Chapter 12

Fenugreek (*Trigonella foenum-graecum* L.): An Important Medicinal and Aromatic Crop

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Abstract

Fenugreek (*Trigonella foenum-graecum* L.) is an annual forage legume and a traditional spice and aromatic crop that has been grown for centuries across the Indian subcontinent. In addition to South Asia, the crop is also grown in some parts of North Africa, Middle East, Mediterranean Europe, China, South East (SE) Asia, Australia, the USA, Argentina and Canada. The plant has been used traditionally in Indian Ayurvedic medicines as well as in traditional Tibetan and Chinese medication for several centuries. Modern research has also demonstrated that fenugreek seed and leaves are useful in the treatment of a number of diseases including successfully reducing blood sugar and blood cholesterol levels in both animals and humans. The plant has recently attracted great interest in the pharmaceutical, nutraceutical and functional food industries due to its rich medicinal properties.

Keywords: fenugreek, *Trigonella foenum-graecum*, spice, medicinal, aromatic, legume, crop

1. Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual forage legume and a traditional spice crop that has been grown for centuries across the Indian subcontinent [1–3]. In addition to South Asia the crop is also grown in some parts of North Africa, Middle East, Mediterranean Europe, China, South East (SE) Asia, Australia, the USA, Argentina and Canada. India is the largest fenugreek producer in the world but due to high internal consumption do not have a major share of the global fenugreek trade [1–3]. The crop has been recommended for the dry and semiarid regions of Asia, Africa and Latin America [4]. The plant has been used traditionally in Indian Ayurvedic medicines as well as in traditional Tibetan and Chinese medication for several centuries. Modern research has also demonstrated that fenugreek seed and leaves are useful in the treatment of a number of diseases including successfully reducing blood sugar and blood cholesterol levels in both animals and human subjects in experimental trials [5]. The crop has the potential to act as a panacea in treatment of diabetic, microbial and cancer disease. The reason behind the rich medicinal properties of fenugreek is due to the presence of a wide diversity of important phytochemicals (diosgenin, trigonelline, fenugreekine, galactomannan and 4-hydroxy isoleucine [4]. Hence, the crop has huge international demand in the associated pharmaceutical, nutraceutical and functional food industries. Being known as a chemurgic crop, fenugreek has a widespread adoption in industrial sectors. Its seeds contain a reliable source of steroid diosgenin, which acts as a supplement in pharmaceutical industry [6].

Furthermore, being a forage legume and a natural nitrogen fixer, it could be easily incorporated in the local crop cycles (short-term rotation) for natural replenishment of soil, for fixation of nitrogen and for feeding the livestock as hay or silage (**Figure 1**). The crop grows well under rainfed conditions and hence cost of production is lower compared to other commercial crops suitable for semiarid regions.

Fenugreek is also well known as a global spice crop grown in all the major continents (depending on soil and climatic conditions) across the globe including parts of North Africa, Mediterranean Europe, Russia, Middle East, China, India, Pakistan, Iran, Afghanistan, parts of Far East and SE Asia, Australia, the USA, Canada and Argentina [7, 8]. India once maintained and still holds the largest fenugreek harvested area in the world [5].

Figure 1. Fenugreek crop in different growth stages (fenugreek seed, seed germination, seedling growth, vegetative-immature and reproductive growth stages of stem formation, podding and physiological ripeness-mature (Image credit: S.K. Basu).

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The crop has been recommended for agricultural production in the dry and semiarid localities of the continents of Asia, Africa and Latin America [3 4, 9, 10]. The plant has been used extensively for centuries as a traditional forage crop in several ancient civilizations across Eurasia. Fenugreek has been reported to be used as an important medicinal herb in Indian Ayurvedic medicinal practices as well in traditional Chinese medication and Tibetan medicines for the treatment of several diseases in humans and also in animals. Ancient Islamic scholars and physicians have also recorded the use of fenugreek in traditional Islamic medicinal practices in ancient texts and scriptures [9]. Modern clinical trials have also established without doubt the efficacy of this medicinal herb in the treatment of several human and animal diseases [5, 8, 11]. Relative frequencies of the major well-known accessions of fenugreek which are produced all over the world are listed out in **Table 1** [1].

Origin	Number of reported fenugreek accessions	*Relative* frequency (%)	Average forage production 1961–2013 (tone/acre) per country
Afghanistan	27	2.48	>550,682
Algeria	2	0.18	3,835,860
Australia	7	0.64	2,868,759
Austria	1	0.09	1,539,297
Azerbaijan	1	0.09	624,045
Canada	54	4.95	>550,682
China	44	4.04	51,489,483
Egypt	16	1.47	>550,682
England	17	1.56	>550,682
Eritrea	1	0.09	>550,682
Ethiopia	152	13.94	>550,682
France	3	0.28	>550,682
Germany	4	0.37	7,445,345
Greece	6	0.55	>550,682
Hungary	3	0.28	>550,682
India	401	36.79	91,881,132
Iran	46	4.22	2,653,431
Iraq	7	0.64	230,685
Israel	3	0.28	>550,682
Italy	4	0.37	9,906,660
Jordan	5	0.46	30,439
Kenya	1	0.09	>550,682
Libya	5	0.46	573,678
Morocco	5	0.46	1,694,057
Nepal	2	0.18	>550,682

Origin	Number of reported fenugreek accessions	*Relative* frequency (%)	Average forage production 1961–2013 (tone/acre) per country
Oman	77	7.06	>550,682
Pakistan	41	3.76	13,819
Poland	2	0.18	7,142,332
Portugal	1	0.09	6,894,828
Romania	15	1.38	694,961
Russia	3	0.28	10,701,727
Slovenia	4	0.37	86,966
South Africa	1	0.09	>550,682
Spain	8	0.73	163,784
Sudan	10	0.92	1,187,407
Sweden	3	0.28	>550,682
Switzerland	2	0.18	3,218,208
Syria	15	1.38	33,113
Taiwan	1	0.09	>550,682
Tunisia	42	3.85	>550,682
Turkey	40	3.67	>550,682
Turkmenistan	1	0.09	>550,682
U.S.A.	3	0.28	>550,682
Ukraine	1	0.09	475,316
Yemen	3	0.28	92,026

Table 1. Origin, number of registered and relative frequency of fenugreek crop distributed across the given countries.

2. Medicinal properties and chemical constituents

Fenugreek leaves and seed are known to have major medicinal properties and have been reported to significantly reduce both blood glucose and cholesterol levels in human and animal subjects in clinical trials around the world [1]. Fenugreek is therefore highly sought after as a chemurgic crop in the local, regional and international pharmaceutical, nutraceutical and functional food industries and markets as a medicinal herb [12]. Fenugreek seed and leaves are a rich source of a wide diversity of medicinally rich phytochemicals like steroidal saponins (diosgenin), fenugreekine (alkaloid), galactomannan (carbohydrate), 4-hydroxy isoleucine (amino acid) among several others [4, 7, 11, 12]. More specifically, fenugreek seed itself contain carbohydrates (45–60%) as in mucilaginous fiber (galactomannans), proteins (20–30%) enriched in tryptophan and lysine, lipids (5–10%) or fixed oil, alkaloids of pyridine type (0.2–0.38%) as in trigonelline; choline (0.5%), and other materials including carpaine and gentianine, flavonoids (apigenin, luteolin, orientin, quercetin, vitexin and isovitexin) and 4-hydroxyisoleucine (0.09%), lysine and histidine, arginine, calcium and iron, saponins

(0.6–1.7%), glycosides such as, yamogenin, tigogenin, neotigogenin and diosgenin (generating steroidal sapogenins on hydrolysis); and sitosterol and cholesterol, vitamins (A, B1, C) and nicotinic acid; n-alkanes and sesquiterpenes (0.015%) known as volatile oils [6]. Fenugreek has been also reported to be rich in antioxidant [13] and antimicrobial properties [14].

3. Agronomy

Agronomic production of fenugreek crop has been well studied and reported in arid and semiarid regions of the world and has been well documented in primary literature [15–17]. Climatic and edaphic environmental (external condition) factors as well as genetic makeup (internal condition) are greatly accounted for metabolic processes in fenugreek crop [18]. It is also believed that the regulation of yield potential in fenugreek is feasible through either breeding programs or modification of cultural treatments [18, 19]. Fenugreek crop growth has been found to be significantly increased by the application of phosphate fertilizer [20]. The plant has indeterminate growth habit and hence mutant population generated through physical and chemical mutating agents have been reported to be successful in generating plants with determinate and fast growing habits [11, 21]. The crop has been found to be attacked by several biological agents like insects, fungi, bacteria and non-biological diseases like micronutrient deficiency, flooding, salinity, stagnant water [22–24].

4. Species, names, origin and distribution

There are noticeable discrepancies in the range of reported species of fenugreek (around 70–97) in the literature [25–29]; however, older taxonomies like Linnaeus have explicitly accentuated on the existence of 260 species [1]. Across the mentioned species of fenugreek, the following are mostly celebrated as for their medicinal and pharmaceutical properties [1]: *T. foenum-graecum, T. balansae, T. corniculata, T. maritima, T. spicata, T. occulta, T. polycerata, T. calliceras, T. cretica, T. caerulea, T. lilacina, T. radiata, T. spinosa*. Among which *T. foenum-graecum* is widely cultivated throughout the world [30]. The genus name, *Trigonella* meaning 'little triangle' resemble the triangular shape of its small yellowish-white flowers. The species name *foenum-graecum* meaning 'Greek hay' in reference to its initial introgression from Greece [1]. To date different indigenous names have been ascribing to the plant depending on the nations, local language and culture on which the crop is grown and/or consumed. For instance, fenugreek in Arabic is called Hulba; in Persian called Shanbalilae; in Greek called Tili, Tipilina, Trigoniskos, Tintelis, Tsimeni and Moschositaro; in Uzbekistani called Boidana, Ul'ba and Khul'ba; in Armenian called Shambala; in Chinese called K'u-Tou; in Ethiopian called Abish; in Japanese called Koroba; in England called fenugreek or Fenigrec; in Pakistani and Indian called Methi; in Italian called Fieno Greco; in Russian called Pazhitnik; and in French called Senegre [30, 31].

Fenugreek is an ancient and multipurpose crop in various geographical latitudes. Species of fenugreek have been identified in the five continents of Asia, Africa, Europa and Australia; being cultivated mostly in North America, West and South Asia, Australia, Russia, Meddle East, North West of Africa. Potential areas for fenugreek production are parts of South East Asia, Japan, Central Asia (Mongolia), wide parts of Africa and South America (**Figure 2**).

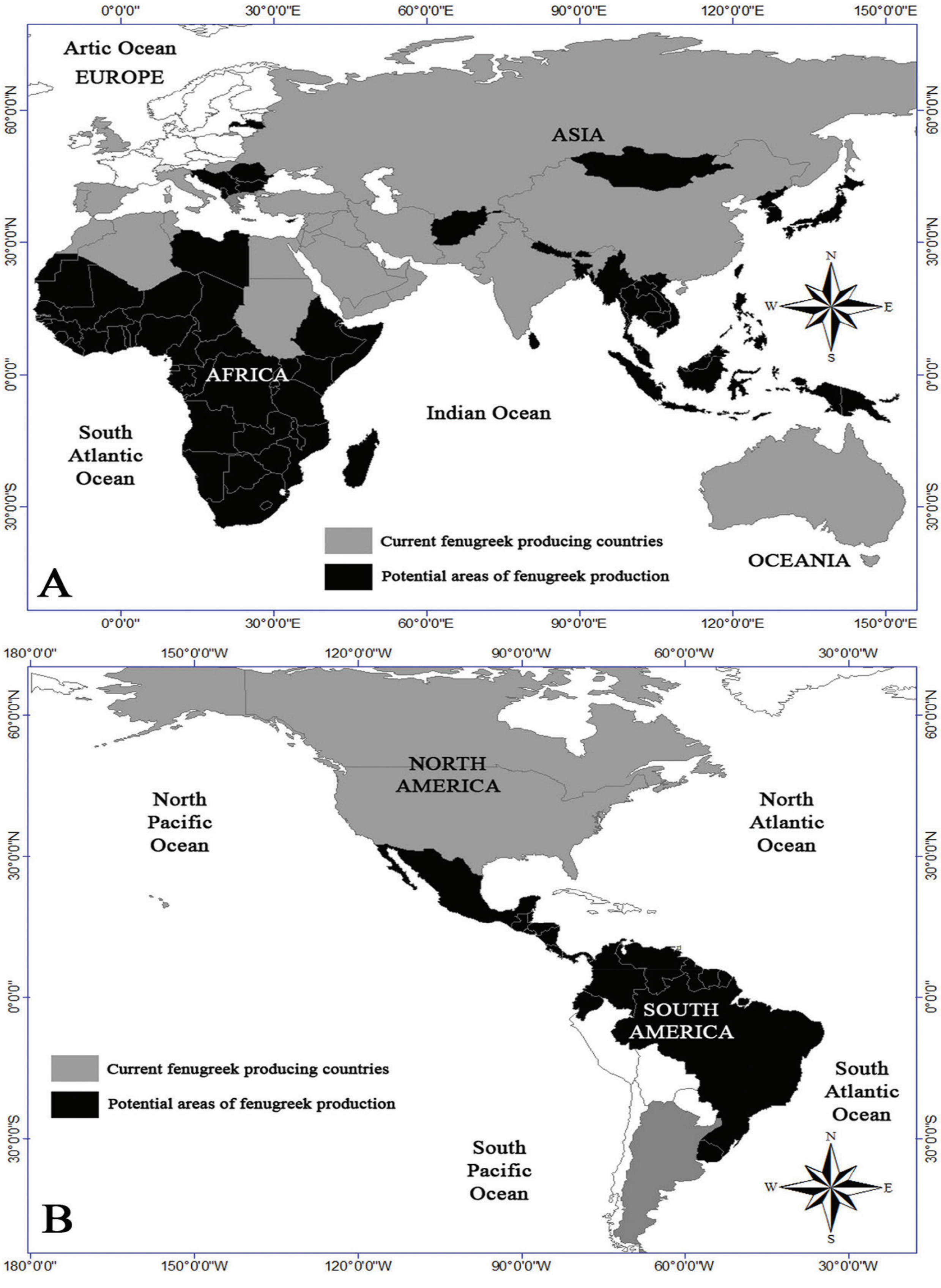

Figure 2. Illustrative map of word (A, B) showing currently grown and potential areas of fenugreek production.

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There are widespread uncertainties regarding the probable ancestry of *T. foenum-graecum* which are still unsolved. Though Vavilov [32] introduced the herb as a native species to the Mediterranean region, on a contradictory statement De Candolle [33] and Fazli and Hardman [34] suggested an Asian root for it. They also declared that fenugreek wildly grows in deserts of Mesopotamia, prolific plains of Persia, in Punjab and Kashmir of Pakistan, in middle Asia, and in southern Europe like Greece, Italy and Spain that take advantage of abundant sunshine. As described earlier by De Candolle [33], it is not a reasonable belief to consider southern Europe as the main origin of fenugreek because if this was the case the plant should be far more common not be remaining inconspicuous in this region.

Many experts unanimously agree that the direct wild ancestor of fenugreek is *T. gladiata* Ste. that differs widely from *T. foenum–graecum* in view of the assemblage of attributes like smaller pod size and abnormal seed tuberculation [35]. So it is likely acceptable to believe that the species T. *foenum graecum* was naturally evolved from *T. gladiata* as it had possibly contributed to prevent the extinction of *T. foenum-graecum* [30].

5. Chromosome number

There are many species in the *Trigonella* genus, many of which are diploid. Based on the Darlington and Wylie [36] reports, haploid chromosome numbers of most species in the *Trigonella* genus is 8, 9, 11 or 14 and diploid chromosome number is $2n = 16$. For example, *T. foenum-graecum, T. balansae, T. corniculata, T. sprunerana* Boiss., *T. monspeliaca* L., *T. uncata, T. anguina, T. stellata, T. astroites, T. gladiata, T. cariensis, T. berythea, T. macrorrhyncha, T. cassia, T. foenum-graecum* and *T. homosa* have $2n = 16$ chromosomes [36–41]. However, there are some exceptions. For instance, *T. geminiflora* from Iran and Asia Minor, *T. grandiflora* from Turkey and *T. hamosa* from Egypt have 44 chromosomes; *T. polycerata* from the Mediterranean and Asia have 28, 30 and 32 chromosomes, and *T. ornithopodioides* from Europe was reported to have 18 chromosomes within its genome [30]. While most species in the *Trigonella* genus have $2n = 16$ chromosomes, the results of some studies show that some of species have undergone several rounds of chromosome duplication and have different diploid number of chromosomes. Singh and Singh [42] found some trisomics along with five double trisomics in an auto-tetraploid population which had 18 ($2n + 1 + 1$) chromosomes. They believed that among 13 species of *Trigonella*, only *T. neoana*, have $2n = 30$ chromosomes. Marc and Capraru [43] studied cytogenetic effects of sodium phosphate on meristematic cells of fenugreek root tips and found that it has a negative effect on the mitotic index. Roy and Singh [44] produced tetraploid fenugreek by treating shoot apices with colchicine and Basu [1] also reported that he had produced tetraploid fenugreek ($2n + 2n = 32$) by treating seeds with colchicine.

There are few studies about the variation in chromosome number in fenugreek. For instance, Raghuvanshi and Joshi [45] and Joshi and Raghuvanshi [46] reported that there is an extra B chromosome in some of the fenugreek genotypes. As far as we know, the presence of this type of chromosome can affect plant growth and development [30]. In some researches, it is observed that same species show different behaviors in terms of having B chromosome. For example, some *T. corniculata* species do not contain B chromosome, while the other species have this chromosome. For instance, Singh [47] and Singh and Singh [42] examined *T. corniculata* in some regions of India and did not observe B chromosomes in it, while Lakshmi et al. [48] reported that *T. corniculata* contained two types of pollen mother cells: one with $2n = 16$ and the other with $2n = 16+$ B chromosomes.

6. Molecular genetic diversity

Knowledge of genetic diversity among plants can help to provide beneficial information in the selection of breeding materials for hybridization programs and mapping quantitative trait loci [49]. Review of literatures show that using DNA markers for investigating the genetic diversity of fenugreek does not have a long history in the world. Dangi et al. [50] studied the genetic diversity of two different species of fenugreek (*T. caerulea* and *T. foenum-graecum*) using Random Amplification of Polymorphic DNA' (RAPD) and Inter Simple -Sequence Repeats (ISSR) markers and showed that the genetic diversity in *T. caerulea* was much more than the other species. They also recommended the using of these two methods for grouping the genotypes and determine the genetic relationship among them.

Sundaram and Purwar [51] evaluated genetic diversity and species relation among two taxonomically *Trigonella* species and 61 accession using 18 RAPD primers. These primers made a total of 141 bands of which 74 were polymorphic. Genetic similarity of the genotypes ranged between 0.66 and 0.90, indicating a moderate to high genetic diversity among the populations. The dendrogram obtained from RAPD primers revealed two main clusters. Each cluster had two separate subgroups. This investigation showed that RAPD marker is a useful tool for the evaluation of genetic diversity and relationship among different *Trigonella* species.

Kumar et al. [52] investigated the genetic diversity of five common fenugreek varieties of India using nine RAPD and seven fluorescently labeled amplified fragment length polymorphism (AFLP) primers. These RAPD primers produced a total of 47 bands in the size range of 200–5000 bp with an average polymorphism of 62.4%. AFLP marker produced a total of 669 bands in the size range of 50–538 bp. The results revealed that RAPD markers were more polymorphic than AFLP markers where the reproducibility of AFLP markers was more than RAPD markers.

Ahari et al. [53] assessed the genetic diversity among and within 20 Iranian fenugreek landraces using AFLP markers. Five AFLP primers combinations used in this study produced a total of 147 bands within the molecular weights ranging from 50 to 500 base pairs of which 87% were polymorphic. The results of polymorphism information content (PIC) showed that there was a high polymorphism existed among Kashan (0.79), Broojerd and Kashan (to 0.93) landraces, which shows the moderate and high genetic diversity among these populations. These results demonstrated high efficiency of AFLP markers for investigation the genetic diversity among Iranian fenugreek populations.

Haliem and Al-Huqail [54] investigated the correlation between biochemical characteristics such as acid phosphatase, and glutamate-oxaloacetate transaminase isozymes, and amino acid composition and molecular variations of seven wild *T. foenum-graecum* L.

accessions using RAPD markers. The molecular analysis revealed that RAPD markers were highly polymorphic (94.12%) and can be used in the differentiation of the genotypes effectively.

Al-Maamari et al. [55] investigated the genetic relationship of 20 Omani fenugreek accessions and compare their relationship with four accessions from Iraq and Pakistan using 6 AFLP primer combinations. A total of 1852 polymorphic loci were produced from these combinations. A high level of genetic diversity (H) was found in Omani populations (0.2146) compared to Pakistani (0.0844) and Iraqi (0.1620) populations. They concluded that the average level of genetic variation among fenugreek populations shows their long history of cultivation and frequent exchange of fenugreek genetic material among regions in Oman.

Hora et al. [56] studied the diversity and phylogenetic relationships of different varieties of fenugreek (eight varieties and six populations) collected from northern India using RAPD and ISSR markers. The high similarity coefficient values suggested a diverse genetic diversity in fenugreek populations in India. They concluded that these two molecular markers (RAPD and ISSR) can be used effectively to evaluate genetic diversity and assess genetic relationship.

7. Mutation breeding

Fenugreek becomes more important economically, agronomically and environmentally, day-to-day all over the world. In recent years, revealing the nutritional and medicinal value of fenugreek, its low soil expectations, and a relatively broad adaptation to the different regions, the scope of its cultivation spread from America to India [7, 30, 57]. For example, this plant has been called as a new species in Canada. There are few fenugreek genotypes that are adapted to the climatic conditions of western Canada. In such cases, mutation breeding can be used to generate new genetic variation in an existing gene pool for a certain trait [58]. Such a mutation breeding can be used for a large number of alleles at the same time to correct a particular trait [59]. Colloquially, mutations called every change in the DNA sequence which ultimately leads to a change in the individual's genotype. Gene mutation is a good affair in plant breeding, because it facilitates the selection [60]. Up to now, mutation breeding has created dramatic changes in the species of legume crops [61–63]. For instance, Mahna et al. [64] used mutation breeding to increase the diosgenin content in *T. corniculata* (a close relative of fenugreek). There are a variety of mutagens (chemicals or irradiation) to make mutations in plants.

According to the researches, it can be concluded that most of the mutations are recessive, can be observed to segregate in a 3:1 ratio in diploid crops like fenugreek [35, 42, 65], and for observation of such mutations, we should wait until the second generations [66]. Vice versa, dominant mutations are rare and can be observed in the first generations [65]. Since fenugreek is self-pollinated and the determinate trait is governed by recessive genes [67], mutation breeding can be used to generate mutant plants with a determinate growth habit without losing beneficial adaptations and other agronomic traits in the base population [68].

Application of mutation breeding in fenugreek is expressed in several studies. There are two major types of mutation: spontaneous and induced. Some varieties of fenugreek have been created through spontaneous mutations [35, 42, 69–70]. RH 3129 variety is produced from spontaneous mutation in a Moroccan cultivar and had high level of diosgenin content and twin pods [35, 69, 71]. In creating new varieties of fenugreek, the effect of induced mutations should not be ignored. RH 3112 cultivar with higher diosgenin content and seed yield and RH 3118 cultivar with higher protein content are two main cultivars which are made by induced mutations [35, 42, 69, 70]. Chemical mutation is also important in the production of new varieties of fenugreek. Basu [1] by inducing the seed of Tristar variety using Ethyl Methane Sulfonate (EMS), produced new population with higher height, seed yield, seed number per pod, biomass yield, total number of pods and number of twin pods.

Also, the results show that the impact of chemical mutation is much more than physical one [18, 68–70, 72–74]. Among chemical mutagens, it is observed that EMS can induce mutation successfully in the fenugreek [35, 69, 71]. Basu [1] studied the effect of different levels of EMS on fenugreek (Tristar variety). He found that EMS by alkylating guanine base and mispairing or mismatch pairing in the genome, effectively induced variation in the fenugreek populations and the mutants which were generated by 300 μM EMS had the best characters.

Also the results of various studies show that more than one genotype should be used in mutation breeding program [60, 74]. This is because different genotypes respond differently to a mutagen.

8. Fenugreek tissue culture

One way of producing variation is tissue culture. Several techniques such as somatic embryogenesis, callus regeneration and micropropagation have been reported in fenugreek [75–77]. Malhotra [78] reviewed various studies on in vitro regeneration and callus induction on fenugreek. Aasim et al. [77] performed a successful in vitro shoot regeneration of fenugreek plants on Murashige Skoog medium (MS) medium containing Thidiazuron (TDZ). The reports show that *T. foenum-graecum* L. hypocotyl explants are most responsive to callus induction and proliferation in tissue culture [79]. El-Nour et al. [80] performed a protocol of callus induction in fenugreek (*T. foenum-graecum* L.) on MS and B5 media supplemented with different types and concentrations of growth regulators were tested in order to obtain the best callus formation. The maximum value of callusing index (2.8) was obtained from MS medium containing 1.5 mg/l, 2,4-D using hypocotyls and cotyledons explants. The maximum callus formation observed in the MS media containing 2.0 mg/l naphthalene acetic acid (NAA) was 3.9 ± 0.08 in hypocotyls segment. The callus was compact in cotyledons and variable in hypocotyls segments and the color was creamy.

Shekhawat and Galston [81] examined different culture media and concluded that medium containing 0.1 mg/L of 6-Benzylaminopurine (BAP), zeatin, glutamine and asparagines was suitable for callus induction and differentiation, rapid cell division and growth. Azam and Biswas [75] believed that callus induction and growth were more successful on MS medium supplemented with naphthalene acetic acid (NAA), 2,4-D, kinetin and coconut water. El-Bahr [82] had a different view; he believes that fenugreek callus had its best growth on MS medium containing 3% sucrose and 2 mg 2,4-D.

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9. Conclusion

Fenugreek (*T. foenum-graecum* L.) is an annual forage legume and a traditional spice crop that has been grown for centuries across the Indian subcontinent. In addition to South Asia, the crop is also grown in some parts of North Africa, Middle East, Mediterranean Europe, China, SE Asia, Australia and the USA, Canada and Argentina. India is the largest fenugreek producer in the world, but due to high internal consumption, do not have a major share of the global fenugreek trade. The crop has been recommended for the dry and semiarid regions of Asia, Africa and Latin America. The plant has been used traditionally in Indian Ayurvedic medicines as well as in traditional Tibetan and Chinese medication for several centuries. Modern research has also demonstrated that fenugreek seed and leaves are useful in the treatment of a number of diseases including successfully reducing blood sugar and blood cholesterol levels in both animals and human subjects in experimental trials. The reason behind the rich medicinal properties of fenugreek is due to the presence of a wide diversity of important phytochemicals (diosgenin, trigonelline, fenugreekine, galactomannan and 4-hydroxy isoleucine).

Hence, the crop has huge international demand in the associated pharmaceutical, nutraceutical and functional food industries. Our globe represents a wide range of agro-ecosystems on the earth with suitable dry, arid and semiarid climatic regimes suitable for the cultivation of fenugreek. Although the crop is crown to a limited amount in potential regions of fenugreek production, namely Africa, Central and South America and Southeast of Asia, but it has the potential to be grown under larger areas as a chemurgic crop with significant economic and commercial potential for the nation. Furthermore, being a forage legume and a natural nitrogen fixer; it could be easily incorporated in the local crop cycles of different geological regions for replenishing the soil naturally. The crop grows well under rainfed conditions and hence cost of production is lower compared to other commercial crops suitable for Iranian agroclimatic regimes.

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