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Fenugreek a multipurpose crop: A review on its biology and production technology

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Abstract
Fenugreek (*Trigonella foenum-graecum* L.), wild or cultivated, is widely distributed throughout the world and belongs to the Fabacecae family. It is an old medicinal plant and has been commonly used as a traditional food and medicine. Fenugreek is known to have hypoglycemic, and hypo-cholesterol effects. It may grow well under diverse and a wide range of conditions; it is moderately tolerant to drought and salinity, and can even be grown on marginal lands in profitable way. Owing to these characteristics and heavy metal remediation potential, fenugreek may well fit several cropping systems. However, efforts should be initiated to develop strategies for improving its biomass production; genetic diversity among different accessions may be mapped, breeding and crop improvement programs may be initiated to improve the biomass and nutritional and functional elements. Recent research has identified fenugreek as a valuable medicinal plant with potential for multipurpose uses and also as a source for preparing raw materials of pharmaceutical industry, especially steroidal hormones. To achieve these goals with regard to sustainable production, we reviewed a summary of biology, cultivation and biotechnology of fenugreek in this paper.

Keywords: *Trigonella foenum-graecum*, cultivation, biotechnology, crop improvement, gaps

Introduction

Fenugreek belongs to Fabaceae family; it was named, Trigonella, from Latin language that means “little triangle” due to its yellowish-white triangular flowers (Flammang *et al*., 2004). It is named as Methi (Hindi, Urdu, Punjabi and Marathi), Hulba (Arabic), Moshoseitaro (Greek), Uluva (Malayalam), Shoot (Hebrew), Dari (Persian), and heyseed in English. Fenugreek (*Trigonella foenum-graecum* L.) is one of the oldest medicinal plants from Fabaceae family originated in central Asia ∼4000 BC (Altuntas *et al*., 2005). It is being commercially grown in India, Pakistan, Afghanistan, Iran, Nepal, Egypt, France, Spain, Turkey, Morocco, North Africa, Middle East and Argentina (Flammang *et al*., 2004, Altuntas *et al*., 2005). Fenugreek seeds contain a substantial amount of fiber (Montgomery, 2009, Meghwal and Goswami, 2012), phospholipids, glycolipids, oleic acid, linolenic acid, linoleic acid (Sulieman *et al*., 2000, Chatterjee *et al*., 2010), choline, vitamin A, B1, B2, C, nicotinic acid, niacin (Leela and Shafeekh, 2008), and many other functional elements.

Fenugreek leaves and seeds are consumed in different countries around the world for different purposes such as medicinal uses (anti-diabetic, lowering blood sugar and cholesterol level, anti-cancer, anti-microbial, etc.), making food (stew with rice in Iran, flavor cheese in Switzerland, syrup and bitter run in Germany, mixed seed powder with flour for making flat bread in Egypt, curries, dyes, young seedlings eaten as a vegetable, etc.), roasted grain as coffee-substitute (in Africa), controlling insects in grain storages, perfume industries, and etc. carbohydrates, mainly mucilaginous fiber (galactomannans), 20-30% proteins high in lysine and tryptophan, 5-10% fixed oils (lipids), pyridine alkaloids, mainly trigonelline (0.2-0.38%), choline (0.5%), gentianine and carpaine, the flavonoids apigenin, luteolin, orientin, quercetin, vitexin and isovitexin, free amino acids, such as 4-hydroxyisoleucine (0.09%), arginine, histidine and lysine. There are some possibilities for increasing the chemical constituents contained in the seed, either during the growing period by using different cultural techniques [9, 10], or during post-harvest treatments by different techniques (enzymes, hormones, etc.) of germination with incubation [11], different conditions of incubation and fermentation [12], by storage [11], by the use of tissue and cell culture (static or suspension) [13, 14] and by biological manipulation of yield [4, 11, 15]. The yield potential of fenugreek can be defined as the total biomass produced or agricultural important part of the crop. The total biomass is a result of the integration of metabolic reactions in the plant. Consequently, any factor influencing the metabolic activity of the plant at any period of its growth can affect the yield [16]. Metabolic processes of fenugreek plants are greatly governed by both internal, i.e. genetic makeup of the plant and external conditions which involve two main factors namely climatic and edaphic environmental factors. The yield potential of fenugreek could be regulated through alternation of genetical make up and reconstitution of genetical structure through breeding programs and/or by modifications of environment through cultural treatments [16, 17]. Despite fenugreek being multipurpose crop, it has not obtained due importance in our cropping pattern and little research work has been done on agronomic and bio technologic aspects of fenugreek, especially in Iran. Thus the objectives of this study are to investigate the cultivation and biotechnology aspects of fenugreek. Species of Trigonella Petropoulos [15] and Basu [18] indicated that older taxonomists like Linnaeus have suggested that as many as 260 species of fenugreek exist. *T. foenum-graecum* (fenugreek) is the only widely cultivated species of the genus Trigonella [15].

Names

The species *Trigonella foenum-graecum*, wild or cultivated, is widely distributed throughout the world, as is indicated by the great number of names it possesses with Arabic, Indian (Sanskrit) and European (Greek and Latin) roots [15, 24]. The genetic name Trigonella, comes from Latin meaning 'little triangle', in reference to the triangular shape of the small yellowish-white flowers. The species epithet foenum-graecum means 'Greek hay' and according to Rosengarten [25] the Romans, who got the plant from Greece where it was a very common crop in ancient times, gave it this name.

Origin and distribution

Different authors have widely divergent opinions about the probable ancestry of *T. foenum-graecum*. Vavilov [27] has suggested that fenugreek is native to the Mediterranean region, while De Candolle [28] and Fazli and Hardman [2] proposed an Asian origin for the crop [26]. De Candolle [28] and Fazli and Hardman [2] notice that fenugreek grows wild in Punjab and Kashmir, in the deserts of Mesopotamia and Persia, in Asia Minor and in some countries in Southern Europe such as Greece, Italy and Spain. De Candolle [28] believes that the origin of fenugreek should be Asia rather than Southern Europe, because if a plant of fenugreek nature was indigenous in Southern Europe Many authors maintain that the direct ancestor of cultivated fenugreek is the wild *T. gladiata* Ste. that differs from *T. foenum-graecum* in respect of the entire aggregate of characters, of which seed tuberculation and the small size of the pods are only the most striking. It is possible that the species *T. foenum-graecum* evolved from *T. gladiata*, which had possibly given rise to some new extinct forms of *T. foenum-graecum* [15, 29].

Botanical description

Fenugreek is also known as one of the oldest medicinal plants recognized in recorded history [26, 30, 31]. Linnaeus has described the species *Trigonella foenum-graecum* first [15, 18, 32]. Fenugreek is an annual dicotyledonous plant belonging to the subfamily Papilionaceae, family Leguminacae (=Fabacecae). A morphological description of the plant is presented by Sinskaya [33], Hutchinson [22], Tutin and Heywod [34], Fazli and Hardman [2], Petropoulos [15, 29], and Basu [18] in Table 2 and Figure 2 [35]. In general, two types of flowering shoots are observed [15, 29]. The common type bears axillary flowers showing an indeterminate growth habit, whereas the less common or so called "blind shoots" have both axillary and terminal flowers, each of which become "tip bearers". Two types of fenugreek flowers also have been described [15, 29]; i.e., cleistogamous (closed) and aneictgamous (open) flowers. However, the majority of fenugreek flowers are cleitogamous; aneictgamous flowers are not common in fenugreek [18]. There are firm indications that there is a linkage of the quantitative character of diosgenin content with the morphological character of the number of pods per node near the top of stem, and that high content of diosgenin is inherited together with the formation of twin pods. So, the phenotype of twin pods in comparison with that of solitary pods is a good index of selection and should provide a reliable basis to predict the performance of their progenies for a higher diosgenin content of seed, from very early generations [15, 18, 29].

Growing period of fenugreek

The time of germination in soil usually varies from 3-10 days. Six to ten days after the fenugreek germination the seedlings produce the first leaf, which is usually simple; there is still no noticeable epicotyl as the first trifoliate leaf is formed after a further 5 – 8 days [15]. Growth is slower under cooler and wetter conditions, and long periods of these conditions may cause a failure of plants to mature for seed harvest. The growth rate of fenugreek is slow at the beginning of the growing season, and leaf development is temperature-dependent [39]. After the seed germination and the first growth of the seedling, follows the main plant growth, which includes the development of stems, flowers, pods and seeds. The fenugreek has an indeterminate growth habit, which means the growth continues from the terminal and axially buds, while the flowering and formation of pods are both in progress [5, 18]. In the cleistogamous flowers of fenugreek there are four distinguished stages of development; flower bud (first stage), main development (second stage), pollination (third stage), fertilization (fourth stage). The pod of fenugreek also has the following four distinguishing stages of development including, length development (first stage), width development (second stage), germ development (third stage), ripening (fourth stage) [15]. However, fenugreek is botanically a short living (4 – 7 months) annual crop.

Ecology

Although the main area cultivated with fenugreek is concentrated in some countries of Asia and Africa, however it has been distributed in many countries throughout the world under different environments. This wide distribution of its cultivation in the world is characteristic of its adaptation to variable climatic conditions and growing environments [15, 29] (Table 4). Duke [5] reports that fenugreek, ranging from cool temperate steppe to wet through tropical very dry forest life zone, is reported to tolerate an annual precipitation of 3.8–15.3 dm and an annual mean temperature of 7.8 – 27.5 °C. There are indications of the possible benefit of colder nights on the sapogenin content of the seed [2]. Depending on the geographical source of the seed its sapogenin content, calculated as diosgenin, varied from 0.8–2.2 percent expressed on a moisture free basis [2]. The highest sapogenin content was found in an Ethiopian sample and the lowest in a sample from Palestine [15]. As a legume crop, it can condition the soil by fixing nitrogen from the atmosphere and can reduce the need for nitrogen fertilizer for subsequent crops. Because fenugreek is a nitrogen-fixing legume, seeds must be inoculated with appropriate Rhizobium species for optimal growth [15, 18, 43].

Cultivation practices

Higher yield per hectare will be obtained through superior varieties and better management practices of production. In the old times, a fenugreek yield of 1 ton of seeds per hectare was considered very well, but nowadays yields of more than 2 tons per hectare are being obtained. The large yields of fenugreek are mainly dependent to suitable cropping and agronomic practices. A significant increase in yields through the suitable use of irrigation and adequate levels of soil fertility could make an immediate and important contribution to farm income [15, 29]. Fenugreek is a dry-land crop which responds even to minimal levels of irrigation. Interest in cultivating fenugreek in temperate climates, has increased because of its rain-fed adaptation [26, 31]. N, P and K fertilizers had a beneficial effect on the fenugreek seed yield, while N and K improved the quality of fenugreek hay [45]. A negative relation between N uptake and diosgenin content was observed [9, 15]. In order to reduce salt accumulation in the soil, small amounts of fertilizers are applied frequently rather than large quantities at longer intervals [15, 29]. Fenugreek is a nitrogen fixing legume. Hence the seed must be inoculated with an appropriate *Rhizobium inoculum* to optimize its growth potential. The most common nodule- forming bacteria associated with *Trigonella foenum-graecum* L. is the Gram negative, aerobic, non-sporulating, rod shaped bacterium, *Rhizobium meliloti* [18, 31]. Abdelgani *et al*. [47] has suggested that inoculation of fenugreek with a suitable strain of Rhizobium can improve quality and amount of seed generated.

Genetic and Breeding

Fenugreek according to Darlington and Wylie [62] has 2n=16 chromosomes, while Joshi and Raghuvanshi [63] have investigated the presence of B-chromosomes. Singh and Singh [64] isolated five double trisomics along with primary trisomics from the progenies of autotriploids, which had 2n+1+1=18 chromosomes. The development of improved varieties of fenugreek with higher diosgenin content in the seed should be obtained at first from the existing populations, cultivated or landraces, using known breeding methods especially those with the induction of mutations [15]. The final goal of a fenugreek breeder is the development of a variety of excellent quality and quantity yield over a wide range of environments [52]. Fenugreek is self-pollinated, but there are opportunities for natural out crossing. The inherent variation in fenugreek is quite immense and so it is grown today in the wide range of climatic conditions of all continents [15, 18]. Allard [65] has suggested that legumes are considered cross-pollinated when more than 10% of them are "out-crossed". On this basis Petropoulos [15, 29] described fenugreek as a rarely cross pollinated plant as its stigma becomes receptive before the anthers mature. Because of this Petropoulos [15, 29] has suggested that cross pollination for breeding purposes, can be done in closed flowers of fenugreek at initiation of the second stage of floral development, when the stamens are lower in position than the stigma, i.e. when the anther are closed but the stigma is receptive to pollination [18]. Consequently selections among world accessions and mutation breeding have been advocated as the best ways to improve the crop [15], and much of the breeding with fenugreek has utilized these two approaches [18, 66].

Biotechnology

Fenugreek tissue and cell cultures have been used for either plant regeneration or for the production of secondary products of economic interest. The first report on the production of spirostane derivatives by Trigonella tissue cultures was published by Khanna and Jain [13] and concerned the establishment of static cultures grown on solid Murashige and Skoog (MS) medium [73] supplemented with 1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) [72]. A first pathway is the incorporation of cholesterol and this pathway is predominant when the precursor is added at subculture. The second pathway involves sidechain cleavage before incorporation and takes place when the sterol is added 10 days after subculture. This fungicide was added (125 µM) to the fenugreek cell cultures, and, after 21 days, the cell growth decreased by about 20% compared to the control cell growth. Furthermore, diniconazole treatment led to a decrease of about 50% of total sterol content, causing an inhibition of sterol biosynthesis at the 14-α-demethylation step leading to accumulation of 14-αmethylΔ8 -sterols. Oncina *et al*. [71] reported on the production of diosgenin by callus cultures of *Trigonella foenum-graecum* L. Leaf, stem and root calli were established and cultured on different soild growth media (MS, White’s basal medium, Gamborg’s B5) [77] supplemented with coconut milk, malt extract and NAA. In all cases, MS medium supplemented with 15% (v/v) coconut milk and 3×10-6M NAA was the most suitable medium for callus growth. Callus cultures of *Trigonella foenum-graecum* contained 3 to 4 times more trigonelline than the seeds of this plant and 12 to 13 times more than the roots and shoots. Even higher levels of this alkaloid were produced by suspension cultures. This high productivity was maintained during successive sub culturing of calli and cell suspensions for eight months. Trigonelline accumulated in callus and suspension cultures with aging [4, 15]. Eight-week-old callus tissue cultures of fenugreek, established from seeds on solid Revised Tobacco (RT) medium and supplemented with 1 mg/l 2,4-D, produced 4.5% trigonelline. In the presence of 0.5 and 1.0 mg/l nicotinic acid, trigonelline increased to 5.25 and 5.01% respectively [75]. Four-week-old callus cultures of *Trigonella foenum-graecum* L. produced 15.6 mg/g dry wt. of trigonelline, which represents 3 to 4 times more trigonelline than the seeds and 12 to 18 times more than the roots and shoots of the parent plants. Four- and 6-week-old suspension cultures produced 38.2 and 44.2 mg/g dry wt., respectively, of trigonelline which is more than twice the amount found in the calli. Proportions of 9- 12% of the trigonelline were released into the solid medium In other way, regeneration of shoots has also been achieved from fenugreek protoplasts. Protoplasts were isolated from the root apices of 48-h-imbibed seeds. The first divisions of root fenugreek protoplasts were observed after a 3–4 day culture and subsequent divisions gave cell colonies. However, a culture of these colonies gave only roots [15, 74].

Conclusion

The usefulness of fenugreek as a commercial and chemurgic crop is now being recognized, not only as a break-crop for cereal areas, where it is a very good soil renovator, but also as forage, medicinal plant, source of diosgenin (the most important raw material for the steroid industry) and other constituents. Our knowledge of agronomic and biotechnology practices which could promote high quality and quantity of fenugreek production under Iranian growth conditions is very limited. There is need to develop an efficient agronomic and biotechnological package for assured production in fenugreek. Widespread cultivation of fenugreek requires that we solve the production problem through proper understanding of the agronomic complexities of growing this crop, and select germplasm. Irradiation and chemical mutagens can be used to produce point mutations in fenugreek. Selection for improved agronomic properties in the world collections is another possible approach that can help solve the seed yield and quality problem.

References

1. Sadeghzadeh-Ahari D, Kashi AK, Hassandokht MR, Amri A, Alizadeh Kh. Assessment of drought tolerance in Iranian fenugreek landraces. Journal of Food, Agriculture & Environment. 2009;7(3&4):414-419.
2. Fazli FRY, Hardman R. The spice, fenugreek (*Trigonella foenum-graecum* L.): Its commercial varieties of seed as a source of diosgenin. Tro. Sci. 1968;10:66-78.
3. Hardman R. Pharmaceutical products from plant steroids. Trop. Sci. 1969;11:196-222.
4. Mehrafarin A, Qaderi A, Rezazadeh Sh, Naghdi Badi H, Noormohammadi Gh, Zand E. Bioengineering of Important Secondary Metabolites and Metabolic Pathways in Fenugreek (*Trigonella foenum-graecum* L.). J of Medicinal Plants. 2010;9(35):1-18.
5. Duke AJ. Handbook of Legumes of World Economic Importance, Plemus Press, New York and London; c1986. p. 345.
6. Hajimehdipoor H, Sadat-Ebrahimi SE, Amanzadeh Y, Izaddoost M, Givi E. Identification and Quantitative Determination of 4-Hydroxyisoleucine in *Trigonella foenum-graecum* L. from Iran. J Medicinal Plants. 2010;9(6):29-34.
7. Budavari S. The merck index: An encyclopedia of chemicals, drugs, and biologicals, 12th ed. Whitehouse Station, N.J. Merk & Co, Inc; c1996.
8. Newall CA, Anderson LA, Phillipson JD. Herbal medicines: A guide for healthcare professionals. London: The Pharmaceutical Press; c1996.
9. Kozlowski J, Nowak A, Krajewska A. Effects of fertilizer rates and ratios on the mucilage value and diosgenin yield of fenugreek. Herba Polonica. 1982;28(3-4):159-70.
10. Mohamed MA. Differences in growth, seed yield and chemical constituents of fenugreek plants (*Trigonella foenum-graecum* L.) due to some agricultural treatments. Egyptian J. of Agronomy. 1990;15(1-2):117-23.
11. Hardman R, Fazli FRY. Methods of screening the genus Trigonella for steroidal sapogenins. Planta Medica. 1972;21:131-138.
12. Elujoba AA, Hardman R. Fermentation of powdered fenugreek seeds for increased sapogenin yields. Fitoterapia. 1985;56(6):368-70.
13. Khanna P, Jain SC. Diosgenin, gitogenin and tigogenin from *Trigonella foenum-graecum* tissue cultures. Lloydia. 1973;36:96-8.
14. Trisonthi P, Baccou JC, Sauvaire Y. Trial to improve production of steroidal sapogenin by fenugreek (*Trigonella foenum-graecum* L.) tissue grown *in vitro*. C.R. Seances Acad. Sci., Ser. D. 1980;291(3):357-60.
15. Petropoulos GA Fenugreek. The genus Trigonella. Taylor and Francis, London and New York; c2002. p. 255.
16. Ahmed MA, Ibrahim OM, Elham AB. Effect of bio and mineral phosphorus fertilizer on the growth, productivity and nutritional value of fenugreek (*Trigonella foenum-graecum* L.) in Newly Cultivated Land. Res. J Agriculture and Biological Sci. 2010;6(3):339-48.
17. Basu SK, Acharya SN, Bandara MS, Friebel D, Thomas JE. Effects of genotype and environment on seed and forage yield in fenugreek (*Trigonella foenum-graecum* L.) grown in western Canada. Australian J. Crop Sci. Southern Cross Journals. 2009;3(6):305-14.
18. Basu SK. Seed production technology for fenugreek (*Trigonella foenum-graecum* L.) in the Canadian. Master of Science Thesis. Department of Biological Sciences University of Lethbridge, Alberta, Canada; c2006. p. 202.
19. Vasil'chenko IT, Bericht uber die Arten der Gattung (SSSR.1:10). Trigonella Trudy Bot. Inst. Akad. Nauk. 1953;17:331-3.
20. Fazli FRY. Studies in steroid-yielding plants of the genus Trigonella, PhD Thesis, University of Nottingham, England; c1967.
21. Hector JN. Introduction to the Botany of Field Crops (Non cereals), Central News Agency Ltd., Johannesburg; c1936. p. 1127.
22. Hutchinson J. The Genera of Flowering Plants. Clarendon Press, Oxford. 1964;1:947-52.
23. Rouk HF, Mangesha H. Fenugreek (*Trigonella foenum-graecum* L.). Its relationship, geography and economic importance, Exper. Stat. Bull. No. 20, Imper. Ethiopian College of Agric. & Mech. Arts; c1963. p. 120-7.
24. Edison S. Spices – research support to productivity. N. Ravi (ed.), The Hindu Survey of Indian Agriculture, Kasturi & Sons Ltd., National Press, Madras; c1995. p. 101.
25. Rosengarten F. The Book of Spices, Livingston, Wynnewood, Penns., USA; c1969. p. 250.
26. Acharya SN, Thomasl and JE, Basu SK. Fenugreek: an "old world" crop for the new world. Biodiversity (Tropical, Conservancy). 2006;7(3&4):27-30.
27. VaviIov NI. Studies in the origin of cultivated plants. Inst. Appl. Bot. Plant Breed. Leningrad. 1926, pp: 289. http://res2.agr.callethbridge/scitech/acharya_e.htm.
28. De Candolle A. Origin of cultivated plants. Hafner, New York; c1964. p. 468.
29. Petropoulos GA. Agronomic, genetic and chemical studies of *Trigonella foenum graecum* L. PhD. Diss. Bath University, England; c1973. p. 145.
30. Lust JB. The herb book. Bantam Books Inc. New York. 1986, p: 1 - 55.
31. Acharya SN, Thomas JE, Basu SK. Fenugreek, an alternative crop for semiarid regions of North America. Crop Sci. 2008;48:841-53.
32. Linnaeus C. Species Plantarum, Silvius, Stockholm; c1753. p. 1200.
33. Sinskaya E. Flora of the Cultivated Plants of the U.S.S.R. XIII. In Perennial Leguminous Plants. Part I: Medicago, Sweet clover, Fenugreek. Israel Programme for Scientific Translations, Jerusalem; c1961. p. 514-70.

34. Tutin TG, Heywood VH. Flora Europaea. Cambridge University Press, Cambridge. 1964;1:452-82.
35. Schoepke T Fenugreek. (*Trigonella foenum-graecum* L.) image, source of Koehler's medicinal plants images. http://pharm1.pharmazie. unigreifswald. de/allgemei /koehler/ koeh-273.jpg
36. Rechinger KH. Flora Iranica (*Trigonella foenum-graecum* L.). No: 157/Dezen, Akademische Druck-u, Verlagsanstalt, Austria; c1984. p. 252.
37. Sadeghzadeh-Ahari D, Hassandokht MR, Kashi AK, Amri A, Alizadeh KH. Genetic variability of some agronomic traits in the Iranian Fenugreek landraces under drought stress and non-stress conditions. African J Plant Sci. 2010;4(2):12-20.
38. Moradi P. Study of genetic diversity of Fenugreek (*Trigonella foenum-graecum* L.) native to Iran. Ph.D. Thesis of Horticulture. Department of Horticulture, Science and Research Branch, Islamic Azad University. Tehran, Iran; c2008. p. 260.
39. McCormick K, Norton R, Eagles HA. Fenugreek has a role in south-eastern Australian farming systems. In Proceedings of Groundbreaking stuff, 13th. Annual Agronomy Conference, Perth, Australia; c2006. p. 639.
40. Dawidar AM, Fayez MBE. Thin-layer chromatographic detection and estimation of steroid sapogenins. Z. Anal. Chem. 1972;259:283-5.
41. Talelis D. Cultivation of Legumes, Agric. College of Athens, Athens (in Greek); c1967. p. 101-5.
42. Yadav GL, Kumawat PD. Effect of organic inorganic, fertilizer and Rhizobium inoculation of yield and yield attributes of fenugreek (*Trigonella foenum-graecum* L). Haryana J Hort. Sci. 2003;32(1-2):147-8.
43. Basu SK, Acharya SN, Thomas JE. Application of phosphate fertilizer and harvest management for important fenugreek (*Trigonella foenum-graecum* L.) seed and forage yield in a dark brown soil zone of Canada. KMITL Sci. Tech. J. 2008;8(1):1-7.
44. Baricevic D, Zupancic A. The impact of drought stress and/or nitrogen fertilization in some medicinal plants. J Herb. Spi. Med. Plants. 2002;9(2-3):53-64.
45. Pareek SK, Gupta R. Effect of fertilizer application on seed yield and diosgenin content in fenugreek. Indian J Agric. Sci. 1981;50(10):746-9.
46. Golez L, Kordana S. Effect of nitrogen, phosphorous and potassium doses, as well as magnesium and calcium fertilisation on a crop yield and uptake of mineral nutrients by *Trigonella foenum-graecum*. Herba Pol. 1979;25(2):121-31, 22.
47. Abdelgani ME, Elsheikh EAE, Mukhtar NO. The effect of Rhizobium inoculation and chemical fertilization on seed quality of fenugreek. Food Chem. 1999;64(3):289-93.
48. Anonymous. Kozieradka pospolita – Instruction of cultivation (*Trigonella foenum-graecum* L.), Instytut Roslin I Przetworow zielarskich, W. Poznaniu; c1987. p. 94.
49. Piper CV. Forage Plants and Their Cultures, The Macmillan Company, New York; c1947. p. 671.
50. Dachler M, Pelzmann H. Heil- und Gew_rzpflanzen, Anbau-Ernte-Aufbereitung, Österreichischer Agrarverlag, Wien; c1989. p. 244.
51. Purohit SS, Vyas SP. Medicinal plant cultivation: A scientific approach (Including processing and financial guidelines). Agrobios (India); c2004. p. 539-42.
52. Bunting ES. Cultivation of fenugreek and some existing its varieties, Univ. of Feed. Lab., Oxford; c1972. p. 101.
53. Billaud C, Adrian J. Fenugreek composition, nutritional value and Physiological properties. Sci. Aliments. 2001;21:3-26.
54. Rizk SG. Atmospheric nitrogen fixation by legumes under Egyptian conditions. II. Grain legumes. J Microbiol. U.A.R. 1966;1(1):33-45.
55. Chhibba IM, Nayyar VK, Kanwar JS. Influence of mode and source of applied iron on fenugreek (*Trigonella corniculata* L.) in a typic ustochrept in Punjab, India. Int. J Agri. Biol. 2007;9(2):254-6.
56. Bismillah Khan M, Aslam Khan M, Sheikh M. Effect of phosphorus levels on growth and yield of fenugreek (*Trigonella foenum-graecum* L.) grown under different spatial arrangements. Int. J Agri. Biol. 2005;7(3):504-7.
57. Slinkard AE. Production fenugreek (*Trigonella foenum-graecum* L.) in Saskatchewan. Saskatchewan Ministry of Agriculture. University of Saskatchewan; c2009. p. 4.
58. Mandam AR, Maiti RG. Efficacy of different herbicides for weed control in fenugreek (*Trigonella foenum-graecum* L.). Environ. Ecol. 1994;12:138-42.
59. Alhadi FA, Yaseen BT, Jabr M. Water stress and giberrellic acid effects on growth of fenugreek plants. Irrig. Sci. 1999;18(4):185-90.
60. Ortuno A, Oncina R, Botia JM, Rio JAD. Distribution and changes of diosgenin during the development of *Trigonella foenum-graecum* plants. Modulation by benzylaminopurine. Food Chem. 1998;63(l):51-4.
61. Alagukannan G, Vijaykumar M. Effect of plant growth substances on yield attributing parameters, yield and quality in fenugreek (*Trigonella foenum-graecum* L.). S. Ind. Hort. 1999;47(1-6):130-3.
62. Darlington CD, Wylie AP. Chromosome atlas of flowering plants, George Allen & Unwin Ltd., London; c1945. p. 164.
63. Joshi S, Raghuvanshi SS. Bchromosomes, pollen germination in situ and connected grains in *Trigonella foenum-graecum*. Beitr. Biol. Pf.I. 1968;44(2):161-6.
64. Singh D, Singh A. Double trisomics in *Trigonella foenum-graecum* L. Crop Improvement. 1976;3(1-2):25-7.
65. Allard RW. Principles of Plant Breeding, J. Hilley and Sons Inc. London; c1960 p. 485.
66. Green JM, Sharma D, Reddy LJ, Saxena KB, Gupta SC, Jain KC, *et al*. Methodology and the progress in the ICRISAT in Pigeon pea Breeding Programme. In Proc. Intern. Workshop on Pigeon peas. Ptancheru; c1981. p. 437-49.
67. Auerbach C. Chemicals and their effects. Proc. Symp. on Mutation and Plant Breeding, Cornell, Nov.– Dec. 1961;25:585-621.
68. Jain SC, Agrawal M. Effect of chemical mutagens on steroidal sapogenin in Trigonella species. Phyto chem. 1987;26(8):2203-6.
69. Roy RP, Singh A. Cytomorphological studies of the colchicine-induced tetraploid *Trigonella foenum-graecum*. Genet. Iber. 1968;20(1-2):37-54.
70. Cerdon C, Rahier A, Taton M, Sauvaire Y. Effect of diniconazole on sterol composition of roots and cell suspension cultures of fenugreek. Phytochem. 1945;39:883-93.

71. Oncina R, Botia JM, Del Rio JA, Ortuno A. Bio production of diosgenin in callus cultures of *Trigonella foenum-graecum* L. Food Chem. 2000;70(4):489-92.
72. Jain SC, Rosenberg H, Stohs SJ. Steroidal constituents of Trigonella occulta tissue cultures. Planta Ned. 1977;31:109-11.
73. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 1962;15:473-97.
74. Christen P. Trigonella species: *In Vitro* Culture and production of Secondary Metabolites. In Nagata T, Ebizuka Y. (Eds.), Medicinal and aromatic plants (Vol. 12). Biotechnology in Agriculture and Forestry 51. Springer; c2002. p. 306-48.
75. Khanna P, Jain SC, Bansal R. Effect of cholesterol on growth and production of diosgenin, gitogenin, tigogenin and sterols in suspension cultures. Indian J Exp. Biol. 1975;13(2):211-3.
76. Brain KR, Williams MH. Evidence for an alternative route from sterol to sapogenin in suspension cultures from *Trigonella foenum-graecum*. Plant Cell Rep. 1983;2:7-10.
77. Gamborg OL. The effects of amino-acids and ammonium on the growth of plant cells in suspension culture. Plant Physiol. 1970;45:372-5.
78. Iribbarren AM, Pomilio AB. components of Bauhinia candicans. J Nat. Prod. 1983;46:752-3.
79. Kuhn A, Gerhard H. The trigonellin and nicotinic acid content of seeds of fenugreek. Arch. Pharm. U. Ber. Deutsch Pharm. Ges. 1943;281:378-9.
80. Joshi JG, Handler P. Biosynthesis of trigonelline. J Biol. Chem. 1960;235:2981-3.
81. Antony A, Gopinathan KP, Vaidyanathan CS. Biosynthesis of trigonelline in root cultures of Fenugreek (*Trigonella foenum-graecum* L). Indian J Exp. Biol. 1975;13:39-41.
82. Radwan SS, Kokate CK. Production of higher levels of trigonellin by cell cultures of *Trigonella foenum-graecum* than by the differentiated plant. Planta. 1980;147:340-4.