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REVIEW

Fenugreek (*Trigonella foenum-graecum* L.) seed: a review of physiological and biochemical properties and their genetic improvement

Peiman Zandi · Saikat Kumar Basu · Leila Bazrkar Khatibani · Morufat Oladimeji Balogun · Matthew Olaleke Aremu · Manorma Sharma · Ashwin Kumar · Ratnabali Sengupta · Xianping Li · Yanshan Li · Sonam Tashi · Abdouli Hedi · William Cetzal-Ix

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Abstract Discovering the complexity of seed structure and function along with a number of vital processes such as seed growth and development, germination are important factors in unlocking the secrets of consistent crop yield. Fenugreek (*Trigonella foenum-graecum* L.), a multi-purpose annual, dryland-adapted, forage, legume crop is cultivated in different parts of the world with great potential for introduction under suitable agro-climatic zones in sub-Saharan Africa and Latin America. Fenugreek seed is used extensively for its medicinal, pharmaceutical and nutraceutical properties. It is effective in the treatment of diabetes, hyperglycaemia (thyroxine-induced type) and hypercholesterolemia. This review discusses seed physiological processes and several important biochemical seed constituent, e.g., steroidal sapogenins (diosgenin), polysaccharide fiber (galactomannan), amino acid (4-hydroxyisoleucine), etc, with important medicinal and pharmacological characteristics impacting human and animal health. However, there are noticeable differences in the quality of several phytochemicals found in fenugreek seed possibly due to variations in plant genotypes and agro-climatic conditions under which the crop is grown. Hence, it is important to note that for consistent seed yield and quality of fenugreek cultivars there is an urgent need for

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P. Zandi, S. K. Basu, L. B. Khatibani, M. O. Balogun, M. O. Aremu, M. Sharma, A. Kumar, R. Sengupta, X. Li, Y. Li, S. Tashi, A. Hedi, W. Cetzal-Ix contributed equally to this work.

P. Zandi (✉)
Department of Agronomy, Takestan Branch, Islamic Azad University, P.O. Box 466, Takestan, Iran
e-mail: z_rice_b@yahoo.com

S. K. Basu
Department of Biological Sciences, University of Lethbridge, Lethbridge, AB T1K 3M4, Canada

L. B. Khatibani
Department of Plant Breeding and Biotechnology, University of Zabol, P.O. Box 98615-538, Zabol, Iran

M. O. Balogun
Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan 900001, Nigeria

M. O. Aremu
Department of Chemical Sciences, Federal University of Wukari, P.M.B. 1020, Wukari, Taraba, Nigeria

M. Sharma · A. Kumar
Department of Botany, University of Rajasthan, Jaipur 302004, Rajasthan, India

R. Sengupta
Department of Zoology, West Bengal State University, Barasat 700126, West Bengal, India

X. Li · Y. Li
Industrial Crop Research Institute, Yunnan Academy of Agricultural Sciences, Kunming 650205, Yunnan, People's Republic of China

S. Tashi
College of Natural Resources, Royal University of Bhutan, Lobesa, Punakha, Bhutan

A. Hedi
Department of Animal Science, Cartgage University, E.S.A 7030 Mateur, Tunisia

W. Cetzal-Ix
Centro de Investigación Científica de Yucatán, A. C. Herbarium CICY, 97200 Mérida, Yucatán, México

continuing efforts in genetic improvements and in developing high yielding, disease and drought-resistant varieties suitable for different agro-climatic conditions. Therefore, in addition to the physico-biochemistry of fenugreek seed different approaches for genetic improvement have also been discussed.

Keywords Fenugreek · *Trigonella foenum-graecum* L. · Seed physiology · Chemical compounds · Phytochemicals · Genotype X environment interaction · Genetic improvement

Abbreviations

4-OH-ILE-C	4-Hydroxyisoleucine content
4-OH-ILE-P	4-Hydroxyisoleucine productivity
ABA	Abscisic acid
AFLP	Amplified fragment length polymorphism
BA	Benzyladenine
BCAA	Branched-chain amino acid
DIOS-C	Diosgenin content
DIOS-P	Diosgenin productivity
GA_3	Gibberellic acid
GE	Genotype X environment
Gal	D-galactopyranosyl
GAL-C	Galactomannan content
GAL-P	Galactomannan productivity
GP	Germination percentage
GR	Germination rate
ISSR	Inter-simple sequence repeat
Man	β-D-mannopyranosyl
NAD	Nicotinamide adenine dinucleotide
RAPD	Random amplification of polymorphic DNA
SSA	Sub-Saharan Africa

Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is a self-pollinating annual forage legume and is widely known for its medicinal, pharmaceutical and nutraceutical properties. In addition to its use as a cover crop and a green manure and forage crop, it is also known widely in different parts of the globe as an important spice crop and a medicinal herb. The crop is mostly grown in the Indian subcontinent, parts of west Asia, Middle East, North Africa, United Kingdom, Russia, Mediterranean Europe, Australia, US and Canada (Acharya et al. 2008). Legumes are important sources of dietary proteins for both human and animals. However, the acceptability and applications of legumes in diets are restricted due to presence of anti-nutritive factors. The nutritional quality is thus compromised by such anti-nutritive factors interacting with the intestinal tract; for example, phytate, tannins and oxalates reducing protein digestibility and efficient absorption of amino acids (Udensi et al. 2005). Several researchers have reported that unless these compounds are actively destroyed by heat or alternate treatments, they can cause negative physiological effects when ingested by both humans and animals (Duhan et al. 2002). It has been clearly demonstrated that by applying specific food processing approaches such as boiling, cooking, sprouting, fermenting and roasting, the anti-nutritional and flatus factors are reduced substantially (ElMaki et al. 2007; Aremu et al. 2010). It is hence important to curb these anti-nutritional factors in the legumes if they are targeted to be exploited effectively as an inexpensive source of dietary protein (Wang and Fields 1978). It will be, therefore, important to investigate the potential anti-nutritional factors in fenugreek crop too for its better utilization as a cheap dietary source protein (Acharya et al. 2008). The multipurpose use of fenugreek seed as a spice in foods or as a condiment in artificial flavoring of maple syrup or in the production of steroid and other hormones for the pharmaceutical, nutraceutical and functional food industries (Basu et al. 2008a), calls for a deeper understanding of the seed structure and compounds. Despite the fact that there are several species of *Trigonella* distributed all over the world, most studies in respect of seed structure and seed physiology have been focused on the popular agronomic species *T. foenum-graecum* L. (Acharya et al. 2006). This review aims at exploring the physiological and biochemical properties of fenugreek seed and the impact of genotype X environment (GE) interactions on the main biologically active compounds available in the seed. The review also investigates different approaches for genetic improvement for consistent improvement in fenugreek seed quantity and quality.

The seed: structure and compounds

Generally, fenugreek seed's dimension constitute 0.3–0.6 cm in length, 0.2–0.4 cm in width and 0.2 cm in thickness (Fig. 1) (Fazli and Hardman 1968; Petropoulos 2002). The seeds are rectangular, square or irregular in shape with a specific concavity on the outer surface separating the radicle from the cotyledon (Slinkard et al. 2009). The seed varies in color from yellowish brown to luteous when mature, but genotypes that miss polyphenolic tannins manifest inconspicuous (yellowish to white) coloration (Basu 2006; Lee 2009). There are also some varieties with the ability to produce mature seeds which are either green or yellowish green in color (McCormick et al. 2009). The

Fig. 1 Fenugreek seed

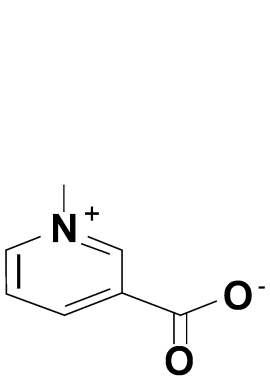
seed coat (testa) that encloses the seed is separated from the embryo by a well-developed semi-transparent endosperm tissue (Fazli and Hardman 1968) which is the main storage organ in mature seeds (Spyropoulos 2002). In the mature seeds, the majority of the endosperm cells are non-living and its cytoplasmic contents are filled with stored reserves (galactomannans) (Petropoulos 2002). More precisely, the aleurone layer that stands across the endosperm and the seed cover consisted of solely unique live cell layer (Reid and Meier 1972; Spyropoulos 2002). The aleurone layer cells are small and thick walled and entail aleurone grains, which disappear during the process of seed germination (Reid and Meier 1972).

Seed chemical compounds

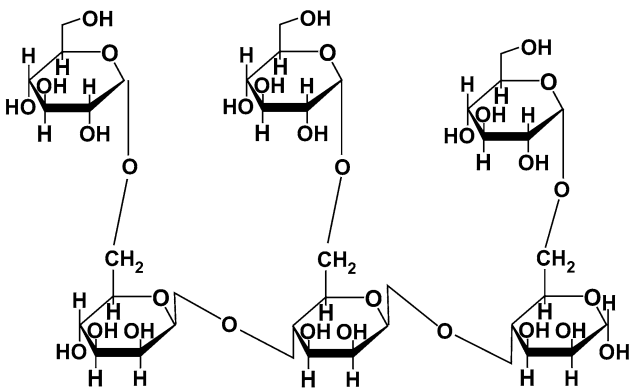
The chemical composition of fenugreek seed constitutes several pyridine alkaloids among which trigonelline (Fig. 2a) is the most substantial (Skaltsa 2002; Mehrafarin et al. 2010). The synthesis of trigonelline in seeds is much less than in pericarps; however, its content inside the seeds is significantly higher than in the pericarps (Mehrafarin et al. 2010). In other word, parts of synthesized trigonelline in pericarps are possibly transmitted to seeds to be utilized at later stages during germination (Zheng et al. 2004). This fact implies that the accumulated trigonelline transforms to nicotinic acid and appears likely to be used up later for nicotinamide adenine dinucleotide (NAD) synthesis (Mehrafarin et al. 2010). The seed chemical constituents (i.e., saponins, fiber, protein, amino acids and fatty acids) vary remarkably (Taylor et al. 2000; Acharya et al. 2006) depending on their varietal and ecological factors (Lee 2009).

Galactomannans (Fig. 2b) or mucilaginous fibers are predominantly hemicellulosic polysaccharides found in seeds of fenugreek and represent ~17–50 % (Raghuram et al. 1994; Kochhar et al. 2006) of dry seed weight. The seed endosperms among legume members such as guar [*Cyamopsis tetragonoloba* (L.) Taub.] and fenugreek are enriched by galactomannan to a large extent (Reid 1985). The galactomannans constitute the bulk of the cell walls of endosperm (Meier and Reid 1977) and their main function is to thicken the surface of these cells (Spyropoulos 2002; Lee 2009). Regardless of only one inconsistent report (Hirst and Jones 1948), the galactomannans have a usual basic structure (Wang et al. 2012). Their basic structure consists of a principal chain of 1,4-linked mannose (β-D-mannopyranosyl) units (Man) which are substituted by single galactose (D-galactopyranosyl) unit (Gal) 1,6-α-linked at C-6 oxygen position (Dilokpimol 2010; Scheller and Ulvskov 2010; Wang et al. 2012). The hydrophilic

Fig. 2 **a** Trigonelline. **b** Galactomannan



A



B

properties of galactose side chains along with their enhanced substitutive degree tend to contribute towards their higher water solubility (Dilokpimol 2010). Galactomannans represent high water binding capacity and thus as a water-soluble agent has better accessibility to enzymatic degradation in comparison to cellulosic microfibrils (Scheller and Ulvskov 2010). The feature of water retention in galactomannan makes it extensively useful as a gum or gelling factor (Dilokpimol 2010). The proportion of Gal to Man units in the galactomannan gums differs among various species and also within the same species (Dea and Morrison 1975; Reid 1985). Indeed, these small differences in the stated ratios are possibly linked to the genotypic or environmental components (Dea and Morrison 1975) and as a whole render to various chemical characteristics such as water holding, thickening, gelling, emulsifying, suspending, binding, and production of films (Srivastava and Kapoor 2005).

The above-mentioned properties make galactomannans to be largely used as multi-purpose materials in different industries (Srivastava and Kapoor 2005). Moreover, they assume to be responsible in proper restrain and relief of diabetes (type 2) in both animals and humans (Vats et al. 2003). This ratio is nearly 1:3 (Dilokpimol 2010) to 1:3.8 (Maier et al. 1993) for tara [*Cesalpinia spinosa* (Molina) Kuntze] gum, 1:1.8 (Maier et al. 1993) to 1:2 (Dilokpimol 2010) for guar gum, 1:3.5–3.9 (Hoefler 2004) to 1:4 (Dilokpimol 2010) for locust bean gum (*Ceratonia siliqua* L.) and around 1:1–1.2 for fenugreek gum (Maier et al. 1993; Petropoulos 2002). The high degree in the weight ratio of d-galactosyl to d-mannosyl in the gum of fenugreek (galactose weight content—50 %) compared to guar gum (33–40 %), tara gum (25 %) and locust bean gum (17–26 %), causes the fenugreek seed galactomannan to be more soluble in comparison to the others (Reid and Meier 1970; Brummer et al. 2003).

Steroidal sapogenins (spirostanols) such as diosgenins are referred to as the foremost sources in the fenugreek seed (Acharya et al. 2008) and can be used widely in pharmaceutical and nutraceutical industries. They are synthesized from cholesterol in almost all plants, although the intermediate steps for their biosyntheses are still unclear (Mehrafarin et al. 2010). It is also determined that the natural formation of glycoside (furostanol) in various plant species occurs by releasing the side chain in steroidal saponins (Tal et al. 1984). The in vitro conversion of these glycosides into spirostanols takes place at C-26 by discarding the glucose molecule (i.e., librating the glycosylated compound) through the hydrolyzing action of glucosidases (Mehrafarin et al. 2010). These results possibly clarify diosgenin biosyntheses from the cholesterol (oxidation at C-26 and cyclization in side-chain sterol) by furostanol in several plant species (Tal et al. 1984). In fenugreek seed, sapogenins are assumed as C_{27} sterols, at specific site where the side branch in cholesterol undergoes transformation to finally generate a spiroketal (spirostane saponins) such as dioscin or a hemiketal (furostane saponins) like protodioscin (Mehrafarin et al. 2010). Diosgenin (Fig. 3a), as a precursor raw material, is involved in the production of human hormones (e.g., testosterone, glucocorticoids, and progesterone) and steroidal drugs (Raghuram et al. 1994), and it has a great effect in the treatment of hypercholesterolemia (Acharya et al. 2008). Fenugreek seeds possibly possess a significant amount of diosgenin content and is suggested to be an applicable alternative plant for diosgenin production rather than wild species of Mexican yam (*Dioscorea* species) (Rosser 1985), because of its shorter growth cycle, reduced cost of production and consistent yield and quality parameters (Fazli and Hardman 1968; Acharya et al. 2008).

The most plentiful free amino acid in fenugreek seed is 4-hydroxyisoleucine (Fig. 3b) (Gupta et al. 1986) that is

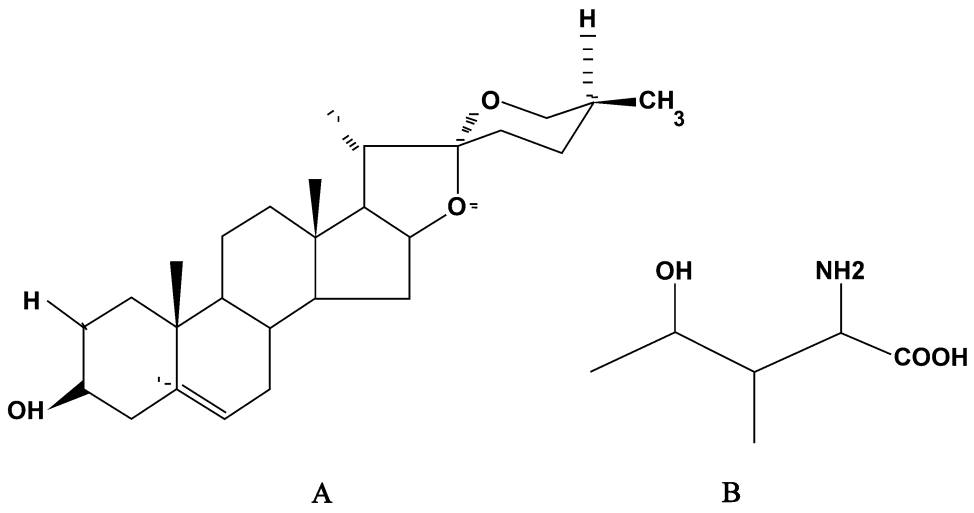


Fig. 3 **a** Diosgenin. **b** 4-Hydroxyisoleucine

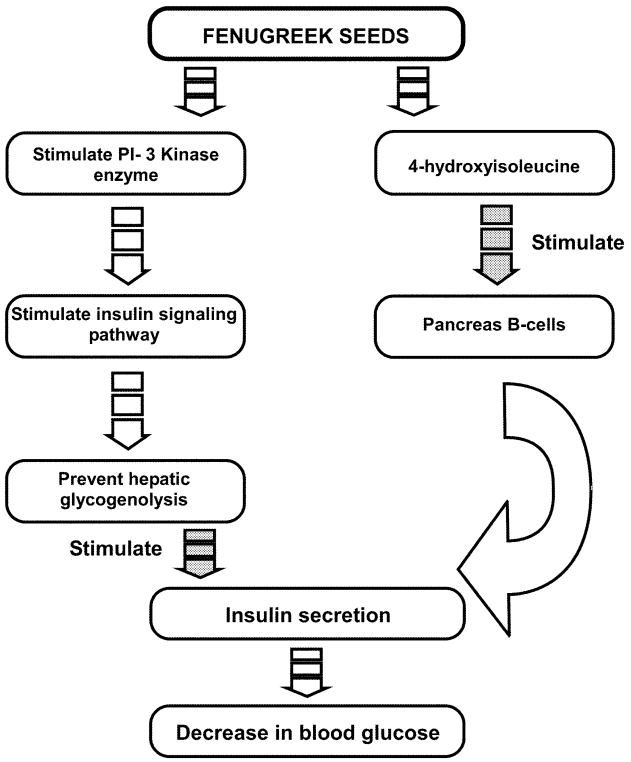


Fig. 4 Mechanism of action of fenugreek seed in controlling diabetes

present in the seed endosperm (Al-Habori and Raman 2002; Skaltsa 2002). Isoleucine, being an underlying branched-chain amino acid (BCAA), found to be the key precursor for synthesis of 4-hydroxyisoleucine and serves as an efficient factor in regulating the insulin secretion in animals (Broca et al. 2000) and making it potentially useful in the control of diabetes (Acharya et al. 2008). According to the literature resources, there are contradictory reports in terms of 4-hydroxyisoleucine concentration in fenugreek seed. These studies demonstrate its content to be around 0.015 % (Narender et al. 2006), 0.09 % (Mehrafarin et al. 2010) and 0.4 % (Hajimehdipoor et al. 2008). Fenugreek seed contains 0.02–0.05 % volatile compounds (Petropoulos 2002). In this group, the major compounds are heptanoic acid, *n*-hexanol, dihydroactiniolide, dihydrobenzofuran, tetradecane, a-muurolene, b-elemene and pentadecane (Leela and Shafeekh 2008). Moreover, the seeds contain around 7 % fixed oil consisting mainly of linoleic, oleic and linolenic acids (Leela and Shafeekh 2008). A word diagram representing the mechanism of action of fenugreek seed in controlling diabetes is shown in Fig. 4.

Galactomannan biosynthesis

It is important to note that galactomannan begins to deposit (secrete) in the endosperm cells after 3–4 weeks of anthesis, and 4–6 weeks later the secretion stops, depending on cultivar and cultivation methods (Reid and Meier 1970). The galactomannan molecular scale distribution in endosperm seems to be more disperse by proceeding in the deposition process (McCleary et al. 1987). However, the deposition continues until nearly all the cytoplasm disappears (Spyropoulos 2002) and the only endosperm cells that are not occupied by galactomannan are the cells of the aleurone layer. In these cells, some galactomannan is infrequently deposited (secreted) on the cell corners as well as at the sidewalls (Meier and Reid 1977). It is accumulated in the net-like enchylema compartment and then released outside the plasmalemma (plasma membrane) span out of the Golgi apparatus participation (Meier and Reid 1977), which basically corresponds to protein modification and its intercellular translocation. The biochemistry of galactomannan synthesis and mobilisation has gained much interest, not just for its biological importance, but conjointly due to the extensive application of galactomannan in different aspects of production of food, pharmaceuticals, cosmetics, paper products, paints and plasters (Shcherbukhin and Anulov 1999).

The enzymatic mechanism of this procedure was first studied by Campbell and Reid (1982), who revealed that the galactomannan deposition in seed endosperm of fenugreek is highly correlated to the activity in the endosperm extracts arising from a galactomannan mannosyltransferase [guanosine 5′-diphosphate (GDP)-mannose]. The special enzyme has been typically checked with the concomitant presence of galactosyltransferase [uridine 5′-diphosphate (UDP)-galactose] (Edwards et al. 1989). In other words, the isolated special mannosyltransferase from the fenugreek seed endosperm is reported to possess UDP-galactose-linked α-D-galactosyltransferase activity (Edwards et al. 1989). These two enzymes which are recognized as membrane-linked glycosyltransferases are responsible for galactomannan biosynthesis (Edwards et al. 1989; Spyropoulos 2002). In other words, the biosynthesis of galactomannan in vivo starts by the parallel activity of mannosyl- and galacto-transferase (Edwards et al. 1989; Spyropoulos 2002). The catalytic activity of mannosyltransferase adds the mannose units to an unspecified interior primer that corresponds to galactomannan synthesis and then the galactosyltransferase inserts the galactose units to recently transmitted mannose unites on the mannan main chain (Reid et al. 1995; Spyropoulos 2002). Fenugreek regulates the Gal to Man ratio in the galactomannan by the enzyme, galactosyltranferase (Reid et al. 1995), so that during the whole seed development period the relative portion of Gal substitution in accumulating galactomannan comparing to Man remained constant (Edwards et al. 1989, 1992). The mechanisms that highlight galactomannan biosynthesis in fenugreek, could lead to the production of transformed

fenugreek plants with the desired quantitative relation of Gal to Man (i.e., 1:4), which are useful for various industrial products (Spyropoulos 2002).

Selective techniques of seed germination

Concerning the economic importance of fenugreek species along with their descending trend for germination percentage (GP) under natural conditions; investigations of different factors such as, hot water, seed wash, sulfuric acid treatment as well as scarification on the germination indices of fenugreek seed seems to be an important issue. The most important factor in reducing GP in *Trigonella* can be related to seed cover hardness which may inhibit entrance of oxygen and water into the seed (Riasat et al. 2005). Mechanical scarification by reducing the seed coat thickness facilitates their infiltration toward seed internal parts (Riasat et al. 2005); for e.g., scarification by emery paper for 2–4 min on *T. corniculata* (L.) L. seed has found to be effective in decreasing seed hardness (Sinha et al. 1993). In a probe on *T. foenum-graecum* L. it was reported that foliar application of gibberellic acid (GA_3) increased GP; meanwhile, the germination rate (GR) decreased (Shahine et al. 1992). They also indicated an inverse trend of GP and GR after application of Paclobutrazol (a plant growth retardant) and Ethrel (plant growth regulator).

Farooq et al. (1985) suggested that gallic acid application decreases the GP. They showed that hot water treatment did not affect the seed and sulfuric acid application more than 2 min would entirely destroy them. According to a study conducted in Iran on determining the best method for the GP enhancement among seven germplasms of *Trigonella*, it was detected that chilling (5 °C), hot water (1, 2, 3 and 5 min), seed wash (24, 48 and 72 h) and sulfuric acid treatment (2, 5 and 15 min) did not influence GP (Riasat et al. 2005). Besides, scarification with emery paper for 2 min on *T. monspeliaca* L., *T. uncata* (Boiss. & Noë), *T. stellata* Forssk., *T. anguina* Delile, *T. astroits* Fisch. & C. A. Mey. and 3–4 min for *T. elliptica* Boiss and *T. spruneriana* Boiss. demonstrated satisfactory results. Furthermore, the highest germination indices were pertained to *T. monspeliaca* L. (Riasat et al. 2005). These results suggest that in addition to GP, the emerging indices have to be considered due to their efficiency in seedlings establishment and other growing stages after germination.

Seed germination

Fenugreek seeds germinate around 10 h after the start of seed imbibition at 25 °C in the dark (Reid and Bewley 1979). The seeds should be at least 95 % pure and have 80 % germination rate (Prasad 2011). In the presence of sufficient moisture, oxygen and heat the seeds follow an epigeal process, so that the emergent cotyledon is pulled from under the soil surface during germination. Within the imbibition process which is demonstrated to be under the control of galactomannan function (Reid and Bewley 1979), water inactively is absorbed by the seed that result in endosperm swelling. As the cells absorb water they elongate, extending the radicle to form a primary root toward the soil, which eventually will develop into secondary roots. Protrusion of the radicle by more than 5 mm is considered a sign of fenugreek seed germination (Petropoulos 2002). A diagrammatic representation of the longitudinal section of a fenugreek seed is presented in Fig. 5.

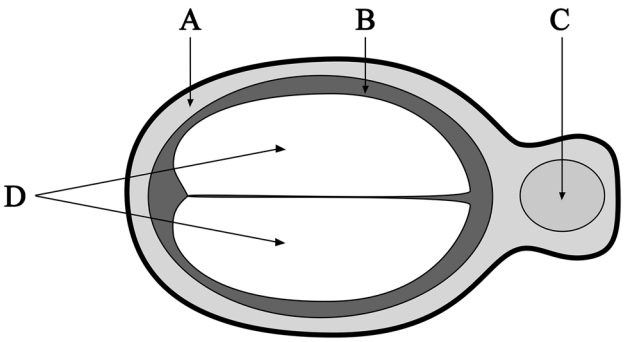


Fig. 5 Diagrammatic representation of the longitudinal section of a fenugreek seed. *A* Testa, *B* endosperm, *C* radicle, *D* cotyledons

Endosperm/embryo reserve mobilisation and inhibiting factors

The mobilization of endosperm galactomannan begins 15 h after imbibition (Zambou and Spyropoulos 1990) through the joint action of three hydrolytic enzymes (i.e., α-galactosidase, endo-β-mannanase, and exo-β-mannanase) synthesized by aleurone cell layers (Meier and Reid 1982; Reid 1985). The regulation of galactomannan mobilization via these enzymes is impacted by both the endosperm (Zambou and Spyropoulos 1990) as well as the embryo (Spyropoulos and Reid 1985; Zambou and Spyropoulos 1990). For instance, the embryo has the potentiality to equilibrate the endospermic factors that inhibits α-galactosidase (Spyropoulos and Reid 1985) and endo-β-mannanase (Malek and Bewley 1991) production. The first two enzymes are synthesized de novo in the endosperm tissue (Reid and Meier 1973; McCleary 1983) on seed germination, but the third enzyme is actively present in the resting endosperm of the dry seed (McCleary 1983).

During the seed imbibition period, the α-galactosidase activity increased; yet, the activity of endo-β-mannanase manifested after about 20 h of imbibition and increased afterwards (Spyropoulos 2002). The increase in hydrolase activities of α-galactosidase and endo-β-mannanase coincided with the decrease in galactomannan content in the endosperm (Spyropoulos and Reid 1988). Prior to germination and concerted with raffinose series oligosaccharides hydrolysis, a little D-galactose released in the endosperm (Zambou and Spyropoulos 1990). Following 20 h of imbibition further D-galactose is built up by galactomannan hydrolysis and with the passage of 48 h imbibition, the whole galactomannan is hydrolyzed (Reid 1971).

Besides, D-mannose presence in the endosperm begins 25 h post-imbibition via the endo- and exo-β-mannanase activity (Spyropoulos and Reid 1988). The ultimate products of galactomannan hydrolysis (i.e., D-galactose and D-mannose) do not accumulate in the endosperm (Zambou and Spyropoulos 1990; Spyropoulos 2002). The products (monosaccharides) immediately transport to the embryo by specific carriers to be later transformed into starch, sucrose and other cell materials (Reid 1971). By exhibiting high degree of specificity relative to the corresponding sugars, these carriers seem to play a decisive role in the setting out of the uptake capacity of these sugars by the fenugreek embryo (Zambou and Spyropoulos 1989, 1990; Spyropoulos 2002).

The D-galactose uptake by fenugreek embryo is achieved through passive non-facilitated diffusion (Ubelmann 1978) that weakens the regulatory role of such monosaccharide in galactomannan hydrolysis (Zambou and Spyropoulos 1989). However, the uptake rate of D-mannose and D-galactose by cotyledons is strongly attributed to the hours of imbibition (Zambou and Spyropoulos 1989). The inhibition of D-galactose uptake by cycloheximide in vitro presumes that the galactose carrier is synthesized de novo during imbibition (Zambou and Spyropoulos 1990; Spyropoulos 2002). According to Spyropoulos (2002), the required metabolic energy for uptake of such monosaccharides is achieved by phosphorylating these sugars for their transformation into cotyledons and to create concentration gradient between both endosperm and the cotyledons. Thus, sequel to galactomannan evanescence in the embryo, gradual appearance of transitory starch and sucrose is expected in great amounts in the embryo (Reid 1971). Although the initiation of starch formation in fenugreek cotyledons is not dependent on the supply of the galactomannan hydrolysates in the embryo; their existence is important for its accumulation (Bewley et al. 1993).

Hence, with smaller volume of incubation medium, the development in enzymatic activity was inhibited (Malek and Bewley 1991). This inhibition could be relieved by 2 h pre-leaching of endosperm prior to the incubation, suggesting that both endosperm and testa have substances with leaching properties; hence diffusion is restricted as the volume in the incubation medium becomes lesser (Spyropoulos 2002). Zambou et al. (1993) demonstrated that the inhibitory action of the leaked substances from both endosperm and testa, first isolated from 5-h imbibed seeds and then incubated for $\geq$2 h during germination, was similar to that of exogenous abscisic acid (ABA) in terms of restrictions in endospermic α-galactosidase production. However, the isolated endosperm and testa from imbibed (15 h) and leached (2 h) seeds did not show any inhibitory behavior after their treatment with these inhibitors for 8 h. They have also shown that the three isolated substances from the whole seed leachate have the same inhibitory action as to substances isolated from endosperm. These substances, however, did not influence the production of such hydrolases in case of endosperm treatment when the galactomannan mobilization was in progress (Zambou et al. 1993).

Reid and Meier (1972) concluded that the isolated endosperm quantitatively converted the galactomannan reserves to its main preliminary components at the same period of galactomannan breakdown in vivo in the intact seeds and within the process and no certain hormonal signals arising from embryo were found to be necessary. It was observed that the externally applied ABA inhibited galactomannan breakdown in isolated endosperms (Reid and Meier 1973). The inhibitory function of ABA when applied to both endosperm and testa inhibited α-galactosidase and endo-β-mannanase production (Reid and Meier 1973) and the ABA content in the endosperm was correlated to its ability pertaining to hydrolyse galactomannan (Malek and Bewley 1991). Excision of the embryonic axis 5 h post-seed sowing, inhibited galactomannan mobilisation rate and reduced the activity and production of endospermic α-galactosidase (Spyropoulos and Reid 1985; Spyropoulos 2002), suggesting that the embryo axis controlled galactomannan mobilisation. It was also detected that the sooner the removal of the embryonic axis prior to the final germination stages, faster such effects could be concluded (Spyropoulos and Reid 1985; Spyropoulos 2002).

The unsuitable impact of embryo axis removal 5 h post-seed sowing on galactomannan hydrolysis and the activity of α-galactosidase was explained when the removed axis was re-added into the seed incubation medium or when the seeds incubation was accompanied with benzyladenine (BA), gibberellic acid (GA_3) or through combination of BA with GA_3 (Spyropoulos 2002), or by the herbicide SAN 9789 (4-chloro-5-(methylamine)-2-(α, α, α -trifluoro-*m*-tolyl)- 3 (2H)-pyridazinone) (Spyropoulos and Reid 1985).

At first, it was understood that the axis by its regulative function determines the inception of α-galactosidase production within the endosperm. Accordingly, the prolonged presence of the axis was believed to be mandatory in the uptake of hydrolysis products of galactomannan and ensured continuous hydrolysis of galactomannan by inhibiting endospermic accumulation (Spyropoulos and Reid 1988). The possible role of the axis is thus believed to be interacting with endogenous inhibitors present in the endosperm and then to function like a sink source for the breakdown products of galactomannan released in the endosperm (Spyropoulos and Reid 1985).

The production of galactomannan-hydrolysing enzymes (i.e., α-galactosidase, endo-β-mannanase, and exo-β-mannanase) and consequently the galactomannan hydrolysis were entirely inhibited by induced water stress over the germinated fenugreek seed, when galactomannan hydrolysis has not started (Zambou et al. 1993). The reduction in the development of enzymatic activities that corresponded to the galactomannan breakdown may have originated by decrease in the removal of diffusible inhibitory substances present in endospermic cell content as pointed out by Spyropoulos and Reid (1988).

The induction of water stress on already 2-h leached seeds gave partial (endo-β-mannanase) and full (α-galactosidase) recovery of the levels of hydrolytic enzymes, which resulted in partial relief of inhibition in galactomannan mobilization (Spyropoulos and Reid 1988). On the other hand, the imposition of water stress on 25-h imbibed seeds where galactomannan breakdown has started, did not impact the production of these major hydrolases; yet, the breakdown of galactomannan was still inhibited by the induced stress (Spyropoulos and Reid 1988). The inhibition of galactomannan breakdown might be an indication of inhibition either in galactomannan hydrolases secretion and/or their diffusion across the aleurone cell wall or in the action of α-galactosidase in situ (Spyropoulos 2002).

In a research on viable protoplasts, isolated from the endosperm tissue of carob, it was found that under moderate water stress conditions (−2.0 MPa) the protoplast products during 5 days incubation continued to supply the carob endosperm hydrolases, i.e., α-galactosidase and endo-β-mannanase (Kontos and Spyropoulos 1995). In other words, the secretion of hydrolase products into incubation medium and their activities were not affected by moderate stress; although, these functions reduced under higher osmotic content of protoplast incubation medium. In addition, experiment on whole carob endosperms has shown that water stress (−1.5 MPa), by reducing the endosperm cell wall porosity, inhibited the diffusion of these hydrolases into the endosperm incubation medium. This demonstrated that the carob endosperm-cell wall-secreted enzyme acted as barrier to manage galactomannan breakdown by regulating the diffusion of galactomannan hydrolases to reach their appropriate site of action (Kontos and Spyropoulos 1995). As regards to fenugreek seed, the porosity in cell walls of aleurone layer could be affected by the imposed water stress such that the diffusion of corresponding enzymes for galactomannan hydrolysis is reduced (Spyropoulos 2002).

Although the germination was completed in the range of 10–14 h after imbibition, the major reserves did not mobilize within the first 24 h (Leung et al. 1981). The mobilisation of "endosperm/embryo" endogenous reserves follows a time period that causes multilateral correlation between the metabolic events with each other and with the completion of germination (Leung et al. 1981). Galactomannan mobilisation process is obviously faster than its deposition in the endosperm cells and starts when the radicle protrudes after nearly 25 h of seed imbibition (Reid 1971). Before the galactomannan hydrolysis has started, a small decline in free sugars within the embryo is observed (Reid 1971). Meanwhile, within the course of galactomannan mobilisation in the endosperm the free sugars were temporarily increased to some extent. This is followed by a series of events: (1) starch accumulation in a large amount, and (2) mobilisation of the storage proteins and a part of the lipid reserves (Bewley et al. 1993). It is important to note that before the start of germination, no starch was detected in the embryo so far; however, under the galactomannan mobilization process, the transient starch content increased in both cotyledons and axis considerably (Reid 1971; Bewley et al. 1993).

At subsequent periods during seedling development, the stored starch in the embryo is remobilised through the action of α-amylase, which is recognized as a single protein band with a native pI of 5.1 after isoelectric focusing (Bewley et al. 1993). The endosperm galactomannan mobilisation was further pursued by the mobilisation of embryo reserves, proteins, lipids and phytates (Leung et al. 1981). Galactomannan hydrolysis was continued by the deposition of starch in the embryo (Spyropoulos 2002). After 30 h of imbibition, proteins in the cotyledons undergo hydrolysis and the resulted amino compounds are concomitantly transferred to the embryonic axis tissues (Leung et al. 1981; Spyropoulos 2002) as the main sink of hydrolysis products (Hodson and Bryant 2012). The observed depletion in the total amount of nitrogen is correlated to the increased activity of proteinase in the cotyledons (Hodson and Bryant 2012). However, the accumulation of amino compounds in the axis commenced much earlier than cotyledons, suggesting an initial increase in the uptake of amino compounds via the embryo axis (Spyropoulos 2002).

The phytate reserve started to deplete from cotyledons and somewhat from the axis after 50 h of imbibition

(Leung et al. 1981; Spyropoulos 2002). The depletion process was apparently associated with the hydrolyzing action of phytase (Leung et al. 1981) which started increasing after about 40 h of imbibition (Spyropoulos 2002). This event is metabolically pursued by stored-lipid hydrolysis accompanied by an increase in the isocitrate lyase activity (Leung et al. 1981). The whole lipid content located in cotyledons is ~7.5–8 % of the dry seed weight (Skaltsa 2002; Spyropoulos 2002). The total lipids consisted of 84.1 % neutral lipids that greatly comprised of triacylglycerols, 10.5 % phospolipids and 5.4 % glycolipids (Skaltsa 2002). The activity of α-galactosidase during embryonic growth is important and the endo-β-mannanase activity enhanced with imbibition time both in the cotyledons and in the axis (Kontos and Spyropoulos 1996). A word diagram representing the process of germination and endosperm reserve mobilization in the fenugreek seed is presented in Fig. 6.

Genotype (G) and environment (E) effects on the seed biochemical constituents

Earlier researchers showed the possibility of different expressions in chemical compositions present in fenugreek seed by similar genotypes as exposed to different locations (Acharya et al. 2006, 2008). These studies demonstrated strong genotype X environment (GE) effect on the production of important fenugreek phytochemicals. A genotype that generates a very high level of a phytochemical at one site may not produce the same level of that specific phytochemical in another location. We should keep in mind that production of phytochemicals are controlled by minor genes or polygenes and so are strongly influenced by environment (Fehr 1998). Recent studies showed highly significant genotype, genotype X year and genotype X location interactions on the diosgenin contents detected in the seed of different genotypes (Acharya et al. 2008).

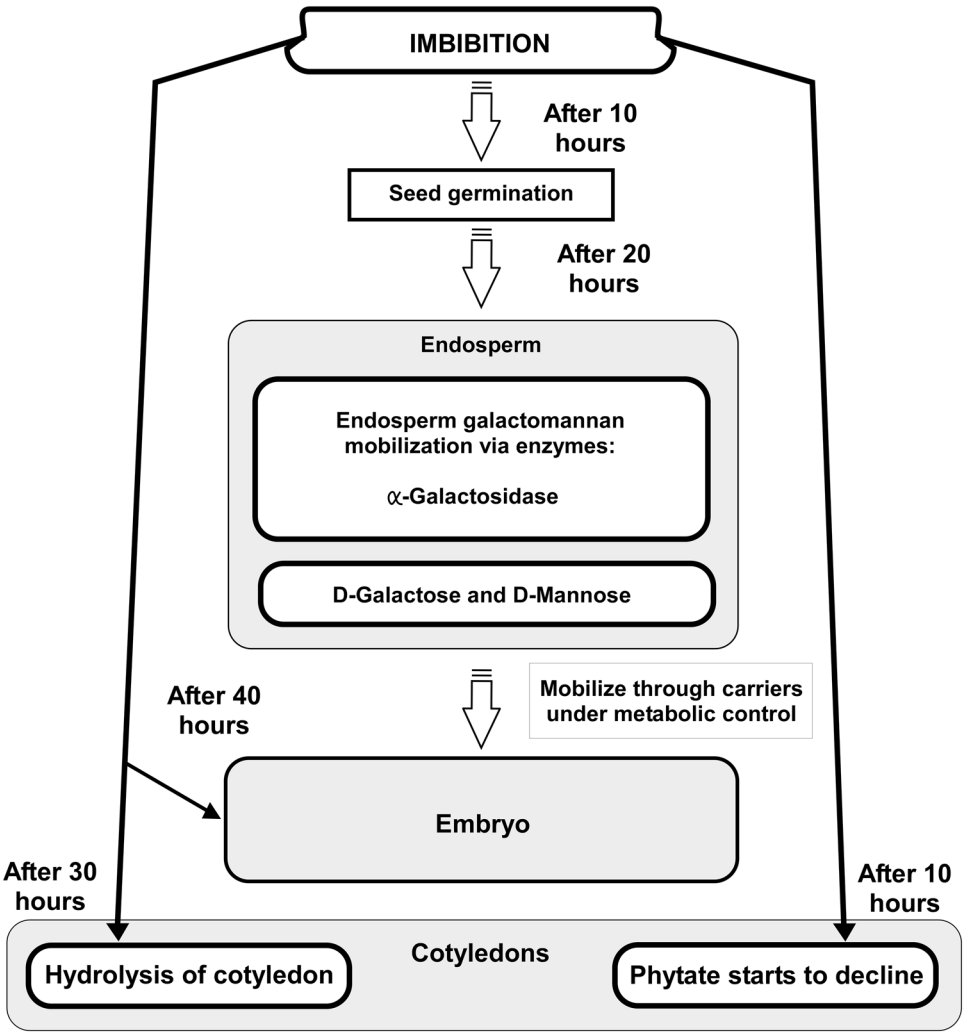


Fig. 6 Seed germination and endosperm reserve mobilization in the fenugreek seed

Earlier studies reported a variation in diosgenin content (0.8–2.2 %, w/w) among 52 fenugreek accessions from 18 different countries (Fazli and Hardman 1968). Furthermore, in a study on *Trigonella* genotypes which was representative of different bio-geographical areas in India, a variation in the seed diosgenin content between 0.33 and 1.9 % was observed as result of GE interaction (Sharma and Kamal 1982). Lee (2009) suggested that environmental factors had lesser impact across total variations observed in the diosgenin content (%), such that they could account for only 6 % of the observed variances based on the specific treatments. This finding may suggest that diosgenin percentage in fenugreek seed is strongly associated to the important genetic factors. It is also concluded from the study that ~70–78 % of the total observed variations for 4-hydroxyisoleucine content (4-OH-ILE-C) and productivity (4-OH-ILE-P), galactomannan content (GAL-C) and productivity (GAL-P), diosgenin content (DIOS-C) and productivity (DIOS-P) was attributed to the main effects of the environment. However, the GE interaction contributed only 15–19 % (15 % accounted for DIOS-P, GAL-P and 4-OH-ILE-C; 16 % accounted for DIOS-C; 17 % accounted for 4-OH-ILE-P; 19 % accounted for GAL-C) to the total variations seen for the seed main chemical constituents (Lee 2009). The findings possibly suggest the greater influence of other involved factors rather than genetic ones in fenugreek crop performance (Sharma and Kamal 1982). In a similar study, Acharya et al. (2007b) detected extensive fluctuations in seed oil contents among 14 fenugreek genotypes grown under greenhouse conditions in western Canada demonstrating genotypic variability for this trait. Overall, it appears that drastic GE effect may be responsible for this observation for fenugreek seed genotypes grown at different environments (Acharya et al. 2007a; Lee 2009).

Improvement programs toward fenugreek seed size

The diploid genotypes of Fenugreek posses $2n = 16$ chromosome numbers; however, other *Trigonella* species can partially deviate from the normal chromosome number (n-8, 9, 14) (Darlington and Wylie 1955). Singh and Singh (1976) isolated primary trisomics plus five double trisomics obtained in a progeny process from the autotetraploid fenugreek by 18 ($2n + 1 + 1$) chromosomes. The presence of ß-chromosomes in fenugreek is highly linked to the increased number of chromosomes in the plant (Joshi and Raghuvanshi 1968). In some lines of *Trigonella* an extra ß-chromosomes have already been reported (Singh and Singh 1976). In addition, there are natural and induced forms of autopolyploid that have been detected in some fenugreek accessions (Roy and Singh 1968).

The somatic chromosomes with higher levels of ploidy tend to increase plant vigor and seed size as a result of larger cell dimensions (Fehr 1998). Colchicine that can be extracted from *Colchicum autumnale* L. inhibits microtubule polymerization via binding with tubulin (Gupta 1972). Its application in genetic studies can induce the polyploidy in plant cell division to withhold chromosomal segregation (Roy and Singh 1968). The importance of this matter lies in the fact that the increase in ploidy levels most cases lead to production of bigger seeds containing higher levels of chemical constituents that were not present in genotypes having smaller seeds (Basu et al. 2007). The production of tetraploid fenugreek ($2n + 2n = 32$) can be done by treating shoot apices (Roy and Singh 1968) or seeds (Basu 2006) with colchicine. The tetraploid plants produced via colchicine treatment on fenugreek seeds [15 min in 0.05 %, (w/v) colchicine] had larger leaves, longer pod length, vigorous growth and larger seed size (Basu 2006; Basu et al. 2007).

Genetic improvement of fenugreek

The creation or assemblage of genetic variations, followed by selection and identification of those that are desirable remain the basis of viable improvement programs. This has been shown for fenugreek especially at the whole plant, morphological and biochemical levels (Acharya et al. 2007b). The reproductive biology of any crop is important in the choice of strategies for its improvement. Fenugreek is self-pollinated, and out crossing is reported to be rare since the stigma is receptive before the anthers mature. This should be an advantage for hybridization breeding. However, fenugreek was reported to be occasionally visited by insects (Basu et al. 2009). There is immense variability in fenugreek, with high level of significant genotype by environment interactions. Even broad sense heritability values changed with environment such that selection within a particular population in a specific environment will be effective (Basu 2006). Genetic improvement of fenugreek should, therefore, be tackled through understanding variations in the amount of metabolites produced in collections as well as variations in the medicinal pathway by which the metabolites act (Al-Habori and Raman 2002).

Even when molecular characterization techniques of Random Amplification of Polymorphic DNA (RAPD), Inter-Simple Sequence Repeat (ISSR), Amplified Fragment Length Polymorphism (AFLP), etc were used, there were no reports on the genetic status of the collections, i.e., whether true-breeding pure line or not. Although elimination of within-accession diversity by mixing DNA from different plants in the same accession was used to

give a complete banding profile of the accession, it is the plant-wise banding profile that will give information on the genetic status of the collections. Consequently, molecular characterization did not agree with morphological characterization in some of the cases although both methods reflected geographic groupings (Fikreselassie et al. 2012; Randhawa et al. 2012). There should be increased focus on the genetic basis of fenugreek breeding to revalidate the breeding strategies. This includes comparison of within- and between-variety variations, re-validation of the reproductive biology of fenugreek, re-establishment of the degree of out crossing using a combination of conventional and molecular approaches. Vegetative propagation through plant tissue culture (Aasim et al. 2009) offers a way of maintaining the uniformity of a particular clone and comparing with a seed propagated relative. Although fenugreek is mostly stored as seed, which remain viable for up to 10 years, the results of the latter studies will give clues as whether it is necessary to keep an in vitro (plantlet) gene bank for fenugreek (Ziv 1991).

Multi-locational trials of existing global diversity for specifically adapted varieties have, therefore, been advocated for genetic improvement. This will require formation of networks among researchers worldwide. In addition, standardization of environments by testing varieties in the controlled in vitro environment will help in providing clues on accessions suited to different environments. In addition, the adaptive or feedback mechanism of metabolic processes can be investigated with more specificity at cell and tissue levels rather than whole plant levels. In elucidating the pathway of lowering cholesterol, it was discovered that the higher the cholesterol in the medium, the higher the diosgenin content (Khanna et al. 1975). Besides selections from world collections, mutation breeding has been used successfully in fenugreek improvement (Basu et al. 2008b). Improved varieties of fenugreek have been released through mutation breeding with the use of chemical and ionizing radiations. Application of mutagens to tissue cultures increased steroidal sapogenins about two–three fold compared to seed plants. The advantage of mutant application to tissue cultures is in its possibility at all levels of cellular organization, ranging from cell, tissues, organs and whole plant. In addition, minimal space is required for evaluation in vitro. Site-targeted mutagenesis (Kunkel 1985) should be exploited in mutation breeding of fenugreek. Also, improving on protocols for somatic embryogenesis, especially in the aspect of rooting, can be explored in generating somaclonal variants with desirable traits. However, efficient rooting, acclimatization and transplanting procedures need to be developed (Basu et al. 2008b).

Conclusion

Efficient fenugreek production is greatly dependent on the seed quality, purity, viability and authenticity. Fenugreek seed should possess $\leq$95 % purity and $\leq$80 % viability for normal germination. Other important factors are adequate moisture, temperature and oxygen. Fenugreek seed follow an epigeal mode of germination in the soil and is regulated by factors such as seed quality and genotype, sowing depth and osmotic potential of the soil. In this review, we have focused on the fenugreek seed structure, seed chemical compounds and germination mechanism as well as galactomannan biosynthesis and degradation. We have explained the important nature and functions of different chemical compound at their site of actions. The most important seed chemicals have been galactomannan, amino acids and diosgenin followed by trigonelline. Galactomannan is a hemicellulosic polysaccharide which thickens the cell walls of endosperm. Higher water retention capacity and additional characteristics make it suitable as a multi-purpose raw material for the relevant industries. The highest amount of diosgenin found in fenugreek seed comparing to other plants has introduced it as a good replacement for diosgenin production which is utilized for treating hypercholesterolemia. The amino acid, 4-hydroxyisoleucine can also be useful in the control of diabetes and regulating insulin secretion in animals and humans. We have also reviewed some results of endosperm incubation in different volumes of medium in the production and activity of galactomannan hydrolases enzymes. Moreover, the inhibitory action of ABA and water stress in galactomannan hydrolysis was also discussed in detail. Finally, the main function of embryonic axis was recognized for decreasing the adverse effects of endosperm inhibitors and for efficient uptake of the hydrolyzing products of galactomannan.

Fenugreek is an annual forage legume mostly produced in the old world and is also known as an important spice crop and a medicinal herb. It is now being screened and investigated widely for its numerous medicinal, pharmaceutical and nutraceutical properties by related industries. Fenugreek seed is a rich source of a number of important phytochemicals such as complex carbohydrates (galactomannan), steroidal sapogenins (diosgenin) and amino acids (4-hydroxyisoleucine) with a good number of physico-biochemical properties that have medicinal values to both humans and animals. The production of the crop has great promises in the dry and semi-arid regions of Africa and Latin America where it will find agro-climatic growing conditions similar to that of its native growing conditions; as observed in the Mediterranean regions of the continents of Europe and Africa, West Asia and the Indian subcontinent in South

Asia. Fenugreek is a predominantly dryland crop suitable for cultivation under rainfed conditions. It could therefore be used either as a forage or spice crop or as a medicinal and aromatic herb under the low input agricultural systems of the sub-Saharan Africa (SSA) and Latin American countries with similar agro-climatic conditions. Fenugreek could turn out to be an important crop in the rural areas of SSA and Latin America due to its immense medicinal values and multi-purpose uses and could transform into an income generating venture for the rural poor. The low cost of production in SSA and Latin America could possibly make it attractive to the growing pharmaceutical and nutraceutical industries as a cheaper source of raw material/products. There is also possible potential of introducing this crop in an experimental basis in some parts of South East Asia with partly similar agro-climatic conditions; however, we do feel that related regions of Africa and Latin America would be more successful with respect to crop productivity and other agronomic potentials of this crop in the comparatively drier regions.

However, it is important to note that due to GE interaction the crop is subjected to fluctuation in both yield and quality of chemicals generated. Hence, there is a need for continuous efforts in genetic improvements and in developing high yielding, drought and disease-resistant cultivars suitable for production in SSA and Latin America. Developing locally adapted cultivars optimized for production and tested for biotic/abiotic resistant in specific agro-climatic regions of the continent could be a viable solution. In short, we believe that this multi-purpose crop has a great future for the SSA and Latin America for the rural poor and small scale producers and farmers spread across the vast continent in suitable agro-climatic zones appropriate for the crop production.

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