Quantification of Bioactive Compound Content in Advanced Fenugreek Lines Selected in Alberta: Levels of Galactomannan

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Abstract

Fenugreek (*Trigonella foenum-graecum* L.) was initially introduced to Alberta as a spice and forage crop. Numerous animal and clinical studies have associated the medicinal properties of the plant with galactomannan, diosgenin and 4-hydroxyisoleucine, the three major bioactive compounds found in fenugreek seed. These bioactive molecules have been demonstrated to regulate plasma cholesterol levels, reduce plasma triglyceride concentrations and stimulate insulin secretion for blood glucose metabolism. Fourteen advanced lines grown satisfactorily in southern Alberta were selected for quantification of these compounds over two harvest seasons. These lines were grown at three locations (Brooks, Bow Island and Lethbridge) in southern Alberta under two growing conditions (rain-fed or irrigated). In this study we report on five different seed lines which possessed a high galactomannan yield during the study; *i.e.*, seed lines F75, L3312, CDC Quatro, F80 and L3308. Seed line F75 was the only line that performed well across all locations and growing conditions. It was observed that seed yield had a greater influence on galactomannan yield compared to seed galactomannan content. This suggests that cultivation of fenugreek plants for neutraceutical purposes should emphasize lines with high seed yield, as galactomannan content does not vary significantly among lines. An understanding of the interaction of bioactive compound content with respective ecotypes will assist in the selection of lines for their economic potential in the functional food and natural health product industry.

Introduction

Fenugreek is a diploid (2n=16) leguminous crop native to the Indian subcontinent and the East Mediterranean region. It has been used for centuries as a medicinal plant as well as a cooking ingredient to impart flavor, aroma and color to foods (Petropoulos, 2002). Fenugreek seeds are known to contain high amounts of dietary soluble fiber (45-50%; 13-20% of which is galactomannan), protein (20-36%), including a free amino acid called 4-hydroxyisoleucine, oil (5-6%), ash (3%), starch (2%), sugar (1%) (Slinkard *et al.*, 2006) and diosgenin, a steroidal compound reported to range between 0.8 and 2.2% on a dry weight basis (Fazli and Hardman 1968; Taylor *et al.*, 1997). Galactomannan, diosgenin, and 4-hydroxyisoleucine are all considered bioactive compounds and have been individually studied using animal and clinical trials to test for potential health benefits. These benefits include regulation of plasma cholesterol levels, reduction of triglyceride levels and insulin stimulation for control of blood glucose levels (Al-Habori and Rahman, 2002).

Galactomannan is a plant polysaccharide which contains a mannan backbone substituted with galactosyl residues; structurally it consists of a 1→4 linked β-D-mannosyl backbone with single-unit galactose side-chains α-linked at the C-6 oxygen (Bhaumick, 2006). Health benefits of galactomanan are mainly associated with the reduction of plasma glucose levels (Sharma, 1986; Madar *et al*., 1988; Madar and Shomer, 1990) and cholesterol concentrations (Sharma *et al*., 1996; Ribes *et al*., 1987). This large molecular polysaccharide has been postulated to regulate plasma glucose levels by delaying gastric emptying and by interfering with glucose absorption in the gut (Madar, 1984). Formation of a physical barrier by galactomannan that may aid inhibition of bile salt absorption in the gut has been associated with fenugreek's hypocholesterolaemic properties (Madar and Shomer, 1990). Fenugreek galactomannan is unique in that it possesses a galactose to mannose ratio of 1:1 (Reid and Meier, 1970)

Little is known about variation in the content of bioactive compounds in the various lines of fenugreek, and the effects of environment and land management practices such as irrigation and dry land farming on bioactive compound production in the plants. This study will hopefully provide insight into the production of bioactive compounds in different fenugreek lines and their ecotypes, as well as providing an estimate of the economic potential for each cultivar when grown under different environmental conditions

Materials and Methods

Fenugreek seeds used in the study were obtained from eleven lines and three cultivars (AC Amber, CDC Quatro and Tristar) grown at three locations (Lethbridge, Brooks and Bow Island), under both rain-fed and irrigated conditions. Each plot was 4.5 m long and consisted of 4 rows spaced 30 cm apart. Lines/cultivars were arranged using a Randomized Complete Block Design (RCBD) with two replicates at each location-condition combination. Moisture accumulation levels for the three locations are shown in Table 1.

Table 1. Moisture accumulation levels for the three locations in 2006.

Location		Accumulation (mm)		
		Precipitation	**Irrigation**	**Total**
Brooks	RF	157.4	-	157.4
	IR	166.0	150.0	316.0
Bow Island	RF	114.8	-	114.8
	IR	151.2	178.3	329.5
Lethbridge	RF	140.5	-	140.5
	IR	140.5	152.4	292.9

* RF = rain-fed; IR = irrigated

Seeds were mechanically harvested and cleaned to remove dirt, dust, stones and weeds. They were placed in a plant dryer for approximately 1 week at 29°C to 32°C to remove surface moisture prior to being stored in a dry and cool environment until use.

Galactomannan content was determined using an enzymatic assay kit purchased from Megazyme International Ltd. This procedure involved hydrolysis of the large molecule to release free galactose into solution. Quantification was achieved through a redox reaction with nicotinamide adenine dinucleotide (NAD+) yielding NADH and the lactone form of galactose, followed by UV-absorbance detection of NADH to calculate the galactomannan content assuming a galactose/mannose ratio of 1:1.

Results and Discussion

Quantification of galactomannan is expressed as galactomannan yield (kg/ha) and was determined as the product of seed yield (kg/ha) * galactomannan content (% dry weight basis). The top performing cultivars / lines are listed in Table 2, along with their respective seed yield and galactomannan contents. F75 was identified as the sole line to perform well across all locations under both growing conditions.

Table 2. Top five performing cultivars / lines across three locations under both growing conditions.

Cultivar/Line	Seed yield (kg/ha)	Galactomannan content (%)	Galactomannan yield (kg/ha)
F75	2653	18.8	493
L3312	2336	19.0	458
CDC Quatro	2357	18.4	447
F80	2244	19.2	433
L3308	2200	18.8	421

The mean galactomannan content for Brooks was 17.6% (rain-fed) and 18.7% (irrigated); for Bow Island 18.0% (rain-fed) and 21.6% (irrigated); and for Lethbridge 15.9% (rain-fed) and 17.1% (irrigated). The average galactomannan content for all locations and growing environments was 18.2%. Seeds from Brooks (rain-fed) produced the highest galactomannan yield (kg/ha) at 589 kg/ha while seeds from Lethbridge (irrigated) gave the lowest galactomannan yield at 123 kg/ha. The effect of growing conditions on the galactomannan content of the eleven lines and three cultivars tested was highly variable.

It was observed that seed yield was a greater influence on total galactomannan yield than the galactomannan concentration of the seed. This may be due to the fact that seed yield is highly dependent on the growing environment and weather variation. Also, the galactomannan content did not vary significantly among lines and cultivars (mean = 18.2%, std. dev. = 2.0%). Based on these observations, selected lines should be recommended for cultivation based on their high seed yield rather than on their galactomannan content, in order to achieve a satisfactory galactomannan yield for commercial extraction.

Galactomannan is concentrated in the seed coat while diosgenin and 4-hydroxyisoleucine is predominantly present in the endosperm. It is hypothesized that the galactomannan content would be higher in a small seed with a large seed coat surface area to volume ratio. Development of a thorough understanding of the effect of agricultural practices on levels of biochemical compounds such as galactomannan will hopefully help expand the economic potential of fenugreek in Canada.

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