could result in genetic variation. Genetic variability is the primary interest in plant improvement because proper management of this diversity can produce a permanent gain in the performance of the character of interest [2]. Estimation of genetic variability is important for the improvement of any crop, but limited previous studies addressed fenugreek importance, applications, and genetic diversity among fenugreek genotypes.

3.1.5. Studies on Genetic Variability of Fenugreek in Ethiopia

A currently different study was conducted on the morphological variability of fenugreek by different scholars. Asebe *et al*. [27], reported on the studies of genetic variability of 36 fenugreek accessions which is collected for quantitative traits: included flowering days, maturity days, Plant height at maturity, number of primary branches, number of secondary branches, Pods number per plant, seeds number per pod, thousand seeds weight, Biological yield per plot and seed yield per plot. Cluster analyses indicated that germplasms collected from the same geographic collection region fell into different cluster groups whereas those collected from different geographic regions tended to be grouped in the same cluster. However, the analysis suggested that there was considerable diversity among the germplasms scoping on improvement through hybridization and selection by crossing germplasms from different clusters.

Million *et al*. [16] evaluated the genetic variability of 143 different fenugreek accessions as well as one commercial variety (Challa). Accordingly, thousand seed weight, number of pods per plant, plant height, number of seeds per plant, number of seeds per pod, seed color, seed shape, seed yield/plant, biomass yield per plot and per plant, and harvest index. The fenugreek accessions were highly variable for several traits, including phenology and yield components. The results obtained in the study indicated single plant or pure line selection for the number of seeds per plant and thousand seed weight may be effective for the improvement of seed yield in fenugreek. As a breeding strategy, recurrent or family selection will be employed for the improvement of traits that have low heritability and genetic advance. The variations among high-performing accessions could provide a basis for a genetically diverse breeding program and provide diversity. Crossing these accessions in a breeding program should result in segregating populations. Therefore, there is a high chance of genetic improvement and of increasing the level of desirable traits in new accessions.

Miheretu Fufa [15], conduct a study on yield and yield-related trait variability analysis on 46 fenugreek accessions at Sinana Agricultural Research Center in 2017. The number of pods per plant, number of pods per plant, and plant height were all highly variable among the accessions. In terms of variability, the accessions had minimal variability for the number of seeds per pod, the number of secondary branches per plant, the seed length, and the number of primary branches per plant. As fenugreek is well distributed in various climatic conditions geographically, it is supposed to have a wide genotypic variability. A similar result was reported by Yimam Ali [4] that seed yield per plot, biomass yield per plot, and harvesting indexes were the first and the most important contributing traits sharing 46% for the total variation in Principal component analysis. The sixty-four fenugreek genotypes were grouped into eight clusters. Genotypes from different locations were grouped and indicated that clustering was based on genetic divergence rather than geographical diversity. Genetic pedigree and selection forces under different environments could cause greater diversity rather than geographical diversity

3.1.6. Breeding Priority in Fenugreek

Fenugreek is grown for multiple uses and breeding programs need to be concerned with the suitability of the product according to its existing uses, such as high diosgenin content of seed for steroidal industry, high protein content for human and animal feeding, high mucilage (galactomannan) content with the appropriate ratio of galactose to mannose for industrial uses and as the case may be for fixed oils, aromatic and spicy substances, as well as pharmaceutical constituents, etc. The ratio of galactose to mannose of the reserve galactomannan of the seed possesses a relative chemotaxonomical value as it varies among the different plant genera of *Leguminosae.* Since fenugreek is a self-pollinated crop, a mutation breeding method can be used to generate mutants with a determinate growth habit.

3.1.7. Breeding Methods for Improvement of Fenugreek

Three methods namely selection, hybridization, and mutation used separately or in combination can be employed for developing improved varieties of fenugreek [17]. Selection is more suitable for the improvement of fenugreek, which possesses a diploid genetic structure, as Busbice *et al.* [5], concluded that under comparable assumptions the response to selection would be more rapid in diploid populations. Hybridization, on the other hand, is a complex and time-consuming process and usually, hundreds of crosses must be made before an individual is found that possesses the combination of characteristics desired [17]. Intra-species hybridization has been used with successful crossing techniques reported by Cornish et al. [10], and Jatasra and Lodhi [12]. Fenugreek readily self-pollinates, and thus the timing of emasculation before pollination is critical [10].

Several mutants of fenugreek from spontaneous mutation (SM) have been isolated and are today in use all over the world [18]. To induce mutation in fenugreek, two methods were applied: gamma-irradiation of isotope Cobalt-60 as chronic rays in an open irradiated field, and acute rays on the dry seeds [14]. Mutation, induced by chemicals or by radiation, has been used to increase the genetic variation in fenugreek in India [19]. Only selection breeding methods are used in Ethiopia for fenugreek improvement from available landraces no other breeding methods have so far reported, hence the breeding system in fenugreek improvement for yield, quality, and medicinal value shift from classical to conventional and modern breeding techniques to exploit the potential of the crop in the country.

3.2. Selection

Selection is considered to be one of the most important methods available for the improvement of diploid species [9]. Petropoulos [17], reported that a "solitary pod" phenotype in fenugreek is dominant to a "twin pod" phenotype and that plants with narrow pods, containing large and rectangular seeds are dominant to phenotypes with wide pods, which contain small and round seeds. Knowledge about dominant and recessively inherited traits is important as it would have a direct impact on the behavior of the progeny of selected plants.

A recessively inherited trait will be fixed in one generation, as was seen by Raghuvanshi and Singh [7] while working on the double pod trait in fenugreek. A trait governed by a dominant gene, on the other hand, can take several generations to fix. Fenugreek accessions from the world collection exhibit extensive phenotypic variability; this variability has a genetic basis, so selection for improved levels of chemical constituents and nutraceutical applications is possible (Acharya et al. 2004b). Raghuvanshi and Singh [7], obtained high heritability estimates in fenugreek when they selected a double pod trait. The double pod trait is known to be genetically linked to diosgenin content and higher seed yields [17].

3.3. Hybridization Techniques

Hybridization involves crossing two or more varieties of genetically different individuals. Common methods of hybridization can involve a 2-parent cross, a 3-parent cross, a 4- parent cross, a back cross, or a complex cross. Emasculation and manual pollination have been used effectively for crossing different lines of fenugreek [17]. Petropoulos [14], suggested that the fenugreek flower should be emasculated at the end of the first floral developmental stage to completely avoid the chances of selfing. Soon after manually pollinating the flowers, a bag should be placed over the fenugreek flowers to avoid any chances of unrestricted outcrossing [10].

For fenugreek improvement, aneuploid chromosome transfer, chromosome addition and substitution, and gene transfer by translocation produced by mutagenesis have all been tried. The basic chemical composition of fenugreek seeds is determined by analyzing their proximate composition. Fenugreek aqueous extract includes active compounds that may be useful as a dietary spice and in the treatment of diseases. This backs up the plant's historic use as a food supplement and in disease control.

3.4. Mutation Breeding

Plant breeding, according to Petropoulos [17], is "managed evolution," and mutation, in addition to selection and recombination, is the most essential means of attaining it. Over the last few decades, mutant breeding has grown in popularity, and interest in it as a crop enhancement method has increased [22]. Soybeans, string beans, French beans, Navy pea beans, haricot beans, peas, and lupines are among the legume crops that have been developed through mutation breeding. Sigurbjornsson and Micke [13], reported the diosgenin content in fenugreek species like *T. corniculata* can be increased through mutation breeding. Mutation breeding is significant when a desirable character is not present in the germplasm that would ordinarily be used as a source for hybridization.

In the greenhouse at Lethirbridge Research Center (LRC), a study was started using Tristar as a base population to look for mutants with desirable and beneficial phonological traits such as determinate growth habits and/or high seed yield. The mutagenic agent was ethyl methane sulfonate EMS, which was utilized at doses of 10, 20, 30, 40, 50, 100, 150, 200, and 300 m. Before applying varied concentrations of EMS, the seeds were pre-soaked in water for 2, 4, 6, 8, 12, 16, and 24 hours. M1 plants were created by planting a treated seed in individual pots using a soil-free mix. After 85 days in a greenhouse with 16-hour days (22 °C) and 8-hour nights (15 °C), the plants were dried with 0.4 percent Reglone solution. The plants were then allowed to dry for 10 d before separating the seed for yield determination. Seeds from selected M1 plants were again seeded in pots and allowed to produce M2 seeds.

3.5. Breeding for Quality Trait

As examples of such an aspect, Lee [13], reported that when 10 genotypes originated from different agro-ecological locations in the world (Afghanistan, India, Iran, Pakistan, and Turkey) were tested under 14 different growing environments (2 years in a total of 14 locations), the contributions of the genotype x environment to the total variation were: 78% vs. 6% for diosgenin content, 11% vs. 70% for galactomannan content, and 7% vs. 78% vs. 6% for 4- hydroxy isoleucine content. These constituents varied from 0.5-0.81, 14.6-17.6, and 0.85-0.99% for diosgenin, galactomannan, and hydroxy isoleucine, respectively. Taylor et al. [25], discovered significant genotype, genotype x year, and year x location interactions effects on diosgenin content in ten accessions from Iran, Ethiopia, Greece, Pakistan, Afghanistan, Spain, Morocco, and Canada in three locations in western Canada over two years. Other proofs of the presence of genotype x environment interaction for many fenugreek seed traits like sapogenin content, seed yield, and flavone content have also been reported. Such high variability in chemical composition resulting in inconsistent results of clinical trials improved fenugreek medical quality possible through suitable breeding programs on low-quality varieties or accessions.

3.6. Improved Variety of Fenugreek in Ethiopia

Only three varieties (Chala, Hund'ol, and Ebbisa) were released in 2016 from regional and federal research institutes (Sinana Agricultural Research Center and Debrezeit Agricultural Research Center) for specific and wider cultivation. Nowadays more than seven varieties (Chala, Hundaol, Ebisa, Burka, Jamma, Wera illu, and Teru) were released for yield potential and oleoresin content, and two varieties (Bishoftu and Chefe) were released for seed quality (white/cream color and black seeded).

Table 2. Released a variety of Fenugreek and their productivity.

	Production quintal/ha				
Variety	**Research field**	**Farmers field**	**Year of Release**	**Releasing Center**	**Breeding technique**
Chala	9-18	8-15	2005	Debrezeit Agricultural Research Center	Selection
Hundaol	12-22	6-8	2006	Sinana Agricultural Research Center	Selection
Ebbisa	13.8		2012	Sinana Agricultural Research Center	Selection
Burka	22.21		2016	Sinana Agricultural Research Center	Selection
Wereillu	11-21	12	2016	Sirinka Agricultural Research Center	Selection
Jamma	12-23	11	2016	Sirinka Agricultural Research Center	Selection
Bishoftu	8-12		2017	Teppi Agricultural Research Center and Debrezeit Agricultural Research Center	Selection

4. Conclusion

Fenugreek is one of the popular multi-purpose crops produced in different parts of the country. Study on genetic variability is the principal concern to the plant improvement to exploit the potential of the crop in the country. So far several studies was conducted on the genetic variability of fenugreek to exploit the potential of the crop in the country. A study on the assessment of genetic variability of fenugreek accession indicated there is a high variability exists among collected accession across different parts of the country for different agronomic and other important traits which is a promising indicator of the crop for further improvement. Even if there is a different breeding technique used for fenugreek improvement only the selective breeding technique is used in the country to develop a variety.

Conflicts of Interest: The authors declare no conflict of interest

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