

**GENOTYPE X ENVIRONMENT IMPACT ON SELECTED  
BIOACTIVE COMPOUND CONTENT OF FENUGREEK  
(*TRIGONELLA FOENUM-GRAECUM* L.)**

**EE LYNN LEE  
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## **Dedication**

To Mom and Dad who are residing in Kuala Lumpur, Malaysia,

Both of you are always the key inspiration and motivation in my pursuit for all things possible. But it is absolutely impossible for me to repay the both of you for all the love and support that have enabled me to comfortably obtain an education far away from home.

Thank you for giving me a blissful childhood and a warm place to call home.

Last but not least, thank you for giving me life.

## Abstract

Fenugreek (*Trigonella foenum-graecum* L.) is a medicinal plant with potential applications in the natural health product industry. In a multi-environmental setting, 10 genotypes were tested across 14 growing environments (using a Randomized Complete Block Design), representing irrigated and rainfed growing conditions in southern Alberta, Canada over two cropping years (2006 and 2007). The objectives of this study were (1) to determine seed yield, plus content and productivity of selected bioactive compounds (galactomannan, diosgenin and 4-hydroxyisoleucine), (2) to assess the impact of growing environment on these variables and (3) to identify promising genotypes for breeding and industrial use. Using principal component and cluster analyses, the study provides insight on the relative influence of growing environments and genes on the biochemical and agronomical traits as well as identifies genotypes based on performance and stability. These are useful as parental materials in cultivar development for the Canadian natural health product industry.

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## List of Abbreviations

4-OH-ILE	4-Hydroxyisoleucine content
4-OH-ILE-P	4-Hydroxyisoleucine productivity
AAFC	Agriculture and Agri-Food Canada
AARD	Alberta Agriculture and Rural Development
AMMI	Additive Main effects and Multiplicative Interaction model
ANOVA	Analysis of variance
BI	Bow Island
BR	Brooks
CDCS	Crop Diversification Centre South
CV	Coefficient of variation
DIOS	Diosgenin content
DIOS-P	Diosgenin productivity
Dry	Rain fed
E	Environment
FFA	Free fatty acid
G	Genotype
G x E	Genotype by environment
GGE	Genotype plus genotype x environment
GLM	Galactomannan content
GLM-P	Galactomannan productivity
HDL	High-density lipoprotein
HFD	High-fat diet

HPLC	High Performance Liquid Chromatography
HSD	Tukey's Honestly Significant Difference test
IMCIN	Irrigation Management Climate Information Network
Irr	Irrigated
LB1	Lethbridge (federal test site)
LB2	Lethbridge (provincial test site)
LDL	Low-density lipoprotein
MET	Multi-environment trial
PITC	Phenyl isothiocyanate
PC1	First principal component
PC2	Second principal component
PCA	Principle Component Analysis
SS	Sum of squares
SY	Seed yield
RCBD	Randomized Complete Block Design
TSW	Thousand seed weight
U / mL	Enzyme activity per millilitre of solution

## 1.0 Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is a self-pollinating, annual leguminous crop (Fig. 1.1), which is native to the Indian subcontinent and the Eastern Mediterranean region (Petropoulos, 2002). It is currently widely cultivated in central Asia, central Europe, northern Africa, North America and parts of Australia, with India being the leading fenugreek producer in the world (Fotopoulos, 2002). The plant is well suited to cool and temperate growing regions with low to moderate rainfall. Fenugreek is adapted to rainfed growing conditions and is suitable for growth within the semi-arid regions of western Canada especially those genotypes of Mediterranean origin due to similarity in day length (Acharya *et al.*, 2008).

Fenugreek, perhaps, is best known for presence of the distinctive, pungent aromatic compounds in the seed (Max, 1992) that impart flavour, color and aroma to foods, making it a highly desirable supplement for use in culinary applications. As a spice, it constitutes one of the many ingredients that make up curry powders (Srinivasan, 2006). In countries such as India, fenugreek leaves are consumed as leafy vegetables in the diet (Sharma, 1986b), while in Ethiopia and Egypt, the plant is used as a supplement in maize and wheat flour for bread-making (Al-Habori and Raman, 2002). In Yemen and Persia, fenugreek represents a key ingredient in the preparation of daily meals among the general population (Al-Habori and Raman, 2002).

Fenugreek also has been used for over two thousand years as a medicinal plant in various parts of the world (Srinivasan, 2006) and may be regarded as the oldest medicinal plant in human history (Lust, 1986 cited in Petropoulos, 2002). As a medicinal plant, use of fenugreek is associated with a wide range of therapeutic applications including its use

as a carminative (prevents flatulence) to its use as an aphrodisiac (Chopra *et al.*, 1982). Reference to fenugreek has been made in Indian Ayurvedic and Traditional Chinese Medicines where it is recognized as a galactagogue or lactation stimulant in women after child birth as well as for its ability to treat wounds and sore muscles (Tiran, 2003). In addition, some plants also possess antibacterial (Thomas *et al.*, 2006), anti-ulcer (Al-Meshal *et al.*, 1985), anti-cancer (Shishodia and Aggarwal, 2006), anthelmintic (antagonistic effect against parasitic worms) (Ghafghazi *et al.*, 1977), and anti-nociceptive (pain-reducing) properties (Javan *et al.*, 1997).

In recent years, laboratory studies and clinical trials have focused on fenugreek as a potential nutraceutical. These studies have shown that fenugreek plants possess immunomodulatory (Bin-Hafeez *et al.*, 2003), hypocholesterolaemic (Sharma, 1986a; Evans *et al.*, 1992; Stark and Madar, 1993), hypoglycaemic (Khosla *et al.*, 1995; Vats *et al.*, 2002), gastro- and hepatoprotective (Pandian *et al.*, 2002; Thirunavukkarasu *et al.*, 2003) and antioxidative (Anuradha and Ravikumar, 2001; Choudhary *et al.*, 2001) properties. Pharmacological properties of fenugreek have been explored to identify a role for the plant in diabetes management (Sharma *et al.*, 1996a, 1996c; Puri *et al.*, 2002) and in cardiovascular health (Petit *et al.*, 1995b; Sauvaire *et al.*, 1996; Hannan *et al.*, 2003), indicating the presence of bioactive compounds in fenugreek, which may be responsible for its health benefits.

Fenugreek has been commercially grown in western Canada since around 1992, shortly after the first Canadian cultivar “AC Amber” was released by the Agriculture and Agri-Food Canada (AAFC) Research Centre in, Morden, Manitoba. This was followed by the release of “CDC Quatro” by the Crop Development Centre (CDC) Saskatoon,

Saskatchewan (Slinkard *et al.*, 2006). In 2002, CDC Canafen (used as a source of galactomannan) and CDC Canagreen (used for forage purposes) were released by the CDC in Saskatoon, and these two cultivars are licensed exclusively to Emerald Seeds Products Limited in Avonlea, Saskatchewan, Canada (Slinkard *et al.*, 2006; Blaine Sudom\*, personal communication, 2008). Fenugreek also was grown in southern Alberta first for seed as a source for spice, but later as a forage crop. The fenugreek crop improvement programs conducted at both the provincial [Alberta Agriculture and Rural Development (ARD), Crop Diversification Centre South (CDCS), Brooks] and federal (AAFC Lethbridge Research Centre) research institutions have selected genotypes based mainly upon high seed and/ or forage yield and early maturity traits, and in 2004, the Lethbridge Research Centre released “Tristar” as the first registered North American forage cultivar.

Accumulated experimental evidence and human trials has supported many of the health, medically related and nutraceutical claims made for fenugreek (Acharya *et al.*, 2007b). This has led to a growing interest in marketing of fenugreek as a natural health product. However, limited information is available regarding the biochemical composition of the genotypes that are being used in these health related applications, raising concern over the ability of the plant to produce desired results (Taylor *et al.*, 2002; Acharya *et al.*, 2004; Thomas *et al.*, 2006). Key bioactive compounds found in fenugreek need to be quantified in order to ensure that the plants being used possess the bioactive compounds essential to produce the desired effects in consumers. Once plants with these attributes have been identified, they can be used for selection of suitable

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\* Blaine Sudom, Chief Operating Officer, Emerald Seeds Products Limited, Avonlea, Saskatchewan, Canada ([www.emeraldseedproducts.com](http://www.emeraldseedproducts.com)).

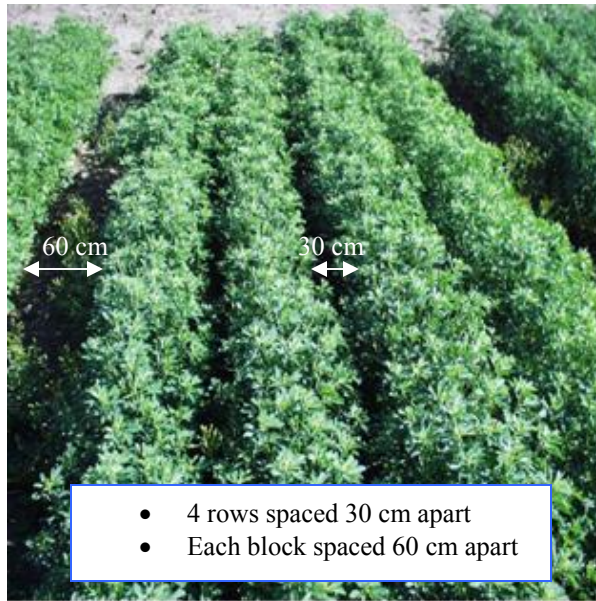
genotypes that can be further developed into cultivars specific for the natural health product processing industry.

Three putative bioactive compounds were examined in these experiments; *i.e.*, galactomannan, diosgenin [(25R)-spirost-5-en-3- $\beta$ -ol] and 4-hydroxyisoleucine. These compounds are among the most frequently identified in the literature for their medicinal and health attributes (Acharya *et al.*, 2007b). However, there are many unknown factors associated with genotype optimization and crop growing environments in relation to the productivity of bioactive compounds in fenugreek.

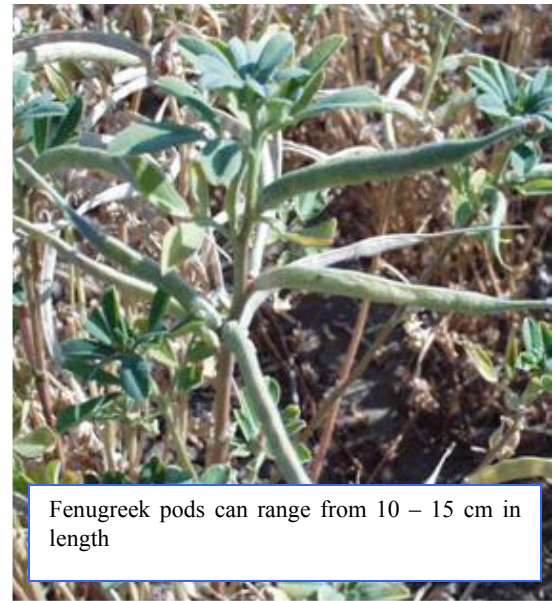
Objectives of this research study project were to:

- i. Determine the content and productivity of selected bioactive compounds (galactomannan, diosgenin and 4-hydroxyisoleucine), and seed yield of ten most promising fenugreek genotypes that have been previously selected based on seed and/or biomass yield and early maturity under the growing conditions in southern Alberta.
- ii. Assess the impact of growing environment on content and productivity of the three bioactive compounds and seed yield of ten fenugreek genotypes.
- iii. Identify the most promising fenugreek genotypes, based on production, productivity and production stability of these selected bioactive compounds, under growing conditions (rainfed and partially irrigated) in southern Alberta.





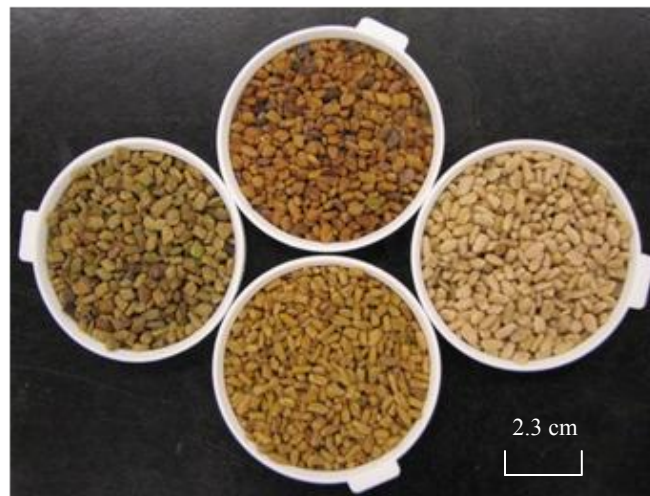
(A)



(B)



(C)



(D)

**Figure 1.1** Foliage (A), pod(s); field-growing (B) and harvested (C), and color and seed size variation of harvested seeds (D) of the fenugreek plant.

## 2.0 Literature review

### 2.1 Taxonomy of fenugreek

Fenugreek (*Trigonella foenum-graecum*) is an annual, self-pollinating legume. Most plants are diploid ( $2n=16$ ) and often used as crops in agriculture (Petropoulos, 2002). Sinskaya (1961), Hutchinson (1964) and Heywood (1967), all have described plants belonging to the genus *Trigonella*. In general, the plants are scented and can be collectively categorized according to their distinct botanical characteristics (Table 2.1).

Taxonomy of the plant is as follows:

Kingdom	<b>Plantae</b>
Class	<b>Magnoliopsida</b>
Order	<b>Fabales</b>
Family	<b>Fabaceae</b>
Genus	<b><i>Trigonella</i></b>
Species	<b><i>foenum-graecum</i></b>

### 2.2 Botanical and physiological aspects of fenugreek

Germinated seeds from fenugreek form a seedling, which eventually develops into stems, flowers, pods and seeds (Petropoulos, 2002). Following swelling of the seed, the radicle emerges from the seed coat, penetrates the soil and initiates primary root development. Release of cotyledons from seed husks soon follows and leads to growth of

**Table 2.1 Characteristics of plants of the genus Trigonella.**

<b>Plant organs/tissues</b>	<b>Characteristics</b>
Leaves	Trifoliate, usually toothed, nerves running out into teeth
Flowers Calyx Corolla Anthers Stigma Ovary	Solitary, pedunculate in axillary heads Teeth may be equal or unequal Yellow, blue or purple Uniform Terminal Sessile, ovules are numerous
Pods	Cylindrical or compressed, linear or oblong, indehiscent or dehiscent with a pronounced beak
Seeds	Tuberculate , cotyledons geniculate

**Source:** Petropoulos (2002)

the first simple leaf, followed by development of the first trifoliate leaf (Petropoulos, 2002). Throughout the plant, the leaves are found in an alternate arrangement, are ovate in appearance and slightly toothed (Slinkard *et al.*, 2006). Stems of the mature fenugreek plant are circular to slightly quadrangular, with a diameter of 0.5 - 1.0 cm. They are erect, hollow and may appear green or pinkish-green due to accumulation of anthocyanin (Petropoulos and Koulombis, 2002).

Flowering of fenugreek starts approximately 35-40 days from the date of sowing, and varies according to plant variety, climate and season in which the seeds were sowed (Petropoulos, 2002). The flowers sit in the leaf axils, are generally paired, but occasionally are solitary. There are two types of flower shoots; one that bears axillary flowers and follows an indeterminate growth habit and the other, referred to as 'blind shoots' that can carry both axillary and terminal flowers which eventually become tip bearers. There are two kinds of flowers; cleistogamous (closed) flowers and aneictogamous (open) flowers. Closed flowers are found on most plants. The keel of these plants remains closed throughout the flower's life, favoring self-pollination. Open flowers, in contrast, offer an abundance of opportunities for cross-pollination, as the corolla remains open continuously. However, these represent less than 1 % of fenugreek flowers (Petropoulos, 2002).

Seed pods from fenugreek plants are long, slender, sickle-shaped and pointed (with a sharp beak at the end). They appear brownish or yellowish brown and can be 10-19 cm in length and 0.2-0.6 cm in width (Ivimey-Cook, 1968 and Duke, 1986 cited in Petropoulos, 2002). Fenugreek plants can be single- (one pod per node) or double-podded (two pods per node projecting in opposite directions). Double-podded varieties appear to

contain higher levels of bioactive compounds (*i.e.* diosgenin; Petropoulos, 2002). Each seed-bearing branch of the plant can produce about 2-8 pods, each carrying roughly 10-20 seeds (Petropoulos and Koulombis, 2002). Fenugreek seeds are surrounded by a seed coat (testa), which is separated from the embryo by the endosperm, the principal storage organ in the seed (Spyropoulos, 2002). Between the seed coat and the endosperm, lies a single-cell layer of living tissue known as the aleurone layer. Galactomannan, a long chain polysaccharide makes up a large portion of the stored reserves in the seed, and is deposited as a cell wall thickening on the surface of cells in the endosperm. Deposition of galactomannan also occurs at the outer walls of the aleurone layer neighboring the seed coat (Spyropoulos, 2002). Seeds are generally about 3-6 mm long, 2-4 mm wide and 2 mm thick (Fazli and Hardman, 1968 cited in Petropoulos, 2002). The seeds have a rectangular or rhomboidal shape with grooves between the radicle and the cotyledon. They are generally yellowish-brown to golden yellow, but new cultivars that lack polyphenolic tannins, appear pale yellowish-white. The weight of a thousand seeds, a common seed quality determinant averages around 15-20 g (Slinkard *et al.*, 2006).

### **2.3 Historical uses of fenugreek**

Fenugreek is one of the oldest known medicinal plants that have been documented in ancient herbal publications, religious scriptures, travel records and anecdotes dating back in human history (Lust, 1986 cited in Petropoulos, 2002). Seeds of the fenugreek plant were found in the tomb of the Egyptian Pharaoh, Tuthankhamun (1333 BC – 1324 BC) and leaves of the fenugreek plant were used as one of the components of holy smoke that the Egyptians used in fumigation and embalming rites (Fazli and Hardman, 1968

cited in Petropoulos, 2002). During the ancient Greek period, fenugreek was cultivated as a forage crop. In ancient Rome, it was used as an aid to induce labor during childbirth and delivery (Yoshikawa *et al.*, 1997). Utilization of fenugreek in Chinese medicine was first introduced during the Song Dynasty (AD 1057). It was used in traditional Chinese medicine as a tonic and treatment for weakness and edema (tissue swelling due to excess lymph fluid) of the legs (Basch *et al.*, 2003). Fenugreek was later introduced to Central Europe at the beginning of the 9<sup>th</sup> century but it was not until the 16<sup>th</sup> century when cultivation of the plant in England was recorded (Petropoulos, 2002).

## **2.4 Modern uses of fenugreek**

One of the major uses of fenugreek seeds and leaves is for medicinal purposes. In India (Basch *et al.*, 2003) it has been used as part of traditional medicine practices. Fenugreek contains a myriad of phytochemicals such as steroids, flavonoids and alkaloids, which have been identified, isolated and extracted by the pharmaceutical industry to serve as raw materials for the manufacture of hormonal and therapeutic drugs (Petropoulos, 2002). Polysaccharides form the mucilage (galactomannan) present in the plant, and are finding wider applications in the food, pharmaceutical, cosmetics, paint and paper industries (Petropoulos, 2002) following the more commonly used locust bean and guar gums, all of which possess high viscosity and neutral ionic properties (Duke, 1986 cited in Petropoulos, 2002). However, fenugreek seed is most commonly used in everyday life as a spice and a seasoning in soups and curries (Duke, 1986 cited in Petropoulos, 2002). In India, fenugreek is consumed as a condiment, and is used as a coloring dye and as a medicinal stimulant to promote lactation in post-partum women and

animals (Fazli and Hardman, 1968 cited in Petropoulos, 2002; Basch *et al.*, 2003).

Another alkaloid, trigonelline that has been extracted from fenugreek contributes to its distinctive odor. Trigonelline can be used in the manufacture of imitation maple syrup and artificial flavoring for licorice, vanilla, rum and butterscotch (Slinkard *et al.*, 2006).

## **2.5 Fenugreek oils**

Extractable oil from fenugreek represents about 6-8% of the seed weight and carries a fetid odor and bitter taste. Its fatty acid composition is listed in Table 2.2 (Suliman *et al.*, 2008). It is reported that the unsaponifiable portion of the oil (3.9 %) contains a lactation-stimulation factor (Srinivasan, 2006). Being strongly scented, the oil is used as an insect repellent for grains and cloths (Duke, 1986 cited in Petropoulos, 2002). In cosmetics, traces of the oil are used in perfumes (Fazli and Hardman, 1968 cited in Petropoulos, 2002).

## **2.6 Fenugreek as a forage crop**

The high forage value of fenugreek is attributed to its rich content of protein, vitamins, and amino acids along with its good digestibility in cattle. The seeds contain diosgenin, a growth and reproduction hormone. The combination of the above factors in fenugreek is thought to improve growth rates and feed utilization efficiency in beef cattle [Alberta Agriculture and Rural Development, 2007]. In a study by Shah and Mir (2004), 20 % (w/w) fenugreek seed was supplemented into a dairy cattle diet and was reported to significantly improve the fatty acid profile in the milk produced ; an increase in the

**Table 2.2 Fatty acid composition of oil extracted from fenugreek seeds.**

<b>Fatty acid</b>	<b>Content (%)</b>
Palmitic acid	11.0
Stearic acid	4.5
Arachidic acid	1.5
Oleic acid	16.7
Linoleic acid	43.2
$\alpha$ -Linolenic acid	22.0

**Source:** Sulieman *et al.* (2008)



polyunsaturated fatty acid (*i.e.* linoleic, linolenic and conjugated linolenic acids) concentrations was observed. The study also found that the fenugreek-fed cattle had a 4 % reduction in blood cholesterol concentration as well as a 19 % decrease in milk cholesterol levels compared to controls, potentially extending health benefits to human consumers of the milk.

## **2.7 Fenugreek as a functional food**

Presence of galactomannan in fenugreek seed accounts for approximately half of its dry weight and is recognized as the principal source of soluble dietary fiber in the plant (AAFC, 2005). Dietary fiber is known to have the potential to reduce risk of cardiovascular disease and to protect against some cancers through the reduction of low-density lipoprotein (LDL) and total cholesterol (AAFC, 2005). In Egypt, supplementation of wheat flour with a small percentage of fenugreek flour has been reported to enhance the nutritional quality of bread as well as its organoleptic characteristics (Bakr, 1997). Addition of fenugreek flour to more commonly used flours for bread making is a common practice in Egypt (Galal, 2001). Sharma and Chauhan (2000) reported improved physicochemical, nutritional and rheological properties of bread made from wheat flour supplemented with fenugreek flour. Galactomannan (mucilage or gum) in fenugreek acts as a thickener or stabilizer in foods such as soups, sauces and ice-cream (Garti *et al.*, 1997, Balyan *et al.*, 2001, Seghal *et al.*, 2002). Currently, the food industry utilizes locust bean gum and guar gum as emulsifiers, viscosity-builders, thickeners and stabilizers. The relatively low cost and ease of growing fenugreek in abundance in Canada makes fenugreek gum a potential candidate for commercial use.

## 2.8 Distribution and cultivation of fenugreek

Fenugreek is native to the Indian subcontinent and the Eastern Mediterranean region. However, it also has been grown in central Asia and in North Africa since early times (Petropoulos, 2002). The extent to which fenugreek has been distributed throughout the world is indicated by the various names it assumes in different countries (Srinivasan, 2005); *i.e.*, *Fenugrec* (French), *Methi* (Hindi), *Bockshorklee* (German), *Fieno greco* (Italian), *Pazhitnik* (Russian), *Alholva* (Spanish), *Koroha/Koroba* (Japanese), *Hulba* (Arabic), *Halba* (Malaya), and *Ku'-Tou / Hu Lu Ba* (Chinese). Other local names for fenugreek in different regions are given in Table 2.3 (Petropoulos, 2002). Fenugreek has been reported as a cultivated crop in all habitable continents of the world. A breakdown of the various countries of each continent that cultivates fenugreek as a crop is listed in Table 2.4.

## 2.9 The fenugreek market

Estimates show that the annually cultivated area of fenugreek is about 57 000 ha, with seed production at 68 000 tons (Petropoulos, 2002). Currently, fenugreek represents an important cash crop in India (the leading producer), Morocco, China, Pakistan, Turkey, Egypt and Ethiopia (Fotopoulos, 2002). India claims to produce 70-80 % of the world's exported fenugreek, followed by Morocco (Fotopoulos, 2002). Other exporting countries include Spain, which supplies major markets in Italy, Tunisia, Turkey, Lebanon and Israel. The major export market for Indian fenugreek appears to be European Union, Japan, United Arab Emirates, Yemen and South Africa (Weiss, 2002).

**Table 2.3 Local names for fenugreek in different countries.**

Language	Local names for fenugreek
Arabic	Hulba, Hulabah, Hhelbah, Hhelbeh
Armenian	Shambala
Azerbaijani	Khil'be, Boil
Chinese	Ku'-Tou, Hu Lu Ba
Croatian	Piskayika, ditelina rogata
Czech	Piskayika, recke seno
Dutch	Fenegriek
Ethiopian	Abish
French	Fenugrec
German	Bockshorklee, Griechisch Heu, Griechisches Heu, Kuhhornklee, Bisamklee
Greek (modern)	Trigoniskos, Tsimeni, Tintelis, Moschositaro, tili, tilipina
Hindi	Methi
Hungarian	Görögszéna
Italian	Fieno greco
Japanese	Koroha , Koroba
Malay	Halba
Persian (Irani)	Schemlit
Polish	Fengrek, Kozieradka
Portuguese	Alforva
Slovak	Seneyka grecka, seno grecka
Spanish	Alholya
Swedish	Bockhornsklover
Russian	Pazhitnik
Uzbekistani	Khul'ba, Ul'ba, Boidana

**Source:** Petropoulos (2002)

**Table 2.4 Cultivation and distribution of fenugreek in the world.**

Continent	Country
Asia	China, India, Iran, Israel, Japan, Lebanon, Pakistan
Africa	Egypt, Ethiopia, Kenya, Morocco, Sudan, Tanzania, Tunisia
Europe	Austria, England, France, Germany, Greece, Portugal, Russia, Spain, Switzerland, Turkey, Ukraine, United Kingdom
North America	Canada, the United States
South America	Argentina
Australia/Oceania	Southwestern and southeastern Australia

**Source:** Petropoulos (2002)

Major importing countries are generally European nations with Germany as the largest importer of fenugreek from India, Morocco and China. Germany roughly imports close to 200 tons of seed annually, mainly using it to flavor food (Fotopoulos, 2002). France, Holland, Spain and the United Kingdom follow closely as major importers of the spice in Europe.

In North America, fenugreek is relatively unfamiliar to most households. However, there is interest among forage breeders to market fenugreek as a potential animal feed (Shah and Mir, 2004). There is also significant development in the nutraceutical industry in which fenugreek is used as a source of bioactive components for health supplements (TSI Health Sciences\*, Emerald Seed Products).

## **2.10 Fenugreek in Canada**

Fenugreek was introduced into Canada as a spice and forage crop. The first commercial cultivar AC Amber was released by the AAFC Research Station at Morden, Manitoba. Shortly after its release, commercial growth of fenugreek was initiated in western Canada. Other cultivars released include CDC Quatro, CDC Canagreen and CDC Canafen, all developed at the Crop Development Centre (CDC), University of Saskatchewan, Saskatoon. Tristar, developed primarily as a forage cultivar by AAFC at Lethbridge, Alberta in cooperation with Alberta Agriculture and Rural Development was released in 2004. Currently, more than 700 acres of land in western Canada are dedicated to the planting of fenugreek for seed production, much of which is concentrated in

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\* TSI Health Sciences is a US-based nutraceutical company that was recently awarded a patent (March 2008) to produce a fenugreek seed extract containing 4-hydroxyisoleucine in its manufacture of Promilin, a health supplement ([www.tsiinc.com](http://www.tsiinc.com)).

southern Saskatchewan (Blaine Sudom<sup>\*</sup>, personal communication, 2008). Emerald Seed Products, a Saskatchewan-based functional food and plant-based natural products manufacturer utilize fenugreek seeds as a source of galactomannan and diosgenin for the natural health products and feed industries, respectively. CDC Canagreen (primarily developed for forage use) and CDC Canafen are licensed exclusively to this organization for these purposes (Blaine Sudom, personal communication, 2008). Emerald Seeds Products is also currently the sole producer of fenugreek extracts as natural health products in Canada. Fenugreek seeds produced in southern Saskatchewan are also sold to producers in Alberta and Manitoba for forage production (Blaine Sudom, personal communication, 2008).

## **2.11 Chemical constituents in fenugreek**

Fenugreek seed contains approximately 4 - 10 % moisture, 6 - 8 % fat, 18 - 30 % protein and 48 - 55 % fiber (Sauvaire *et al.*, 1976; Sharma *et al.*, 1986b; Vats *et al.*, 2003; Srinivasan, 2006), depending on varietal and ecological factors. A comprehensive list of chemical components found in fenugreek leaves and seed is provided in Table 2.5.

Hemavathy and Prabhakar (1989) published a detailed report on the lipid composition of fenugreek seeds. In their report, total lipids extracted from dry seeds were 7.5 %, which agrees with other values given in the literature (Srinivasan, 2006). Fractionation of the lipids extracted indicates that the seed contains 84.1 % neutral lipids (composed mainly of triacylglycerols), 5.4 % glycolipids and 10.5 % phospholipids. A

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<sup>\*</sup> Blaine Sudom, Chief Operating Officer, Emerald Seed Products Limited, Avonlea, Saskatchewan, Canada ([www.emeraldseedproducts.com](http://www.emeraldseedproducts.com))

**Table 2.5 Chemical composition of fresh fenugreek leaves and mature seeds.**

<b>Component</b>	<b>Fresh fenugreek leaves (100 g)</b>	<b>Fenugreek seeds (100 g)</b>
Moisture	86.0 g	
Protein	4.4 g	30 g
Fat	1.0 g	7.5 g
Fiber	1.0 g	50 g
Sapogenins		2 g
Diosgenin		
Yamogenin		
Gitogenin		
Neogitogenin		
Yuccagenin		
Tigogenin		
Sarsasapogenin		
Smilagenin		
Trigonelline		380 mg
Ca	395 mg	160 mg
Mg	67 mg	160 mg
P	51 mg	370 mg
Fe	16.5 mg	14 mg
Na	76 mg	19 mg
K	31 mg	530 mg
Cu	0.26 mg	33 mg
S	167 mg	16 mg
Cl	165 mg	165 mg
Mn		1.5 g
Zn		7.0 mg
Cr		0.1 mg
Choline	1.35 g	50 mg
Vitamin C	52 mg	50 mg
B-carotene	2.3 mg	96 µg
Thiamine	40 µg	340 µg
Riboflavin	310 µg	290 µg
Nicotinic acid	800 µg	1.1 mg
Folic acid		84 µg

**Source:** Srinivasan (2006)

breakdown of the fatty acid composition shows that linoleic acid is the predominant fatty acid, followed by  $\alpha$ -linolenic acid and oleic acid.

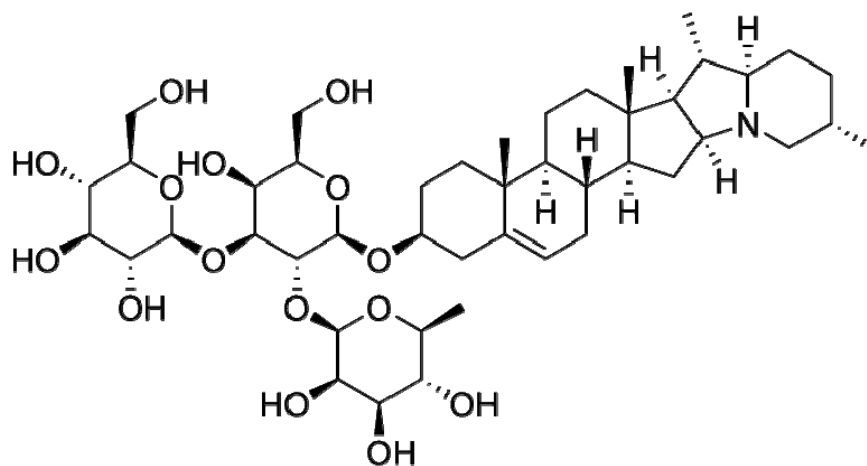
Some of the biological and pharmacological attributes of fenugreek are ascribed to the abundance of chemical constituents it contains; *i.e.*, its steroids (saponins/sapogenins), alkaloids, polyphenolic compounds, volatile compounds and amino acids, in particular 4-hydroxyisoleucine (Skaltsa, 2002; Srinivasan, 2006).

The bitter flavor of fenugreek is most likely due to the presence of saponins (4 -8 %) and alkaloids (~ 1 %). Consumption of these constituents appears to contribute to gastric stimulation, increased acidity and improved appetite (Srinivasan, 2006).

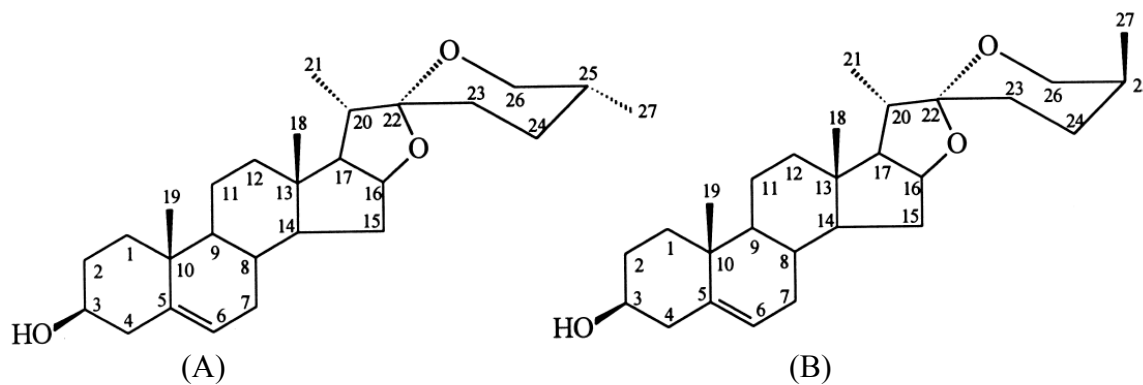
### 2.11.1 Steroids

Fenugreek contains various steroidal sapogenins with diosgenin ( $\Delta^5$ , 25 $\alpha$ -spirostan-3 $\beta$ -ol) being the major component. Sapogenins are the aglycone portions of the of plant-based steroid-derivative saponins, containing 6-C rings with 2 to 3 side chains substitutable either with methyl or hydroxyl groups (Skaltsa, 2002). Saponins are amphipatic glycosides (Fig. 2.1), containing both hydrophilic glycoside and lipophilic triterpene moieties, therefore capable of producing soap-like foaming properties.

Diosgenin, a 27-C steroidal compound (Fig. 2.2) is currently used as a raw material for the manufacture of oral contraceptives and sex hormones by the pharmaceutical industry (Skaltsa, 2002). It is traditionally extracted from wild Mexican and Asian yam species, *Dioscorea*. Utilization of fenugreek seeds as an alternative source of diosgenin was proposed in the 1950s in recognition of the increasing demand for raw steroids (Marker *et al.*, 1947 and Fazli and Hardman, 1968 cited in Skaltsa, 2002;



**Figure 2.1** Chemical structure of solanine, exemplifying the amphipathic properties of a typical steroidal saponin.



**Figure 2.2** Chemical structures of diosgenin (A) and yamogenin (B) showing epimerism at C-25. Source: Pires *et al.* (2002).



Bhatnagar et al., 1975). It is important to note that fenugreek seeds contain no free sapogenins (Sauvaire and Baccou, 1978), and that they occur as complex glycosides (saponins) which are released following enzymatic treatment or acid hydrolysis (Blunden and Hardman, 1963 cited in Skaltsa, 2002). Fenugreek sapogenins also occur in various forms due to stereochemistry and functional groups substitution. Those that have been reported in extracts from fenugreek seeds are diosgenin, yamogenin, tigogenin, neotigogenin, yuccagenin, lilagenin, gitogenin, neogitogenin, sarsapogenin and smilagenin (Gupta et al., 1986b; Cornish et al., 1983; Skaltsa, 2002). Yamogenin is the (25S)-epimer of diosgenin (Fig. 2.2) and occurs at a ratio of 2:3 with diosgenin in the seed, acid-hydrolyzed extract (Skaltsa, 2002)

### **2.11.2 Polyphenolic compounds**

Polyphenols possess anti-oxidative attributes, which may prevent some forms of chronic disease. Gupta and Nair (1999) reported that fenugreek is generally rich in polyphenols (100 mg g<sup>-1</sup>). The phenylpropanoid pathway occurs ubiquitously across the plant kingdom and has been shown to be involved in the biosynthesis of important secondary plant metabolites such as flavonoids and lignins (Dixon and Sumner, 2003).

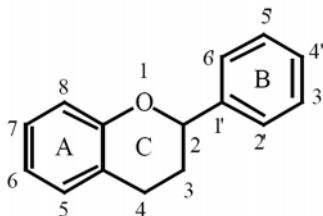
Flavonoids consist of several classes; *i.e.*, the flavones, flavonones, flavonols, flavanols (flavan-3-ols), isoflavones, proanthocyanidins and anthocyanins. They exhibit strong antioxidative effects *in vitro* and have been largely associated with prevention of oxidative damage in biological systems, which can confer protection against cardiovascular disease and cancer (Erdman *et al.*, 2005). Flavonoids have a basic three ring chemical structure; *i.e.*, two aromatic rings (A and B) coupled with a three-carbon

oxygenated heterocyclic ring (C) (Fig. 2.3). Among the many flavonoids reported in fenugreek, quercetin, luteolin, vitexin and kaempferol appear to be the most common (Parmar *et al.*, 1982; Jain *et al.*, 1992; Huang and Liang, 2000; Skaltsa, 2002). Quercetin and kaempferol are flavonols (Fig. 2.4); luteolin is a flavone (Fig. 2.5) while vitexin occurs as a glycosylated flavone (Fig. 2.6).

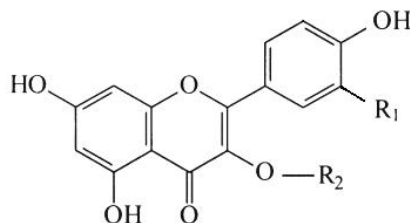
Isoflavanoid phytoalexins are also reported to occur in fenugreek in the form of the pterocarpan, medicarpin (Fig. 2.7) and maackiaian (Ingham, 1981). These compounds play a key role in maintaining plant health in the event of microbial invasion; they are absent in healthy plants and their production is only induced upon microbial attack (Skaltsa, 2002).

Polyphenolics are also potent antioxidants that scavenge for free radicals and protect against oxidation. Common phenolic compounds isolated from fenugreek are scopoletin, coumarin, chlorogenic and caffeic and p-coumaric acids (Figs. 2.8 and 2.9) (Skaltsa, 2002; Acharya *et al.*, 2007b). Aqueous extracts of germinated fenugreek seeds containing  $17.5 \text{ mg mL}^{-1}$  quercetin and  $64.4 \text{ mg mL}^{-1}$  gallic acid equivalents per gram of fenugreek powder and was shown to exhibit significant antioxidant activity and offered greater protection against oxidation compared to other extracts (Dixit *et al.*, 2005).

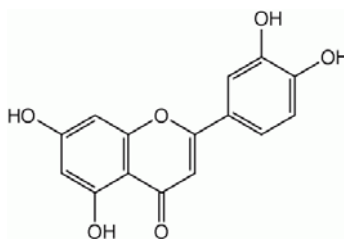
The protective action of fenugreek seed polyphenols has been investigated in rats on lipid peroxidation using aqueous extracts and was found to exert gastroprotective effect on gastric ulcer (Pandian *et al.*, 2002) and prevent ethanol-induced toxicity in the liver and the brain (Thirunavukkarasu *et al.*, 2003). Recently, Kaviarasan *et al.* (2006) reported on the cytoprotective effect of a polyphenolic extract of fenugreek seeds against ethanol-induced damage in human Chang liver cells. The protective effects were found



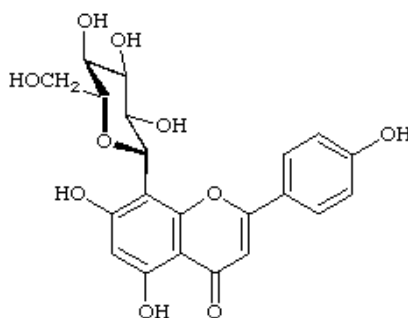
**Figure 2.3 Basic chemical structure of a flavonoid.**



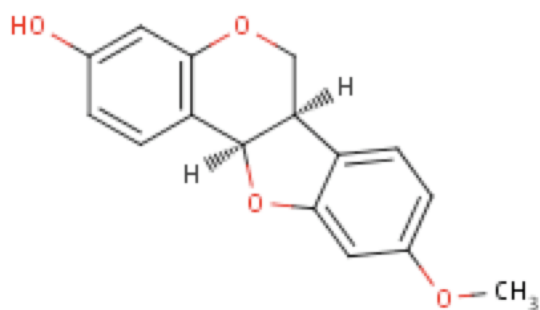
**Figure 2.4 Chemical structure of a flavonol; quercetin when R<sub>1</sub> = OH, R<sub>2</sub> = H; kaempferol when both R<sub>1</sub> and R<sub>2</sub> = H. Source: Dubber and Kanfer (2004).**



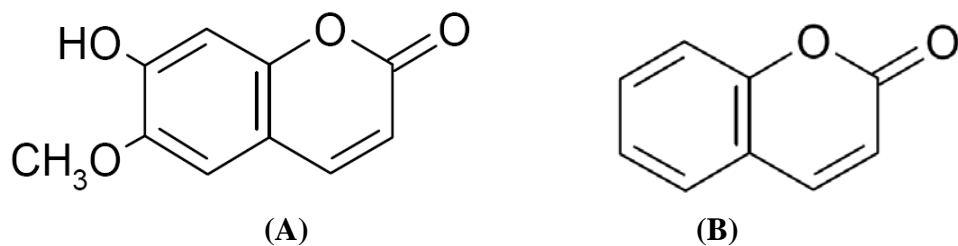
**Figure 2.5 Chemical structure of luteolin, a flavone.**



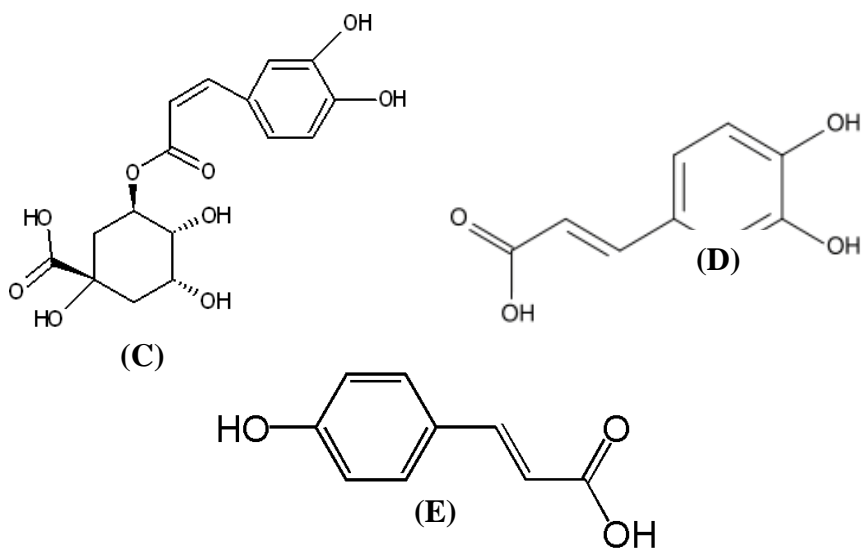
**Figure 2.6 Chemical structure of vitexin (8-C- $\beta$ -D-glucosyl 5, 7, 4'-trihydroxyflavone). Source: chemBlink (2008).**



**Figure 2.7 Chemical structure of the pterocarpan, medicarpin.** Source: European Bioinformatics Institute (2008).



**Figure 2.8 Chemical structures of (A) scopoletin and (B) coumarin.**



**Figure 2.9 Chemical structures of (C) chlorogenic acid, (D) caffeic acid and (E) *p*-coumaric acid.**

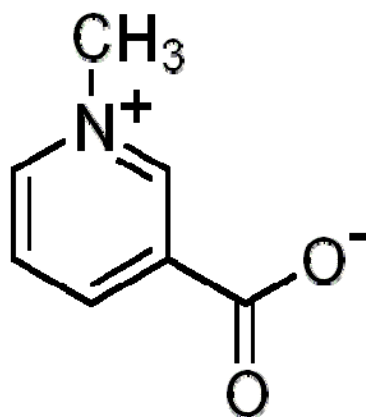
comparable to a silymarin, a known hepatoprotective agent.

### **2.11.3 Alkaloids**

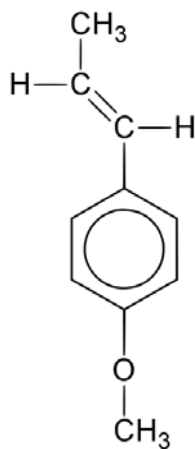
Trigonelline (Fig. 2.10), a methylbetaine derivative of nicotinic acid (Skaltsa, 2002) is one of the major alkaloids found in fenugreek seeds. This compound (chemical formula  $C_7H_7NO_2$ ) has been reported to exert mild hypoglycemic (Shani *et al.*, 1974; Marles and Farnworth, 1994) and anti-pellagra [pellagra is a disease caused by lack of dietary niacin (vitamin B3) and the amino acid tryptophan] effects (Bever and Zahnd, 1979).

### **2.11.4 Volatile components**

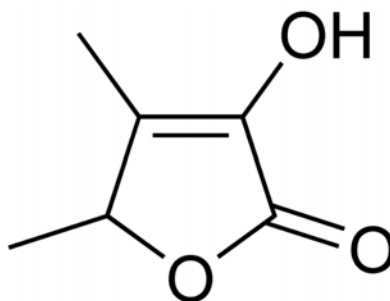
Volatile constituents contribute to the aroma and flavor of fenugreek. Anethol (Fig. 2.11), an aromatic compound found mostly in anise, camphor and fennel, also occurs in fenugreek and produces a licorice-like aroma (Aggarwal and Shishodia, 2006; Acharya *et al.*, 2007b). Mazza *et al.* (2002) have identified 175 volatile constituents in Sicilian fenugreek seeds, which include carbonyls, sesquiterpene hydrocarbons, alcohols, heterocyclic, and furan compounds. Sotolone (3-hydroxy-4,5-dimethyl-2(5H)-furanone (Fig. 2.12) has been identified as the principal component contributing to the flavor of fenugreek (Hatanaka, 1992; Blank *et al.*, 1997). These compounds together impart the burnt sugar, curry or maple syrup flavour, which is characteristic of fenugreek (Monastiri *et al.*, 1997).



**Figure 2.10** Chemical structure of trigonelline.



**Figure 2.11** Chemical structure of anethol.



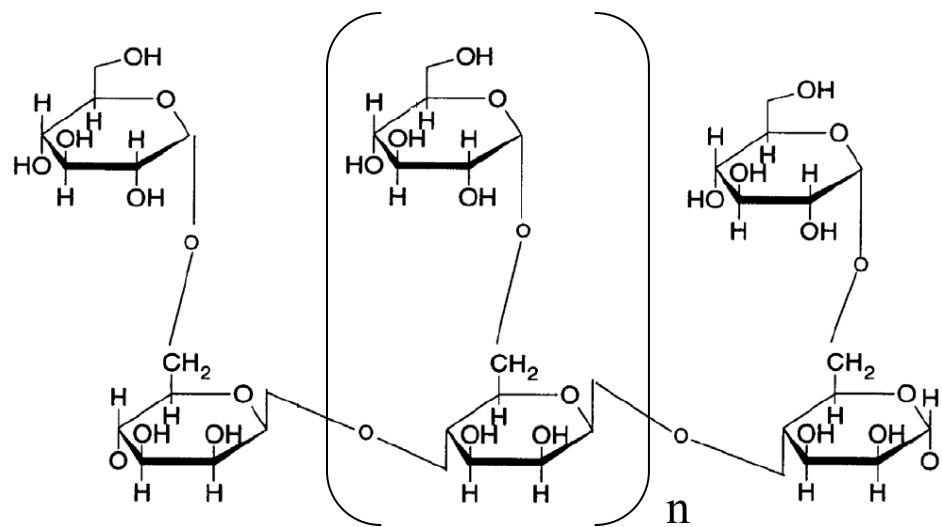
**Figure 2.12** Chemical structure of sotolone, the principal flavour constituent in fenugreek.

## 2.12 Bioactive Compounds and their Biochemistry

Various clinical (Bhardwaj *et al.*, 1994; Sharma *et al.*, 1996a; Vajifder *et al.*, 2000; Sowmya and Rajyalakshmi, 1999; Abdel-Barry *et al.*, 2000; Gupta *et al.*, 2001) and animal studies (Sauvaire *et al.*, 1991; Evans *et al.*, 1992; Thirunavukkarasu *et al.*, 2003; Anuradha and Ravikumar, 2001; Puri *et al.*, 2002; McAnuff *et al.*, 2002) conducted using fenugreek have identified numerous potential health benefits for consumption of fenugreek, and have drawn much attention to fenugreek as a potential functional food and natural health product or ingredient therein. Among the plethora of bioactive compounds found in fenugreek, the three major chemical constituents, galactomannan, diosgenin and 4-hydroxyisoleucine have, by far superseded the rest as being the most frequently studied health-promoting factors for humans.

### 2.12.1 Galactomannan

Galactomannan (Fig. 2.13) represents the major polysaccharide found in fenugreek seeds and accounts for approximately 17 – 50 % of the dry seed weight (Petropoulos, 1973 and Duke, 1986 cited in Petropoulos, 2002; Kochhar *et al.*, 2006). It is an integral component of the cell wall which is found concentrated around the seed coat (Spyropoulos, 2002). Galactomannans are structurally composed of a 1→4 beta-D-mannosyl backbone substituted by a single galactose unit  $\alpha$ -linked at the C-6 oxygen (Bhaumick, 2006). Fenugreek galactomannans are unique relative to other commonly used galactomannans such as those found in guar and locust beans. They contain a galactose to mannose ratio of 1:1. This high degree of galactose substitution renders the



**Figure 2.13** Chemical structure of fenugreek galactomannan (adapted from Bhaumick, 2006)



molecule relatively more soluble compared to galactomannans from guar or locust bean, which has a galactose to mannose ratio of 1:2 and 1:4, respectively (Reid and Meier, 1970; Brummer *et al.*, 2003).

**Enzyme specificity was shown to influence the degree of galactose substitution during galactomannan formation. It has been shown that substitution on the mannan backbone is determined by the existing galactose-substitution pattern** on to the nearest mannosyl residues (Reid *et al.*, 2003). Biosynthesis of galactomannan is attributed to the cooperative actions of both D-mannosyltransferase and D-galactosyltransferase. Mannosyltransferase was shown to require divalent metal cations such as  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Mn}^{2+}$  for its activity, whereas galactosyltransferase has a specific requirement for  $\text{Mn}^{2+}$ .

The formation of (1→4) β-linked mannan by D-mannosyltransferase is independent of the presence of UDP-D-galactose, while the activity of D-galactosyltransferase is highly dependent on the transfer of D-mannosyl residues from GDP-D-mannose.

Mannosyltransferase extends the linear (1→4)-β-linked D-mannan backbone towards the non-reducing terminus, while galactosyltransferase transfers a (1→6)-α-D-galactosyl residue onto the growing mannan chain from the same end.

The soluble nature of galactomannan fiber from fenugreek has been linked to numerous human health benefits, mainly in the reduction of plasma glucose levels which has an antidiabetic effect (Sharma 1986b; Madar *et al.*, 1988; Madar and Shomer, 1990). This has been demonstrated in animal models where diabetes was experimentally induced in dogs, rats, mice and rabbits using either alloxan or streptozocin (Zanosar®) which kill insulin-producing beta cells in the pancreas of mammals (Ribes *et al.*, 1986; Swanston-

Flatt *et al.*, 1989; Grover *et al.*, 2002; Mondal *et al.*, 2004; Hannan *et al.*, 2007). Hannan *et al.* (2007) also have demonstrated that the soluble dietary fiber (SDF) portion of fenugreek can significantly improve glucose homeostasis in type 1 and type 2 diabetes by delaying carbohydrate digestion and absorption. They have also suggested that the SDF fraction may enhance insulin action in type 2 diabetes as indicated by the improvement of oral glucose tolerance in these test subjects.

Monnier *et al.* (1978) and Jenkins (1979) reported that addition of soluble fiber to the diet of diabetics reduced blood glucose during an oral glucose tolerance test (OGTT). The OGTT is a blood test that is used to assess metabolism of sugar in test subjects. Individuals are required to fast prior to consuming a fixed amount of glucose. Their blood is later tested at designated time intervals in order to determine if it will reach unusually high glucose levels (American Diabetes Association, 2008). Johnson and Gee (1980) reported that use of soluble fiber from fenugreek resulted in inhibition of glucose absorption in the intestine. It has been postulated that fenugreek galactomannan may regulate plasma glucose levels by delaying gastric emptying and by interfering with glucose absorption in the gut (Madar, 1984; Al-Habori and Rahman, 1998; Nahar *et al.*, 2000). Sharma (1986b) suggested that the delay in carbohydrate absorption induced by a diet rich in fenugreek fiber could reduce insulin requirements. Dietary fibers such as galactomannan are able to reduce postprandial glycaemia and exhibit great potential in diabetes management (Jenkins *et al.*, 2000; Basch *et al.*, 2003).

In several small-scale clinical trials, fenugreek seeds have been found to reduce fasting serum glucose levels in patients exhibiting both acute and chronic hyperglycaemia (Basch *et al.*, 2003). Cooked and defatted fenugreek seeds (containing soluble fiber)

prevented a rise in blood glucose levels in these patients. This was attributed to hypoglycaemic properties of fenugreek gum present in the seeds which were not lost during the cooking process (Srinivasan, 2006). Sharma *et al.* (1990) and Sharma and Raghuram (1990) also have reported that reduced levels of fasting blood glucose, improved glucose tolerance and reduced glucose excretion in both insulin-dependent (type 1) diabetic and non-insulin dependent (type 2) diabetic patients respectively, following a 10-day trial in which the subjects were given 100 g of defatted fenugreek powder. Pahwa (1990) conducted a study using both diabetic and non-diabetic test subjects and reported a significant hypoglycaemic effect only in diabetic individuals following fenugreek consumption prior to glucose loading (1 g kg<sup>-1</sup> body weight). However, in a later study by Nahar *et al.* (1992), a similar blood glucose lowering effect was observed in healthy human subjects given the soluble fiber fraction as a single dose. A double-blind, placebo-controlled study involving 25 type 2 diabetic patients was conducted by Gupta *et al.* (2001) to evaluate the effects of fenugreek on control of blood glucose levels and insulin resistance. Diabetic and normal patients were separated into groups, and they received an alcoholic extract of fenugreek seeds and 'usual care' (dietary discretion and exercise), respectively. It was reported that both groups experienced a reduction in fasting blood glucose levels without significant differences, but significantly lower levels for blood glucose and insulin were observed in the diabetic group. This suggests that fenugreek seed extracts and a discrete diet/ exercise habit may be equally effective in the control of blood glucose and insulin levels (Basch *et al.*, 2003).

Amin *et al.* (1987) reported that fenugreek seeds exerted hypoglycaemic effects by inhibiting the activities of  $\alpha$ -amylase and sucrase. More recent studies have also

associated the therapeutic effectiveness of fenugreek with beneficial counter-changes in enzyme activity in glucose and lipid metabolism (Gupta *et al.*, 1999; Raju *et al.*, 2001). They assessed the effect of fenugreek whole seed powder administered to alloxan-induced diabetic rats on glycolytic, gluconeogenic and nicotinamide adenine dinucleotide phosphate (NADP)-linked lipogenic enzymes in liver and kidney tissues. A favourable restoration of activity of these enzymes to control levels was reported following fenugreek treatment, resulting in stabilization of glucose homeostasis. A potential therapeutic role for fenugreek in the treatment of type-1 diabetes was suggested. Recently, Hannan *et al.* (2007) looked at the inhibitory effects of a SDF fraction from fenugreek on the activity of digestive enzymes *in vivo*. Their findings suggest that the reduction in sucrose absorption seen following fenugreek treatment may be related to inhibition of disaccharidase (sucrase) activity in the gut. Although the mode of action for SDF in diabetes management is still not well understood in human test subjects, fenugreek galactomannan, as exemplified by the various animal and clinical studies, can be an effective support therapy in the control of this disease.

Fenugreek galactomannan has also been shown to have hypocholesterolaemic properties in diabetic dogs and non-diabetic rats (Sharma, 1986a; Ribes *et al.*, 1987). In humans, Sharma *et al.* (1991) found that treatment of 15 hyperlipidemic adults who were fed 100 g of defatted fenugreek daily over a three-week period had lower blood cholesterol levels. These test subjects reported lower than baseline values for triglyceride and low-density lipoprotein (LDL) cholesterol. Sowmya and Rajyalakshmi (1999) conducted a study to assess the effect of germinated fenugreek seed consumption in human subjects on cholesterol levels. It was revealed that germination drastically

increased the soluble fiber content in fenugreek seeds. Consumption of these seeds brought about a significant reduction in total and LDL cholesterol levels, although no significant changes were observed for high density-lipoprotein (HDL), very low-density lipoprotein (VLDL) cholesterol and triglyceride levels. In a similar study by Gupta *et al.* (2001), the diabetic group showed a significant decrease in serum triglyceride levels and a similar increase in high-density lipoprotein (HDL) levels when compared to normal test subjects. A more recent study by Venkatesan *et al.* (2003) investigated the hypocholesterolemic effects of a unique dietary fiber ('Fibernat'), which is a combination of fenugreek seed powder, guar gum and wheat bran, in rats. The study examined lipid metabolism and cholesterol homeostasis by determining the activity of hepatic triglyceride lipase (HTGL) and uptake of atherogenic lipoproteins (LDL and VLDL) by the hepatic apo B, E receptor. Fibernat was successful in increasing both apo B and E receptor expression in the liver and activity of HTGL, giving rise to a marked hypocholesterolemic effect and establishment of an improved cholesterol homeostasis in Fibernat-fed rats.

It has been suggested that formation of a physical barrier in the gut by the viscous fraction of fenugreek may aid in inhibition of bile salt absorption in the intestine or may possibly cause intra-luminal binding of cellular receptors, resulting in increased fecal extraction of bile acids and neutral sterols. In both cases, existing cholesterol is converted to bile acids, hence decreasing plasma cholesterol levels (Madar and Shomer, 1990; Venkatesan *et al.*, 2003). This has been demonstrated in a recent study by Dakam *et al.* (2007), which looked at the physiological properties of fenugreek galactomannan in rats. Using male abino Wistar rats, they divided the animals into three groups according

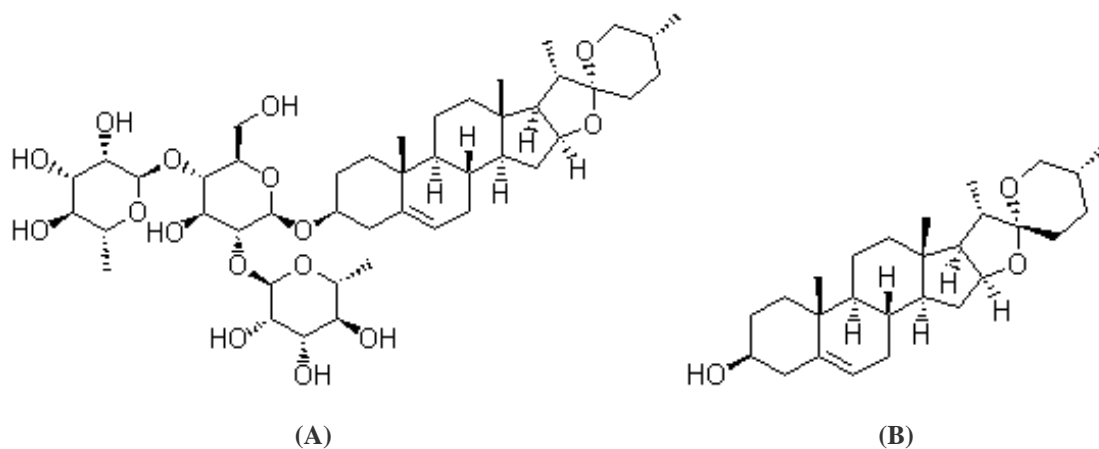
to their diet over a period of 4 weeks; *i.e.*, galactomannan (gum), gum + sodium bicarbonate, gum + albumin. The researchers reported a significant decrease in plasma total cholesterol in all three groups, with the greatest decrease in LDL-cholesterol for the gum + albumin group. They hypothesized that the combination of albumin (protein) with galactomannan contributed to the viscosity associated with the gum solutions administered, and consequently may offer better protection against coronary heart disease.

### 2.12.2 Diosgenin

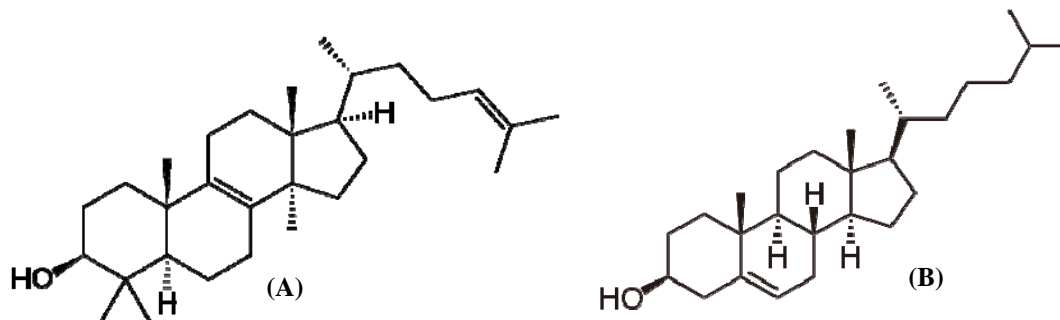
Diosgenin is a 27-carbon steroidal compound with the chemical name (25R)-spirost-5-en-3- $\beta$ -ol (Fig. 2.14) and is one of the many saponin glycosides found in fenugreek seed, also derived from yams (*Dioscorea* spp.) (Sauvaire and Baccou, 1978). It is currently an important raw material for the manufacturing of pharmaceutical hormones and steroids such as estrogen, progesterone, testosterone and glucocorticoids (Skaltsa, 2002). Diosgenin occurs naturally as a glycosylated compound in fenugreek, and can be liberated by acid hydrolysis (which removes three carbohydrate residues) of the steroidal saponin, dioscin (Fig. 2.14) (Dewick, 1997; Sauvaire *et al.*, 1991).

Diosgenin is synthesized as part of the mevalonate pathway in the biosynthesis of steroids (C<sub>18</sub>-C<sub>30</sub>). Steroids are modified triterpenoids which contain a tetracyclic ring structure of lanosterol (Fig. 2.15) but lack the three methyl groups at C-4 and C-14 (Dewick, 1997). Lanosterol is a precursor to the biosynthesis of plant cholesterol, which in turn is the most fundamental structure of a plant steroid. Steroidal diosgenin is formed

by modification of the side chain of cholesterol, in which a spiroketal structure is formed at C-22, yielding a non-polar compound with 6 carbon rings.



**Figure 2.14** Chemical structure of dioscin (A) and diosgenin (B).



**Figure 2.15** Chemical structure of lanosterol (A), the precursor to the biosynthesis of cholesterol (B) and other phytosterols in plants.

Hydrolyzed chemical extracts (*i.e.*, in ethanol/ methanol) of fenugreek seeds yield a mixture of steroidal sapogenins (Marker *et al.*, 1947 cited in Skaltsa, 2002). These extracts contain major sapogenins such as diosgenin, yamogenin, tigogenin, neotigogenin, smilagenin and sarsapogenin, and minor compounds such as yuccagenin, gitogenin and neogitogenin (Fazli and Hardman, 1971; Gupta *et al.*, 1986; Sauvaire *et al.*, 1996; Taylor *et al.*, 1997; Petropoulos, 2002). Depending upon biogeographic origins, genotypes and environmental factors, reported diosgenin contents in fenugreek vary between 0.3 and 2.0% (Fazli and Hardman, 1968 cited in Petropoulos, 2002; Puri *et al.*, 1976; Sharma and Kamal, 1982; Taylor *et al.*, 2002). It has been demonstrated that fenugreek with similar genotypes grown in different environments and geographical locations, can possess significantly different plant chemical compositions (Acharya *et al.*, 2006; Thomas *et al.*, 2006; Taylor *et al.*, 1997) resulting from genotype x environment interactions.

Atherosclerosis (the narrowing of blood vessels) is associated with high levels of LDL- and VLDL-cholesterol and is a major cause of cardiovascular disease (Srinivasan, 2006). Therefore, a reduction in LDL-cholesterol is considered essential to reducing the risk of a heart disease. Laguna *et al.* (1962; cited in Sauvaire *et al.*, 1991) reported that diosgenin treatment was able to lower plasma cholesterol concentrations in chickens and rabbits fed with cholesterol. In another study, diabetic rats were fed with steroidal saponin extracts from fenugreek and a reduction in both VLDL and LDL total cholesterol was observed (Sauvaire *et al.*, 1996). Ribes *et al.* (1987) treated diabetic dogs with extracts of germinated fenugreek seeds containing large amounts of protein (52.8 %) and saponins (7.2 %), and reported a significant decrease in triglycerides and plasma



cholesterol concentrations in these animals. Further analysis using extracts containing a saponin concentration as high as 22% (w/v) produced a sharp decrease in LDL-cholesterol levels whereas a high protein-containing extract (70.5%) did not produce similar effects. Steroidal saponins and/or sapogenins (diosgenin) have been studied by Sauvaire *et al.* (1991) and were specifically implicated, either alone or synergistically in the hypocholesterolemic effect of fenugreek seeds in diabetic dogs.

Stark and Madar (1993) investigated the hypocholesterolemic potential of an ethanol extract from defatted fenugreek seeds *in vitro* as well as *in vivo* in hypercholesterolemic rats. *In vitro*, the purified ethanol extract exhibited the ability to inhibit bile salt absorption in a dose-dependent manner. Reductions in plasma cholesterol levels (18-26%) and lower concentrations of liver cholesterol were observed *in vivo*. This study attributed the decrease in cholesterol absorption observed to an interaction of saponins with bile salts in the digestive tract. These findings seem to agree with those of Bhat *et al.* (1985) and Sharma (1984), in which an increase of both fecal weight and bile acid excretion was observed in treated subjects following consumption of a fenugreek-enriched diet.

It has been suggested that the hypocholesterolemic effect of diosgenin can be attributed to inhibition of cholesterol absorption, and its capacity to increase biliary cholesterol secretion and to increase neutral sterol excretion in feces (Cayen and Dvornik, 1979 cited in Al-Habori and Raman, 2002; Uchida *et al.*, 1984; Ulloa and Nervi, 1985). It is also postulated that formation of large mixed micelles containing bile salts and saponins in the gut inhibits the absorption of these molecules; hence they are lost in the feces. This would lead to a subsequent increase in conversion of cholesterol to bile acids

in the liver, thereby lowering blood and hepatic cholesterol levels (Al-Habori and Raman, 2002).

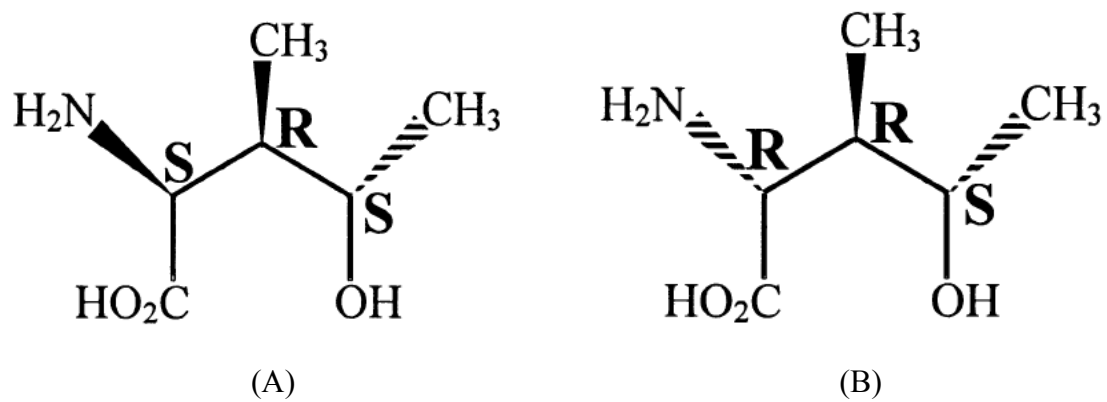
Saponins also appear to selectively inhibit the growth of tumor cells by arresting their growth within the cell cycle and inducing them to initiate apoptosis or programmed cell death (Francis *et al.*, 2002). Other studies have specifically linked diosgenin with cancer prevention (Raju *et al.*, 2004; Liagre *et al.*, 2005; Aggarwal and Shishodia, 2006). It was shown that diosgenin is needed in the inhibition and/or activation of key proteins (i.e., bcl-2 and caspase-3) in mediating apoptosis in human colon cancer cells (Raju *et al.*, 2004). Liagre *et al.* (2005) has elucidated a mechanism of diosgenin-induced apoptosis in human erythroleukemia cells, and Shishodia and Aggarwal (2006) have identified a chemopreventative role for diosgenin against bone cancer through growth suppression and apoptosis induction in cancer cells. Diosgenin also has been reported as a potential therapeutic chemical that can be used to treat dementia in drug abusers with HIV infection and a history of intravenous drug abuse (Turchan-Cholewo *et al.*, 2006).

### **2.12.3 4-Hydroxyisoleucine**

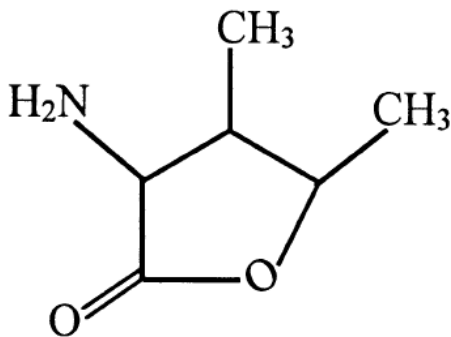
4-Hydroxyisoleucine is the most abundant free amino acid (up to 80 % of the total free amino acids) in fenugreek seeds (Fowden *et al.*, 1973; Sauvaire *et al.*, 1984). A recent study by Hajimehdipour *et al.* (2008) determined the content of 4-hydroxyisoleucine to be 0.4% in Iranian fenugreek seeds, while a previous publication reported a content of just 0.015% in Indian fenugreek seeds (Narender *et al.*, 2006). The stereochemistry of this rare amino acid was subsequently described by Alcock *et al.* (1989) and is only found in specific plants such as those of the genus, *Trigonella*.

4-Hydroxyisoleucine exists in two isomeric forms; the major isomer has a (2S, 3R, 4S) configuration (Fig. 2.16) which accounts for 90 % of the total 4-hydroxyisoleucine found in the seeds, while the minor isomer has a (2R, 3R, 4S) configuration (Fig. 2.16) (Broca *et al.*, 2000). Under specific conditions, such as high acidity, the linear form of 4-hydroxyisoleucine may cyclicize into the lactone form (Fig. 2.17) (Broca *et al.*, 2000). This unique amino acid has been shown to possess both hypoglycemic and insulinotropic properties *in vitro* and *in vivo* using animal and human models, making it a potential candidate as a antidiabetic agent (Sauvaire *et al.*, 1998; Broca *et al.*, 1999). Branched-chain amino acids such as valine, leucine and isoleucine (Fig. 2.18) are produced exclusively in plants. Since humans lack the enzymes needed to synthesize these amino acids, they represent essential amino acids that must be consumed as part of the human diet (Binder *et al.*, 2007).

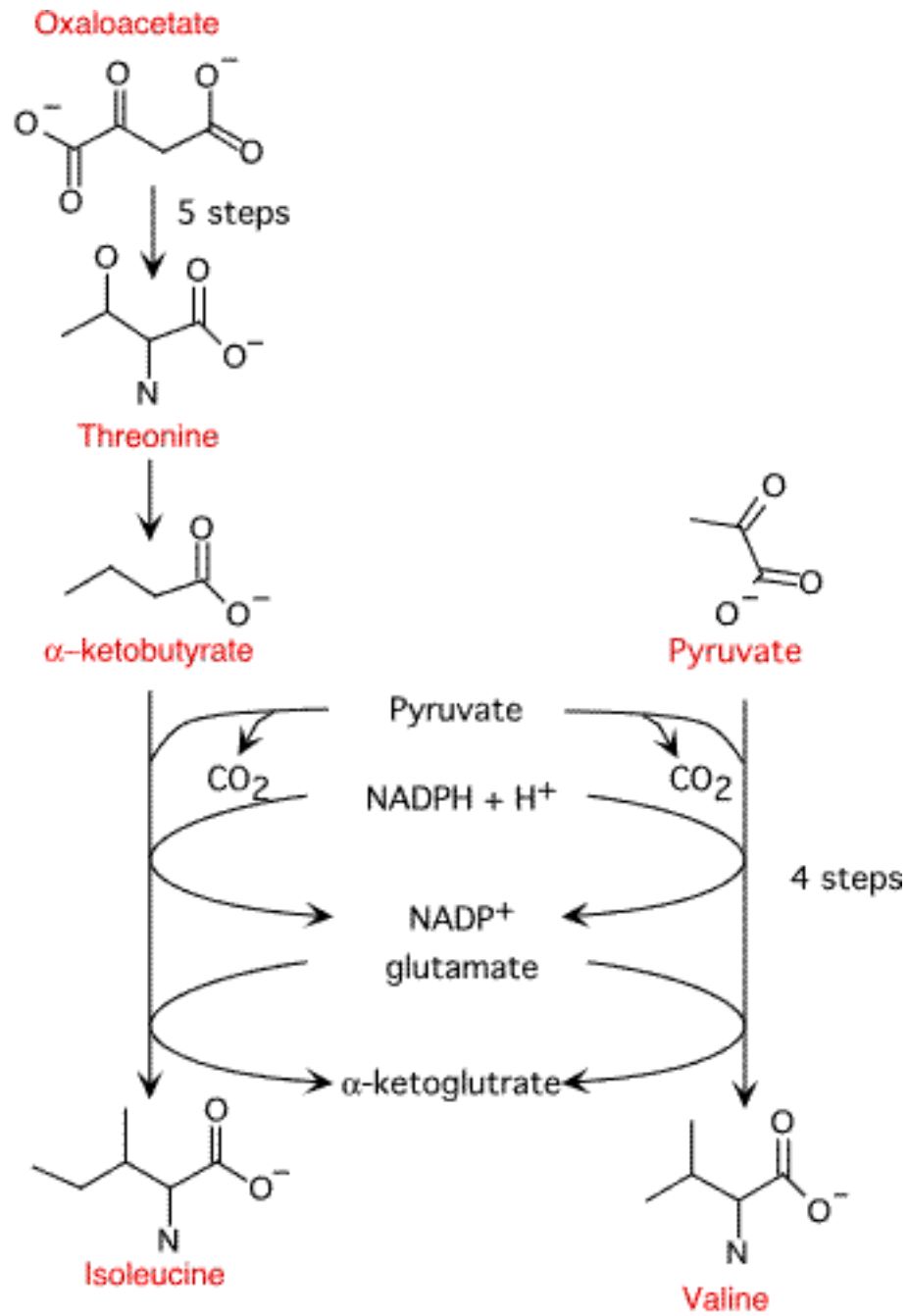
4-Hydroxyisoleucine is a branched-chain amino acid that is produced almost exclusively in plants of the genus *Trigonella*. As its name suggests, 4-hydroxyisoleucine is formed through the hydroxylation of isoleucine (Haefel  *et al.*, 1997). Formation of this amino acid in fenugreek is attributed to the activity of isoleucine hydroxylase, a dioxygenase that catalyzes the attachment of one atom of an oxygen molecule to C-4 of the amino acid, while the other oxygen atom is incorporated into 2-oxoglutarate to yield succinate and carbon dioxide (Haefel  *et al.*, 1997). The same study also identified 2-oxoglutarate, ascorbate, iron (II) and oxygen as essential co-factors and co-substrates that contribute towards the functionality of the enzyme. It is important to note that the linear



**Figure 2.16** The two isomers of 4-hydroxyisoleucine; major isomer (2S, 3R, 4S) (A) and minor isomer (2R, 3R, 4S) (B). Source: Broca et al. (2000).



**Figure 2.17** The lactone form of 4-hydroxyisoleucine. Source: Broca et al. (2000).



**Figure 2.18 Biosynthesis of branched-chain amino acids, isoleucine and valine.**  
**Source:** Paustian (2000).

form of 4-hydroxyisoleucine is necessary for it to be biologically active. The lactone form, which has a significantly altered chemistry, is inactive (Broca *et al.*, 2000). Also, it has been indicated that full methylation (full branching along the carbon skeleton), at carbon- $\alpha$  in the S-configuration and carbon- $\gamma$  hydroxylation are required for its insulinotropic activity (Broca *et al.*, 2000; Bhaumick, 2006).

Treatment of subjects with 4-hydroxyisoleucine has been shown to result in insulin stimulating effects in animals, hence giving it anti-diabetic properties (Hillaire-Buys *et al.*, 1993; Petit *et al.*, 1995a; Sauvaire *et al.*, 1996). Sauvaire *et al.* (1996) reported that 4-hydroxyisoleucine treatments produced a concentration dependent insulin response both *in vitro* in cell cultures and *in vivo* in fasted dogs. They also showed that 4-hydroxyisoleucine treatments were effective after oral administration and improved oral glucose tolerance. The amino acid was shown to stimulate pancreatic  $\beta$  cells to produce insulin directly in rats and human subjects. It was found that this effect was glucose-dependent and occurred only at moderate (8.3 mM) to high concentrations (16.7 mM) (Sauvaire *et al.*, 1998). Broca *et al.* (1999) studied the effects of 4-hydroxyisoleucine in normal and type-2 diabetic rats through intravenous and oral glucose tolerance tests. A single intravenous administration of 4-hydroxyisoleucine partially restored a glucose-induced insulin response, while a subchronic administration of the compound reduced basal hyperglycemia and basal insulinemia, and significantly improved glucose tolerance in type 2 diabetic rats. They concluded that 4-hydroxyisoleucine imparted anti-diabetic effects through direct pancreatic  $\beta$  cell stimulation.

Obesity is a condition in which lipid accumulates excessively in the adipose tissue. Aside from genetic factors, this disease is also caused by various environmental

factors such as a high-fat diet (Weiser *et al.*, 1997). If uncontrolled, obesity may be a risk factor for chronic diseases such as diabetes, hyperlipidemia and hypertension. Sharma *et al.* (1990) first reported a lipid lowering effect for fenugreek in type 1 diabetic patients. They found a significant reduction in total serum cholesterol, LDL- and VLDL-cholesterol and triglycerides, indicating that fenugreek can be effective in the management of diabetes. In a placebo-controlled study by Bordia *et al.* (1997), fenugreek was found to significantly reduce blood lipids without affecting HDL-cholesterol in patients with both coronary heart disease and type 2 diabetes. In another study, whole fenugreek seed powder fed to alloxan-treated diabetic rats was shown to improve glucose homeostasis by altering glucose and lipid metabolic enzyme activities, indicating a possible therapeutic potential of fenugreek for type 1 diabetes (Raju *et al.*, 2001). Hannan *et al.* (2003) used the soluble dietary fiber fraction of fenugreek to study its effect on lipidemia in type 2 diabetic models. A lack of 4-hydroxyisoleucine in these extracts may have contributed to failure in this study to identify a lipid lowering principle, even though positive therapeutic effects for fenugreek in the control of heart disease were identified.

Handa *et al.* (2005) conducted a study using obese mice that were fed a high-fat diet supplemented with 0.3-1.0% fenugreek seed extract (containing 20% 4-hydroxyisoleucine). It was found that fenugreek seed extract significantly reduced total adipose tissue and liver weights and resulted in a decrease in liver triglyceride levels. They repeated the experiments with similar parameters using 4-hydroxyisoleucine alone and reported a decrease in plasma triglyceride levels following corn oil administration (a method that would promote an increase in blood triglyceride levels in the test subjects). Their results identified 4-hydroxyisoleucine as an effective agent in fenugreek seed

extracts that has potential for use in obesity prevention. In another study, Narender *et al.* (2006) used a high-fat diet (HFD) fed to dyslipidemic hamsters to examine the anti-dyslipidemic properties of fenugreek. The HFD caused a 2 to 5-fold elevation in plasma triglycerides, total cholesterol, HDL-cholesterol, glycerol and free fatty acids (FFA) in the animals. However, following treatment with 4-hydroxyisoleucine (50 mg kg<sup>-1</sup> b.w.), a significant decrease in plasma triglycerides (33%), total cholesterol (22%) and an increase in HDL-cholesterol (8.7%) was observed. These results were similar to those obtained when fenofibrate, a triglyceride lowering drug was used under similar conditions (Koyama *et al.*, 2004). A 14% reduction in FFA and a 4.9% decrease in glycerol levels were also reported. The study concluded that 4-hydroxyisoleucine possessed both hyperglycemic as well as anti-dyslipidemic properties, which allowed it to decrease plasma triglycerides, cholesterol and free fatty acid levels in the test subjects.

## **2.13 Safety/ Toxicology**

Due to its documented historical and traditional use as a spice and medicinal herb in various parts of the world, fenugreek has been granted “Generally Recognized As Safe” (GRAS) status by the U.S. Food and Drug Administration (U.S. Food and Drug Administration, 2006). However, caution is warranted in patients who are allergic to fenugreek (Patil *et al.*, 1997). Ohnuma *et al.* (1998) have reported that a patient with an allergy to curry powder containing fenugreek responded with severe bronchospasms, wheezing and diarrhea. Tiran (2003) reported that a significant number of patients had an allergenic reaction to fenugreek in a skin test-patch. Transient diarrhea, flatulence and dizziness have also been reported as side effects of fenugreek consumption (Sharma *et al.*,



1996a; Abdel-Barry *et al.*, 2000). Bartley *et al.* (1981) suggested that fenugreek consumption can elicit a maple syrup-like odor in urine and may potentially cause the misdiagnosis of maple syrup urine disease.

There also is potential of chemical components in fenugreek to interact with other drugs during patient medication (Ernst *et al.*, 2001). Bioactive components in fenugreek such as dietary fiber (*i.e.*, galactomannan) and steroids (*i.e.*, diosgenin), which have the potential to affect glucose and cholesterol levels in humans, could pose risk to individuals with health problems. Consumption of fenugreek can lead to hypoglycemia; hence proper blood glucose monitoring may be necessary for patients prior to use of fenugreek as a dietary supplement but significant clinically harmful adverse effects due to consumption of fenugreek as a food or medicinal supplement have not been reported (Basch *et al.*, 2003).

Most reports on the toxicity of fenugreek consumption were obtained from studies with laboratory animals. Shlosberg and Egyed (1983) reported that ruminants that consumed fenugreek appeared to become myopic. Nakhla *et al.* (1991) also reported finding pathological abnormalities in the liver and kidney, along with reduced body weight and increased concentrations of uric acid in the blood in Sudanese chicks that had been fed fenugreek. Panda *et al.* (1999) have reported a decrease in body weight due to the inhibition of triiodothyronine (T<sub>3</sub>) production in mice and rats. Fenugreek preparations contain coumarin or its derivatives, which are anti-coagulants that prevent blood clotting (Lee *et al.*, 2000; Lambert and Cormier, 2001). This will increase the risk of internal bleeding in patients who are taking blood-clotting drugs such as warfarin, as the coumarin in fenugreek may increase the activity of these drugs. Use of fenugreek

during pregnancy is also discouraged as saponins in the plant may stimulate uterine contractions leading to premature abortion of the fetus as observed in early animal studies (Abdo and al-Kafawi, 1969; Basch *et al.*, 2003). In an animal study, an acute oral LD<sub>50</sub> was reported to be more than 5 g kg<sup>-1</sup> in rats and an acute dermal LD<sub>50</sub> was found to be more than 2g kg<sup>-1</sup> in rabbits (Opdyke, 1978; Basch *et al.*, 2003). In another animal study, the diets of mice and rats were supplemented with 1-10 % debitterized fenugreek powder, an approach which likely removes many of the saponins. No signs of toxicity or mortality in laboratory animals that received acute and subchronic regimens were observed (Muralidhara *et al.*, 1999). Rao *et al.* (1996) conducted a nutritional and safety evaluation of fenugreek over a short term in weanling rats. The rats' diet was supplemented with 5-20% fenugreek powder over a 90-day period; the studies concluded that fenugreek appeared to be non-toxic in doses administered to the animals, within that time frame. However, a recent evaluation conducted by Kassem *et al.* (2006) reported a potential anti-fertility effect of fenugreek in rabbits. Through a diet containing 30 % fenugreek seeds, these researchers reported a significant anti-fertility effect in female rabbits and a toxicity effect in male rabbits. A significant reduction in developing fetuses was observed in females at 20 days of gestation and a histo-pathological assessment of the testis in male rabbits revealed damaged seminiferous tubules and interstitial tissues.

## **2.14 Crop genotype assessment: Biometrical aspects**

### **2.14.1 Genotype x environment interaction**

Most crop breeding studies which aim at selecting genotypes that are superior in traits of agronomic interest (e.g., high-yielding or disease resistant plants) face two fundamental research problems: interaction and noise (Gauch and Zobel, 1996). Without interaction, a particular crop genotype would perform similarly all over the world, and without noise an experiment would not require replications, as the results would be indifferent. However, in a practical world, crop trials require a multi-environment design as variation in crop response to growing conditions (*i.e.*, agro-climatic regions) and yearly variation does occur (Yan *et al.*, 2001; Yang *et al.*, 2005). Replication of experimental trials is also necessary to establish a valid data set where statistical differences among the results can be rationally determined (Gauch and Zobel, 1997).

The ultimate goal of crop breeders is to identify superior genotypes that thrive in all environments. This objective is usually hampered by a phenomenon known commonly as the “genotype x environment interaction.” Such an interaction can cause similar sets of cultivars to acquire different performance rankings at different growing locations, resulting in inconsistent performance of genotypes across different environments (DeLacy *et al.*, 1990). This makes cultivar evaluation meaningless if it cannot be characterized over a large range of test sites to include varying regional climatic characteristics. In the face of limited resources and the increasing need for more cost-effective cultivar testing (Yang *et al.*, 2005), yield-maximizing crop breeding programs in the past decades have focused on identifying genotypes that are stable across

homogenous growing environments (targeted sites with similar biotic, abiotic and management conditions) (Yan *et al.*, 2000). Consequently, this warrants multi-environment trials (MET) in which a number of genotypes are tested over a range of environmental conditions (Mohammadi *et al.*, 2007).

### **2.14.2 Multi-environment trials**

In most multi-environment trials, main environment effect account for about 80% of the total variation observed while genotype and genotype x location effects account for about 10% of the total variation observed (Gauch and Zobel, 1996; Yan *et al.*, 2000). Similar to generic crop traits such as seed yield or plant height, levels of plant structural components and secondary metabolites can vary among and within species due to genotypic differences (Amar *et al.*, 2008). In addition, selection for superior genotypes can be complicated by the presence of an environment interaction factor, which can cause genetically similar plant lines to perform differently when grown under varying environmental conditions (DeLacy *et al.*, 1990). The international wheat trial by Braun *et al.* (1996) exemplifies this concept, in which they identified 12 regional locations in various developing countries across continents, grouped according to their latitude, soil conditions and agro-climatic characteristics, and collectively known as “mega environments.” Gauch and Zobel (1996) defined mega environments as, “a portion of a crop species’ growing region with a homogenous growing environment that causes some genotypes to perform similarly.”

### 2.14.3 Yield stability analysis

Gauch and Zobel (1997) demonstrated the concept of mega environments mentioned above using an Additive Main effects and Multiplicative Interaction (AMMI) model to analyze a MET data set for maize (*Zea mays* L.). Their “which-wins-where” methodology assisted in identifying facilitating locations (mega environments) for genotypes with superior crop performance. The AMMI model incorporates the additive portion of the variance using an analysis of variance (ANOVA) for the genotype main effect and later applies a principal component analysis (PCA) to identify multiplicative interaction effects (Gauch and Zobel, 1996). An AMMI biplot can be generated based on the first and second principle component scores for genotype and environment plotted against each other to analyze the interaction effect from the ANOVA model. The biplot, based on the concept of matrix multiplication was first developed by Gabriel (1971) and has evolved into a data visualization tool that is highly cost-effective in yield trials for determining mega environments and identifying winning genotypes (Yan *et al.*, 2007).

An arguably equivalent analysis resembling AMMI is the genotype plus genotype x environment (GGE) biplot technique by Yan (1999). The term GGE is a contraction of genotype (G) + genotype-environment interaction (GE) and is based on the assumption that the environment main effect, although large is irrelevant to genotype evaluation; hence it is removed from the data. Only G and GE effects, when considered simultaneously, are useful for meaningful genotype evaluation (Yan and Kang, 2003). The basic model for a GGE biplot is given as:

$$\hat{Y}_{ij} = \mu + \alpha_i + \beta_j + \Phi_{ij} \quad \text{[Equation 1]}$$

or

$$\hat{Y}_{ij} - \mu - \beta_j = \alpha_i + \Phi_{ij} \quad \text{[Equation 2]}$$

where  $\hat{Y}_{ij}$  is the expected yield of genotype  $i$  in environment  $j$ ,  $\mu$  is the grand mean of all observations,  $\alpha_i$  is the main effect of genotype  $i$ ,  $\beta_j$  is the main effect of environment  $j$ , and  $\Phi_{ij}$  is the interaction between genotype  $i$  and environment  $j$ . The model then further partitions G and GE into two multiplicative terms:

$$\hat{Y}_{ij} - \mu - \beta_j = g_{i1}e_{1j} + g_{i2}e_{2j} + \varepsilon_{ij} \quad \text{[Equation 3]}$$

where  $g_{i1}$  and  $e_{1j}$  are the primary scores for genotype  $i$  and environment  $j$ , respectively,  $g_{i2}$  and  $e_{2j}$  are the secondary scores for genotype  $i$  and environment  $j$ , respectively, and  $\varepsilon_{ij}$  is the residue not explained by the primary and secondary effects. The plotting of  $g_{i1}$  against  $g_{i2}$ , and  $e_{1j}$  against  $e_{2j}$  in a single scatter plot, ultimately constructs the GGE biplot (Yan and Kang, 2003).

The “which won where” view is one key feature of the GGE biplot and consists of an irregular polygon with perpendicular lines drawn from the biplot origin, dividing the polygon into sectors (hypothetical mega environments) with winning cultivars located at the vertices of the polygon, within each sector (Yan *et al.*, 2000; Yan *et al.*, 2002). This technique has been widely adapted and used by plant breeders and agronomists worldwide for crops including durum wheat (Letta *et al.*, 2008), rice (Tabien *et al.*, 2008), lentil (Sabaghnia *et al.*, 2008), barley (Dehghani *et al.*, 2006) and corn (Samonte *et al.*, 2005) to identify superior cultivars and facilitate identification of environmental factors.

Both the AMMI model and GGE biplot analysis are very useful tools for analysis of MET data. However, these models are only valid for trials with fixed effects, as the ANOVA portion of the models utilizes the General Linear Model (Equation 1),

which is incapable of making an inference of results that reflect random effects (Yang, 2008). Since in the present study, genotype and growing environment were considered as fixed and random factors, respectively, the application of either the AMMI or the GGE biplot models to elucidate, particularly the interaction component would therefore be statistically inappropriate. Despite that, Yan *et al.* (2001) has emphasized the usefulness of the biplot technique such as the GGE biplot analysis to provide graphically informative and highly convenient figures for visual identification of high-yielding genotypes and their potential environments.

## **3.0 Materials and Methods**

### **3.1 Fenugreek seed material**

The ten fenugreek genotypes used in this study were of *Trigonella foenum-graecum* L. Seeds of these genotypes originated from different agro-ecological locations in the world, including Afghanistan, India, Iran, Pakistan, and Turkey (Table 3.1). They were obtained from USDA/ARS in Pullman, Washington and further selected for high seed yield and/or forage yield as well as early maturity traits at CDCS, Brooks, Alberta, Canada with the exception of Tristar (selected at AAFC, Lethbridge, Alberta, Canada), L3312 (obtained from Plant Gene Resources of Canada, Saskatoon, Saskatchewan, Canada), Quatro MP 30 (selected at the Crop Development Centre, Saskatoon, Saskatchewan, Canada), AC Amber (selected at AAFC, Morden, Manitoba, Canada) and X92-32-23T (a zero-tannin line from the collection of the University of Saskatchewan, Saskatoon, Saskatchewan, Canada).

### **3.2 Growing environments**

The multi-environment study was conducted over two cropping years (2006 and 2007) at three locations; *i.e.*, Brooks, Bow Island and Lethbridge in southern Alberta, where both rainfed and irrigated growing conditions of the Brown soil zones in western Canada were represented. In 2007, another site within the Lethbridge area was added to the study (Lethbridge – Provincial). For statistical purposes, the year x growing condition x location contribution is considered as a growing environment (Lin and Binns, 1991),



**Table 3.1 Fenugreek genotypes and their origins, used in the study.**

<b>Fenugreek genotypes/ cultivar</b>		<b>Origin</b>
Tristar		Selected at Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada
Quatro MP 30		Line of the Crop Development Centre, Saskatoon, Saskatchewan, Canada
AC Amber (Amber)		Selected at Agriculture and Agri-Food Canada, Morden, Manitoba, Canada
F17	Seeds obtained from the United States Department of Agriculture/ Agricultural Research Station in Washington, and lines have been selected for prairie growth at the Crop Diversification Centre South, Brooks, Alberta, Canada	Iran
F75		Afghanistan
F96		Italy
F86		Afghanistan
X92-23-32T (X92)		Origin unknown (Obtained from the University of Saskatchewan, Saskatoon, Canada)
Indian Temple (Ind Temp)		Imported from India (Indian origin)
L3312		Obtained from Plant Gene Resources Canada, Saskatoon, Saskatchewan, Canada

which produced a multi-environment study with a total of fourteen growing environments.

The soil type and growing conditions of test sites are given in Table 3.2. The fenugreek genotypes were seeded into 4.5 m or 6.0 m long plots with four or six rows spaced 30 cm or 20 cm apart, depending upon the location, at a seeding rate between 27 - 33 kg ha<sup>-1</sup>, depending upon seed size of the genotype, to receive a target plant population density of 130 seeds m<sup>-2</sup>. The plots were arranged in a Randomized Complete Block Design with two replicates at each growing environment. The crop was grown using cultural practices recommended by Alberta Agriculture and Rural Development. At maturity, after eliminating borders, depending upon the location, individual plot area of 4.2m<sup>2</sup> or 7.2 m<sup>2</sup> at each test site was mechanically harvested, the seed dried in a ventilated dryer at 32 °C for several days to bring the seed moisture levels to about 6 %, and plot seed weights and 1000-seed weights were determined.

### **3.2.1 Brooks**

Brooks, Alberta (50° 33' N and 111° 55' W, Ele. 758 m) is located about 168 km east of Calgary along the Trans-Canada Highway (Environment Canada, 2008). Orthic Brown Chernozem is the dominant soil type in this area (Wyatt *et al.*, 1939). The annual average maximum and minimum temperatures for year 2006 were 13.3 °C and -0.8 °C, respectively, and for year 2007 were 12.7 °C and -1.5 °C, respectively. The estimated

**Table 3.2 Descriptions of the fourteen growing environments used in this study.**

Environment		Location description	Soil type	Mean max and min temperatures May to September	Available Water May to Sept.	Reference	
2006	Brooks	Irrigated (BR-Irr-06)	50° 31' 23" N and 111° 47' 19" W	Brown Chernozem, silt soil	24.7 °C / 8.4 °C	316 mm	Wyatt <i>et al.</i> , 1939; IMCIN, 2008
		Rainfed (BR-Dry-06)				157 mm	
	Bow Island	Irrigated (BI-Irr-06)	49° 52' 5" N and 111° 21' 58" W	Orthic Brown Chernozem	24.4 °C / 9.1 °C	330 mm	
		Rainfed (BI-Dry-06)				115 mm	
	Lethbridge	Irrigated (LB1-Irr-06)	49° 41' 39" N and 112° 45' 42" W	Orthic Dark Brown Chernozem	31.5 °C / 1.8 °C	293 mm	
		Rainfed (LB1-Dry-06)				141 mm	
2007	Brooks	Irrigated (BR-Irr-07)	49° 44' 2" N and 111° 27' 36" W	Brown Chernozem, silt soil	23.8 °C / 7.9 °C	165 mm	Wyatt <i>et al.</i> , 1939; IMCIN, 2008
		Rainfed (BR-Dry-07)				85 mm	
	Bow Island	Irrigated (BI-Irr-07)	50° 31' 23" N and 111° 47' 19" W	Orthic Brown Chernozem	24.1 °C / 8.8 °C	407 mm	
		Rainfed (BI-Dry-07)				107 mm	
	Lethbridge (Federal)	Irrigated (LB1-Irr-07)	49° 42' 21" N and 112° 45' 44" W	Orthic Dark Brown Chernozem	30.1 °C / 2.4 °C	337 mm	
		Rainfed (LB1-Dry-07)				83 mm	
	Lethbridge (Provincial)	Irrigated (LB2-Irr-07)	49° 41' 10" N and 112° 44' 38" W	Orthic Dark Brown Chernozem	30.1 °C / 2.4 °C	105 mm	
		Rainfed (LB2-Dry-07)				80 mm	

annual total precipitation for 2006 and 2007 was 241.5 mm and 292.3 mm, respectively [Irrigation Management Climate Information Network (IMCIN), 2008]. The GPS coordinates for the test sites in Brooks are 50° 31' 23" N and 111° 47' 19" W.

### **3.2.2 Bow Island**

Bow Island Alberta (49° 44' N and 111° 27' W, Ele. 817 m) is located about 322 km southeast of Calgary on the Alberta Highway 3 (Environment Canada, 2008). The predominant soil type in this area is Brown Chernozem and silty soil. The average annual maximum and minimum temperatures for 2006 were 13.8 °C and 0.4 °C, respectively, and for 2007, 13.1 °C and 0.0 °C, respectively. Total precipitation for 2006 and 2007 was recorded at 230.8 mm, and 304.2 mm, respectively (IMCIN, 2008). The GPS coordinates for the test sites in Bow Island are 49° 52' 5" N and 111° 21' 58" W.

### **3.2.3 Lethbridge (Federal test site)**

Lethbridge, Alberta (49° 45' N and 112° 45' W, Ele. 929 m) is located approximately 216 km southeast of Calgary, situated on both sides of the Oldman River (Environment Canada, 2008). The soil type predominant in this area is Orthic Dark Brown Chernozem (Wyatt *et al.*, 1939). The annual average maximum and minimum temperatures for 2006 were 14.0 °C and -0.6 °C, respectively, and for 2007 were 12.8 °C and 0.1 °C, respectively. The total precipitation recorded for 2006 and 2007 was 284.9 mm and 288.2 mm, respectively (IMCIN, 2008).

### **3.2.4 Lethbridge (Provincial test site)**

In 2007, another site referred to as Lethbridge (Provincial) was added to the study. The site bears the location coordinates 49° 41' 10" N and 112° 44' 38" W, Ele. 903 m (Environment Canada, 2008). Soil type was the same as that found at the Lethbridge (Federal) site. The annual average maximum and minimum temperatures as well as total precipitation for both 2006 and 2007 were similar to those given for the Lethbridge (Federal) site.

## **3.3 Agronomic and biochemical traits evaluated in the study**

Eight agronomical and biochemical traits assessed in this study include mean seed weight, expressed as thousand seed weight (TSW, in g), seed yield (SY as  $\text{kg ha}^{-1}$ ), galactomannan content (GLM as %), galactomannan productivity (GLM-P as  $\text{kg ha}^{-1}$ ), diosgenin content (DIOS as %), diosgenin productivity (DIOS-P as  $\text{kg ha}^{-1}$ ), 4-hydroxyisoleucine content (4-OH-ILE as %) and 4-hydroxyisoleucine productivity (4-OH-ILE-P as  $\text{kg ha}^{-1}$ ). The individual bioactive compound productivity was calculated by multiplying the respective bioactive compound content (%) by seed yield ( $\text{kg ha}^{-1}$ ).

For the biochemical analyses, approximately 100 g of whole seed was ground in a household coffee grinder for 15 seconds. The ground material was visually inspected to ensure a homogenous sample. The powdered fenugreek seed was transferred into envelopes (unsealed) and stored at room temperature until use. Prior to sub-sampling, the sample material in the envelope is stirred to provide a representative sample.

### 3.4 Estimation of galactomannan content

Galactomannan contents (GLM) for mature seed were determined using a commercially available assay kit (Megazyme Int., Bray, Ireland). The protocol utilized sequential enzymatic hydrolysis of galactomannan molecules to release free galactose into solution. Quantification was achieved through a redox reaction with nicotinamide adenine dinucleotide (NAD<sup>+</sup>) yielding NADH and the lactone form of galactose, followed by UV detection ( $\lambda = 340$  nm) of NADH to calculate the galactomannan content assuming a galactose/mannose ratio of 1:1. Galactomannan contents were measured in percentages in relation to dry seed material with a 5% moisture content basis. Measurements were carried out in duplicate along with a control sample using carob powder with a given galactomannan content of 36% (“as is” basis). Recovery of the control sample averaged 89.50% (n = 26) with a coefficient of variation (CV) of 1.37 %, on a day-to-day basis. Each of the selected fenugreek genotypes was replicated in the field (20 samples), and each replication was analyzed in duplicates (40 measurements). Approximately 100 g of whole fenugreek seed (approximately 100 mg of powdered sample) material was initially extracted with aqueous ethanol (80% v/v) to remove galactosyl sucrose oligosaccharides, which can be a source of non-galactomannan derived galactose residues. The ethanol-treated sample was then suspended in 8 mL of acetate buffer (100 mM, pH 4.5) and placed in a heating block (Multi-Block, Labline Instruments, IL) at 90 °C for 30 seconds followed with vigorous vortexing before returning the sample to the heating block for another 30 seconds, followed by more vigorous vortexing. The sample was returned to the heating block for another 4 minutes to complete galactomannan hydration. The samples were then allowed to cool to 40 °C in

a water bath prior to addition of 10  $\mu\text{L}$   $\beta$ -mannanase ( $450 \text{ U mL}^{-1}$ ), followed by incubation at  $40 \text{ }^\circ\text{C}$  for 1 hour, with intermittent stirring. The hydrolyzed solution was quantitatively transferred to a 25 mL volumetric flask, and made up to volume with distilled water. The solution (10 mL) was centrifuged at 4000 rpm for 10 minutes, and the supernatant was used in the final enzymatic procedure. The supernatant (200  $\mu\text{L}$ ) was transferred to a cuvette containing 100  $\mu\text{L}$  acetate buffer and 20  $\mu\text{L}$  of  $\alpha$ -galactosidase ( $150 \text{ U mL}^{-1}$ ) and  $\beta$ -mannanase ( $50 \text{ U mL}^{-1}$ ), in combination and was incubated at  $40 \text{ }^\circ\text{C}$  to effect complete hydrolysis of the galactomannan molecule. Tris/hydrochloric acid buffer (100  $\mu\text{L}$ ), nicotinamide adenine dinucleotide (NAD<sup>+</sup>) solution (100  $\mu\text{L}$ ) were mixed with 2.3 mL of distilled water and then added to a cuvette. The absorbance of the solution was determined at 340 nm, against a blank solution using a Spectronic 1201 diode array spectrophotometer (Milton Roy Co., Rochester, NY). The final enzymatic step was conducted with the addition of 20  $\mu\text{L}$  of  $\beta$ -galactose dehydrogenase ( $200 \text{ U mL}^{-1}$ ) to yield NADH and D-galactono-1,4-lactone.

### **3.5 Estimation of diosgenin content**

This analytical method was developed with modifications based on the published methods of Wu and Wu (1991), Ortuño *et al.* (1998), Taylor *et al.* (2002) and Trivedi *et al.* (2007). In principle, the analysis involves hydrolysis of naturally occurring glycosylated dioscin to yield free diosgenin in solution, followed by repeated extractions with and subsequent evaporation of a non-polar solvent. The dry residue was redissolved in methanol prior to analysis with an Agilent 100 HPLC system (Agilent Technologies

Inc. Canada, Mississauga, ON) equipped with a photodiode array detector. Diosgenin contents were measured in percentages in relation to dry seed material with a 5% moisture content basis.

Each fenugreek genotype with 2 replicates was measured in duplicate. Sample analysis was executed along with the analysis of a reference sample. The day-to-day variation expressed as coefficient of variation (CV) for diosgenin content of the reference sample was 3.79% (n = 82), while the CV for duplicate measurements of fenugreek samples was 3.47% (n = 20). Average accuracy of the method in terms of spike recoveries (diosgenin standard) was 82% (n = 33). Approximately 100 mg of powdered seed material was hydrolyzed with 3 mL of 1 M sulfuric acid in 100% ethanol for 30 minutes at 100 °C in a heating block (Multi-Block, Labline Instruments, IL). After hydrolysis, the solution was diluted with 2 mL of distilled water. Repeated extraction of diosgenin was carried out using 2 mL of hexane as solvent. The accumulated hexane extract (~ 6 mL) was then washed with 2 mL of 0.1 M sodium hydroxide to neutralize the free fatty acids, followed by another 1 mL of distilled water wash to remove any remaining hydrophilic molecules. The washed hexane extract was quantitatively transferred to a recovery flask (50 mL), and evaporated to dryness with a rotary evaporator (Büchi Rotavapor-R, Brinkmann Instruments Canada Ltd., Toronto, ON) at 50 °C. The dry residue was redissolved in 2 mL of methanol and an aliquot of the reconstituted solution was filtered through a 0.45 µm polypropylene membrane filter (Fisher Scientific Canada, Ottawa, ON) prior to HPLC analysis. Reversed-phased chromatography was executed using an isocratic separation with a 5 µm particle size, 4.6 mm (i.d.) x 150 mm Luna C18 column (Phenomenex, Torrance, CA), at 14 °C, with a



flow rate of  $1.0 \text{ mL}^{-1}$ , and 94% methanol mobile phase. Detection of the compound was achieved at 214 nm using a photodiode array detector. Quantitation of diosgenin in the sample was based upon the peak area of authentic diosgenin (Steraloids Inc., Newport, RI) as an external standard. The retention time of diosgenin was approximately 9.9 min using this method. The separation of diosgenin from other structurally similar sapogenins is unlikely based on the efficiency of an HPLC system. The diosgenin peak observed would rationally include a variety of fenugreek sapogenins. The total sapogenin content of fenugreek seeds were reported as diosgenin for the purpose of this study.

### **3.6 Estimation of 4-hydroxyisoleucine content**

This analytical method was developed with modifications based upon a protocol acquired from Chromadex Inc., Irvine, CA. This method involves initial extraction of free 4-hydroxyisoleucine with aqueous methanol, followed by phenylisothiocyanate (PITC) derivatization to enable detection of the compound with a photodiode array detector at 254 nm following a gradient elution with an Agilent 1100 HPLC system (Agilent Technologies Inc. Canada, Mississauga, ON). 4-Hydroxyisoleucine content (4-OH-ILE) was measured on a percentage basis in relation to dry seed material with 5 % moisture content. Each fenugreek line with 2 replicates was measured in duplicate. Sample analysis was carried out along with the analysis of a reference sample. The day-to-day variation expressed as coefficient of variation (CV) for 4-OH-ILE of the reference sample was 2.27 % ( $n = 32$ ) and the average CV for duplicate measurements of fenugreek samples was 2.02 % ( $n = 22$ ). The determined accuracy for the method in terms of spike recovery (4-hydroxyisoleucine standard) was 114 % ( $n = 4$ ).

Approximately 300 mg of powdered sample material was extracted with 3 mL aqueous methanol (50 % v/v) for 15 minutes on a magnetic stirrer at 600 rpm. This was followed by another 15 minutes of extraction in a Bransonic 220 sonicator (Branson Ultrasonics Corporation, Danbury, CT), to effect complete solubilization of the compound. The methanol extract (1 mL) was then mixed with 2.5 mL of coupling solution [67 % acetonitrile, 20 % water and 13 % triethylamine (Sigma-Aldrich, Oakville, ON)] and 50  $\mu$ L PITC (Sigma-Aldrich, Oakville, ON) in a 25 mL volumetric flask for compound derivatization. Methanol (15 mL) was added to the treated solution and was made up to volume with distilled water. The solution was filtered through a 0.45  $\mu$ m polypropylene membrane prior to HPLC analysis. Separation of the analytes was achieved using 0.1 % phosphoric acid and acetonitrile (B) as the mobile phase through a gradient elution (t = 0 min, 20 % B; t = 24 min, 80 % B; t = 25 min, 0 % B for 1.5 min). A 20  $\mu$ L sample size was injected into a 5  $\mu$ m, 4.6 x 150 mm Luna C18 column (Phenomenex, Torrance, CA), at 30 °C, with a flow rate of 1.5 mL min<sup>-1</sup>. Retention time of 4-hydroxyisoleucine in the column was approximately 6.6 minutes using this method. The compound was quantified using an external standard curve based on authentic 4-hydroxyisoleucine (purchased from ChromaDex Inc.).

## 3.7 Statistical analysis

### 3.7.1 Analysis of main effects and assessment of associations among traits

Treatment (genotype and growing environment) effects on thousand seed weight (TSW), seed yield (SY), galactomannan content (GLM), diosgenin content (DIOS), 4-hydroxyisoleucine content (4-OH-ILE) and their respective productivity (GLM-P, DIOS-P, 4-OH-ILE-P; ) were subjected to Analysis of Variance (ANOVA) in a Randomized Complete Block Design (RCBD) using PROC MIXED (SAS Institute, Cary, NC). In the ANOVA model, the factor, genotype was considered as fixed, whereas replicate and growing environment were considered as random factors. The treatment effects were further partitioned into main and interaction effects of genotype and growing environment for individual traits to assess their relative contributions to the total variation.

Whenever the main effects of genotype and growing environment were significant at  $p \leq 0.05$ , treatment means were compared for significance using Tukey's Honestly Significant Difference test. The critical value,  $w$  (Equation 4) was calculated using differences between all pairs of means, at a  $p \leq 0.05$ .

$$w = q_{\alpha}(p, f_e) s_{\bar{y}} \quad \text{[Equation 4]}$$

where  $q_{\alpha}$  can be obtained from a table of "upper percentage points of the studentized range" originally produced by May (1952) cited in Steel and Torrie (1980) in which  $q_{\alpha} = (\bar{Y}_{\max} - \bar{Y}_{\min}) / s_{\bar{y}}$ ,  $p = t =$  number of treatments,  $f_e$  is the error in the degrees of freedom and  $s_{\bar{y}}$  is the error in the mean of the squares. Means were assigned similar superscripts if they were not significantly different from each other at a 95% confidence

level. Means that were not assigned superscripts are significantly different from all other means at a 95% confidence level.

### **3.7.2 Interaction effect analysis**

In multivariate statistics, data dimensions reduction can be achieved using cluster analysis by simplifying the pattern of responses and by grouping both genotypes (G) and environments (E) into more homogenous categories. The classification of genotypes or environments according to similar response patterns is essential for improving efficiency, especially in breeding programs. The procedure for cluster analysis (XLSTAT, Addinsoft, New York, NY) was used to identify homogenous groups (clusters) for genotypes based on selected trait performance. The clusters were grouped so that differences *between* subsets of clusters are due to significant G x E interactions (Lin *et al.*, 1986); no interactions were observed *within* the subsets. Similarity of clusters was based on Pearson correlation coefficients (*r*-values), while agglomeration of the clusters was achieved using an unweighted pair group average method, where the distance between two clusters is calculated as the average distance between all pairs of objects in two separate clusters.

The genotype plus genotype x environment (GGE) biplot methodology described by Yan (1999) was used to visualize winning genotypes and their environmental niches. This procedure grouped homogenous environments based on a similar genotype response and identifies the best performing genotypes at their most influential environments for the traits studied. The biplot is constructed by plotting principal component scores for both genotype (entries) and environment (testers) simultaneously to create a two-dimensional diagram, using the first principal component (PC1) scores as the abscissa (horizontal

axis), and the second principal component (PC2) scores as the ordinate (vertical axis). The total variation relative to GGE explained by the biplot is given as percentages represented by PC1 and PC2, and is presented in the upper-left corner of the diagram. Entries (*i.e.*, genotypes) are in lowercase and testers (*i.e.*, environments or traits) are in uppercase. “Entries” which have a high PC1 score (positive) indicate superior performance while those that have a PC2 score close to zero indicate stability (Yan and Kang, 2003). The polygon-view or the “which won where” view of the GGE biplot presents a polygon drawn by connecting all the genotypes located farthest from the biplot origin, so that all genotypes are contained in the polygon. Perpendicular lines to each side of the polygon are drawn from the biplot origin dividing the polygon into sectors. Genotypes positioned at the vertices of the polygon are referred to as vertex genotypes. In relation to their distance to the biplot origin, these vertex genotypes also have the longest vectors in their respective directions, which are a measure of their responsiveness to environments. Genotypes positioned at the vertices are therefore the most responsive, while those contained in the polygon are less so, in their respective directions. Moreover, genotypes located close to the biplot origin responded similarly in all environments and hence were not at all responsive to the environments. The vertex genotype is the highest-yielding genotype in all environments that share the sector with it.

The nature of association among the traits considered in this study was determined by performing a Pearson correlation analysis using MINITAB Statistical Software (Minitab Inc., State College, PA). To provide a graphical representation of the association of traits, the GGE biplot analysis was also performed to provide a “which won what” view which eventually provided information for the respective trait profiles of

each genotype. The distance of an environment from the biplot origin is referred to as the tester's vector and its length is a direct indication of the environment's ability to discriminate among the genotypes. For example, a relatively short vector implies that the environment generates similar response among genotypes and any observed differences for genotypes tested in that environment may be a reflection of experimental noise, and hence may not be reliable. The angle of separation between testers can also estimate the correlation among them; an acute angle ( $\alpha < 90^\circ$ ), obtuse angle ( $\alpha > 90^\circ$ ) and right angle ( $\alpha = 90^\circ$ ) shows positive, negative and no correlation between them, respectively (Yan *et al.*, 2001).

## **4.0 Results**

### **4.1 Impact of genotypes and growing environments on selected agronomical and biochemical traits of fenugreek**

This study was conducted at six locations in 2006 and eight locations in 2007. These locations and test years were collectively considered ‘growing environments’, consequently producing a total of fourteen growing environments for this study. At each growing environment, ten fenugreek genotypes were evaluated for eight selected agronomic and biochemical traits namely mean seed weight expressed as 1000-seed weight (TSW), seed yield (SY), galactomannan content (GLM), galactomannan productivity (GLM-P), diosgenin content (DIOS), diosgenin productivity (DIOS-P), 4-hydroxyisoleucine content (4-OH-ILE) and 4-hydroxyisoleucine productivity (4-OH-ILE-P). The relative impact and significance of the growing condition and genotype for the selected traits were assessed by performing an Analysis of Variance (ANOVA) using a mixed model where the genotype and the growing environment were considered fixed and random factors, respectively.

#### **4.1.1 Main effects of genotype and growing environment on selected traits**

A summary of the ANOVA results indicating degrees of freedom and sums of squares for growing environment (E), genotype (G) and G x E interactions and their significance for individual traits is given in Table 4.1. Results indicate that the main effects of G, and E and the G x E interaction effect are significant for all traits measured at  $p \leq 0.01$ . The relative impact of the effects of G and E, and the G x E interaction on

evaluated traits is given in Table 4.2, considering the total variation due to treatment equals 100%. Results indicated that the main effect of E had the highest contribution to the total variation due to treatments for all the traits tested, which varied from 63% for TSW to 78% for 4-OH-ILE, except for DIOS where the main effect of E contributed only 6%. With the exception for DIOS, the contribution of G for other traits tested, varied from 7% (for 4-OH-ILE) to 24% (for TSW) of the total variation due to treatment. The main effect of G for DIOS accounted for 78% of the total variation (Table 4.2). Main effects of genotype and growing environment on the mean performance of the evaluated traits are given in Table 4.3. A Tukey's Honestly Significant Difference (HSD) test was performed to separate treatment means, based on their statistical significance.

#### **4.1.1.1 Thousand seed weight**

On average, the mean seed weight expressed as 1000-seed weight (TSW) significantly varied among genotypes with a range of 12.9 g for F75 to 17.6 g for AC Amber. AC Amber produced the heaviest seed among all the genotypes tested and the seed was also significantly heavier than all the genotypes tested (Table 4.3). The fenugreek genotype F75 produced the smallest seed in terms of mean seed weight and it was statistically comparable with those of F17 and Quatro MP 30. The TSW of Tristar was statistically comparable with those of Quatro MP 30, F17 and F86. TSW of L3312 was statistically comparable with those of Ind Temp and F96. Moreover, TSW of F96 was statistically comparable with those of X92 and Ind Temp with a range of 15.1 g



**Table 4.1 Mean squares for thousand seed weight, seed yield, galactomannan content and productivity, diosgenin content and productivity, and 4-hydroxyisoleucine content and productivity.**

Source of variation	df	Mean Squares							
		TSW (g)	SY (kg ha <sup>-1</sup> )	GLM (%)	GLM-P (kg ha <sup>-1</sup> )	DIOS (%)	DIOS-P (kg ha <sup>-1</sup> )	4-OH-ILE (%)	4-OH-Ile-P (kg ha <sup>-1</sup> )
Replication within									
environment	14	2.9**	232122**	1.4 <sup>NS</sup>	8780 **	0.002 <sup>NS</sup>	9.7**	0.049**	36.1**
Environment (E)	13	113.7**	14360270**	97.1**	538377**	0.014**	512.0**	0.613**	813.9**
Genotype (G)	9	61.4**	2540376**	21.6**	105739**	0.247**	79.5**	0.084**	206.1**
G x E	117	2.7**	311964*	2.9**	12358**	0.003**	11.4**	0.012**	21.6**
Error	126	0.8	91914	1.4	2944	0.001	4.0	0.007	6.8
Coefficient of variation (%)		6.0	20.8	7.3	21.7	6.6	22.5	9.1	20.3

\*\* Denotes significance at  $p \leq 0.01$ , <sup>NS</sup> Not significant at  $p = 0.05$ , *df*, degrees of freedom, TSW, thousand seed weight; SY, seed yield; GLM, galactomannan content; GLM-P; galactomannan productivity; DIOS, diosgenin content; DIOS-P, diosgenin productivity; 4-OH-ILE, 4-hydroxyisoleucine content; 4-OH-ILE-P, 4-hydroxyisoleucine productivity.

**Table 4.2 Contribution of genotype, environment and genotype x environment effects to the total variance due to treatments observed for 8 selected traits evaluated in this study.**

<b>Source of variation</b>	<b>Sum of Squares (SS)</b>	<b>SS (%)</b>
<b>Thousand Seed Weight (g)</b>		
E	1478	63
G	552	24
G x E	318	14
<b>Total</b>	<b>2348</b>	<b>100</b>
<b>Seed Yield (kg ha<sup>-1</sup>)</b>		
E	186683514	76
G	22863390	9
G x E	36499818	15
<b>Total</b>	<b>246046722</b>	<b>100</b>
<b>Galactomannan Content (%)</b>		
E	1263	70
G	195	11
G x E	350	19
<b>Total</b>	<b>1807</b>	<b>100</b>
<b>Galactomannan Productivity (kg ha<sup>-1</sup>)</b>		
E	6998901	74
G	951649	10
G x E	1445876	15
<b>Total</b>	<b>9396426</b>	<b>100</b>
<b>Diosgenin Content (%)</b>		
E	0.18	6
G	2.23	78
G x E	0.45	16
<b>Total</b>	<b>2.86</b>	<b>100</b>
<b>Diosgenin Productivity (kg ha<sup>-1</sup>)</b>		
E	6657	77
G	716	8
G x E	1329	15
<b>Total</b>	<b>8701</b>	<b>100</b>
<b>4-Hydroxyisoleucine Content (%)</b>		
E	7.98	78
G	0.76	7
G x E	1.51	15

Total	10.25	100
<b>4-Hydroxyisoleucine Productivity (kg ha<sup>-1</sup>)</b>		
E	10580	71
G	1855	12
G x E	2527	17
Total	14962	100

**Table 4.3 Main effects of genotype and growing environment on the mean performance of 8 selected traits of fenugreek grown in southern Alberta<sup>§</sup>.**

	Traits							
	TSW (g)	SY (kg ha <sup>-1</sup> )	GLM (%)	GLM-P (kg ha <sup>-1</sup> )	DIOS (%)	DIOS-P (kg ha <sup>-1</sup> )	4-OH-ILE (%)	4-OH-ILE-P (kg ha <sup>-1</sup> )
<b>Genotype</b>								
Tristar	13.7 <sup>bc</sup>	1586 <sup>bc</sup>	16.6 <sup>def</sup>	277.0 <sup>b</sup>	0.53 <sup>ab</sup>	8.4 <sup>bc</sup>	0.99 <sup>d</sup>	14.7 <sup>de</sup>
Quatro MP 30	13.1 <sup>ab</sup>	1733 <sup>bc</sup>	16.5 <sup>cdef</sup>	301.5 <sup>bc</sup>	0.66 <sup>c</sup>	11.6 <sup>c</sup>	0.91 <sup>abc</sup>	14.8 <sup>de</sup>
AC Amber	17.6 <sup>g</sup>	1198 <sup>a</sup>	14.6 <sup>a</sup>	186.1 <sup>a</sup>	0.81 <sup>g</sup>	9.8 <sup>cd</sup>	0.95 <sup>bcd</sup>	10.5 <sup>ab</sup>
F17	13.0 <sup>ab</sup>	1541 <sup>b</sup>	16.9 <sup>def</sup>	272.4 <sup>b</sup>	0.50 <sup>a</sup>	7.5 <sup>ab</sup>	0.92 <sup>abcd</sup>	13.7 <sup>cd</sup>
F96	15.1 <sup>ef</sup>	1617 <sup>bc</sup>	15.6 <sup>abc</sup>	265.1 <sup>b</sup>	0.61 <sup>d</sup>	9.7 <sup>cd</sup>	0.88 <sup>ab</sup>	13.0 <sup>cd</sup>
F75	12.9 <sup>a</sup>	1814 <sup>c</sup>	17.6 <sup>f</sup>	324.8 <sup>c</sup>	0.59 <sup>cd</sup>	10.9 <sup>de</sup>	0.98 <sup>cd</sup>	16.6 <sup>e</sup>
X92	15.7 <sup>f</sup>	995 <sup>a</sup>	16.1 <sup>bcd</sup>	164.1 <sup>a</sup>	0.75 <sup>f</sup>	7.6 <sup>ab</sup>	0.85 <sup>a</sup>	8.3 <sup>a</sup>
Indian Temple	15.3 <sup>ef</sup>	959 <sup>a</sup>	15.5 <sup>ab</sup>	150.7 <sup>a</sup>	0.62 <sup>d</sup>	5.9 <sup>a</sup>	0.99 <sup>d</sup>	8.7 <sup>a</sup>
L3312	14.7 <sup>de</sup>	1620 <sup>bc</sup>	17.1 <sup>ef</sup>	295.7 <sup>bc</sup>	0.53 <sup>ab</sup>	8.6 <sup>bc</sup>	0.90 <sup>ab</sup>	13.8 <sup>cd</sup>
F86	14.0 <sup>cd</sup>	1547 <sup>b</sup>	16.3 <sup>bcd</sup>	265.2 <sup>b</sup>	0.57 <sup>bc</sup>	8.8 <sup>bc</sup>	0.85 <sup>a</sup>	12.0 <sup>bc</sup>
Critical value, <i>q</i>	0.74	256.11	1.01	45.84	0.03	1.69	0.07	2.21
<b>Environment</b>								
BR-Irr-06	14.7 <sup>c</sup>	2722 <sup>g</sup>	17.4 <sup>fgh</sup>	475.3 <sup>g</sup>	0.57 <sup>a</sup>	15.3 <sup>g</sup>	0.83 <sup>cd</sup>	23.7 <sup>g</sup>
BR-Dry-06	17.4 <sup>f</sup>	3102 <sup>h</sup>	18.2 <sup>h</sup>	573.2 <sup>h</sup>	0.64 <sup>cde</sup>	19.3 <sup>h</sup>	0.81 <sup>bc</sup>	25.6 <sup>g</sup>
BI-Irr-06	15.0 <sup>e</sup>	2407 <sup>f</sup>	17.9 <sup>gh</sup>	442.8 <sup>g</sup>	0.59 <sup>ab</sup>	14.0 <sup>g</sup>	0.84 <sup>cd</sup>	20.4 <sup>f</sup>
BI-Dry-06	13.5 <sup>e</sup>	1599 <sup>e</sup>	21.5 <sup>i</sup>	344.9 <sup>f</sup>	0.66 <sup>de</sup>	10.6 <sup>f</sup>	0.68 <sup>a</sup>	11.5 <sup>cde</sup>
LB1-Irr-06	14.5 <sup>de</sup>	772 <sup>b</sup>	15.5 <sup>cd</sup>	120.1 <sup>bcd</sup>	0.62 <sup>bcd</sup>	4.8 <sup>bc</sup>	0.81 <sup>bc</sup>	6.6 <sup>ab</sup>
LB1-Dry-06	17.4 <sup>f</sup>	1438 <sup>de</sup>	17.0 <sup>efgh</sup>	247.3 <sup>e</sup>	0.67 <sup>c</sup>	9.5 <sup>f</sup>	0.91 <sup>de</sup>	13.6 <sup>e</sup>
BR-Irr-07	11.9 <sup>a</sup>	1157 <sup>cd</sup>	13.6 <sup>ab</sup>	156.9 <sup>cd</sup>	0.59 <sup>ab</sup>	6.8 <sup>cde</sup>	0.88 <sup>cd</sup>	10.3 <sup>cd</sup>
BR-Dry-07	12.9 <sup>bc</sup>	365 <sup>a</sup>	12.8 <sup>a</sup>	46.8 <sup>a</sup>	0.60 <sup>abc</sup>	2.2 <sup>a</sup>	1.26 <sup>i</sup>	4.6 <sup>a</sup>
BI-Irr-07	17.6 <sup>fg</sup>	2209 <sup>f</sup>	16.1 <sup>de</sup>	356.9 <sup>f</sup>	0.62 <sup>bcd</sup>	13.4 <sup>g</sup>	0.82 <sup>c</sup>	18.3 <sup>f</sup>
BI-Dry-07	12.3 <sup>ab</sup>	1390 <sup>de</sup>	16.9 <sup>efg</sup>	236.3 <sup>e</sup>	0.62 <sup>bcd</sup>	8.5 <sup>ef</sup>	0.73 <sup>ab</sup>	10.2 <sup>cd</sup>
LB1-Irr-07	18.5 <sup>g</sup>	873 <sup>bc</sup>	16.2 <sup>def</sup>	142.5 <sup>bcd</sup>	0.63 <sup>bcd</sup>	5.4 <sup>bcd</sup>	1.04 <sup>fg</sup>	9.2 <sup>bc</sup>
LB1-Dry-07	12.2 <sup>ab</sup>	604 <sup>ab</sup>	15.4 <sup>cd</sup>	92.8 <sup>ab</sup>	0.61 <sup>abc</sup>	3.6 <sup>ab</sup>	1.20 <sup>hi</sup>	7.2 <sup>ab</sup>
LB2-Irr-07	13.6 <sup>cd</sup>	1143 <sup>cd</sup>	14.5 <sup>bc</sup>	167.0 <sup>d</sup>	0.62 <sup>bcd</sup>	7.0 <sup>de</sup>	1.12 <sup>gh</sup>	12.7 <sup>de</sup>
LB2-Dry-07	11.4 <sup>a</sup>	671 <sup>ab</sup>	15.0 <sup>cd</sup>	100.7 <sup>abc</sup>	0.59 <sup>ab</sup>	4.0 <sup>ab</sup>	0.98 <sup>ef</sup>	6.6 <sup>ab</sup>
Critical value, <i>q</i>	0.92	321.33	1.26	57.51	0.04	2.12	0.09	2.77

<sup>§</sup> TSW, thousand seed weight; SY, seed yield; GLM, galactomannan content; GLM-P; galactomannan productivity; DIOS, diosgenin content; DIOS-P, diosgenin productivity; 4-OH-ILE, 4-hydroxyisoleucine content; 4-OH-ILE-P, 4-hydroxyisoleucine productivity. Means that share similar superscripts within the same column under either genotype main effect or environment main effect are not significantly different from each other (Tukey's HSD at  $p \leq 0.05$ ).

to 15.7 g, which represents a heavy seed category among the tested genotypes. AC Amber produced the heaviest seed among all the genotypes tested, and TSW of AC Amber was significantly higher than those of all the tested fenugreek genotypes.

On average, TSW among environments varied significantly with a range of 11.4 g (for LB2-Dry-07) to 18.5 g for (LB1-Irr-07). The growing environment LB2-Dry-07 produced the smallest mean seed weight, but it was statistically comparable with the TSW observed at BR-Irr-07, BI-Dry-07 and LB1-Dry-07. TSW observed at LB1-Irr-07 was statistically comparable with that of BI-Irr-07. TSW observed at BR-Dry-07 was statistically comparable to that of LB2-IR-07, but it was significantly lower than those of BR-Irr-06, BR-Dry-06, BI-Irr-06, LB1-Irr-06 and BI-Irr-07.

#### **4.1.1.2 Seed yield**

On average, fenugreek genotypes evaluated under various growing environments in southern Alberta produced significantly different ( $p < 0.01$ ) seed yields (SY) with a range of 959 kg ha<sup>-1</sup> for Ind Temp to 1814 kg ha<sup>-1</sup> for F75 (Table 4.3). SY of F75 was statistically comparable to those of Tristar, Quatro MP 30, F96, and L3312. SY of Ind Temp was statistically comparable to those of X92 (995 kg ha<sup>-1</sup>) and AC Amber (1198 kg ha<sup>-1</sup>).

SY among environments varied significantly with a range from 365 kg ha<sup>-1</sup> for BR-Dry-07 to 3102 kg ha<sup>-1</sup> for BR-Dry-06 (Table 3.3). Both BR-Irr-06 (2722 kg ha<sup>-1</sup>) and BR-Dry-06 (3102 kg ha<sup>-1</sup>) produced the highest SY among all environments and were found to be significantly higher than those observed at all the other growing environments tested. SY at BR-Dry-07 was statistically comparable to that of LB1-Dry-

07 (604 kg ha<sup>-1</sup>) and LB2-Dry-07 (671 kg ha<sup>-1</sup>). LB1-Dry-06, BR-Irr-07, BI-Dry-07 and LB2-Irr-07 (1143 – 1438 kg ha<sup>-1</sup>) produced statistically comparable SY but they were significantly lower than those of BI-Irr-07 (2209 kg ha<sup>-1</sup>) and BI-Irr-06 (2407 kg ha<sup>-1</sup>).

#### **4.1.1.3 Galactomannan content**

On average, the seed galactomannan content of fenugreek genotypes varied significantly ( $p \leq 0.01$ ) with a range of 14.6 % for AC Amber to 17.6 % for F75 (Table 4.3). F75 produced the highest GLM among the genotypes tested and it was statistically comparable to that of Tristar, Quatro MP 30, F17 and L3312, with a range of 16.5 % to 17.1 %. The GLM of AC Amber was statistically comparable with that of Ind Temp and F96, but was significantly lower than those of X92 and F86.

On average, the GLM content among growing environments significantly varied with a range of 12.8 % for Br-Dry-07 to 12.5 % for BI-Dry-06 (Table 4.3). The GLM at BI-Dry-06 was significantly higher than those of all the other growing environments tested. The GLM observed at LB1-Irr-06 (15.5 %) was statistically comparable with those observed at BI-Irr-07, LB1-Irr-07, LB1-Dry-07 and LB2-Dry-07, within a range of 15.0 % to 16.2 %. BR-Irr-06, BR-Dry 06, BI-Irr-06 and LB1-Dry-06 produced statistically comparable GLM with a range of 17.0 % to 18.2 % (Table 4.3).

#### **4.1.1.4 Galactomannan productivity**

Galactomannan productivity (GLM-P) was calculated by multiplying SY by GLM. On average, GLM-P of fenugreek genotypes tested varied significantly with a

range of 150.7 kg ha<sup>-1</sup> for Ind Temp to 324.8 kg ha<sup>-1</sup> for F75. The GLM-P of Ind Temp was statistically comparable to that of AC Amber and X92. Genotypes Tristar, F17, F96 and F86 reported statistically comparable GLM-P, ranging from 265.1 kg ha<sup>-1</sup> to 277.0 kg ha<sup>-1</sup>, while GLM-P reported for Quatro MP 30 and L3312 were statistically comparable to that of F75.

The GLM-P among growing environments significantly varied with a range of 46.8 kg ha<sup>-1</sup> for BR-Dry-07 to 573.2 kg ha<sup>-1</sup> for BR-Dry-06. The GLM-P observed at LB1-Irr-06 was statistically comparable with those observed at BR-Irr-07, LB1-Irr-07 and LB2-Dry-07, with a range of 100.7 kg ha<sup>-1</sup> to 156.9 kg ha<sup>-1</sup>. The GLM-P observed at LB1-Dry-06 was statistically comparable with that observed at BI-Dry-07. The GLM-P observed at BI-Dry-06 was comparable with that observed at BI-Irr-07. On average, BR-Irr-06 and BI-Irr-06 observed statistically comparable GLM-P, ranging from 442.8 kg ha<sup>-1</sup> to 475.3 kg ha<sup>-1</sup> but these were significantly lower than that observed at BR-Dry-06 at 573.2 kg ha<sup>-1</sup>.

#### **4.1.1.5 Diosgenin content**

On average, DIOS of seeds among the ten fenugreek genotypes varied significantly with a range of 0.50 % for F17 to 0.81 % for AC Amber (Table 4.3). DIOS of Tristar was statistically comparable to those of F17, L3312 and F86, within a range of 0.50 % to 0.57%. Genotypes F96, F75 and Ind Temp produced comparable DIOS but these were significantly lower than those of Quatro MP 30, AC Amber and X92.

On average, a relatively narrow variation in DIOS was observed among environments, with a range of 0.57 % to 0.67 % (Table 4.3). BR-Irr-06, on average,

produced seed with the lowest DIOS, although this was statistically comparable to DIOS observed at BI-Irr-06, BR-Irr-07, BR-Dry-07, LB1-Dry-07 and LB2-Dry-07. On average, the environments Br-Dry-06, LB1-Irr-06, BR-Dry-07, BI-Irr-07, BI-Dry-07, LB1-Irr-07, LB1-Dry-07 and LB2-Irr-07 produced statistically comparable DIOS within a range of 0.60 % to 0.64 %. The environment LB1-Dry-06 on average produced the highest DIOS, although this was also statistically comparable to those observed at BR-Dry-06 and BI-Dry-06.

#### **4.1.1.6 Diosgenin productivity**

Diosgenin productivity (DIOS-P) was calculated by multiplying SY by DIOS. On average, DIOS-P among genotypes significantly varied with a range 5.9 kg ha<sup>-1</sup> for Ind Temp to 11.6 kg ha<sup>-1</sup> for Quatro MP 30 (Table 4.3). The DIOS-P for Ind Temp was statistically comparable to those of F17 and X92, with a range of 5.9 kg ha<sup>-1</sup> to 7.6 kg ha<sup>-1</sup>. The DIOS-P of Tristar (8.4 kg ha<sup>-1</sup>) was statistically comparable to those of L3312, F86, AC Amber and F96, but was significantly lower than those of F75 and Quatro MP 30.

On average, a larger variation was observed for DIOS-P among environments, with a range of 2.2 kg ha<sup>-1</sup> to 19.3 kg ha<sup>-1</sup> for BR-Dry-07 and BR-Dry 06, respectively. DIOS-P observed at BR-Dry-07 was statistically comparable to those of LB1-Dry-07 and LB2-Dry-07, but was significantly lower than that of LB1-Irr-06 (4.8 kg ha<sup>-1</sup>) and LB1-Irr-07 (5.4 kg ha<sup>-1</sup>). BI-Dry-06, LB1-Dry-06 and BI-Dry-07 reported comparable DIOS-P, within a range of 8.5 kg ha<sup>-1</sup> to 10.6 kg ha<sup>-1</sup>. The DIOS-P observed at BI-Dry-07, BI-Irr-06 and BR-Irr-06 were statistically comparable, ranging from 13.4 kg ha<sup>-1</sup> to 15.3 kg



ha<sup>-1</sup>. However, these values were significantly lower than that observed at BR-Dry-06 (19.3 kg ha<sup>-1</sup>).

#### **4.1.1.7 4-Hydroxyisoleucine content**

The 4-OH-ILE of fenugreek genotypes varied significantly with a range of 0.85% to 0.99% (Table 4.3). 4-OH-ILE observed in genotypes X92, F86, F96, L3312, Quatro MP 30 and F17 was statistically comparable, ranging from 0.85 % to 0.92 % (Table 4.3). 4-OH-ILE observed in AC Amber was comparable to that of F75, Tristar and Ind Temp (0.99 %).

A larger variation in 4-OH-ILE was observed among environments, ranging from 0.68 % to 1.26 % for BR-Dry-07 and BI-Dry-06, respectively (Table 4.3). Within a range of 0.81 % to 0.88 %, the 4-OH-ILE observed at BR-Dry-06, BR-Irr-06, BI-Irr-06, LB1-Irr-06, BR-Irr-07 and BI-Irr-07 were statistically comparable. On average, 4-OH-ILE observed at LB1-Dry-06 (0.91 %) was comparable to that of LB2-Dry-07 (0.98 %) but was significantly lower than that of LB1-Irr-07 (1.04 %). The 4-OH-ILE observed at LB2-Irr-07 (1.12 %) was comparable to that observed at LB1-Dry-07 (1.20 %), but was significantly lower than that observed at BR-Dry-07 (1.26 %). While rainfed growing environments such as BR-Dry-07 and LB1-Dry-07 produced low SY at 365 kg ha<sup>-1</sup> and 604 kg ha<sup>-1</sup>, respectively (Table 4.3), relatively higher 4-OH-ILE (1.20-1.26 %) was observed when compared to the other environments tested (Table 4.3).

#### **4.1.1.8 4-Hydroxyisoleucine productivity**

4-Hydroxyisoleucine productivity (4-OH-ILE-P) was calculated by multiplying SY by 4-OH-ILE. On average, the 4-OH-ILE-P among fenugreek genotypes varied significantly with a range of 8.3 kg ha<sup>-1</sup> for X92 to 16.6 kg ha<sup>-1</sup> for F75 (Table 4.3). The 4-OH-ILE-P for X92, Ind Temp and AC Amber were statistically comparable falling within a range of 8.3 kg ha<sup>-1</sup> to 10.5 kg ha<sup>-1</sup>. At, 4-OH-ILE-P for the F86 (12.0 kg ha<sup>-1</sup>) was comparable to that of F96, F17 and L3312 (Table 3.3). 4-OH-ILE-P was highest in F75, and it was statistically comparable to that of Tristar and Quatro MP 30 at 14.7 kg ha<sup>-1</sup> and 14.8 kg ha<sup>-1</sup>, respectively.

A greater variation in 4-OH-ILE-P was observed among environments with a range of 4.6 kg ha<sup>-1</sup> to 25.6 kg ha<sup>-1</sup> (Table 4.3). The 4-OH-ILE-P observed at BR-Dry-07 was statistically comparable to those observed at LB1-Irr-06, LB1-Dry-07 and LB2-Dry-07, ranging from 4.6 kg ha<sup>-1</sup> to 7.2 kg ha<sup>-1</sup>. LB1-Irr-07 produced 9.2 kg ha<sup>-1</sup>, which was comparable to those of BI-Dry-07, BR-Irr-07, and BI-Dry-06, but was significantly lower than that of LB2-Irr-07 (12.7 kg ha<sup>-1</sup>). The 4-OH-ILE-P observed at BI-Irr-07 (18.3 kg ha<sup>-1</sup>) was comparable to that of BI-Irr-06 (20.4 kg ha<sup>-1</sup>), but was significantly lower than that observed at BR-Irr-06 (23.7 kg ha<sup>-1</sup>) and BR-Dry-06 (26.5 kg ha<sup>-1</sup>).

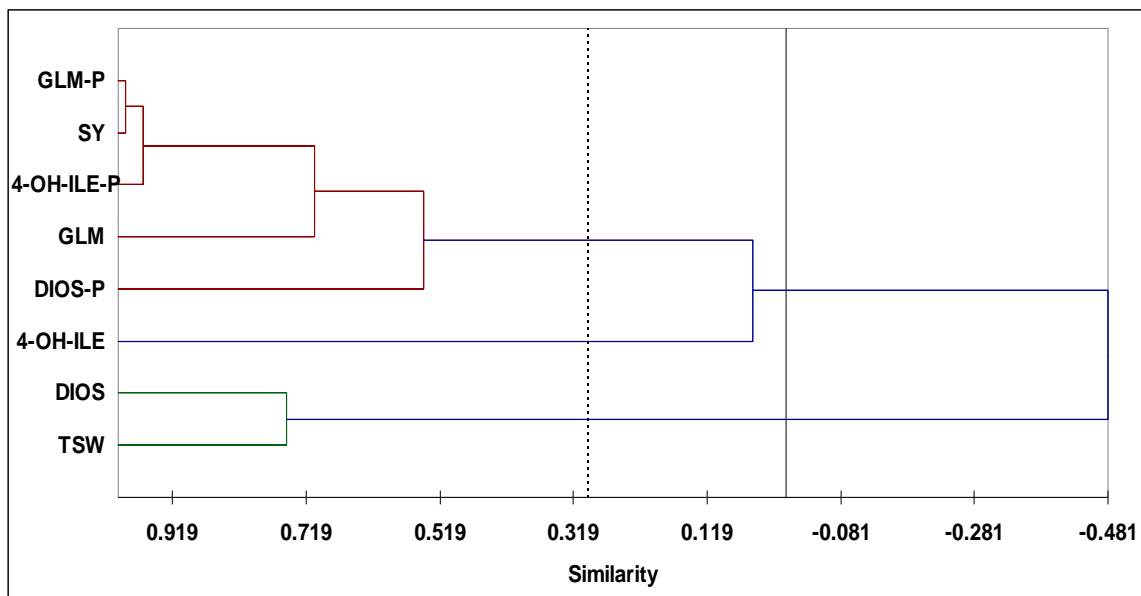
#### **4.1.2 Genotype x environment interaction effects**

Although the G x E interaction effect contributed only 14-19 % (Table 4.2) the total variation observed for each of the eight selected agronomic and biochemical traits, it is still considered significant as further analysis of this component would provide

statistical information to rank and group genotypes, based on performance under different growing environments.

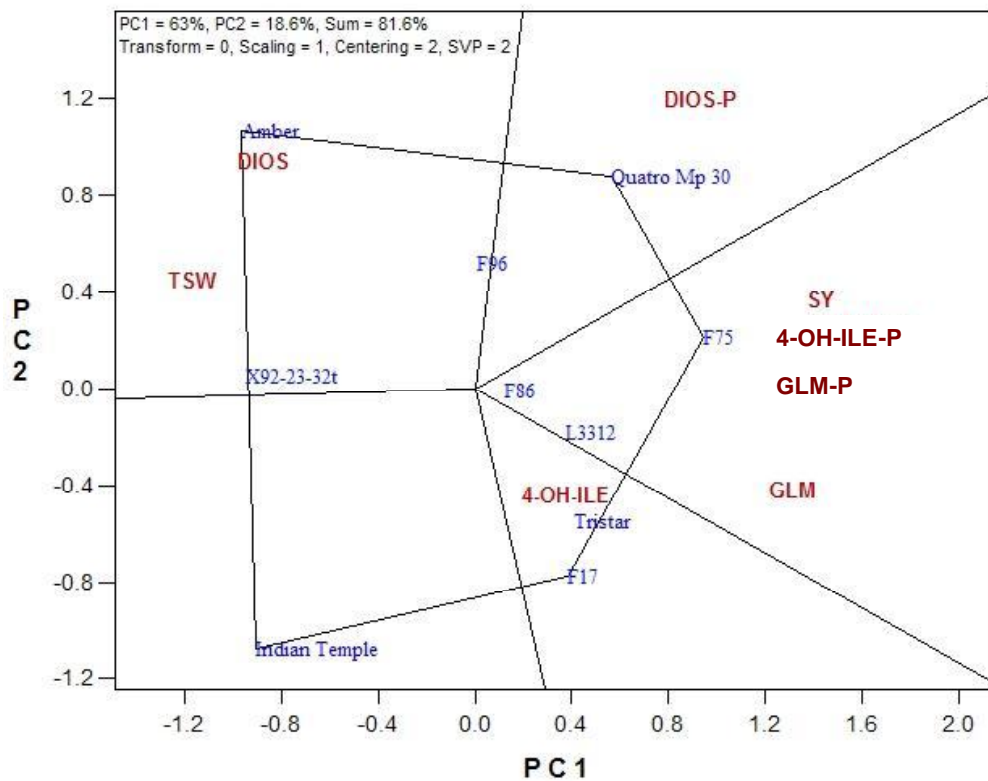
An Agglomerative Hierarchical Cluster analysis (XLSTAT, Addinsoft, New York, NY) was carried out on means of selected traits of fenugreek genotypes grown across 14 environments (Fig 4.1). Response analysis indicated that GLM-P, SY and 4-OH-ILE-P are closely associated to each other ( $r > 0.92$ ), whereas DIOS and TSW were weakly associated to each other ( $r > 0.72$ ). In general, the analysis produced three distinct clusters; where GLM-P, SY, 4-OH-ILE-P and GLM formed a cluster, DIOS and TSW formed another cluster, and 4-OH-ILE was too different to be shared by any one cluster, and hence formed a cluster on its own (Fig. 4.1).

The genotype x environment interaction effects for eight agronomic and biochemical traits evaluated in this study were further analyzed using the GGE biplot methodology (Yan, 1999). The “which won what” view of the biplot identifies the winning genotypes for each of the selected agronomic and biochemical traits as indicated by the vertices of the polygon. Perpendicular lines originating from the biplot origin divide the polygon into sections containing winning genotypes with their corresponding traits. Based on performance, F75 was the most promising genotype in terms of SY, GLM-P and 4-OH-ILE-P, and F17 was the best performing for 4-OH-ILE (Fig. 4.2). Although genotype AC Amber was the best performer in terms of DIOS, genotype Quatro MP 30 was better in terms of DIOS-P, as a result of its high seed yielding capacity. Other genotypes located within the polygon were not as discriminatory for the traits studied (Fig 4.2).



**Figure 4.1 Dendrogram depicting hierarchical clustering of the 8 traits based on the means of 10 fenugreek genotypes tested across 14 growing environments.<sup>§</sup>**

<sup>§</sup> Similarity of clusters is based on Pearson correlation coefficients; agglomeration of the clusters based on unweighted pair-group average. Similar color groups represent distinct clusters. Dotted line represent truncation point for clusters. TSW = 1000-seed weight; SY = seed yield; GLM = galactomannan content; GLM-P = galactomannan productivity; DIOS = diosgenin; DIOS-P = diosgenin productivity; 4-OH-ILE = 4-hydroxyisoleucine; 4-OH-ILE-P = 4-hydroxyisoleucine productivity.



**Figure 4.2** The "which won what" view of the "genotype x trait" GGE biplot for all 8 traits of 10 fenugreek genotypes tested across 14 environments.<sup>8</sup>

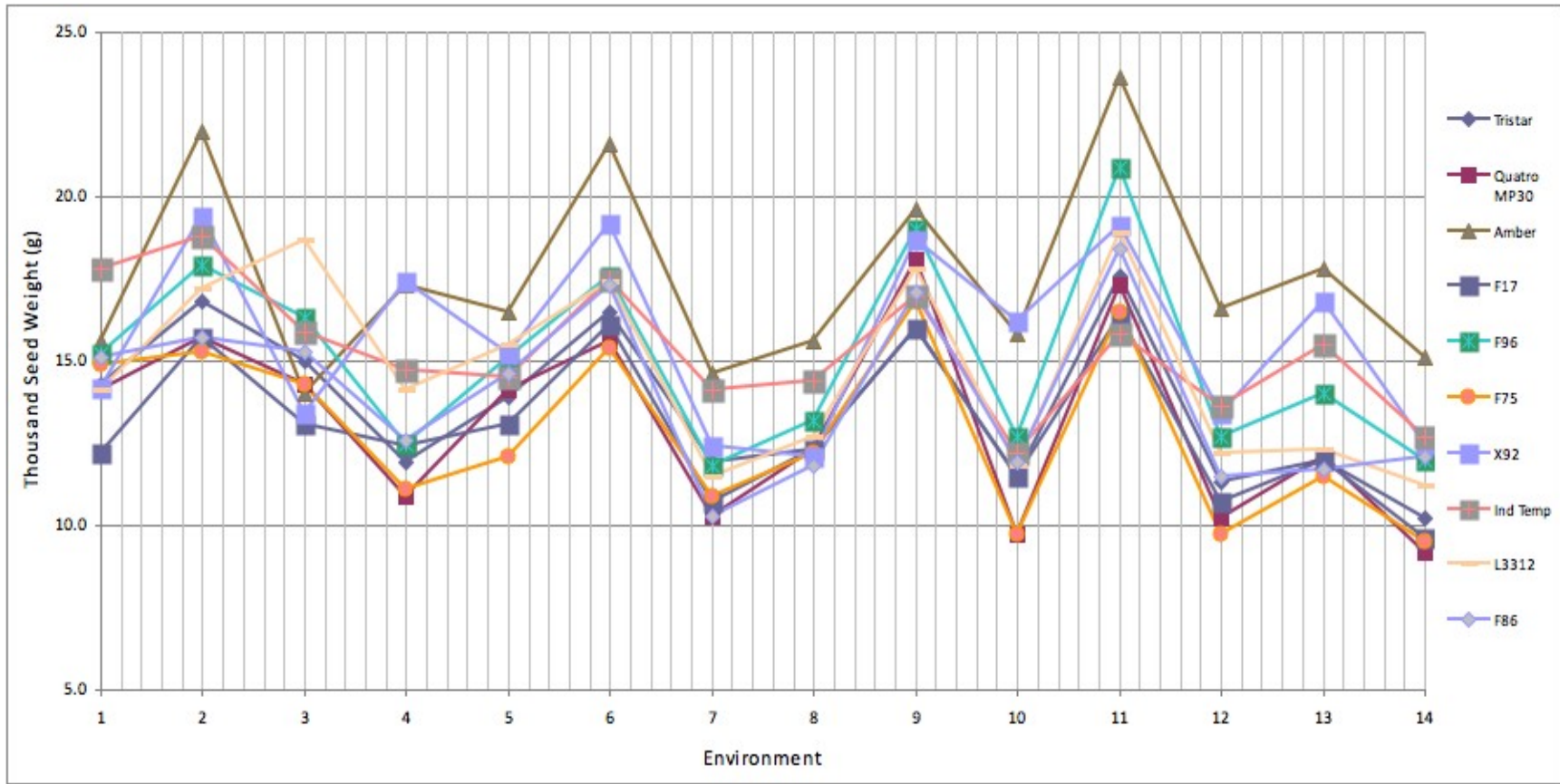
<sup>8</sup> Traits parameters are in uppercase and genotypes are in lower case. PC 1 and PC 2 are primary and secondary effects, respectively. TSW = 1000-seed weight; SY = seed yield; GLM = galactomannan content; GLM-P = galactomannan productivity; DIOS = diosgenin content; DIOS-P = diosgenin productivity; 4-OH-ILE = 4-hydroxyisoleucine; 4-OH-ILE-P = 4-hydroxyisoleucine productivity.

#### 4.1.2.1 Thousand seed weight

The G x E interaction effect for TSW accounted for 14% of the total variation due to treatments (Table 4.2). The interaction effect for TSW due to treatments is graphically represented in a scatter diagram (Fig. 4.3; for numerical values, see Appendix II). Performance-stable genotypes should theoretically produce parallel lines (lack of cross-overs) when plotted against the growing environments in the absence of a G x E interaction. Even though the growing environments are discrete variables, lines drawn connecting the data points are intended to exhibit the crossover interactions. Among the genotypes tested, only genotype AC Amber demonstrated fairly good stability, almost always ranking first in terms of TSW (few cross-overs) (Fig. 4.3).

Hierarchical clustering of genotypes according to their response patterns in terms of TSW is presented in a dendrogram in Fig. 4.4. The hierarchical cluster analysis indicates that genotypes F96, Tristar, F75, Quatro MP 30, F86, F17 and L3312 have formed one cluster, generally representing smaller seed weights. AC Amber and X92, which had larger seed weights, together form a distinct cluster while Ind Temp remained in a cluster on its own (Fig. 4.4).

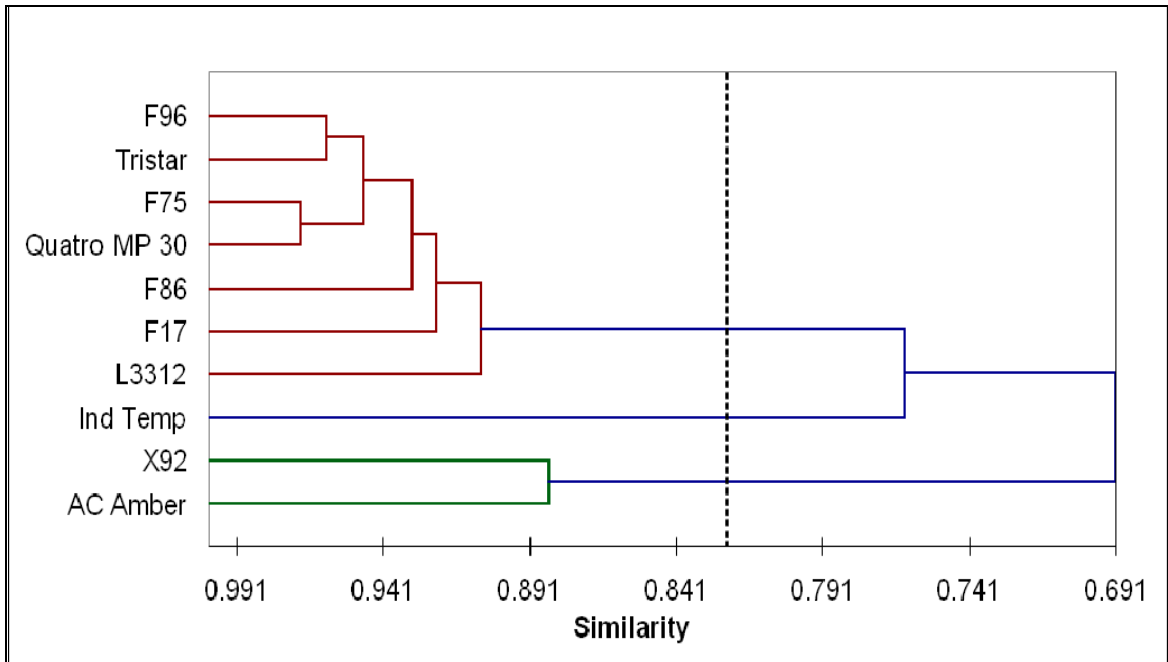
The GGE biplot analysis on TSW is presented in Fig. 4.5, in a “which won where” view. The analysis shows that TSW was most responsive in Ind Temp, F75, Quatro MP 30, F96 and AC Amber, given their position at the vertices of the polygon. However, the location of F75 at the farthest left of the polygon (low PC 1 score) indicates poor performance (low yielding) while AC Amber located at the farthest right of the polygon (high PC 1 score), surrounded by the majority of growing environments indicates superior performance (a winning genotype) in those environments [2 (BR-Dry-



**Figure 4.3 A scatter plot of mean thousand seed weight (g) and growing environments (numbered 1 to 14) to illustrate the presence of genotype x environment interaction for 10 fenugreek genotypes tested across 14 environments in southern Alberta.<sup>8</sup>**

<sup>8</sup> Environment 1 = BR-Irr-06; 2 = BR-Dry-06; 3 = BI-Irr-06; 4 = BI-Dry-06; 5 = LB1-Irr-06; 6 = LB1-Dry-06; 7 = BR-Irr-07; 8 = BR-Dry-07; 9 = BI-Irr-07; 10 = BI-Dry-07; 11 = LB1-Irr-07; 12 = LB1-Dry-07; 13 = LB2-Irr-07; 14 = LB2-Dry-07.

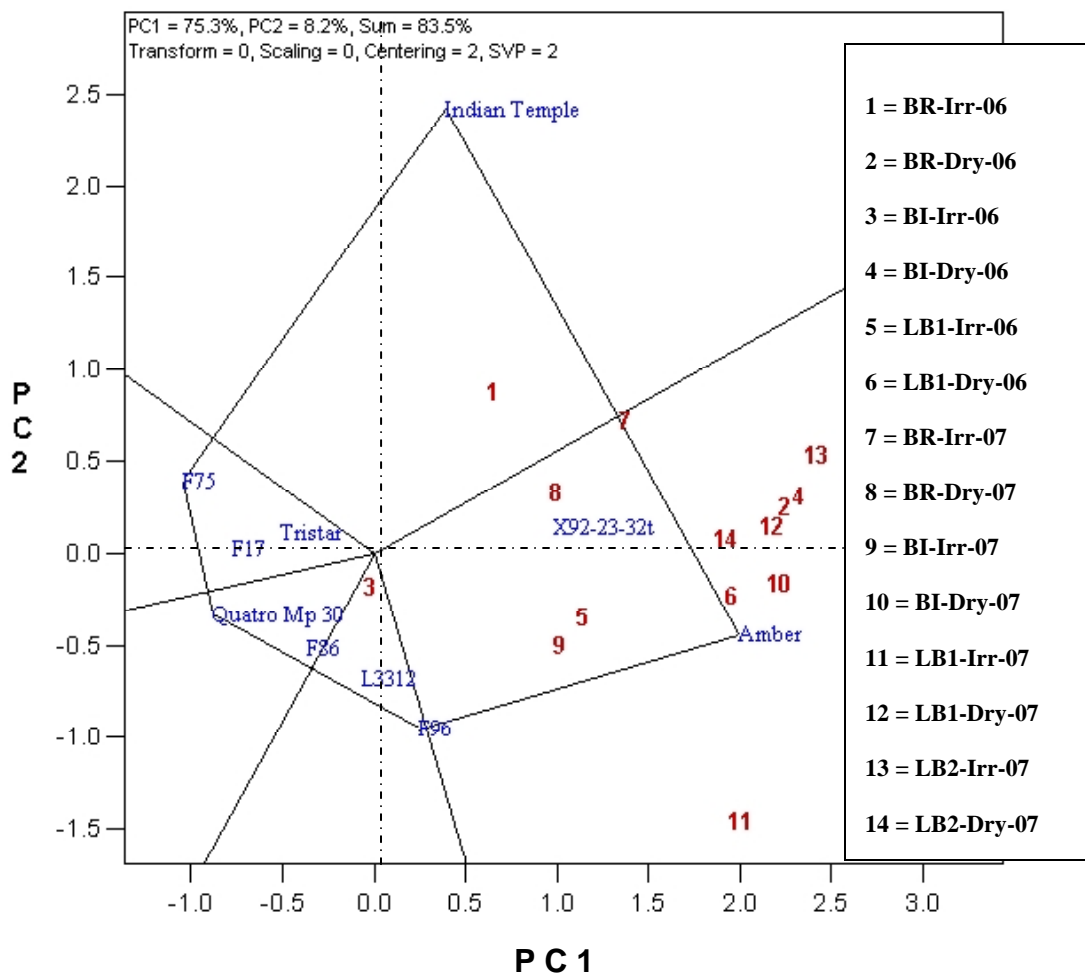
Note: Environments are considered discrete variables, lines drawn connecting the data points are intended to exhibit the crossover interactions.



**Figure 4.4 Dendrogram depicting the hierarchical clustering of 10 fenugreek genotypes tested in 14 environments according to thousand seed weight (g).<sup>8</sup>**

<sup>8</sup> Similarity of clusters is based on Pearson correlation coefficients; agglomeration of the clusters based on unweighted pair-group average. Similar color groups represent a distinct cluster. The dotted line represents the truncation point for clusters.





**Figure 4.5** The “which won where” view of the GGE biplot for mean thousand seed weight (g) of 10 fenugreek genotypes tested across 14 growing environments.<sup>8</sup>

<sup>8</sup> PC 1 denotes Principal Component 1, and PC 2 denotes Principal Component 2. Genotypes are in blue, growing environments are numbered 1 to 14, in red.

06), 4 (BI-Dry-06), 6 (LB1-Dry-06), 10 (BI-Dry-07), 12 (LB1-Dry-07), 13 (LB2-Irr-07), 14 (LB2-Dry-07)]. The TSW trait was fairly stable in these environments, as indicated by the low PC 2 scores (small deviation from PC 2 axis), with the exception of Ind Temp, which had the greatest deviation on the PC 2 axis (Fig. 4.5).

#### **4.1.2.2 Seed yield**

The main effect of growing environment for SY contributed 76 % to the total variation due to treatment, while the main genotypic effect contributed only a mere 9% (Table 4.2). Moreover, the G x E interaction effect was higher than that of genotype at

15 %. The G x E interaction effect for SY is graphically represented in Fig. 4.6. A scatter diagram of SY was plotted against environments (Fig. 4.6; for numerical values see Appendix II) to illustrate the crossover interaction characteristic of these multi-environment trials, where ranking of genotypes in terms of productivity changed across environments. In this analysis, genotypes with higher seed yield stability should theoretically produce parallel lines across environments, suggesting a lack of interaction with the environment. Even though growing environments are considered discrete variables, lines drawn connecting the data points are intended to exhibit the crossover interactions. SY assessed over the different environments tested produced significant crossover (i.e. F86) and changes in genotype rankings (Fig. 4.6); indicating the presence of a G x E

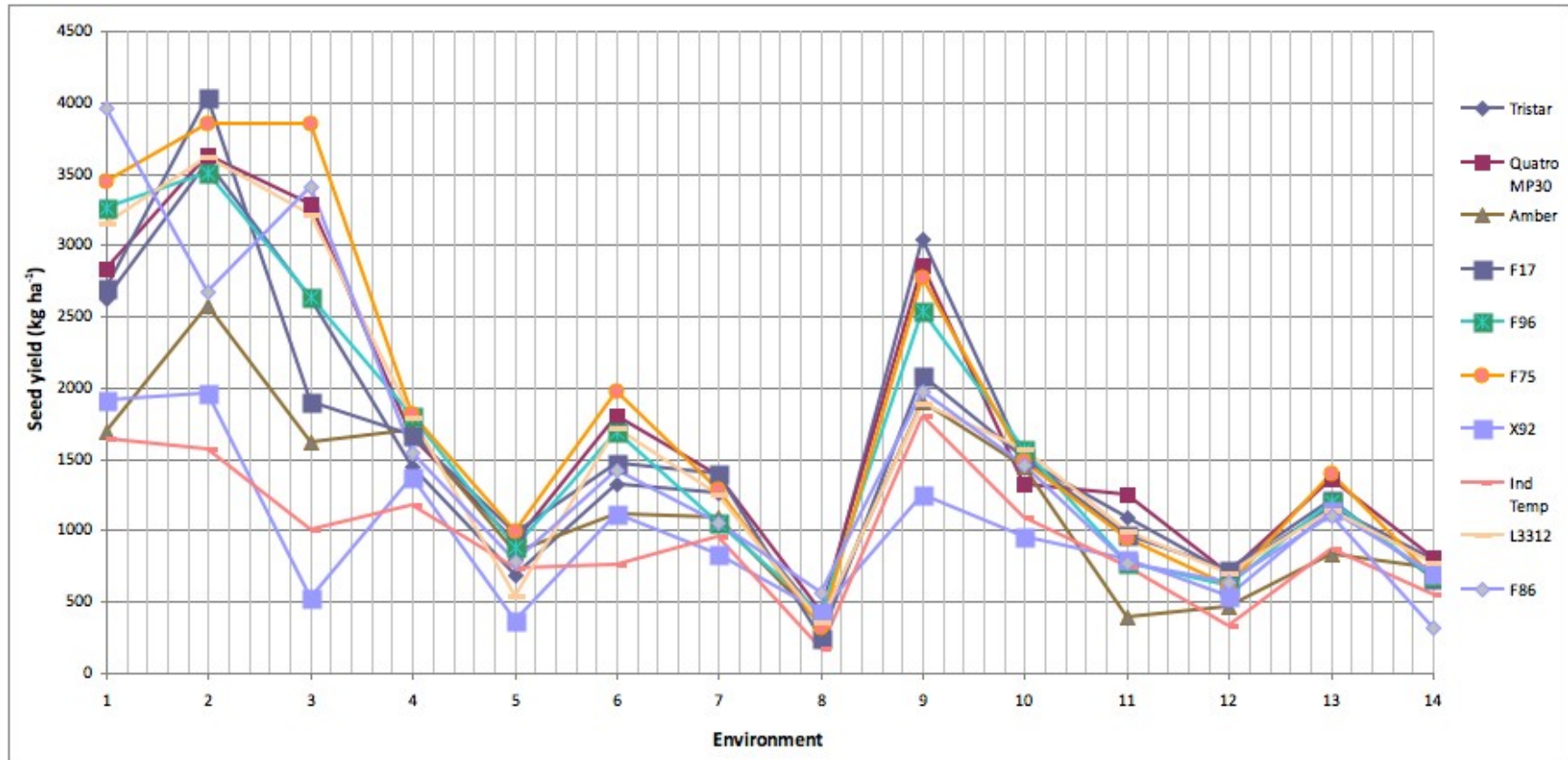
interaction. In contrast, genotypes X92 and Ind Temp were shown to be consistently low-yielding (stable).

The hierarchical cluster analysis of genotype for SY was performed to group genotypes based on similarities in their genotype response pattern (Fig. 4.7). This analysis indicates that F75, Quatro MP 30, F96, L3312 and Tristar have formed one cluster whereas F17, AC Amber, F86, Ind Temp and X92 formed distinct and separate clusters; *i.e.*, each remained in a cluster on its own.

The GGE biplot analysis on SY (Fig. 4.8) shows the most responsive genotypes (F17, X92, F86 and F75) based on their locations at the vertices of the polygon, and the most discriminating environments [1 (BR-Irr-06), 2 (BR-Dry-06), 3 (BI-Irr-06) and 9 (BI-Irr-07)], given their furthest distances from the biplot origin. F75 is identified as possessing the best performing genotype based on its high PC 1 score (located furthest right) while X92 was the lowest seed yielding genotype with the lowest PC 1 score (located furthest left). However, these genotypes were considered stable (consistent performance) as their PC 2 scores were close to zero.

#### **4.1.2.3 Galactomannan content**

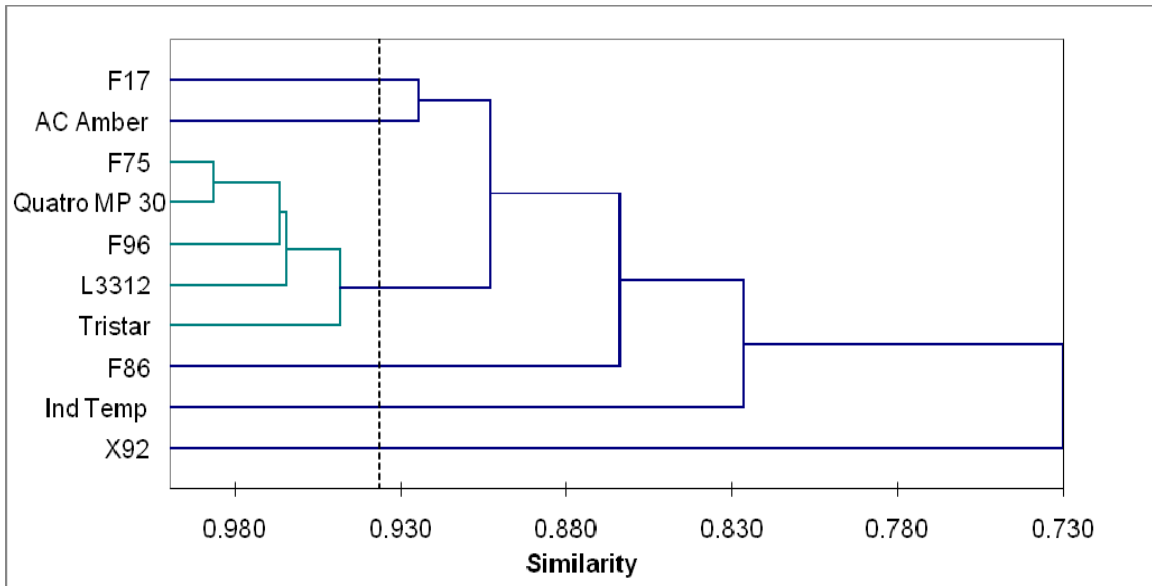
The G x E interaction effect on GLM accounted for 19 % of the variation, due to treatments (Table 4.2) .The interaction effect on GLM is graphically represented in Fig. 4.9. A scatter diagram of GLM was plotted against



**Figure 4.6 A scatter plot of mean seed yield (kg ha<sup>-1</sup>) and growing environments (numbered 1 to 14) to illustrate the presence of genotype x environment interaction for 10 fenugreek genotypes tested across 14 environments in southern Alberta.<sup>8</sup>**

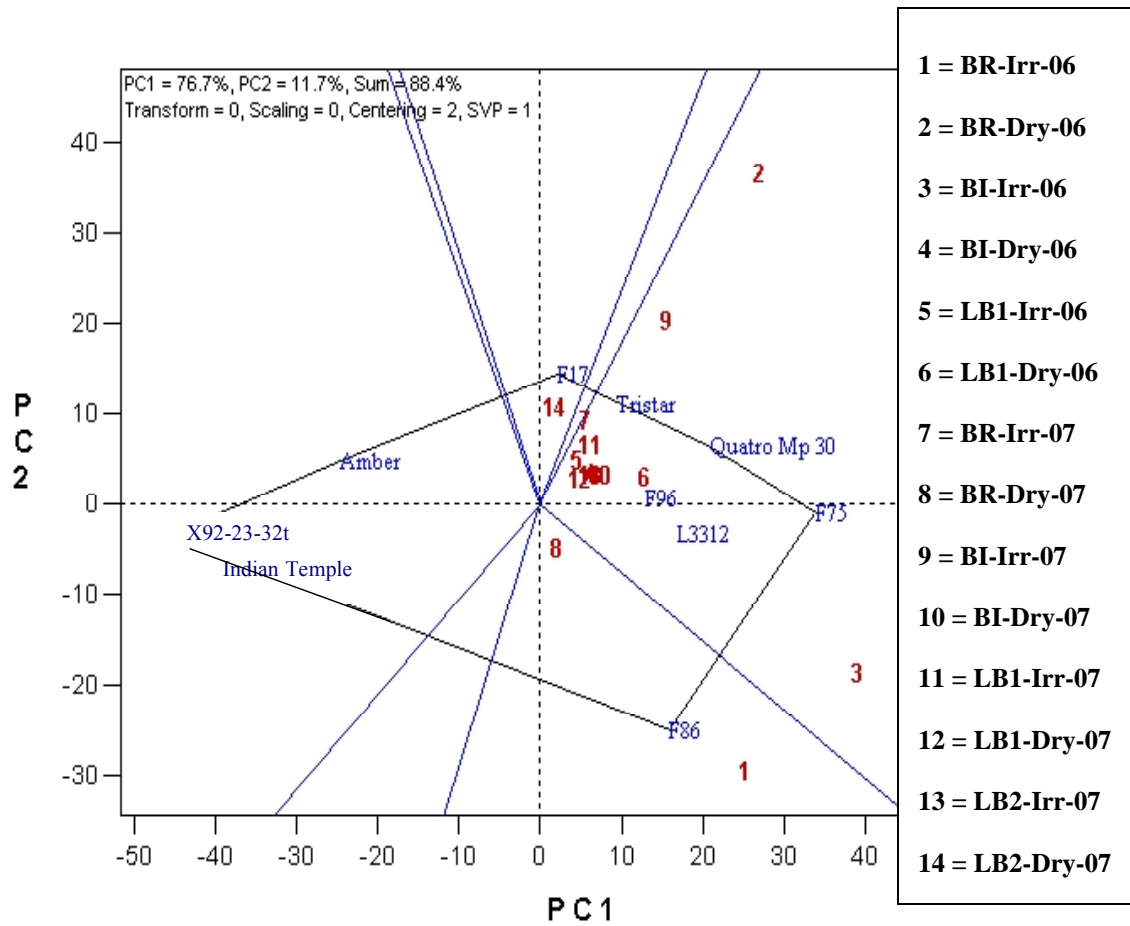
<sup>8</sup> 1 = BR-Irr-06; 2 = BR-Dry-06; 3 = BI-Irr-06; 4 = BI-Dry-06; 5 = LB1-Irr-06; 6 = LB1-Dry-06; 7 = BR-Irr-07; 8 = BR-Dry-07; 9 = BI-Irr-07; 10 = BI-Dry-07; 11 = LB1-Irr-07; 12 = LB1-Dry-07; 13 = LB2-Irr-07; 14 = LB2-Dry-07.

Note: Environments are considered discrete variables, lines drawn connecting the data points are intended to exhibit the crossover interactions.



**Figure 4.7 Dendrogram depicting the hierarchical clustering of 10 fenugreek genotypes tested in 14 environments based on their mean seed yield ( $\text{kg ha}^{-1}$ ).<sup>§</sup>**

<sup>§</sup> Similarity of clusters is based on Pearson correlation coefficients; agglomeration of the clusters based on unweighted pair-group average. Similar color groups represent a distinct cluster. The dotted line represents the truncation point for clusters.



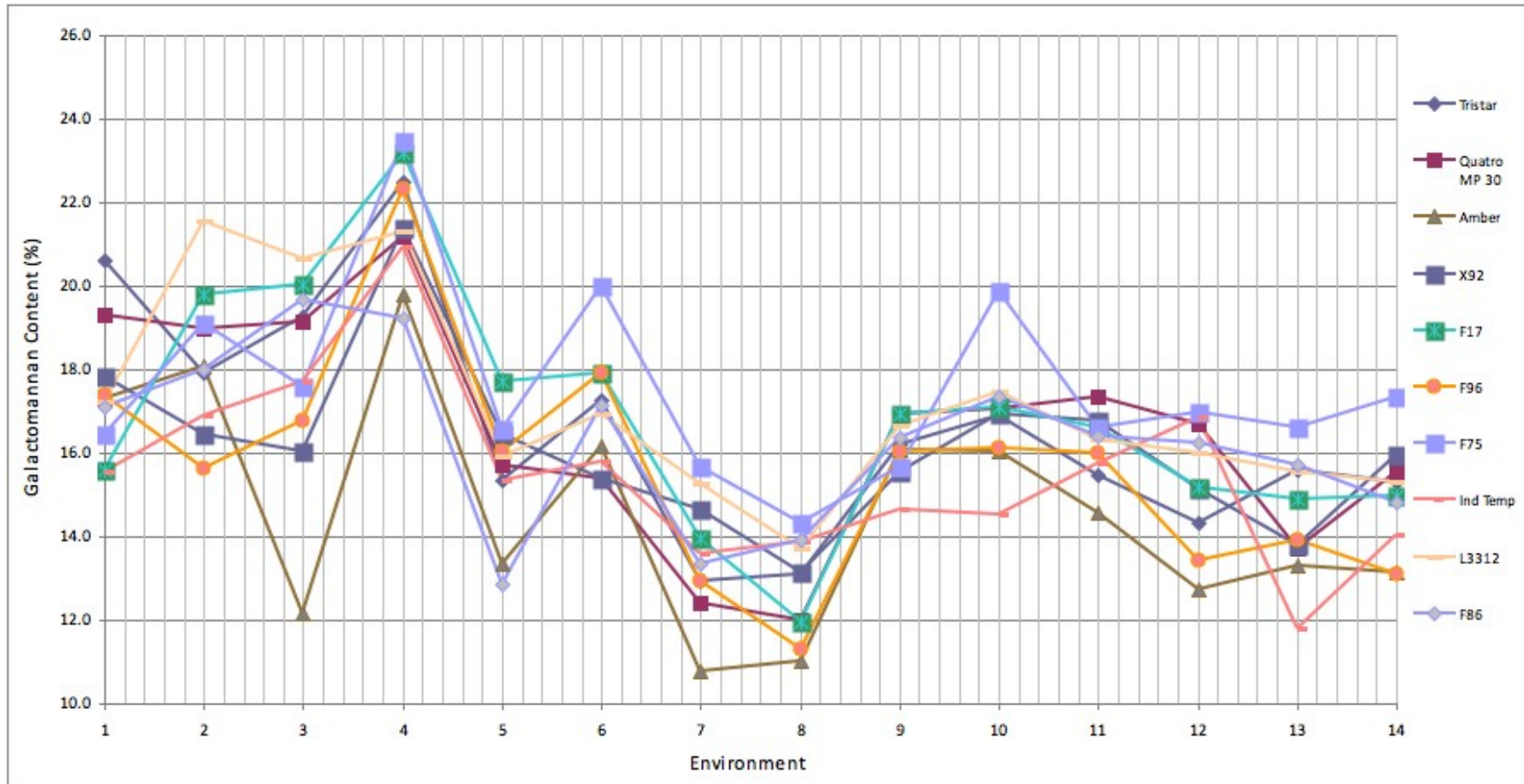
**Figure 4.8** The “which won where” view of the GGE biplot for mean seed yield ( $\text{kg ha}^{-1}$ ) of 10 fenugreek genotypes tested across 14 environments.<sup>§</sup>

<sup>§</sup> PC 1 denotes Principal Component 1 and PC 2 denotes Principal Component 2. Genotypes are in blue, growing environments are numbered 1 to 14, in red.

the growing environments (Fig. 4.9; for numerical values see Appendix II). Significant crossover was observed among genotypes as response to the growing environments in terms of GLM. F75 and AC Amber were observed to produce fewer crossovers among the genotypes tested. Even though growing environments are considered discrete variables, lines drawn connecting the data points are intended to exhibit the crossover interactions.

Hierarchical cluster analysis performed for GLM indicates that genotypes F96, Tristar, X92 and Quatro MP 30 have formed one cluster, and L3312, F17 and Ind Temp formed another cluster. AC Amber, F75 and F86 were too different in their response patterns to share any one cluster (Fig. 4.10).

The GGE biplot analysis for GLM determined that the most responsive genotypes were AC Amber, L3312, F75 and F86, based on their positions at the vertices of the polygon (Fig. 4.11). The most favorable environments for F75 to produce higher GLM include 4 (BI-Dry-06), 6 (LB1-Dry-06), 3 (BI-Irr-06), 10 (BI-Dry-07), 7 (BR-Irr-07), 14 (LB2-Dry-07) and 13 (LB2-Irr-07), all located at the top right sector of the biplot (Fig. 4.11). AC Amber was the poorest performing genotype in terms of GLM given its low PC 1 score but was also the most stable (low PC 2 score) among the responsive genotypes.

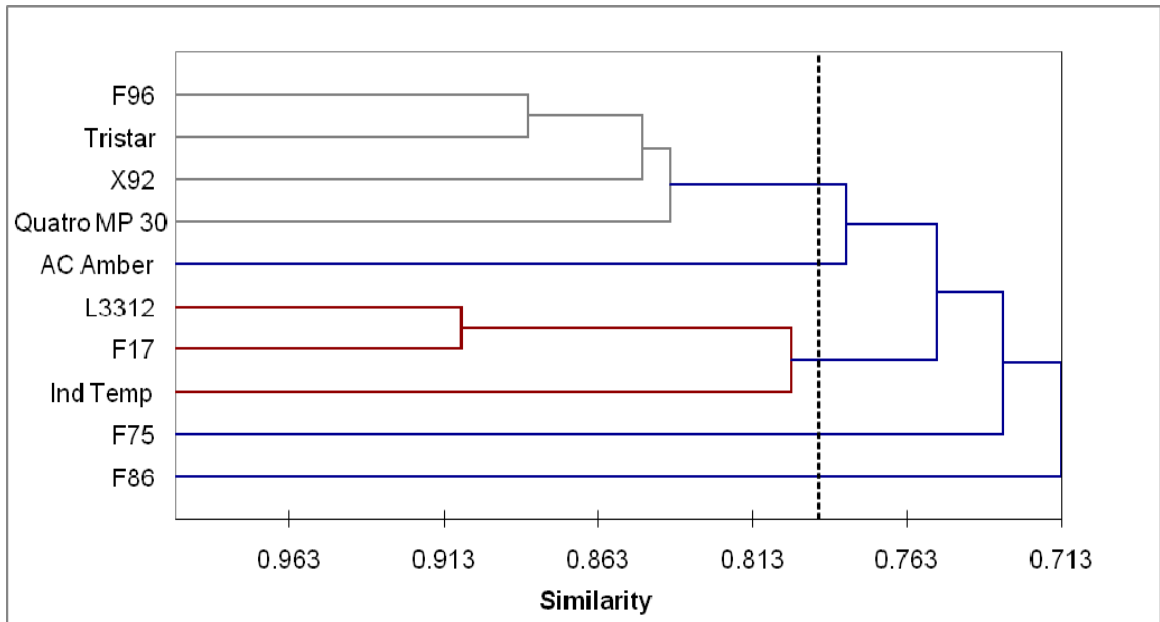


**Figure 4.9** A scatter plot of galactomannan content (%) and growing environments (numbered 1 to 14) to illustrate the presence of genotype x environment interaction for 10 fenugreek genotypes tested across 14 environments in southern Alberta.<sup>8</sup>

<sup>8</sup> Environment 1 = BR-Irr-06; 2 = BR-Dry-06; 3 = BI-Irr-06; 4 = BI-Dry-06; 5 = LB1-Irr-06; 6 = LB1-Dry-06; 7 = BR-Irr-07; 8 = BR-Dry-07; 9 = BI-Irr-07; 10 = BI-Dry-07; 11 = LB1-Irr-07; 12 = LB1-Dry-07; 13 = LB2-Irr-07; 14 = LB2-Dry-07.

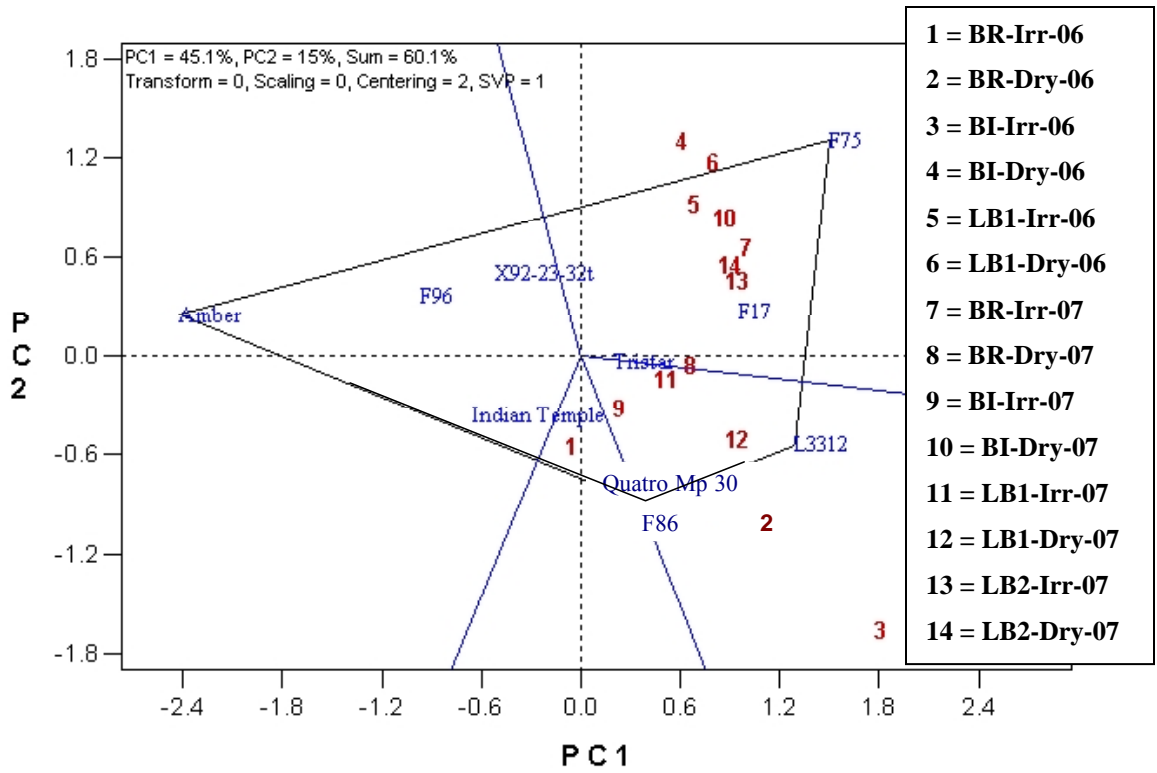
Note: Environments are considered discrete variables, lines drawn connecting the data points are intended to exhibit the crossover interactions.





**Figure 4.10 Dendrogram depicting the hierarchical clustering of 10 fenugreek genotypes tested in 14 environments based on their mean galactomannan content(%).<sup>§</sup>**

<sup>§</sup> Similarity of clusters is based on Pearson correlation coefficients; agglomeration of the clusters based on unweighted pair-group average. Similar color groups represent a distinct cluster. The dotted line represents the truncation point for clusters.



**Figure 4.11** The GGE biplot for mean galactomannan content (%) of 10 fenugreek genotypes tested across 14 environments.<sup>§</sup>

<sup>§</sup> PC 1 denotes Principal Component 1 and PC 2 denotes Principal Component 2. Genotypes are in blue, growing environments are numbered 1 to 14, in red.

#### **4.1.2.4 Galactomannan productivity**

The G x E interaction effect on galactomannan productivity (GLM-P) accounted for 15% of the total variation observed due to treatments (Table 4.2). For the numerical values representing the interaction effect on GLM-P, see Appendix II.

The GGE biplot analysis shows that genotypes F86, Ind Temp, X92, AC Amber, F17 and F75 were the most responsive genotypes in terms of GLM-P, as indicated by their positions at the vertices of the polygon (Fig. 4.12). Results show that F75 was the best performing genotype, as it is situated on the furthest right of the biplot (high PC 1 score). 2 (BR-Dry-06) was the most discriminative environment for F75, given its location furthest away from the biplot origin inside its sector. Similarly, 1 (BR-Irr-06) and 3 (BI-Irr-06) were the best environments for F86, indicated by their locations furthest away from the biplot origin within the F86 sector.

#### **4.1.2.5 Diosgenin content**

The G x E interaction effect on diosgenin content (DIOS) contributed 16 % of the total variation due to treatments (Table 4.2). The interaction effect on DIOS of the fenugreek genotypes is graphically represented in Fig. 4.13. Even though the environments are considered discrete variables, lines drawn connecting the data points are intended to exhibit the crossover interactions. The scatter plot shows crossover interactions among genotypes across growing environments for DIOS (Fig. 4.13; for numerical values see Appendix II). However, this change was relatively subtle compared to the crossover interactions observed for other traits as indicated in Figs. 4.3, 4.6, 4.9. Among the

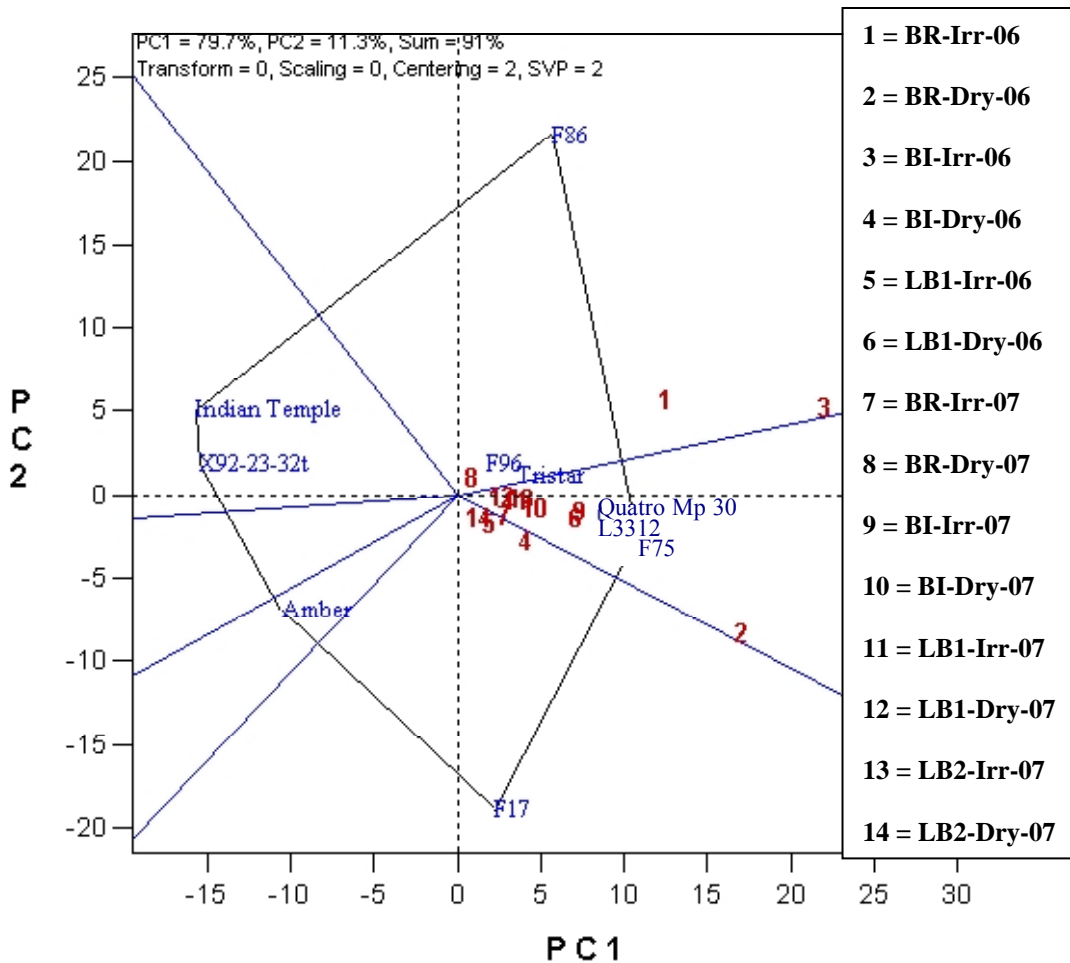
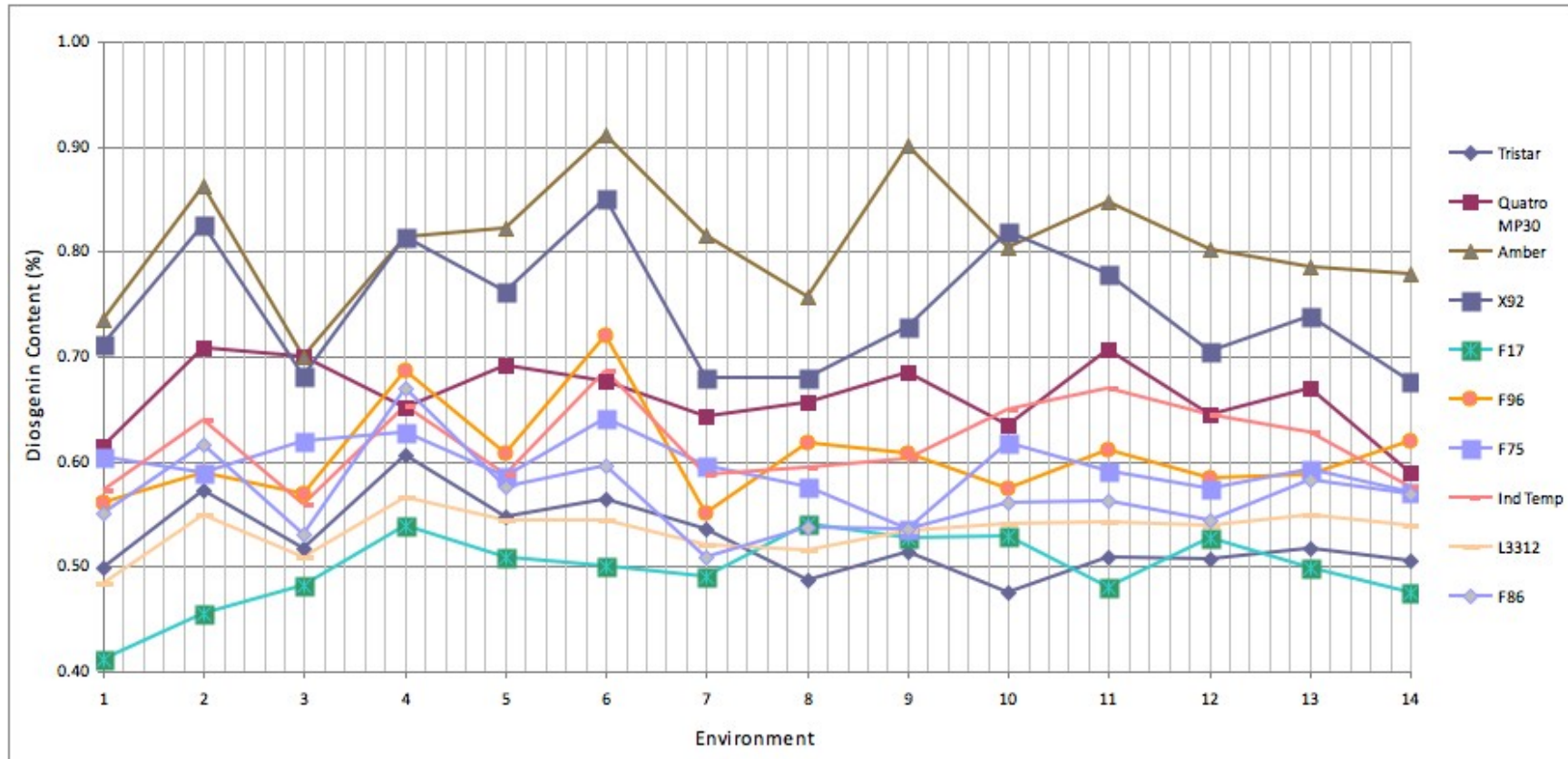


Figure 4.12 The "which won where" view of the GGE biplot for mean galactomannan productivity ( $\text{kg ha}^{-1}$ ) of 10 fenugreek genotypes tested across 14 environments.<sup>§</sup>

<sup>§</sup> PC 1 denotes Principal Component 1 and PC 2 denotes Principal Component 2. Genotypes are in blue, growing environments are numbered 1 to 14, in red.



**Figure 4.13** A scatter plot of mean diosgenin content (%) and growing environments (numbered 1 to 14) to illustrate the presence of genotype x environment interaction for 10 fenugreek genotypes tested across 14 environments in southern Alberta.<sup>§</sup>

<sup>§</sup> Environment 1 = BR-Irr-06; 2 = BR-Dry-06; 3 = BI-Irr-06; 4 = BI-Dry-06; 5 = LB1-Irr-06; 6 = LB1-Dry-06; 7 = BR-Irr-07; 8 = BR-Dry-07; 9 = BI-Irr-07; 10 = BI-Dry-07; 11 = LB1-Irr-07; 12 = LB1-Dry-07; 13 = LB2-Irr-07; 14 = LB2-Dry-07.

Note: Environments are considered discrete variables, lines drawn connecting the data points are intended to exhibit the crossover interactions.

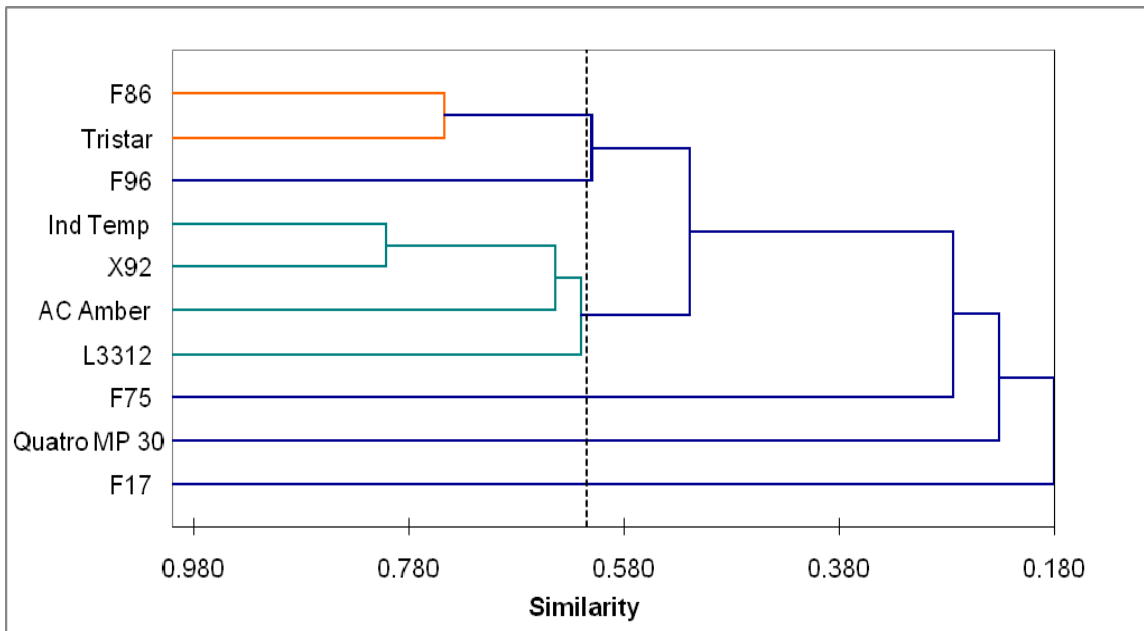
genotypes tested, AC Amber and X92 which observed high DIOS also had minimal crossover interactions across growing environments suggesting that these genotypes not only had high diosgenin-producing capacity but also good stability.

Hierarchical cluster analysis performed for DIOS of fenugreek genotypes showed that F86 and Tristar have formed one cluster, while Ind Temp, X92, AC Amber, and L3312 have formed another cluster. F96, F75, Quatro MP 30 and F17 were too different from each other in their response patterns to consider placing them in the same cluster; hence they were placed in individual clusters (Fig. 4.14).

The GGE biplot analysis shows AC Amber and X92 as the most promising genotypes in terms of DIOS in all growing environments except at 12 (LB1-Dry-07), indicated by their high PC 1 scores and close proximity to all but one environment within the sector (Fig. 4.15). Although AC Amber was superior to X92 in terms of DIOS, the lower PC 2 score of X92 indicates better stability (consistency) compared to that of AC Amber (Fig 4.15).

#### **4.1.2.6 Diosgenin productivity**

Diosgenin productivity (DIOS-P) was similar to the trend exhibited by SY; *i.e.*, 77 % of the total variation for DIOS-P was attributed to the environment main effect while only 8 % of the total variation was contributed by the genotype main effect (Table 4.2). The remaining 15 % of the variation was attributed to the G x E interaction effect. For numerical values representing the G x E interaction for DIOS-P, see Appendix II.



**Figure 4.14 Dendrogram depicting the hierarchical clustering of 10 fenugreek genotypes tested in 14 environments based on their mean diosgenin content (%).<sup>§</sup>**

<sup>§</sup> Similarity of clusters is based on Pearson correlation coefficients; agglomeration of the clusters based on unweighted pair-group average. Similar color groups represent a distinct cluster. The dotted line represents the truncation point for clusters.

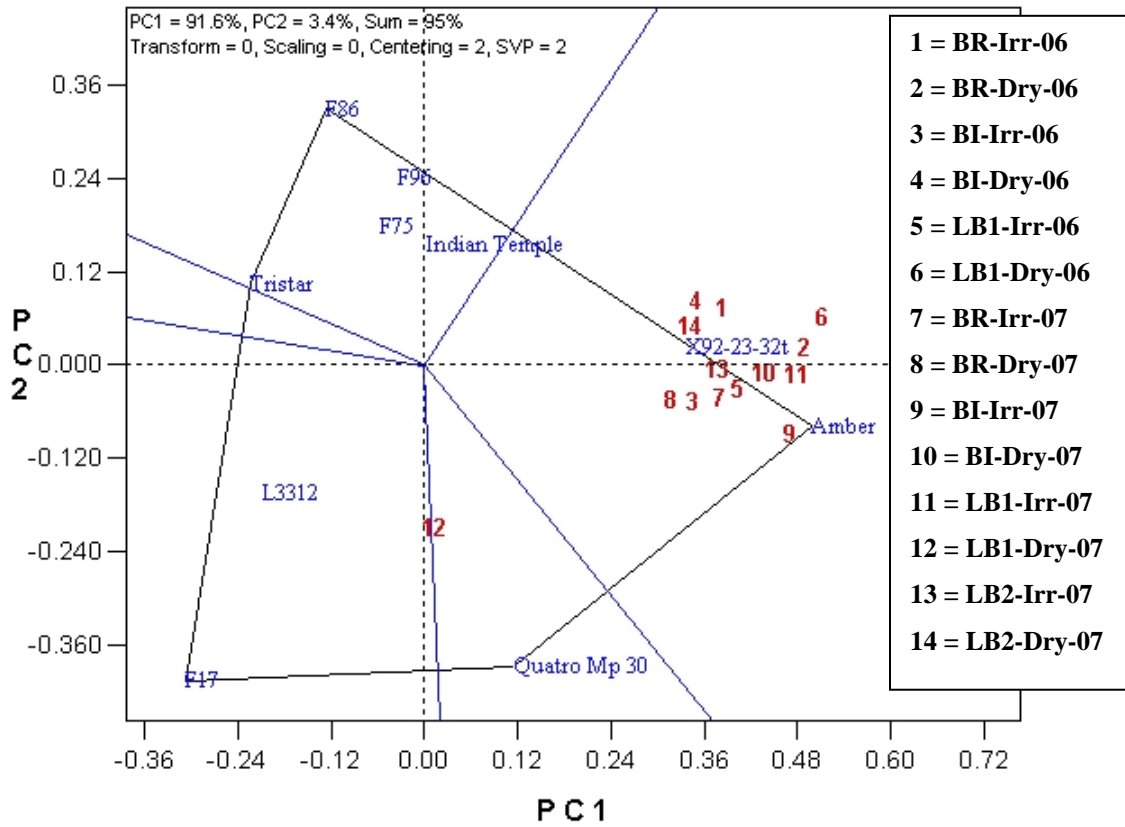


Figure 4.15 The "which won where" view of the GGE biplot for mean diosgenin content (%) of 10 fenugreek genotypes tested across 14 environments.<sup>§</sup>

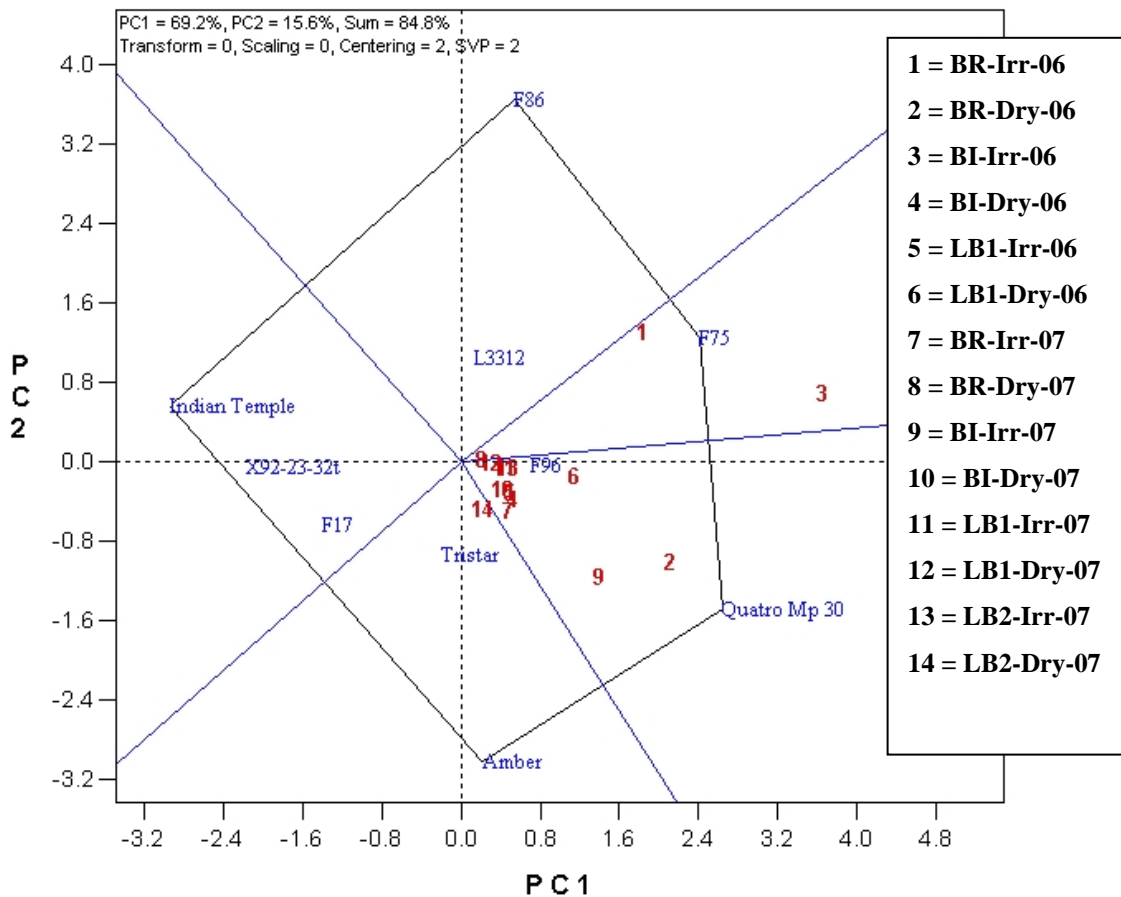
<sup>§</sup> PC 1 denotes Principal Component 1 and PC 2 denotes Principal Component 2. Genotypes are in blue, growing environments are numbered 1 to 14, in red.



The GGE biplot analysis performed for DIOS-P identified F86, Ind Temp, AC Amber, Quatro MP 30 and F75 as the most responsive genotypes given their positions at the vertices of the polygon (Fig. 4.16). Most environments except for Br-Irr-06 (1), BI-Irr-06 (3), Br-Dry-06 (2) and BI-Irr-07 (9) were not discriminative of the genotypes; *i.e.*, most environments on the biplot are seen in close proximity to the origin (Fig. 4.16). F75 was most promising genotype at Br-Irr-06 and BI-Irr-06 while Quatro MP 30 was the best performing genotype at all environments positioned within its sector; this excludes 1 (BR-Irr-06), 3 (BI-Irr-06) and 14 (LB2-Dry-07). Although Quatro MP 30 was superior to F75 in terms of DIOS-P, F75 was shown to be relatively more stable as indicated by its lower PC 2 score compared to that of Quatro MP 30 (Fig 4.16).

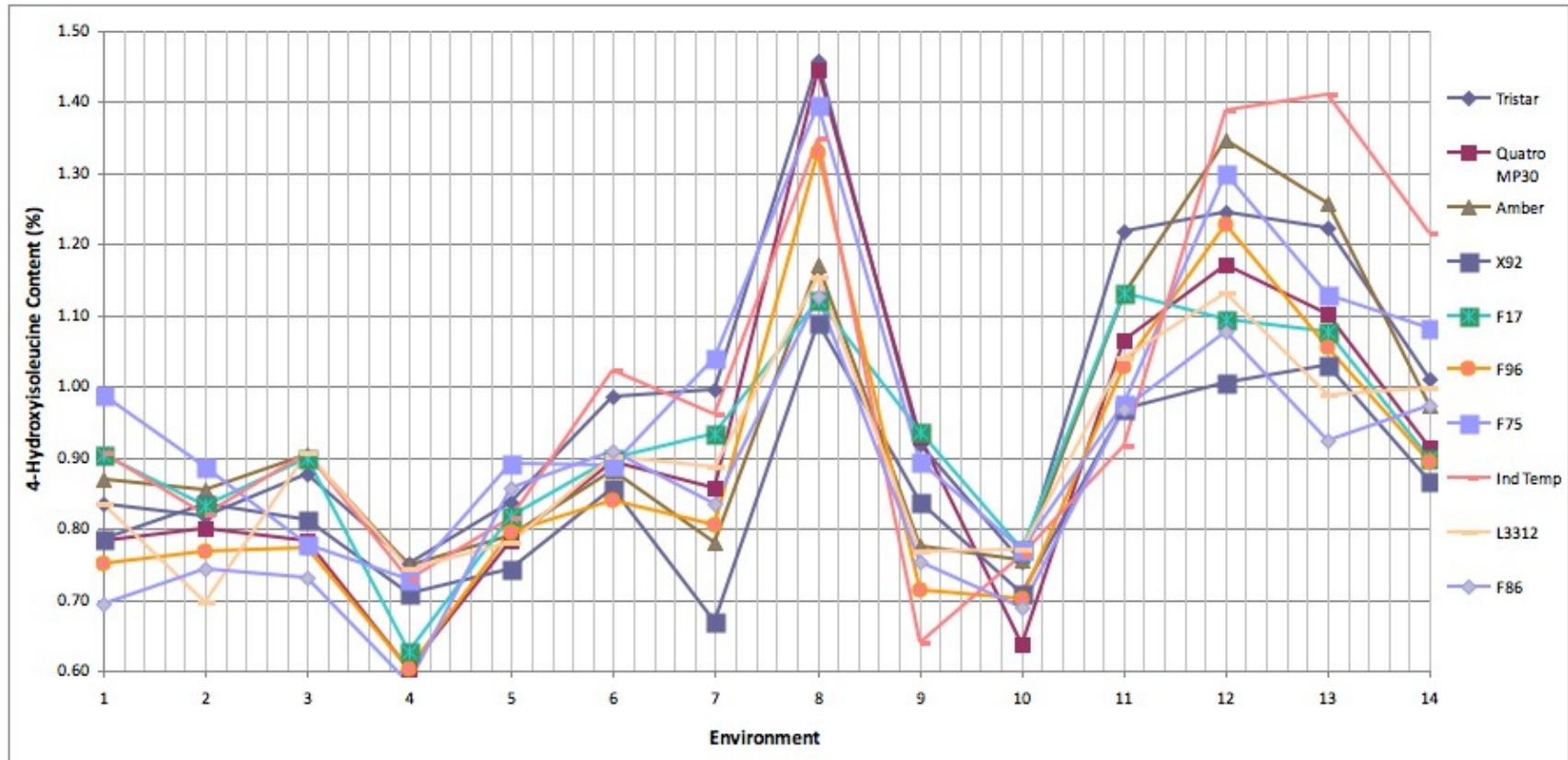
#### **4.1.2.7 4-Hydroxyisoleucine content**

The G x E interaction effect on 4-hydroxyisoleucine content (4-OH-ILE) accounted for 15 % of the total variation observed (Table 4.2). The interaction effect is graphically represented in Fig. 4.17. Even though growing environments are considered discrete variables, lines drawn connecting the data points are intended to exhibit the crossover interactions. The presence of an interaction between genotypes and the growing environments for seed 4-OH-ILE was indicated by the extensive change in genotype rankings (crossovers) across environments (Fig. 4.17; for numerical values see Appendix II). While crossovers for the other genotypes may not be clearly observed, Ind Temp can be seen to demonstrate poor stability, indicated by its significant change in ranking in terms of 4-OH-ILE (Fig 4.17).



**Figure 4.16** The "which won where" view of the GGE biplot for mean diosgenin productivity ( $\text{kg ha}^{-1}$ ) of 10 fenugreek genotypes tested across 14 environments.<sup>§</sup>

<sup>§</sup> PC 1 denotes Principal Component 1 and PC 2 denotes Principal Component 2. Genotypes are in blue, growing environments are numbered 1 to 14, in red.



**Figure 4.17** A scatter plot of 4-hydroxyisoleucine content (%) and growing environments (numbered 1 to 14) to illustrate the presence of genotype x environment interaction effect for 10 fenugreek genotypes tested across 14 environments in southern Alberta.<sup>8</sup>

<sup>8</sup> Environment 1 = BR-Irr-06; 2 = BR-Dry-06; 3 = BI-Irr-06; 4 = BI-Dry-06; 5 = LB1-Irr-06; 6 = LB1-Dry-06; 7 = BR-Irr-07; 8 = BR-Dry-07; 9 = BI-Irr-07; 10 = BI-Dry-07; 11 = LB1-Irr-07; 12 = LB1-Dry-07; 13 = LB2-Irr-07; 14 = LB2-Dry-07.

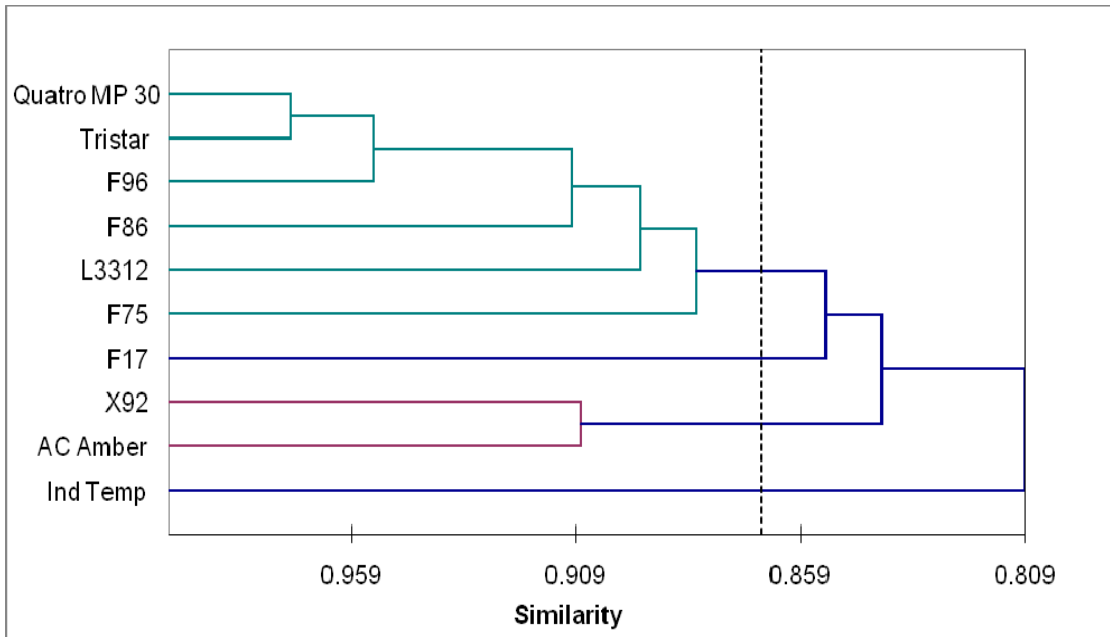
Note: Environments are discrete variables, lines drawn connecting the data points are intended to exhibit the crossover interactions.

Hierarchical cluster analysis performed on 4-OH-ILE identified that Quatro MP 30, Tristar, F96, F86 and L3312 have formed one cluster, while X92 and AC Amber have formed another cluster. F17 and Ind Temp were too different in their response patterns to be placed in the same cluster; hence each formed a cluster by itself (Fig. 4.18).

The GGE biplot analysis performed for genotypes and environments on 4-OH-ILE identifies Tristar, Quatro MP 30, X92 and Ind Temp as the most responsive genotypes, given their positions at the vertices of the polygon (Fig. 4.19). Tristar was the promising genotype in all environments positioned within its sector; this includes 5 (LB1-Irr-06), 2 (BR-Dry-06), 11 (LB1-Irr-07), 7 (BR-Irr-07) and 8 (BR-Dry-07). Similarly, Ind Temp was the winning genotype in all environments located within its sector, which includes 14 (LB2-Dry-07), 12 (LB1-Dry-07) and 13 (LB2-Irr-07), while Quatro MP 30 was identified as the winning genotype at 9 (BI-Irr-07), the only environment located within its sector. Because it is located at the largest distance from the biplot origin, Ind Temp was considered the most responsive, but was also the most unstable genotype, indicated by its high PC 2 score. In contrast, the F75 genotype with a comparable mean 4-OH-ILE to Ind Temp and Tristar had a relatively lower PC 2 score, indicating better stability (Fig 4.19).

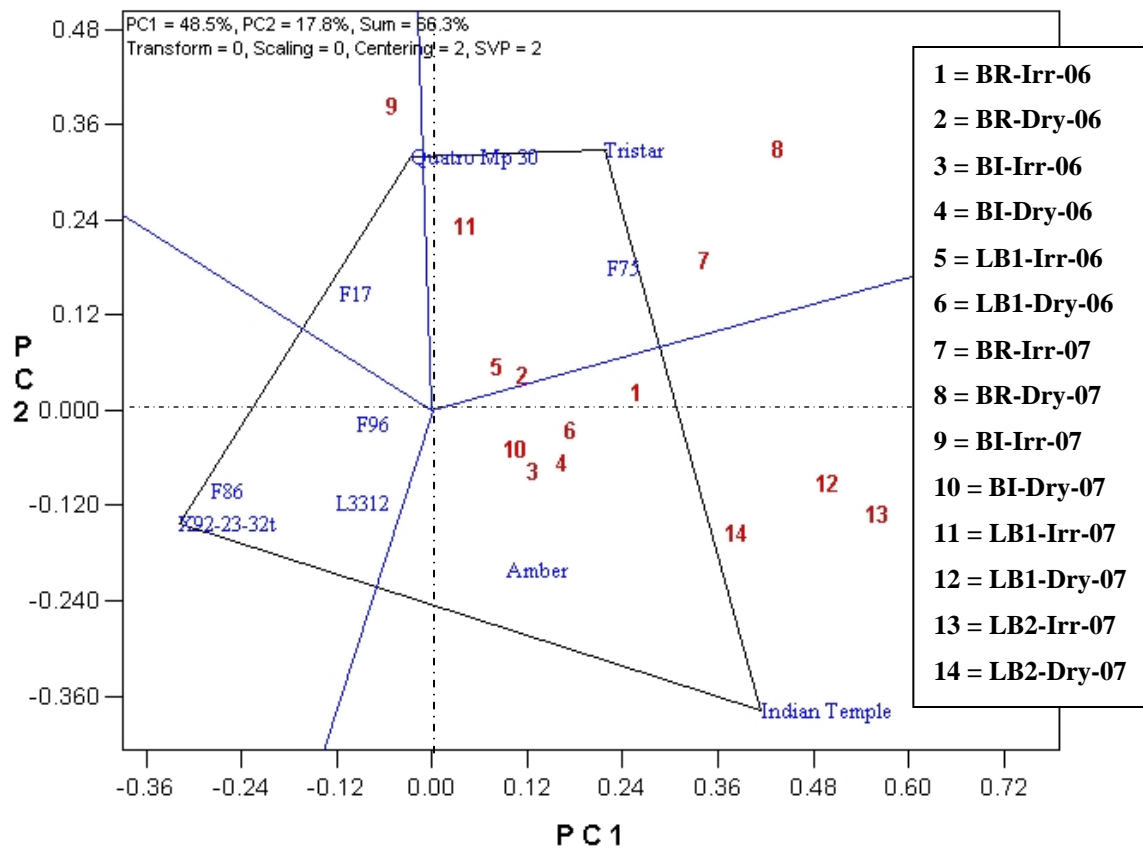
#### **4.1.2.8 4-Hydroxyisoleucine productivity**

Following the trend of the other productivity traits, the main effect of environment on 4-hydroxyisoleucine productivity (4-OH-ILE-P) accounted for 71 % of the total variation due to treatments, whereas only 12 % of the total variation was



**Figure 4.18 Dendrogram depicting the hierarchical clustering of 10 fenugreek genotypes tested in 14 environments based on their 4-hydroxyisoleucine content (%).<sup>§</sup>**

<sup>§</sup> Similarity of clusters is based on Pearson correlation coefficients; agglomeration of the clusters based on unweighted pair-group average. Similar color groups represent a distinct cluster. The dotted line represents the truncation point for clusters.



**Figure 4.19** The "which won where" view of the GGE biplot for mean 4-hydroxyisoleucine content (%) of 10 fenugreek genotypes tested across 14 environments.<sup>§</sup>

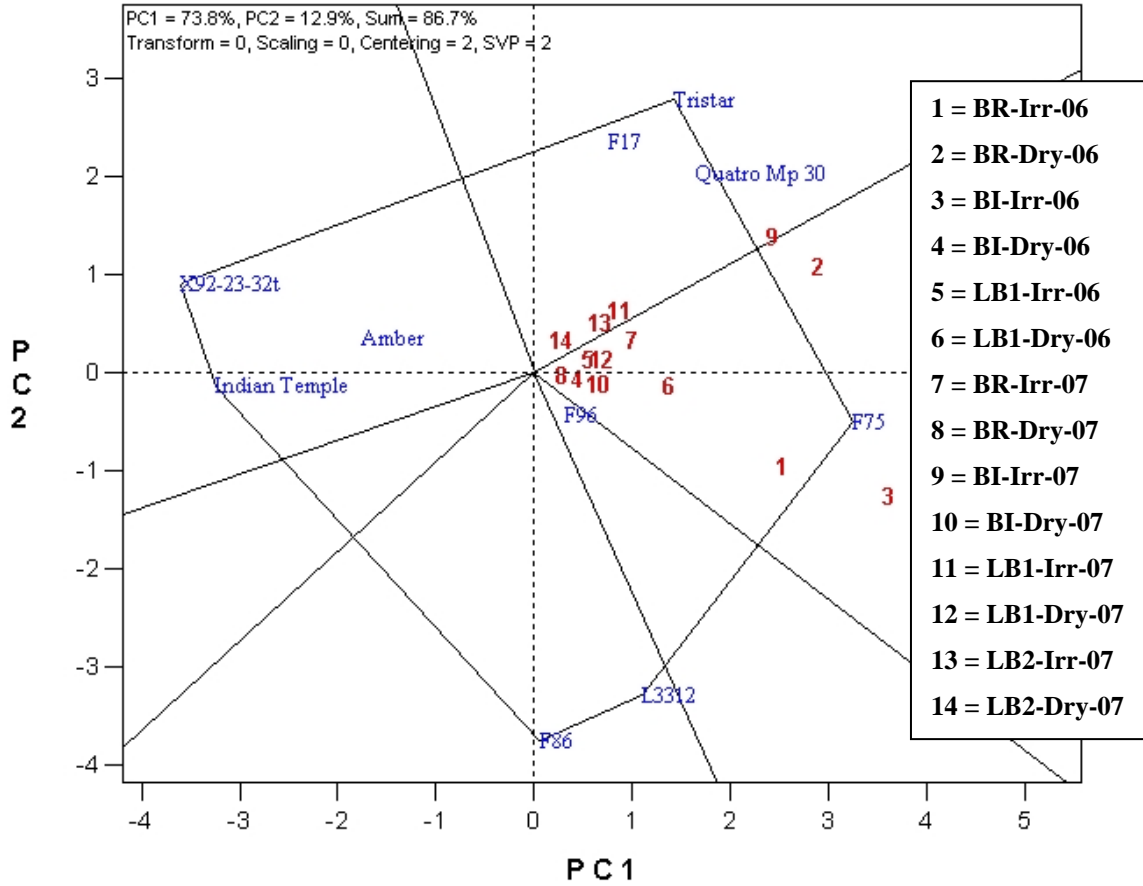
<sup>§</sup> PC 1 denotes Principal Component 1 and PC 2 denotes Principal Component 2. Genotypes are in blue, growing environments are numbered 1 to 14, in red.

contributed by genotype main effect (Table 4.2). The remaining 17 % of the variability was attributed to the G x E interaction effect. For numerical values representing the G x E interaction for 4-OH-ILE-P, see Appendix II.

The GGE biplot analysis for 4-OH-ILE-P on genotypes and environments identified Tristar, X92, Ind Temp, F86, L3312 and F75 as the most responsive genotypes, given their positions at the vertices of the polygon (Fig. 4.20). In terms of 4-OH-ILE-P, all environments except 1 (BR-Irr-06), 2 (BR-Dry-06), 3 (BI-Irr-06) and 9 (BI-Irr-07) were not discriminative of the genotypes tested as indicated by their close proximity to the biplot origin. F75 was the superior genotype for 4-OH-ILE-P in terms of 4-OH-ILE-P as well as stability, given its high PC 1 and low PC 2 (close to zero) scores.

## **4.2 Trait profiles of genotypes and the association of traits evaluated in the study**

Pearson correlation coefficient values (*r*-values) representing associations among the selected eight traits examined are given in Table 4.4. Results indicate a strong negative correlation between TSW and SY ( $r = -0.712$ ). On average, the DIOS in the seeds was shown to respond positively with TSW ( $r = 0.749$ ), and negatively with GLM ( $r = -0.698$ ). The productivity of the three biochemical traits, GLM-P, DIOS-P and 4-OH-ILE-P was positively and highly correlated to SY as indicated by their *r*-values; *i.e.*, 0.989, 0.700 and 0.960, respectively.



**Figure 4.20** The "which won where" view of the GGE biplot for mean 4-hydroxyisoleucine productivity ( $\text{kg ha}^{-1}$ ) of 10 fenugreek genotypes ested across 14 environments.<sup>§</sup>

<sup>§</sup> PC 1 denotes Principal Component 1 and PC 2 denotes Principal Component 2. Genotypes are in blue, growing environments are numbered 1 to 14, in red.

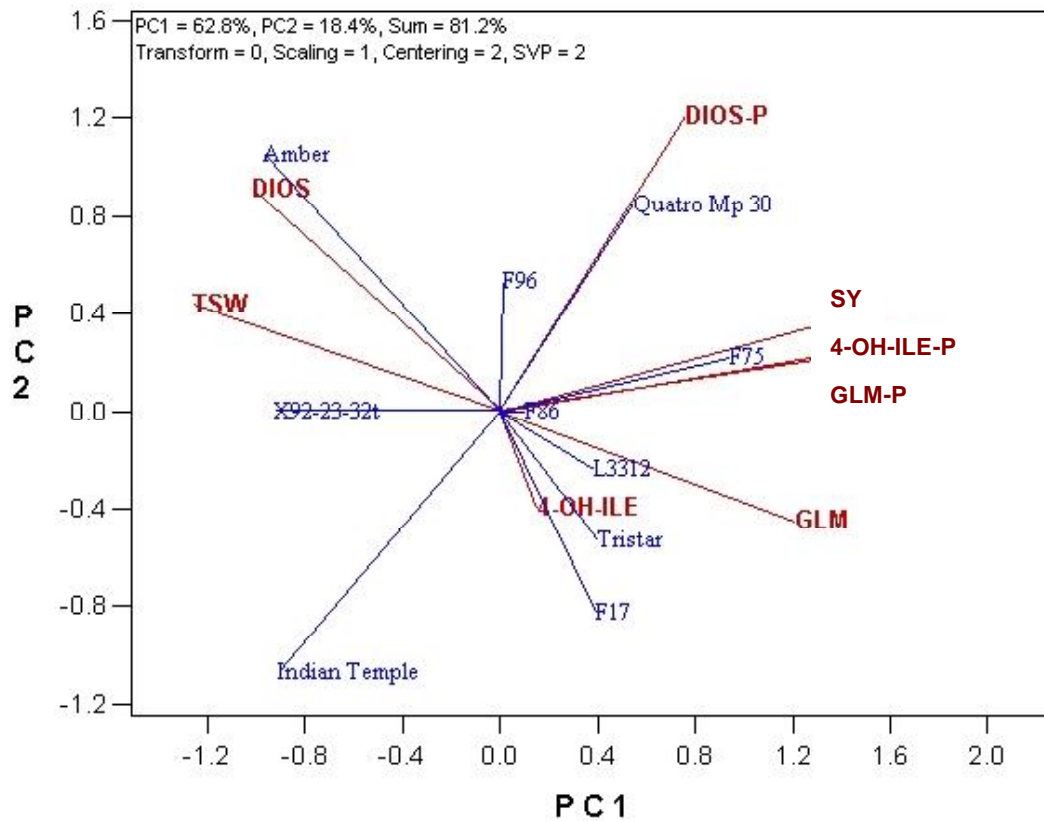


This association can also be observed visually in a biplot analysis; *i.e.* positive or negative associations can be visualized by looking at the angles between trait vectors. DIOS-P was positively correlated with the amount of diosgenin found in seeds (acute angle,  $\alpha < 90^\circ$ ) and negatively correlated with TSW (obtuse angle,  $\alpha > 90^\circ$ ) (Fig. 4.21). TSW and GLM were significantly and negatively correlated to each other (straight angle,  $\alpha \approx 180^\circ$ ). The angles between trait vectors for SY, GLM content and 4-OH-ILE-P were smaller than  $90^\circ$ , indicating a strong positive correlation among these traits (Figure 4.21). The associations among trait profiles (strengths and weaknesses) of genotypes are also indicated (Fig. 4.21). A close association between trait and genotype vectors was observed for genotype AC Amber and DIOS indicating that AC Amber was the best performing genotype for producing the steroid diosgenin. However, Quatro MP 30 was the best yielding genotype in terms of DIOS-P. On average, fenugreek genotype F75 was the best genotype in terms of SY, 4-OH-ILE-P and GLM-P. Tristar appeared to be the best genotype in terms of 4-OH-ILE. None of the genotypes tested was clearly indicated as being superior in terms of GLM (Fig. 4.21).

**Table 4.4 Correlation coefficient (*r*) values among means of 8 traits assessed in 10 fenugreek genotypes grown across 14 environments.**

	TSW	SY	GLM	GLM-P	DIOS	DIOS-P	4-OH-ILE
SY	<b>-0.712</b>						
GLM	<b>-0.850</b>	<b>0.658</b>					
GLM-P	<b>-0.760</b>	<b>0.989</b>	<b>0.753</b>				
DIOS	<b>0.749</b>	-0.563	<b>-0.698</b>	-0.619			
DIOS-P	-0.230	<b>0.700</b>	0.186	<b>0.640</b>	0.190		
4-OH-ILE	-0.092	0.009	0.039	0.014	-0.128	-0.064	
4-OH-ILE-P	<b>-0.740</b>	<b>0.960</b>	<b>0.709</b>	<b>0.964</b>	-0.574	<b>0.647</b>	0.252

§ TSW, thousand seed weight; SY, seed yield; GLM, galactomannan content; GLM-galactomannan productivity; DIOS, diosgenin content; DIOS-P, diosgenin productivity; 4-OH-ILE, 4-hydroxyisoleucine content; 4-OH-ILE-P, 4-hydroxyisoleucine productivity. Values in bold are significantly different from zero ( $p \leq 0.05$ ).



**Figure 4.21 Genotype x trait biplot for 10 fenugreek genotypes tested across 14 growing environments.<sup>8</sup>**

<sup>8</sup>Traits parameters are in uppercase and genotypes are in lowercase. PC 1 and PC 2 are primary and secondary effects, respectively. TSW = 1000-seed weight; SY = seed yield; GLM = galactomannan content; GLM-P = galactomannan productivity; DIOS = diosgenin content; DIOS-P = diosgenin productivity; 4-OH-ILE = 4-hydroxyisoleucine; 4-OH-ILE-P = 4-hydroxyisoleucine productivity.

## 5.0 Discussion

### 5.1 Thousand seed weight

Thousand seed weight (TSW), generally referred to as the average seed weight is an essential yield component trait in crop studies as it can assist producers in accounting for seed size variation (Ellis, 1991). The average seed weight in turn becomes useful to determine seeding rates and combine losses, and in some cases can help indicate seed quality (*i.e.*, plumpness or shrinkage) resulting from various treatments. Seed size and TSW can vary among species of the same crop plant, from year to year, as well as among growing environments. A simple scatter plot of TSW against growing environments provides a visual representation of the crossover interaction effect of genotype response (change in genotype rankings) when tested across varying environments. A larger seed weight is usually associated with larger seed and may extend benefits in terms of better seed survival, faster seedling growth and higher reproduction rates (Halpern, 2004). It was recently shown that concentrations of biochemical components such as isoflavones and phenolic compounds in large, medium and small-sized soybean seeds not only correlated with seed size but were also occasionally influenced by seed size x site interactions (Lee *et al.*, 2008). For this study, TSW and the production of galactomannan, diosgenin and 4-hydroxyisoleucine were expected to be correlated, as larger seeds generally carry out metabolism at greater rates, hence inherently become more equipped biochemically (Ellis, 1991; Grieve and Francois, 1992; Kidson and Westoby, 2000). Significant correlation coefficient values negatively associating TSW with galactomannan and positively with diosgenin provided strong evidence that a relationship

between seed size and these biochemical characteristics do exist. This suggests that levels of galactomannan tend to be lower in larger seeds while levels of diosgenin increase with increasing seed weight (or size). Galactomannan content may be lower in larger seeds due to a larger surface area to volume ratio as this structural compound is generally found deposited around the seed coat. On the contrary, diosgenin is mainly concentrated around the seed embryonic section, thus a larger seed with a larger embryo is expected to have greater diosgenin content. The dendrogram (Fig 4.1) shows the grouping of diosgenin content and TSW into one cluster indicating the relationship mentioned above; i.e. seeds with higher TSW (larger seeds), also had higher diosgenin content. However, this hypothesis needs to be further investigated.

The biplot analysis (Yan *et al.*, 2001) identifies genotype with high mean performance by large values of primary effects (PC 1) and stable genotypes by values close to zero for secondary effects (PC 2). In a similar way, the biplot analysis can also help discern the most discriminative growing environments among genotypes for high performance and stability. For TSW, AC Amber had the largest seed weight, represented by its high primary effects, but was relatively unstable compared to X92 and the low-performance genotypes, F17 and Tristar (all of which displayed small secondary effects). The genotype Indian Temple, although ranked third in average weight, was the most unstable indicating inconsistent performance. Perpendicular rays from the origin divided the biplot into 5 sectors, with the majority of the growing environments located outside the polygon near AC Amber, indicating a possible mega environment. Also, rainfed-growing environments (even numbered) predominantly occupied this sector, suggesting that high seed weight may correlate to low moisture conditions (stress environments).

## 5.2 Seed yield

The fenugreek genotypes selected for this study were previously adapted for growth in arid regions of Western Canada, and were bred for high seed yields and/or biomass production as well as for early maturity over the last decade. In recent years, a modern genomics approach for crop improvement has been widely explored (Varshney *et al.*, 2006, Vij and Tyagi, 2007; Tuberosa and Salvi, 2008) as these methods would allow for more precise crop modification over relatively shorter periods of time (Basu *et al.*, 2007). For fenugreek, Basu *et al.* (2008) adopted a mutation breeding approach using ethyl methane sulfonate to improve seed yield and plant growth habit of Tristar, one of the genotypes tested in the current study.

Yield stability is one the major concerns for crop breeders in multi-environmental trials (Piepho, 1999) because genotype x environment interactions often can be an impediment to stable crop production (Yang, 2007). In this study, significant genotype x environment interaction on seed yield was indicated in the change in genotype rankings across growing environments (Fig 4.6). Seed yield was found to be the predominant factor in the productivity of galactomannan ( $r = 0.99$ ), diosgenin ( $r = 0.70$ ) and 4-hydroxyisoleucine ( $r = 0.96$ ). Hierarchical clustering of the dependent variables; seed yield and all three compound productivity data for genotypes, grouped these traits together within a single cluster, indicating their similar response patterns. The dendrogram produced for seed yield using genotypic means grouped F75, Quatro MP 30, F96, L3312 and Tristar into one cluster. Based on performance means and the GGE biplot analysis, these same genotypes also had the best seed yields. In the biplot, F75 was shown as the most promising genotype, with Bow Island Irrigated 2006 as its niche environment.

Among the highest yielding genotypes, F75, L3312 and F96 were the most stable, as exhibited by their high primary effects and close to zero secondary effects (Fig. 4.8). Despite their low yields, X92 and Indian Temple were also stable genotypes, suggesting that they are potential parental materials in view that they were high in diosgenin and 4-hydroxyisoleucine contents, respectively. Bow Island-irrigated 2006 and Bow Island-irrigated 2007 were the most valuable in differentiating among genotypes in terms of seed yield; while Brooks-irrigated 2006 and Brooks-rainfed 2006 were ineffective due to their high secondary (PC 2 scores) effects. Both Bow Island-irrigated 2006 and Bow Island-irrigated 2007 were sites with favorable soil moisture conditions and consequently produced the highest seed yields among the growing environments tested.

### **5.3 Galactomannan productivity**

Galactomannans represent a major group of polysaccharide fiber in fenugreek seeds found concentrated in the seed coat surrounding the endosperm (Meier and Reid, 1977). Genetic variability of fenugreek for galactomannan content can range from 17 - 50 % of dry seed weight (Petropoulos, 1973 cited in Petropoulos, 2002; Meier and Reid, 1977; Duke, 1986; Kocchar *et al.*, 2006; Srichamroen *et al.*, 2008). Levels of galactomannan in seed could also vary according to biogeographic origins; *i.e.* fenugreek of Indian origin had higher galactomannan content compared to that from Ethiopia, and fenugreek from the Mediterranean area was generally inferior producers of the bioactive compound (Petropoulos, 1973 cited in Petropoulos, 2002). In this study, F75 the genotype of Afghan origin performed significantly better in terms of seed yield compared to the genotypes from Iran (F17) and India (Indian Temple).

Unique, relative to other commonly used galactomannans such as those found in guar and locust beans, fenugreek galactomannan contains a galactose to mannose ratio of 1:1 (Reid and Meier, 1970). The high degree of galactose substitution renders the molecule relatively hydrophilic (Brummer *et al.*, 2003), making galactomannan a good emulsifier and stabilizer in oil-water mixtures as well as a good thickener for use in foods (Slinkard, 2002). Apart from its industrial potential, the soluble nature of galactomannan fiber from fenugreek has been shown to exert anti-diabetic (Sharma, 1986b; Madar *et al.*, 1988) and hypocholesterolemic effects (Sharma, 1986a; Venkatesan *et al.*, 2003) by reducing plasma glucose levels (Madar and Shomer, 1990) and inhibiting bile salt absorption in the gut (Dakam *et al.*, 2007).

In the present study larger seeds were shown to accumulate lower amounts of galactomannan ( $r = -0.85$ ), and vice versa. Genotypes with lower average seed weight (smaller seeds) such as F75, Quatro MP 30 and L3312 had both high seed yields and galactomannan contents. The positions of these genotypes at polygon vertices in the biplot analysis (Fig. 4.12) also indicate their high responsiveness to environment niches. Among the best yielding genotypes, L3312 was identified as being the most stable. Genotypes F75, Quatro MP 30 and L3312 had the highest galactomannan productivity, and were shown to be both the best performing and most stable genotypes for this trait while Brooks-rainfed 2006 was the most discriminating environment. Interestingly, a significant negative correlation between diosgenin and galactomannan contents ( $r = -0.698$ ) was observed; this is also shown in the “genotype by trait” biplot (Fig 4.21) where DIOS and GLM are separated by an obtuse angle ( $\alpha > 90^\circ$ ). This suggests that



there is a trade-off relationship between these two compounds in the seed, since there was no one genotype that prevailed for both traits.

Environmental effects (E) accounted for 70 – 74 % of the total variation for galactomannan content (%) and its productivity ( $\text{kg ha}^{-1}$ ), while genotype (G) and G x E interaction effects accounted for only 10 - 11 % and 15 – 19 %, respectively. This indicates that environments have a greater influence on the production of these traits in fenugreek, and suggests that non-genetic factors are involved in crop performance (Yang and Baker, 1991; Gallacher, 1997). Under some circumstances, it may be more cost and time effective to propose improving agronomic systems rather than attempting genetic manipulations for the purpose of increasing crop performance for this trait (R.C. Yang\*, personal communication, 2008). However, since a significant positive correlation was observed between galactomannan productivity and both seed yield and galactomannan content, it is clear that productivity of this compound is reliant on the synergism of these traits. Therefore, it is imperative to use an extremely stable, high seed-yielding genotype such as F75 and another such as F17 that contains fairly high levels of galactomannan to as parents to develop fenugreek lines has both yield performance and stability.

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\* R.C. Yang is an Adjunct Professor of Statistical Genomics and Quantitative Genetics with the Department of Agricultural, Food and Nutritional Science at the University of Alberta (Edmonton, Alberta, Canada).

## 5.4 Diosgenin productivity

Steroidal sapogenins such as diosgenin are extensively used as raw materials by both the pharmaceutical and nutraceutical industries for the manufacture of steroidal drugs and medicinal extracts (Raghuram *et al.*, 1994; Skaltsa, 2002). Traditionally diosgenin has been extracted from wild Mexican and Asian yam species. However, with increasing demand for raw steroids, fenugreek seeds have been proposed as a viable alternative source of diosgenin due to the plant's shorter growing cycle, lower production costs and consistent quality (Fazli and Hardman, 1968 cited by Petropoulos, 2002). Significant variability in biochemical and physical attributes among fenugreek genotypes due to genotype x environment (G x E) interactions has been identified (Acharya *et al.*, 2006). Depending upon biogeographic origins, genotypes and environmental factors, reported diosgenin contents in fenugreek vary between 0.3 and 2.0 % (Fazli and Hardman, 1968; Puri *et al.*, 1976; Sharma and Kamal, 1982; Taylor *et al.*, 2002). Taylor *et al.* (2002) specifically evaluated the diosgenin contents of selected fenugreek genotypes grown in western Canada and found G x E interaction effects to be significant in explaining the variation observed. In the current study, AC Amber and X92 were identified as the best performing genotypes in terms of diosgenin content which is consistent with the findings reported by Taylor *et al.* (2002), where these two genotypes were found to contain the highest levels of diosgenin in seeds among the selected fenugreek genotypes that were grown in western Canada.

Genotypes used for this fenugreek study were previously selected based primarily on high seed and/or biomass production as well as early maturity. From this study, high diosgenin-producing genotypes such as AC Amber and X92 were identified, which can

be used to provide potential parental materials for future genotype development. A significant and positive association was observed between diosgenin content and average seed weight ( $r = 0.75$ ), while a negative correlation was observed between levels of diosgenin and galactomannan ( $r = -0.70$ ). This suggests that diosgenin levels in seeds tend to increase with increasing seed weight (or size) due to the presence of a larger embryonic area, where the complex precursors (furostanol glycosides) of sapogenins are concentrated in the seed (Skaltsa, 2002).

Hierarchical clustering of genotypes may provide a good indication of how similar the genotypes responded to varying growing conditions when tested across different environments. In terms of diosgenin content, the cluster analysis grouped F86 and Tristar in one cluster, and Indian Temple, X92, AC Amber and L3312 into another cluster. F96, F75, Quatro MP 30 and F17 were too different from each other to be grouped together into a single cluster. Such groupings, although not applicable at this point of the study, allow for better predictions of the performance of similarly clustered genotypes in future trials. The GGE biplot analysis clearly showed the superiority of AC Amber and X92 as the best performing genotypes, dominating at all environments except for Lethbridge (Fed)-rainfed 2007. X92 also demonstrated high stability, as indicated by its low absolute secondary (PC 2) effects. The potential for fenugreek to be used as a source of diosgenin is ultimately dependent on productivity of the compound; this study indicates that diosgenin productivity is directly related to seed yield. This observation is supported by the biplot analysis for diosgenin productivity where the high seed-yielding genotypes F75 and Quatro MP 30 occupied the far right vertices of the polygon, winning at Brooks-irrigated 2006 (1) and Bow Island-rain fed 2006 (3), and Brooks-rain fed 2006 (2) and

Bow Island-irrigated 2007 (9), respectively. Other tested environments were located near the biplot origin, and hence were less discriminative for genotypic contributions (Yan *et al.*, 2001).

Environmental effects accounted for only 6% of the total variation in diosgenin productivity. This strongly suggests the presence of significant genetic control for diosgenin production in fenugreek seeds and that perhaps one or a few major genes may influence this trait (R.C. Yang, personal communication, 2008). In this case, there is greater justification for genetic manipulation of genotypes for diosgenin productivity, which may be more effective when combined with agronomic improvements. In this study, a stable but low seed yielding genotype such as X92, which contains high levels of diosgenin, and high seed yielding genotypes such as F75 and Quatro MP 30 may be excellent parental material for the development of superior genotypes with high diosgenin content and productivity.

## **5.5 4-Hydroxyisoleucine productivity**

4-Hydroxyisoleucine is the most abundant free amino acid in fenugreek seeds (Sauvaire *et al.* 1984; Gupta *et al.*, 1998,). Evidence suggests that 4-hydroxyisoleucine has anti-diabetic (Petit *et al.*, 1995a; Sauvaire *et al.*, 1996; Broca *et al.*, 1999) and anti-obesity properties in animal studies (Handa *et al.*, 2005; Narender *et al.*, 2006). These findings are generating considerable interest in fenugreek by pharmaceutical and nutraceutical industries (Basch *et al.*, 2003, Acharya *et al.*, 2008;). Levels of fenugreek 4-hydroxyisoleucine content was also shown to vary according to the biogeographic origins

of the crop; *i.e.* Hajimehdipoor *et al.*, (2008) reported a 4-hydroxyisoleucine content of 0.4% in Iranian fenugreek seeds, while Narender *et al.* (2006) reported a 4-hydroxyisoleucine content of just 0.015 % in Indian fenugreek seeds. In this study, 4-hydroxyisoleucine contents among genotypes tested across different growing environments averaged ~ 1 %.

Although 4-hydroxyisoleucine is present in the seed endosperm (Al-Habori and Raman, 2002; Skaltsa, 2002), no relationship was found between its level in seed and the other trait parameters measured, except for 4-hydroxyisoleucine productivity ( $r = 0.65$ ). Mean 4-hydroxyisoleucine content among genotypes (RSD = 5.9 %) did not vary as much as that observed among environments (RSD = 18.9 %), indicating that environment contributed more to the variability observed. Tristar, F75 and Ind Temp were identified as the best performing genotypes in terms of 4-hydroxyisoleucine. Hierarchical clustering of genotypes based on 4-hydroxyisoleucine contents grouped Tristar, F75, Quatro MP 30, F96, F86 and L3312 together under one cluster, while AC Amber and X92 were grouped into another cluster. These groupings represent a good indication of how similar the genotypes responded (in terms of 4-hydroxyisoleucine content) to the varying growing conditions when tested across different environments. Consequently, genotypes Quatro MP 30, F96, F86 and L3312, which reported significantly lower means than Tristar and F75, are a poor choice for breeding material to increase 4-hydroxyisoleucine contents in fenugreek. Results from the hierarchical cluster analysis determined that Ind Temp too different in its response pattern to be clustered with other any of the other genotypes hence formed a cluster by itself. This was also observed in the biplot analysis for 4-hydroxyisoleucine content (Fig 4.19) where Ind Temp was shown to have the highest

primary effects (best yield) but also the highest secondary effects, indicating significant instability. Among the high performing genotypes, F75 with its close to zero secondary effects was determined to be the most stable. As with the other bioactive compounds evaluated, the potential of fenugreek as a source of 4-hydroxyisoleucine relies on the productivity of this compound. Seed yield and 4-hydroxyisoleucine productivity were strongly positively correlated ( $r = 0.96$ ), indicating that seed yield contributed significantly to productivity of this compound. The genotype Ind Temp, despite being very stable in terms of 4-hydroxyisoleucine productivity (low secondary effects), had low primary (PC 1) effects indicating low yield (Fig. 4.20). Consequently, this genotype is considered unsuitable for farm-scale commercialization. The complementary trait profiles of Ind Temp and F75 suggest that these genotypes are potential parental materials for increasing 4-hydroxyisoleucine contents in fenugreek cultivar development.

## **5.6 Crop productivity potential**

The genotypes chosen for this study were previously adapted for growth in semi-arid regions of western Canada, and were selected for high seed and/or forage yield as well as for early maturity. This trait profile is essential as the number of frost-free days in this region average only about 100 days (Acharya *et al.*, 2006). Therefore, these genotypes already represent an elite germplasm prior to the commencement of the study. To ensure a genetically diversified collection of starting materials, the genotypes were selected based on their various geographic origins. The generation of varying growing environments, manipulated by the combination of year x location x growing conditions

was crucial in providing a multi-environment platform for the selection of high performing genotypes with high stability.

This study aims to identify potential fenugreek genotypes with high levels of galactomannan, diosgenin and 4-hydroxyisoleucine productivity with stable yield. However, due to genetic variation and interaction with growing environments, it was not possible to identify any one genotype that prevailed for all traits. The GGE biplot presenting “which won what” for the 8 traits assessed in 10 fenugreek genotypes provides a visual display of the relationship of genotypes with each of the traits, as well as the relationship between traits (Fig. 4.21). Fenugreek genotypes AC Amber, Quatro MP 30, F75, F17 and Ind Temp were identified as the most responsive genotypes for all traits due to their locations at the vertices of the polygon. While AC Amber was the best genotype in terms of diosgenin content, Quatro MP 30 was superior in terms of diosgenin productivity. The most stable genotypes for 4-hydroxyisoleucine content were F17 and Tristar but productivity overall for 4-hydroxyisoleucine and galactomannan as well as seed yield was best in F75.

From this two-year study conducted on ten selected fenugreek genotypes tested across fourteen growing environments, several promising genotypes were identified as parental material for future breeding and selection efforts. As this fenugreek study focuses on selecting potential genotypes for the functional food and nutraceutical industry, genotypes which demonstrated high productivity of galactomannan (F75, Quatro MP 30 and L3312), diosgenin (Quatro MP 30 and F75) and 4-hydroxyisoleucine (F75) are the most useful for future crop improvement efforts. The genotype, F75 is of Afghan origin and demonstrated superior stability and high performance for all yield-

related traits. Genotypes such as Tristar and L3312 were originally selected as lines for forage purposes meant for the animal feed industry. However, this study managed to identify new functionalities for these genotypes, extending their trait profiles. The zero-tannin X92 genotype is a unique experimental line that has a cream-colored seed coat. This phenotype may be desirable in large-scale processing to create a light-colored product, which may have a greater consumer appeal. Also, despite its low seed yield, X92 reported excellent stability and performance for diosgenin content. Low yield performance is often associated with stability (static stability) as demonstrated by X92; due the lack of space for fluctuations (Lin *et al.*, 1986). It would be worthwhile to explore the genetic basis for such stability as well as the underpinning chemistry for the trade-off between tannin and diosgenin. Despite demonstrating environmental instability, Ind Temp reported one of the highest mean 4-hydroxyisoleucine contents (1.46 %), and was on average among the best performing genotypes for this trait. Therefore, this genotype may provide suitable genetic material for the development of superior fenugreek lines with high 4-hydroxyisoleucine productivity.



## 6.0 Future prospects

Fenugreek has been commercially grown in western Canada for only 16 years, dating back to 1992 with the release of the first cultivar “AC Amber” by Agriculture and Agri-Food Canada (AAFC) in Morden, Manitoba. Although the fenugreek crop has traditionally been grown for seed to supply the spice industry, fenugreek cultivars have been developed and released in western Canada since the release of “AC Amber” for specific purposes; *i.e.* CDC Canafen used mainly for galactomannan extraction, and CDC Canagreen and Tristar grown mainly for forage use. However, primary selection criteria of these newly developed cultivars were based on high seed and/or forage yield as well as for early maturity.

With accumulation of more experimental evidence in support of the nutraceutical properties of fenugreek, there is growing interest in marketing fenugreek as a natural health product in Canada. Therefore, there is a need to evaluate the biochemical productivity of fenugreek to allow for selection of suitable genotypes that may be further developed into cultivars specific for the natural health product industry. In this study, the productivity of three bioactive compounds (galactomannan, diosgenin and 4-hydroxyisoleucine) was assessed by testing 10 fenugreek genotypes across 14 growing environments (year x location x growing condition).

Yield stability for crops is usually hampered by genotype x environment interaction effects, causing genotypes to respond differently when grown at different locations and even in different years. In view of this counter-productive phenomenon and the increasing need for more cost-effective cultivar testing, crop improvement programs

require a multi-environmental design with greater focus on identifying yield-stable genotypes with satisfactory performance across homogenous growing environments.

In this study, genotype, environment and their interaction effects were significant for all trait parameters evaluated. Environmental effects accounted for more than 60 % of the total variation observed for each trait, with the exception for diosgenin content. The high variance proportion explained by environmental effects indicates a greater influence of environmental factors on the productivity of these traits. In this case, under some circumstances, it may be more cost and time-effective to improve trait performances in genotypes by embracing an agronomic approach (enhancing field-related systems; *i.e.* available moisture, crop row-spacing, fertilizer application). However, in situations where genetic factors are dominant, such as the case observed for diosgenin content (environmental effects accounted for only 6 % of the total variance), it may be worthwhile exploring a molecular genetics approach (identification of contributing genes) combined with agronomic improvements to increase crop performance.

Productivity of the bioactive compounds was evaluated based not only on high mean performance but also productivity stability (Table 6.1). Genotypes that demonstrated the least genotype x environment interaction were the most stable and are desirable in crop improvement programs; *i.e.* F75 that had high seed yield, galactomannan content and productivity. However, genotypes that were high performing on average, but were unstable across environments could be equally useful as sources of unique genes for future crossbreeding efforts; *i.e.* Ind Temp which had a high 4-hydroxyisoleucine content. Likewise, genotypes that have high stability and high

performance, but low productivity due to low seed yield may also serve as good parental material for future cultivar development; *i.e.* X92 which had a high diosgenin content.

Homogenous growing environments that have similar biotic and abiotic stresses can be termed, “mega environments” when they produce similar genotype rankings. These are also useful for selecting and/or discriminating among responsive genotypes. Mega environment studies would be useful in the long run to identify similarly contributing environments. This would allow us to recommend cultivars according to their environmental profiles, and from a more academic perspective, to avoid duplicating test locations for future breeding studies. This fenugreek study is based on a two-year data set and hence, is not adequate for decisive identification of mega environments (may require at least three years to identify); genotype rankings must be repeatable on average for three consecutive years (Yan and Kang, 2003).

There is growing interest in the medicinal properties of fenugreek due to contributing science-based evidence implicating some of the principal biochemical components likely responsible for its health effects. However, most clinical researchers studying the effects of fenugreek do not consider the genetic and environmental response variability of the crop. Biotic and abiotic stresses may exert pressure on growing plants and cause variation in their chemical and physical properties. Occasionally, contradicting drug efficacy results from different clinical studies using allegedly similar testing material may be attributed to these variations. Attention given to this aspect would lead to better agreeability and could lead to dosage standardization among clinical trials.

**Table 6.1 Clusters of fenugreek genotypes based on the magnitude and stability of agronomic and biochemical traits evaluated in this study.**

<b>Agronomical/ biochemical trait</b>	<b>Magnitude of performance</b>	<b>Stability of performance</b>
Thousand seed weight	AC Amber	X92-23-32T
Seed yield	F75, Quatro MP 30, L3312, F96	F75, F96, L3312
Galactomannan content	F75, F17, Quatro MP 30, L3312	Tristar, F17, F96
Galactomannan productivity	F75, Quatro MP 30	Tristar, Quatro MP 30, F96, L3312, F75
Diosgenin content	AC Amber, X92-23-32T	X92-23-32T
Diosgenin productivity	Quatro MP 30	X92-23-32T, F96, Indian Temple, F17
4-Hydroxyisoleucine content	Indian Temple, Tristar, F75, AC Amber	F96
4-Hydroxyisoleucine productivity	F75, Quatro MP 30, Tristar	Indian Temple, F96, AC Amber, F75

Through this study, we now have a better understanding of the trait profiles of these ten genotypes that were previously selected in southern Alberta mostly on the basis of high seed yield and early maturity traits. The multi-environment design in this study in which fenugreek genotypes were tested across 14 different growing environments was essential in evaluating their stability in yield performance. Assessment of the genotype (G) and environment (E) main effects as well as the G x E interaction effects provided valuable information on the relative contribution of these effects on the traits evaluated in this study. In terms of diosgenin content, it is unusual in crop trials that environmental effects contributed only 6 % towards the total variation observed for this trait. This will provide a strong basis for future studies of the genetic basis of diosgenin production in fenugreek. Determination of the bioactive compound content in the ten fenugreek genotypes enabled selection of those that were productive in one or more of the biochemical components studied. Stable genotypes, *i.e.* those with the least genotype x environment interaction, were also identified and can be further selected with high yielding genotypes for future crop improvement studies. The preliminary identification of the potential genotypes (Table 6.1) in this study represents a vital progressive step towards the selection and subsequent recommendation of fenugreek cultivars as a source of natural health products to producers and processors in Alberta.

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## Appendix I

**Mean maximum and minimum temperatures at the test sites from May to September in 2006 and 2007.**

	Mean maximum/ minimum temperatures (°C)				
Test sites	May	June	July	August	September
2006 Brooks	20.0 / 5.7	24.0/ 10.2	29.8/ 12.1	27.1/ 8.9	22.8/ 5.0
2006 Bow Island	20.5 / 6.4	23.6/ 10.8	29.5/ 12.8	27.4/ 9.7	21.1/ 5.7
2006 Lethbridge	30.9/ -4.6	30.9/ 5.4	32.4/ 7.5	33.1/ 3.9	30.0/ -3.2
2007 Brooks	19.4 / 4.6	24.0/ 8.6	31.1/ 13.2	25.4/ 9.1	19.3/ 3.9
2007 Bow Island	19.2/ 5.7	23.9/ 9.8	31.3/ 14.2	26.7/ 10.1	19.6/ 4.3
2007 Lethbridge	26.7/ 0.3	27.8/ 5.1	34.4/ 6.4	32.7/ 2.3	28.9/ -2.1

**Source:** Irrigation Management Climate Information Network (IMCIN), 2008.

## **Appendix II**

Numerical Values for Productivity of the Ten Fenugreek Genotypes Grown in Different  
Environments across Southern Alberta

Mean thousand seed weight (g) of ten fenugreek genotypes grown at fourteen environments in southern Alberta.

Genotype	Environment													
	BR-Irr-06	BR-Dry-06	BI-Irr-06	BI-Dry-06	LB1-Irr-06	LB1-Dry-06	BR-Irr-07	BR-Dry-07	BI-Irr-07	BI-Dry-07	LB1-Irr-07	LB1-Dry-07	LB2-Irr-07	LB2-Dry-07
Thousand seed weight (g)														
<b>Tristar</b>	14.3	16.8	15.0	11.9	13.9	16.5	11.9	12.3	16.0	11.5	17.6	11.3	12.0	10.2
<b>Quatro</b>	14.1	15.7	14.3	10.9	14.1	15.6	10.3	12.3	18.1	9.7	17.3	10.2	12.1	9.2
<b>MP 30</b>	15.6	22.0	14.0	17.3	16.5	21.6	14.6	15.6	19.6	15.8	23.6	16.6	17.8	15.1
<b>AC</b>	12.2	15.7	13.1	12.4	13.1	16.1	10.7	12.3	16.0	11.5	16.4	10.7	12.0	9.6
<b>Amber</b>	15.2	17.9	16.3	12.5	15.1	17.6	11.8	13.2	19.0	12.7	20.9	12.7	14.0	12.0
<b>F17</b>	14.9	15.3	14.3	11.1	12.1	15.4	10.9	12.2	16.8	9.7	16.5	9.7	11.5	9.5
<b>F96</b>	14.2	19.4	13.4	17.4	15.2	19.2	12.4	12.1	18.7	16.2	19.1	13.4	16.8	12.5
<b>F75</b>	17.8	18.8	15.9	14.7	14.5	17.5	14.1	14.4	17.0	12.2	15.8	13.6	15.5	12.7
<b>X92</b>	14.1	17.2	18.7	14.1	15.5	17.4	11.5	12.7	17.8	11.8	18.9	12.2	12.3	11.2
<b>Ind Temp</b>	15.1	15.7	15.3	12.6	14.6	17.3	10.3	11.8	17.1	11.9	18.4	11.5	11.7	12.1
<b>L3312</b>														
<b>F86</b>														

Mean seed yield (kg ha<sup>-1</sup>) of ten fenugreek genotypes grown at fourteen environments in southern Alberta.

Genotype	Environment													
	BR-Irr-06	BR-Dry-06	BI-Irr-06	BI-Dry-06	LB1-Irr-06	LB1-Dry-06	BR-Irr-07	BR-Dry-07	BI-Irr-07	BI-Dry-07	LB1-Irr-07	LB1-Dry-07	LB2-Irr-07	LB2-Dry-07
	Seed yield (kg ha <sup>-1</sup> )													
<b>Tristar</b>	2618	3584	2629	1448	681	1319	1264	378	3037	1492	1093	698	1163	802
<b>Quatro</b>	2839	3634	3286	1658	918	1806	1382	434	2856	1329	1256	698	1359	808
<b>MP 30</b>	1696	2575	1620	1705	848	1110	1089	343	1906	1443	391	468	840	735
<b>AC</b>	2693	4040	1905	1670	979	1475	1397	248	2079	1511	969	729	1214	662
<b>Amber</b>	3264	3511	2637	1806	883	1693	1053	419	2533	1572	773	618	1203	671
<b>F17</b>	3443	3852	3847	1813	994	1969	1288	314	2766	1481	944	613	1394	678
<b>F96</b>	1912	1958	527	1377	364	1119	838	426	1253	955	802	545	1146	703
<b>F75</b>	1645	1569	1006	1180	734	756	962	175	1801	1093	746	328	871	558
<b>X92</b>	3146	3619	3210	1788	543	1713	1248	351	1892	1570	989	703	1140	776
<b>Ind</b>	3965	2677	3404	1540	780	1424	1050	562	1968	1454	768	642	1100	323
<b>Temp</b>														
<b>L3312</b>														
<b>F86</b>														



Mean galactomannan content (%) of ten fenugreek genotypes grown at fourteen environments in southern Alberta.

Genotype	Environment													
	BR-Irr-06	BR-Dry-06	BI-Irr-06	BI-Dry-06	LB1-Irr-06	LB1-Dry-06	BR-Irr-07	BR-Dry-07	BI-Irr-07	BI-Dry-07	LB1-Irr-07	LB1-Dry-07	LB2-Irr-07	LB2-Dry-07
Galactomannan content (%)														
<b>Tristar</b>	20.6	17.9	19.3	22.5	15.3	17.3	12.9	13.1	16.2	16.9	15.5	14.3	15.6	15.3
<b>Quatro</b>	19.3	19.0	19.1	21.2	15.7	15.4	12.4	12.0	16.9	17.1	17.4	16.7	13.7	15.6
<b>MP 30</b>	17.3	18.1	12.2	19.8	13.4	16.2	10.8	11.0	16.1	16.1	14.6	12.7	13.3	13.2
<b>AC</b>	15.6	19.8	20.0	23.2	17.7	17.9	14.0	11.9	17.0	17.1	16.6	15.2	14.9	15.0
<b>Amber</b>	17.4	15.6	16.8	22.3	16.0	17.9	12.9	11.3	16.0	16.1	16.0	13.4	13.9	13.1
<b>F17</b>	16.4	19.1	17.6	23.5	16.6	20.0	15.7	14.3	15.7	19.9	16.6	17.0	16.6	17.4
<b>F96</b>	17.8	16.5	16.0	21.4	16.4	15.4	14.6	13.1	15.6	16.9	16.8	15.1	13.8	16.0
<b>F75</b>	15.6	16.9	17.7	21.0	15.3	15.8	13.6	13.9	14.6	14.5	15.8	16.9	11.8	14.0
<b>X92</b>	17.2	21.5	20.7	21.3	15.9	16.9	15.3	13.7	16.6	17.5	16.3	16.0	15.5	15.3
<b>Ind</b>	17.1	18.0	19.7	19.2	12.8	17.2	13.3	13.9	16.3	17.3	16.4	16.2	15.7	14.8
<b>Temp</b>														
<b>L3312</b>														
<b>F86</b>														

Mean galactomannan productivity (kg ha<sup>-1</sup>) of ten fenugreek genotypes grown at fourteen environments in southern Alberta.

Genotype	Environments													
	BR-Irr-06	BR-Dry-06	BI-Irr-06	BI-Dry-06	LB1-Irr-06	LB1-Dry-06	BR-Irr-07	BR-Dry-07	BI-Irr-07	BI-Dry-07	LB1-Irr-07	LB1-Dry-07	LB2-Irr-07	LB2-Dry-07
Galactomannan productivity (kg ha <sup>-1</sup> )														
<b>Tristar</b>	540.2	642.8	506.3	325.9	104.5	227.8	163.6	49.5	491.5	252.3	168.9	100.0	181.5	122.8
<b>Quatro</b>	548.3	689.5	628.6	351.1	144.3	277.7	171.4	52.1	484.0	226.7	218.2	116.4	186.5	125.8
<b>MP 30</b>	293.5	465.8	197.3	337.5	113.3	179.6	117.4	37.8	306.6	231.6	57.0	59.6	111.8	96.8
<b>AC</b>	420.0	799.6	381.9	387.1	173.6	264.4	195.0	29.6	352.4	258.1	161.1	110.8	180.7	99.2
<b>Amber</b>	567.5	549.3	442.3	403.3	141.6	303.4	136.1	47.4	406.1	253.1	123.6	83.0	167.2	87.8
<b>F17</b>	565.8	736.6	676.3	425.3	164.4	393.8	201.6	45.0	433.7	294.6	157.0	104.1	231.3	117.6
<b>F96</b>	341.0	322.2	84.6	294.8	59.8	172.1	122.7	56.0	194.9	161.5	134.5	82.5	158.0	112.3
<b>Ind</b>	255.9	265.3	178.3	247.3	112.5	119.5	130.7	24.3	263.6	158.8	117.7	55.3	102.7	78.2
<b>Temp</b>	542.0	779.3	663.0	380.7	86.5	290.2	190.7	48.1	314.9	274.0	161.5	112.6	177.2	118.7
<b>L3312</b>	678.4	481.3	669.8	296.0	100.2	244.4	140.1	78.2	321.7	252.0	126.1	104.2	172.8	47.9
<b>F86</b>														

Mean diosgenin content (%) of ten fenugreek genotypes grown at fourteen environments in southern Alberta.

Genotype	Environments													
	BR-Irr-06	BR-Dry-06	BI-Irr-06	BI-Dry-06	LB1-Irr-06	LB1-Dry-06	BR-Irr-07	BR-Dry-07	BI-Irr-07	BI-Dry-07	LB1-Irr-07	LB1-Dry-07	LB2-Irr-07	LB2-Dry-07
	Diosgenin Content (%)													
<b>Tristar</b>	0.50	0.57	0.52	0.61	0.55	0.56	0.54	0.49	0.51	0.48	0.51	0.51	0.52	0.51
<b>Quatro</b>	0.61	0.71	0.70	0.65	0.69	0.68	0.64	0.66	0.69	0.63	0.71	0.65	0.67	0.59
<b>MP 30</b>	0.74	0.86	0.70	0.81	0.82	0.91	0.82	0.76	0.90	0.80	0.85	0.80	0.79	0.78
<b>AC</b>	0.41	0.45	0.48	0.54	0.51	0.50	0.49	0.54	0.53	0.53	0.48	0.53	0.50	0.47
<b>Amber</b>	0.56	0.59	0.57	0.69	0.61	0.72	0.55	0.62	0.61	0.57	0.61	0.58	0.59	0.62
<b>F17</b>	0.60	0.59	0.62	0.63	0.59	0.64	0.60	0.58	0.53	0.62	0.59	0.57	0.59	0.57
<b>F96</b>	0.57	0.64	0.56	0.65	0.59	0.69	0.59	0.60	0.60	0.65	0.67	0.64	0.63	0.58
<b>F75</b>	0.71	0.83	0.68	0.81	0.76	0.85	0.68	0.68	0.73	0.82	0.78	0.70	0.74	0.68
<b>Ind</b>	0.48	0.55	0.51	0.57	0.54	0.54	0.52	0.51	0.53	0.54	0.54	0.54	0.55	0.54
<b>Temp</b>	0.55	0.62	0.53	0.67	0.58	0.60	0.51	0.54	0.54	0.56	0.56	0.54	0.58	0.57
<b>X92</b>														
<b>L3312</b>														
<b>F86</b>														

Mean diosgenin productivity (kg ha<sup>-1</sup>) of ten fenugreek genotypes grown at fourteen environments in southern Alberta.

Genotype	Environments													
	BR-Irr-06	BR-Dry-06	BI-Irr-06	BI-Dry-06	LB1-Irr-06	LB1-Dry-06	BR-Irr-07	BR-Dry-07	BI-Irr-07	BI-Dry-07	LB1-Irr-07	LB1-Dry-07	LB2-Irr-07	LB2-Dry-07
	Diosgenin Productivity (kg ha <sup>-1</sup> )													
<b>Tristar</b>	13.1	20.5	13.6	8.8	3.7	7.4	6.8	1.8	15.6	7.1	5.6	3.5	6.0	4.1
<b>Quatro</b>	17.4	25.7	23.0	10.8	6.4	12.2	8.9	2.8	19.6	8.4	8.9	4.5	9.1	4.8
<b>MP 30</b>	12.5	22.2	11.3	13.9	7.0	10.1	8.9	2.6	17.2	11.6	3.3	3.8	6.6	5.7
<b>AC</b>	11.1	18.4	9.2	9.0	5.0	7.4	6.9	1.3	11.0	8.0	4.7	3.9	6.1	3.1
<b>Amber</b>	18.3	20.7	15.0	12.4	5.4	12.2	5.8	2.6	15.4	9.0	4.7	3.6	7.1	4.2
<b>F17</b>	20.8	22.7	23.8	11.4	5.8	12.6	7.7	1.8	14.8	9.2	5.6	3.5	8.3	3.9
<b>F96</b>	13.6	16.2	3.6	11.2	2.8	9.5	5.7	2.9	9.1	7.8	6.2	3.8	8.5	4.8
<b>F75</b>	9.4	10.0	5.6	7.7	4.3	5.2	5.7	1.0	10.9	7.1	5.0	2.1	5.5	3.2
<b>X92</b>	15.2	19.9	16.3	10.1	3.0	9.3	6.5	1.8	10.1	8.5	5.4	3.8	6.3	4.2
<b>Ind Temp</b>	21.9	16.5	18.1	10.3	4.5	8.5	5.3	3.0	10.6	8.2	4.3	3.5	6.4	1.8
<b>L3312</b>														
<b>F86</b>														

Mean 4-hydroxyisoleucine content (%) of ten fenugreek genotypes grown at fourteen environments in southern Alberta.

Genotype	Environment													
	BR-Irr-06	BR-Dry-06	BI-Irr-06	BI-Dry-06	LB1-Irr-06	LB1-Dry-06	BR-Irr-07	BR-Dry-07	BI-Irr-07	BI-Dry-07	LB1-Irr-07	LB1-Dry-07	LB2-Irr-07	LB2-Dry-07
4-Hydroxyisoleucine Content (%)														
<b>Tristar</b>	0.83	0.82	0.88	0.75	0.84	0.99	1.00	1.46	0.92	0.75	1.22	1.24	1.22	1.01
<b>Quatro</b>	0.78	0.80	0.78	0.60	0.78	0.89	0.86	1.45	0.93	0.64	1.06	1.17	1.10	0.91
<b>MP 30</b>	0.87	0.86	0.90	0.75	0.79	0.88	0.78	1.17	0.78	0.76	1.13	1.35	1.26	0.97
<b>AC</b>	0.90	0.83	0.90	0.63	0.82	0.90	0.93	1.12	0.94	0.77	1.13	1.10	1.08	0.90
<b>Amber</b>	0.75	0.77	0.77	0.60	0.79	0.84	0.80	1.33	0.71	0.70	1.03	1.23	1.06	0.90
<b>F17</b>	0.99	0.89	0.78	0.73	0.89	0.89	1.04	1.40	0.89	0.77	0.98	1.30	1.13	1.08
<b>F96</b>	0.79	0.84	0.81	0.71	0.74	0.86	0.67	1.09	0.84	0.71	0.97	1.01	1.03	0.87
<b>F75</b>	0.91	0.82	0.91	0.73	0.82	1.02	0.96	1.35	0.64	0.76	0.92	1.39	1.41	1.22
<b>X92</b>	0.83	0.70	0.91	0.74	0.78	0.90	0.89	1.15	0.77	0.77	1.04	1.13	0.99	1.00
<b>Ind</b>	0.69	0.74	0.73	0.58	0.86	0.91	0.83	1.13	0.75	0.69	0.97	1.08	0.92	0.97
<b>Temp</b>														
<b>L3312</b>														
<b>F86</b>														

Mean 4-hydroxyisoleucine productivity (kg ha<sup>-1</sup>) of ten fenugreek genotypes grown at fourteen environments in southern Alberta.

Genotype	Environment													
	BR-Irr-06	BR-Dry-06	BI-Irr-06	BI-Dry-06	LBI-Irr-06	LBI-Dry-06	BR-Irr-07	BR-Dry-07	BI-Irr-07	BI-Dry-07	LBI-Irr-07	LBI-Dry-07	LB2-Irr-07	LB2-Dry-07
4-Hydroxyisoleucine Productivity (kg ha <sup>-1</sup> )														
<b>Tristar</b>	23.0	30.1	23.6	11.4	6.0	13.7	12.6	5.5	27.9	11.2	13.3	8.7	14.2	8.1
<b>Quatro</b>	23.4	29.8	26.3	10.5	7.6	17.0	11.9	6.3	26.6	8.5	13.4	8.2	15.0	7.4
<b>MP 30</b>	15.5	22.6	15.2	13.4	7.1	10.3	8.5	4.0	14.8	10.9	4.4	6.3	10.6	7.2
<b>AC</b>	25.6	34.5	17.6	11.0	8.4	14.0	13.0	2.8	19.5	11.6	11.0	8.0	13.1	6.0
<b>Amber</b>	25.8	27.6	20.9	11.5	7.4	15.0	8.5	5.6	18.1	11.0	7.9	7.6	12.7	6.0
<b>F17</b>	35.8	35.0	30.7	13.9	9.3	18.4	13.4	4.4	24.7	11.4	9.2	8.0	15.7	7.3
<b>F96</b>	15.8	16.8	4.4	10.3	2.9	10.1	5.6	4.6	10.5	6.8	7.8	5.5	11.8	6.1
<b>F75</b>	15.7	13.2	9.3	9.0	6.3	8.1	9.2	2.4	11.5	8.4	6.8	4.6	12.3	6.8
<b>X92</b>	27.6	25.8	29.8	14.0	4.5	16.2	11.1	4.0	14.5	12.1	10.3	8.0	11.3	7.7
<b>Ind Temp</b>	28.9	20.4	26.2	9.5	7.0	13.6	8.8	6.3	14.8	10.0	7.4	6.9	10.2	3.1
<b>L3312</b>														
<b>F86</b>														