Chapter 19
Biotechnological Approaches for Genetic Improvement of Fenugreek (*Trigonella foenum-graceum* L.)

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Abstract Fenugreek (*Trigonella foenum-graecum* L.) is one of the important medicinal plants of ancient medicinal systems due to its high nutraceutical and pharmaceutical properties. Seeds and leaves of Fenugreek contain phytochemicals like diosgenin and trigonelline. It is a cultivated plant of the modern world for medicinal uses, an edible vegetable, and a forage plant. Advancement in industrial and biotechnological techniques for the isolation of phytochemicals increase the demand of Fenugreek, and its breeding programs are based on improving the secondary metabolites compared to other uses. Recent advancement in modern biotechnological approaches enables researchers to develop elite cultivars of desired traits in a short time. Application of modern techniques like artificial mutations under in vitro conditions, characterization using molecular markers, and development of successful plant tissue culture techniques, genetic transformation techniques, and functional genomics studies have significant potential to improve Fenugreek traits. The study highlights the application of biotechnological approaches used for the development of elite Fenugreek traits for the researchers for future breeding programs. Furthermore, the research gap and areas to improve research have been highlighted in this present study.

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Abbreviation

2,4-D	2,4-dichlorophenoxyacetic acid
B5	Gamborg medium
EMS	Ethyl-methanesulfonate
IAA	Indole acetic acid
IBA	Indole-3-butyric acid
IPA	Indole-3-propionic acid
MAS	Marker-assisted selection
MMS	Methyl-methanesulfonate
MS	Murashige and Skoog medium
NAA	α-Naphthaleneacetic acid
NaN_3/SA	Sodium azide
OD	Optical density
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PGRs	Plant growth regulators
QTL	Quantitative trait locus
TDZ	Thidiazuron
UV	Ultravoilet
WP	Woody plant
ϒ-rays	Gamma rays

19.1 Introduction

Plants have a key role in human diets due to providing nutrition and biochemicals that are used as medicines; humans know the use of these herbs to cure acute or chronic diseases since ancient times. Fenugreek (*Trigonella foenum-graecum* L.) is one of the oldest plants used by different human civilizations due to its high medicinal, nutraceutical, and pharmaceutical properties and also used as an edible or spice herb. It is considered as one of the most important ingredients in old medicinal systems like Indian Ayurvedic medicines and Chinese/Tibetan medications (Zandi et al. 2017).

Fenugreek is an annual dicotyledonous herb of the Fabaceae family (subfamily – Papilionoideae). The genotypes of Fenugreek possess a wide variability in morphology growth and development, seed size and color, plant biomass, and seed production. The plant has short stature with 40–60 cm high glabrous stem, trifoliate leaves with ovate leaflets (Acharya et al. 2006a), and twin flowers. There are two types of plants; the early mature plants take 80–90 days, while late-maturing plants

Table 19.1 Major bioactive compounds of Fenugreek seeds

Compound	Chemical name	References
Carbohydrates	Galactomannans	Meghwal and Goswami (2012)
Proteins	Lecithin, albumin	Naidu et al. (2011)
Lipids	Unsaturated lipids, phospholipids, glycolipids	Chatterjee et al. (2010)
Free amino acids	4-Hydroxyisoleucine, lysine, histidine, arginine	Isikli and Karababa (2005)
Vitamins	A, B1, B2, C, nicotinic acid, niacin	Leela and Shafeekh (2008)
Minerals	Zn, P, Mn, Fe, Ca	Al-Jasass and Al Jasser (2012)
Coumarins	Trimethyl coumarin, trigocoumarin, methyl coumarin	Raju et al. (2001)
Flavonoids	Vitexin, vecenin-1, quercetin, orientin, tricin, saponaretin, naringenin, luteolin, lilyn, kaempferol, isoorientin, apigenin, isovitexin, 7-O-D glucopyranoside	Blumenthal et al. (2000)
		Suavare et al. (2000)
		Naidu et al. (2011)
		Meghwal and Goswami (2012)
Phenolics	Vanillic acid, gentisic acid, gallic acid	Rababah et al. (2011)
Saponins	neotigogenin, diosgenin, fenugrin, hederagin, glycoside, gitogenin, foenugracin, tigogenin, smilagenin, yamogenin, yuccagenin, trigonoesides, sarsasapogenin	Raju et al. (2004)
Volatile oils	N-alkanes, sesquiterpenes	Meghwal and Goswami (2012)

take 100–115 days from germination to maturity (Petropoulos 2002). Seeds are available in different color range of olive green, brown, cinnamon, or lighter green in color with seed size of 3.5–6 mm long and 2.5–4 mm wide (Petropoulos 2003).

Fenugreek seed is the most attractive part of the plant due to different bioactive compounds (Taylor et al. 2000; Acharya et al. 2006b). However, the amount of these chemicals is associated with genotypes or ecological factors which consequently affect the minor- or polygenes which in turn affect the phytochemical production (Fehr 1998; Acharya et al. 2008). Major bioactive compounds and chemicals contained in Fenugreek seeds are given in Table 19.1.

Fenugreek has high nutritional values due to presence of macro- and microelements and dietary food fiber in leaves and seed (Thomas et al. 2011). Fresh leaves are used as vegetable (Balch 2003), and dried leaves are utilized as flavoring agent (Olaiya and Soetan 2014). Fenugreek seeds are the major constituent of oily pickles for special flavor in the Indian subcontinent (Najma et al. 2011) and for bread making in Egypt as a staple food (Mehrafarin et al. 2011). Seeds are rich in proteins

Table 19.2 Pharmacological and therapeutic uses of Fenugreek

Disease/disorders	References
Aging	Kaviarasan et al. (2004)
Anemia	Mahmoud et al. (2012)
Antibacterial	Premnath et al. (2011)
Antifertility	Aswar et al. (2010)
Antimicrobial	Jasim et al. (2017)
Anti-obesity	Kumar and Bhandari (2015)
Antioxidant	Ktari et al. (2017)
Antiulcer	Al-Meshal et al. (1995)
Cancer/anticarcinogenic	Mohamed et al. (2015)
Diabetes	Jiang et al. (2017)
Hypercholesterolemia/antilipidemic	Sharma and Choudhary (2016)
Immunodeficiency/immunomodulatory	Moradi Kor and Moradi (2013)
Indigestion and flatulence	Sauvare et al. (2000)
Inflammation	Piao et al. (2017)
Kidney disorders	Belguith-Hadriche et al. (2013)
Myocardial infarction	Panda et al. (2013)
Skin irritation	Meghwal and Goswami (2012)

(Naidu et al. 2011), carbohydrates (Najma et al. 2011), minerals (Hegazy and Ibrahim 2009; Jani et al. 2009), and vitamins (Sharma 1986). However, the use of Fenugreek leaves after boiling, frying, or steaming resulted in depletion of certain vitamins (Leela and Shafeekh 2008). Fenugreek is an important constituent in food processing as food adhesive, stabilizer and emulsifier (Jani et al. 2009), food gum (Sowmya and Rajyalakshmi 1999), alcoholic beverages (Jani et al. 2009), bread making (Raju et al. 2001, Meghwal and Goswami 2012), and food preservative (Betty 2008).

Fenugreek seeds are rich in phytochemicals having certain pharmacological properties and are highly effective against diseases. The major use of Fenugreek as medicinal plant includes lactation, stimulant, and condiment (Betty 2008) in India, treating labor pain by Romans, leg weakness and edema by Chinese (Yoshikawa et al. 2000), and diabetes by Africans and Asians (Miraldi et al. 2001). Other folkloric uses for Fenugreek include anemia (Kaviarasan et al. 2004), kidney disorders (Xue et al. 2011; Belguith-Hadriche et al. 2013), skin irritation (Suavare et al. 2000; Meghwal and Goswami 2012), and other diseases like arthritis, chronic cough, dropsy, epilepsy, gout, liver disorders, paralysis, piles, and respiratory disorders (Ahmadiani et al. 2001; Tayyaba et al. 2001; Kaviarasan et al. 2004; Amin et al. 2005), whereas the use of modern technologies enables humans to use these bioactive compounds more efficiently against different diseases and disorders (Table 19.2).

19.2 Genetic Resources of Fenugreek

Fenugreek or *Trigonella* has a large number of species throughout the world, and around 260 species have been reported (Petropoulos 2002; Basu 2006). Some of these *Trigonella* species are cultivated like *T. balansae*, *T. calliceras*, *T. spicata*, *T. occulta*, *T. lilacina*, *T. corniculata*, *T. spinosa*, *T. caerulea*, *T. radiata*, *T. maritima*, *T. cretica*, *T. polycerata*, and *T. foenum-graecum.* The most cultivated among these species is *T. foenum-graecum* due to its high demand for its bioactive compounds used for medicinal purposes (Petropoulos 2002). These *Trigonella* species and landraces show their distribution in Australia, Asia, Africa, and Europe. The major Fenugreek-producing countries are India, China, Turkey, Pakistan, Spain, India, France, Morocco, Egypt, and Ethiopia (Petropoulos 2002; Acharya et al. 2008), whereas the crop is still cultivated as a minor crop in North America (Canada and the United States) (Fig. 19.1).

The word *Trigonella* means "little triangle" due to triangular-type resemblance of leaves, and *foenum-graecum* means "Greek hay" due to its early introgression from Greece (Basu 2006). However, there are different theories about the exact ancestors and origin of *T. foenum-graecum* besides of its cultivation since 4000 BC (Acharya et al. 2008). The probable ancestors of *T. foenum-graecum* are *T. gladiata* (Petropoulos 1973) and *T. gladiate* (Petropoulos 2002). Regarding center of origin of Fenugreek, different geographical regions have been reported by different researchers. Duke et al. (1981) reported the Mediterranean region as the center of origin for Fenugreek. Earlier, De Candolle (1964) and Fazli and Hardman (1968) reported Asian regions (Mesopotamia, Persia, Punjab, and Kashmir) and European

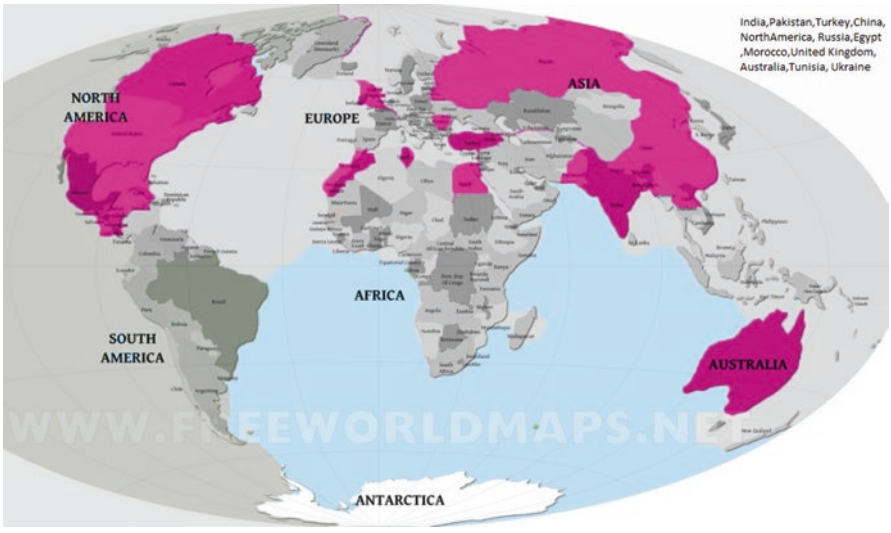

Fig. 19.1 Geographical distribution of Fenugreek (*T. foenum-graecum*) – pink color shows the presence of Fenugreek

regions (Greece, Italy, and Spain) as an origin of Fenugreek, whereas Turkey was reported as a center of origin for Fenugreek (Dangi et al. 2004) due to the presence of a number of Fenugreek genotypes. Currently, 51 species of Fenugreek have been recorded in Turkey having endemic *Trigonella* species with distribution in the Black Sea and Mediterranean region.

Germplasm collection of any plant/crop has significant impact on its improvement through conventional breeding or application of modern biotechnological tools. The germplasm collection of Fenugreek is available at the Plant Gene Resources of Canada, Saskatoon in Canada (Acharya et al. 2006b); National Bureau of Plant Genetic Resources, India (Basu et al. 2014); Longerenong Agricultural College and University of Melbourne, Australia (McCormick et al. 2009); and USDA ARS Plant Introduction Station, Washington.

Genetic improvement of any crop can be accomplished through the application of traditional or modern biotechnological approaches. The commercial use of Fenugreek all over the world is mainly based on its bioactive compounds like diosgenin, and there is dire need to improve Fenugreek germplasm for steroidal industry and for food industry (Petropoulos 2002; Basu 2006; Acharya et al. 2008) with more yield. Currently, breeding programs for Fenugreek improvement include selection (Prajapati et al. 2010, Basu et al. 2014), hybridization (Acharya et al. 2008), and mutation (Rajoriya et al. 2016) used singly or in combination of more than one method (Mehrafarin et al. 2011). It is difficult to develop new cultivars by hybridization under field conditions due to self-pollinated nature of Fenugreek (Petropolous 2002; Acharya et al. 2008).

Application of modern biotechnological approaches enables researchers to develop elite cultivars of desired traits in a short time. Therefore, modern techniques like induced mutations under in vitro conditions (Petropolous 2002), characterization using molecular markers, development of successful plant tissue culture techniques (Aasim et al. 2009), and genetic transformation techniques have significant potential to improve Fenugreek traits. In recent years, researchers are focusing on the isolation and utilization of secondary metabolites of Fenugreek due to advancement in industrial and biotechnological techniques. On the other hand, work related to its genetic or biotechnological improvement is also confined to secondary metabolites rather than its improvement for human consumption, food processing, or as forage plant. The study highlights the application of biotechnological approaches used for the development of elite Fenugreek traits with main focus on its phytochemicals and molecular genetic diversity.

19.3 Mutation Breeding of Fenugreek

The importance of medicinal metabolites of Fenugreek along with its nutritional values opens a new window for its cultivation worldwide. Furthermore, low agronomic practices, soil fertility, and adaptation to wide climatic regions are the major factors for its distribution (Petropolous 2002; Acharya et al. 2006a, b; Montgomery

et al. 2006). However, there is still need to breed/develop new varieties which can cope with local climate and can produce more yield with higher concentrations of nutraceutical compounds in order to increase its economic value. Mutation breeding is one of the most commonly practiced techniques for generating genetic variation in existing gene pool of economic plants (Toker et al. 2007) up to a certain extent (Fehr 1993) and can facilitate selection in the local environment (Yadav et al. 2007). Furthermore, a large number of alleles can be manipulated for a particular trait (Chopra 2005). The final result of this mutation breeding is recessive and segregated as 3:1 ratio in diploid plants like Fenugreek, and there is need to check the results up to the second generation (Micke and Donini 1993).

Mutagens (physical or chemical) are playing a vital role for the development of new traits with more yield and quality by developing resistance to biotic or abiotic stresses. In Fenugreek breeding program, mutagens have been applied for the development of genetic variability for desirable traits. Mutation breeding in Fenugreek is carried out through spontaneous mutation or induced mutation (Petropolous 1973; Singh and Singh 1976; Laxmi and Datta 1987) using chemicals or physical mutagens like radioactive rays. There are few examples of development of Fenugreek varieties through spontaneous mutation (Petropolous 1973; Singh and Singh 1976; Laxmi et al. 1980; Laxmi and Datta 1987) like RH 3129 variety that was developed from spontaneous mutation of Moroccan cultivar having twin pods and high diosgenin contents (Laxmi et al. 1980; Petropolous 2002).

The first study reported for Fenugreek mutants by using chemical mutagens was reported by Auerbach (1961), and the seeds were obtained successfully, whereas colchicine treatment to shoot apex resulted in tetraploid plants (Roy and Singh 1968). Further studies using physical mutagens (UV irradiation, ϒ-rays) or chemical mutagens (ethyl-methanesulfonate (EMS), methyl-methanesulfonate (MMS), and sodium azide (NaN_3 or SA) have been successfully reported by different researchers. Anis and Wani (1997) reported the meiotic abnormalities (bridges, cytomixis, laggards, nondisjunction, precocious movement of chromosomes stickiness, univalents, etc.) due to caffeine treatment. Similar types of effects were also observed on the morphological characters of Fenugreek which was directly proportional to caffeine dosage.

Sodium azide (SA/NaN_3) is one of the most commonly used chemical mutagen for developing Fenugreek mutants. Siddiqui et al. (2007) applied 0.1, 0.2, 0.3, 0.4, and 0.5% NaN_3 at 24 ± 1 °C for 72 h on root tip cells of *T. foenum-graecum* for cytogenetic changes. Application of NaN_3 resulted in decreased seed germination (%), radicle length, and mitotic index, whereas increased chromosome stickiness, bridge formation, precocious separation, and lagging chromosomes were also observed. Prabha et al. (2010) checked 1.5 mM, 3.0 mM, and 4.5 mM of sodium azide for 4, 6, and 8 h and recommended the 4.5 mM sodium azide applied for 4 h for selecting new genotypes with higher seed yield of Fenugreek. Kapoor and Srivastav (2010) treated seeds with sodium azide and obtained as tetraploid and mixoploid in M_2 generation with meiotic abnormalities. Further study of M_3 generation revealed the decreased RAR in tetraploid and reduced chiasma per chromosome for both types of mutants.

Ethyl-methanesulfonate (EMS) is another mutagen that has been used for seed quality and production (Basu 2006). Earlier, Chaudhary and Singh (2001) obtained determinate mutant by treating Rmt 1 (indeterminate variety) with 0.10% EMS for 4 h and rose up to M3 generation. Their results revealed the significant difference on plant growth and insect infestation compared to Rmt 1 and UM 305 (spontaneous mutant). Basu et al. (2008) successfully developed Fenugreek mutants with early maturity, determinate growth, and high seed yield by employing EMS.

Besides using single mutagen, a number of studies reflected the use of more than one chemical mutagen or comparison of physical and chemical mutagens. The first report on comparison of multiple chemical mutagens was reported by Jain and Agarwal (1987). They obtained two- to fourfold steroidal sapogenins (diosgenin and tigogenin) in seeds and plants of *T. foenum-graecum* when treated with chemical mutagens (EMS, MMS, and NaN3) at low concentrations. Contrarily, treated with higher concentrations resulted in decreased sapogenins contents, whereas Agarwal and Jain (2015) treated *T. foenum-graecum* and *T. corniculata* seeds with different concentrations of EMS, MMS, and NaN_3 for steroidal sapogenins production. Both steroidal sapogenins were enhanced with the application of all three mutagens with maximum augmentation at 0.1 M EMS.

The studies related to comparison of physical and chemical mutagens by researchers reported the variable effects of these mutagens on developing Fenugreek mutagens. Gadge et al. (2012) treated the Fenugreek seeds with UV radiation and ethidium bromide at different doses and reported ethidium bromide as better mutagen compared to UV or UV+ ethidium bromide. Bashir et al. (2013a) applied ϒ-rays, EMS, and SA at different dose/concentration on M_1 and M_2 generations. Their conclusion was decreased mutagenic effectiveness with the increased mutagen dose/concentration, and mutagenic efficiency of mutagens was EMS > SA > ϒ-rays. In another study, Bashir et al. (2013b) reported decreased germination percentage, seedling height, percent survival, and pollen fertility with increased dose/concentration of the mutagens. They also concluded that EMS treatments were more superior to ϒ-rays and SA in inducing pollen sterility (EMS > ϒ-rays>SA). Recently, Rajoriya et al. (2016) investigated the mutagenic effect of ϒ-rays, EMS, and SA and obtained M_1 and M_2 generations. Their results revealed decreased germination, seedling height, and plant survival with increased doses/concentrations of mutagens. Comparing mutagens, ϒ-ray treatment was more detrimental than other mutagens on plant growth and survival. Application of physical and chemical mutagens in these studies revealed the variable effects of mutagens on newly developed mutants. The difference in results might be due to difference in genotypes, mutagens, concentration, and mode of application. However, it is important to note that multiple mutagens have been employed for the development of new superior mutants in recent years.

19.4 Molecular Genetic Diversity of Fenugreek

In the modern era of the twenty-first century, exploiting the natural biodiversity for novel alleles in order to improve the production, quality, nutritional value, and adaptation to different geographical regions has immense importance in modern breeding programs. Increasing human population and demand for nutrition can be coped with the application of modern plant breeding for elite crops with high yield. However, scarcity of local genetic material and use of elite cultivars resulted in erosion of genetic material and brought the crops/plants to an endangered level. Therefore, there is a need to save these endangered landraces by using biotechnological techniques for conserving the elite genes which control the yield and quality for the coming future.

Exploitation of phenotype and genotype variations in order to characterize and managing genetic diversity and germplasm collection of different plant species have been done during the last few decades. Advancement in genome mapping and sequencing methods provide a toolbox for researchers/scientists to explore the structure and function of the genome of desired organism (Baloch et al. 2017). Molecular markers enable to measure direct genetic diversity and allow to proceed further beyond indirect diversity measures, based mainly on morphological traits or geographical origin of that species. Currently, different marker systems are available for the monitoring of genetic diversity, and these molecular markers have been employed for the determination of genetic diversity of Fenugreek (Table 19.3).

Table 19.3 An overview of molecular markers used for genetic diversity of Fenugreek

Molecular markers	No of accessions/genotypes/varieties etc	References
RAPD	17 varieties	Sundaram and Purwar (2011)
	61 accessions	Choudhary et al. (2013)
	7 accessions	Haliem and Al-Huqail (2014)
	5 cultivars	Modi et al. (2016)
	48 genotypes	Mamatha et al. (2017)
AFLP	20 landraces	Ahari et al. (2014)
	24 accessions	Al-Maamari et al. (2014)
ISSR	49 accessions	Randhawa et al. (2012)
RAPD/ISSR	17 accessions	Dangi et al. (2004)
	30 genotypes	Tomar et al. (2014)
	8 varieties and 6 populations	Hora et al. (2016)
RAPD/AFLP	5 varieties	Kumar et al. (2012)

19.4.1 Random Amplified Polymorphic DNA (RAPD) Markers for Fenugreek Genetic Diversity

Studies on Fenugreek regarding the application of molecular markers revealed the use of RAPD markers more than other markers like AFLP, ISSR or comparison of two markers like ISSR/RAPD or RAPD/AFLP markers. RAPD primers (18) for assessing the genetic diversity and species relation of two taxonomically *Trigonella* species and 61 accessions were reported by Sundaram and Purwar (2011). They recorded a total of 141 bands, and 74 were polymorphic with 66–100% polymorphic band range with an average of 52.85%. Genetic similarity values of 0.66–0.90 showed the moderate to high genetic variability, whereas these populations were divided into two main clusters with two separate subgroups. Choudhary et al. (2013) evaluated the genetic variability of 17 varieties using morphological and 17 RAPD markers and recorded 57.66% polymorphism. They also divided these 17 varieties into two major clusters with 12 varieties in cluster-I and 5 varieties in cluster-II. Similarly, these varieties were also distributed into two major clusters on the basis of morphological dendrogram. It was interesting to note that morphological analysis of some varieties was not accordingly to RAPD analysis due to environmental factors.

Haliem and Al-Huqail (2014) analyzed the correlation between biochemical characteristics and genetic variation of seven wild accessions of Fenugreek collected from different ecogeographical regions by using RAPD markers. The results of molecular analysis revealed high polymorphism (94.12%), whereas 90.00 and 93.75% total polymorphism values were recorded for acid phosphatase and glutamate-oxaloacetate transaminase. Modi et al. (2016) analyzed the 5 *Trigonella* cultivars to assess the genetic diversity by using 11 RAPD primers. They reported a total of 80 bands of 200–3060 bp size, of which 66 were polymorphic with 82.50% polymorphism. They also reported Jaccard's similarity coefficient of 0.266–0.615 and constructed a dendrogram which revealed two clusters. Mamatha et al. (2017) analyzed the genetic diversity of 48 *Trigonella* genotypes by using 30 RAPD markers which yielded 119 bands of 50.00–91.66% polymorphism with 79.21% polymorphism, whereas polymorphism information content (PIC) value was ranged 0.66–0.90, and these genotypes were clustered into 10 groups at 0.75 similarity coefficient.

19.4.2 Amplified Fragment Length Polymorphism (AFLP) Markers for Fenugreek Genetic Diversity

There are only two reports which revealed the use of AFLP markers for Fenugreek. Twenty Fenugreek accessions collected from different parts of Oman with 4 accessions from Iraq and Pakistan were compared by Al-Maamari et al. (2014). They employed 6 AFLP markers and attained 1852 polymorphic loci from 24 accessions.

The highest genetic diversity (H) of 0.2146 was recorded for Omani populations as compared to 0.0844 (Pakistan) and 0.1620 (Iraq). Their results proved the cultivation of Fenugreek for long time with frequent exchange of genetic material among Fenugreek accessions cultivated in Oman. Another study by Ahari et al. (2014) revealed the use of 20 landraces of Iranian Fenugreek genetic diversity with the help of AFLP markers. They obtained 147 bands with 50–500 bp size and 87% polymorphism by using 5 AFLP primers. The results of PIC were scored 0.79 (Kashan), 0.93 (Broojerd), and 0.93 (Kashan), whereas genetic similarity coefficient was scored 44–94% among landraces.

19.4.3 Inter Simple Sequence Repeat (ISSR) Markers for Fenugreek Genetic Diversity

There is only a single report regarding use of ISSR markers for assessing the genetic diversity of Fenugreek. Randhawa et al. (2012) analyzed the 49 accessions of Fenugreek collected from different locations using 19 morphometric and 186 ISSR markers. The morphometric data classified the accessions into two clusters with ~65% similarity. Initial screening with 100 ISSR primers resulted in 21 polymorphic primers, and these 21 primers generated 186 amplicons with 92.4% polymorphism, whereas 47 accessions were classified as single group with ~65% similarity on the basis of cluster analysis.

19.4.4 ISSR/RAPD Markers for Fenugreek Genetic Diversity

Most of the studies on molecular genetic diversity of Fenugreek have the use of two markers for assessing and comparing the genetic diversity. Dangi et al. (2004) studied genetic diversity of 17 accessions of *T. foenum-graecum* and 9 accessions of *T. caerulea* collected from different parts of the world by using ISSR, RAPD, and ISSR+RAPD markers. Their results revealed the distribution of accessions from different geographical regions of both species into different groups. They also reported higher genetic similarity indices of *T. caerulea* compared to *T. foenum-graecum.* Similarly, molecular and biochemical characterization of ten Fenugreek accessions was reported by Harish et al. (2011) using ISSR and RAPD markers.

A study by Tomar et al. (2014) using 30 RAPD and 20 ISSR markers and 30 Fenugreek genotypes yielded 250–1300 bp products, whereas a relatively higher proportion of polymorphic bands were recorded for RAPD (76.78%) compared to ISSR (68.08%). The dendrogram constructed for RAPD and ISSR revealed the classification of genotypes into two main groups. Recently, Hora et al. (2016) checked the genetic diversity of 8 varieties and 6 populations of Fenugreek collected from Northern India by using 100 ISSR and 400 RAPD markers. The polymorphism

among different Fenugreek varieties and populations was recorded 42.91% for RAPD and 55.66% for ISSR markers. They also reported the effective use of cluster analysis for unraveling the genetic variation within the accessions and use of RAPD and ISSR markers for assessing the genetic diversity and genetic relationship.

19.4.5 RAPD/AFLP Markers for Fenugreek Genetic Diversity

Nine RAPD and 17 fluorescently labeled AFLP primers for assessing the genetic diversity of 5 varieties are cultivated in India by Kumar et al. (2012). They reported 47 bands with 200–5000 bp size and average polymorphism of 62.4% for RAPD markers, whereas 669 bands with 50–538 bp size were amplified for AFLP primer combinations (PCs). The mean genetic diversity (Nei's 1973) of 23.83% (RAPD) and 2.1% (AFLP) was recorded across all loci. Results also revealed more polymorphism for RAPD markers compared to AFLP markers, whereas reproducibility and authentication of AFLP markers were more compared to RAPD markers.

The studies on molecular markers reflected the use of these markers for optimization of genetic diversity by using a single marker or comparison of two markers for same number of accessions, genotypes, varieties, etc. In all these studies, researchers used variable number of accessions/genotypes/varieties collected from their own region or other regions of the world. In general, there is need to use more detailed work with more focus on using a number of accessions/genotypes/varieties for future studies to select target-specific superior traits on the basis of molecular markers for specific geographical region with more yield and quality.

19.5 In Vitro Cell/Tissue Culture of Fenugreek

Fenugreek is an important medicinal plant that contained bioactive compounds like alkaloid, saponins, choline, steroidal sapogenins trigonelline, trigocoumarin, and trimethyl (Aasim et al. 2014). Although, fenugreek varieties developed all over the world have better morphological characteristics, wide geographical adaptation, and more yield, the primary objective of these efforts made to date to improve Fenugreek is based on these bioactive compounds. It is very important to understand the variations that occur in metabolites production or medicinal pathway (Al-Habori and Raman 2002) for genetic improvement of Fenugreek.

Plant cell and tissue culture techniques provide direct production of elite plants or induction of callus, cell suspension cultures, somatic embryogenesis, or genetic transformation (Aasim et al. 2014) for the production of economically important diosgenin and trigonelline (Oncina et al. 2000; Ramesh et al. 2010). The results of different researches show the advantage of isolation of secondary metabolites through in vitro cell culture compared to whole plant or seeds taken from field conditions. Furthermore, the cells or plants taken from in vitro culture are consistent

and elite in nature due to being grown under controlled environment. Furthermore, application of different chemicals/enzymes/organic compounds or controlled change in culture conditions more efficiently makes it possible to change the metabolite concentration. There are a number of reports available which highlight the use of different plant tissue culture techniques like callus culture, cell suspension culture, protoplast culture, and organogenesis under in vitro production which have been employed for genetic improvement and phytochemical production.

19.5.1 In Vitro Cell Suspension Culture of Fenugreek

Cell suspension culture is the most common technique used for the synthesis of secondary metabolites. Furthermore, it also allows the researchers to check the efficacy of different chemicals or organic compounds on cell growth and subsequently secondary metabolites production of economic plants. This technique was first employed by Cerdon et al. (1945) in Fenugreek, and they reported 20% decreased cell growth when culture medium was provided with 125 μM diniconazole compared to control after 21 days of culture. The reduction in plant growth due to diniconazole treatment resulted in 50% decreased total sterol contents. Later on, Khanna et al. (1975) gained more sapogenin contents by adding cholesterol in the suspension culture medium. Positive bearings of mevalonic acid on steroidal sapogenin synthesis during cell suspension cultures of Fenugreek tissues were reported by Trisonthi et al. (1980). Similarly, application of cholesterol in cell suspension culture also resulted in enhanced sapogenin contents (Brain and Williams 1983). A clear correlation between copper and de novo synthesis of medicarpin (isoflavonoid pterocarpans) using cell suspension culture has been reported by Tsiri et al. (2009), whereas 37% more trigonelline contents have been reported by adding nicotinic acid in the cell suspension culture of Fenugreek (Ramesh et al. 2010).

19.5.2 In Vitro Protoplast Culture of Fenugreek

The studies about protoplast culture of Fenugreek are limited and used for both in vitro isolation of secondary metabolites and shoot regeneration. The first study on protoplast culture was reported by Shekhawat and Galston (1983), and they successfully gained green calli and leafy shoots. They used mesophyll protoplasts taken from leaf explant followed by culture on medium enriched with 0.1 mg/l 6-Benzylaminopurine (BAP) and Zeatin. Christen (2002) successfully developed protoplast culture using root apices explant, but they failed to convert it into shoots. However, successful shoots induction from protoplast taken from root apices were reported by Petropoulos (2002) and Mehrafarin et al. (2010). They also achieved more trigonelle contents from callus that were 3–4-folds more than seeds and 12- to 13-folds more than roots and shoots.

19.5.3 In Vitro Callus Culture of Fenugreek

Callus culture is an important technique used for plant proliferation, somatic embryogenesis, cell suspension culture, protoplast culture, and isolation of secondary metabolites in Fenugreek. Most of these studies on callus culture of Fenugreek were used or developed for secondary metabolites isolation rather than shoot/plant proliferation. Callus induction using different explants, plant growth regulators, and culture conditions proved to be more economic and efficient for secondary metabolites production compared to seeds. Joshi and Handler (1960) reported the importance of nicotinic acid and s-adenosylmethionine for trigonelline production enriched with additional adenosine triphosphate (ATP) and $MgCl_2$ in the culture medium. Their results revealed three- to fourfold more trigonelline contents compared to seeds. They also reported 12- to 13-fold more trigonelline contents from callus culture than roots or shoots culture. Khanna and Jain (1973) reported higher steroidal contents (diosgenin, gitogenin, tigogenin) and spirostane derivatives from callus culture using 1 mg/l 2,4-D on agar solidified MS medium. The best culture time for the production of these metabolites were optimized as 6-week-old callus cultures. Radwan and Kokate (1980) attained more trigonelline contents (15.6 mg/g of dry wt) after 4 weeks of culture which were 3- to 4-folds more than seed and 12- to 18-folds more than roots/shoots culture, whereas increased trigonelline contents were also recorded on medium supplemented with 10 mg/l 2,4-D, IAA, IPA, and NAA.

Higher trigonelline contents from calli compared to in vivo culture using different explants were presented by Ahmed et al. (2000). The trigonelline contents under in vivo conditions were recorded as 0.45 mg/g (leaves), 0.21 mg/g (stems), and 0.29 mg/g (roots), whereas trigonelline contents from calli were recorded as 0.61 mg/g (leaves), 0.30 mg/g (stems), and 0.40 mg/g (roots). Oncina et al. (2000) also used calli of different explants for diosgenin production and obtained 2.2 mg/g of dry wt. (leaf), 0.74 mg/g (stem), and 0.60 mg/g (root) from 45-day-old calli. Rezaeian (2011) reported increased callus induction with increase in 2,4-D and achieved maximum callus induction from shoot apical meristem explant after 45 days of culture, whereas diosgenin contents were high in leaf calli compared to shoot or root callus. Variable effects of mannitol and sodium chloride on calli growth and secondary metabolites levels were reported by Hussein and Aqlan (2011). The highest total chlorophyll and protein contents from callus culture (2.727 mg/g) compared to 0.789 mg/g from in vitro regenerated shoots and 0.421 mg/g from fresh callus were recorded (Prabakaran and Ravimycin 2012). Recently, importance of harvesting time, type of media, and plant organ on the concentration of diosgenin of Fenugreek was highlighted by Ciura et al. (2015). The highest content of diosgenin was recorded from leaves compared to stems, roots, and callus culture. They also reported the highest content of diosgenin between the 21st and 38th day of growth. Alalwani and Alrubaie (2016) checked the effects of PEG and combination of PEG+magnetic water (0% PEG+1000G, 3% PEG +1000G, 6% PEG +1000G, 9% PEG +1000G) on the production of trigonelline from callus of *T. foenum-graecum* L. Provision of 1 mg/L BA +1 mg/L 2,4-D was optimized for callus induction, whereas

9% PEG and 9% PEG +1000G magnetic water resulted in maximum trigonelline contents from callus

Besides use of callus for secondary metabolites production, a number of studies revealed the successful use of different explants and culture conditions for callus induction. Shekhawat and Galston (1983) reported 0.1 mg/l of BAP, zeatin, glutamine, and asparaginase in the culture medium as best for callus induction and differentiation, rapid cell division, and growth, whereas Azam and Biswas (1989) reported MS medium enriched with NAA, 2,4-D, kinetin, and coconut water for callus induction and growth of Fenugreek. El-Bahr (1989) reported MS medium enriched with 3% sucrose and 2 mg 2,4-D for optimum callus induction of Fenugreek. Seyedardalan and Mahmood (2013) reported direct somatic embryogenesis using hypocotyls. MS medium containing 3 mg/l picloram+0.5 mg/l BAP was optimal for globular embryos induction followed by 2 more weeks for maturation. Abd Elaleem et al. (2014) successfully developed callus from cotyledons and hypocotyls explants. MS and B5 media augmented with 2,4-D and NAA resulted in 100% callus induction.

In recent years, number of studies highlighted the successful callus induction using different explants and culture conditions but failed to obtain shoot induction from induced callus. Aasim et al. (2010) achieved callus induction from hypocotyl explant but failed to get shoots from induced calli. El-Nour et al. (2013) induced calli by using 8- to 20-day-old cotyledonary node and hypocotyl explants cultured on MS and B5 media containing different PGRs. They achieved maximum callusing index value (2.8) from MS medium enriched with 1.5 mg/l, 2,4-D using hypocotyls and cotyledons explants. In another study, El-Nour et al. (2015) successfully achieved callus induction of Fenugreek using cotyledons and hypocotyl explants cultured on MS medium containing 0.5 mg/l Kin with different concentrations of 2,4-D and NAA. Among explants, hypocotyl explant was more responsive than cotyledon for callus induction. The highest mean callus index for hypocotyl (3.50 ± 0.15) and cotyledon (2.41 ± 0.18) was recorded on medium enriched with 4.0 mg/l NAA+ 0.5 mg/l Kin and 1.0 mg/l 2, 4-D, respectively, after 6 weeks of culture. In both studies, they failed to induce shoots from calli.

19.5.4 In Vitro Organogenesis/Regeneration of Fenugreek

In vitro organogenesis of Fenugreek is one of the greatest challenges for researchers to develop reliable and reproducible protocol, although a number of studies on in vitro regeneration through direct or indirect organogenesis or direct or indirect somatic embryogenesis have been reported for Fenugreek. But these studies have major drawbacks like difficulties in propagation, rooting, and adaptation which make this plant recalcitrant in nature. Therefore, callus induction or somatic embryogenesis employing different techniques like cell suspension culture, callus induction, or protoplast culture for secondary metabolites production are more preferable compared to organogenesis. Although, reports are available which reflect the

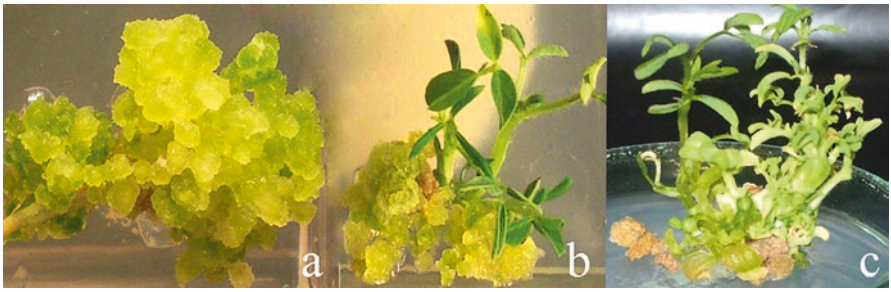

Fig. 19.2 Callus induction and shoot regeneration from hypocotyl and cotyledon node explant. (**a**) Callus induction on hypocotyl explant; (**b**) shoot regeneration using BAP-NAA; (**c**) and kinetin (Aasim et al. 2010)

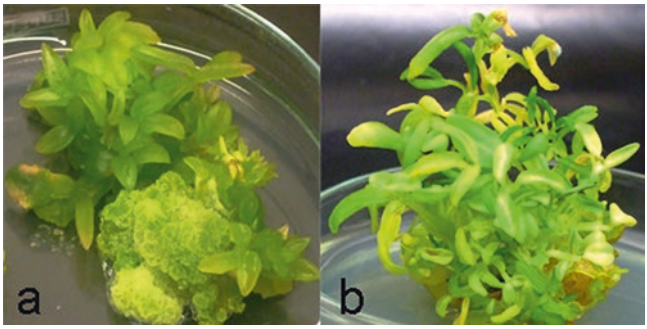

Fig. 19.3 Shoot regeneration from cotyledon node explant. (**a**) Hyperhydric shoots on MS medium supplemented with TDZ and (**b**) normal shoots on MS medium supplemented with TDZ-IBA (Aasim et al. 2010)

development of protocol in order to gain plants/plantlets under in vitro for further studies like genetic transformations. Khawar et al. (2004) successfully obtained in vitro regenerated shoots induction from apical meristem but failed to get rooted plantlets.

Different explants (cotyledonary nodes, leaves, and hypocotyl) of Fenugreek cultured on different PGRs like TDZ-IBA, BAP-NAA, and kinetin were tested by Aasim et al. (2010). There was no shoot regeneration from hypocotyl explants on any medium, but cotyledonary node explants responded well to BAP-NAA, kinetin (Fig. 19.2), and TDZ-IBA (Fig. 19.3) to induce multiple shoots. Among these PGRs, TDZ-IBA induced more number of shoots compared to others. However, they did not achieve rooted plantlets, and no acclimatization was performed.

Afsharie et al. (2011) checked the efficacy of different basal medium salts, PGRs, and explants (stem segments, embryos, and hypocotyls) for in vitro regeneration potential of Fenugreek. Their results revealed that both B5 or MS medium with 2.5 mg/l BAP + 0.5 mg/l NAA were optimum for somatic embryogenesis and 1.5 mg/l BAP + 0.5 mg/l NAA for shoot regeneration. Prabakaran and Ravimycin

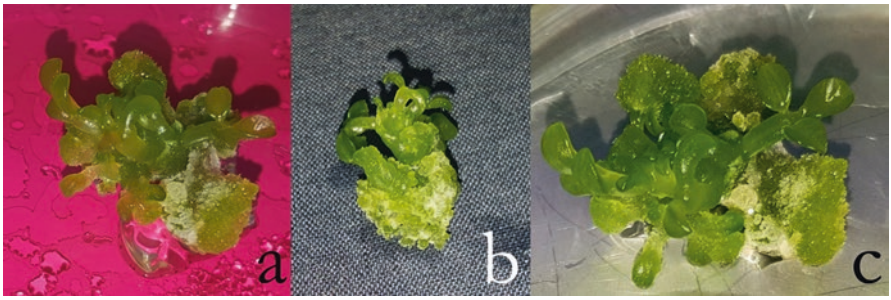

Fig. 19.4 Multiple shoot regeneration of Fenugreek (*Trigonella foenum-graecum* L.) using cotyledonary node explants, (**a**) callus induction, (**b**) shoot induction, and (**c**) multiple elongated shoots (Taşbaşi et al. 2017)

(2012) reported successful use of shoot tip explants for multiple shoot induction of Fenugreek. They achieved a maximum of two and four shoots from medium supplemented with 1.0 mg/l BA and 0.5 mg/l Kin, respectively, after 30 days of culture. However, no information about rooting and acclimatization of in vitro regenerated shoots was provided.

Indirect organogenesis through somatic embryogenesis was reported by Al-Mahdawe et al. (2013) using cotyledonary node explants. The process involved callus induction>somatic embryogenesis>secondary somatic embryos and embryoids>rooting> plantlets. Although they achieved plantlets, no information was given about plantlets transferred to soil. Pant et al. (2013) used different explants (leaf, stem, root, cotyledonary node, and hypocotyl) of Fenugreek on media supplemented with different PGRs. They achieved maximum shoot induction from leaf and stem explant cultured on medium containing 0.5 ppm BAP, whereas maximum shoots from cotyledonary node were achieved from medium supplemented with 0.1 ppm TDZ. Vaezi et al. (2015) cultured hypocotyl and cotyledon explants on MS medium provided with 2,4-D and Kin for callus induction followed by subculture to medium containing BAP and NAA for shoot induction. 5.0 mg/l BAP + 5.0 mg/l NAA was found best for maximum number of shoots per explant from hypocotyl explant.

Recently, two studies on in vitro regeneration of Fenugreek have been reported about the efficacy of sucrose concentration, explants age, and explant type (Taşbaşi et al. 2017; Kavci et al. 2017). Cotyledonary nodes and leaf explants taken from 18- to 20-day-old c seedlings were cultured on Phytagel-solidified MS medium with different sucrose concentrations (1.5, 3.0, 4.5, and 6.0%) and TDZ (0.40, 0.80, and 1.20 mg/l) + 0.20 mg/l NAA. Both explants induced 100% callus but no shoot induction from leaf explants, whereas a maximum of 18.75 shoots/shoot buds were achieved from MS medium enriched with 0.40 mg/l TDZ + 0.20 mg/l NAA and 1.5% sucrose concentration (Fig. 19.4-Taşbaşi et al. 2017).

In another study, Kavci et al. (2017) used 10- and 20-day-old cotyledonary node explants and cultured on Gelrite-solidified MS medium containing TDZ (0.40, 0.80, and 1.20 mg/l + 0.20 mg/l NAA) and different sucrose concentrations (1.5, 3.0, 4.5,

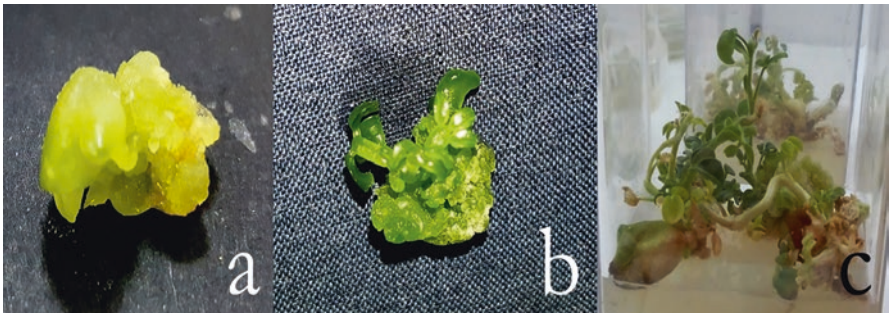

Fig. 19.5 Multiple shoot regeneration of Fenugreek (*Trigonella foenum-graecum* L.) using cotyledonary node explants, (**a**) callus induction and (**b, c**) multiple shoot induction (Kavci et al. 2017)

and 6.0%). They reported callus induction followed by somatic embryogenesis (100%) after 4 weeks of culture followed by development of shoot buds and shoots. Twenty-day-old explants were more effective than 10-day-old explants. A maximum number of shoots/shoot buds were recorded on medium containing 0.80 mg/l TDZ + 0.20 mg/l NAA + 4.5% sucrose. Burdak et al. (2017) inoculated shoot apex explant of different genotypes using different growth variants. Maximum callus induction frequency was recorded on MS medium supplemented with 0.5 mg/l BAP + 0.5 mg/l 2,4-D, whereas de novo shoot regeneration was achieved after subculturing of calli to 0.5 mg/l BAP-containing medium followed by rooting on medium supplemented with 0.2 mg/l IAA (Fig. 19.5).

Application of plant cell and tissue culture techniques in Fenugreek revealed the significance and superiority of this biotechnological tool. Different techniques like in vitro cell suspension culture, protoplast culture, and callus induction have been reported more advantageous for metabolites compared to seed and plant. On the other hand, few reports also reflected the successful use of callus for somatic embryogenesis and shoot induction. The study also reveals the successful in vitro organogenesis from different explants and culture conditions. However, information about rooting and adaptation is very rare or not provided which shows the recalcitrant nature of *Trigonella* plant and challenge for researchers to develop reproducible and complete plant tissue culture protocol for the application of other biotechnological techniques for its improvement. Development of in vitro regeneration of Fenugreek plantlets will allow researchers to incorporate genes of interest through genetic transformation studies.

19.6 Genetic Transformation Studies in Fenugreek

Genetic transformation of desired trait or gene to medicinal plant in order to obtain economically and medicinally important bioactive molecules or compounds has been common in the past years. However, there are few studies which successfully

report the use of *Agrobacterium tumefacien*s or *Agrobacterium rhizogenes* in Fenugreek. *A. rhizogenes* has been used for the production of hairy roots of Fenugreek in order to produce important secondary metabolites like diosgenin. Although the number of these studies are very low, they revealed the successful use of different *A. rhizogenes* strains for hairy root production followed by production of diosgenin (Merkli et al. 1997) or trigonellin (Raheleh et al. 2011) contents.

Merkli et al. (1997) established successful hairy root culture of *Trigonella foenum-graecum* L. using *Agrobacterium rhizogenes* strain A4 by infecting 2-week-old stems of sterile plantlets. They checked the root growth and diosgenin contents of hairy roots cultured on different mediums like WP, MS, and B5 for 35 days. The maximum growth (606 mg) with maximum growth index (80) was recorded from WP medium containing 3% sucrose, whereas maximum diosgenin content (0.040% dry weight) was achieved from on-half WP liquid medium with 1% sucrose compared to control (0.024% dry weight). Raheleh et al. (2011) used different *A. rhizogenes* (A4, 9126 and 15,834) and two different techniques for infection (cocultivation and injection) for checking the transformation efficacy and trigonellin production of two Iranian masses of *T. foenum-graecum.* (Zanjan and Borazjan). They achieved 100% hairy root production from all strains, whereas 26% transformation efficiency was recorded by injection method. They also achieved the highest trigonelline amounts of 14.89 (Borazjan – 28 days) and 14.03 mM g-1 DW (Zanjan – after 7 days).

Besides using *A. rhizogenes* for hairy root and secondary metabolites production, it has been used for gene function or expression. Shahabzadeh et al. (2013) evaluated the transformation frequency using *A. rhizogenes* strain K599 harboring a *GFP* gene. They inoculated the leaf and stem explants taken from two different ecotypes (Karaj and Bushehr) with three different OD_{600} concentrations (0.8, 1.2, and 1.6). Stem explant induced more hairy roots (8.09) with 81.3% transformation frequency compared to leaf explant, whereas a maximum of 8.76 transgenic hairy roots, 79.76% transformation frequency, and 0.77 d^{-1} growths rate of transgenic roots were recorded at OD_{600} of 1.2 for K599 strain. Their results reflected the importance of genotype, type of strain, explant, and inoculation condition for successful production of transgenic hairy roots for subsequent secondary metabolites production in Fenugreek.

Besides the use of *A. rhizogenes*, there is single report available on the use of *A. tumefaciens* for genetic transformation in Fenugreek by Khawar et al. (2004). They inoculated 1-week-old cotyledon, root, and hypocotyl explants with oncogenic *A. tumefaciens* strain A281 harboring β-glucuronidase (GUS) gene. Tumors induced with GUS gene were expressed by histochemical analysis, and presence of *uidA* gene was successfully confirmed by PCR amplification. There is no report available which highlights the use of economically important gene like insect or herbicide resistance genes in Fenugreek. Similarly, use of other technologies for genetic transformation like biolistic or protoplast is not available. This might be due to lack of proper tissue culture protocol, rooting problems, and transformation efficiency.

19.7 Genomic Studies of Fenugreek

A limited number of functional genomic studies of Fenugreek have been reported to date irrespective of large number of studies about isolation, characterization, and clinical studies of diosgenin and other bioactive compounds of Fenugreek. However, studies related to genes responsible for the biosynthesis of these phytochemicals are very rare. Similarly, a limited number of studies about genome sequencing are available to date. The first study about de novo transcriptome analysis, diosgenin pathway, and genes responsible for diosgenin biosynthesis in *T. foenum-graecum* was reported by Vaidya et al. (2012). They used sequencing messenger ribonucleic acid (RNA) aided with a SOLiD 4 Genome Sequencing Analyzer for transcriptome analysis. They obtained a total of 42 million high-quality reads, and de novo assembly was performed using Velvet at different *k*-mer, Oases, and CLC Genomics Workbench, which yielded 20,561 transcript contigs, and 18,333 transcript contigs were annotated functionally. About 6775 transcripts were found related to plant biochemical pathways including the diosgenin biosynthesis pathway according to Kyoto Encyclopedia of Genes and Genomes pathway mapping.

Chaudhary et al. (2015) investigated the effects of methyl jasmonate (MeJA) on diosgenin biosynthesis and gene expression of six Fenugreek varieties. Application of 0.01% MeJA significantly increased diosgenin levels from 0.5%–0.9% to 1.1%–1.8% within 12-day-old seedlings, whereas MeJA also upregulated the expression of two pivotal genes of the mevalonate pathway, the metabolic route leading to diosgenin: 3-hydroxy-3-methylglutaryl-CoA reductase (*HMG*) and sterol-3-β-glucosyl transferase (*STRL*). Increased gene expression of *HMG* and *STRL* genes was recorded for Gujarat Methi-2 and Kasuri-2 variety. They concluded the use of MeJA as a promising elicitor for diosgenin production by Fenugreek plants.

Ciura et al. (2017) reported the first report on the next-generation sequencing of cDNA-RDA products of Fenugreek. They used methyl jasmonate for elicitation and cholesterol and squalene as precursor feeding for enhancement of sterols and steroidal sapogenins of in vitro grown plants for representational difference analysis of cDNA (cDNA-RDA). Differential, factor-specific libraries were subjected to the next-generation sequencing for identifying genes responsible for diosgenin biosynthesis. Approximately 9.9 million reads were obtained, trimmed, and assembled into 31,491 unigenes with an average length of 291 bp. Functional annotation and gene ontogeny enrichment analysis was achieved by aligning all unigenes with public databases. They identified the novel candidate genes responsible for diosgenin biosynthesis and validated their expression by using quantitative RT-PCR analysis. Their results revealed the biosynthesis of diosgenin from cycloartenol via cholesterol. These results open the new window for the breeders and researchers to understand the biosynthesis pathway, genes responsible for biosynthesis, and genome sequence to find more functional genes responsible for plant growth and production of bioactive compounds of Fenugreek.

19.8 Conclusion

Fenugreek is an underutilized plant all over the world where it is used for various purposes based on the demand of the community that ranged from its use as vegetable to spice and medicinal plant. The wide distribution of plants in different geographic regions has wide genetic variability, but studies related to its genetic variability are very limited. Although extensive work related to artificial mutation using physical and chemical mutagens have been reported under in vitro conditions for its bioactive compounds, there is also need to do more work on its agronomic characterization and acclimatization to different environmental conditions. Similarly, different plant tissue culture protocols have been employed successfully with aim to improve the major bioactive compounds contents. But success about the development of in vitro grown plantlets is still the challenge for the researchers for the application of modern biotechnological tools like genetic transformation studies to incorporate genes of interests. The main drawback of Fenugreek is the availability of limited work related to its functional genomics, gene expression studies, genome sequencing, and other plant omics. There is also a need to explore the potential of plant by applying biological tools like QTL or MAS in order to identify the potential genes for future conventional or modern breeding programs for developing elite cultivars against biotic or abiotic stresses to improve yield and nutraceutical values. The potential of Fenugreek as medicinal plant has been exploited well compared to its other uses. There is also a need to exploit the potential of Fenugreek as forage crop and edible uses by developing new cultivars with the aid of biotechnology.

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