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Seed production technology for fenugreek (Trigonella foenum-graecum L.) in the Canadian prairies

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SEED PRODUCTION TECHNOLOGY FOR FENUGREEK (*Trigonella foenum-graecum* L.) IN THE CANADIAN PRAIRIES

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DEDICATION

This work is dedicated to the memories of my beloved grandmother Ms. Radharani Basu, who passed away in India while I was doing this research work in Lethbridge, AB Canada. Until her death in February, 2005 she was a constant source of love, inspiration and encouragement for me. Although she did not have formal education she always took great interest in my research work and showered me with her blessings whenever I needed them most.

ABSTRACT

Fenugreek (*Trigonella foenum-graecum* L.) is an annual legume mainly used as a spice crop in many parts of the world. "Tristar" is a new forage cultivar that requires ~120 days to produce mature seed in western Canada where only ~100 frost-free days are available. The goal for this study was to reduce maturity duration for the crop through a series of studies on the genetics and agronomic aspects of fenugreek. This two year study suggests that: 1) mutation breeding using Tristar seed as a base population could be successfull; 2) multi-location trials using world accessions exhibited genotype X environment interaction; 3) swathing of plants before freezing temperatures set in; 4) application of phosphate fertilizer increased seed yield and; 5) foliar sprays of chemicals can be used for production of high quality seed. In this study some short duration, high yielding and determinate lines of fenugreek were produced improving the potential for use of fenugreek and the economics of beef production in western Canada.

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LIST OF ABBREVIATIONS

AAFC	Agriculture and Agri-Food Canada
AAFRD	Alberta Agriculture, Food and Rural Development
AB	Alberta
AVOVA	Analysis of Variance
BC	Before Christ/British Columbia
CDCN	Crop Diversification Center North
CDCS	Crop Diversification Center South
cm	centimeter
CV	Coefficient of Variation
df	Degrees of freedom
DM	Dry Matter
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
EMDIP	Electron Microscopy and digital Image Processing Laboratory
EMS	Ethyl Methane Sulfonate
F	F value
g	gram
G:M	Galactose:Mannose
GA_3	Gibberellic acid
h	hour
ha	hectare
IAA	Indole acetic acid

kg	kilogram
km	kilometer
L	liter
LRC	Lethbridge Research Center
LSD	Least Significant Difference
M	Molar
m	meter
mg	milligram
MH	Maleic Hydrazide
min	minutes
mM	milli Molar
mm	millimeter
MS	Mean Square
MSL	Mean Sea Level
M_{0-7}	Mutation Generation(s), subscript represent the number of generation
n	Basic chromosome number
N	North
NAA	2-1-napthylacetic acid
NHP	Natural Health Products
P	Phosphate
p	Probability level
PGRC	Plant Genetic Resources of Canada
ppm	Parts Per Million

r	Correlation coefficient
Pr	Probability
psi	Per square inch
RCBD	Randomized Complete Block Design
SDF	Soluble Dietary Fibers
SE	Standard Error
SEM	Scanning Electron Microscopy/Scanning Electron Micrograph
UK	United Kingdom
USDA	United States Department of Agriculture
UV	Ultra Violet
W	West
W/V	Weight/Volume
W/W	Weight/Weight
WFT	Western Flower Thrips
°C	Degree Celsius/Degree Centigrade
2,4-D	2,4 dichlorophenoxyacetic acid

Chapter One: Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual forage legume crop. The species name "*foenum-graecum*" means "Greek hay" indicating its use as a forage crop in the past. Fenugreek is believed to be native to the Mediterranean region (Petropoulos 2002), but now is grown as a spice in most parts of the world. It is reported as a cultivated crop in parts of Europe, northern Africa, west and south Asia, Argentina, Canada, United States of America (USA) and Australia (AAFRD 1998; Edison 1995; Fazli and Hardman 1968; Petropoulos 2002). India is the leading fenugreek producing country in the world (Edison 1995). The first North American fenugreek forage cultivar "Tristar" was released for use in western Canada in 2004 by Agriculture and Agri-Food Canada (AAFC). Tristar fenugreek was developed from a line L3314 (formerly PI–138687), originally collected in 1940 from Iran.

Fenugreek is regarded as the oldest known medicinal plant in recorded history (Lust 1986). Its seed and leaves have medicinal value, and have been used to reduce blood sugar and lower blood cholesterol in humans and animals (Dahanukar *et al.* 2000). In parts of Asia, the young plants are used as "pot herbs" and the seed as a spice or herbal medicine.

Fenugreek has many features that will benefit Canada's agri-food industry, one of them being its potential for use as a forage crop. The crop is adapted to rain-fed growing conditions and should grow well within the semi-arid regions of western Canada. It is a legume and can be incorporated into short term crop rotations (Moyer *et al.* 2003) to replenish nitrogen within the soil. It also can produce high quantity (Mir *et al.* 1993) and high quality forage (Mir *et al.* 1998), can be grown for hay or silage (Mir *et al.* 1998),

does not cause bloat in cattle (Mir *et al.* 1997), and contains animal growth promoting substances such as diosgenin not present in other forage legumes (Mir *et al.* 1997).

However, most fenugreek cultivars like Tristar variety currently in use in western Canada, suffer from lack of consistency in quality seed production every year due to an indeterminate growth habit. Due to this habit plants grow continuously at the shoot apex, or tip, and continue producing new shoots, flowers and pods. When grown in tropical climates the duration to development of mature plants (maturity) for fenugreek is reported to be 130-140 days (Petropoulos 2002). Tristar fenugreek takes about 120 days to produce mature seed in a temperate climate such as that found on the Canadian prairies where only ~100 frost free days are available for crops to mature. The indeterminate growth habit in fenugreek has been reported to be a monogenic recessive trait (Choudhury and Singh 2001). Plants transport essential photosynthetates to the apical part of the plant during periods of active growth (apical dominance). Indeterminate plants keep producing new apical buds as a result of which seed maturation occurs over a long period and the mutirity is inconsistent. However, apical growth in determinate plants will be arrested quickly making the seed pods to mature early. This has the potential to produce high quality seed within the limited growing period available in Canadian prairies.

Widespread cultivation of fenugreek requires that we solve the seed production problem through proper understanding of the agronomic complexities of growing this crop, and select new early maturing germplasm. These objectives can be addressed in three ways. Since fenugreek is a self-pollinated crop, a mutation breeding method can be applied to generate mutants with a determinate growth habit that mature earlier than

indeterminate types and have high seed yield and quality. Petropoulos (2002) has used alkylating agents to produce point mutations in fenugreek that mature early. Selection for improved agronomic properties such as early maturity in the world collections is another possible approach that can help solve the seed yield and quality problem. Our knowledge of agronomic practices which could promote high quality and quantity of fenugreek seed production under Canadian prairie growth conditions is very limited. There is need to develop an efficient agronomic package for assured seed production in fenugreek. To achieve these goals the following specific experiments were conducted:

(I) A mutation breeding study was conducted using a Tristar cultivar as the base population and ethyl methane sulfonate (EMS) as the mutagen. Specific objectives of this study were to:

  (A) Develop fenugreek mutants with a determinate growth habit, high seed yield and seed quality.

  (B) Determine through cytological studies if the EMS generated mutants possess gross chromosome abnormalities that may cause a sterility problem.

  (C) Assess the potential for use of tetraploid fenugreek to produce more vigorous plants with better forage traits.

(II) World accessions of fenugreek were evaluated under rain-fed and irrigation field conditions to select for early maturing, high yielding lines with potential for use in western Canada.

(III) A complete agronomic package for quality seed production in fenugreek was developed. Specific objectives of this study were to:

(A) Assess adaptability of fenugreek as a crop for use in different agro-climatic zones of western Canada.

(B) Determine the effect of phosphate fertilizers on plant seed yield and maturity.

(C) Determine if swathing the fenugreek crop before combining will help recover mature seed.

(D) Assess the effect of gibberellic acid (GA_3) and six other chemical foliar sprays (ferrous sulphate, calcium chloride, cupric sulphate, magnesium sulphate, ammonium sulphate and ammonium molybdate) on seed yield and maturity under irrigated field conditions.

(E) Identify potential insect pests and their effect on fenugreek growth.

In these studies we show that development of fenugreek cultivars with early maturity and a determinate growth habit is possible. Selected lines with high seed yield and quality were developed that mature during the short growing season found on the Canadian prairies. We anticipate that this investigation will help to improve the economics of beef and dairy production in western Canada and, will lead to more sustainable cropping practices. This study will provide new knowledge needed to facilitate widespread use of fenugreek as a new forage crop as well as a crop with potential for nutraceutical use in Canada.

Chapter Two: Background

2.1. History of the crop

Fenugreek (*Trigonella foenum-graecum* L.) is an annual crop belonging to the legume family. Although grown as a spice in most parts of the world (Figure 2.1.1.), the species name "*foenum-graecum*" means "Greek hay" indicating its use as a forage crop in the past (Petropoulos 2002). Saraswat (1984) recovered carbonized fenugreek seed from a Rohira village in the Sangrur district of Punjab, India indicating its use in trade by people of the Harappan civilization as far back as 2000 -1700 B.C. Fenugreek also is known as one of the oldest medicinal plants recognized in recorded history (Lust 1986).

2.2. Origin, distribution and different species

Different authors have widely divergent opinions about the probable ancestry of *T. foenum-graecum*. Vavilov (1926, 1951) has suggested that fenugreek is native to the Mediterranean region, while De Candolle (1964) and Fazli and Hardman (1968) proposed an Asian origin for the crop and Dangi *et al.* (2004) suggested that *T. caerulea* (Figure 2.2.1. A) and *T. foenum-graecum* both originated in Turkey (Figure 2.2.1. B). Confusion over the origins of fenugreek led Sinskaya (1961) to suggest that locating the direct wild ancestor of *T. foenum-graecum* is debatable. Indigenous species have been reported (Allen and Allen 1981; Ivimey-Cook 1968; Petropoulos 2002; Phitos and Damboldt 1985; Polunin 1988) on the continents of Asia (6 species), Europe (5 species), Africa (1 species) and Australia (1 species). Fenugreek also is reported as a cultivated crop in parts of Europe, northern Africa, west and south Asia, North and South America and Australia (AAFRD 1998; Edison 1995; Ethiopia: Country Report 1996; Fazli and Hardman 1968;

Jongebloed 2004; Petropoulos 2002; Rosengarten 1969; Rouk and Mangesha 1963; Smith 1982) (Table 2.2.1.).

The exact number of species of fenugreek also has been debated. Petropoulos (2002) indicated that older taxonomists like Linnaeus have suggested that as many as 260 species of fenugreek exist. In contrast, about 128 species of fenugreek were reported by Vasil'chenko (1953), 97 by Fazli (1967) and 70 by Hector (1936), Hutchinson (1964) and, Rouk and Mangesha (1963). A total of 18 different species of fenugreek (*Trigonella*) currently are recognized in the primary literature (Table 2.2.2.)

Figure 2.1.1. World map showing distribution of fenugreek (*Trigonella foenum-graecum* L.) Map not to scale.

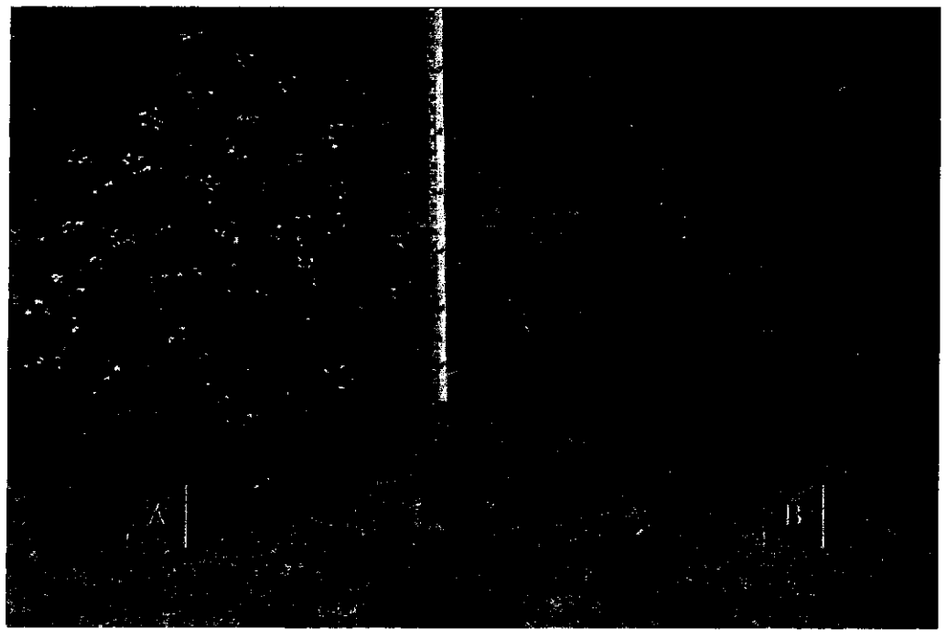


Figure 2.2.1. A. ***Trigonella caerulea and*** **B.** ***Trigonella foenum-graecum***

Table 2.2.1. Distribution of fenugreek (*Trigonella foenum-graecum* L.) by country and continent.

Continents	Country(s)
Europe	Austria, France, Germany, Greece, Portugal, Russia, Spain, Switzerland, Turkey and United Kingdom (UK)
Africa	Egypt, Ethiopia, Kenya, Morocco, Sudan, Tanzania and Tunisia
Asia	China, India, Iran, Israel, Japan, Lebanon and Pakistan
South America	Argentina
North America	Canada and the United States
Australia	Parts of Australia

Table 2.2.2. Common species of the *Trigonella* genus according to Petropoulos (2002).

Genus	Species
Trigonella	*T. foenum-graecum, T. anguina, T. arabica, T. caerulea, T. corniculata, T. cariensis, T. rigida, , T. suavissima, T. torulosa, T. spinosa, T. polycerata, T. radiata , T. platycarpos, T. hamosa, T. cretica, T. occulta, T. arcuata,* and *T. striata*

2.3. Botanical perspective of fenugreek

Fenugreek (*Trigonella foenum-graecum* L.) is an annual dicotyledonous plant belonging to the subfamily Papilionaceae, family Leguminacae (=Fabacecae). A morphological description of the plant (Fazli and Hardman (1968), Hutchinson (1964), Petropoulos (1973, 2002), Sinskaya (1961) and Tutin and Heywod (1964)) is presented in Table 2.3.1. In general, two types of flowering shoots are observed (Petropoulos 1973, 2002). The common type bears axillary flowers showing an indeterminate growth habit, whereas the less common or so called "blind shoots" have both axillary and terminal flowers, each of which become "tip bearers". Two types of fenugreek flowers also have been described (Petropoulos 1973, 2002); *i.e.*, cleistogamous (closed) and aneictgamous (open) flowers. However, the majority of fenugreek flowers are cleitogamous; aneictgamous flowers are not common in fenugreek.

Table 2.3.1. Morphological characteristics of fenugreek.

Morphological characteristics	Description, color and texture	Dimensions
1. Plant habit	Erect or prostrate, straight or profusely branched	20-130 cm in length
2. Stem	Circular to slightly quadrangular, greenish, often characterized by pinkish colour due to anthocyanin accumulation under field condition	0.5-1 cm in diameter
3. Leaf	Simple and trifoliate, distinctly petiolate, stipulate; leaf lamina oval or orbicular with an entire margin. The petioles and leaf lamina varies form greenish to pinkish in the field	1.5-4.5 cm X 0.8-1.5 cm
4. Petiole	Pale green, pubescent, often anthocyanin tinged	Very small; 0.5-1.1 mm
5. Flower	Yellow when young but white on maturity	1.6 - 2.2 cm
6. Calyx	Campanulate, pale green, pubescent	6 - 8 mm
7. Individual sepal	Pale green, pubescent	13 – 19 mm
8. Corolla	Papilionaceous, white, papery	1.5 - 1.9 cm
9. Standard/ Vexillum/Banner	White, papery	1.5 - 1.8 cm
10. Keels/Carina	White, papery	6 - 10 mm
11. Wings/Alae	White, papery	4.5 - 5.5 mm
12. Anther lobes	Bright yellow, rectangular	1 - 1.5 mm X 0.4- 0.5 mm
13. Filament	Hyaline, tubular	1. 7 - 1.9 mm
14. Ovary	Deep green, glaucous	1.8 - 2.5 mm
15. Stigma	Pale green, glaucous	1.5 - 2.1 mm
16. Style	Pale green/hyaline glaucous	0.2 - 0.5 mm
17. Pollen grain	Oval (70-90 %) to circular, orbicular, ellipsoidal grains (10-30 %). Hyaline; stained pink or red when treated with 0.5 % aceto-carmine	0.032 - 0.042 mm X 0.025 -0.027 mm
. Ratio of terminal to axillary flowers	All flowers yellow when immature and white when matured	Extremely rare, however the ratio varies as 1:8/1:10/ 1:11/1:13
. Number of pods per plant and pod dimensions	Pods brownish or yellowish brown with mucronate tips.	2-8/plant 9.5-18.6cm X 0.2-0.4 cm
20. Seed	Rectangular to oval in shape with deep grooves between the radicle and cotyledon. Varies in colour form pale brown to golden yellow	10-20/pod 3-5 mm X 2-3 mm

The high frequency of cleistogamous flowers each with a protective papilionaceous corolla may have lead to development of fenugreek as a predominantly self-pollinated plant (Figure 2.3.1.). Allard (1960) and, Darlington and Wylie (1945) have classified the plants as self- and cross pollinated based on observations of open and closed flowers. Del'Gaudio (1952, 1953) on the other hand, suggested that the plants were self-fertile based on detailed investigations on floral physiology of fenugreek plants. Cross pollination may occur in open flowers of fenugreek. Allard (1960) has suggested that legumes are considered cross-pollinated when more than 10 % of them are "out-crossed". On this basis Petropoulos (1973, 2002) described fenugreek as a rarely cross pollinated plant as its stigma becomes receptive before the anthers mature. Because of this Petropoulos (1973, 2002) has suggested that cross pollination for breeding purposes, can be done in closed flowers of fenugreek at initiation of the second stage of floral development, when the stamens are lower in position than the stigma, *i.e.* when the anthers are closed but the stigma is receptive to pollination.

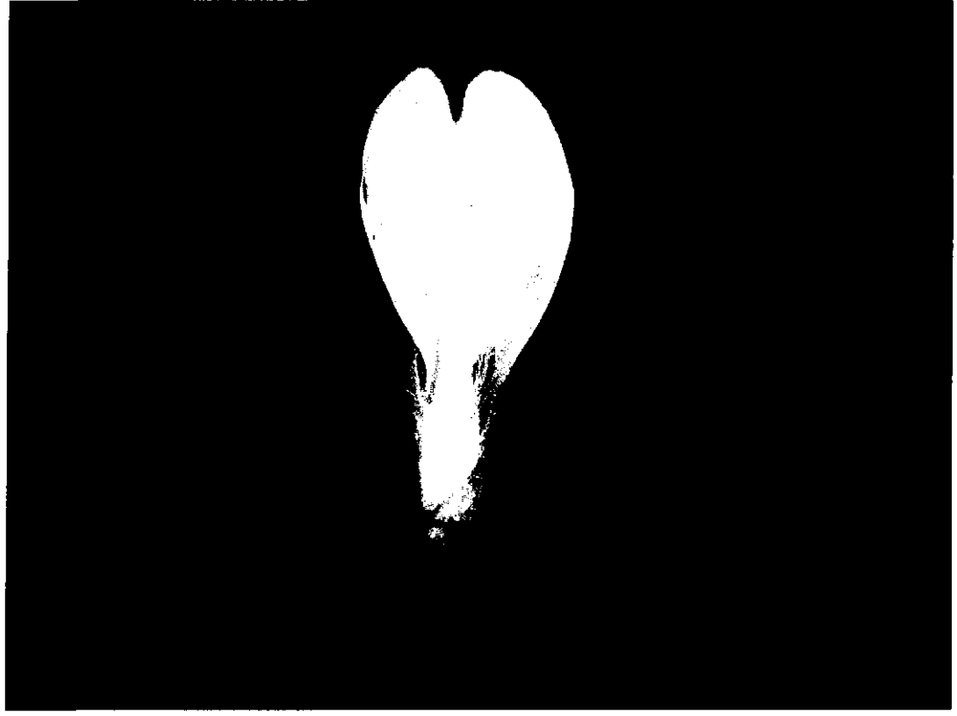


Figure 2.3.1. Enlarged view of the papilionaceous corolla of the fenugreek flower.

2.4. Cytogenetic studies and variability in fenugreek lines

According to Darlington and Wylie (1945) the haploid chromosome number (n) of *Trigonella* can be 8, 9, 11 or 14. Most species including *Trigonella foenum-graecum* L. are diploids with $2n = 16$ chromosomes. However, *T. hamosa* from Egypt was found to have 16 and 44 chromosomes; *T. geminiflora* (from Persia in Asia Minor) and *T. grandiflora* (from Turkestan) both have 44 chromosomes; *T. polycerata* (from the Mediterranean region of south west Asia) has 28, 30 and 32 chromosomes and, *T. ornithopodioides* is reported to have 18 chromosomes. These data suggest that some *Trigonella* species have undergone several rounds of chromosome doubling and rediploidization through gene and chromosome elimination.

Singh and Singh (1976) isolated five double trisomics from *Trigonella* along with primary trisomics from the progeny of autotetraploids which have a chromosomal constitution of $2n+1+1=18$. Roy and Singh (1968) also have produced tetraploid fenugreek by treating shoot apices with colchicine. Joshi and Raghuvanshi (1968) have in addition, demonstrated that chromosome number in fenugreek can increase through the presence of B-chromosomes.

Varieties of fenugreek currently available have not been subjected to intensive selection through modern breeding programs, and still express a high level of variability among genotypes present in world collections (Acharya *et al.* 2004b; Moyer *et al.* 2003; Taylor *et al.* 1997; Taylor *et al.* 2002; Huang and Liang 2000). These genotypes differ in morphology, growth habit, biomass and seed production capability as well as in the chemical constituents found in the seed; e.g., variation in saponins, fibre, proteins, amino acids and fatty acid content is observed. These observations suggest that evolution in

fenugreek has given rise to variation in phenotype of the plants which can be used in selective breeding (Acharya *et al.* 2004b).

2.5. Nutraceutical properties of fenugreek

Fenugreek has been referred to as a medicinal herb both in Indian Ayurvedic and traditional Chinese medicines (Tiran 2003). Ancient literature, religious scripture, travel records and anecdotes from different continents and from different periods of human history, record a wide variety of medicinal properties associated with fenugreek (Lust 1986). Medicinal uses vary from wound-healing to bust enhancement and, from promotion of lactation in weaning mothers, to its use as a sex stimulant or aphrodisiac (Petropoulos 2002; Tiran 2003).

Although Fugh-Berman (2003) has suggested that there is little clinical evidence to support these claims, fenugreek leaves and seed have been used extensively to prepare extracts and powders for medicinal use (Basch *et al.* 2003). A number of important chemicals with medicinal value have been found in fenugreek seed and leaves (Petropoulos 2002). Some of these are presented in Table 2.5.1.

In general, fenugreek contains three important chemical constituents with medicinal value; *i.e.* 1) steroidal sapogenins; 2) galactomannans and 3) isoleucine. These constituents have placed fenugreek among the most commonly recognized "nutraceutical" or health food products (Srichamroen *et al.* 2005). Some medicinal properties attributed to fenugreek are presented in Table 2.5.2.

Table 2.5.1. Major chemical constituents of fenugreek seed and leaves.

Broad group of chemicals	Name of individual constituent members	References
Major polysaccharides	Galactomannans (consisting of galactose and mannose in the ratio of 1:1)	Petropoulos (2002)
Major steroidal sapogenins	Diosgenin[(25 R)-spirost-5-ene-3β-ol](Figure 2.5.1. F); yamogenin, tigogenin (Figure 2.5.1. E), neotigogenin, smilagenin and sarsasapogenin	Fazli and Hardman (1968); Sauvaire *et al.* (1991); Taylor *et al.* (1997)
Dihydroxy steroidal sapogenins (minor sapogenins)	Yuccagenin, gitogenin and neogitogenin	Taylor *et al.* (1997)
Spirostanol saponins	Graecunin-B, C, D, E and G	Varshney and Jain (1979)
Triterpenoids		Shang *et al.* (1998)
N-compound	Trigonelline (methylbetaine derivative of nicotinic acid) (Figure 2.5.1. D)	Petropoulos (2002)
Flavonoids	Kaemfrol, afroside, quercetin, isoquercitrin, vitexin, isovitexin, orientin, and luteolin	Huang and Liang (2000); Petropoulos (2002)
Isoflavonoid phytoalexins	Medicarpin, maackiaian, vestitol and sativan	Petropoulos (2002)
Kaempferol glycosides	Lilyn	Han *et al.* (2001); Petropoulos (2002)
Phenolic compounds	Scopoletin (Figure 2.5.1. C), chlorogenic, caeffic acids, p-coumaric acids, hymercromone, coumarin (Figure 2.5.1. B) and trigocoumarin	Petropoulos (2002).
Stercal sapogenin-peptide ester	Fenugreekine	Ghosal *et al.* (1974).

Table 2.5.2. Fenugreek and its associated medicinal properties.

Medicinal properties reported	References
Anti-diabetic and cholesterol lowering properties	Devi *et al.* (2003); Hannan *et al.* (2003); Thakaran *et al.* (2003); Vats *et al.* (2003), Chaturvedi and Pant (1988); Suboh *et al.* (2004); Thompson Coon and Ernst (2003); Venkatesan *et al.* (2003)
Anti- hyperthyroidism	Tahiliani and Kar (2003a)
Against thyroxine-induced hyperglycaemia	Tahiliani and Kar (2003b)
Anti-cancer effects	Devasena and Menon (2003)
Gastro-protective effect	Pandian *et al.* (2002)
Antioxidant property	Raskin *et al.* (2002)
Antinociceptive property	Javan *et al.* (1997)
Antimicrobial property	Bhatti *et al.* (1996)
Anthelmintic property	Ghafgazi *et al.* (1980)
Anti-sterility and anti-androgenic effects	Kamal *et al.* (1993)
Wound healing property	Taranalli and Kuppast (1996)
Anti-inflammatory and antipyretic actions	Ahmadiani *et al.* (2001)

2.5.1. Steroidal sapogenins

Fenugreek seed is an important source of steroidal sapogenins such as diosgenin which are used extensively by both pharmaceutical and nutraceutical industries (Srichamroen *et al.* 2005). Diosgenin is often used as a raw precursor for the production of steroidal drugs and hormones such as testosterone, glucocorticoids and progesterone (Fazli and Hardman, 1968; Raghuram *et al.* 1994; Srichamroen *et al.* 2005). McAnuff *et al.* (2002) reported that steroidal sapogenins are effective agents for the treatment of hypocholesterolemia, a disorder often associated with diabetes.

At present natural diosgenin is procured chiefly from the tubers of certain wild species of Mexican yam (*Dioscorea* species). However this process is both time consuming and costly, requiring several years before the yam tubers grow to a size where they possess a sufficient concentration of diosgenin to be used as source of commercial and pharmaceutical reagent (Rosser 1985). Fenugreek may be a viable alternative for production of diosgenin because of its shorter growing cycle, lower production costs and, consistent yield and quality (Hardman 1969; Petropoulos 1973).

2.5.2. Galactomanans

Galactomannans are a major polysaccharide species found in fenugreek seeds and, represent ~50% of the seed weight (Raghuram *et al.* 1994). They make up an integral component of the cell walls in the seed endosperm (Meier and Reid 1977). Their structure is composed of a 1→4 linked β-D-mannosyl backbone with single unit galactose side-chains, α-linked at the O-6 oxygen. Fenugreek galactomanan is considered unique due to a 1:1 to 1.2:1 ratio of galactose to mannose (G:M) molecules (Andrews et al. 1952; Petropoulos 2002). The high ratio of galactose substitution contributes to the

hydrophilic properties associated with the galactomannans in fenugreek seed and, is important in determining the overall biological value of the galactomannans in fenugreek (Srichamroen *et al.* 2005).

Fenugreek galactomannans appear to be beneficial for control of type 2 diabetes in both animals (Puri *et al.* 2002; Raju *et al.* 2001; Ribes *et al.* 1986; Tayyaba *et al.* 2001; Vats *et al.* 2002; Vats *et al.* 2003) and humans (Madar *et al.* 1988; Puri *et al.* 2002; Raghuram *et al.* 1994; Sharma 1986; Sharma and Raghuram 1990; Sharma *et al.* 1996) by reducing hyperglycemia in these individuals. They have a high water-binding capacity and are able to form highly viscous solutions at relatively low concentrations, which appears to reduce glucose absorption in the digestive tract (Raghuram *et al.* 1994). According to Ramesh *et al.* (2001) fenugreek gum (galactomanan) is under-exploited in our global food industry.

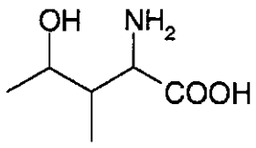
2.5.3. Isoleucine

The amino acid isoleucine is a precursor of 4-hydroxyisoleucine (Figure 2.5.1. A) which is known to regulate the secretion of insulin in animals (Broca *et al.* 2000; Sauvaire *et al.* 1998). Most hypoglycaemic and anti-hyperglycaemic effects of fenugreek are attributed to the gastrointestinal effect of dietary fiber and systemic effects of amino acids like 4-hydroxyisoleucine present in the seed (Madar 1984; Moorthy *et al.* 1989; Petropoulos 2002; Ribes *et al.* 1986; Sauvaire *et al.* 1996).

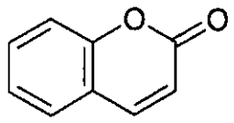
2.6. Functional food aspects of fenugreek

Legume consumption is known to have a beneficial or protective effect in diabetes, hypercholesterolaemia and coronary heart disease, as well as protecting against obesity and menopause (Mazur *et al.* 1998; Madar and Stark 2002). Specialty crops like

fenugreek are attracting the attention of producers to meet manufacturing demands for "functional food" additives and Natural Health Products (NHP) in Canada (Fitzpatrick 2004). Mansour and El-Adawy (1994) recommended that fenugreek seed could be added to foods like ground meat and baked goods, not only as nutritional supplements but also as a potential functional food. A summary showing studies associated with functional foods developed with fenugreek additives is presented in Table 2.6.1.



A.



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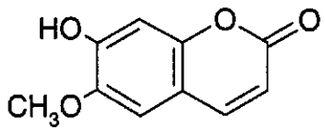

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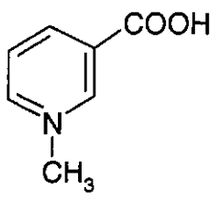

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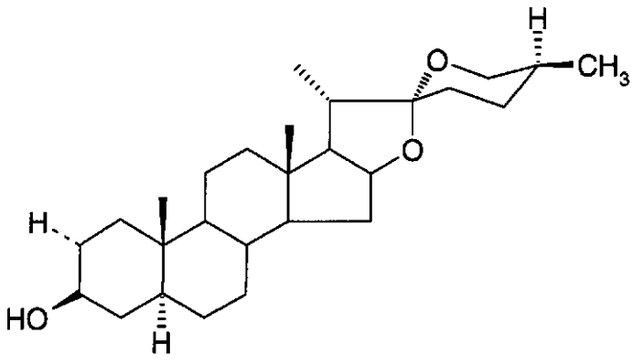

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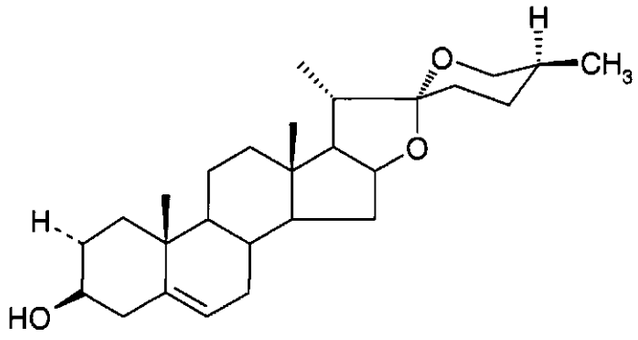


F.

Figure 2.5.1. Structures of important chemical constituents detected in fenugreek A. 4-Hydroxyisoleucine; B. Coumarin; C. Scopoletin; D. Trigonelline; E. Tigogenin and F. Diosgenin.

Table 2.6.1. Summary of research progress with respect to the functional food aspect of fenugreek.

Functional food aspect of fenugreek	References
Fenugreek seed has protein, lipid, cellulose starch and ash. It is also reported to be rich in calcium, iron and β-carotene.	Sauvaire *et al.* (1976)
Fenugreek seed endosperm is characterized by soluble fiber. Approximately half the dry weight of fenugreek seed has Soluble Dietary Fibers (SDF), an edible dietary fiber	Aspinall (1980)
Boiling of fenugreek seed results in loss of crude proteins, total sugars, ash but subsequently increases the level of crude fibers; without any significant effect on lipid levels.	Ismail (1996)
Good quality wheat breads with high nutritional characteristics and higher acceptability have been produced in Egypt by supplementation of 4 % fenugreek flour.	(Bakr 1997)
Cooking fenugreek leaves in a pressure cooker improve retention of essential chemical nutrients such as ascorbic acid and beta-carotene	Yadav and Sehgal (1997)
Fenugreek galactomanans as food emulsifiers	Gatri *et al.* (1997)
Fenugreek seed contain sufficient ascorbic acid	Riddoch *et al.* (1998)
Nutritional quality of lactic acid fermented fenugreek leaves	Gupta *et al.* (1998)
Fenugreek fibers, psyllium husk and wheat bran could be used as dietary supplements to increase roughage in the human diet	Al-Khalidi *et al.* (1999)
Supplementation of wheat flour with ground debittered fenugreek improves the physicochemical, nutritional and rheological properties	Sharma and Chauhan (2000)
Suitability of the development of food products based on millets, legumes and fenugreek seed in diabetic diet	Pathak *et al* (2000)
Use of corn bread mixed with a small amount of fenugreek (3 %) or with wheat flour (30 %) is used as staple food in Egypt	Galal (2001)
Fenugreek contains 23-26 % protein, 6-7 % fat and 58 % carbohydrate and ~25 % dietary fiber.	USDA (2001)
Properties of fenugreek mucilage and its use in ice cream as a stabilizer	Balyan *et al.* (2001)
Nutritional value and physiological properties of fenugreek.	Billaud and Adrian (2001)
Improvement of oxidative stability of raw food products like eggs by fenugreek	Armitage *et al.* (2002)
Fenugreek seed mucilages can be used as viscosity builders	Seghal *et al.* (2002)
Supplementation of fenugreek flour in rice bran improved the physical and sensory properties and qualities of breads and cookies	Sharma and Chauhan (2002)
Reduction in phytic acid levels and simultaneous *in vitro* increase with respect to calcium and iron with higher temperature and longer fermentation duration in case of fenugreek supplemented Indian bread	Bhatia and Khetrapaul (2002)
Analysed the composition of raw, soaked and germinated seed and found that nutritional quality of fenugreek seed could be improved with subsequent reduction of bitterness through processing	Hooda and Jood (2003a.)
Physiological, rheological and organoleptic characteristics of wheat-fenugreek supplemented blends showed increase in protein and fat contents	Hooda and Jood (2003b.)

2.7. Agricultural aspects of the crop

Fenugreek is a speciality crop in western Canada (Acharya *et al.* 2004b.) It can benefit the producer a number of ways. As a legume crop it can condition the soil, fixing valuable nitrogen from the atmosphere thereby reducing the need for nitrogen fertilizers, and effectively reducing production costs. As a rain-fed crop, its water requirements are low; use of fenugreek can reduce the costs of irrigation and save water. A decreased need for application of fertilizers to the soil, has potential to decrease contamination of surface waters by runoff from agricultural fields. This has potential to reduce eutrophication of surface waters and limit contamination of ground water sources (Acharya *et al.* 2004a; Basu *et al.* 2004). These properties also make fenugreek a useful legume crop for incorporation into short term rotations (Moyer *et al.* 2003). In addition, it can produce high quantity (Mir *et al.* 1993) and high quality forage (Mir *et al.* 1998), can be grown for hay or silage (Mir *et al.* 1998), does not cause bloat in cattle (Mir *et al.* 1997), and contains animal growth promoting substances such as diosgenin not present in other forage legumes (Mir *et al.* 1997).

2.7.1. Cultural requirements for fenugreek

Agronomic research conducted in different agro-climatic zones in India suggest that optimum productivity can be obtained when fenugreek seed is spaced 20 to 30 cms apart and planted in early October or November to optimize crop productivity (Baswana and Pandita 1989; Bhatt 1988; Gill *et al.* 2001; Korla and Saini 2003). Other agronomic practices known to maximize fenugreek yield involve optimization of sowing dates and forage cutting (Lal *et al.* 2003), and use of minor irrigation (Bhatt 1993; Kumar *et al.* 2000; Ram and Verma 2000; Sheoran *et al.* 2000). Well-drained loam soils (Rosengarten

1969) with a pH of 8-8.5 are favored by the crop. According to Petropoulos (1973) heavy and wet soils limit crop growth. Potash has been used to adjust soil pH to increase nutrient uptake of fenugreek (Yadav and Kumawat 2003).

Fenugreek is a dry-land crop which responds even to minimal levels of irrigation (Acharya *et al.* 2004a; Mir *et al.* 1993; Moyer *et al.* 2003). Interest in cultivating fenugreek in temperate climates, such as that found in western Canada, has increased because of its rain-fed adaptation (Acharya *et al.* 2004b). Baričevič and Zupančič (2002) from Slovenia, reported lower diosgenin yield from draught stressed fenugreek cultivars. However, when grown under optimal irrigation regime (35 % depletion of available soil water) diosgenin yield increased in comparison to normal irrigated plants, suggesting that the plant does well under minimal irrigation.

2.7.2. Application of soil inoculums, fertilizers, organic manures and sludge

Use of organic and inorganic fertilizers, farmyard manure, nitrogen and phosphorus have been found to be effective in increasing fenugreek yield (Detoroja *et al.* 1995; Khiriya and Singh 2003; Yadav and Kumawat 2003). Abd-Ala and Omar (1998) reported that application of wheat straw and fungi (*Sinorhizobium meliloti, Trichoderma harzianum, Aspergillus niger* and *Chaetonium globosum*) promote nodulation, nodule efficiency and fenugreek growth under saline soil conditions.

Fenugreek is a nitrogen fixing legume. Hence the seed must be inoculated with an appropriate *Rhizobium* inoculum to optimize its growth potential. The most common nodule- forming bacteria associated with *Trigonella foenum-graecum* L. is the Gram negative, aerobic, non-sporulating, rod shaped bacterium, *Rhizobium meliloti* (Subba Rao

and Sharma 1968). Abdelgani *et al.* (1999) has suggested that inoculation of fenugreek with a suitable strain of *Rhizobium* can improve quality and amount of seed generated.

The effects of gamma-irradiated sludge on the growth and yield of fenugreek have been reported by Pandya *et al.* (1991). These workers identified a statistically significant increase in the yield of fenugreek plants grown in irradiated sludge (for 45-90 days), compared to the control. Their research suggests that there is a promising positive effect of recycling irradiated sludge for agricultural application and fenugreek can serve as an efficient tool in the process of reclamation.

2.7.3. Application of plant growth regulators

Alhadi *et al.* (1999) found that application of gibberellic acid to the seed before sowing causes a slight change in the growth characteristics and physico-biochemical properties of standing fenugreek crops under water deficient conditions. Ortuno *et al.* (1998) reported a considerable increase in diosgenin content in young leaves (20 mg g^{-1} dry weight) in fenugreek seeds treated with benzylaminopurine. Alagukannan and Vijaykumar (1999) reported that application of 2-1-napthylacetic acid (NAA) to seed increased the number of seeds per pod and, seed size in fenugreek, while application of maleic hydrazide (MH) and 2,4 dichlorophenoxyacetic acid (2,4-D) on seed increased the seed protein content.

2.7.4. Disease organisms affecting fenugreek

Fungal, bacterial, viral and insect mediated diseases are reported to be associated with considerable lowering of forage and seed yield in fenugreek and hence is a serious agronomic concern (AAFRD 1998; Fogg *et al.* 2000; Jongebloed 2004; Petropoulos 1973, 2002; Prakash and Sharma 2000). Some workers have also reported physiological

diseases due to mineral deficiencies that are associated with lowering of forage yield in fenugreek (Sinskaya 1961; Petropoulos 1973). Hence, fenugreek diseases are broadly classified on the basis of their pathogenicity into two broad groups: biological (fungal, bacterial, viral, insect mediated diseases) and mineral nutrient deficiency.

A. Fungal diseases: The two most common fungal diseases infecting fenugreek are *Cercospora* leaf spot and powdery mildew (AAFRD 1998). Prakash and Sharma (2000), Petropoulos (2002) and Jongebloed (2004) have reported powdery mildew on fenugreek, caused by *Erysiphe polygoni*. Additional studies from Australia (Jongebloed 2004) have shown that yield of fenugreek can be seriously affected by blight disease caused by *Cercospora traversiana* and wilt caused by *Fusarium oxsysporum* and *Rhizoctonia solani*. In addition, other well known fungal diseases associated with fenugreek are collar rot, leaf spot and pod spot diseases (Petropoulos 2002).

B. Bacterial diseases: Fogg *et al.* (2000) reported a bacterial leaf spot in fenugreek which was caused by *Pseudomonas syringae* pv. *syringae* in New Jersey, USA. It also has been suggested that the bacterium *Xanthomonas alfalfa* can infect fenugreek (Petropoulos 2002).

C. Viral diseases: Petropoulos (2002) reported that Bean Yellow Mosaic Virus, Alfalfa Mosaic Virus, Cow Pea Mosaic Virus, Soybean Mosaic Virus, Pea Mosaic Virus, Potato Virus A and Y, and Clover Vein Mosaic Virus are common viral infections of fenugreek. These viral

diseases have been associated with moderate loss of seed and forage yield.

D. Insect diseases: Lucy (2004) reported that in Australia insects such as thrips, pod-borers and heliothis can cause serious damage to forage yield in fenugreek. Root rot by the soil borne nematode *Meloidogyne incognita,* which causes the death of immature plants has also been reported from Australia (Jongebloed 2004).

E. Mineral nutrient deficiency: Fenugreek is reported to be sensitive to mineral deficiencies (Petropoulos 1973). It has been suggested that yellowing of some fenugreek plants under field conditions is connected to probable mineral deficiencies, in particular to elements like boron, magnesium, manganese or potassium deficiency (Sinskaya 1961). Physiological diseases have been reported to be associated with early death and loss of forage and seed yield in fenugreek (Petropoulos 2002).

2.8. Breeding of fenugreek:

Fenugreek is a diploid ($2n = 16$) crop adapted to self pollination (Darlington and Wylie 1945). However, there are opportunities for improvement through cross pollination (Petropoulos 2002). Artificial crossing is difficult and complicated (Fehr 1993) in this self-pollinating crop and hence, is less successful (Busbice 1975). Selection and mutation breeding have been advocated for fenugreek (Fehr 1993; Sinskaya 1961; Petropoulos 1973; Raghuvanshi and Singh 1981). Most of the recent breeding work on fenugreek has focused on these two approaches (Green *et al.* 1981; Petropoulos 1973, 2002;

Sigurbjornsson 1983). However, hybridization also has been reported to be successful in this crop (Cornish *et al.* 1983; Edison 1995, Saleh 1996).

2.8.1. Selection

Selection is a simple but important method used in traditional plant breeding. It involves phenotypic selection of "outstanding" lines and elimination of "poor performers" or those with "undesirable" or "unsuitable" characteristics (Petropoulos 2002). Selection is considered to be one of the most important methods available for improvement of diploid species (Busbice *et al.* 1975). Petropoulos (2002) reported that a "solitary pod" phenotype in fenugreek is dominant to a "twin pod" phenotype, and that plants with narrow pods, containing large and rectangular seed are dominant to phenotypes with wide pods, which contain small and round seed. Knowledge about dominant and recessively inherited traits is important as it would have a direct impact on behaviour of the progeny of selected plants. A recessively inherited trait will be fixed in one generation as was seen by Raghuvanshi and Singh (1981) while working on the double pod trait in fenugreek. A trait governed by a dominant gene on the other hand can take several generations to fix.

Fenugreek accessions from the world collection exhibit extensive phenotypic variability; this variability has a genetic base, and so selection for improved levels of chemical constituents and nutraceutical applications is possible (Acharya *et al.* 2004b). Raghuvanshi and Singh (1981) obtained high heritability estimates in fenugreek when they selected for a double pod trait. The double pod trait is known to be genetically linked to diosgenin content and higher seed yields (Petropoulos 2002; Ahmed *et al.* 1989).

2.8.1.1. Single Plant Selection:

This procedure is sometimes known as "pedigree" or "pure line selection" and is one of the two methods that are used when improvement through selection is practiced. The other method is mass selection. Single plant selection is recognized as one of the best methods for breeding of new fenugreek lines since the crop is predominantly self-pollinated and there is no "inbreeding depression" reported for the crop so far (Petropoulos 2002). In this method, individual plants are selected for exhibiting superior traits. This method has proven useful for selection of highly heritable traits such as seed size, color, growth habit and the seed number per pod (Del' Gaudio 1953; Green *et al.* 1981; Petropoulos 1973, 2002; Saleh 1996).

2.8.1.2. Mass Selection

This method also is suggested for breeding of fenugreek although, it is mostly suitable for cross-pollinating crops (Petropoulos 2002). The method involves selection of a number of plants with desirable characteristics (as in individual plant selection) but in this case, seeds are mixed and bulked together. Superior individuals also are selected in subsequent generations while poor ones are eliminated. The process is repeated for several generations until the plants become uniform and stable in the desired characteristics (Fehr 1993; Petropoulos 1973, 2002; Saleh 1996).

2.8.2. Mutation Breeding

According to Fehr (1993) genetic variability is very important for improvement of crop characters. One method to induce genetic variability is through induced mutations. Mutations can either occur spontaneously or may be induced artificially and, are a

valuable tool for crop improvement. Petropoulos (2002) suggested that plant breeding is a "controlled evolution" and in addition to selection and recombination, mutation is the most important way of achieving it. During the past decades mutation breeding has gained importance, and interest in it as an effective tool for crop improvement has risen dramatically (Dubinin 1961; Fehr 1993; Manha *et al.* 1994; Petropoulos 2002).

According to Gaul (1961), Petropoulos (1973) and Singh and Singh (1974), most induced mutations are recessive and, can be observed to segregate in a 3:1 genetic ratio in diploid crops like fenugreek. Among the legume crops that have been developed by mutation breeding, the most prominent are soybeans, string beans, French beans, Navy pea beans, haricot beans, peas and lupines (Sigurbjornsson and Micke 1974; Sigurbjornsson 1983). Manha *et al.* (1994) indicated that diosgenin content in species of fenugreek like *T. corniculata* can be increased through mutation breeding. Mutation breeding is important when a desirable character is not available in the germplasm that normally could be used as a source for hybridization and selection. Although the frequency of desirable genetic changes from artificial mutagenesis is usually quite low, the probability of success can be increased by screening higher numbers of mutants for the desired phenotypic traits (Fehr 1993).

Several approaches can be used to generate mutants. Petropoulos (1973) irradiated open fields of fenugreek with chronic levels of γ-rays from Co^{60}, and used acute levels of radiation to expose dry seeds. However, use of ionizing radiation requires special facilities to reduce the risk to human health, and to avoid uncontrolled chromosome breakage and induction of high levels of chromosome aberrations in the plants (Petropoulos 2002). A number of workers and research groups have used chemical

mutagenesis to produce successful mutations in fenugreek; the most commonly used chemical mutagen in their studies was ethyl methane sulphonate (Jain and Agarwal 1987, 1994; Laxmi *et al.* 1980; Laxmi and Datta 1987; Petropoulos 2002; Singh and Raghuvanshi 1980).

2.8.3. Hybridization

One of the important steps in the process of cultivar development is incorporation of genetic variability for desirable traits into a population. Fehr (1993) suggested that this can be achieved by hybridization of parents with different genetic backgrounds. Hybridization involves crossing two or more varieties of genetically different individuals. Common methods of hybridization can involve a 2-parent cross, a 3-parent cross, a 4-parent cross, a back cross, a complex cross, a convergent cross and/or a polycross (Fehr 1993). Emasculation and manual pollination has been used effectively for crossing different lines of fenugreek (Petropoulos 2002). Petropoulos (1973) suggested that the fenugreek flower should be emasculated at the end of the first floral developmental stage to completely avoid chances of selfing. Soon after manually pollinating the flowers, a bag should be placed over the fenugreek flowers to avoid any chances of unrestricted out-crossing (Cornish *et al.* 1983).

Hybridization, a process which occurs in the natural world can be extremely tedious and labor intensive when practiced artificially. At times hundreds of crosses need to be made in order to generate a successful hybrid, and to find individual plants that possess unique and desirable combinations of characters. This is one of the most common methods in use and, many fenugreek varieties have been produced by this method

(Cornish et al. 1983; Edison 1995; Petropoulos 1973, 2002; Saleh 1996). However, this method has a major disadvantage in that undesirable character combinations often are created; these can be difficult to select out and can take many generations of selection or back crossing to eliminate.

Chapter Three: Genetic Studies on Fenugreek

3.1. Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual crop belonging to the legume family. This crop is believed to be native to the Mediterranean region and is now widely cultivated in India, China, northern and eastern Africa, parts of Europe and Argentina (Petropoulos 2002). India leads the world in fenugreek production, producing 70-80% of the global export (Edison 1995). The first North American fenugreek forage cultivar "Tristar" was released for use in western Canada in 2004 by Agriculture and Agri-Food Canada (AAFC). Tristar fenugreek was developed from a line L3314 (formerly PI–138687), originally collected in 1940 from Iran. Fenugreek is useful for incorporation into short term crop rotations (Moyer *et al.* 2003). It can produce high forage yield and high quality forage (Mir *et al.* 1998), can be grown for hay or silage (Mir *et al.* 1998), does not cause bloat in cattle (Mir *et al.* 1997), and contains animal growth promoting substances such as diosgenin not present in any other forage legumes (Mir *et al.* 1997).

Although, Tristar fenugreek is capable of producing high forage yield, it suffers from lack of consistency in seed quality and yield every year due to its indeterminate growth habit and long duration for maturity. Based on evaluation of 72 fenugreek lines Chandra *et al.* (2000) concluded that plant height, dry weight and number of pods are the three most important seed yield components in fenugreek. They strongly recommended use of these traits as selection parameters for improving fenugreek.

In tropical climates maturity duration for fenugreek is reported to be 130-140 days (Petropoulos 2002). Although, Tristar fenugreek has been selected for growth in a temperate climate, this cultivar possesses an indeterminate growth habit and takes about 120 days to produce a high proportion of mature seed. This maturity duration is a problem when the plants are grown under prairie conditions where only ~100 frost free days are available for crop maturity. Indeterminate plants exhibit apical dominance where they produce new branches and leaves at the apex (apical dominance) delaying pod filling and seed maturity in the lower pods on the plants and resulting in an inconsistent level of high quality seed production.

The determinate growth habit in fenugreek has been reported to be a monogenic recessive trait (Choudhury and Singh 2001). Among the germplasm tested in western Canada, very few exhibited a determinate growth habit. The few lines that were determinate in growth habit produced low above ground biomass and seed yield, and so were considered unsuitable for commercial production in this area. In addition, the small inconspicuous flowers from fenugreek only had a 4 % success rate when crossed (Choudhury and Singh 2001) and, did not look encouraging as a route for improvement of the crop.

According to Fehr (1993) mutation breeding is important when a desirable character is not available in germplasm that normally can be used as a source of hybridization and selection. The indeterminate growth habit variant has been reported for all species of fenugreek (Petropoulos 2002). While introduction of desirable genetic changes by artificial mutagenesis is known to be low, the probability of success can be increased by screening high numbers of mutants for desirable phenotypic traits (Fehr

1993). Use of this approach has made mutation breeding increasingly popular in recent times as an effective tool for crop improvement. It is considered to be an efficient means of supplementing existing germplasm for cultivar improvement in breeding programs all over the world (Dubinin 1961).

Crop improvement through mutation breeding has been achieved by use of different techniques; e.g., temperature treatment, long-term seed storage, generation of genetic variability by tissue culture, physical mutagenesis using both ionizing radiation sources (X-rays, γ-rays) and non-ionizing radiation sources (β-particles, α-particles, neutrons, protons and deuterons), as well as chemical treatments. The most commonly used chemical mutagens are methyl methane sulfonate, ethyl methane sulfonate (EMS), diethyl sulfate, ethyleneimine, methyl nitroso urea, ethyl nitroso urethane and ethyl nitroso urea (Manual of Mutation Breeding 1977).

Mutations induced by radiation have been explored in many crops, e.g., rice, wheat and soybean (Yang 1987); rapeseed (Uslu 1997); buckwheat, black mappe, peas and turnips (Danno *et al.* 1985); and safflower (El-Gayer and Heyab 1986; Uslu 1997). Petropoulos (1973) has produced mutants in fenugreek by irradiating open fields with chronic levels of γ-rays generated by Co^{60}, and by treating dry seed with acute levels of radiation. However, use of ionizing radiation requires special facilities to reduce the risk to human health, and to avoid uncontrolled chromosome breakage and induction of high levels of chromosome aberrations in the plants (Petropoulos 2002). Stimulatory effects of low dosages of radiation and, inhibitory effects of higher dosages on some morphological and yield characteristics of crops such as safflower have been reported, suggesting that

restricted use of radiation for mutation breeding may be applicable in some cases (Ramachandran and Goud 1983).

Some legume crops that have been improved through mutation breeding are soybeans, string beans and French beans (Sigurbjornsson and Micke 1974); Navy pea beans and haricot beans (Sigurbjornsson 1983); peas and fenugreek (Petropoulos 1973, 2002); and lupines (Gaul 1961). A majority of induced mutations in these plants are recessive, and can be observed to segregate in a 3:1 ratio in diploid crops like fenugreek (Gaul 1961; Petropoulos 1973; Singh and Singh 1974). Manha *et al* (1994) reported an increase in the level of steroidal sapogenin (diosgenin) in *Trigonella corniculata* following chemical mutagenesis using dimethyl and diethyl sulfate. In another study, Jain and Agarwal (1994) reported a 2-3 fold increase in steroidal diosgenin after treating fenugreek maintained in tissue culture with either UV light, 0.1 M ethyl methane sulfonate (EMS) or 0.025 M methyl methane sulfonate.

Chemical mutagens have been known to induce genetic variability in crop plants (Laxmi and Datta 1987). Improvement in both qualitative and quantitative characters of crop species following treatment with mutagens is well recognized (Sigurbjornsson 1977). Tanaka (1969) detected enhanced seed protein in mutant rice cultivars, while Doll (1975) reported a higher lysine content and better amino acid balance in mutant barley. Examples of plants where application of EMS has been used successfully are: safflower (Fernandez and Munoz 1987; Mallikarjunaradhaya 1978; Ramachandran and Goud 1983; Sahu and Kumar 1977), *Medicago* (Varma Penmetsa and Douglas 2000), *Arabidopsis* (Henikoff and Comai 2003) and opium poppy (Chauhan and Patra 1993).

Since fenugreek is a predominantly self pollinated crop and, the determinate trait is governed by recessive genes (Choudhury and Singh 2001) we believe that mutation breeding can be used to generate mutant plants with a determinate growth habit without losing beneficial adaptations and other agronomic traits of the base population. Based on literature surveys (Jain and Agarwal 1987; Laxmi *et al.* 1980; Laxmi and Datta 1987; Manual of Mutation Breeding 1977) and, success reported on use of the chemical mutagen EMS (an alkylating agent) for fenugreek improvement with respect to steroidal sapogenins and oil constituents (Petropoulos 1973, 2002; Singh and Raghuvanshi 1980; Zhu *et al.* 2003), EMS was used in the present study to improve seed yield and quality in fenugreek. Moreover, EMS is known to produce pleiotropic effects showing modification in more than one character possibly due to mutations induced at different loci (Gottschalk 1976) and, was expected to be useful in improving more than the intended seed yield in this crop. The principal objective of this mutation study was to develop early maturing fenugreek mutants with a determinate growth habit, high seed yield and seed quality.

3.2. Materials and Methods

3.2.1. Greenhouse study

Tristar fenugreek seed from the Agriculture and Agri-Food Canada (AAFC), Lethbridge Research Centre (LRC) Forage Laboratory were presoaked in distilled water for 4 h (for effective imbibition) and then treated with EMS (Sigma-Aldrich) at concentrations of 10, 20, 30, 40, 50, 100, 150, 200 and 300 mM made up in distilled water. Each concentration of EMS was prepared in 200 ml of distilled water and 100 seeds (M_0) were treated for 2, 4, 6, 8, 12, 16 and 24 h separately to increase the probability of generating maximum number of mutants with desirable characters. Fifty treated seeds from each concentration and treatment time combination were planted individually in the greenhouse in 6 inch plastic pots containing a non-sterile, soil-free mix (LRC Soiless mix/Cornell mix) and were designated as M_1 plants. Separate control blocks comprised of 50 plants of untreated Tristar fenugreek were planted for comparison of survival rates, maturity duration and, seed and forage yield for three successive generations (M_1-M_3) in the greenhouse. The soil free mix was composed of a 3.8 cubic feet bale of sphagnum peat moss, a 18.6 kg bag of medium horticultural grade vermiculite, 1000 g of calcium carbonate flour, 1500 g of 18-6-12 Osmocote (Southern Agricultural Insecticides, Inc.), 1200 g 0-21-0, 20 g of "Fritted" trace elements, 15 g of 13.2 % (W/W) chelated iron, 7 g of 14 % (W/W) chelated zinc and 30 L of washed mortar sand (The Scotts Co.).

These plants were grown for 85 days in a greenhouse set to cycle with 16 h day (22 °C) and 8 h nights (15 °C) after which the plants were desiccated with a 0.4 % (W/V) Reglone (Syngenta Crop Protection Canada Inc.) solution along with 0.23 % (W/V) of

AG-SURF (Interprovincial Cooperative Ltd.) as a surfactant. The plants were allowed to dry for 10 days before separating the seed for yield determination. The rate of survival was compared with the control and, various morphological and reproductive parameters of the EMS generated mutants were recorded. Plants were selected on the basis of number of pods, number of double pods, seed yield, above ground biomass (forage yield), early maturity and a determinate growth habit. Fifty progeny each of selected M_1 and M_2 plants were grown in the greenhouse using the same growing conditions. The dry weight of the above ground biomass and various components of seed yield were monitored to identify desirable mutants.

Fifteen M_2 plants were selected to grow M_3 progeny using a divergent selection (top five high yielding, five intermediate yielding and bottom five seed yielding lines). Fifty seeds from each of these fifteen lines were then individually seeded in the greenhouse. Another selection cycle (based on the performance of the lines in the M_3 generation) was initiated by selecting the top 10 high yielding lines from M_3 for producing an M_4 generation in the same manner as described above.

3.2.2. Field study

3.2.2.1. Materials

Seeds from M_1 bulk (top 32 lines), M_2 (top 12 selected lines), M_3 (top 19 selected lines) and M_4 (top 35 selected lines) selected on the basis of selection criteria as discussed in subsection 3.2.1 were seeded in LRC research plots under irrigation.

3.2.2.2. Study site

The field trial site was located at Lethbridge (49° 45' N; 112° 45' W), Alberta, Canada located about 200 kilometers south east of Calgary on the Oldman River. It has an elevation of 900 m MSL (Harsh 1985) and is characterized by an Orthic Dark Brown Chernozem soil type (Wyatt *et al.* 1939). Lethbridge is located on semi-arid lands and is dependent on irrigation for successful crop production (Harsh 1985). It has a moderate continental climate with warm winters and mild summers. The annual average maximum temperature is 12.1 °C and the minimum is -1.0 °C. The average annual snowfall is 160 cm and annual average precipitation is 262 mm (Wyatt *et al.* 1939). The GPS coordinates of the irrigated field plots at the LRC were 49° 42' 24.98" N and 112° 45' 47.77" W.

3.2.2.3. Experimental design

Seed of all M generations were seeded in the LRC irrigation field (50 mm X 4) on May 25, 2005 using a Split-Plot Design with two times replicated Randomized Complete Block Design (RCBD), randomized within each plot. In each case plots were seeded using a custom built forage seeder and consisted of a 3 m long single row plot spaced 1 m apart with 120 seeds for the M_1 generation and 50 seeds for M_2, M_3 and M_4 generations due to shortage of seed for the advanced generations. Each generation was seeded in a separate block with separate control plots. Tristar control plots had 120 seeds per 3 m row. After one month (June 29, 2005) the plots were scored for proportion of the row with plants. The plots were hand harvested on October 7 and 12, 2005 due to inclement weather conditions. After drying the material for one week indoors, the dry weight of the above ground biomass was determined for each row. The seeds were separated from rest of the plant, cleaned and weighed to determine the seed yield and 1000 seed weight for

each line used in the experiment. Since all the lines did not have the same number of plants, the seed yield was adjusted using the relative proportion of surviving plants. The equation used for this purpose was:

Adjusted Yield = [(Observed Yield X 100) / % stand as measured on June 29].

The lines showing an average stand height of less than 15 cm and maturing within 90 days were considered to be "determinate" in growth habit. The lines with a determinate growth habit, high forage and seed yield, early maturity, and stable performance were identified.

3.2.3. Scanning Electron Microscopic (SEM) studies

A comparative SEM study of the floral structures, pollen grains and seed surface of normal Tristar fenugreek and mutant plants was done at the Electron Microscopy and Digital Image Processing Laboratory (EMDIPL) of LRC using a SEM-Hitachi S-570 electron microscope. The magnifications used were 50, 150, 500 and 2500 X. The samples were fixed in 4 % glutaraldehyde (Canemco, St. Laurent, Quebec) in sodium phosphate buffer (Sigma Aldrich) for 24 h and washed 3 X in sodium phosphate buffer for 10 minutes each. Then the samples were dehydrated using a graded series of ethanol (Sigma Aldrich) at 35, 50, 70, 85, 95 and 100 % respectively with 3 X wash for 10 minutes each. The samples were then dried in liquid CO_2 in a critical point drier at a critical pressure of 1073 psi and a temperature of 31.3 °C (Polaron, Watfod England, Serial number 1318385S2) to remove any trace of moisture. Finally the specimens were mounted on SEM pint type specimen stubs AS 101-0 (Canemco-Marivac, Canada), sputter coated with gold on a sputter coater (Denton Vaccum, Desk II) and then observed under the electron microscope.

3.2.4. Statistical analysis

The mutation breeding study was not amenable to statistical manipulation as the objective was to identify mutants with desired characteristics. However, means and standard errors for the four generations were calculated using Agrobase 99 (Agronomix Software, Inc. 1999) software. A frequency distribution of the seed yield for M_2-M_4 generations was done using Microsoft Excel® software.

3.3. Results

3.3.1. Greenhouse study

The EMS treated plants representing the three successive generations (M_1-M_3) exhibited a distinct pattern of survival rates compared to their respective control groups (Table 3.3.1.1.). A representative sample of the EMS generated M_3 plants growing in the greenhouse is shown in Figure 3.3.1.1.

The survivability levels decreased from low to high concentrations of EMS (10-300 mM) in all three generations. The survivability among different treatment times under each individual concentration of EMS used also decreased from shorter to longer treatment times *i.e.*, from 2-24 hours. These observations indicate that combinations of longer treatment times with higher dosages of EMS applied to the base population of Tristar fenugreek (M_0 seed) generated higher levels of lethal mutants, across the three generations. The rate of survival among the mutant plants varied from 7.8–84.7 % in the M_1 generation, to 49.5–86.3 in the M_2, and 90.9–96.4 % in the M_3 generation (Table 3.3.1.1.).

A number of recessive mutants showing dwarfness, albinism, virina xanthescens and other abnormal phenotypes (double pods or twin pods compared to normal single pods, terminal or apical flowers compared to normal axillary flowers) were observed (Figure 3.3.1.2.). However, many of these plants died before maturity and the poor seed and forage yielding plants were not carried forward because of a strong selection criterion for high seed yield in the study. The number of detrimental mutants was reduced significantly in the population of elite plants used in the M_3 generation before the progeny were subjected to field trials in the M_4 generation.

Table 3.3.1.1. Percent seedling survival in M_1, M_2 and M_3 generations when grown under greenhouse conditions in 2004 and 2005.

Concentration (mM)	M_1 (2004)	M_2 (2004)	M_3 (2005)
Control	96.3	98.2	97.5
10	84.7	86.3	96.4
20	79.2	78.5	95.2
30	68.2	68.8	93.4
40	41.2	66.6	93.2
50	28.8	64.3	92.4
100	30.1	55.8	91.3
150	17.4	51.9	91.5
200	12.6	50.8	91.7
300	7.8	49.5	90.9

Figure 3.3.1.1. EMS generated M_3 fenugreek plants (single plant per pot) growing in the LRC greenhouse. These M_3 plants were derived from selected M_2 plants with high yield performance and determniate growth habit, plants showing earliness and determinate growth habit were marked with red tape. (Spring, 2005).

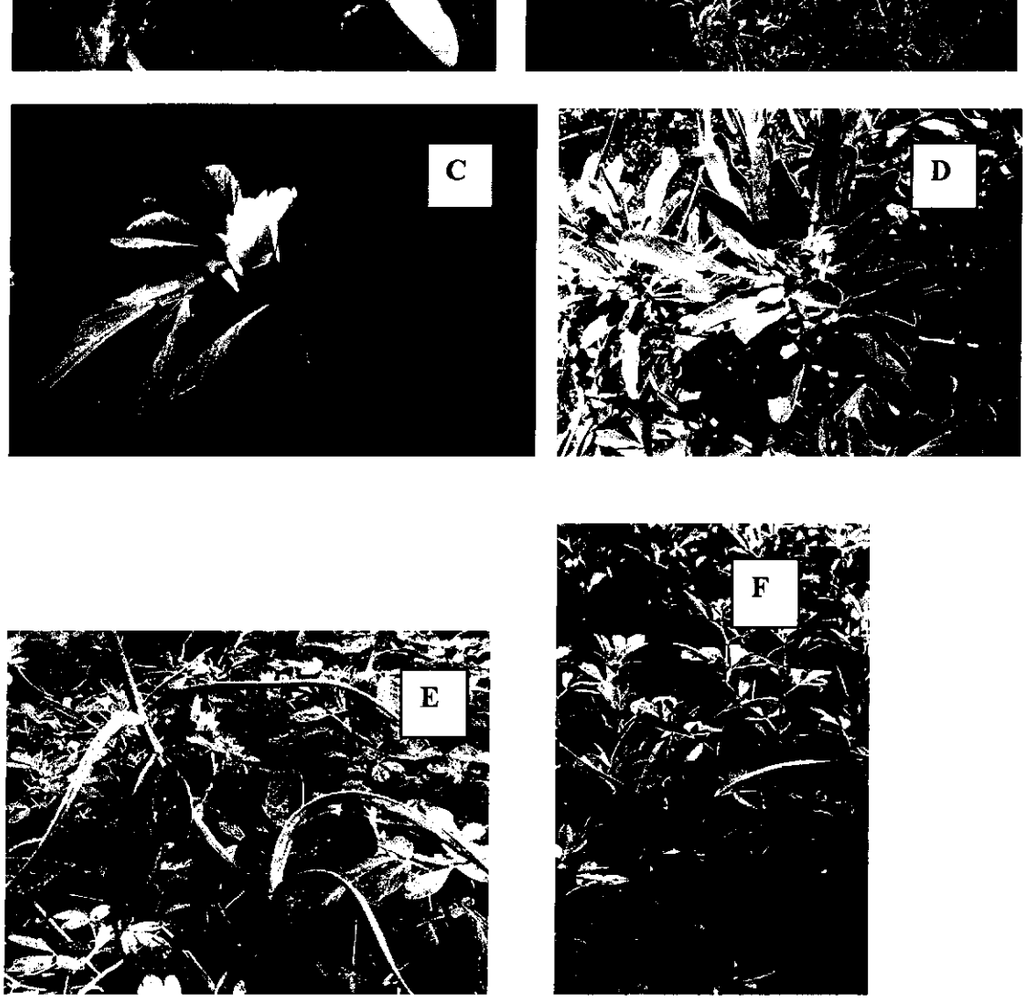


Figure 3.3.1.2. (A) Albino and Xantha leaves in a mutant fenugreek plant growing in the greenhouse; (B) normal fenugreek with green foliage; (C) a mutant showing apical flowers; (D) a normal plant with axillary flowers; (E) fenugreek mutant with double pods and (F) normal plants showing single pods.

The greenhouse study also indicated a wide variability in the morphometric and reproductive parameters of mutant plants; *i.e.*, above ground height and biomass, internode length, number of nodes and pods (single, double and total), length of pods and seed size of the EMS generated mutant plants (Tables 3.3.1.2., 3.3.1.3. and 3.3.1.4.). The level of segregation in the traits examined (Tables 3.3.1.2., 3.3.1.3. and 3.3.1.4.) was reduced with each advanced generation, showing indications of stabilization by M_4.

It is encouraging to note that mutants produced in this study and grown under greenhouse conditions showed signs of a high proportion of double or twin pods, improvement in pod length, seed yield and seed size across the different EMS concentrations from M_1 to M_3. However, the M_3 plants (Table 3.3.1.4.) showed lower above ground biomass compared to that of the M_2 plants (Table 3.3.1.3.).

Table 3.3.1.2. Ranges of different morphometric parameters of the M_1 plants (single plant per pot) generated in greenhouse, measured after 95 days of seeding when the plants were sprayed with desiccant (2004 spring).

Conc. (mM)[1]	Height (cm)	Inter-node length (cm)	No. of nodes	Total no. of pods	No. of single pods	No. of double pods	Pod length (cm)	Dry weight (g)[2]	Seed no.[3]	1000 seed weight (g)[4]
C*	19.7-78.2	1.3-3.7	18-23	9-16	10-17	-	0.9-5.8	2.3-13.6	15-129	1.2-2.8
10	18.8-67.6	0.9-7.6	6-21	6-25	0-14	0-12	0.3-15.9	1.6-10.5	9-336	0.4-4.2
20	30.3-81.2	0.9-16.1	8-19	4-18	0-13	0-7	1.3-19.8	1.6-10.3	68-271	0.6-4.1
30	31.3-74.4	1.8-17.1	9-24	6-21	0-17	0-7	0.5-19.5	1.6-7.7	56-306	0.3-2.7
40	32.4-72.2	2.0-7.5	11-27	5-22	0-17	0-6	0.4-21.6	2.2-9.4	59-324	0.5-2.8
50	30.2-65.8	2.2-12.3	5-24	5-27	1-15	0-7	0.2-21.1	2.1-8.1	3-176	0.03-3.4
100	33.2-72.7	1.9-13.1	12-23	6-36	1-26	0-9	0.7-24.3	0.1-7.7	5-190	0.01-2.5
150	28.7-75.1	0.8-43.9	11-29	8-67	1-29	0-19	0.6-21.6	1.2-11.6	4-289	0.05-2.4
200	25.7-73.6	0.5-8.6	7-26	4-90	0-36	0-27	0.2-18.8	1.4-36.8	5-1352	0.01-10.1
300	7.5-35.7	1.1-5.9	4-26	0-18	0-7	0-6	0.8-11.4	0-4.4	0-173	0-1.7

* Untreated control

[1] Concentarions of EMS expressed in millimolar (mM)

[2] Above ground biomass of individual plant

[3] Number of seeds from the pods produced in a single plant

[4] Seed size (weight of 1000 seed)

Table 3.3.1.3. Ranges of different morphometric parameters of the M_2 plants (single plant per pot) generated in greenhouse, measured after 95 days of seeding when the plants were sprayed with desiccant (2004 summer).

Conc. (mM)[1]	Height (cm)	Inter-node length (cm)	No. of nodes	Total no. of pods	No. of single pods	No. of double pods	Pod length (cm)	Dry weight (g)[2]	Seed no.[3]	1000 seed weight (g)[4]
C*	29.5-68.3	1.1-4.1	14-33	15-22	4-16	-	1.8-2.8	2.5-10.3	18-183	1.1-2.6
10	11.6-42.4	1.1-5.3	11-44	10-38	4-23	1-13	2.6-6.9	3.18-11.8	98-388	1.2-4.6
20	6.7-45.6	1.1-5.3	13-56	2-52	4-34	2-15	1.5-8.6	5.6-19.6	89-556	1.2-6.1
30	16.7-67.3	1.3-6.3	18-51	17-52	3-32	2-14	2.3-11.2	3.0-17.3	98-467	1.2-5.2
40	6.9-45.6	1.1-4.8	9-41	5-37	1-28	0-8	3.5-13.7	4.1-19.0	124-756	1.6-8.3
50	33.9-83.4	1.6-6.3	28-77	23-82	2-44	3-23	1.7-11.5	4.6-22.4	0-654	1.4-5.8
100	11.9-45.3	1.2-4.1	9-39	7-41	3-22	0-13	3.2-9.1	4.5-13.7	0-667	1.2-5.0
150	9.1-35.1	0.5-3.4	6-29	4-27	4-21	0-5	2.5-10.8	3.5-11.8	125-636	1.5-5.3
200	17.5-48.9	1.1-5.3	15-54	10-58	3-34	2-18	3.2-12.8	1.1-22.6	122-552	1.8-5.9
300	5.6-28.9	1.2-2.8	3-31	5-48	3-23	0-14	1.9-13.2	5.7-38.5	163-759	1.1-9.8

* Untreated control

[1] Concentarions of EMS expressed in millimolar (mM)

[2] Above ground biomass of individual plant

[3] Number of seeds from the pods produced in a single plant

[4] Seed size (weight of 1000 seed)

Table 3.3.1.4. Ranges of different morphometric parameters of the M_3 plants (single plant per pot) generated in greenhouse, measured after 95 days of seeding when the plants were sprayed with desiccant (2005 spring).

Conc. (mM)[1]	Height (cm)	Inter-node length (cm)	No. of nodes	Total no. of pods	No. of single pods	No. of double pods	Pod length (cm)	Dry weight (g)[2]	Seed no.[3]	1000 seed weight (g)[4]
C*	36.5-73.2	1.2-3.5	22-38	15-22	10-13	-	0.8-2.5	4.5-12.3	20-176	1.1-4.8
10	34.5-70.6	1.3-2.9	19-31	16-37	6-19	4-15	1.2-9.3	3.5-10.8	26-133	1.2-4.8
20	38.6-76.3	1.3-4.2	21-48	20-51	12-33	4-18	1.5-8.7	4.7-12.3	29-146	1.3-6.9
30	35.7-75.8	1.5-6.3	26-71	25-49	22-38	6-22	1.6-9.2	5.3-14.8	33-159	1.3-6.7
40	44.5-68.9	1.2-5.8	31-77	29-67	26-43	9-26	1.6-8.7	5.5-16.8	28-166	1.2-5.9
50	55.9-86.8	1.3-5.3	36-81	33-73	14-51	6-25	1.8-12.2	6.1-17.3	6-162	1.1-6.1
100	37.9-70.5	1.3-5.2	32-56	41-77	16-44	8-19	5.5-11.1	4.6-10.6	66-421	1.5-6.4
150	38.9-71.2	1.4-3.9	26-44	26-41	11-23	6-16	4.2-9.9	3.4-10.3	23-228	1.6-7.2
200	37.8-61.3	1.3-3.3	22-58	19-57	7-25	4-19	4.3-14.8	4.9-15.1	24-316	1.6-8.7
300	9.7-15.2	1.2-5.3	16-21	8-21	0-9	2-8	13.5-22.4	2.1-10.6	65-362	1.7-11.7

* Untreated control
[1] Concentarions of EMS expressed in millimolar (mM)
[2] Above ground biomass of individual plant
[3] Number of seeds from the pods produced in a single plant
[4] Seed size (weight of 1000 seed)

No distinct pattern in earliness (maturity) was detected in M_1 and M_2 generations when compared to their respective control groups. The plants from the different treatment groups exhibited a random pattern of maturation; *i.e.*, some plants matured early while others matured late. Variation in maturity among the M_4 lines grown under 2005 irrigation field conditions is shown in Figure 3.3.1.3.

However, in M_3 a distinct pattern with respect to early maturing plants was identified when compared to the control. Plants originally treated with 10-50 mM EMS were late maturing; while those originally treated with 100-200 mM EMS matured at the same time as the untreated control group. Plants treated with 300 mM EMS matured a week earlier than the control. The seed size from the 300 mM EMS treatment improved over the three generations; i.e., the 1000 seed weight was 0-1.7 g in M_1 (Table 3.3.1.2.), 1.1–9.8 g in M_2 (Table 3.3.1.3.) and 1.7–11.7 g in M_3 (Table 3.3.1.4.). The plant height of the mutant plants was reduced considerably in M_3 (9.7–15.2 cm, Table 3.3.1.4.) compared to M_1 (7.5–35.7 cm, Table 3.3.1.2.) and M_2 (5.6–28.9 cm, Table 3.3.1.3.). These observations indicate that the mutant plants generated with 300 mM EMS produced the best possible combination of characters among the mutant plants. The M_4 seed produced from the M_3 plants grown in the greenhouse showed that selection for early maturity and high seed yield was effective. Some lines (particularly the 300 mM treatment group) exhibited all of the desirable characteristics; *i.e.*, a determinate growth habit (apical growth in determinate plants is arrested quickly making the seed pods mature early. This has the potential to produce high quality seed within the limited growing period available on the Canadian prairies.), early maturity, high seed yield and quality.

Figure 3.3.1.3. M_4 lines growing under LRC irrigated field conditions (Summer, 2005) showing variation in maturity relative to control Tristar plants. The maturity duration increases from left to right.

3.3.2. Field study

Under field conditions M_1 to M_4 plants flowered (50 % flowering in each replicate) between July, 15th and 22nd while plants from the control group began flowering on July, 22 nd. Initiation of pod filling (50 % of the plants in a row showing pod initiaon) in most of the plants from the M_1 to M_4 generations was observed around August 16th to 23rd, in comparison to August, 23rd to 29th for the control group. In general, a majority of the mutant plants showed early flowering and early maturity relative to the untreated Tristar fenugreek.

The field study showed a distinct pattern in plant habit and associated yield characteristics among M_1 to M_4 plants (Tables 3.3.2.1., 3.3.2.2., 3.3.2.3. and 3.3.2.4.). The M_4 plants showed less variability (Table 3.3.2.4.) compared to the variability observed for growth parameters and yield characteristics in earlier (M_1, M_2 and M_3) generations (Tables 3.3.2.2., 3.3.2.3. and 3.3.2.4.). The mean seed yield and forage yield values for M_1 and control plants was 1947 and 8723 kg ha $^{-1}$ and, 247.6 and 811.8 kg ha $^{-1}$ respectively. The mean seed size (based on 1000 seed weight) for the untreated control was found to be much lower (18.1 g) than the mean M_1 seed size (23.6 g) (Table 3.3.2.1.). The mean seed yield (1210 kg ha $^{-1}$), forage yield (6948 kg ha $^{-1}$) and seed size (23.4 g) in M_2 also were higher than the control values (236.1 and 802.5 kg ha $^{-1}$, and 19.1 g respectively) (Table 3.3.2.2.). Similarly, the mean forage yield, seed yield and seed size in M_3 and M_4 were higher than the respective mean control values (Tables 3.3.2.3. and 3.3.2.4.).

All of the lines included in the M_1 generation were shorter in height (mean = 20.4 cm) than the untreated control plants (mean = 36.7 cm) (Table 3.3.2.1.). The M_2 , M_3 and

M_4 plants were also shorter than their respective controls (Tables 3.3.2.2., 3.3.2.3., 3.3.2.4.). The proportion of determinate lines increased in successive generations, *i.e.*, none were seen in M_1 (Table 3.3.2.1.), only one (Line 125) in M_2 (Table 3.3.2.2.), eight in M_3 (Table 3.3.2.3.) and 14 were determinate in M_4 (Table 3.3.2.4.). Line numbers 88-34, 131-19, 227-22, 227-36, 340-6 and 340-10 exhibited a determinate growth habit, as well as high performance (with respect to forage and seed yield) in both M_3 and M_4 generations, indicating the effectiveness of the selection regime in the greenhouse.

Table 3.3.2.1. Mean growth ± SE (where appropriate) and yield parameters of M_1 plant lines (bulk) grown under LRC irrigation (2005).

Line numbers[1]	% seedling Survival[2]	Height (cm)[3]	Forage yield ($kgha^{-1}$)[4]	Seed yield ($kgha^{-1}$)	1000 seed wt. (g)[5]
Control	**45±0.5**	**36.7±2.2**	**811.8±55.0**	**247.6±21.3**	**18.1±0.3**
10mM-2h	39	20.0	10184	2499	23.6
10mM-4h	48	18.5	10798	2100	23.4
10mM-6h	45	22.0	10372	2332	23.1
10mM-8h	42	22.5	10110	2125	22.7
20mM-2h	38	21.5	6981	1717	23.2
20mM-4h	40	19.0	6505	1301	24.2
20mM-6h	44	21.5	6998	2220	24.5
20mM-8h	42	21.5	8941	2406	22.9
30mM-2h	44	19.5	6819	1193	23.3
30mM-4h	42	18.5	11312	3149	23.2
30mM-6h	41	20.5	7891	2069	23.9
30mM-8h	38	22.5	7704	2084	23.5
40mM-2h	41	22.0	6529	2050	23.8
40mM-4h	45	17.0	8997	1753	23.3
40mM-6h	40	21.5	13041	3049	23.1
40mM-8h	40	20.5	8389	1653	23.8
50mM-2h	40	21.0	6686	1513	24.1
50mM-4h	45	21.0	8879	3028	24.9
50mM-6h	42	22.0	6088	1363	24.2
50mM-8h	39	22.5	7780	1021	23.6
100mM-2h	43	21.0	7918	1414	23.3
100mM-4h	33	21.5	10968	1807	23.9
100mM-6h	40	19.0	8148	2223	24.6
100mM-8h	37	22.0	6739	1690	23.6
150mM-2h	29	19.0	23832	3538	22.8
150mM-4h	42	20.5	7977	1610	22.8
150mM-6h	40	18.0	7653	2579	24.6
150mM-8h	33	17.5	7079	653	23.2
200mM-2h	43	21.5	6978	1922	22.9
200mM-4h	44	20.5	8827	1449	23.4
200mM-6h	40	18.0	6542	2126	24.0
200mM-8h	47	19.5	5476	684	24.1
Combined Mean	**40.8**	**20.4**	**8723**	**1947**	**23.6**
± SE	**0.7**	**0.3**	**613**	**119**	**0.1**

[1] **Line numbers in this case refer to bulked seed of all M_1 selected plants from each initial treatment of Tristar fenugreek base population (M_0). Only treatment combinations with sufficient amount of seed for two replications were included in the field trial.**
[2] **Percentage of emerging seedlings counted when they were ~1 cm in height in the field.**
[3] **Heights of plants measured on 104 days from the day of seeding.**
[4] **Above ground biomass of 2 m row measured before seed separation.**
[5] **Seed size (weight of 1000 seed) determined on each row.**

Table 3.3.2.2. Mean growth ± SE (where appropriate) and yield parameters of M_2 selected lines grown under LRC irrigation (2005). Lines with a determinate growth habit are highlighted.

Line numbers[1]	% seedling Survival[2]	Height (cm)[3]	Forage yield ($kgha^{-1}$)[4]	Seed yield ($kgha^{-1}$)	1000 seed wt. (g)[5]
Control	**44±1.6**	**24.6±2.2**	**802.5±12.4**	**236.1±11.2**	**19.1±0.5**
1	35	16.5	13646	2440	22.9
12	45	20.5	6598	1122	23.6
60	46	15.0	7746	2063	23.7
75	44	19.5	6880	1234	24.1
81	43	18.0	6191	1481	23.3
125*	**47**	**14.5**	**4476**	**1048**	**23.7**
131	43	16.5	5912	930	23.6
134	42	20.0	3518	493	24.2
162	43	17.5	7468	1138	22.8
191	34	20.0	8945	917	22.7
256	47	15.0	5058	422	22.8
242	40	17.5	6939	1233	23.1
Combined Mean ± SE	**42.4 1.2**	**17.5 0.6**	**6948 806**	**1210 181**	**23.4 0.1**

*** Line with a determinate growth habit**

[1] Line numbers in this case refer to M_2 selected plants from high yielding and early maturing M_1 lines. Only treatment combinations with sufficient amount of seed for two replications were included in the field trial.

[2] Percentage of emerging seedlings counted when they were ~1 cm in height in the field.

[3] Heights of plants measured on 104 days from the day of seeding.

[4] Above ground biomass of 2 m row measured before seed separation.

[5] Seed size (weight of 1000 seed) determined on each row.

Table 3.3.2.3. Mean growth ± SE (where appropriate) and yield parameters of M_3 selected lines grown under LRC irrigation (2005). Lines with a determinate growth habit are highlighted.

Line numbers[1]	% seedling Survival[2]	Height (cm)[3]	Forage yield ($kgha^{-1}$)[4]	Seed yield ($kgha^{-1}$)	1000 seed wt. (g)[5]
Control	**47±0.8**	**23.4±2.1**	**833.5±22.1**	**241.7±11.3**	**19.3±1.2**
48-4	42	21.0	14179	3691	23.8
131-1	38	15.0	8178	1277	22.2
131-19*	**44**	**13.0**	**7303**	**898**	**23.2**
134-5	45	16.5	9660	1557	22.8
225-2	43	18.5	11608	3383	23.5
88-34*	**46**	**14.0**	**7392**	**1161**	**22.5**
88-37*	**44**	**14.5**	**9247**	**1676**	**23.0**
227-11	39	19.0	11984	2290	23.1
227-12	46	18.5	7600	1267	22.7
227-22*	**42**	**14.0**	**8056**	**1404**	**22.9**
227-36*	**38**	**14.5**	**5873**	**617**	**21.9**
242-9	45	17.0	8689	2528	22.7
267-13	42	17.5	10446	2684	23.6
309-19*	47	**13.5**	**7539**	**892**	**22.5**
340-5	44	17.5	9189	2557	22.9
340-6*	**45**	**13.0**	**6525**	**991**	**23.1**
340-10*	**45**	**14.0**	**6122**	**1076**	**23.7**
340-12	45	18.5	7247	1753	23.6
340-15	42	15.0	7579	1955	23.1
Combined Mean	**43.2**	**16.03**	**8653**	**1771**	**23.00**
± SE	**0.6**	**0.5**	**432**	**159**	**0.1**

***Lines with a determinate growth habit**

[1] Line numbers in this case refer to M_3 selected plants from high yielding and early maturing M_2 lines. Only treatment combinations with sufficient amount of seed for two replications were included in the field trial.

[2] Percentage of emerging seedlings counted when they were ~1 cm in height in the field.

[3] Heights of plants measured on 109 days from the day of seeding.

[4] Above ground biomass of 2 m row measured before seed separation.

[5] Seed size (weight of 1000 seed) determined on each row.

Table 3.3.2.4. Mean growth and yield parameters of M_4 selected lines grown under LRC irrigation (2005). Lines with a determinate growth habit are highlighted.

Line numbers[1]	% seedling Survival[2]	Height (cm)[3]	Forage yield ($kgha^{-1}$)[4]	Seed yield ($kgha^{-1}$)	1000 seed wt. (g)[5]
Control	**46±1.3**	**23.3±1.6**	**607.5±52.1**	**152.8±18.4**	**19.4±0.6**
48-4-7	42	17.5	8255	1825	24.2
131-19-1*	**27**	**14.0**	**8535**	**388**	**22.0**
134-5-3*	**28**	**14.5**	**3966**	**211**	**24.1**
88-34-2*	**44**	**13.5**	**7052**	**845**	**23.2**
88-34-3*	**39**	**14.0**	**6834**	**643**	**22.9**
88-34-4	47	18.5	7942	1179	23.6
88-34-6	42	16.5	6802	1447	23.3
88-34-8*	**46**	**13.5**	**6454**	**862**	**21.9**
88-34-16	45	15.0	7760	1183	23.6
225-2-7*	**45**	**13.0**	**9418**	**2319**	**23.6**
225-2-17	45	15.0	9276	2126	23.6
227-11-5*	**26**	**14.5**	**13883**	**1891**	**23.5**
227-11-6	32	17.0	9181	1171	22.6
227-11-17*	**29**	**13.5**	**6733**	**794**	**23.5**
227-12-4*	**41**	**14.5**	**5129**	**589**	**23.2**
227-12-13	34	15.0	9453	1147	23.4
227-22-12	31	17.0	11630	2045	22.5
227-22-15*	**40**	**13.5**	**7891**	**795**	**22.2**
227-36-2*	**41**	**17.0**	**7348**	**1097**	**23.7**
309-19-3	39	16.0	9806	1491	24.3
309-19-11	43	16.5	8112	1063	23.6
340-6-3	28	16.5	11731	1754	23.4
340-6-6*	**40**	**14.0**	**7570**	**1149**	**23.4**
340-6-12	45	16.0	7323	1335	22.7
340-6-15	31	15.0	7956	1462	22.2
340-10-2	42	16.0	5892	909	23.8
340-10-6*	**43**	**14.0**	**7432**	**1234**	**22.4**
340-10-10	44	19.0	8196	1063	22.3
340-10-12	36	18.0	11906	2021	22.9
340-10-14*	**42**	**14.0**	**4530**	**348**	**23.0**
340-10-17	42	15.0	7015	1145	23.0
Combined Mean	**38.6**	**15.6**	**7958**	**1141**	**23.08**
± SE	**1.1**	**0.3**	**311**	**80**	**0.1**

***Lines with a determinate growth habit**

[1] Line numbers in this case refer to M_4 selected plants from high yielding and early maturing M_3 lines. Only treatment combinations with sufficient amount of seed for two replications were included in the field trial.

[2] Percentage of emerging seedlings counted when they were ~1 cm in height in the field.

[3] Heights of plants measured on 109 days from the day of seeding.

[4] Above ground biomass of 2 m row measured before seed separation.

[5] Seed size (weight of 1000 seed) determined on each row.

In addition to 14 determinate lines in the M_4 generation there were several indeterminate but high yielding lines in this generation (Table 3.3.2.4.). Among the promising indeterminate, high seed yielding lines are 225-2-17 (2126 kg ha $^{-1}$), 227-22-12 (2045 kg ha $^{-1}$), 340-10-12 (2021 kg ha $^{-1}$), 48-4-7 (1825 kg ha $^{-1}$) and 340-6-3 (1754 kg ha $^{-1}$). The high seed yielding determinate lines are 225-2-7 (2319 kg ha $^{-1}$), 227-11-5 (1891 kg ha $^{-1}$), 340-10-6 (1234 kg ha $^{-1}$) and 340-6-6 (1149 kg ha $^{-1}$). A number of M_4 lines although considered determinate in this study had poor seed yield such as 131-19-1 (388 kg ha $^{-1}$), 134-5-3 (211 kg ha $^{-1}$) and 340-10-14 (348 kg ha $^{-1}$) and so were not considered useful for breeding purposes (Table 3.3.2.4.).

The frequency distribution of the mean seed yield (kg ha^{-1}) of M_2, M_3 and M_4 populations is shown in Figure 3.3.2.1. This figure shows that the frequency of high yielding lines in M_3 is higher than the frequency of high yielding lines in M_2 and M_4. The mean seed yield for M_3 (1771 kg ha $^{-1}$) was also higher than M_4 (1166 kg ha $^{-1}$) and M_2 (1210 kg ha $^{-1}$). A majority of the lines yielded between 750 to 2000 kg ha^{-1} for the three generations included in this figure. It is important to note that there were a few lines from the M_2 and, M_3 generations that yielded over 4000 kg ha^{-1} and within the lines some plants were variable for growth and seed yield characteristics.

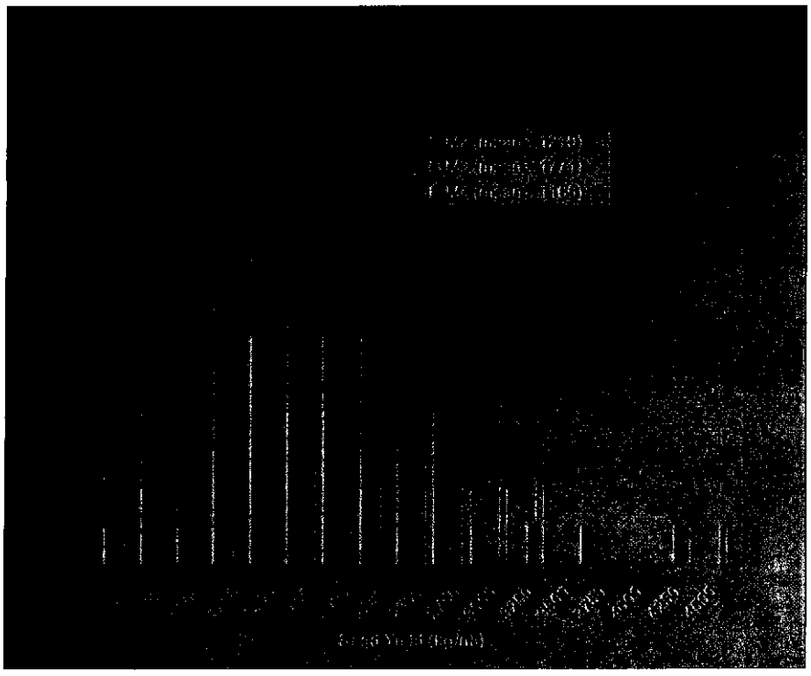
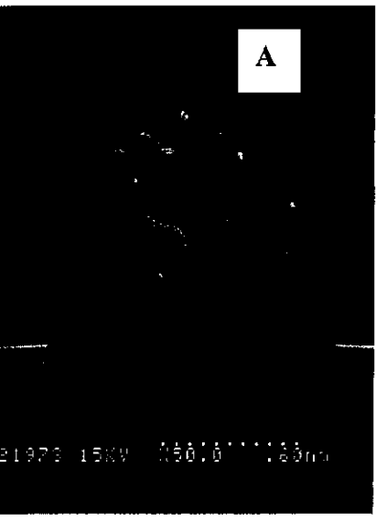


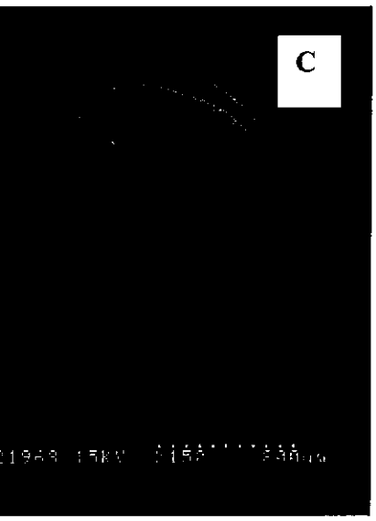
Figure 3.3.2.1. Frequency distribution of the mean seed yield (kg ha^{-1}) of M_2, M_3 and M_4 populations.

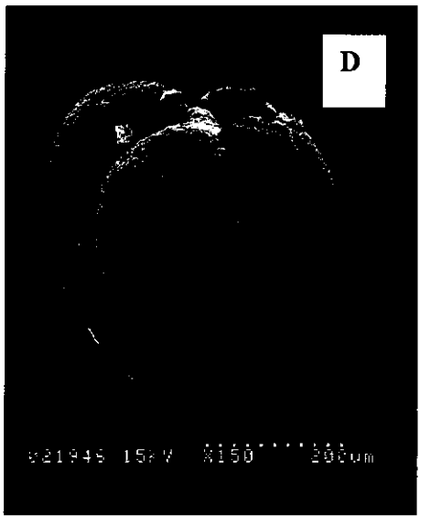
A comparative SEM study of the floral structures using 20 randomly selected normal and mutant flowers (androecium, gynoecium and anther lobes), pollen grains and seed surfaces from normal Tristar fenugreek and mutant plants showed no striking differences. This indicated that the mutation(s) induced did not drastically alter the reproductive and morphological structures of the EMS generated mutants (Figure 3.3.2.2.). Hence chances of getting sterile pollen and/or abnormal seed were considered low in these mutants.

The number of lethal mutants detected under field conditions was much lower in comparison to that observed in the greenhouse. Those that did germinate died well before flowering, naturally eliminating these plants from contributing towards the existing gene pool. Such plots were eliminated from our study to avoid unnecessary introduction of a detrimental seed source into the high yielding and early maturing elite lines.


A


B


C


D

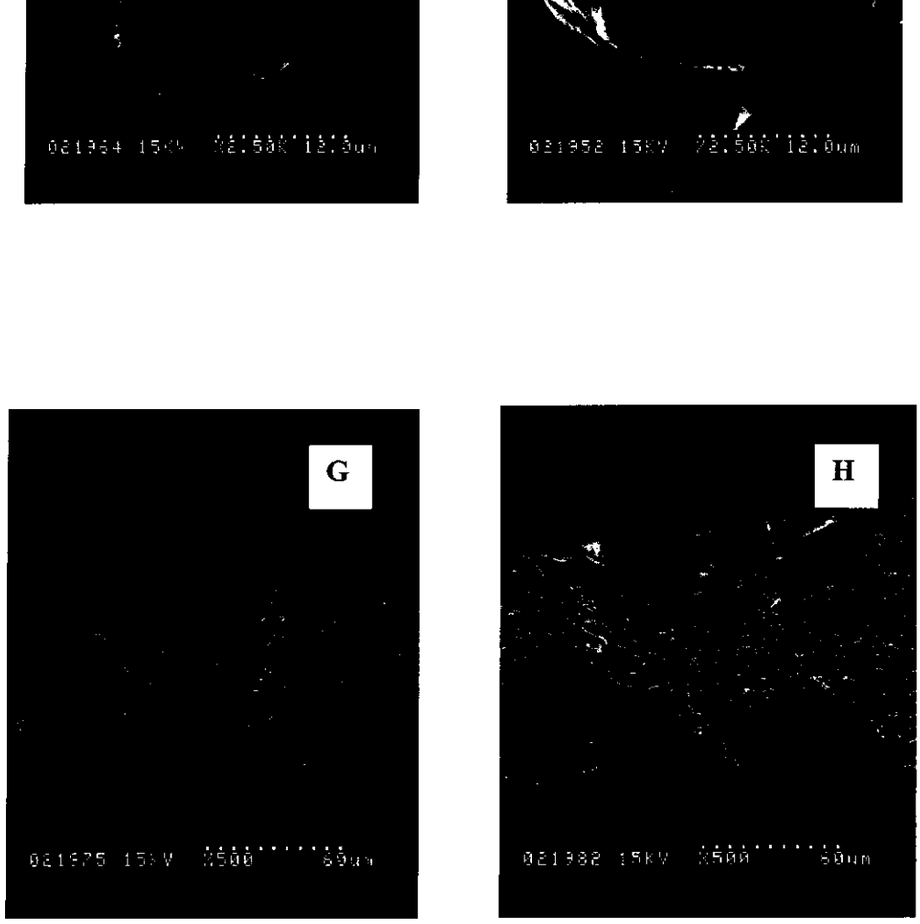


Figure 3.3.2.2. Side by side presentation of representative SEM pictures of mutant (left) and normal Tristar (right) (A) and (B) androecia at 50 X; (C) and (D) anther lobes at 150 X; (E) and (F) pollen grains at 2500 X; and (G) and (H) seed surfaces at 500 X.

3.4. Discussion

In addition to allelic mutations, small deletions and other chromosomal rearrangements are induced by EMS; most importantly EMS alkylates guanine bases and leads to mispairing or mismatch pairing within the genome. In these particular types of mutations, alkylated G bases pair with T in place of C, thereby inducing point mutations due to G/C to A/T transitions (Henikoff and Comai 2003). So, it is possible that a single mutation in a gene has the potential to activate morphometric and reproductive changes in the Tristar base population; further selection of mutant plants through a number of generations could eliminate deleterious mutations, resulting in a positive effect of chemical mutagenesis induced by EMS.

Seeds treated with EMS were observed to have produced mutants of different kinds (Tables 3.3.1.2., 3.3.1.3. and 3.3.1.4.). This may have resulted from a pleiotropic effect of mutated genes or mutations at different loci as was observed by Gottschalk (1976). A number of morphological mutations have been reported in legume plants (Sjodin 1971) and several of these mutations exhibited modifications in more than one character. Mutants with desirable seed yield components expressed the traits for four successive generations in greenhouse and field conditions indicating high heritability for the traits. This could have been due to the fact that a change in a single base pair in the DNA is involved in this case as was observed by Singh and Singh (1974) for a spontaneous "green-trailing" fenugreek mutant. They also suggested that in such cases there is a high possibility that the gene involved could have pleiotropic effects or that the trait(s) involved is governed by multiple genes with tight linkage that is being transmitted as a single unit. Similar results have been reported by Saini *et al.* (1974) for sterile

mutants of *Phaseolus aureus*, another related legume crop. They reported that although many characters were affected, only single gene differences were detected. The results of the present study indicate that multiple traits have been affected, but whether this is due to single gene or multiple genes or multiple genes acting as one functional gene complex is not clear at this stage. (Details of a cytogenetic study of root tip chromosomes of untreated plants and mutant lines are discussed in Chapter Five).

In this study a comparison of the pollen structure of normal and mutant plants did not show any significant difference (Figure 3.3.1.2.). Selected EMS induced mutants from Tristar fenugreek did not show drastic changes, suggesting that minor changes in a single gene or a gene complex were introduced in the treated plants. More importantly the selected mutants in the present study could be viable genetic material for improvement in fenugreek seed yield. Some of the mutant lines showed a determinate growth habit and these mutants could be double recessives as suggested by Choudhury and Singh (2001) for fenugreek.

The selection criteria used in this study resulted in a reduction in the segregation of poor performing phenotypes or deleterious alleles for the traits being examined during each generation of growth for the EMS generated mutants. Continued selection for these criteria is expected to further stabilize the selected traits in generations M_6 and M_7. It is interesting to note that mutants produced in this study showed signs of a high proportion (60-75 %) of double or twin pods, indicative of high diosgenin content in the seed (Petropoulos 2002). A significant decline of important chemical constituents has been reported by Manha *et al.* (1994) for EMS treated virina xanthescens mutants of *Trigonella corniculata*. However, the selection process used and death of abnormal types

may have eliminated most of the deleterious mutants from our genetic stock and so were not clearly visible in M_4.

Higher dosage and treatment time combinations for the EMS treated plants showed higher death and reduced yield in the M_1 plants under the greenhouse conditions. Similar observations have been reported for fenugreek seed by Jain and Agarwal (1994). They reported that callus cultures did not grow on EMS concentrations above 0.1 M. Singh and Raghuvanshi (1980) used 0.5, 0.7 and 1 % diethyl sulphate with 0.5 % dimethyl sulfoxide to induce plant mutations; these treatments resulted in delayed germination of the plants and an inverse correlation between germination and dose of the mutagen. It appears that in our study careful selection for four successive generations increased the seed yield and associated reproductive and morphometric parameters even in plants representing higher dosage and treatment time combinations for EMS. In fact, our elite lines (eg., line numbers from the series 48, 88, 131, 134, 225, 227, 242, 267, 309 and 340) exhibited high seed and forage yield and, occasionally a determinate growth habit, derived mostly from combinations of higher dosages of EMS (50-300 mM) and a wide range of treatment times (2-24 h). The above trend is also supported by earlier studies on maize (Chandra Sekhar and Reddy 1971), barley (Heiner *et al.* 1960) and rice (Siddiq *et al.* 1968). Chandra Sekhar and Reddy (1971) reported an increase in pollen sterility with use of increasing dosages of chemical muatagens in maize, whereas, Heiner *et al.* (1960) reported reduced survival of barley mutants on treatment with diethyl sulphate and irradiation. Siddiq *et al.* (1968) also has reported that use of increased concentrations of EMS in conjunction with DMSO (as an effective adsorbent) resulted in a proportionate decrease on germination and survival of rice mutants.

Another important observation from this study was the lower forage and seed yield obtained in the field trial for M_2, M_3 and M_4 generations compared to M_1 in spite of the strong selection pressure applied for higher seed yield under the greenhouse conditions. This may be due to differential seeding rate used for the field trial. For M_1 120 seeds per 3 m row were used compared to 50 seeds per 3 m row for M_2, M_3 and M_4. For M_1 bulk seed was used for each line in order to provide an ample seed supply. Seed for the advanced generations were in short supply as the selected lines were grown in the greenhouse; only 50 seeds were planted in the 3 m row for each line. In fact some high yielding lines from these generations could not be tested under field conditions due to a shortage of seed for these lines. This may have affected the overall mean yield values in these three generations. It is important to note that controls planted within the blocks also had 50 seeds per 3 m row and, these rows also had a lower seed and forage yield compared to controls (120 seed row $^{-1}$) in the M_1 block. In this case plant growth could not compensate for a very low seeding rate during the short growing season experienced in western Canada. Although direct comparison of M_2, M_3 and M_4 is not possible in this case, it seems that forage and seed yield increased from M_2 to M_3 and then went down in M_4. This may have resulted due to the fact that all lines could not be included in M_4 due to a shortage of seed.

The height of the mutant plants also showed a progressive decrease from M_2 to M_4 generations compared to their respective controls, adding another reason for decrease of seed and forage yield in the M_4 generation. It is important to note that one of the selection criteria was the determinate nature of the plant. This selection increased the frequency of determinate types in advanced generations and this may have been linked

with low forage and seed yield. A more detailed study is necessary to verify these observations. The 2005 field trial showed that (Figure 3.3.2.1.) the mean seed yield for M_4 (1166 kg ha $^{-1}$) was lower than that of M_2 (1210 kg ha $^{-1}$) and M_3 (1711 kg ha $^{-1}$). This may have been due to the fact that selection based on a determinate growth habit and earliness in maturity may have also reduced the plant size, consequently reducing forage and seed yield of the selected plants in advanced generations. The mean height of the lines included for the three generations indicated a progressively decreasing trend from 18 cm in M_2 to 16 cm in M_3 and then 15.5 cm in M_4.

The present study indicated that mutagenesis using EMS was able to generate a large amount of variability in the fenugreek population and many mutant lines showed important traits that were rare among world collections. The accessions considered adapted to western Canada differed in their ability to produce good quality seed within the 90 to 100 frost-free days normally available for crop growth in this region. Therefore, mutation breeding is an appropriate and valuable method for fenugreek improvement. The agronomically superior lines identified in this study, need to be tested in multi-location trials in coming years to determine their performance under a wide range of growing conditions. These trials will also determine their adaptation and the genotype X environment interactions they may exhibit. The present study objectives were to generate variability in the seed yield components using a cultivar adapted to western Canada; the results indicate that this was achieved. Generation of variability and selection for desirable traits resulted in identification of some mutants that can be used as germplasm for improvement of this crop. Further studies should be done to determine if there is any change in the chemical constitution of the plant that may have resulted from pleiotropic

effects of the mutations affecting seed traits. This study not only successfully induced variability for seed characteristics in fenugreek but, also identified mutants with high seed and forage yield along with earliness and a determinate growth habit.

Chapter Four: Agronomic Studies on Fenugreek

4.1. Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual, self pollinating, legume crop, currently grown in India and west Asia, north Africa, Mediterranean Europe, Australia, Argentina, United States of America (USA) and Canada (AAFRD 1998; Edison 1995; Fazli and Hardman 1968; Jongebloed 2004; Petropoulos 2002; Rosengarten 1969; Rouk and Mangesha 1963; Smith 1982). Fenugreek seed is used as a spice, in artificial flavoring of maple syrup, as a condiment and in the production of cortisone and other hormones (Duke 1981; Jorgensen 1988). India is the largest fenugreek producing country, contributing towards $3/4^{th}$ share of the global market (Edison 1995). The first North American forage fenugreek cultivar Tristar was released in 2004 and will be available for commercial seed production in 2006. Tristar was developed by Agriculture and Agri-Food Canada (AAFC) at the Lethbridge Research Centre (LRC) in close collaboration with Alberta Agriculture, Food and Rural Development (AAFRD) (Acharya *et al.* 2004b).

Fenugreek is a rain-fed crop but responds well to minimum application of irrigation (Acharya *et al.* 2004a; Basu *et al.* 2004; Bhatt 1993; Kumar *et al.* 2000; Ram and Verma 2000; Sheoran *et al.* 2000; Verma *et al.* 1990). It could be an useful legume for incorporation into short term rotations with other crops (Moyer *et al.* 2003). Fenugreek produces high forage yield and quality and can be grown efficiently for hay or silage (Mir *et al.* 1993; Mir *et al.* 1998) in the Canadian prairies. Fenugreek is a bloat-free annual legume, having animal growth promoting substances (steroidal sapogenins like diosgenin), not present in other forage legumes (Mir *et al.* 1997).

Agronomic studies conducted in different agro-climatic zones of India and in some parts of Egypt suggest that optimum productivity of fenugreek can be enhanced when seed is spaced 20 to 30 cm apart and planted in early October or November (Baswana and Pandita 1989; Bhatt 1988; Gill *et al.* 2001; Korla and Saini 2003; Mohamad 1990). Randhawa *et al.* (1996) reported highest yield of the crop in western India with a seeding rate of 30 kg ha^{-1}. Well-drained loam soils (Rosengarten 1969) are favored by this crop and heavy and wet soils limit crop growth (Petropoulos 1973). The crop prefers faintly alkaline soil with a pH range of 8-8.5. Potash has been used to adjust soil pH to increase nutrient uptake of fenugreek (Yadav and Kumawat 2003). The application of organic and inorganic fertilizers, farmyard manures, nitrogen and phosphorus has been found to be effective in increasing fenugreek yield (Detoroja *et al.* 1995; Khiriya and Singh 2003; Yadav and Kumawat 2003). Lal *et al.* (2003) reported that optimization of sowing dates and forage cutting maximize fenugreek yield.

Fenugreek is a nitrogen fixing legume. Hence the seed needs to be inoculated with an appropriate *Rhizobium* inoculum to optimize this potential (Abdelgani *et al.* 1999). The most common nodule-forming bacteria associated with fenugreek are the Gram negative, aerobic, non-sporulating, rod shaped bacterium, *Rhizobium meliloti* (Subba Rao and Sharma 1968). Abd-Ala and Omar (1998) reported that application of wheat straw and certain fungi promotes nodulation, nodule efficiency and growth of the crop under saline soil conditions.

The toxic effect of heavy metals like cadmium, lead and zinc on the growth of fenugreek was reported by Dang *et al.* (1990). The phytotoxicity was found to be highest with cadmium and lowest with zinc. Application of a foliar spray of phosphorus sources

on another legume crop chickpea (*Cicer arietinum* L.) was reported to have improved yield characteristics and seed protein content (Khan and Samiullah 2002). However, such application was not evaluated on fenugreek.

Alhadi *et al.* (1999) found that the application of gibberellic acid before sowing causes a slight change in the growth parameters and physico-biochemical characteristics of a standing fenugreek crop under water deficient conditions. Khan (1997) reported that spray application of gibberellic acid @ 50 mg L^{-1} resulted in an increase in the number of pods, seed yield, and harvest index in mustard (*Brassica juncea* L.). Jain and Agarwal (1988) reported that external application of plant hormones such as auxins (*ie.* 2-4 dichlorophenoxyacetic (2,4-D), indole acetic acid (IAA), 2-1-napthylacetic acid (NAA) and gibberellic acid (GA_3) resulted in an increased level of steroidal sapogenins in two species of fenugreek (*T. corniculata* and *T. foenum-graecum*). The highest level of increase in steroidal sapogenin content was found to be associated with an external 100 ppm GA_3 application. Application of 2,4-D to fenugreek seed at lower concentrations (1-2 ppm) was found to be effective in enhancing fruit and seed maturity, vegetative growth and embryo organization (Hariharn and Unnikrishnan 1983).

Ortuno *et al.* (1998) reported a considerable increase in diosgenin content in young leaves (20 mg g^{-1} dry weight) in fenugreek seeds treated with benzylaminopurine. Alagukannan and Vijaykumar (1999) studied the effects of different plant growth substances on yield performance of fenugreek. They found that NAA increased the number of seeds per pod and seed size; while maleic hydrazide (MH), 2-4-D, and NAA increased seed protein content. A recent study on a related genus, *Vigna radiata* (green gram) in India, showed a significant increase in biomass production, flowering and seed

yield following application of a foliar spray of nitrogen, phosphorus, potassium and magnesium salts (Dey and Srivastava 2004).

Fenugreek is a late maturing crop, requiring 130-140 days to complete its maturation (Petropoulos 2002). Tristar matures in about 120 days under western Canada prairie conditions (Acharya *et al.* 2004b). Standard cultural practices for growing fenugreek in the prairies of western Canada are not available. So, the primary goal for this study was to develop a suitable agronomic package for growing this crop in western Canada. The specific objectives of the current study were to determine the:

(i) Effect of growing conditions (rain-fed and irrigation) on forage and seed production.

(ii) Effect of climatic zone (locations) in western Canada on forage and seed production.

(iii) Effect of harvesting methods (Swath versus direct combine) on seed production.

(iv) Effect of phosphate fertilizer application on seed and forage production.

(v) Effect of GA_3 and other foliar (chemical) sprays on forage and seed yield as well as maturity duration.

4.2. Materials and Methods

4.2.1. Fenugreek seed material

The lines used in this study were taken from LRC seed collections and fenugreek seed collected from spice markets in India (Appendix I). The LRC collections included seed from the Crop Diversification Centre, North (source Dr. S. Blade, Edmonton), Plant Gene Resources of Canada (PGRC), Saskatoon and some personal collections of Dr. Acharya (Lethbridge, Alberta) from India. All of the lines used were *T. foenum-graecum* L., with the exception of lines L3673 and L3674 which were *T. caerulea.*

4.2.2. Environments

The multi-location trial was conducted at three locations, *i.e.,* at Lethbridge, Brooks and Creston. At Lethbridge the trials were planted under two growing conditions, rain-fed and irrigation and these were considered as two separate environments. The trials were planted over two years (2004 and 2005) and the years were also considered different environments. The locations, growing conditions and years provided eight environments (4 locations X 2 years) in total; each of these was considered a random effect for statistical purposes.

Lethbridge: Lethbridge (49° 45' N and 112° 45' W) is located in southern Alberta (AB), Canada and is about 216 km south-east of Calgary. It is situated on both sides of the Oldman River with an average elevation of 900 m MSL (Harsh 1985). The soil type of the area is Orthic Dark Brown Chernozem (Wyatt *et al.* 1939). Lethbridge is situated in a semi-arid zone, with a moderate continental type climate characterized by mild summers and warm winters (Harsh 1987). The average annual maximum and minimum temperatures are 12.1 °C and - 1.0 °C respectively. The annual average snowfall is around

160 cm and the average annual precipitation is about 262 mm. The 2005 GPS coordinates for the LRC irrigated field was (49° 42' 24.98" N and 112° 45' 47.77" W) and for the rain-fed field was (49° 42' 19.80" N and 112° 45' 59.02" W).

Brooks: Brooks (50° 35' N and 111° 55' W) is also located in southern Alberta, Canada, about 168 km east of Calgary on the Trans Canada Highway, with an elevation of 758 m MSL (Harsh 1985). The soil type is Orthic Brown Chernozem (Wyatt *et al.* 1939). With the development of an extensive irrigation system this region has become a highly productive livestock, grain and vegetable producer that is a part of the Eastern Irrigation District in Alberta (Harsh 1985). Brooks is also characterized by a mild continental climate (Harsh 1985). The average annual maximum and minimum temperatures are 11.3 °C and - 2.4 °C respectively. The average annual rainfall is 43.3 mm and snowfall is around 79 mm. The multi-location trial was conducted on rainfed plots at the CDCS, Brooks. The GPS coordinates for the plots were at 50° 31' 23.14" N and 111° 47' 18.84" W.

Creston: Creston (49° 10' N and 116° 31' W) is in British Columbia (BC), Canada, 760 km east of Vancouver. It has a temperate climate, which plays an important role in BC agriculture due to its relatively long growing season. The elevation of Creston is 762 m MSL and the soil type is a stone-free alluvial deposit of Carbonated Rego Gleysol (composed of silt loam and silty clay loam) with poor to moderately poor drainage (Wittneben and Sprout 1971). Creston experiences good crop growing weather without the high or low temperature extremes typical of many regions in Canada. The summers in Creston are warm and sunny. The winters are mild as a result of Pacific systems crossing British Columbia and moderating the influence of Kootenay Lake

(Harsh 1985). The average annual maximum and minimum temperatures are 12.9 °C and 3.1 °C respectively. The average annual rainfall is 454 mm and the average annual snowfall is 1406 mm (Wittneben and Sprout 1971). The trials were grown under rainfed conditions of Creston with GPS coordinates of the plots 49 ^{0} 06 ' 27.39 " N and 116 ^{0} 34 ' 05.62 " W.

Since all of the lines used in the field trials conducted did not have the same number of plants, the seed and forage yields were adjusted based on the proportion of surviving plants (stand growth percentage) in both years. The equation used for this purpose:

Adjusted yield = [(Observed yield X 100) / % stand after one month of growth in the field]

The plants showing an average stand height of less than 15 cm and maturing within 90 days after seeding were considered to be 'determinate' in growth habit. In field trials, standard *Rhizobium* legume soil inoculants (The Nitragin Company, USA) were used to optimize legume plant growth. The code for this particular inoculant was "N" and the dosage applied was @ 0.3 g 120 seed^{-1}.

For weed control Edge (Dow AgroScience Canada Inc.), Odyssey (BASF Canada), and Embutox 625 (Nufarm Canada) were used in the field experiments and Reglone (Syngenta Crop Protection Canada Inc.) desiccant was used for the seed yield trials.

4.2.3. Experimental design

4.2.3.1. Study of growing conditions

In 2004, depending on the seed supply 83 and 65 fenugreek accessions were seeded under irrigation and rain-fed conditions, respectively. Based on their yield performance in 2004, 73 lines were selected and seeded both under irrigation (50 mm X 4) and rain-fed conditions at LRC in 2005. For this study 120 seeds from each line were planted in a 2m long row and the rows were spaced 1 m apart. Each row was arranged as in a two times replicated Randomized Complete Block Design (RCBD) under each growing condition in 2004 and 2005. Unfortunately only 45 accessions were common among the two years and the two growing conditions and so, a fixed model ANOVA including these 45 accessions was done on the seed and forage yield and 1000 seed weight.

4.2.3.2. Study on effect of locations

For this study five genotypes including Tristar and Amber fenugreek were used. Each plot consisted of 10 rows (18 cm apart) and was 1.8 X 6 m^2 in size; the plots were arranged as in a five times replicated RCBD in each environment. The environments were Lethbridge rain-fed and irrigation (50 mm X 4) for 2004 and 2005; and rainfed conditions in Brooks for 2004 and 2005 and, rainfed conditions in Creston for 2005. The seeding rate for each line in all of the environments was 15 kg ha^{-1}. The seeding was during the first week of May and, the plots were harvested for forage yield in the first week of August and, desiccated for seed harvested in the last week of September. Due to fungal infestations of the plants at Creston in 2004, seed yield could not be determined. A preliminary study indicated powdery mildew caused by the fungus *Erysiphe* can be

detrimental for fenugreek in wet environments (unpublished data). The seeding and harvesting dates for the Brooks trial in 2004 and 2005 were May 5 and October 7, and May 10 and October 5, respectively. The seeding and harvesting dates for the 2005 Creston trial were May 7 and October 12 respectively. Details about LRC seeding, harvesting dates and herbicide or desiccant spray dates are presented in Appendix II.

The data obtained were subjected to a mixed model ANOVA using environment as random and the genotypes as fixed effects.

4.2.3.3. Study on harvest method

To determine the effect of harvest method on seed production the study was conducted using five genotypes including Tristar and Amber fenugreek, under two growing conditions; *i.e.*, rain-fed and irrigation (50 mm X 4) over two years (2004 and 2005) (considered four environments). The plot size used was 1.8 m X 6 m plot with a 18 cm row spacing. The plots were arranged as in a four times replicated RCBD in each environment. The seeding rate used was 15 kg ha^{-1}. Details about seeding and harvesting dates and desiccant spray dates are presented in Appendix II.

The data obtained were subjected to a mixed model ANOVA using environment as random and the genotypes as fixed effects.

4.2.3.4. Study of effect of phosphate fertilizer

This study was conducted under rain-fed and irrigated (50 mm X 4) conditions at LRC in 2004 and 2005 (considered four environments) using Tristar fenugreek. An initial soil survey was done in each year to make sure that the field selected for the trial had low phosphate levels. The soil survey indicated that the average phosphate level for 2004 plot varied between 36-37 kg ha^{-1}, wheras that in 2005 varied between 24-44 kg ha^{-1}. The plot

size for these experiments under both rain-fed and irrigated conditions was 1.8 m X 6 m with an 18 cm spacing between rows. The seeding rate used was 15 kg ha^{-1}. The rate of phosphate fertilizer (Westco Fertilizer, Edmonton) used was 0, 30, 40, 50 and 60 kg ha^{-1}. The plots were arranged as in a four times replicated RCBD in each environment. Details about seeding and harvesting dates and desiccant spray dates are presented in Appendix II.

The data obtained were subjected to a mixed model ANOVA using environment as random and the rate of phosphate application (P rate) as fixed effects.

4.2.3.5. Study on GA_3 and other foliar sprays

To determine the effect of GA_3 and foliar sprays on fenugreek seed and, forage production and maturity, a greenhouse trial was conducted at LRC. For the GA_3 experiment five Tristar fenugreek plants were planted in six inch plastic pots containing non-sterile, soil-free mix (LRC Soiless mix/Cornell mix). The soil free mix was composed of 3.8 cubic feet bale of sphagnum peat moss, 18.6 kg bag of medium horticultural grade vermiculite, 1000 g of calcium carbonate flour, 1500 g of 18-6-12 Osmocote (Southern Agricultural Insecticides, Inc.), 1200 g 0-21-0, 20 g of "Fritted" trace elements, 15 g of 13.2 % (W/W) chelated iron, 7 g of 14 % (W/W) chelated zinc and 30 L of wash mortar sand (The Scott C.). Each treatment (concentration and growth stage of application) was replicated twice including the untreated controls. The plants were sprayed with 90 % pure GA_3 (Sigma-Aldrich) at five concentrations (0, 30, 60, 90 and 120 ppm) at four growth stages of the plants (flowering, post-flowering, pod-emergence and post-pod emergence). Observations for each parameter were made when 50 % of the plants in a plot reached each of the four growth stages mentioned above. The

experiment also contained two more treatments (GA_3 only and GA_3 mixed with 0.3 % (W/V) surfactant AG-SURF) (Interprovincial Cooperative Ltd).

In a separate study other chemicals were used as foliar sprays. The chemicals in this case were ferrous sulphate (Sigma-Aldrich), calcium chloride (Sigma-Aldrich), cupric sulphate (Sigma-Aldrich), magnesium sulphate (Sigma-Aldrich), ammonium sulphate (Sigma-Aldrich) and ammonium molybdate (Sigma-Aldrich). The concentration used was 10 mM mixed with 0.3 % (W/V) surfactant (AG-SURF) and one untreated control. In this case, each six inch diameter plastic pot containing soil-free mix had 2 plants and all of the treatments were replicated thrice including the control. For this experiment spraying was done only at the flowering stage.

All of the pots in both trials, GA_3 and foliar spray, were arranged in the LRC greenhouse as in a two times replicated randomized complete block design. After 95 days in a greenhouse set to cycle 16 h days (22 °C) and 8 h nights (15 °C) the plants were desiccated with a 0.4 % (W/V) Reglone (Syngenta Crop Protection Canada Inc.) solution along with 0.23 % (W/V) of AG-SURF as surfactant. The sprayer used for this purpose was an E-Z Sprayer (Vaporisateur) (Wal-Mart Canada Inc.). The plants were allowed to dry for 10 days before separating the seed for yield determination. All morphological, reproductive and growth parameters of the treated plants against the control were monitored and recorded. The experiment was conducted in Spring of 2005.

The GA_3 and foliar spray field trials were seeded under irrigated conditions (50 mm X 4) in 2005. For this purpose, 120 Tristar fenugreek seeds were planted in single 3 m row plots and the plots were spaced 1 m apart. The treatments were applied as in a two times replicated RCBD. The concentrations of GA_3 and the stages at which the treatments

were applied were the same as was described for the greenhouse study. The concentration of the chemicals and the sprayer used in the foliar spray experiment in the field was also the same as that described for the greenhouse study.

The GA_3 and foliar spray trial had a common replicated control, sprayed with 0.3 % (W/V) AG-SURF only to compare the seed yield attributes but was not included in the statistical analysis due to poor yield.

All of the plots were hand harvested in 2005. After drying the material for one week indoors, the dry weight of individual rows was recorded. Then the seeds were separated from the rest of the plant, cleaned and weighed to determine the total and 1000 seed weight (seed size) for each individual line used in the experiment. Details about LRC seeding, harvesting dates and herbicide or desiccant spray dates are presented in Appendix II.

The data obtained were subjected to a fixed effects model ANOVA. The controls grown under greenhouse conditions and in field trials for the GA_3 experiments were used only for visual comparison of the maturity duration (earliness) and yield attributes and were not part of the statistical analysis as most control plants did not bear any mature seed during the 95 days for whch the experiments were run.

4.2.4. Statistical analysis

A mixed model ANOVA (environment effect was considered random and the other effects were fixed) was used for analyzing the data on studies dealing with growing conditions, locations, harvest methods, and application of phosphate fertilizers. A fixed model ANOVA was used for data analysis of the study on growing conditions and GA_3 and other chemical spray trials. For all analyses the Agrobase 99 (Agronomix Software,

Inc. 1999) software was used. All the means and standard errors used in this study were also generated using the same software.

4.3. Results and Discussion

4.3.1. Study on growing conditions

The location effect was significant for forage yield (Table 4.3.1.1.). For seed yield year, genotype, year X location and year X genotype interactions were significant. Year effect only was significant for 1000 seed weight. Fenugreek is known to be adapted to rain-fed growing conditions in western Canada, but its biomass production can be increased by application of minimal irrigation in dry areas such as the southern part of Alberta (Mir *et al.* 1993). This study confirmed earlier observations in this regard. Seed yield of this crop is influenced by environment more than forage yield and in this study even the interaction effect of year X genotype was highly significant. This indicated that improvement in seed yield would be difficult and would require selection using multiple locations and years.

Since the year effect was highly significant for seed yield and 1000 seed weight, correlation coefficients (r) were calculated using seed yield and 1000 seed weight with growing conditions (rain-fed and irrigation) for the two years separately. Data from 65 common lines grown under the two growing conditions in 2004 and 73 lines in 2005 were used for the analysis. The r values for 2004 were 0.54 and 0.61 ($p < 0.05$) for seed yield and 1000 seed weight, respectively and for 2005 were 0.62 and 0.67, respectively ($p < 0.05$). The significant r values indicated that the growing conditions (rain-fed and irrigation) within a year played a relatively small role in seed yield and seed size determination for these genotypes.

A wide variability in forage and seed yield was observed for the world accessions grown under irrigation and rain-fed conditions at LRC in 2004 and 2005 (Appendix III-

VI). The cultivar Tristar was not among the top five producers under rain-fed or irrigated conditions in 2004 and 2005 (Table 4.3.1.2.). The accession X92-23-3 was among the top five seed yielding lines under irrigation in 2004 and 2005, while accession L3308 was among the top five high seed yielding lines under rain-fed conditions in both years. Among the low yielding lines, accession L3068 and L3674 were common under both rain-fed and irrigated conditions. It is important to note that L3673 and L3674 belong to a different species of fenugreek (*T. caerulea*) and were found to be consistently low yielding under the two growing conditions used during the two test years.

A wide range of variability with respect to seed and forage yield was observed among different world accessions at the two locations and two years. For example, the accessions PI 143504 and L3312 were consistent with their high seed yield under both irrigation and rain-fed conditions in 2004, whereas accession L3720, which is among the top yielding lines under irrigation (2391 kg ha^{-1}) was not as good in seed yield (1731 kg ha^{-1}) under rain-fed conditions in 2004 (Appendix III and IV). Even among these three lines the forage yield varied considerably within a range of 8475-15580 kg ha^{-1}. Similarly in 2005 accessions ZT-5, PI 143504, L3312 and Amber were among the top seed yielding lines under both irrigation and rain-fed conditions (Appendix V and VI). On the other hand, accession X92 -23-3 showed a wide fluctuation in seed yield under irrigation (6739 kg ha^{-1}) and rain-fed (2289 kg ha^{-1}) conditions (Appendix V and VI). Forage yield among these lines varied between the two locations ranging from 7978-19548 kg ha^{-1}. (Appendix III-VI).

In 2004 the seed yield of Tristar (1478 kg ha^{-1}) was above the mean seed yield, but the forage yield (11671 kg ha^{-1}) was less than the mean value under irrigation

(Appendix III). However, both seed yield (2268 kg ha^{-1}) and forage yield (10235 kg ha^{-1}) of Tristar under rain-fed conditions in 2004 was higher than the mean value (Appendix IV). In 2005, under the two growing conditions (Appendix V and VI), the seed and forage yield of Tristar was higher than the cumulative means. However, the seed size of Tristar was smaller than that of the mean values of all accessions under both growing conditions in the two test years. The fluctuations in the performance of some lines under varied environmental conditions indicated presence of a genotype X environment interaction. On the other hand stable performance of some of the lines was characteristic of their genetic influence. This information is important for use of appropriate genetic material in crossing programs for developing cultivars with higher seed yield, shorter maturity duration and most importantly stable yield performance. Fenugreek accessions from the world collection exhibit extensive phenotypic variability; this variability has a genetic base, and so selection for improved seed yield is possible (Acharya *et al.* 2004a.). Raghuvanshi and Singh (1981) obtained high heritability estimates in fenugreek when they selected for a double pod trait. The double pod trait is known to be linked to diosgenin content and higher seed yields (Petropoulos 2002; Ahmed *et al.* 1989).

The seed yield performance of the lines under rain-fed conditions (Appendix III) was better than those under irrigation (Appendix IV) in 2004. Of all the lines included in this study only two, L3172 and L3177 exhibited a determinate nature under both conditions in the two test years (Appendix III, V and VI). The seed yield of the determinate lines however was considerably less than that of Tristar. A comparative growth habit of an indeterminate and determinate line is shown in Figure 4.1. In 2004, the mean seed yield (1254 kg ha^{-1}) under irrigation conditions was lower than that under rain-

fed conditions (1990 kg ha^{-1}). However, in 2005, the mean seed yield was higher under irrigation (2283 kg ha^{-1}) than under rain-fed conditions (1828 kg ha^{-1}) (Table 4.3.1.2.). The field trial for world accessions growing under LRC rain-fed conditions (2004) is shown in Figure 4.3.1.1.

The better performance of rain-fed over irrigation conditions in 2004 is probably associated with higher rates of unusual precipitation that year. In 2004 the total rainfall during the growing season (May to September) was 286 mm, well over the 30 year average for this period (241.8 mm). Additional precipitation may have been detrimental to the crop under irrigation, as it may have induced a higher cooling effect. This may have slowed the growth and hence the maturity of the crop under irrigation in 2004. In 2005, the mean yield for seed and forage was higher as expected under irrigation in comparison to the rain-fed conditions. The total rainfall for the growing season (May to September) in 2005 was 102.8 mm, much lower than that of 2004 (286 mm) (Basu *et al.* 2004). Another observation from this study was the identification of only two lines with determinate growth habit among the 83 tested. This was expected as the determinate growth habit in fenugreek is a monogenic recessive trait; hence an indeterminate growth habit is the general norm (Choudhury and Singh 2001). A side by side comparison of an indeterminate line and a determinate line grown under LRC irrigation conditions 2005 is shown in Figure 4.3.1.2.

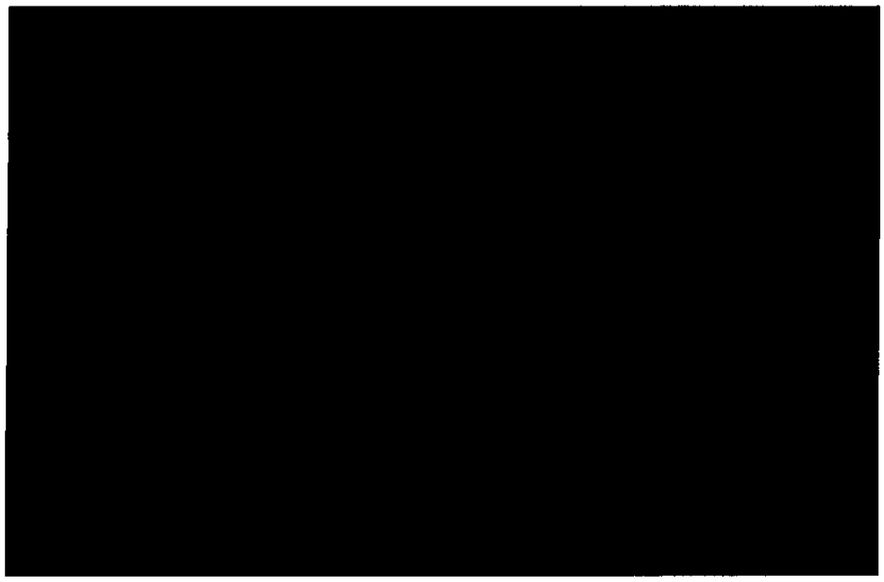


Figure 4.3.1.1. Variability in height and stand establishment of world accessions when grown under LRC rain-fed conditions in summer 2004.

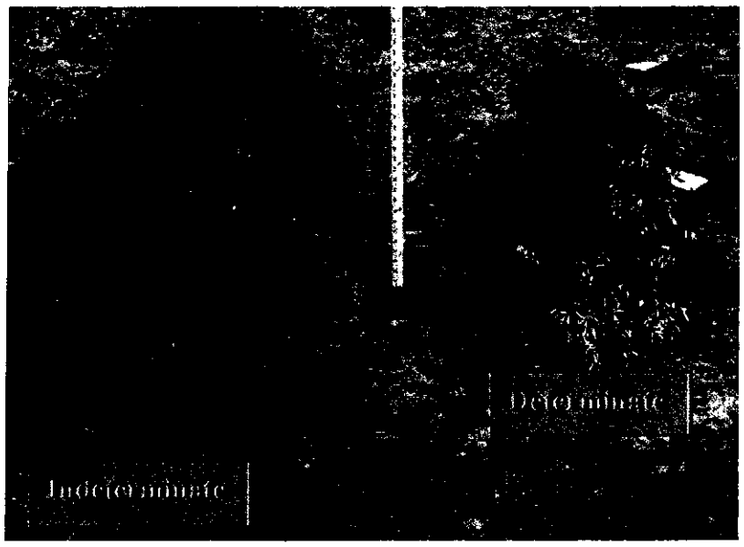


Figure 4.3.1.2. An indeterminate (Tristar) and a determinate line (L3172) growing side by side at LRC under irrigation conditions in 2005. The determinate line is showing signs of early maturity, however, the seed and forage yield was lower compared to Tristar fenugreek controls.

Table 4.3.1.1. Mean square (MS), degrees of freedom (df) and probability (Pr) of F value for forage yield, seed yield and 1000 seed weight as determined by a fixed model ANOVA. For this purpose, 45 genotypes were tested at two locations (LRC rain-fed and irrigation) over two years (2004 and 2005).

		Forage yield (kg ha^{-1})		Seed yield (kg ha^{-1})		1000 Seed Weight (g)	
Source	**df**	**MS**	**Pr of F**	**MS**	**Pr of F**	**MS**	**Pr of F**
Total	359						
Replication	1	1144.0	0.060	84.4	0.335	0.3	0.042
Year	1	261.1	0.367	1545.0	0.000	22.7	0.000
Location	1	19843.1	0.000	102.8	0.287	0.0	0.616
Genotype	44	457.3	0.054	279.7	0.000	0.09	0.265
Year*Location	1	1746.3	0.020	4149.0	0.000	0.08	0.310
Year*Genotype	44	315.6	0.499	156.7	0.006	0.1	0.135
Location*Genotype	44	363.6	0.274	74.9	0.766	0.06	0.710
Year*Location*Genotype	44	383.5	0.203	82.8	0.624	0.05	0.883
Residual	179	319.2		90.5		0.07	
CV		18.0		16.0		6.0	
R^2		0.6		0.6		0.7	

Table 4.3.1.2. Mean seed yield (kg ha^{-1}) of five top and bottom lines from world accessions studied at two locations (LRC rain-fed and irrigation) over two years (2004 and 2005). A Grand Mean (±SE) for each group was calculated using all of the accessions.

2004 Irrigation		2005 Irrigation		2004 Rain-fed		2005 Rain-fed	
Accessions	**Seed yield**	**Accessions**	**Seed yield**	**Accessions**	**Seed yield**	**Accessions**	**Seed yield**
PI 211636	2881	X92-23-3	6739	PI 143504	4119	L3708	7015
L3312	2621	L3068	6358	L3308	3952	ZT-5	3866
L3671	2452	PI199264	4315	F18	3937	L3308	3273
L3720	2391	L3375	4300	PI 138687	3320	QUATRO	3079
X92-23-3	2374	AMBER	4057	F86	3207	L3683	2979
L3678	261	L3679	1032	L3708	1032	L3714	994
L3673	253	L3682	948	L3704	884	L3695	975
L3674	137	L3678	798	L3702	823	L3068	958
L3068	127	L3680	568	L3710	491	L3698	949
L3675	19	L3674	169	L3068	377	L3674	195
Mean	**1254**		**2283**		**1990**		**1828**
SE	**64**		**111**		**104**		**98**

4.3.2. Study on effect of location on seed and forage production

The multi-environment study indicated a highly significant effect of environment on both forage and seed yield while only genotypic effects were significant for forage yield (Table 4.3.2.1). The plants were infected with fungal pathogens at Creston, BC in 2004, and this reduced the seed and forage yield by 60 %; this data was not used in the analysis. Due to this, the data set for the four locations was incomplete, forcing us to include only seven environments *i.e.*, 2004 and 2005 Lethbridge rain-fed (rainfed) and irrigation, 2004 and 2005 Brooks rainfed and, the 2005 Creston rainfed data. Although large variations in seed yield were noticed for the genotypes tested for the years and locations, a statistically significant effect for the interaction could not be determined. Highest seed yield was observed in Tristar (1249 kg ha^{-1}) closely followed by F70 (1245 kg ha^{-1}). The lowest yield was observed in the case of Amber (1103 kg ha^{-1}).

Usually 2-3 years are necessary to acclimate new plant species to a new environment, and then these species are subjected to further breeding manipulations to address the needs of local farming communities (Detoroja *et al.* 1995). However this approach is crop specific, and several crops or plant species may have to be subjected to different types of acclimation depending upon their adaptability (Fehr 1993). The wide variability in yield performance due to the environmental variation and associated micro-environmental impact is an important consideration for a cultivar development program in fenugreek. In this experiment genotypes that were selected for their ability to produce a good amount of forage in western Canada were included and so these genotypes were grown in the area for 3-4 years. Therefore, variation in seed and forage yield cannot be

attributed strictly to lack of acclimatization. The data indicates that development of fenugreek cultivars with wide adaptation (for the entire western Canada) will be difficult and breeding programs may have to develop cultivars for specific environmental conditions to maximize yield performance. Widely adapted cultivars (Co-1, R Mt-1, Pusa Early Bunching) as was observed by Edison (1995) in India will have to be selected on the basis of multi-location and multi-year trials with some sacrifice in yield performance in specific agro-climatic zones.

Tristar and F70 fenugreek produced significantly more forage yield than the rest of the genotypes used in this study (Table 4.3.2.2.). This was observed earlier in other studies (Acharya *et al.* 2004a; Basu *et al.* 2004).

Table 4.3.2.1. Mean square (MS), degrees of freedom (df) and probability (Pr) of F value for forage and seed yield as determined by a mixed model ANOVA[1]. For this purpose, five genotypes were considered fixed and seven environments were considered random.

		Forage yield		Seed yield	
Source	**df**	**MS**	**Pr of F**	**MS**	**Pr of F**
Total	174				
Replications	4			40.8	
Environment	6	885171264.4	0.000	7535.3	0.000
Genotypes	4	9106124.8	0.000	21.5	0.311
Environment*Genotypes	24	2551981.7	--	15.5	--
Residual	136	1271287.1		17.8	
CV		15.0		13.9	
R^2		0.9		0.9	

[1] ANOVA was done on square root transformed data.
For the interaction term there is no direct F test and so the probability is not shown.
To determination Environment and Genotype F values, MS for the Environment*Genotypes calculation was used as the denominator.

Table 4.3.2.2. Mean forage yield (kg ha^{-1}) of the five genotypes when grown in seven environments.

Level	Forage yield (kg ha^{-1})[1]
Tristar	8083.1 a
F70	8002.2 a
F86	7381.2 b
Amber	7183.3 b
F80	6921.1 b

[1] Means followed by same letter are not significantly different at ($p=0.05$) according to a LSD test.

4.3.3. Swath versus combine study

The statistical analysis of the data on swath versus the direct combine study on fenugreek seed yield showed a significant effect of genotype and highly significant effect of environment and harvest methods (Table 4.3.3.1.). The interaction effect of genotype X harvest method was not significant.

The four environments produced a mean seed yield of 1582, 1289, 539 and 338 kg ha^{-1} in 2004 irrigation, in 2004 rain-fed, 2005 irrigation and 2005 rain-fed, respectively. At Lethbridge the 2004 growing season was better for seed production than the 2005 growing season and this was reflected by their respective mean seed yields of 1435 and 438 kg ha^{-1}.

Among the genotypes F70 produced a significantly higher seed yield than the others included in the test. The seed yield of F70 was recorded as 1068 kg ha^{-1}, followed by F86 (1003 kg ha^{-1}). Superiority of F70 in seed production (1245 kg ha^{-1}) was observed earlier in studies conducted using other locations (Acharya *et al.* 2004a, Basu *et al.* 2004; Mir *et al.* 1993).

Between the two seed harvest methods swathing yielded significantly ($p < .001$) more seed (975 kg ha^{-1}) than direct combine methods (898 kg ha^{-1}). This is expected as swathing is normally practiced as a method of seed harvest to force rapid seed maturity and to reduce loss due to seed shatter (Petropoulos 1973). This practice will help the fenugreek seed harvest as it expedites seed maturation in crops having an indeterminate growth habit.

Table 4.3.3.1. Mean square (MS), degrees of freedom (df) and probability (Pr) of F value for seed yield (kg ha^{-1}) from a swath versus combine study as determined by ANOVA[1]. For this purpose, five genotypes (fixed effect) were tested in four environments (random effect) using a mixed model.

Source	df	MS	Pr of F
Total	119		
Replication	3	49.5	
Environment	3	3050.4	0.000
Genotype	4	44.3	0.002
Environment*Genotype	12	18.1	--
Harvest method (HM)	1	165.6	0.000
Environment*HM	3	73.1	--
Genotype*HM	4	8.5	0.472
Environment*Genotype*HM	12	6.2	--
Residual	77	9.5	
CV		10.6	
R^2		0.9	

[1] ANOVA was done on square root transformed data.

For the interaction term there is no direct F test and so the probability is not shown.

For the determination of the F values for Genotype, Harvest Method and Genotype*Harvest Method, Environment*Genotype, Environment*Harvest Method and Environment*Genotype*Harvest Method were used as denominators, respectively.

4.3.4. Fertilizer trial

The ANOVA results indicate a highly significant ($p<0.001$) effect of environment and rate of phosphate application on both forage and seed yield (Table 4.3.4.1.). Effect of P (phosphate) on yield attributes of fenugreek was observed earlier (Petropoulos 2002). The interaction effect for phosphate rate X genotype was significant ($p<0.05$) for forage and seed yield. The genotypes only showed significant effects for seed yield.

Since the interaction effect of P rate X genotype was significant the mean values for each treatment combination are presented in Table 4.3.4.2. This table indicates that high seed yield was obtained when 40 to 50 kg ha^{-1} of P was applied. For high forage yield most genotypes required 50 to 60 kg ha^{-1} of P fertilizer. Many Indian fenugreek genotypes produced significantly higher levels of seed and forage yield when increasing levels of P_2O_5 (up to 52.8 kg ha^{-1}) were applied (Khiriya and Singh 2003; Yadav and Kumawat 2003; Ram and Verma 2000). Sheoran *et al.* (2000) observed a 28.8 % increase in seed yield in response to application up to 60 kg ha^{-1} P.

This study indicates that the LRC plots used did not have high P levels (as indicatd by our pre-trial soil surveys conducted in each year of study) and so the response to P was noticeable, unlike the study in Punjab, India where Randhawa *et al.* (1996) observed no fenugreek response to phosphate application if the soil was rich in elemental P. Fenugreek roots have the ability to trap high levels of phosphorus and use it for growth and development of the plant (Randhawa *et al.* 1996). But, if the soil is already rich is P, addition of P fertilizer will not be effective. For this study although different plots were used for the two years and the locations, the P levels were low according to our soil test and so we observed a pronounced effect. Our results are similar to results obtained in

studies conducted in other environments on phosphate application and yield attributes of fenugreek (Khiriya and Singh 2003; Yadav and Kumawat 2003).

During the two years of this study reduction in maturity duration was not observed due to P application in the LRC plots. This was also supported by Sheoran *et al.* (2000) in a study conducted in India where floral initiation and maturity were not affected after P application.

Table 4.3.4.1. Mean square (MS), degrees of freedom (df) and probability (Pr) of F value for forage (kg ha^{-1}) and seed yield (kg ha^{-1}) on phosphate fertilizer trials as determined by ANOVA[1]. For this purpose, five genotypes (fixed effect) were tested in four environments (random effect) using a mixed model.

		Forage yield		Seed yield	
Source	**df**	**MS**	**Pr of F**	**MS**	**Pr of F**
Total	399				
Replication	3	167.5		10.4	
Environment	3	27886.6	0.000	13092.9	0.000
Phosphate rate	4	159.1	0.000	654.1	0.000
Environment* P rate	12	89.6	--	148.9	--
Genotype	4	36.1	0.147	52.6	0.0002
Environment*Genotype	12	44.8	--	50.4	--
P rate* Genotype	16	39.6	0.022	19.9	0.005
Environment*P rate* Genotype	48	46.1	--	19.9	--
Residual	297	21.1		9.2	
CV		8.9		12.0	
R^2		0.9		0.9	

[1] ANOVA was done on square root transformed data.
For the mixed model ANOVA there was no direct F test used for the interactions that included environment and, so the probabilities are not shown.
For the determination of the F values of P rate, Genotype and P rate*Genotype, Environment*P rate, Environment*Genotype and Environment*P rate*Genotype were used as denominators, respectively.

Table 4.3.4.3. Mean seed and forage yields (kg ha^{-1}) for genotypes and phosphate levels (P rate) in kg ha^{-1} from a study conducted under rain-fed and irrigated conditions at LRC in Summer 2004 and 2005.

Genotypes	P-rate (Seed yield)[1]				
	0	30	40	50	60
F70	480	755	796	877	693
F80	647	805	969	830	795
F86	461	769	802	870	819
Amber	541	823	988	892	815
Tristar	415	786	942	782	746
Genotypes	P-rate (Forage yield)[2]				
	0	30	40	50	60
F70	3279	2706	3116	2862	3072
F80	2758	2830	2931	3149	3126
F86	2915	2870	2964	2830	3265
Amber	3060	2721	2824	2581	2834
Tristar	2990	2791	2775	2730	3237

[1] **Overall mean = 764, LSD = 43.6 and CV = 12.2**
[2] **Overall mean = 2929, LSD = 143.1 and CV = 8.9**

4.3.5. GA_3 and foliar spray study

Our greenhouse study in spring 2005 indicated a significant effect of GA_3 on the growth, maturity and seed yield of fenugreek (Table 4.3.5.1). In fact all the effects (surfactant, GA_3 concentration (rate) and growth stage at which GA_3 was applied) including the interaction effects were highly significant for seed yield. All of the plants treated with GA_3 regardless of concentration matured at least a week earlier than the control groups (the control groups were desiccated on 95^{th} day). There was considerable increase in the height (Figure 4.3.5.1), dry matter and seed yield of treated plants compared to the controls. The effect was particularly noticeable when GA_3 was sprayed along with the surfactant. The mean seed yield for GA_3 with surfactant was 8.9 g pot^{-1} compared to 6.4 g pot^{-1} for GA_3 without surfactant.

Addition of surfactant had a major impact on the growth, maturity, dry matter and seed yield of the plants in the greenhouse. This was expected as the addition of surfactant ensures proper spread and absorption of GA_3 on the plant surface improving its incorporation into the plant tissue compared to GA_3 sprayed alone. However, our field trials indicate that only rate X stage interaction was not significant (Table 4.3.5.2.).

The stage of growth at which GA_3 was applied had a significant impact on seed yield in both greenhouse (Table 4.3.5.1.) and field trials (Table 4.3.5.2.) a detailed look at the mean values was done for the growth stages. From the observations made in the greenhouse study it seems that GA_3 application after the post pod emergence stage is helpful for increasing seed yield (Table 4.3.5.3.). The field study however, showed that early application of GA_3 was more helpful in increasing seed yield (Table 4.3.5.4.). This may have been due to the fact that the growing conditions in the field are very different

from that of the greenhouse. Less number of replicates (2) in the experimental design could be another reason for observing such variation in the results between greenhouse and the field trial. Use of a larger number of replications (improved error control) may resolve this problem in future experiments.

Most reports on legume crop responses to gibberrelin are limited to *Pisum sativum* L. (Ross *et al.* 1997). Shoots from these plants elongate in response to hormone treatment, possibly because of inactivation of genes suppressing an elongation-specific plant pathway (Ross *et al.* 1997). GA_3 is known to induce a normal growth habit (elongation) in dwarf pea varieties and single gene dwarf maize mutants and promote growth and elongation in rice, wild oat, wheat, lettuce and cucumber and, floral promotioin in crops grown under non-inductive daylength conditions (Phinney, 1956). Lang (1965) also has suggested that floral promotion by GA_3 may be due to its effect on stem elongation. It is important to note that reports on studies involving GA_3 on fenugreek are scanty and are mostly restricted to plant tissue culture. The current study on use of GA_3 as a foliar spray applied to fenugreek is relatively small and, needs to be confirmed through more in depth research.

Table 4.3.5.1. Mean square (MS), degrees of freedom (df) and probability (Pr) of F value for seed yield for plants treated with GA_3 in the LRC greenhouse (Spring, 2005) as determined by a fixed effects model ANOVA[1]. For this purpose, four concentrations of GA_3 (rates) were tested on four growth stages of the Tristar fenugreek plants, with and without surfactant.

Source	df	MS	Pr of F
Total	63		
Replication	1		
Surfactant	1	68.0	0.000
Rate	3	43.9	0.000
Growth stage	3	27.0	0.000
Surfactant*Rate	3	44.3	0.000
Surfactant*Growth stage	3	5.2	0.000
Rate*Growth stage	9	2.8	0.000
Surfactant*Rate*Growth stage	9	5.9	0.000
Residual	31	0.4	
CV		3.4	
R^2		0.9	

[1] ANOVA was done on square root transformed data

Figure 4.3.5.1. GA_3 treated fenugreek plants growing in the LRC greenhouse (Spring, 2005): 1. Treated with 120 ppm; 2. Control; and 3. Treated with 30 ppm.

Table 4.3.5.2. Mean square (MS), degrees of freedom (df) and probability (Pr) of F value for seed yield of plants treated with GA_3 in the LRC irrigation field (Summer, 2005) as determined by a fixed effects model ANOVA[1]. For this purpose, four concentrations of GA_3 (rates) with surfactant were sprayed at four growth stages of the Tristar fenugreek plants.

Source	df	MS	Pr of F
Total	31		
Replication	1		
Rate	3	2.9	0.353
Growth stage	3	6.7	0.080
Rate*Growth stage	9	4.1	0.184
Residual	16	2.50	
CV		8.2	
R^2		0.6	

[1] ANOVA was done on square root transformed data

Table 4.3.5.3. Mean seed yield (g per pot) [1] of Tristar plants grown in the LRC greenhouse in Spring 2005 and sprayed at four growth stages with four concentrations of GA_3 along with surfactant.

	GA_3 concentrations (ppm)				
Growth stages (GS)	**30**	**60**	**90**	**120**	**Mean for GS**
Flowering	6.9	8.6	4.9	8.2	7.1
Pod Emergence	8.1	8.8	5.0	7.4	7.3
Post Pod emergence	9.6	9.6	6.6	8.7	8.6
Pod Filling	9.4	9.9	6.7	8.5	8.6
Mean for GA_3 conc.	8.5	9.2	5.8	8.2	7.9

[1] LSD for the experiment (at p=0.05) is 0.42.

Table 4.3.5.4. Mean seed yield (kg ha^{-1}) [1] of GA_3 treated Tristar fenugreek plants at different stages of growth under LRC irrigation (Summer, 2005).

Growth stages	Seed yield (kg ha^{-1})
Flowering	210.0 a
Pod Emergence	200.3 a
Pod Filling	180.9 b
Post Pod Emergence	180.1 b

[1] Means followed by same letter were not significantly different at (p=0.05) when compared using a LSD test.

The results from the chemical spray experiment conducted in spring 2005 in the greenhouse indicated a highly significant effect of chemicals sprayed on the seed yield of fenugreek (Table 4.3.5.5.). For this experiment only one concentration of each chemical (10 mM mixed with 0.3 % (W/V) surfactant) was used at the flowering stage of the plants. All the chemical treatments were significantly different from the control (Table 4.3.5.6). Among the chemicals magnesium sulphate had the largest positive effect on seed yield while ammonium molybdate had a significantly lower yield than the control.

The treated plants matured a week and a half earlier than the control group (the control group was desiccated on 95^{th} day). The average height, dry weight, number of pods per plant and seed yield was higher in all of the treated plants compared to the control (Table 4.3.5.7.), with the exception of the plants treated with ammonium molybdate where the seed yield was even lower than the control. However, variation in the colour of the harvested seed from the plants treated with different chemicals and untreated Tristar was noticeable (Figure 4.3.5.2.).

Table 4.3.5.5. Mean square (MS), degrees of freedom (df) and probability (Pr) of F value for seed yield of the Tristar fenugreek plants treated with different chemicals (six + control) in the LRC greenhouse (Spring, 2005) as determined by a fixed effects model ANOVA[1].

Source	df	MS	Pr of F
Total	13		
Replication	1	0.004	0.124
Chemicals	6	0.267	0.000
Residual	6	0.001	
CV		1.9	
R^2		0.9	

[1] ANOVA was done on square root transformed data

Table 4.3.5.6. Mean seed yield[1] of Tristar fenugreek plants treated with seven chemicals (six + control) under LRC greenhouse Spring 2005.

Chemicals	Seed yield (g)
Magnesium sulphate	5.6 a
Calcium chloride	4.7 b
Ammonium sulphate	3.4 c
Cupric sulphate	3.3 c
Ferrous sulphate	2.8 d
Control	2.3 e
Ammonium molybdate	1.7 f

[1] Means followed by same letter were not significantly different at ($p=0.05$) when compared using a LSD test.

Table 4.3.5.7. Mean values[1] for days to maturity, stand height (cm), dry weight (g), and 1000 seed weight (g) of Tristar fenugreek plants per pot treated with six chemicals and the untreated control under LRC greenhouse (Spring, 2005).

Chemicals	No. of days to maturity[2]	Stand height[3]	Dry weight[4]	1000 seed weight[5]
Control	95.0 a	33.5 f	8.5 c	10.8 d
Ferrous sulphate	92.3 a	50.0 c	10.9 b	13.0 b
Calcium chloride	93.0 a	58.3 b	12.2 b	11.1 c
Cupric sulphate	91.3 b	39.1 d	13.0 a	11.2 c
Magnesium sulphate	90.6 b	52.7 c	11.8 b	14.9 a
Amonium sulphate	92.3 a	61.9 a	13.5 a	11.1 c
Ammonium molybdate	93.6 a	41.1 e	8.1 c	9.8 e

[1] Means followed by same letter word not significantly different at ($p=0.05$) when compared using a LSD test.
[2] Number of days taken by the plants in a pot to reach maturity or when desiccant was sprayed (control)
[3] Heights at harvest (95 days after seeding) of plants per pot
[4] Above ground biomass of plants per pot
[5] Seed size as determined by weight of 1000 seed of plants per pot

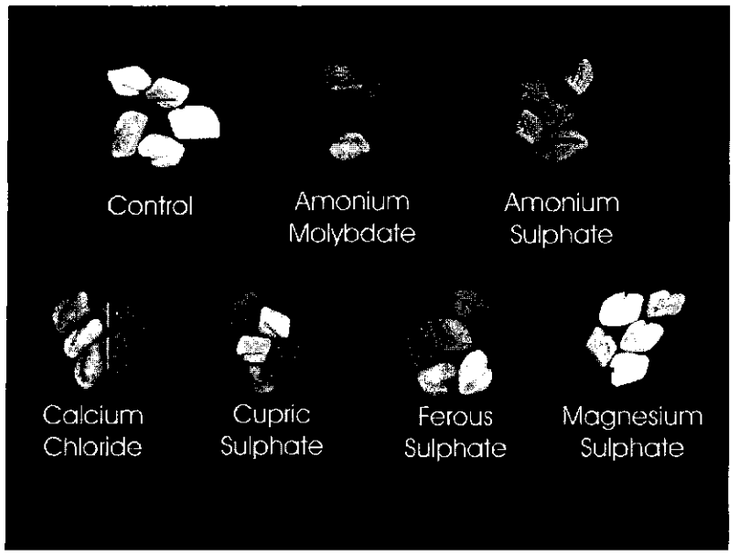


Figure 4.3.5.2. Seed colour of Tristar fenugreek treated with different chemical sprays and untreated control grown under the LRC greenhouse (Spring, 2005).

The seed yield from the field trial did not show a significant effect of chemicals (Table 4.3.5.8.). But the treated plants matured about two weeks earlier (13 days) than the untreated control. Effect of some of these chemicals on maturity, earliness and seed yield performance in fenugreek was observed earlier by De and Srivastava (2003) on another legume crop *Vigna radaiata*. A closer look at the mean seed yield values for the treatments revealed large differences among the chemical treated plants and untreated control (Table 4.3.5.9.). There was a 66 % difference between the cupric sulphate treatment and the control plants but, this difference was not found to be significant. This may have been due to the fact that only two replications were used under variable field conditions. Furthermore, greater variability under field conditions in comparison to the highly controlled environment of the greenhouse may have been responsible for the differences.

Another observation from these chemical spray trials was the fact that ammonium molybdate treated plants performed poorly under greenhouse conditions but, out yielded the control under field conditions. The most probable reason for this may be the use of *Rhizobium* inoculant in the field trial. The nitrogen-fixing enzyme nitrogenase of the nodulating *Rhizobium* has molybdenum as the essential cofactor of the enzyme complex (Petropoulos 2002; Subba Rao and Sharma 1968). Availability of molybdenum in the soil in the case of the field trial seems to have a beneficial effect on nitrogen fixation and hence general health and yield performance of the plants in the field. But since a soil-free mix was used as the growth medium in the greenhouse, there was no chance of microbial nitrogen fixation. Instead the application of molybdenum salt had a detrimental effect on the plants.

Table 4.3.5.8. Mean square (MS), degrees of freedom (df) and probability (Pr) of F value for forage yield of Tristar fenugreek plants treated with different chemicals (six + control) grown under irrigation at LRC (Summer, 2005) as determined by a fixed effects model ANOVA[1].

Source	df	MS	Pr of F
Total	13		
Replication	1	7.3	0.105
Chemicals	6	4.6	0.169
Residual	6	2.0	
CV		7.5	
R^2		0.7	

[1] ANOVA was done on square root transformed data

Table 4.3.5.9. Mean seed yield (kg ha^{-1}) [1] of Tristar fenugreek plants treated with different chemicals (six + control) under irrigation at LRC (Summer, 2005).

Chemicals	Seed yield (kg ha^{-1})
Cupric sulphate	413.0 a
Ammonium sulphate	396.8 a
Ammonium molybdate	395.5 a
Calcium chloride	376.7 a
Magnesium sulphate	370.2 a
Ferrous sulphate	361.4 a
Control	247.6 b

[1] Means followed by same letter are not significantly different at ($p=0.05$) when compared using a LSD test.

Chapter Five: Cytogenetic Studies on Fenugreek

5.1. Introduction

Most diploid fenugreek (*Trigonella foenum-graecum* L.) genotypes possess $2n$ = 16 chromosomes (Darlington and Wylie 1961). According to Darlington and Wylie (1945) the haploid chromosome number (n) of *Trigonella* can vary; some plants with n = 8, 9, 11 and 14 chromosomes have been identified. In contrast, *T. hamosa* from Egypt was found to have 16 and 44 chromosomes; *T. geminiflora* from Persia in Asia Minor and *T. grandiflora* from Turkestan have 44 chromosomes; *T. polycerata* from the Mediterranean region of south west Asia has 28, 30 and 32 chromosomes; while *T. ornithopodioides* is reported to have 18 chromosomes (Darlington and Wylie 1945).

Other types of variation in chromosome number also have been noted for fenugreek. Joshi and Raghuvanshi (1968) found extra B-chromosomes in some fenugreek lines. Presence of B chromosome is known to modify the growth of some plants (Petropoulos 2002). In addition; natural and chemically induced forms of autoploidy also appear to be tolerated within the species. Singh and Singh (1976) identified five fenugreek lines with double trisomics ($2n$+1+1) along with primary trisomics ($2n$+1) from the progeny of natural autotetraploids. Roy and Singh (1968) also produced tetraploid plants by treating fenugreek shoot apices with colchicine. Increased ploidy levels often result in bigger seed which may contain higher levels of chemical constituents and other valuable morphometric traits not present in genotypes with smaller seeds.

Treatment of seed with chemical mutagens such as ethyl methane sulfonate (EMS) has the potential to cause chromosome damage, resulting in individual mutations, as well as addition or loss of chromosomes from cells. In addition, natural and chemically induced forms of autoploidy also can cause chromosome number to vary (Petropoulos 2002).

The objective of this study was to determine if new fenugreek genotypes produced through mutation breeding, possessed a normal chromosome complement. In addition, to increase plant and seed size, Tristar fenugreek plants were treated with colchicine to double the chromosome number; plants possessing a tetraploid genotype were selected from the survivors and then selected for increased vigour, forage and seed yield. A cytogenetic analysis of the untreated control and selected mutant plants was done.

5.2. Materials and Methods

5.2.1. Plant material used

The Tristar fenugreek seed used in this study were taken from the Forage Research Laboratory of the Lethbridge Research Center (LRC), Agriculture and Agri-Food Canada (AAFC), Lethbridge, Alberta, Canada.

5.2.2. Assessment of chromosome number

Seeds (fifty) from mutant plants (M_3) which had been propagated for three generations were placed on moist filter paper and then kept at room temperature (23 °C) for 3 days to allow germination. Roots 2-3 cm long were placed in vials containing ice water and incubated over crushed ice in a cold room at 5 °C. After 28 h root tips were fixed in a 3:1 mixture of 95 % ethanol: glacial acetic acid (Sigma-Aldrich) for 24 h, and then stained in a mixture of 45 % acetic acid (Sigma-Aldrich) mixed with 2-3 drops of 1 % (W/V) acetocarmine (Sigma-Aldrich) for 1 minute. A total of 30 mitotic stages of colchicine treated plants were examined for each mutant line under a compound microscope.

5.2.3. Colchicine treatment

Tristar fenugreek seeds were placed on moist filter paper and kept at room temperature (23 °C) for 3 days to allow germination. After five days germinated seeds with roots 2-4 cm long were treated with 0.05, 0.1 and 0.2 % (W/V) colchicine (Sigma-Aldrich) mixed with Dimethyl Sulfoxide (DMSO) (Sigma-Aldrich) to promote effective absorption (Table 5.2.3.1).

Table 5.2.3.1. Mixture for colchicine treatments used for the Tristar fenugreek seed.

Colchicine conc. (%W/V)	DMSO (µL)
0 (Control)	0
0.2	400
0.1	200
0.05	120

Sets of 50 germinated seeds each were treated for 15, 30, 45 and 60 minutes in each colchicine concentration used in the experiment. The treated seeds were thoroughly washed (6X) with running tap water and then placed in labeled 8.5 cm diameter plastic petri plates. Fifty seeds from each treatment including untreated (control) seed were potted in labeled 6-inch green plastic pots containing a non-sterile, soil-free mix (LRC Soiless mix/Cornell mix). The soil free mix was composed of a 3.8 cubic foot bale of sphagnum peat moss, 18.6 kg bag of medium horticultural grade vermiculite, 1000 g of calcium carbonate flour, 1500 g of 18-6-12 Osmocote (Southern Agricultural Insecticides, Inc.), 1200 g 0-21-0, 20 g of "Fritted" trace elements, 15 g of 13.2 % chelated iron, 7 g of 14 % chelated zinc and 30 L of washed mortar sand (The Scott C.). The treated seed was grown in labeled plastic pots which were transferred to a greenhouse set to cycle 22/15 °C (day/night) temperature with 16 h days. Over the next 10 weeks growth of the surviving seedlings was monitored. Root tips from the surviving treated plants (10 weeks old) were collected and their ploidy assessed as described above.

After 100 days of growth in the greenhouse, the plants were desiccated with 0.4 % (W/V) Reglone (Syngenta Crop Protection Canada Inc.) solution along with a 0.23 % (W/V) surfactant (AG-SURF) (Interprovincial Cooperative Ltd.). Plants were then allowed to dry for 10 days before separating the seed for determination of seed yield.

5.3. Results and Discussion

5.3.1. Comparison of chromosome number in EMS treated lines

About 90 % of the cells from the EMS treated plants (M_3) possessed a normal chromosome number of $2n = 16$ (Figure 5.3.1.1.) in comparison to the untreated control group where 100 % of the cells were diploid ($2n = 16$). It was interesting to note that about 10 % of the mitotic cells observed in the EMS treated plants were mutated and contained cells with abnormal ($2n \neq 16$) chromosome numbers *i.e.,* the EMS treated plants possessed some cells with chromosome numbers ranging from 11 to 20 (Table 5.3.1.1.). It appears that selection for stable performance and agronomically suitable traits for three generations (for details refer to Chapter Three) may have contributed to elimination of most plants possessing a high number of cells with an abnormal chromosome number.

No evidence of chromosome translocations was observed. Noticeably long or short chromosomes or chromosome bridges between cells were not seen. Similarly, no evidence for chromosome endoreduplication was observed in the mutant lines. Major changes in chromosome numbers ranged from -5 ($2n = 11$) to + 4 chromosomes ($2n = 20$) in the mutant lines (Table 5.3.1.1. and Figure 5.3.1.2.) and, only could be accounted for through addition or deletion of chromosomes. A diploid chromosome number of $2n = 16$ is reported for *Trigonella foenum-graecum* L. (Darlington and Wylie 1961). No cells with a chromosome number close to $2n = 32$ were observed in our study, indicating that the chromosome number had not doubled during generation of the variants that we observed. Endoredupliaction has not been reported in *Trigonella foenum-graecum* L. to date.

However, Darlington and Wylie (1945) have reported variation in chromosome number in other species of *Trigonella* as discussed previously in section 5.1.

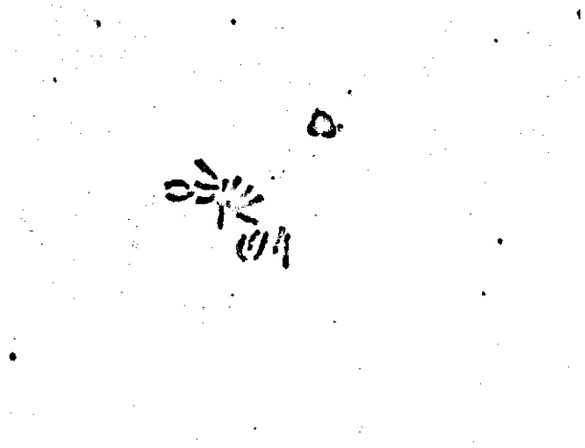


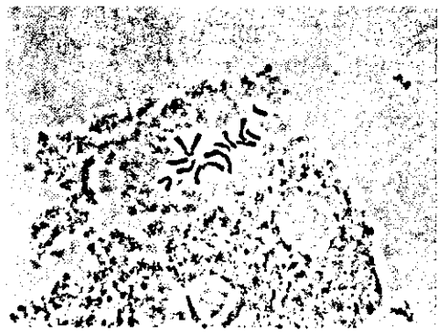
Figure 5.3.1.1. A representative chromosome squash from a normal mitotic cell in Tristar fenugreek (*2n* =16).

Table 5.3.1.1. Variation in chromosome number of representative EMS generated M_3 mutant lines of fenugreek in the greenhouse.

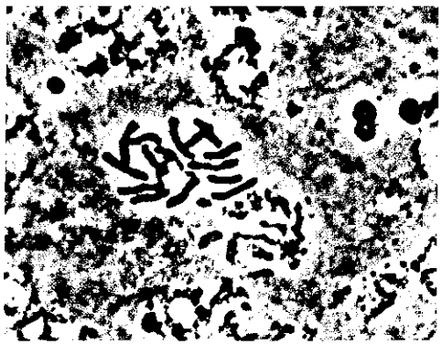
Line numbers of M_3 mutant plants*	% of cells with a normal ($2n = 16$) chromosome number**	Abnormal chromosome numbers observed	% of cells with an abnormal chromosome number**
48-4	94.8	$2n = 12 / 14 / 20$	5.2
131-1	92.9	$2n = 13 / 18 / 19 / 20$	7.1
131-19	90.0	$2n = 11 / 14 / 18$	10
134-5	94.8	$2n = 11 / 15$	5.2
88-34	93.0	$2n = 12 / 14 / 15$	7.0
88-37	93.8	$2n = 11 / 13 / 17 / 20$	6.2
225-2	94.0	$2n = 11 / 12 / 15$	6.0
227-11	94.7	$2n = 13 / 18$	5.3
227-12	92.2	$2n = 11 / 13 / 17 / 20$	7.8
227-22	93.5	$2n = 13 / 18 / 19 / 20$	6.5
227-36	91.8	2n = 11 / 13 / 18	8.2
267-13	94.2	2n = 12 / 14 / 20	5.8
242-9	94.5	$2n = 11 / 15 / 17$	5.5
309-19	92.7	$2n = 15 / 17 / 19$	7.3
340-5	90.6	$2n = 11 / 14 / 17 / 18$	9.4
340-6	94.4	$2n = 11 / 15 / 19$	5.6
340-10	94.5	$2n = 11 / 12 / 14 / 20$	4.5
340-12	90.5	$2n = 11 / 14 / 20$	9.5
340-15	92.3	$2n = 11 / 13 / 17 / 20$	7.7
340-19	95.0	$2n = 12 / 14 / 15$	5.0

* Only top yielding lines (single plant pot^{-1}) with respect to forage (above ground biomass) and seed yield under greenhouse conditions were used in the chromosome study

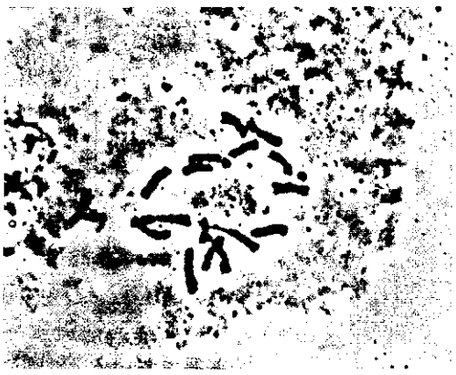
** A total of 30 mitotic stages of colchicine treated plants were examined for each line.



A. ($2n$ = 12, Line no. 267-13)



B. ($2n$ = 17, Line no. 242-9)



C. ($2n$ = 15, Line no. 88-34)

Figure 5.3.1.2. (A-C). Representative chromosome squash from 3 different lines of EMS treated Tristar fenugreek plants that were selected for stable growth characteristics over three generations following mutagenesis.

5.3.2. Identification of tetraploid seedlings derived from colchicine treated Tristar seed

All seedlings treated with colchicine exhibited a high level of mortality throughout the 10 weeks of growth in the greenhouse following the treatment. The highest levels of surviving plants containing cells with a doubled chromosome number ($2n = 32$) were observed for the seedlings treated for 15 minutes in 0.05 % colchicine (Figure 5.3.2.1.). No chromosome doubling but a 100 % survival rate was observed in the untreated control group (10 weeks old) *i.e.* no evidence of chromosome endoreduplication was observed in the control plants. The percent of survival among different concentrations of colchicine treated plants is shown in Figure 5.3.2.2. Flowering and fruiting were observed in all of the surviving plants. However, across all treatment times and colchicine concentration combinations, stunted growth accompanied by shriveled leaves was observed. Some of the surviving plants were dwarf, extremely late in flowering and, accompanied by very low yields. Flowering and pod formation in some of the treated plants were about a week earlier than for the untreated control plants.

Considerable variability with respect to morphometric and reproductive parameters was noticed among plants obtained from the different treatments (Table 5.3.2.1.). Most cells in the colchicine treated plants had double the normal number of chromosomes ($4n = 32$) found in Tristar fenugreek and were tetraploid (Figure 5.3.2.3.). Some of these plants had larger leaves, longer pod lengths and greater height in comparison to control Tristar plants (Figure 5.3.2.4.).

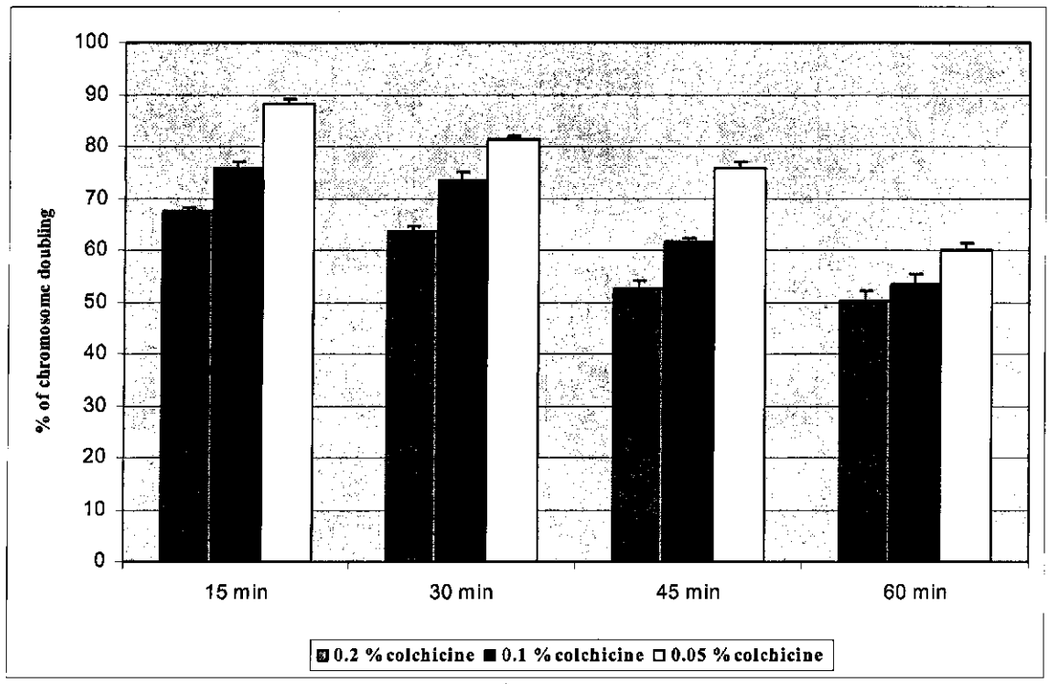


Figure 5.3.2.1. Proportion of cells with $4n$=32 chromosomes in plants that were treated with 0.05, 0.1 and 0.2 % (W/V) of colchicine for 15, 30, 45 and 60 minutes after ten weeks of growth in the greenhouse. Control plants did not show variation in chromosome number from $2n$ = 16.

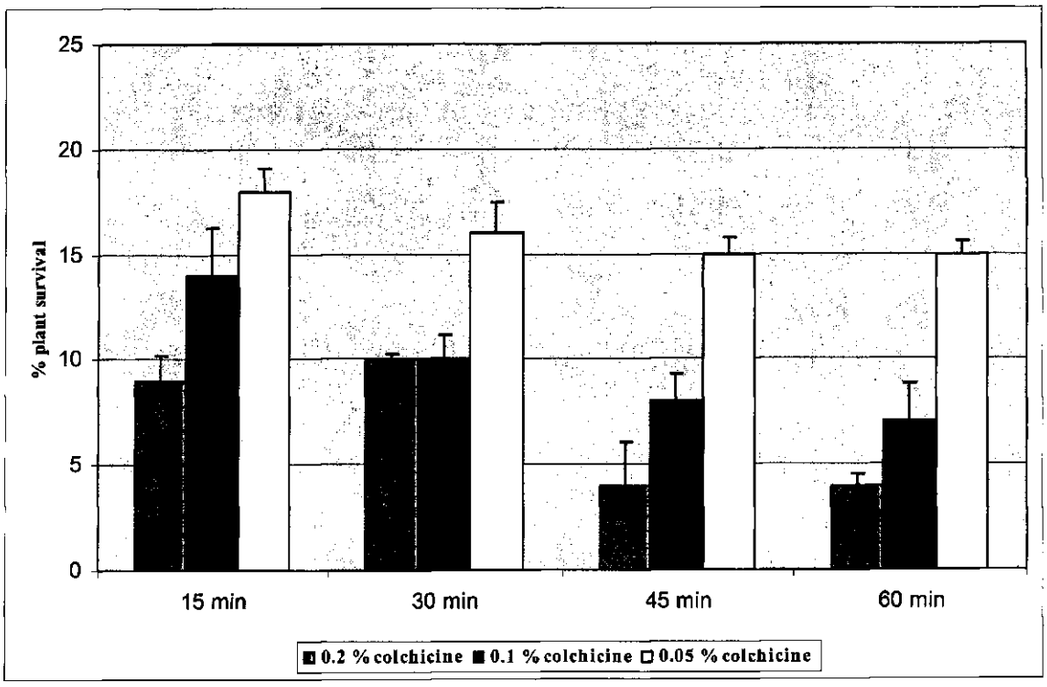


Figure 5.3.2.2. Percent survival of Tristar fenugreek seedlings after treatment with 0.05, 0.1 and 0.2 % (W/V) colchicine for 15, 30, 45 and 60 minutes after ten weeks of growth under greenhouse conditions. Control plants exhibited 100 % survival rate.

Table 5.3.2.1. Range in morphometric parameters for surviving plants (single plant / pot) grown in the greenhouse, after 50 seedlings were treated with 0.05, 0.1 and 0.2 % (W/V) colchicine for 15, 30, 45 and 60 min each.

Times (min)	Height (cm.)[1]	Inter-node length (cm)	No. of nodes	Total no. of pods	No. of single pods	No. of double pods	Pod length (cm)	Dry weight (g)[2]	Seed no.[3]	1000 seed weight (g)[4]
Control*	9.6-16.5	0.6-2.1	4-13	8-9	4-9	0-2	6.9-16.4	12.3-21.5	68-116	43.9-72.1
0.2 % Colchicine										
15	14.6-22.3	1.1-2.3	11-18	7-11	3-6	1-3	13.7-18.6	16.9-22.6	79-111	60.3-64.5
30	8.9-29.3	1.2-1.6	6-19	5-9	3-6	1-2	13.5-18.4	12.6-24.7	88-112	60.7-70.9
45	12.8-23.6	1.1-2.1	10-19	4-12	2-6	1-3	13.5-18.1	16.8-24.7	88-123	52.1-67.8
60	16.4-23.5	0.9-1.3	15-19	5-12	3-7	1-3	12.2-18.6	18.5-22.4	83-111	57.2-71.1
0.1 % Colchicine										
15	18.6-24.5	0.9-1.4	15-21	5-12	3-6	1-4	14.1-17.3	18.5-24.5	89-117	57.7-66.2
30	13.6-23.4	0.8-1.6	11-23	6-9	3-6	1-3	13.4-17.9	16.1-23.4	87-116	58.1-62.1
45	12.5-24.5	0.8-1.4	16-21	6-10	4-6	1-3	13.9-17.3	16.9-24.2	77-121	57.1-72.7
60	10.6-23.4	0.9-1.5	9-24	5-9	3-7	1-2	12.5-16.3	18.8-22.3	98-116	56.1-64.7
0.05 % Colchicine										
15	13.7-24.2	0.8-1.3	11-22	7-11	3-8	1-4	12.4-16.7	19.8-23.4	89-112	58.4-64.1
30	16.9-23.8	0.7-1.8	17-22	8-11	2-7	1-4	13.9-16.3	18.5-23.4	97-118	57.6-61.3
45	17.6-22.8	0.9-1.4	16-21	5-9	3-7	1-2	12.7-15.9	18.9-23.6	88-113	57.5-63.6
60	16.5-22.6	0.5-1.2	14-21	5-12	3-7	1-3	14.7-16.3	18.7-24.5	97-122	50.0-58.4

* Untreated control; [1] Heights of plants measured at 100 days from the day of seeding after being sprayed with dessicant; [2] Above ground biomass of individual plants; [3] Number of seeds from pods produced by individual plants and; [4] Seed weights (weight of 1000 seeds) calculated for an individual plant.

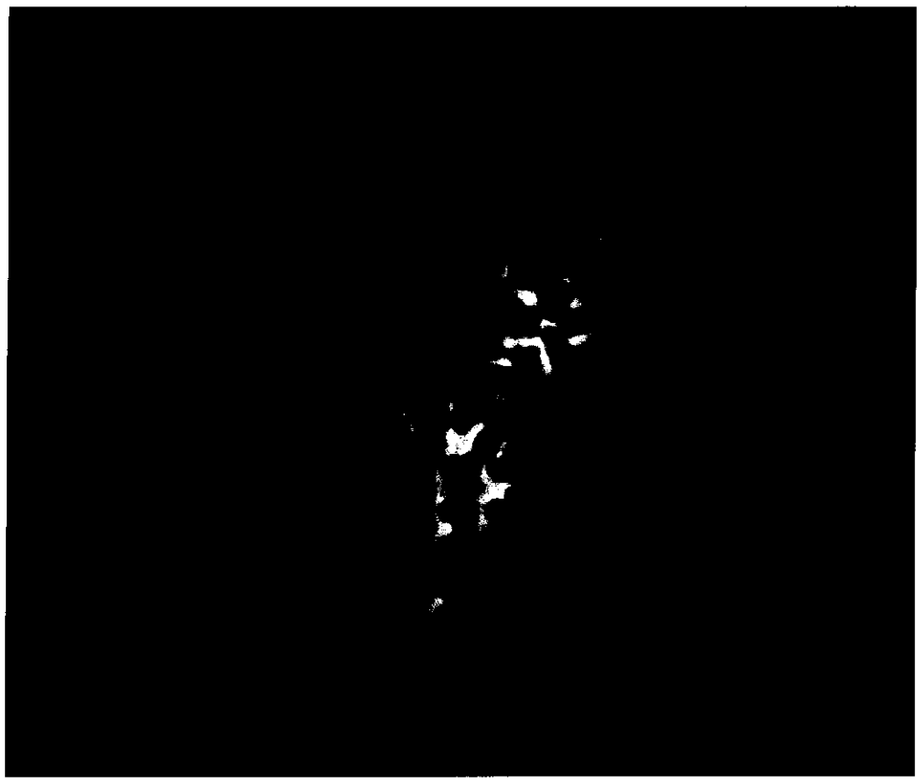


Figure 5.3.2.3. Mitotic metaphase of a tetraploid ($4n = 32$) cell of Tristar fenugreek after colchicine treatment.

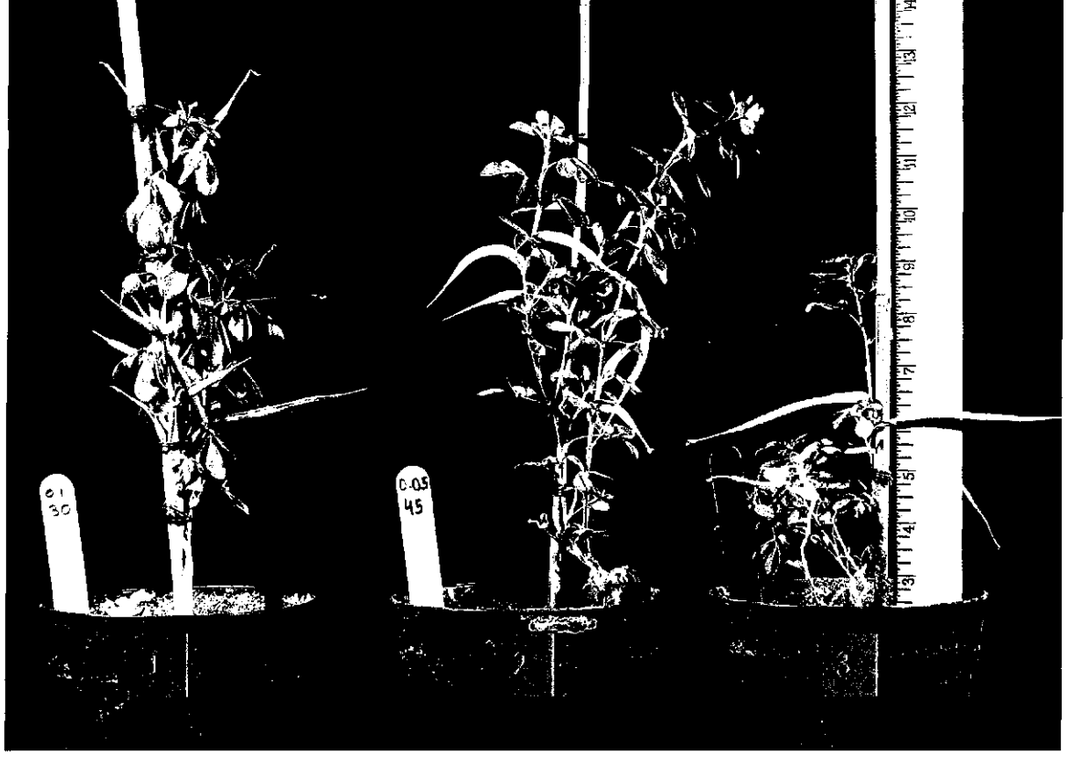


Figure 5.3.2.4. Tristar fenugreek treated (1) with 0.1 % (W/V) colchicine for 30 minutes, tetraploid ($4n = 32$); (2) with 0.05 % (W/V) colchicine for 45 minutes, tetraploid ($4n = 32$); and (3) normal diploid ($2n = 16$) Tristar fenugreek (untreated control).

5.4. Conclusions

About 90 % of root tip cells examined from EMS mutated fenugreek plants possessed a normal chromosome number $2n = 16$. The selected mutants continued to produce some abnormal gametes but, were not sterile and did not appear to express abnormal or atypical plant characteristics. The EMS treated plants were selected for high seed yield and a determinate growth habit and have potential to be used for production of new and, improved fenugreek cultivars.

Chromosome doubling was observed in fenugreek seeds treated with colchicine. The ideal concentration of colchicine for successful chromosome doubling in fenugreek (*Trigonella foenum-graecum* L.) was 0.05 % and, the duration of treatment time was 15 minutes. The tetraploid plants generated showed a wide variation with respect to morphometric and reproductive parameters; these properties could serve as an important basis for selection of better seed and forage yield in the future.

Chapter Six: Study on Potential Insect Pests of Fenugreek

6.1. Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual crop belonging to the legume family. Dried leaves of this crop have been reported to have insecticidal properties and in India they are effectively used in storing grains and cereals in traditional old-fashioned granaries (Petropoulos 2002). However, thrips have been identified as one of the pests of fenugreek in the United Kingdom, Greece and Australia (Petropoulos 2002). Plant bugs of the genus *Lygus* and *Adelophocoris* [*A. lineolatus* (Goeze)] (Heteroptera: Miridae), alfalfa weevils and other common prairie pests of alfalfa are well documented (Cárcamo *et al.* 2003; Philip and Mengersen 1989). However, nothing is known about their incidence in fenugreek. Alfalfa is a well known forage crop and is also botanically closely related to fenugreek, belonging to the same subfamily (Papilionaceae) under the family Leguminacae (=Fabaceae). *Lygus* bugs are generalist pests of both alfalfa and canola in the prairies whereas alfalfa plant bugs are only considered serious pests of seed alfalfa (Cárcamo *et al.* 2003).

Fenugreek can produce high forage yield and quality, does not cause bloat in ruminants and is a source of steroidal sapogenins not present in other forage legumes (Petropoulos 2002). Commercial seed for the first forage cultivar "Tristar" developed for western Canada is expected to be available in the fall of 2006. Before the crop is used extensively, it was important to recognize the insect pests that could damage the crop under greenhouse and field conditions. The objectives of this preliminary study were:

i. To identify potential greenhouse and field insect pests of fenugreek.

ii. To compare common insect pests and beneficial groups of insects found in fenugreek with those found in alfalfa.

iii. To perform a comparative survey of ladybird beetle in fenugreek and alfalfa.

iv. To make an assessment of the nature of insect infestations and insect damage in fenugreek.

6.2. Materials and Methods

6.2.1. Greenhouse study

The greenhouse plants were swept twice every week for 10 minutes with a standard small indoor sweep net and the insects were identified and preserved in 70 % alcohol. Potential insect pests affecting growth of fenugreek in the greenhouse were observed, and damage patterns on the crop recorded, particularly with respect to thrips, aphids and fungus gnats (sciarid flies). The sweeping was repeated 3X per week after treatment with chemical control agents Success 480 SC (Dow AgroScience Canada Inc) and Pirliss® 50 DF (Zeneca Agro) to identify changes in number of insects sampled. Every month (May through September) during 2004 and 2005, sampled insects particularly thrips were transferred in plastic vials from the greenhouse to the field crops at different growth stages (flowering, post-flowering, fruiting and maturity) to study the effects of thrip infestations under field conditions.

6.2.2. Field study

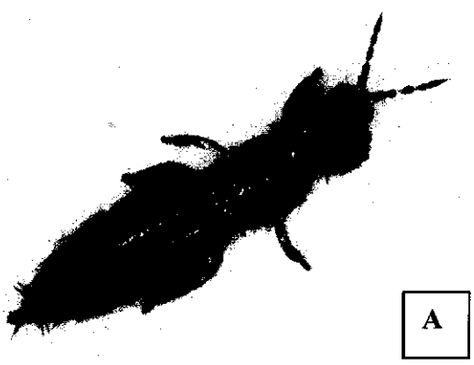
Irrigated fenugreek plots and adjacent alfalfa plots were sampled by using a sweep net to determine the number and types of potential pests and their natural enemies in the fields. The sampling time for each crop was 15 minutes of sweeping single rows (6m) of fenugreek and 15 minutes of sweeping in a nearby alfalfa block (36 m X 38 m). Five plots (36 m X 38 m) of each crop were sampled by taking 50 sweeps per plot on September 2, 2005. After collection, each sample was put in glass vials containing 70 % alcohol. These samples were separated, mounted, labelled and identified into different insect species using standard taxonomic keys and by comparison with similar specimens

from the insect museum at Lethbridge Research Center (LRC), Agriculture and Agri-Food Canada (AAFC), Lethbridge, Alberta, Canada. Identified specimens were stored as voucher specimens for future reference work at the LRC insect museum. A t-test was done to compare mean values of different herbivore and predatory species of insects sampled ($p = 0.05$) for the fenugreek and alfalfa crops using the statistical software SYSTAT (version 10.2).

A sex ratio pattern study on *Lygus* bug species on fenugreek and alfalfa crops was conducted based on August 19, 2005 sampling. A separate ladybird beetle diversity study on the two dates was also conducted by making 50 sweeps in representative fenugreek and alfalfa blocks (36 m X 38 m) with 5 replicates in each case. Only the ladybird beetle species were recorded in the latter case. All important species included in the studies were photographed using a Nikon Coolpix 995 camera.

6.3. Results and Discussion

Major insect pests of fenugreek observed under greenhouse conditions were western flower thrips (*Frankliniella occidentalis* Pergande) also known as WFT (Figure 6.3.1. A); pea aphid (*Acyrthosiphon pisum* (Harris)) (Figure 6.3.1. B); and dark winged fungus gnat (Figure 6.3.1. C) belonging to the Sciaridae family. Although fungus gnats were observed to be associated with fenugreek under greenhouse conditions, no serious reduction in yield was noticed. Predatory mites like *Hypoaspis s*pp. and nematodes like *Steinernema feltiae* (Filipjev) have been recommended as biocontrol agents against fungus gnats (Greer and Driver 1999; Greer and Driver 2000; Greer 2005; Harper and Roberts 1988; Palumbo 1998).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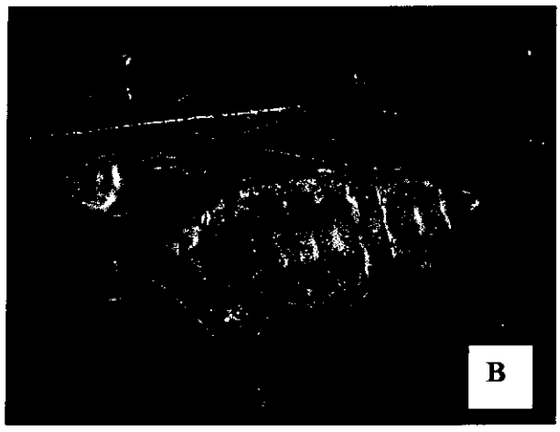
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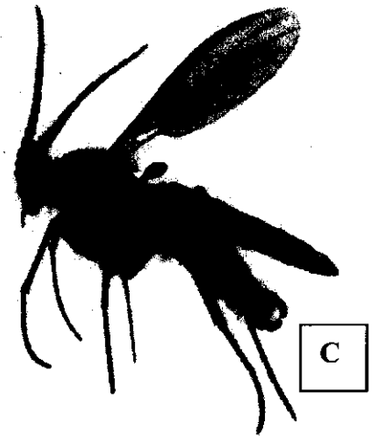
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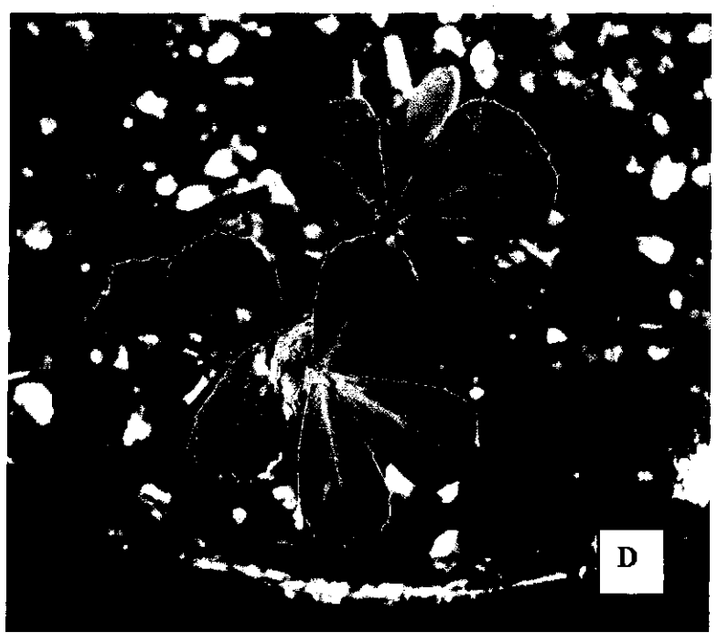
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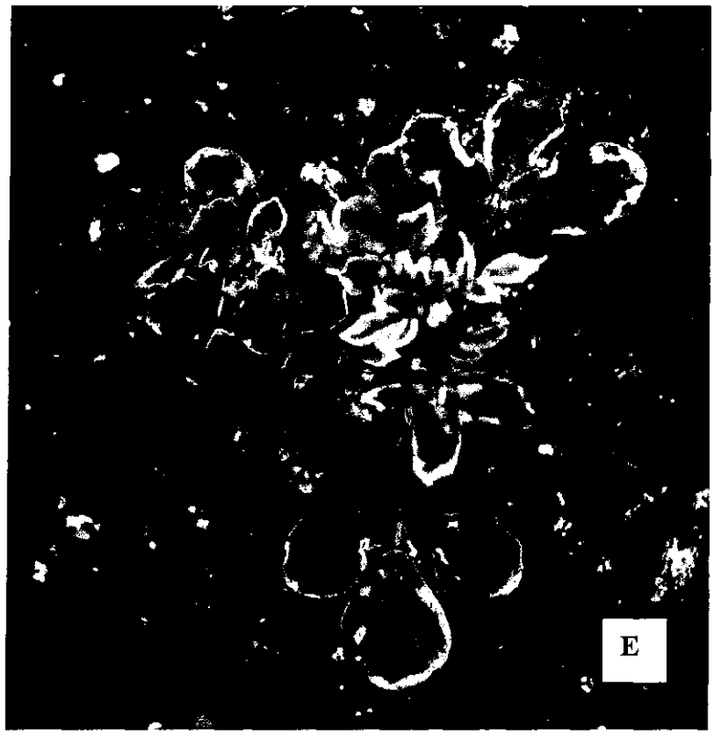
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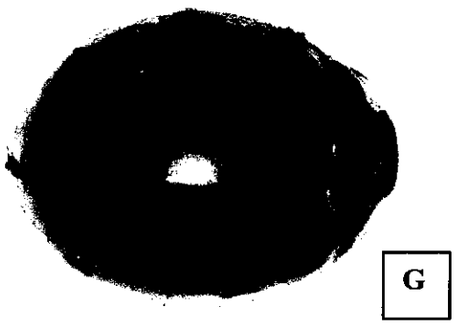


Figure 6.3.1. A. Western flower thrip (*Frankliniella occidentalis* Pergande); B. Pea aphid (*Acyrthosiphon pisum* (Harris)); C. Dark winged fungus gnat; D. Healthy fenugreek seedling; E. Thrips damaged fenugreek seedling; F. *Lygus* bug *(Lygus keltoni* (Schwartz & Foottit)); G. Seven spotted ladybird beetle (*Coccinella septempunctata* L.) and H. Parenthesis ladybird beetle (*Hippodamia parenthesis* (Say))

The most serious damage to the plants was inflicted by WFT in the greenhouse, and this is the first report of a thrip infestation on fenugreek in North America. However, deliberate transfer of WFT from greenhouse grown plants to field grown fenugreek plants, did not produce noticeable damage at any growth stages (flowering, post-flowering, fruiting and maturity) of the plants.

The physical effect of WFT on fenugreek was characterised by the presence of white patches on the leaf surfaces. Presence of such markings is an indication of a serious thrip infestation and is normally followed by stunted growth, reduced vigour and dwarfing of the plant. This reduction in plant growth resulted in weaker stems and curly leaves that dropped off at an early stage of plant development (pre-flowering) leading to death of the plant (Figure 6.3.1. D & E). Thrips appear to be the most serious pest of fenugreek in the greenhouse. Success 480 SC, a natural metabolite derived from a bacteria containing spinosad @ 480g L^{-1} applied at the rate of 1.5-2.0 mL 10 mL^{-1} of water was found to be an effective agent against thrip infestations, while treatment with Pirliss® 50 DF applied at the rate of 1.5 tablespoons $4L^{-1}$ of water was found to be effective against aphids in the case of fenugreek. Both potentially harmful (herbivores) and beneficial (predators/parasitoids) insects were present in the fenugreek and alfalfa plots (Table 6.3.1).

Table 6.3.1. Abundance of insects in fenugreek and alfalfa

Species	Fenugreek			Alfalfa		
	August[1]	September[2]		August[1]	September[2]	
	Total #	Mean	SE	Total #	Mean	SE
I. HERBIVORES						
Lygus bug (nymph)	38	112.6*	4.47	17	79.8	2.44
Lygus bug (adult)	32	189.0*	3.91	21	13.8	2.11
Alfalfa plant bug (nymph)	0	15*	1.76	79	24.4	1.29
Alfalfa plant bug (adult)	0	4.4*	0.51	116	14.0	2.17
Alfalfa looper	18	9.8	1.89	24	9.6	2.01
Alfalfa weevil	11	13.4	1.69	37	13.2	1.65
Sitona sp.	26	20.0	1.70	77	22.0	1.30
Aphids	66	75.4*	8.26	589	276.4	34.14
Grasshoppers	12	5.2	0.86	29	2.6	0.51
II. PREDATORS						
Ladybird beetle (larvae)	21	39.4*	4.15	0	13.8	1.65
Ladybird beetle (adult)	26	65.8*	7.14	14	27.0	4.35
Nabidae	19	28.6*	2.69	62	19.0	2.37
Syrphid	9	12.8*	1.59	16	3.2	0.86
Parasitic hymenoptera	128	139.8*	10.29	368	99.0	10.98

[1] Sampling for each crop on August 19, 2005 consisted of sweeping for 15 minute single row plots of fenugreek and 15 minutes in alfalfa block in an adjacent plot.
[2] Sampling for each crop on September 2, 2005 consisted of sweeping 50 times in each of 5 plots 36 m X 38 m in size.
* Mean value showing significant difference at $p = 0.05$ with respect to t-test between insects sampled in fenugreek and alfalfa plots on September 2, 2005

A detailed analysis of the *Lygus* species (Figure 6.3.2) revealed 4 species: *Lygus keltoni* (Schwartz & Foottit) (Figure 6.3.1. F) was found to be the most dominant followed by *Lygus elisus* Van Duzee and *Lygus borealis* (Kelton) whereas *Lygus lineolaris* (Palisot de Beauvois) was extremely rare. The sex ratio of the *Lygus* species also varied between fenugreek and alfalfa (Figure 6.3.3.). The two species, *L. borealis* and *L. elisus* showed an equal number of males and females on alfalfa (Figure 6.3.3. B) but a higher number of females on fenugreek (Figure 6.3.3. B). By contrast, the most dominant species *L. keltoni*, had an equal number of males and females on fenugreek (Figure 6.3.3. A) but a higher proportion of males in alfalfa (Figure 6.3.3. B). Therefore the overall male-female ratio in the case of *Lygus* bugs was found to be dominated by males in alfalfa and females in fenugreek.

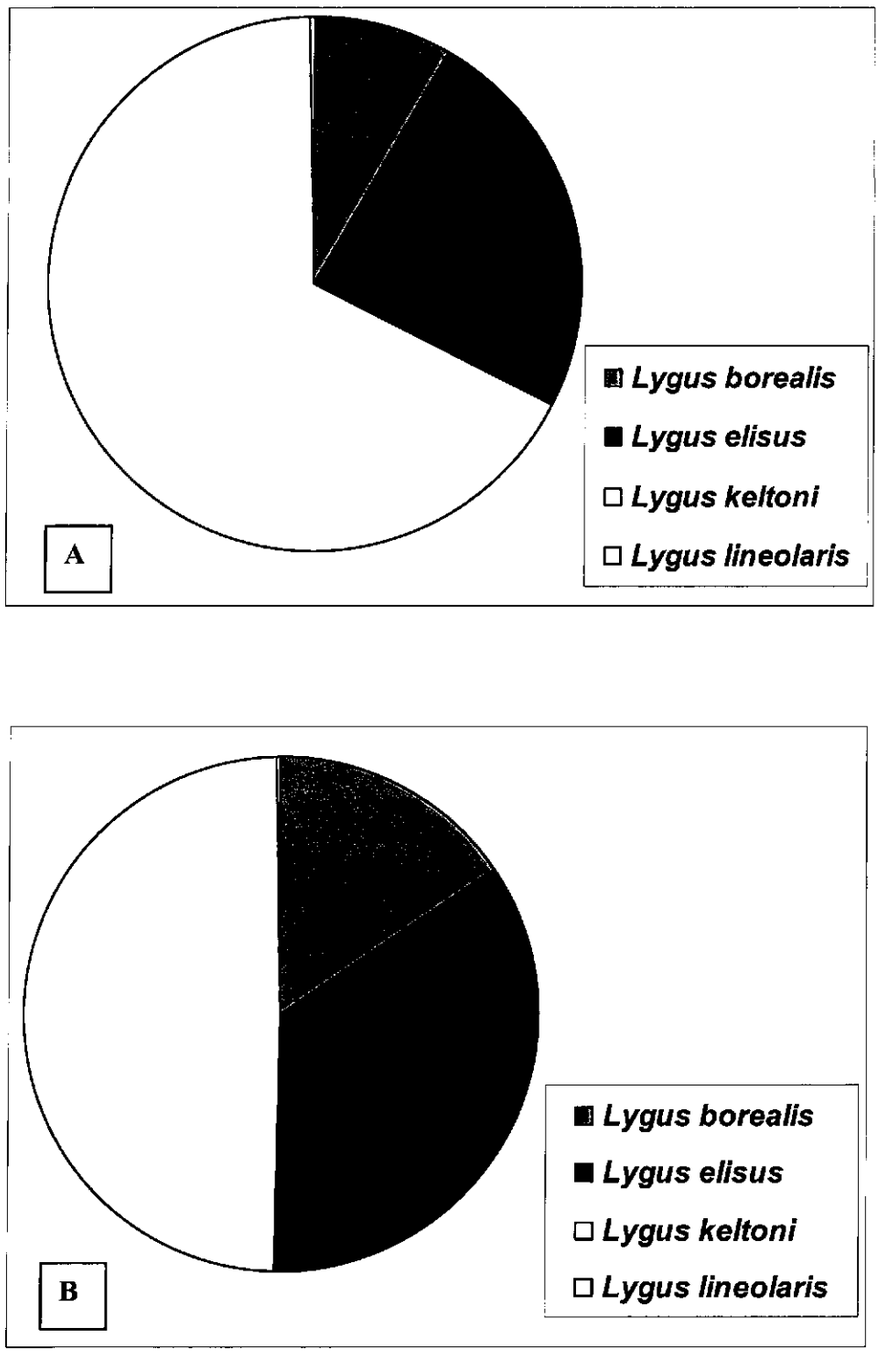
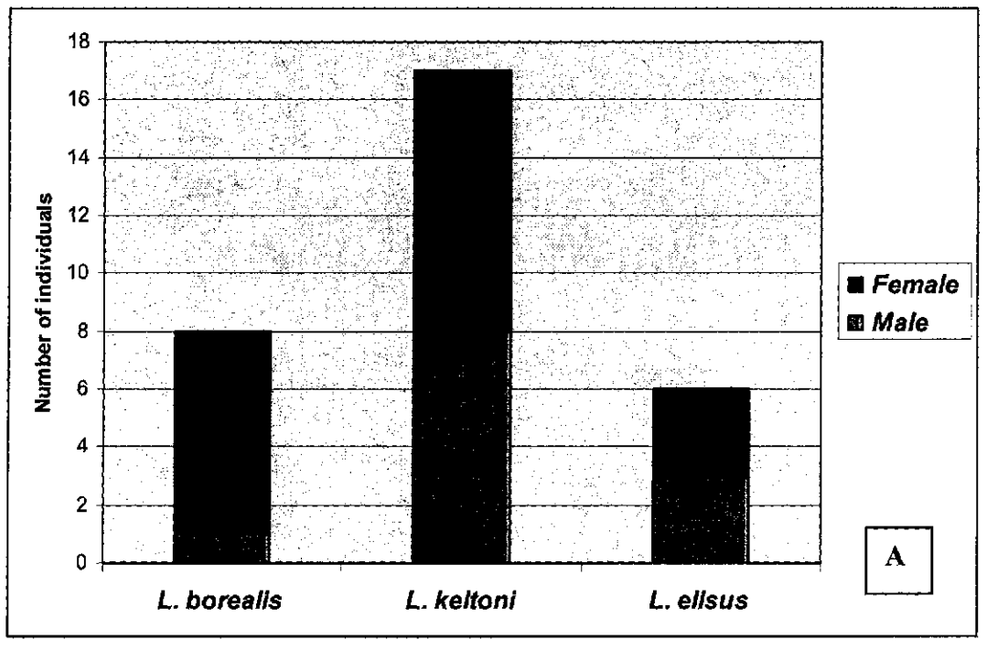


Figure 6.3.2. Proportion of the four *Lygus* bug species in irrigated fenugreek (A) and alfalfa (B) plots at LRC on the basis of September 2, 2005 sampling.



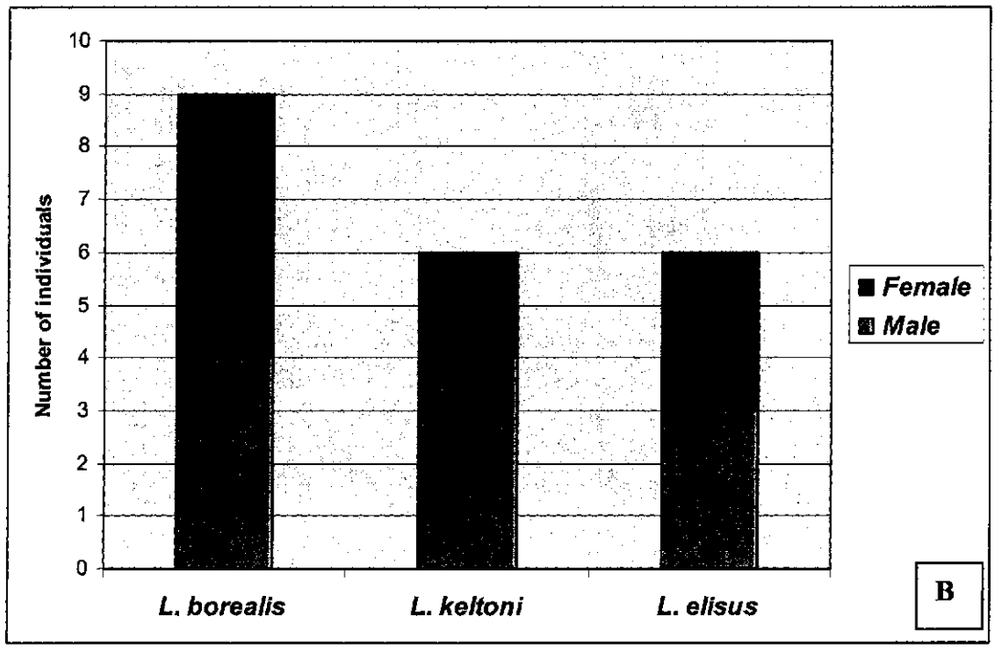


Figure 6.3.3. Proportion of male and female members in *Lygus* bug species observed in irrigated LRC plots in fenugreek (A) and alfalfa (B) on August 19, 2005.

A comparative analysis of plant bugs indicated that *Lygus* bugs are the dominant taxon in fenugreek (Figure 6.3.4.). The presence of nymphs of both species detected in fenugreek suggests that the plant bugs are not just migrating from nearby fields to fenugreek plots but that they are actually surviving and thriving on the fenugreek crop. Although we found most of the dominant alfalfa pests to be present in fenugreek we detected no incidence of serious insect damage in any fenugreek plot. This could be attributed to the insecticidal properties reported earlier for fenugreek (Petropoulos 2002). Alternatively it could be due to plant tolerance to the levels of insect pressure observed in our fields.

Fenugreek has been reported to be a weaker competitor with weeds compared to alfalfa (Moyer *et al.* 2003). The weed infestation pattern in fenugreek (with respect to the most prevalent weed species) observed in the irrigation plots at Lethbridge, Alberta in 2004 and 2005 is presented in Figure 6.3.5. It is possible that the higher diversity and number of insects in fenugreek may be due to the presence of weeds in the fenugreek plots and not the crop itself. Lack of insect damage to fenugreek may have resulted from the insects feeding on the weeds instead of fenugreek. Although *Lygus* bugs are generalist herbivores, some species have been reported to prefer certain plant species such as Brassicaceous weeds (Hatfield *et al.*1983). The density of ladybird beetles in this study was found to be higher in fenugreek than alfalfa which may be associated with a reduction in the aphid population in the crop (Figure 6.3.6.).

A fluctuation with respect to ladybird beetle species composition was noticed in samples collected in August and September (Figure 6.3.7). The 13-spotted ladybird beetle (*Hippodamia tredecimpunctata tibialis* (Say)), a native species and the 7-spotted ladybird

beetle (*Coccinella septempunctata* L.) (Figure 6.3.1. G), an introduced species were most dominant in August. However, parenthesis ladybird beetles (*Hippodamia parenthesis* (Say)), another native species (Figure 6.3.1. H) was found to be most dominant in the September sampling. In our study the population size of convergent ladybird beetle was not found to be dominant on both sampling dates. The scientific and corresponding common names of all the species of ladybird beetle and weed species studied are included in table 6.3.2.

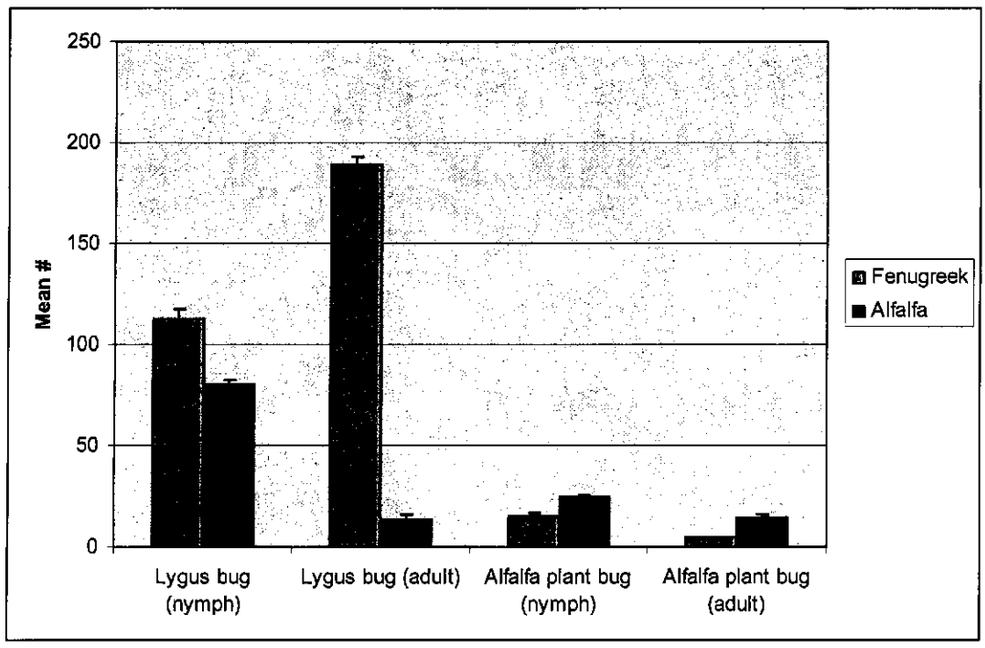


Figure 6.3.4. Mean (SE bars) of plant bugs in irrigated alfalfa and fenugreek plots at LRC on the basis of September 2, 2005 sampling.

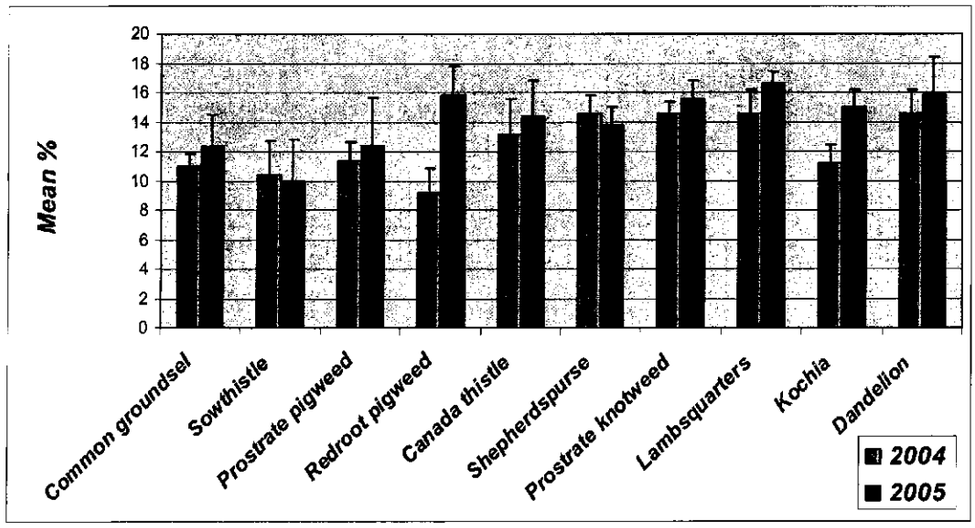


Figure 6.3.5. Mean proportion (SE bars) of common weeds in irrigated fenugreek plots at LRC in 2004 and 2005.

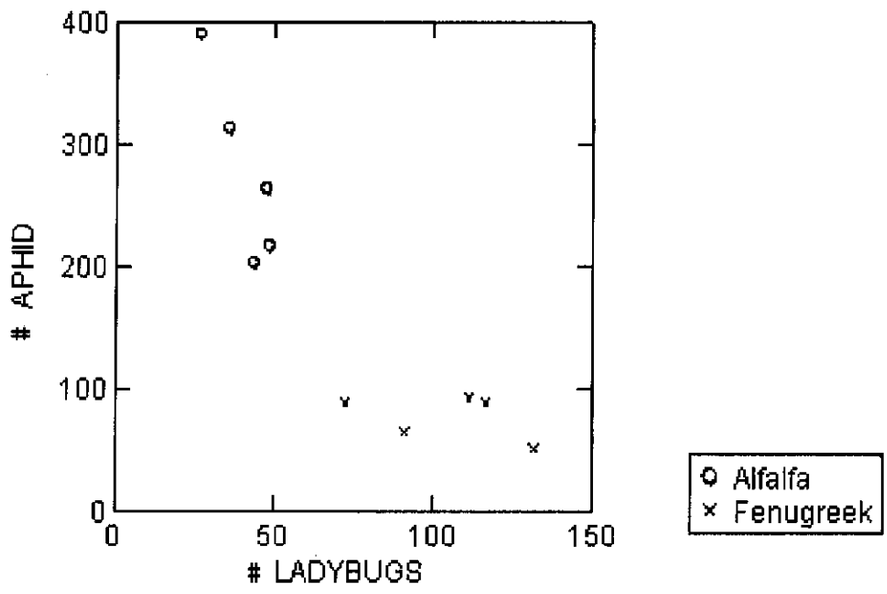
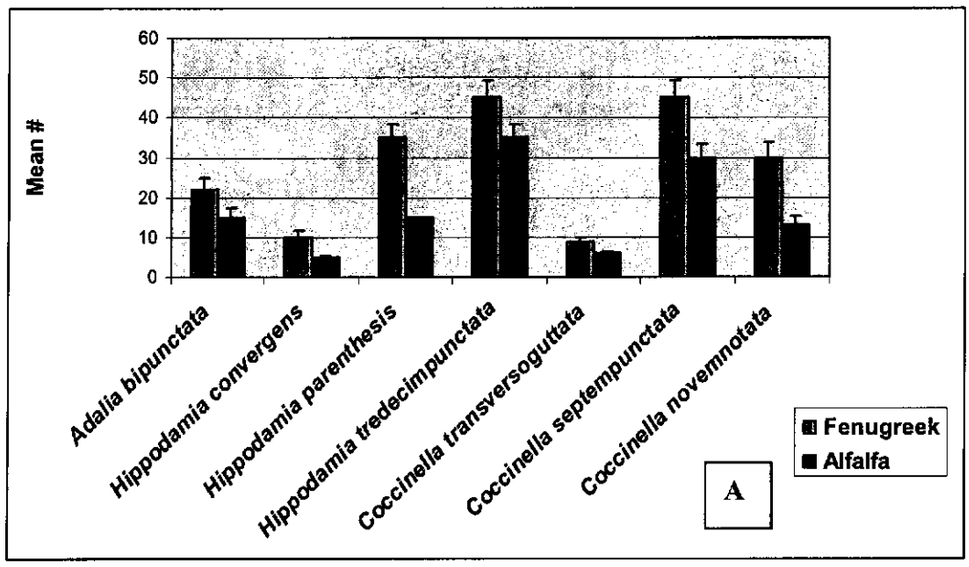


Figure 6.3.6. Number of ladybird beetles and aphids in fenugreek and alfalfa plots ($r = -0.89, p < 0.05$).



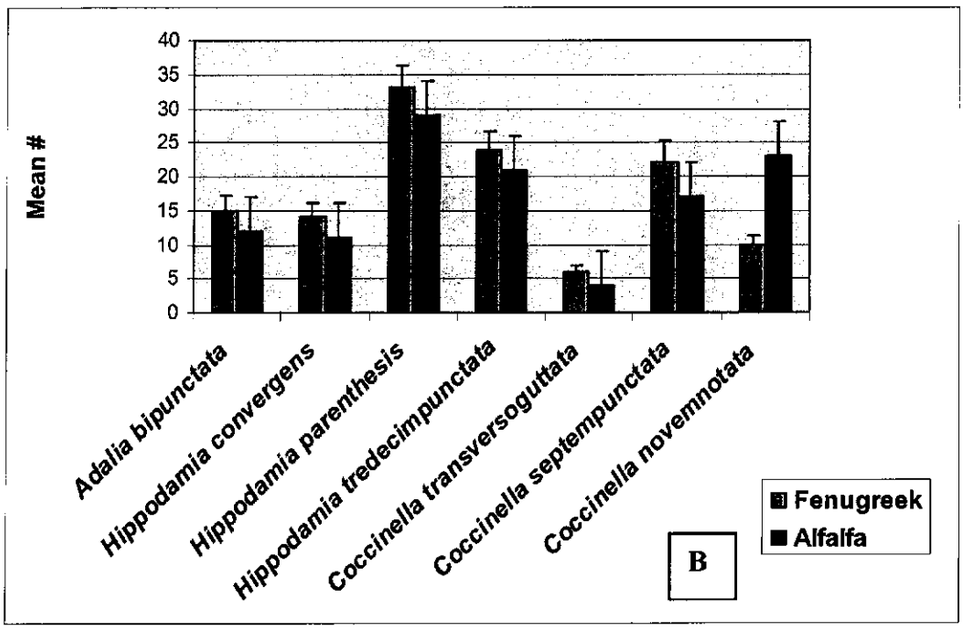


Figure 6.3.7 A. Number of ladybird beetles for each species in alfalfa and fenugreek grown under irrigation at LRC when sampled on August 19, 2005.

B. Number of ladybird beetles for each species in alfalfa and fenugreek grown under irrigation at LRC when sampled on September 2, 2005.

Table 6.3.2. Scientific names of the ladybird beetles and weed species studied.

A. Ladybird beetles	
Common name	**Scientific names**
Two-spotted lady bird beetle	*Adalai bipunctata* (L.)
Convergent lady bird beetle	*Hippodamia convergens* Guerin
Seven spotted lady bird beetle	*Coccinella septempunctata* L.
Parenthesis lady bird beetle	*Hippodamia parenthesis* (Say)
Transverse lady bird beetle	*Coccinella transversoguttata* (Falderman)
Nine spotted lady bird beetle	*Coccinella novemnotata* Herbst
Thirteen-spotted lady bird beetle	*Hippodamia tredecimpunctata tibialis* (Say)
B. Weed species	
Common name	**Scientific names**
Prostrate pigweed	*Amaranthus blitoides* S.Wats., Family- Amaranthanceae
Redroot pigweed	*Amaranthus retroflexus* L., Family- Amaranthanceae
Common groundsel	*Senecio vulgaris* L., Family-Asteraceae
Sowthistle	*Sonchus oleraceus* L., Family-Asteraceae
Dandelion	*Taraxacum officinale* Webber in Wiggers, Family-Asteraceae
Canada thistle	*Cirsium arvense* (L.) Scop., Family-Asteraceae
Shepherdspurse	*Capsaella bursa-pastoris* (L.) Medic, Family-Brassicaceae
Kochia	*Kochia scoparia* (L.) Schrad, Family-Chenopodiaceae
Netseed lambsquarters	*Chenopodium berlandieri* Moq., Family-Chenopodiaceae
Prostrate knotweed	*Polygonum aviculare* L., Family- Polygonaceae

6.4. Conclusions

Insect pest damage to fenugreek in western Canada was relatively low in 2005. However, in the long run the major pests of alfalfa could turn out to be important pest sources for fenugreek. Based on the 2005 studies the most serious pest seems to be WFT in the greenhouse. Among other potential pests to be considered seriously for long term studies are *Lygus* bugs, and to a lesser extent alfalfa plant bugs and aphids. Along with potential pests, fenugreek also attracted a number of beneficial predators such as ladybird beetles and parasitoid wasps. This may be beneficial for the crop in the long run as we could see a pattern of lower number of aphids and higher number of ladybird beetles in fenugreek compared to alfalfa. Further detailed studies using caged insects on plants are required to expand our knowledge of the insect plant interactions with this novel crop.

Chapter Seven: Thesis Summary

Fenugreek (*Trigonella foenum-graecum* L.) is traditionally used as a spice crop and a "pot herb" in parts of Asia, Europe, Africa, North and South America and Australia. This crop is now being developed as an annual forage legume crop in western Canada. "Tristar" is the first North American fenugreek forage cultivar. However, Tristar does not produce high quality seed every year due to an indeterminate growth habit and/or long maturity duration. It takes about 120 days to produce mature seed in a temperate climate such as that found on Canadian prairies where only ~100 frost free days are available for crops to mature. Widespread use of fenugreek requires a solution to the seed production problem in this crop which can be achieved through development of a proper understanding of the physiology of the plant, its interaction with the environment and, selection for new early maturing cultivar that can consistently produce high yield and quality seed under prairie growth conditions. To achieve this goal a series of studies on the genetics and agronomic aspects of fenugreek was conducted.

Mutation breeding using a Tristar base population and ethyl methane sulfonate (EMS) as the mutagen was done to generate early maturing plants with a determinate growth habit and high seed yield. Plants were selected for four generations (M_1-M_4) to stabilize the traits. Chromosomal studies on these plants indicated that they maintained a normal chromosome complement with $2n = 16$. Tristar plants also were treated with colchicine to double the chromosome number ($4n = 32$); this produced more vigorous plants with better above ground biomass than the base population. Moreover, a comparative SEM study of the floral structures (androecium, gynoecium and anther lobes), pollen grains and seed surface of normal Tristar fenugreek and mutant plants

showed no striking differences. This suggests that the mutation(s) induced did not drastically alter the reproductive and morphological structures of the EMS generated mutants.

The present study indicates that mutagenesis using EMS was able to generate a large amount of variability in the fenugreek population and many mutant lines showed important traits that were rare among world collections. The accessions considered adapted to western Canada differed in their ability to produce good quality seed within the 90 to 100 frost-free days normally available for crop growth in this region. Therefore, mutation breeding is an appropriate and valuable method for fenugreek improvement. The agronomically superior lines identified in this study, need to be tested in multi-location trials in coming years to determine their performance under a wide range of growing conditions. These trials will also determine their adaptation and the genotype X environment interactions they may exhibit.

Generation of variability and selection for desirable traits resulted in identification of some mutants that can be used as germplasm for improvement of this crop. Further studies should be done to determine if there is any change in the chemical constitution of the plant that may have resulted from pleiotropic effects of the mutations affecting seed traits. This study not only successfully induced variability for seed characteristics in fenugreek but, also identified mutants with high seed and forage yield along with earliness and a determinate growth habit.

Variability in seed yield of various world accessions of fenugreek also were examined under field conditions over a two year period and under two growing conditions (rain-fed and irrigation). The study indicated that year, genotype, year X

location and year X genotype interactions were significant for seed yield. Seed yield of this crop is influenced by environment more than forage yield and in this study even the interaction effect of year X genotype was highly significant. This further indicated that improvement in seed yield would be difficult and would require selection using multiple locations and years.

Multi-location trials conducted in southern Alberta and within the interior of British Columbia over two years indicated a highly significant effect of environment on both forage and seed yield while only a genotypic effect was significant for forage yield. Fenugreek is known to be adapted to rain-fed growing conditions in western Canada, but its biomass production can be increased by application of minimal irrigation in dry areas such as the southern part of Alberta. The data indicates that development of fenugreek cultivars with wide adaptation (for the entire western Canada) will be difficult and breeding programs may have to develop cultivars for specific environmental conditions to maximize yield performance.

Agronomic study on selected fenugreek cultivars revealed a significant effect of genotype and a highly significant effect of environment and harvest method on seed and forage yield. The interaction effect of genotype X harvest method was not significant. The study showed that swathing produced significantly higher seed yield than direct combine methods. This was expected as swathing is normally practiced as a method of seed harvest to force uniform and rapid seed maturity and to reduce loss due to seed shatter (Petropoulos 1973). This practice will help the production of high quality seed in high biomass producing cultivars such as Tristar which has an indeterminate growth habit.

Use of phosphate fertilizer showed a highly significant effect of environment and rate of phosphate application on both forage and seed yield. The interaction effect for phosphate rate X genotype was significant for forage and seed yield. The genotypes only showed significant effects for seed yield. Hence the study indicates that application of phosphate fertilizer have a promotive effect on the yield attributes of fenugreek under western Canadian agro-climatic zone.

In a separate greenhouse study, applications of gibberellic acid and six different chemical (foliar) sprays (ferrous sulphate, calcium chloride, cupric sulphate, magnesium sulphate, ammonium sulphate and ammonium molybdate) to Tristar plants exhibited significant effects on both seed yield and duration to maturity. However, no significant effect was observed for the treatments under the field conditions. This may have been due to the fact that the growing conditions in the field were very different from that of the greenhouse. Less number of replicates (2) in the experimental design could be another reason for observing such variation in the results between greenhouse and the field trial. Use of a larger number of replications (improved error control) may resolve this problem in future field experiments.

From the observations made in the greenhouse study it seems that GA_3 application after the post pod emergence stage is helpful for increasing seed yield. The field study however, showed that early application of GA_3 was more helpful in increasing seed yield. It is also important to note that reports on studies involving GA_3 on fenugreek are scanty and are mostly restricted to plant tissue culture. Moreover, most reports of gibberellin mutants within the legume family are limited to *Pisum sativum* L. (Ross *et al.*

1997). This study on the GA_3 foliar spray on fenugreek is new and, needs to be confirmed through more in depth research.

Plant bugs of the genus *Lygus* and *Adelophocoris,* alfalfa weevils and other common prairie pests of alfalfa are well documented (Cárcamo *et al.* 2003; Philip and Mengersen 1989). However, nothing is known about their incidence in fenugreek. Alfalfa is a well known forage crop and is also botanically closely related to fenugreek. *Lygus* bugs are generalist pests of both alfalfa and canola on the prairies whereas alfalfa plant bugs are only considered serious pests of seed alfalfa (Cárcamo *et al.* 2003). Before fenugreek is used extensively, it was important to recognize the insect pests that could damage the crop under greenhouse and field conditions. Western Flower Thrips were identified as insect pests in the greenhouse while, *Lygus* bugs and, to a lesser extent alfalfa plant bugs and aphids under field conditions exhibited potential to damage the crop. Along with potential pests, fenugreek also attracted a number of beneficial predators such as ladybird beetles and parasitoid wasps. This may be beneficial for the crop in the long run as we could see a pattern of lower number of aphids and higher number of ladybird beetles in fenugreek compared to alfalfa.

Fenugreek is a speciality crop in western Canada (Acharya *et al.* 2004b.) It can benefit the producer in a number of ways. As a legume crop it can condition the soil, fixing valuable nitrogen from the atmosphere and reduce the need for nitrogen fertilizers, effectively reducing production costs. A decreased need for application of N fertilizer to the soil, has the potential to decrease contamination of surface waters by runoff from agricultural fields. This has potential to reduce eutrophication of surface waters and limit contamination of ground water sources (Acharya *et al.* 2004a; Basu *et al.* 2004). These

properties also make fenugreek a useful legume crop for incorporation into short term rotations (Moyer *et al.* 2003). As a rain-fed crop, its water requirements are low; use of fenugreek will allow the saved water to be used for other crops or increasing the area under irrigation with the same volume of water. In addition, it can produce high quantity (Mir *et al.* 1993) and high quality forage (Mir *et al.* 1998), can be grown for hay or silage (Mir *et al.* 1998), does not cause bloat in cattle (Mir *et al.* 1997), and contains animal growth promoting substances such as diosgenin not present in other forage legumes (Mir *et al.* 1997).

High yielding and early maturing fenugreek cultivars adapted to the western Canadian climate could prove to be a big boost to the export enrichment of the Canadian forage industry as it has the potential to increase forage yield many fold in subsequent years. In this study some short duration, high yielding and determinate lines of fenugreek were identified for the short growing season found on the Canadian prairies. These lines will need to be acclimatized for few more years before they are released. Therefore, this study on fenugreek has the potential to improve the economics of beef production in western Canada due to lower production cost and assured seed yield due to superior quality of the lines and could hence lead to a more sustainable agriculture on the Canadian prairies.

Fenugreek seed is an important source of steroidal sapogenins such as diosgenin which are used extensively by both pharmaceutical and nutraceutical industries (Srichamroen *et al.* 2005). Diosgenin is often used as a raw precursor for the production of steroidal drugs and hormones such as testosterone, glucocorticoids and progesterone (Fazli and Hardman, 1968; Raghuram *et al.* 1994; Srichamroen *et al.* 2005). McAnuff *et*

al. (2002) reported that steroidal sapogenins are effective agents for the treatment of hypocholesterolemia and diabetes. Legume consumption is known to have a beneficial or protective effect in diabetes, hypercholesterolemia and coronary heart disease, as well as protecting against obesity and menopause (Mazur *et al.* 1998; Madar and Stork 2002). Specialty crops like fenugreek are attracting the attention of producers to meet manufacturing demands for "functional food" additives and Natural Health Products (NHP) in Canada (Fitzpatrick 2004). Hence the crop has the potential to develop into a health crop or "nutraceutical" in the long run in addition to being a forage crop, bringing economic benefits for farmers in western Canada.

Fenugreek is a new crop to North America that is being recognized in western Canada as having positive commercial, agricultural and environmental potential. New cultivars of this crop are now being made available to the Canadian producers through direct introductions. In our collections there is ample genetic variability from which selection for important agricultural, food and medicinal traits can be made. Some of these genotypes differ radically in morphology, growth habit, biomass and seed production capability as well as in chemical constituents found in the seed; e.g., the saponin, fibre, protein, amino acid and fatty acid content. This variability often is overlooked and could dramatically impact clinical and nutraceutical applications of the crop. We have evidence that genetic variability and, genotype X environment interactions play a significant role in this crop for traits such as forage and seed yield as well as in the chemical constitution of the seed. Adequate information on how genotype and genotype X environmental interactions can be managed for medicinal purposes needs to be developed. Clinical studies need to be conducted using locally grown seed to ensure public about its

usefulness as a medicinal plant. New cultivars of fenugreek with improved seed yield and enhanced levels of chemical constituents need to be developed for improving efficiency of its use both for cattle and human. Fenugreek, a traditional "Old World" crop, appears to have a future in the "New World" through application of breeding of novel, new and regionally adapted varieties.

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APPENDICES

Appendix I: The fenugreek world accessions, their corresponding sources, and origin. Seed used in this study are identified by line or name given to the seed in our collection, source of the seed and origin of the seed. All the lines used were *Trigonella foenum-graecum* L., with the exception of lines L3673* and L3674* which were *T. caerulea*.

Line	Source	Origin
9095	PGRC	CDC Saskatchewan
AMBER	AAFC – Lethbridge, Canada	Eston Saskatchewan
F18	CDC South, Canada	Iran
F70	CDC South, Canada	Turkey
F80	CDC South, Canada	India
F86	CDC South, Canada	Afghanistan
L3068	AAFC – Lethbridge, Canada	Uttar Pradesh, India
L3172	India	India
L3177	India	India
L3312	PGRC, Canada	Hamadan, Iran
L3375	China	China
L3671	PGRC, Canada	Washington, U.S.
L3672	PGRC, Canada	Germany
L3673*	PGRC, Canada	Unknown
L3674*	PGRC, Canada	United Kingdom
L3675	PGRC, Canada	Vienna, Austria
L3676	PGRC, Canada	Poland
L3677	PGRC, Canada	Germany
L3678	PGRC, Canada	Germany
L3679	PGRC, Canada	United Kingdom
L3680	PGRC, Canada	Geneva, Switzerland
L3681	PGRC, Canada	Romania
L3682	PGRC, Canada	France
L3683	PGRC, Canada	CDC Saskatchewan
L3684	PGRC, Canada	CDC Saskatchewan
L3685	PGRC, Canada	CDC Saskatchewan
L3689	Unknown	India
L3690	Gujarat, India	India
L3691	Hyderabad, Andhra Pradesh, India	India
L3692	Chennai, Tamil Nadu, India	India
L3693	Rajasthan, India	India
L3694	Lucknow, Uttar Pradesh, India	India
L3695	New Delhi, India	India
L3696	Guwahati, Assam, India	India
L3697	Amritsar, Punjab, India	India
L3698	Madhya Pradesh, India	India
L3699	Bangalore, Karnataka, India	India
L3700	Kolkata,	India

	West Bengal, India	
L3701	Mumbai, Maharashtra, India	India
L3702	Bhubaneswar, Orissa, India	India
L3703	Rajasthan, India	India
L3704	Amritsar, Punjab, India	India
L3705	New Delhi, India	India
L3706	Kolkata, West Bengal, India	India
L3707	Gujarat, India	India
L3708	Hyderabad, Andhra Pradesh, India	India
L3709	Mumbai, Maharashtra, India	India
L3710	Varanasi, Uttar Pradesh, India	India
L3711	Lucknow, Uttar Pradesh, India	India
L3712	Pushkar, Rajastahn, India	India
L3713	Bhopal, Madhya Pradesh, India	India
L3714	Chennai, Tamil Nadu, India	India
L3715	Imphal, Manipure, India	India
L3716	Guwahati, Assam, India	India
L3717	Bangalore, Karnataka, India	India
L3718	Bhubaneshwar, Orissa, India	India
L3719	Srinagar, Jammu and Kashmir, India	India
L3720	Rajasthan, India	India
L3721	Rajasthan, India	India
NGC 2001	Grocery store – Edmonton, Canada	Unknown
PI 229626	CDC – North, Canada	Unknown
PI 138687	PGRC, Canada	Shiraz, Iran
PI 143504	PGRC, Canada	Hamadan, Iran
PI 195691	PGRC, Canada	Ethiopia
PI 199264	PGRC, Canada	Greece
PI 211636	PGRC, Canada	Afghanistan
PI 269994	PGRC, Canada	Pakistan
PI 577711	PGRC, Canada	Meknes, Morocco
PI 577713	PGRC, Canada	Madrid, Spain
QUATRO	PGRC, Canada	CDC Saskatchewan
TRISTAR	PGRC, Canada	Shiraz, Iran
X92-23-3	PGRC, Canada	CDC Saskatchewan
ZT-5	PGRC, Caanada	CDC Saskatchewan

Appendix II: The seeding, harvesting and the spraying dates for the agronomic study (2004-2005) at LRC plots.

1. Rain-fed world accessions 2004

Seeding Date	Harvest Date	Spraying Dates
May 4, 2004	October 11, 2004	May 2, 2004 - Edge June 21, 2004 – Odyssey Sept 15, 2004 - Reglone

2. Irrigation world accessions 2004

Seeding Date	Harvest Date	Spraying Dates
May 20, 2004	September 20, 2004	May 15, 2004 - Edge June 21, 2004 – Odyssey Sept 15, 2004 – Reglone

3. Fertilizer trials 2004

Seeding Date		Harvest Date		Spraying Dates
Irrigation	May 20, 2004	Forage yield	August 20, 2004	May 15, 2004 – Edge June 21, 2004 – Odyssey Sept 15, 2004 Reglone
Rain-fed	May 3, 2004	Forage yield	August 20, 2004	May 2, 2004 - Edge June 21, 2004 – Odyssey Sept 15, 2004 – Reglone
Irrigation	May 20, 2004	Seed yield	October 6, 2004	May 15, 2004 - Edge June 21, 2004 – Odyssey Sept 15, 2004 – Reglone
Rain-fed	May 3, 2004	Seed yield	October 6, 2004	May 2, 2004 - Edge June 21, 2004 – Odyssey Sept 15, 2004 – Reglone

4. Swath vs. Combine 2004

Seeding Date		Harvest Date	Spraying Dates
Irrigation	May 20, 2004	October 6, 2004	May 15, 2004 - Edge June 21, 2004 – Odyssey Sept 15, 2004 – Reglone

Rain-fed	May 3, 2004	October 6, 2004	May 2, 2004 - Edge June 21, 2004 – Odyssey Sept 15, 2004 - Reglone

5. Rain-fed world accessions 2005

Seeding Date	Harvest Date	Spraying Dates
May 25, 2005	October 12, 2005	May 3, 2005 - Edge June 24, 2005 - Odyssey Sept 19, 2005 - Reglone

6. Irrigation world accessions 2005

Seeding Date	Harvest Date	Spraying Dates
May 25, 2005	October 14 , 2005	May 10, 2005 - Edge June 26, 2005 - Odyssey Sept 19, 2005 - Reglone

7. Fertilizer trials 2005

Seeding Date		Harvest Date		Spraying Dates
Irrigation	May 20, 2005	Forage yield	September 9, 2005	May 10, 2005 - Edge June 26, 2005 – Odyssey July 19, 2005 – Embutox 625 Sept 19, 2005 - Reglone
Rain-fed	May 9, 2005	Forage yield	September 9 , 2005	May 3, 2005 - Edge June 24, 2005 – Odyssey July 19, 2005 – Embutox 625 Sept 19, 2005 - Reglone
Irrigation	May 20, 2005	Seed yield	October 10, 2005	May 10, 2005 - Edge June 26, 2005 – Odyssey July 19, 2005 – Embutox 625 Sept 19, 2005 - Reglone
Rain-fed	May 9, 2005	Seed yield	October 10, 2005	May 3, 2005 - Edge June 24, 2005 – Odyssey July 19, 2005 – Embutox 625 Sept 19, 2005 - Reglone

8. Swath vs. Combine 2005

Seeding Date		Harvest date	Spraying Dates
Irrigation	May 9, 2005	October 10 , 2005	May 10, 2005 - Edge June 26, 2005 – Odyssey July 19, 2005 – Embutox 625 Sept 19, 2005 - Reglone
Rain-fed	May 20, 2005	October 10 , 2005	May 3, 2005 - Edge June 24, 2005 – Odyssey July 19, 2005 – Embutox 625 Sept 19, 2005 - Reglone

9. GA_3 plots under irrigation 2005

Seeding Date	Harvest date	Spraying Dates
May 25, 2005	October 7, 2005	May 10, 2005 - Edge June 26, 2005 - Odyssey Sept 19, 2005 - Reglone

10. Chemical treated plots under irrigation 2005

Seeding Date	Harvest date	Spraying Dates
May 25, 2005	October 7, 2005	May 10, 2005 - Edge June 26, 2005 - Odyssey Sept 19, 2005 - Reglone

Appendix III: Mean DM yield (kg ha^{-1}), seed yield (kg ha^{-1}) and seed size (g) of world accessions grown under irrigation at LRC in 2004. Mean values of yield attributes for Tristar and the determinate lines are highlighted.

Accessions	DM yield (kg ha^{-1})	Seed yield (kg ha^{-1})	1000 seed weight (g)
PI 211636	11382	2881	19
L3312	14063	2621	19
L3671	35510	2452	18
L3720	12296	2391	22
X92-23-3	11389	2374	20
PI 143504	15580	2342	21
PI 9095	11055	2061	19
L3538	14143	2004	19
L3308	13228	1920	18
F86	10060	1839	18
Amber	12004	1829	20
PI 199264	10782	1821	17
ZT-5	12505	1809	17
F80	9952	1796	18
L3375	11589	1768	14
Quatro	9999	1744	19
PI 269994	11014	1707	17
PI 138687	12848	1696	20
L3676	22323	1607	19
L3699	12646	1536	21
Tristar	**11671**	**1478**	**14**
L3707	8231	1468	22
L3721	11730	1461	22
L3692	8377	1460	15
L3717	11854	1413	24
F70	7667	1389	22
L3716	9411	1388	19
L3705	12320	1368	15
PI577711	7991	1359	18
L3700	10503	1344	20
F18	9654	1333	18
L3698	11554	1289	22
L3697	11566	1285	17
L3703	11989	1258	16
L3690	8847	1248	19
L3685	28600	1230	22
PI 577713	7660	1135	21
NGC 2001	10033	1122	23
L3706	9843	1119	19
L3709	9282	1100	16

L3695	12217	1088	21
L3704	7151	1055	17
L3693	10704	1054	20
PI 195691	8994	1041	19
L3711	9805	1035	22
L3712	24058	1028	20
L3710	11173	1027	22
L3719	9471	1023	19
L3701	9248	999	21
L3696	10056	997	18
L3715	8962	991	23
L3708	10485	989	16
L3172*	**7345**	**939**	**18**
L3713	10138	937	19
L3691	10226	917	18
L3682	8738	890	17
L3694	9514	840	19
L3683	12953	809	19
L3714	8443	807	20
L3718	8835	737	17
L3679	13223	707	15
L3677	8421	665	16
L3177*	**13778**	**649**	**15**
L3702	9000	642	18
L3684	22753	622	13
L3672	9055	597	16
L3681	11466	524	16
L3680	5963	442	14
L3678	7837	261	15
L3673	10937	253	14
L3674	6824	137	13
L3068	15160	127	13
L3675	12965	19	4
Combined Mean	**12306**	**1254**	**19.1**
± SE	**785.7**	**64**	**0.3**

Appendix IV: Mean DM yield (kg ha^{-1}), seed yield (kg ha^{-1}) and seed size (g) of world accessions grown under rain-fed at LRC in 2004. Mean values of yield attributes for Tristar is highlighted.

Accessions	DM yield (kg ha^{-1})	Seed yield (kg ha^{-1})	1000 seed weight (g)
PI 143504	11651	4119	19
L3308	15413	3952	20
F18	13167	3937	23
PI 138687	9732	3320	20
F86	8070	3207	21
L3312	10906	3131	19
F70	9613	2980	23
L3703	11614	2881	17
L3713	16870	2564	18
L3707	9343	2465	26
L3705	10480	2313	21
Amber	9456	2298	23
Tristar	**10235**	**2268**	**17**
L3693	5377	2216	20
L3718	11866	2194	15
L3690	6966	2143	24
F80	6121	2137	21
PI 9095	10966	2043	18
L3696	9754	1992	18
L3689	10679	1982	18
L3711	8556	1943	17
L3691	7293	1938	19
L3692	5446	1927	18
L3721	7783	1919	20
L3714	10569	1841	20
L3709	10257	1758	16
L3694	15752	1755	19
L3720	8475	1731	18
L3701	8811	1706	17
L3700	7550	1659	20
L3717	9383	1638	18
L3715	6505	1552	19
L3697	9496	1550	20
L3719	9955	1536	20
L3695	8678	1424	18
L3699	8869	1407	21
L3698	6946	1187	18
L3716	8653	1175	18
L3706	5756	1142	24
L3712	4077	1117	20

NGC 2001	9185	1059	18
L3708	5256	1032	20
L3704	7934	884	20
L3702	4979	823	16
L3710	5097	491	18
L3068	7830	377	18
Combined Mean	**9073.2**	**1990**	**19.3**
± SE	**410.3**	**104**	**0.3**

Appendix V: Mean DM yield (kg ha^{-1}), seed yield (kg ha^{-1}) and seed size (g) of world accessions grown under irrigation at LRC in 2005. Lines with * were identified as determinate lines. Mean values of yield attributes for Tristar and the determinate lines are highlighted.

Accessions	DM yield (kg ha^{-1})	Seed yield (kg ha^{-1})	1000 seed weight (g)
X92-23-3	19548	6739	25
L3068	19338	6358	24
PI199264	13185	4315	25
L3375	20840	4300	24
AMBER	18799	4057	23
ZT-5	20600	4051	23
F70	14681	3994	25
L3683	12528	3963	23
L3312	16878	3945	23
F18	19528	3793	22
L3689	10677	3736	25
TRISTAR	**16440**	**3544**	**22**
PI211636	18723	3289	23
QUATRO	15067	3157	24
PI269994	12865	3146	24
PI143504	11625	2879	23
L3308	14328	2853	23
L3705	13951	2700	23
L3708	12707	2575	25
L3671	14484	2496	24
L3719	19269	2475	24
L3703	12907	2473	25
L3691	12029	2435	23
L3712	13518	2395	24
L3676	11259	2358	23
L3538	15105	2287	24
F80	10325	2248	24
L3685	13006	2245	24
L3714	11973	2220	25
L3700	10525	2167	25
PI138687	15479	2163	23
L3711	12569	2144	23
PI195691	10513	2131	23
L3716	9940	2061	24
L3707	9099	2039	25
L3697	12494	2033	25
L3692	10740	1982	24
L3704	10644	1974	24
L3177*	**10524**	**1964**	**24**

F86	15726	1944	24
L3706	11674	1923	25
PI9095	10452	1834	26
L3715	10198	1745	24
L3172*	**9753**	**1736**	**24**
L3721	11862	1708	24
L3684	12397	1700	23
L3695	10585	1687	24
L3699	11155	1686	25
L3710	10066	1663	24
L3681	10361	1648	24
L3720	10658	1608	25
L3713	10680	1600	23
L3702	8655	1580	25
L3690	10454	1560	23
L3717	10501	1547	25
L3694	8944	1541	24
L3693	9875	1539	25
NGC 2001	12266	1520	23
PI577713	8494	1509	23
L3718	9616	1485	24
L3709	10499	1477	24
PI577711	10967	1446	23
L3698	11108	1398	23
L3672	8587	1344	24
L3675	8165	1285	24
L3701	11043	1272	25
L3677	8791	1263	23
L3696	9231	1208	24
L3679	7078	1032	24
L3682	8608	948	24
L3678	6882	798	25
L3680	5788	568	24
L3674	8805	169	23
Combined Mean	**12173**	**2283**	**24**
± SE	**375.3**	**111.3**	**0.1**

Appendix VI: Mean DM yield (kg ha^{-1}), seed yield (kg ha^{-1}) and seed size (g) of world accessions grown under rain-fed at LRC in 2005. Lines with * were identified as determinate lines. Mean values of yield attributes for Tristar and the determinate lines are highlighted.

Accessions	DM yield (kg ha^{-1})	Seed yield (kg ha^{-1})	1000 seed weight (g)
L3708	36464	7015	24
ZT-5	12230	3866	23
L3308	12127	3273	23
QUATRO	7431	3079	23
L3683	8741	2979	23
L3678	9144	2873	23
L3677	9720	2808	23
PI211636	9389	2755	23
AMBER	8201	2706	22
F70	8243	2676	23
L3375	9167	2514	24
F80	10322	2447	23
PI143504	9777	2427	24
L3312	8111	2388	23
PI269994	9624	2348	23
F18	10224	2346	22
TRISTAR	**13154**	**2335**	**22**
X92-23-3	7978	2289	25
L3671	8191	2272	22
PI199264	6655	2195	23
PI138687	8352	2178	23
L3689	7108	2178	23
L3691	8894	2145	22
L3707	7026	1963	23
L3721	7064	1937	23
PI577711	8836	1887	23
L3172*	**8556**	**1840**	**24**
PI577713	8828	1781	23
L3679	7030	1754	23
L3693	7317	1741	23
L3692	8729	1732	23
L3713	8090	1731	23
NGC 2001	9301	1727	22
L3690	7177	1722	23
L3672	7069	1710	24
L3676	8793	1698	22
L3684	7003	1672	23
L3538	8353	1665	23

L3703	7971	1560	23
L3697	7862	1505	23
L3716	8950	1474	24
L3701	8466	1473	23
L3712	8759	1467	23
L3177*	**5789**	**1452**	**23**
PI9095	7263	1445	24
L3719	6238	1444	24
F86	4316	1421	23
L3681	7252	1410	22
L3711	6711	1400	22
L3704	7884	1400	23
L3720	7921	1316	23
L3699	5753	1270	21
L3718	7106	1243	23
L3682	6839	1239	22
L3694	8699	1232	24
L3717	6677	1222	23
L3696	7562	1201	23
L3705	7581	1188	23
L3685	6559	1188	23
L3700	7257	1179	24
L3706	7539	1177	24
L3675	6577	1160	24
L3709	7213	1153	24
L3715	6230	1150	24
PI195691	6741	1129	23
L3710	7591	1118	22
L3702	6353	1070	23
L3680	6683	1062	23
L3714	7672	994	24
L3695	7693	975	24
L3068	5984	958	24
L3698	8590	949	23
L3674	6060	195	17
Combined Mean	**8339**	**1828**	**23.4**
± SE	**409**	**98**	**0.1**