EFFECT OF ENVIRONMENTAL AND MANAGEMENT FACTORS ON GROWTH AND SEED QUALITY OF SELECTED GENOTYPES OF *CAMELINA SATIVA* L. CRANTZ

by

Yunfei Jiang

Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
January 2013

DALHOUSIE UNIVERSITY

FACULTY OF AGRICULTURE

The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled "EFFECT OF ENVIRONMENTAL AND MANAGEMENT FACTORS ON GROWTH AND SEED QUALITY OF SELECTED GENOTYPES OF *CAMELINA SATIVA* L. CRANTZ" by Yunfei Jiang in partial fulfilment of the requirements for the degree of Master of Science.

Dated: January 30, 2013

Supervisor: _____

Readers: _____

_____

_____

DALHOUSIE UNIVERSITY

DATE: January 30, 2013

AUTHOR: Yunfei Jiang

TITLE: EFFECT OF ENVIRONMENTAL AND MANAGEMENT FACTORS ON GROWTH AND SEED QUALITY OF SELECTED GENOTYPES OF *CAMELINA SATIVA* L. CRANTZ

DEPARTMENT OR SCHOOL: Faculty of Agriculture

DEGREE: MSc CONVOCATION: May YEAR: 2013

_____

Signature of Author

Table of contents

List of Tables

List of Figures

Abstract

Key aspects of the basic agronomy *Camelina sativa* were evaluated under controlled environment conditions and at multiple field locations in 2011 and 2012. Camelina is a highly adaptable crop. It germinates well even under low water availability and has a great potential for yield compensation. The line CDI007 was the most promising genotype with the highest yield potential, the lowest glucosinolate content, and the highest tolerance to downy mildew. The optimum N rate for seed yield varied by year and location: 100 kg N/ha at Truro and Canning in 2011, 120-150 kg N/ha at Canning, Truro and New Glasgow, 160-200 kg N/ha at Fredericton in 2012. N was positively correlated with protein content, but negatively correlated with oil content. Application of sulphur increased protein content at all of the sites and yield at some of the sites. In general, camelina response to S was maximized when N was sufficient.

List of Abbreviations and Symbols Used

AAFC	Agriculture and Agri-Food Canada
ACP	Acyl carrier protein
B	Boron
CDI	Crop Development Institute
DM	Downy mildew
FAS	Fatty acid synthase
FID	Flame ionization detector
GC	Gas chromatography
GS	Glucosinolates
ICMS	Integrated Crop Management Services
K	Potassium
N	Nitrogen
NB	New Brunswick
NIR	Near inferred spectroscopy
NS	Nova Scotia
P	Phosphorous
PAR	Photosynthetically active radiation
PE	Prince Edward Island
PEG	Polyethylene glycol
PGRC	Plant Gene Resources of Canada
S	Sulphur
SK	Saskatchewan
TKW	Thousand Seed Weight
WP	Water potentials

Acknowledgements

I would like to begin by thanking the most influential person in my academic life, my supervisor, Dr. Claude Caldwell. I would like to express my deep appreciation to Dr. Claude Caldwell, for his valuable guidance, continuous encouragement and persistent help. My deep gratitude extends to my committee members - Dr. Rajsekaran R. Lada and Dr. Kevin Falk, for their advice and inspiration. I would like to express my sincere thanks to Dr. Nancy Pitts for her academic and spiritual support, and to Dr. Tess Astatkie for his guidance and help in my thesis data analyses.

Special thanks go to CDI Scientific Officer, Doug MacDonald, our lab technician Jili Li, and all of the CDI summer students and assistants – Dustin MacLean, Charlene Locke, Caitlin Congdon, Min Gong, Xueming Gao, Edward MacDonell, Nancy Loomer, Mannfred Boehm, Libiao Gao, Yare Aden, Qin Xu, William Hannah, Longfeng Weng, Zhuhui Ye, Wenjun Lin, Ying Lin, Zili Lai, Yanping Huang and Xiaoling Huang. This thesis would not have been possible without their help. I would like to thank Dr. Rong Zhou for allowing me to visit Quality Lab of Agriculture and Agri-Food Canada in Saskatoon where I learned valuable information pertaining to gas chromatography.

I would like to take this opportunity to express my sincere thanks to many people who have lent their hands during my study life at Dal-AC. They include Dr. Samuel Asiedu, Shannon Kilyanek, Cara Kirkpatrick, Velda Doucette, Dr. Nancy McLean, Mark Mason, Margaret Rovers, Fred Manley, Krista MacLeod, Elaine MacInnis, Sherree Miller, Jolene Reid, Erin MacPherson, Verna Mingo and Jennifer MacIsaac.

In addition, I would like to thank the OEC staff from Fujian Agriculture and Forestry University, especially Mr. Zhengliang Guo and Dr. Songliang Wang, who offered their assistance and allowed me to start this venture - my M.Sc. application.

I would like to acknowledge the financial support provided by Genome Atlantic.

I am grateful to Dustin MacLean for helping me get through the difficult times. I am also indebted to Jili and Jili's family for their support, generous care and the home feeling they provided to me during my period of study.

Last but not least I would like to thank my family and friends for providing a loving environment for me. My parents, Meihua Jiang and Yixi Lin, and my siblings, Yunpeng Jiang and Yunquan Lin are particularly supportive.

Chapter 1: Introduction

1.1 General Literature Review

Camelina sativa L. Crantz, a member in the *Brassicaceae* (*Cruciferae*) family, is also called "wild flax", "German sesame", "Gold of pleasure" and "false flax" since it has a similar appearance to flax early in its growth (Zubr, 1997; Zubr, 2003a). The chromosome number of *Camelina sativa* has been reported to be n=6 or 14 or 2n=12, 26 or 40; the latter (2n=40) is considered to be the most common (Schnell and Davis, 2011). Camelina possesses a hexaploid genome according to the ancestral blocks (Parkin et al., 2012). Seed and capsules of *Camelina sativa* ssp. *C. linicola* (Schimp. and Spenn.) were found in archaeological excavations from the Bronze Age in Scandinavia and Western Europe, which indicated camelina seed was used as an ingredient in bread and porridge 2000 years ago (Zubr, 1997; Hatt, 1937 in Zubr, 2003a). The cultivation of camelina declined in Europe due to farm subsidy programs which preferred the major commodity grain and oilseed crops (Ehrensing and Guy, 2008). There is currently a renewed interest in camelina due to its favorable agronomic traits and potentially large number of uses. It is drought-tolerant (Putnam et al., 1991), cold-tolerant (McVay and Khan, 2011) and nutrient-use efficient, and a short growing season crop; its oil potentially offers excellent health benefits and nutritional value to humans and animals. It also has been used in the bio-fuel and lubrication industry (Agarwal et al., 2010; Paulsen et al., 2011).

Camelina can be cultivated as an annual summer crop or winter annual crop. The seeds do not have seed dormancy (Ehrensing and Guy, 2008). Thousand seed weight of camelina ranges from 0.8 to 1.8g based on different genotypes, growth conditions and

plant nutrients (Zubr, 1997). Camelina is self-pollinating (Plessers et al., 1962; Zubr, 1997; Mulligan, 2002). When sown as a summer annual in the Northern mid-Western U.S., the growing season was about 80-100 days (Putnam et al., 1991), but the length of the growing season is reportedly longer (approximately 120 days) in Northern and Central Europe (Zubr, 1997). Winter-sown camelina is more susceptible to disease and less competitive with weeds in Wales (Zubr, 1997). Harvested seed should be stored with less than 8% moisture (Grady and Nleya, 2010 in "Camelina Plant Guide", 2011).

Field work conducted by Urbaniak et al. (2008b) suggested seeding rates ranging from 400 to 600 seeds/m^2 would likely be best for camelina cultivation in the Maritime region of Canada. It can adapt to different climatic and soil conditions except for heavy clay and organic soils (Zubr, 1997). Camelina did not perform well on wet and poorly drained soils in some trials in Pennsylvania (Hunter, 2010 in "Camelina Plant Guide", 2011). Camelina has been reported to be able to grow without fertilizer application, but would then rely on the nutrient levels in the soil (Schnell and Davis, 2011). The nutrient requirement of the crop is moderate to low; e.g., approximately 30 kg P/ha, 50 kg K/ha should be incorporated before sowing, and 100 kg N/ha is the optimum N rate (Zubr, 1997). The content of camelina oil was positively correlated with P application, which increased from 39.1% without P application to 42.1% with 60 kg/ha of P_2O_5 (Imbrea et al., 2011).

1.1.1 N Application

Some papers suggested camelina requires lower N input than other competitor crops such as canola and sunflower (Putnam et al., 1991). Depending on the soil fertility, residual level of nutrients and weather conditions, the optimum N supply is about 100

kg/ha in Denmark (Zubr, 1997). For winter genotypes, N fertilizer should be used early in the spring in order to avoid the loss of N in the soil, and at the four to six-leaf stage for summer genotypes (Zubr, 1997). The optimum N input for camelina was found to be 100-125 kg/ha. This results in yields ranging from 800-2500 kg/ha in Nova Scotia (MacDonald and Li, 2010). According to Agegnehu and Honermeier (1997), the yield of camelina of all treatments ranged from 1160 kg/ha in 1994 to 1800 kg/ha in 1995 in Germany. The maximum yield of all treatments was 2280 kg/ha (120 kg/ha N, 400 seeds/m^2). Under N deficiency, camelina plants are thin and very upright and the leaves are small and pale yellow-green. Also, pods tend to ripen prematurely and there are fewer pods and seed bearing branches. Therefore, N is an important nutrient for a high yield (Agegnehu and Honermeier, 1997).

According to Urbaniak et al. (2008a), the yield of camelina has been shown to enhance by increasing N, but the increase was not obvious when the application of N exceeded 60 kg/ha in Nova Scotia and 80 kg/ha in PEI. Also, the oil content decreased with higher N. Almost all the fatty acids, except for erucic acid in camelina increased or decreased relative to the different levels of N. In contrast, plant height, total N content in plant tissue and seed protein content increased (Urbaniak et al., 2008a). Urbaniak et al. (2008a) demonstrated camelina cultivar selection and applied N levels are important factors in obtaining optimum yield.

Camelina could be grown successfully with N levels of 100 kg/ha (Zubr, 2003b). Crowley and Frohlich (1998) found that camelina yields reached a peak by using 75 kg/ha N. However, the incidence of plant disease (*Botrytis*) was intensified with increased N rates but no obvious oil content changes were observed. It was found that N

levels of 100 kg/ha led to yield increases in 58% of camelina, and decreases in oil content in Romania (Zubr, 2003b). Research done by Agegnehu and Honermeier (1997) showed that increasing N levels from 60 to 130 kg/ha resulted in a 30% yield increase and a dramatic decline in oil concentration with a corresponding increase in protein. In general, there are many inconclusive and somewhat contradictory results in references to N application in camelina production in different areas of the world.

1.1.2 S Application

S is necessary for plant growth and physiological functions including chlorophyll formation, protein and vitamin development, resistance to cold and water stress (Gardner et al., 1985). According to the Nova Scotia Department of Agriculture, an S level with over 40 kg/ha is enough for plant normal growth ("Understanding the soil test report", 2011). Some crops such as canola and cabbage require higher amounts of S (Sharifi et al., 2010). Studies showed that the increase application of S fertilizer enhanced the seed oil content of several crops such as flax and soybeans (Gardner et al., 1985). The symptoms of S deficiency include stunting, general yellowing and thin stems, which are similar to those of N deficiency (Gardner et al., 1985).

Generally, plant performance responding to N application could reach the optimum level only when S supply was sufficient; and that response to S could be maximized only when N was sufficient (Hu and Sparks, 1992). S application was critical for high seed yield and oil yield for oilseed crops (Losak et al., 2011). The increase of S application increased oil content but not seed yield of Montana trials (Enrensing and Guy, 2008). Under S deficiency, the increasing levels of N could accelerate the deficiency symptoms and decrease production (Janzen and Bettany, 1984).

1.1.3 B Application

B is an essential micronutrient for plant growth and development, but in high concentrations it can be toxic to plants (Banks, 1990; Yau and Ryan, 2008). B plays an important role in the synthesis of uracil - one of the RNA bases and in cellular development such as cell division, differentiation, maturation and respiration (Jones, 1998). B affects cell development by regulating sugar transport and polysaccharide formation (Gardner et al., 1985). It develops a polyhydroxy complex with sugars that are more steadily translocated through cellular membranes compared with the non-borate sugars (Sinha, 2000). B is crucial for pollen germination and growth, and it also promotes the stability of pollen tubes (Jones, 1998). B is relatively immobile in plants and is transported mainly in the xylem; therefore, uptake of B by young plant tissue relies on current absorption (Gardner et al., 1985). B exists as the borate anion (BO_3^{3-}) in soil solution. The total B in the soil ranges from approximately 20 to 200 ppm, and the available amount for plant absorption ranges from 1 to 5 ppm (Jones, 1998) and is affected by soil pH. The optimum amount for maximum B availability is 5.5-7.0 ppm.

B deficiency decreased the yield of mustard because of excessive accumulation of phenols and auxins, which resulted in early necrosis of the growing apex and then inhibited plant growth (Sinha et al., 2000). Effects of different levels of B (0, 1.5, 3.0, and 4.5 kg N/ha) were tested on soybean (Seguin and Zheng, 2006). Combined S (0, 7.5, 15 and 30 kg/ha) and B (0, 2.5, 5.0, and 7.5 kg/ha) were evaluated on sesame. Gypsum (18% S) and borax (11% B) were used as S and B sources. The highest yield was achieved with the highest application of 30 kg S/ha and 7.5 kg B/ha. This indicated both S and B are important in the production of oilseed crops. S promotes primordial floral initiation,

which leads to an increasing number of capsules per plant and seeds per capsule (Mathew and George, 2011).

1.1.4 Water Stress and Its Effect on Seed Germination

Camelina is relatively tolerant to drought and strongly competitive for water. Camelina may be a valuable alternative crop to wheat or cotton in the southwestern US or other areas where water may be limited for plant growth (French et al., 2009). According to Vollmann et al. (1996), a serious water stress during the flowering stage reduced plant development and yield potential of camelina, while sufficient rainfall at the seed filling phase led to an increase in seed oil content. French et al. (2009) examined water use efficiency of camelina; they monitored water consumption and irrigation requirements in central Arizona from December 2006 through to April 2007. Camelina was grown in a 1.3-ha field in a randomized complete block design including 32 plots repeated with 4 levels of water depletion (40%, 55%, 65% and 75%). Six additional plots of camelina with 85% soil water depletion were used to evaluate water stress. A significant drop in seed yield (as much as 76%) was observed where camelina experienced serious water deficits (85% water depletion). The results showed that seasonal water consumption of camelina varied from 333 to 423 mm (French et al., 2009). Average yield for the camelina crop was 1000 ± 310 kg/ha. Average total oil content was 41.4 ± 3.8% by weight (French et al., 2009). The water use efficiencies of camelina and canola were 72 and 78 kg of seed for each inch of cumulative water use (Hergert et al., 2011).

Seed germination involves a series of biological events, which are affected by many factors including temperature, light and availability of soil water. Among these, the available soil water is considered to be the most important factor for affecting seed

germination (Sy et al., 2001). Germination is initiated by water imbibition, followed by enzymatic metabolism of storage nutrients and radicle protrusion (Gao et al., 1999). Water, as a solvent, provides a fluid medium where substrates diffuse to active sites and the conformational changes of enzymes happen, which is essential to catalytic activity (Yang et al., 2010). It has been reported that germination was strongly limited by water stress at extremely high or low temperatures (Zheng et al., 2004). Water stress may also decrease seedling establishment and growth of surviving seedlings (Shao et al., 2008). Research done by Yang et al. (2010) showed decreased water potential led to a delay and lower germination percentage of Piceaasperata. A similar phenomenon was also found in the studies of lentils (Zheng et al., 2005). Polyethylene glycol (PEG) has been used to create a range of water potential regimes. PEG cannot pass through materials made of plant membrane because of its high molecular weight (4,000 or more), thus it has been used to keep experimental media at predetermined water potential values. It was reported that increasing moisture stress delayed the initiation of germination and slowed shoot and root elongation (Rono, 1994).

1.1.5 Oil Quality

According to Zubr (2009), camelina oil for human consumption can be produced by pressing seed in a screw press without any pre-treatment. In contrast, seed must be crushed or milled before pressing when the oil is produced industrially on a large scale. For pressing oil in the laboratory, the seeds are heated to 35-45 ^{o}C by friction in the screw expeller. Furthermore, the temperature of the expeller should be maintained at 80 ^{o}C in order to decrease lipases and increase the release of oil-soluble substances including tocopherols, phenols and phytosterols (Zubr, 2009). The temperature during pressing can

reach up to 100 ^{o}C (Zubr, 1997). In order to be considered "cold-pressed", the temperature during processing cannot exceed 80 degrees Fahrenheit (27 degree Celsius) ("What is cold pressed oil", 2012).

The average oil content of camelina seed was 43.3% (Zubr, 2003a). The concentration of unsaturated fatty acids in camelina oil is approximately 90%. A comparison of fatty acid profiles of camelina and canola (*Brassica napus L.*) is shown in Table 1. The percentage of polyunsaturated linoleic acid (18:2, n-6) (LA) and α-linolenic acid (18:3, n-3) (ALA) is about 50% of the total fatty acids with C18:3, 37.8% and C18:2, 14.8% (Zubr, 2003a). The n-3 polyunsaturated fatty acids play a key role in brain and eye development and prevention of heart disease (Nettleton, 1991). Diets which are rich in ALA are beneficial for the prevention of coronary events and cardiac deaths (Zubr, 2003a). The concentration of erucic acid (22: 1, n-9) in the oil is about 3% (Zubr, 1997; Zubr, 2003a), which has many of the same uses as mineral oils. Erucic acid is used as a superior lubricant in tribology. Erucic acid has been associated with myocardial lipidosis and heart lesion in laboratory rats ("Erucic acid in food", 2003). Myocardial lipidosis decreases the contractile force of the heart muscle. Camelina also possesses approximately 14% oleic acid, which may hinder the progression of adrenoleukodystrophy that is a fatal disease affecting the brain and adrenal glands (Rizzo et al., 1986). Camelina oil is richer in γ-tocopherol (72 mg/100g) compared to linseed oil (52 mg/100g) and rapeseed oil (51 mg/100g), respectively (Schwartz et al., 2008) and is beneficial for cell regeneration, skin elasticity and slenderness recovery (Vollmann, 1996). Camelina oil is regarded to be a good remedy for stomach and duodenal ulcers, the treatment of burns, wounds and eye inflammations (Rode, 2002).

Table 1.1: Comparison of lipid profiles of camelina, canola (*Brassica rapa* L.), linseed and sunflower

Fatty acid profile	Camelina (%)	Canola (%)	Linseed (%)	Sunflower (%)
C18:1 (n-6) (oleic)	14.6	55.9	16.2	4.0
C18:2 (n-6) (linoleic)	17.2	21.7	14.7	16.5
C18:3 (n-3) (linolenic)	38.1	14.2	59.6	72.4
C22:1 (n-9) (erucic)	2.5	0	0.9	0

(Falk and Klein-Gebbinck, 2009; Enrensing and Guy, 2008)

Karvonen et al. (2002) found that a daily intake of 30 g of camelina oil for six weeks was helpful in terms of serum cholesterol level and serum fatty acid composition in human subjects. Although camelina oil proved to be more oxidatively stable than linseed oil, it is less stable than rapeseed, olive, corn, sesame and sunflower oils (Eidhin et al., 2003). Compared to flaxseed oil, camelina oil has a lower concentration of α - linolenic acid and saturated fatty acids. However, camelina seed has a higher content of tocopherols (7000 mg/100g) than flax seed, which makes camelina oil more oxidatively stable (Karvonen et al., 2002). Karvonen et al. (2002) studied the effects of α-linolenic acid rich camelina oil on serum lipids and on the fatty acid content of total lipids compared with rapeseed and olive oils. The results showed that the percentage of α-linolenic acid in camelina oil was 2.5 times and 4 times higher than rapeseed oil and olive oil, respectively.

Eidhin and O'Beirne (2010a) compared the oxidative stability of camelina and sunflower which is commonly used as domestic and commercial edible oil. The main fatty acids in sunflower oil are 48-74% linoleic, 10-14% oleic acid, 4-9% palmitic acid and 1-7% stearic acid ("British Pharmacopoeia Commission", 2005). The results showed that in comparison to sunflower oil, omega-3 rich camelina oil had a good oxidative

stability and acceptability in salad dressing and mayonnaises, but was not stable enough for deep frying (Eidhin and O'Beirne, 2010a).

Peiretti et al. (2007) conducted research on the use of different levels of camelina seed in diets for fattening rabbits. Control (0%), 10% and 15% of camelina seeds were blended in iso-nitrogenous and iso-caloric diets; the results showed that no significant differences were observed in rabbits' live weight, live weight gain, feed consumption, feed efficiency, carcass yield or the percentages of edible organs. On the other hand, the polyunsaturated fatty acids in the longissimus dorsi muscle and peritoneal fat were enhanced with the increase of camelina seeds, and the percentage of saturated fatty acids declined. These results indicated that using camelina meal as a partial replacement was effective in lowering the saturation, atherogenic and thrombogenic indices and was consequently beneficial on the nutritional quality of rabbit meat for human consumption without significant adverse effects on growth performance and carcass characteristics.

The presence of 2-sec-butyl-3-methoxy pyrazne, aldehydes and alcohols and S compounds like 2, 4, 5 -trithiahexane and 1-utene-4-isothiocyanato in camelina oil may contribute to the unique aroma of the oil (Sampath, 2009). Under optimal conditions (darkness and low temperature 5 ^{o}C), camelina oil can be stored for 9 years without losing its nutritional quality (Zubr, 2003a). The oil must be stored in light proof containers. It has also been reported that the shelf life of crude (unrefined) camelina oil is 12-24 months, and that of refined oil (refined, bleached and deodorized) is 6-9 months (Sampath, 2009).

1.1.6 Protein

Camelina has a relatively rich protein content with 29.1% on a seed basis, which is approximately 3.6% more than canola (*Brassica rapa L.*) (Falk and Klein-Gebbinck, 2009). Crude protein averaged 45.2% of fat-free dry matter (Zubr, 2003a). Camelina contains a wide range of amino acids, and its comparison with selected oilseed crops are outlined in Table 2. Amino acids including histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine are essential for human health. Amino acids such as arginine, cystine and tyrosine are required by infants and growing children (Imura and Okada, 1998).

Table 1.2: Percent of total amino acids in selected oilseeds

Amino acids	Symbol	Camelina (%)	Rape (%)	Soya (%)	Flax (%)
Alanine	ALA	4.6	4.0	4.8	5.5
Arginine	ARG	8.2	6.7	7.5	11.1
Aspartic acid	ASP	8.7	6.6	12.7	12.4
Cystine	CYS	2.1	3.0	1.3	4.3
Glutamic acid	GLU	16.4	18.1	19.0	26.4
Glycine	GLY	5.4	4.7	4.5	7.1
Histidine	HIL	2.6	3.1	3.2	3.1
Isoleucine	ILE	4.0	4.1	3.1	5.0
Leucine	LEU	6.6	6.3	7.3	7.1
Lysine	LYS	5.0	6.5	6.1	4.3
Methionine	MET	1.7	1.7	1.3	2.5
Phenylalanine	PHE	4.2	3.5	5.0	5.3
Proline	PRO	5.1	6.0	6.0	5.5
Serine	SER	5.0	4.0	5.6	5.9
Threonine	THR	4.3	4.5	4.2	5.1
Tryplophan	TRY	1.2	0	1.3	1.7
Tyrosine	TYR	3.0	2.4	3.9	3.1
Valine	VAL	5.4	6.0	3.2	5.6

(Zubr, 2003a)

Due to high protein content in camelina seed and meal, camelina meal has been used in animal feed (Pilgeram, 2008). Researchers of Bio-based Products Institute in Montana State University have established that camelina is suitable for livestock feeds which in turn enrich eggs, dairy and meat products. At present, camelina products are not approved as livestock feed ingredients in Canada. Furthermore, individual types of camelina ingredients (e.g., camelina oil, camelina meal and seeds) will require separate approval to feed livestock in Canada (Canadian Food Inspection Agency, 2012). Generally Recognized as Safe (GRAS) has been achieved for camelina meal commercialization in the United States (Church, 2010).

1.1.7 Glucosinolates

Glucosinolates (GS) are secondary plant metabolites commonly found in *Brassica* species such as broccoli, cabbage and oilseed rape. These sulfur-containing compounds comprise at least 120 anionic thioglycosides (Yan and Chen, 2007). The chemical structures of GS consist of a β-thioglucose moiety, a sulfonatedoxime moiety and a variable side chain derived from one of eight amino acids (Wink, 1999). In general, the GS concentration is 0.1% or less in fresh plant parts such as stems and leaves (Wink, 1999). These moderate levels of GS do not lead to health problems in animals and humans (Wink, 1999). Based on the toxic properties and pungent taste of GS, they are often regarded as anti-nutritional factors (Wink, 1999). GS and the degradation products are precursors of compounds with goitrogenic action in animals and humans. These compounds block the uptake of iodine, which lead to modification of thyroid function. For example, one of the major GS of rapeseed, 2-hydroxy-3-butenyl GS, can be

hydrolyzed to form an oxazolidine-2-thione which leads to goiter formation and effects animal nutrition negatively (Wink, 1999).

GS are one of the possible restricting factors for camelina commercialization. The GS content of camelina is approximately 24 μmol/g, and ranging from 13.2 to 36.2 μmol/g dry seed within different genotypes (Schuster and Friedt, 1998). There are three major camelina GS: 9-methylsulfinylnonyl-glucosinolate, 10-methylsulfinyldecyl-glucosinolate (glucocamelinin) and 11-methylsulfinylundeyl-glucosinolate. Glucocamelinin (10-methyl-sulfinyl-decyl-glucosinolate) is the major GS of camelina, which accounts for approximately 65% of the total GS (Schuster and Friedt, 1998). The content of 9-methylsulfinylnonyl-glucosinolate was higher than that of 11-methylsulfinylundeyl-glucosinolate for most of the analyzed genotypes of camelina (Schuster and Friedt, 1998). According to Imbrea et al. (2011), however, camelina seed and meal can be used directly as animal feed due to its low content of GS (Imbrea et al., 2011).

At present, knowledge of the rich protein in camelina residue makes it a potential ingredient for poultry diets (Ryhänen et al., 2007). Ryhänen et al. (2007) tested the effect of camelina expeller cake (CSEC) on performance and meat quality of broilers in Finland. The results showed that the use of camelina in broiler feed had no adverse effects on the sensory quality of the meat. However, CSEC contains a high ratio of GS, varying between 21 and 34 μmol/g, which reduced the feed intake and growth of broiler chickens (Ryhänen et al., 2007). Even though GS themselves are not toxic to animals, the breakdown of GS has been shown to have toxic effects in animals because of their enzymatic metabolic products (Schuster and Friedt, 1998). In comparison with *Brassica*

species, the GS content of camelina is relatively low (Ryhänen, et al., 2007; Antonious et al., 2009).

1.1.8 Weed, Insects and Disease Control

Camelina can be grown in an environmentally-friendly way without excessive application of herbicides or pesticides (Zubr, 2009). Winter genotypes germinate before many weed species and they are competitive when seeded at high density (Ehrensing and Guy, 2008). Camelina has allelopathic properties and many weeds are inhibited until leaf drop starts in the crop (Ehrensing and Guy, 2008). Perennial weeds such as field bindweed, Canada thistle, and skeleton-weed may be hard to control (Ehrensing and Guy, 2008, and "Camelina Plant Guide", 2011).

Camelina is tolerant to the Group I acetyl CoA carboxylase (ACCase) inhibitors and dinitroaniline herbicides including trifluralin, ethalfluralin and pendimethalin (John et al., 2007, 2008 and 2011 in Schnell and Davis, 2011). Trifluralin is a pre-emergent herbicide, which has been applied to annual grasses and broad leaf weeds in camelina fields. A rate of 1.5 kg/ha Trifluralin was recommended (Zubr, 1997). Trifluralin is registered for use on oilseed and fiber production crops in Canada ("Trifluralin", 2009). The pre-emergence herbicide Ethalfluralin (5% granular, 20 kg/ha fall applied) was used in camelina fields in Saskatoon in 2001 (Gugel and Falk, 2006). In the United States, Poast (sethoxydim) has been registered to control grass weeds in camelina (Schenell and Davis, 2011). Pre-seeding tillage is recommended to control weeds in camelina (Schenell and Davis, 2011; Zubr, 1997). However, camelina was reported to be susceptible to some herbicides, and it was a weak competitor with weeds several decades ago (Anderson and Olsson, 1950 in Plessers et al., 1962).

Gugel and Falk (2006) compared camelina with various *Brassica* species in Western Canada and they found that camelina was more tolerant to flea beetle (*Phyllotretacruciferae*, Goeze) infestations than *Brassica* oilseeds. Also, camelina was observed to be highly resistant to blackleg (*Leptosphaeriamaculans*), and alternaria black spot (*Cochliobolussativus*) (Seguin-Swartz et al., 2009). It is susceptible to sclerotinia stem rot (*Sclerotiniasclerotiorum*), clubroot, white rust, and aster yellows (Seguin-Swartz et al., 2009). Genotypes with resistance to sclerotinia stem rot, brown girdling root rot and downy mildew have been found (Seguin-Swartz et al., 2009).

Camelina cultivation can be grown in rotation with wheat, barley, peas and lentils. Camelina production after similar crops including canola, mustard, and other *Brassica* crops is problematic, as seeds from previous crops in the field lead to volunteer problems (Grady and Nleya, 2010 in "Camelina Plant Guide", 2011). It is recommended to choose fields with less weed pressure and good mechanical or chemical fallow operations ("Camelina Plant Guide", 2011). Dense plant establishment is also a good weed control mechanism ("Camelina Plant Guide", 2011).

Seeds from plants infected with downy mildew should not be used for planting in subsequent years since downy mildew is a seed-borne fungal disease ("Camelina Plant Guide", 2011). Control of downy mildew can be achieved by dryland production, limited irrigation, and low plant density which allows greater air movement (McVay and Lamb, 2008).

1.1.9 Variety Selection

Calena is a European variety which performed well in early regional trials (Ehrensing and Guy, 2008). Epona (winter variety) and Celine (spring variety) were

released by Group Limagrain in France (Ehrensing and Guy, 2008). Blaine Creek and Suneson were released by the plant breeders in Montana State University in 2007. Blaine Creek is rich in omega-3 fatty acids and is a short season and high-yielding variety. Suneson is an average-yielding and mild-season variety which has 2-3% higher oil than Blaine Creek (Ehrensing and Guy, 2008). Platte is a spring variety which substituted Cheyenne by Blue Sun Biodiesel in 2009. It performs relatively well in dry conditions (less than 500mm annual precipitation) ("Camelina Plant Guide", 2011). SO-40, SO-50, and SO-60 were released by the Sustainable Oils Company in 2010. They are spring genotypes with high yields in various environmental conditions ("Camelina Plant Guide", 2011).

1.2 Statement of Goals

This study evaluated key aspects of the basic agronomy of camelina as an oilseed crop under controlled environmental conditions and in the field. Testing locations included two sites in NS and one each in PEI, NB, and SK in 2011 and 2012. Water stress and plant nutrients such as N, S and B were tested to determine the response of different genotypes of camelina to environmental and management factors in terms of germination, growth, yield and seed quality. Seed quality factors analyzed were fatty acids, protein content, oil content and glucosinolates. In order to achieve the overall goals, experiments with the following objectives were conducted:

(1) Determine the effects of water stress on seed germination and early growth of 29 lines of camelina;

(2) Determine the effects of (a) N, (b) S and B, (c) N, S and genotype on the growth, yield, and seed quality of camelina under controlled environmental conditions;

(3) Using a two year field study at five locations, evaluate the effects of N and genotype on the growth, yield and seed quality of camelina;

(4) Using a field study in 2012 at five sites across Canada, examine the effects of combined N and S on growth, yield and seed quality of two elite lines of camelina.

Chapter 2: Effects of Water Stress on Seed Germination and Early Growth of *Camelina sativa* L. Crantz

2.1 Introduction

Seed germination involves a series of biological events, which are affected by many factors including temperature, light and available soil water. Among these, the available soil water is considered to be the most important (Sy et al., 2001). The process of germination is initiated by water imbibition, followed by enzymatic metabolism of storage nutrients and radicle protrusion (Gao et al., 1999). Water, as a solvent, provides a fluid medium in which substrates diffuse to active sites and the conformational changes of enzymes occur, which are essential to catalytic activity (Yang et al., 2010). It has been reported that germination is strongly limited by water stress at extremely high or low temperatures (Zheng et al., 2004). Water stress may also decrease seedling establishment and growth of surviving seedlings (Shao et al., 2008). Research done by Yang et al. (2010) showed decreased water potential led to a delay and reduced germination in *Piceaasperata*. A similar result was found in lentils (Zheng et al., 2005). It was reported that increasing moisture stress can delay the initiation of germination and slow shoot and root elongation (Rono, 1994).

Polyethylene glycol (PEG) has been used to create a range of water potential regimes (Rono, 1994). PEG cannot pass through materials made of plant membrane because of its high molecular weight (4,000 or more), so it has been used to keep experimental media at predetermined water potential values. According to the van't Hoff equation, the theory about the quantity of PEG 8000 calculation is $\Psi_{\pi} = -cRT$, ($t = T - 273\ ^{o}C$) (Rono, 1994). Where R is the universal gas constant (8.32 J mol^{-1} K^{-1}), T is the absolute temperature (in degrees Kelvin, or K), and c is the solute concentration of the

solution, expressed as osmolality (moles of the total dissolved solutes per liter of water - mol/L). The minus sign indicates that dissolved solutes reduce the water potential of a solution relative to the reference state of pure water (Rono, 1994).

The hypothesis of this study was that different genotypes would differ in germination and early growth response to water stress. The objective of this study was to examine, using two controlled environment studies, the effects of water stress on seed germination and early growth of camelina and to screen camelina genotypes using this germination test under various water potential regimes.

2.2 Materials and Methods

2.2.1 Materials

(1) The first experiment used 25 camelina lines to test seed germination under water stress (see Table 1). The seeds were provided by Plant Gene Resources of Canada (PGRC), Agriculture and Agri-Food Canada (AAFC), Saskatoon.

Table 2.1: Numerous coding of 25 genotypes of camelina

CN 113673	CN 113681	CN 113685	CN 113689	CN 113690
CN 113695	CN 113696	CN 113701	CN 113704	CN 113707
CN 113710	CN 113715	CN 113716	CN 223723	CN 113726
CN 113728	CN 113729	CN 113738	CN 113739	CN 113746
CN 113749	CN 113752	CN 113756	CN 113758	CN 113759

(2) The second experiment used four advanced camelina lines to evaluate germination and early growth under water stress. They were CDI002, CDI005, CDI007 and CDI008. Lines were provided by Dr. Kevin C. Falk, a camelina breeder at AAFC Saskatoon Research Centre.

2.2.2 Methods

(1) The Petri-dish Method

The experiment was designed as a two-factor factorial Completely Randomized Design (CRD) (25 genotypes * 6 water potentials). It was conducted in a seed germination chamber (Conviron, CMP3244). Water potential was set from zero (de-ionized water) to -3.0 MPa. PEG8000 (0, 1.94, 3.87, 5.81, 7.74 and 9.68g) was dissolved in water (1L) to make solutions that had water potentials of 0, -0.6 MPa, -1.2 MPa, -1.8 MPa, -2.4 MPa and -3.0 MPa, respectively. Fifty camelina seeds from each genotype, replicated three times, were placed on filter papers, and then were put onto petri dishes. PEG solution was added to the petri dishes, covering the seeds, and then they were placed into the seed germination chamber for a week at 25 ^{o}C, relative humidity 85% and no light. All the experimental units were randomized.

(2) The Growth Pouch Method

This experiment was designed as a two-factor factorial Completely Randomized Design (CRD) (4 lines * 6 water potential). It was done in a seed germination chamber (Conviron, CMP3244). Water potential was set from zero (de-ionized water) to -3.0 MPa. PEG8000 (0, 1.94, 3.87, 5.81, 7.74 and 9.68g) was dissolved in water (1L) to make solutions that had water potentials of 0, -0.6 MPa, -1.2 MPa, -1.8 MPa, -2.4 MPa and -3.0 MPa, respectively. Different levels of PEG solutions were added to growth pouches with 20 ml per pouch. Ten camelina seeds from each line, replicated four times, were placed on the small holes in the growth pouches. They were placed into the seed germination chamber for 10 days. The germination conditions were 25 ^{o}C, relative humidity 85%, and

no lighting during the first 24 hours. After germination, the conditions of the chamber were 20 ^{o}C, relative humidity 85% and 80 watts of light from 0800 to 1159; and 15 ^{o}C, relative humidity 85% and no light from 0000 to 0800. All the experimental units were randomized.

2.3 Data Collection

2.3.1 The Petri Dish Method

(1) Number of germinated seeds was counted each day with 24 hour intervals until all seeds germinated.

(2) Root length and the combination of root and shoot length were measured, and shoot length was calculated after 3 days. Nine samples were selected randomly from each treatment (per genotype per water potential).

(3) Biomass was measured: all germinated seedlings were weighed and counted, and then placed into envelopes. The seedlings were dried in a hot air oven at 80 ^{o}C for 48 hours, and were immediately moved to a desiccator and were cooled; dried seedlings were again weighed.

2.3.2 The Growth Pouch Method

(1) Number of germinated seeds was counted after 24 hour intervals until all seeds germinated.

(2) Root length in all experimental units was measured after 3, 6 and 9 days.

(3) Fresh weight of seedlings from each growth pouch was weighed.

2.4 Statistical Analysis

Minitab 16 Statistical Software (Minitab Inc., USA, 2012) was used in the analysis of data. General linear model was used to examine whether different genotypes and different concentrations of PEG affected camelina growth (root, root & shoot and biomass). The significant level was set to be 0.05. Independence, Constant Variance and Normality of the Residuals are the assumptions of ANOVA. If there were significant differences in the means, significance tests were conducted. SAS 9.3 (SAS Institute Inc., Cary NC, USA, 2012-2013) was applied when there were significant effects of factors on the targeted responses. Proc Mixed with Tukey test was used.

Kruskal-Wallis non-parametric test was used when the data distribution was not normal and it could not be made normal by transformations (the data of seed germination). Non-parametric test used the median and not the mean. There was a significant difference between at least two of the median values, Mann-Whitney tests of all the possible pairs of medians were analyzed to find out where the differences were.

2.5 Results

2.5.1 The Petri-dish Method (25 lines)

(1) Seed Germination Percentage

The data of seed germination percentage were not normally distributed after transformations, so Kruskal-Wallis was used to analyze the data. The p-value was larger than 0.999, hence, no significant difference of germination percentage among these 25 genotypes was observed. The seed germination percentages of all of the genotypes and

different levels of water stress (from 0 MPa to -3.0MPa) were high ranging from 95.35%-100% (Table 2.2).

Table 2.2: Median of germination percentage of 25 genotypes with 6 water potentials

Lines	0 MPa	-0.6 MPa	-1.2 MPa	-1.8 MPa	-2.4 MPa	-3.0 MPa
673	100.00	100.00	100.00	100.00	100.00	100.00
681	100.00	100.00	100.00	100.00	100.00	100.00
685	100.00	100.00	100.00	100.00	100.00	98.39
689	100.00	97.96	95.35	100.00	100.00	100.00
690	100.00	100.00	100.00	100.00	100.00	100.00
695	100.00	97.83	100.00	100.00	100.00	100.00
696	100.00	100.00	98.11	100.00	100.00	98.15
701	98.33	100.00	100.00	100.00	100.00	100.00
704	100.00	100.00	100.00	98.04	97.67	100.00
707	100.00	100.00	100.00	100.00	100.00	98.33
710	100.00	100.00	100.00	97.96	100.00	100.00
715	100.00	98.55	100.00	100.00	100.00	100.00
716	100.00	100.00	100.00	100.00	100.00	100.00
723	100.00	100.00	98.46	100.00	100.00	100.00
726	100.00	100.00	100.00	100.00	100.00	100.00
728	100.00	100.00	100.00	100.00	100.00	100.00
729	100.00	100.00	100.00	100.00	98.65	100.00
738	100.00	100.00	100.00	100.00	100.00	100.00
739	100.00	96.30	100.00	100.00	97.44	100.00
746	100.00	100.00	97.22	100.00	100.00	100.00
749	100.00	100.00	98.63	100.00	98.53	100.00
752	100.00	100.00	100.00	100.00	98.59	100.00
756	97.37	100.00	100.00	100.00	100.00	100.00
758	100.00	100.00	100.00	100.00	100.00	98.28
759	100.00	100.00	100.00	98.16	100.00	100.00

(2) Root Length after 3 days of Seeding

Genotype and water potential independently had significant effects on the root length (cm) after 3 days of seeding (Table 2.3). CN113710 had the longest roots with

3.2cm, which was not significantly different from CN11673, CN11685, CN11690, CN11695, CN11696, CN11704, CN11707, CN11726, CN11738, CN11739, CN11749 and CN11759. In contrast, CN113729 had the shortest roots among the 25 genotypes (Figure 2.1). Plants without water stress had significantly higher root lengths than plants with -1.8 and -2.4 MPa water potential (Table 2.4). Root length decreased with the reduction of water potential from 0 MPa to -1.8 MPa, and then increased slightly with water potential from -1.8MPa to -3.0 MPa (Figure 2.2).

Table 2.3: ANOVA table of root length after 3 days

Effect	F-value	P-value
Genotype (G)	**8.33**	**<.0001**
Water potentials (WP)	**3.87**	**0.0021**
G*WP	1.05	0.356

(no transformation)

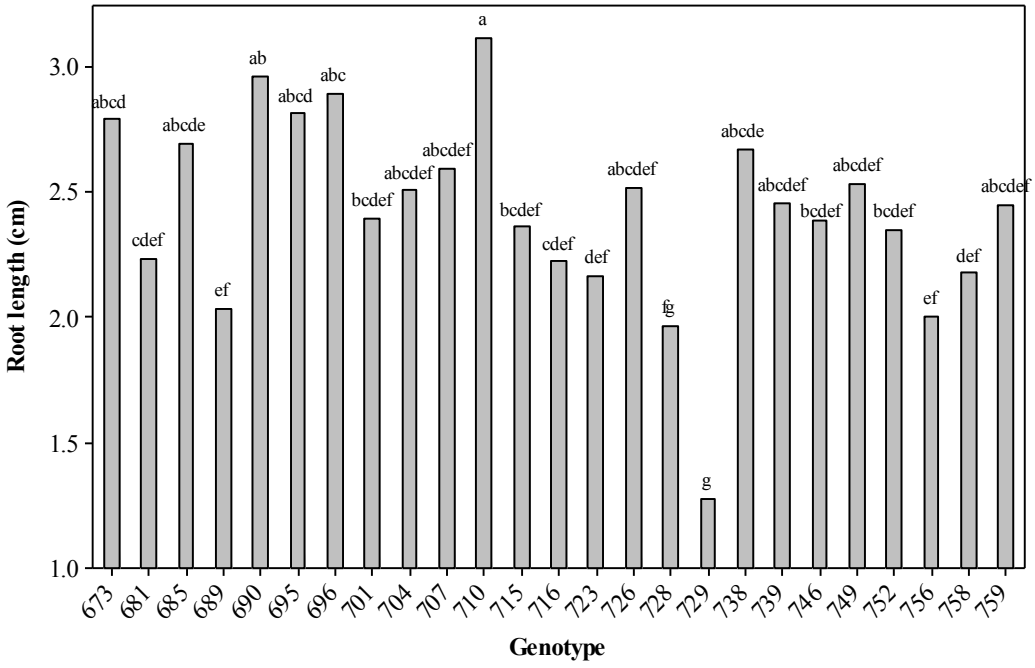


Figure 2.1: Effects of genotype on root length (cm) over all water potentials after 3 days

(Means with a common letter are not significantly different at the 5% level)

Table 2.4: Effect of water potential on root length after 3 days of seeding of 25 lines

Water potential (MPa)	Root length (cm)
-3.0	2.4 ab
-2.4	2.3 b
-1.8	2.3 b
-1.2	2.4 ab
-0.6	2.5 ab
0	2.6 a

(Means with a common letter are not significantly different at the 5% level)

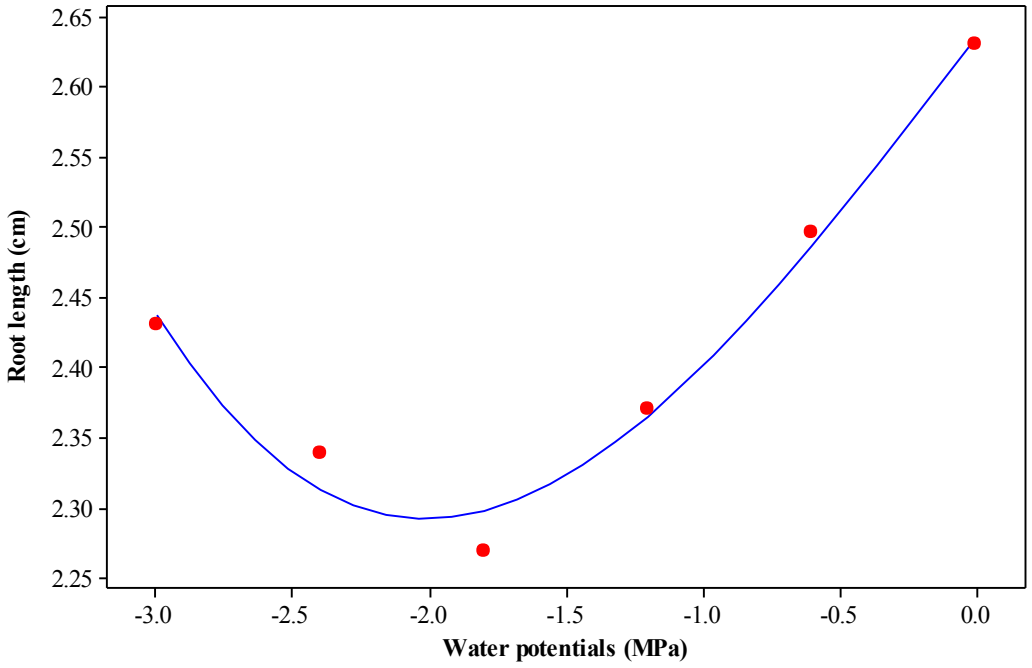


Figure 2.2: Regression analysis of effects of water potential on root length after 3 days

(each dot represents mean of 100 samples)

Y=2.635+0.2524WP-0.00289WP**2-0.02172WP**3 with R-Sq(adj)=94.9%

(3) Total Root and Shoot length after 3 days of seeding

Genotype and water potential independently had significant effects on the total root and shoot length (Table 2.5). CN11729 had the shortest roots among the 25

genotypes (Figure 2.3). CN11710 had the longest roots, which was not significantly different from CN11673, CN11685, CN11690, CN11695, CN11696, CN11704 and CN11738 (Figure 2.3). The total root and shoot length increased with an increase in water potential (Figure 2.4 and Table 2.6). The average root and shoot length at water potential 0 MPa was 4.3 cm, which was not significantly different from the mean length (4.00 cm) at water potential -0.6 MPa, but significantly higher than the root length when water potential continued to decrease from -1.2 MPa to -3.0 MPa (Table 2.6).

Table 2.5: ANOVA table of total root and shoot length after 3 days

Effect	F-value	P-value
Genotype (G)	**13.46**	**<.0001**
Water potentials (WP)	**10.47**	**<.0001**
G*WP	1.18	0.1291

(no transformation)

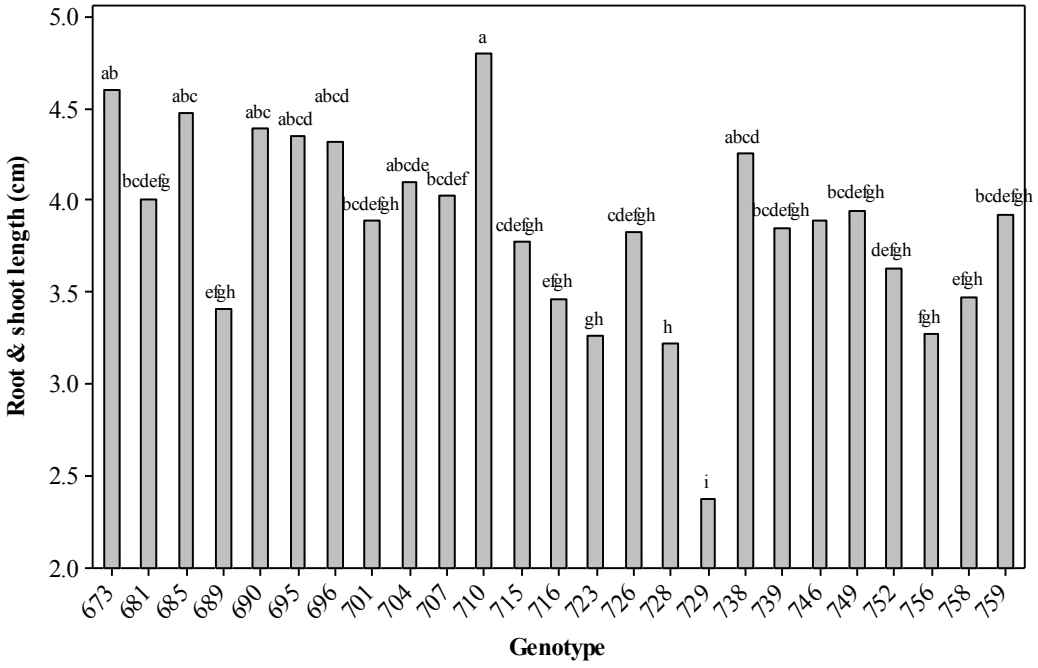


Figure 2.3: Effects of genotype on total root and shoot length after 3 days

(Means with a common letter are not significantly different at the 5% level)

Table 2.6: Effect of water potential on the total length of root and shoot of 25 lines

Water potentials (MPa)	Root and shoot length (cm)
-3.0	3.7 c
-2.4	3.7 bc
-1.8	3.7 c
-1.2	3.8 bc
-0.6	4.0 ab
0	4.3 a

(Means with a common letter are not significantly different at the 5% level)

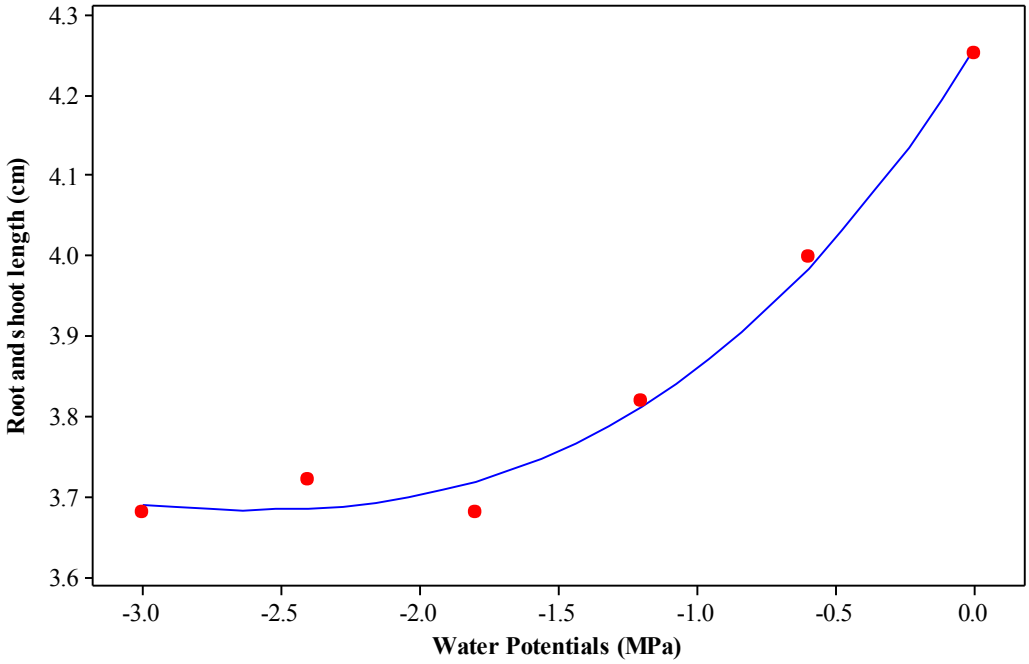


Figure 2.4: Regression analysis of water potential on root and shoot length after 3 days

(each dot represents mean of 100 samples)

Y=4.258+0.5511WP+0.1681WP**2+0.01587WP**3 with R-Sq(adj)=96.8%

(4) Biomass Measurement

This experiment failed to accurately measure the biomass of camelina seedlings. This was because the wrong envelopes were coupled with seedlings that were too small

and therefore were too light. In this experiment, empty envelopes (W_1) were weighed first. The camelina seedlings from each petri dish were counted and put into those envelopes, and then envelopes with fresh weight of seedlings were weighed (W_2). The next step was to place all the envelopes with seedlings into an 80℃ hot air oven for 48 hours (W_3). However, W_3 was found to be smaller than W_1. This was probably due to the envelopes having absorbed moisture in the air before being used.

2.5.2 The Growth Pouch Method (4 advanced lines)

(1) Seed Germination Percentage

The data of seed germination percentage were not normally distributed after transformations, so Kruskal-Wallis was used to analyze the data. The calculated p-value was 0.998 and therefore, no significant difference in germination percentage among these four genotypes was detected. The seed germination percentages of all of the genotypes and different levels of water stress (from 0 MPa to -3.0MPa) were relatively high with median values of 90% and 100% (Table 2.7).

Table 2.7: Median of effects of genotype and water potential on germination

	0 MPa	-0.6 MPa	-1.2 MPa	-1.8 MPa	-2.4 MPa	-3.0 MPa
CDI002	100	100	100	100	90	100
CDI005	100	100	100	100	100	100
CDI007	100	100	100	100	100	100
CDI008	100	100	100	100	100	100

(2) Root Length Measurements

Genotype had a significant effect on the root length after both 3 and 6 days of seeding (Table 2.8). After 3 days of seeding, CDI007 showed the longest root length;

CDI008 had the shortest root length, which was not significantly different from CDI005 (Figure 2.5). After 6 days of seeding, CDI007 had the longest root length, which was not significantly different from CDI002; CDI008 had the shortest root length (Figure 2.5).

Table 2.8: ANOVA table of root length after 3 and 6 days

Effect	3 days		6 days	
	F value	P value	F value	P value
Genotype (G)	22.5	<.0001	16.84	<.0001
Water potentials (WP)	2.01	0.087	1.69	0.1479
G*WP	1.43	0.157	1.25	0.2601

(no transformation)

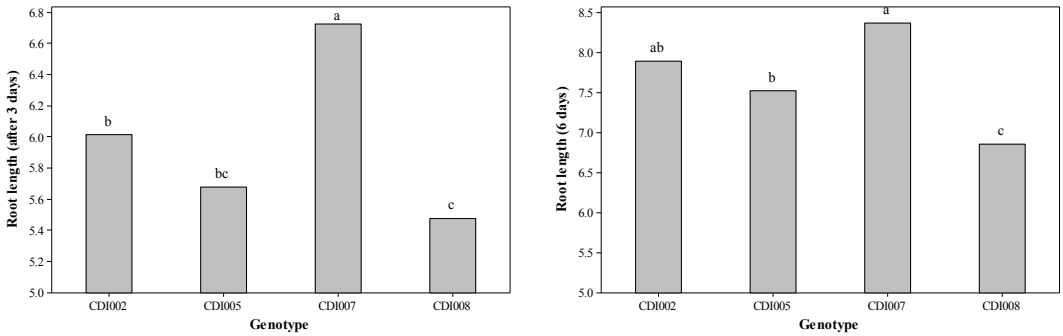


Figure 2.5: Effects of genotype on root length after 3 and 6 days of seeding
(Means with a common letter are not significantly different at the 5% level)

(3) Fresh Weight Measurement

Genotype had a significant effect on the fresh weight of seedlings (Table 2.9). The value of R-Sq was 43.4%, which indicated the proportion of the variability in fresh weight that was explained by variability in lines and/or water potential. The fresh weight of CDI002 was the highest but was not significantly different from that of CDI007, but was significantly different from that of CDI005 and CDI008 (Figure 2.6).

Table 2.9: ANOVA table of effects of genotype and water potentials on fresh weight of seedlings after 10 days

Effect	F-value	P-value	R-Sq
Genotype (G)	9.73	<.0001	
Water potentials (WP)	1.51	0.197	43.73%
G*WP	1.28	0.237	

(no transformation)

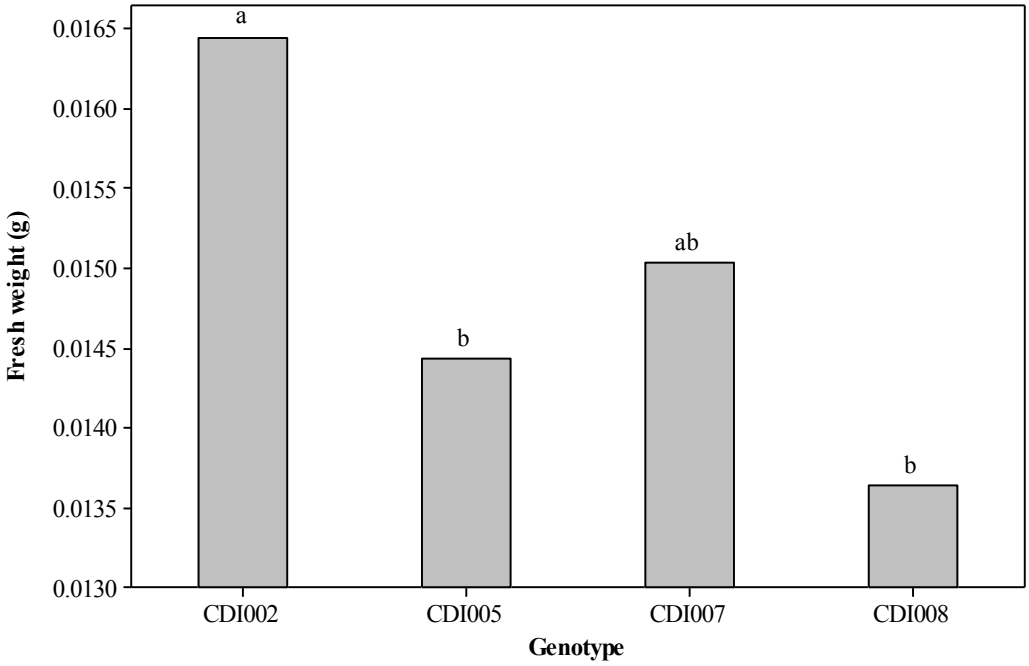


Figure 2.6: Effect of genotype on fresh weight of camelina seedling
(Means with a common letter are not significantly different at the 5% level)

2.6 Discussion

2.6.1 The Petri-dish Method

The germination percent of different genotypes was relatively high throughout the stress range (0 MPa to -3.0 MPa). According to Gardner et al. (1985), permanent wilting percentage for different species of crop plants ranged from -1.5 MPa to -5.0 MPa, but was often arbitrarily set at -1.5 MPa, since very little water was available from -1.5 MPa

to -3.0 MPa. The experiment showed that camelina germinated even under high moisture deficit.

In general, the root and/or shoot length decreased with the reduction of water potential from 0 MPa to -3.0 MPa. The reason for this was that the soil moisture level of field capacity was generally optimum for germination. Plant germination slows the growth rate when soil moisture comes close to the wilting point. Under less than optimum water content partial imbibition and slow germination would occur (Gardner et al. 1985).

2.6.2 The Growth Pouch Method

The results obtained in the growth pouch method were not as clear as those observed in the petri-plate method. Genotype had a significant effect on root length after both 3 and 6 days, but water potential did not. CDI007 performed the best in terms of root length and CDI008 performed the worst after both 3 and 6 days. Genotype had a significant effect on the fresh weight after 10 days, but water potential had no significant effect on fresh weight. Fresh weight of CDI002 was the biggest and that of CDI008 was smallest. CDI002, CDI005, CDI007 performed better than CDI008 in both root length and fresh weight.

2.7 Conclusion

All 29 selected genotypes of camelina germinated well (above 90%) despite external water potential having decreased to -3.0 MPa. However, the root and shoot length of seedlings decreased with the decline in water potential. In the petri-dish method, CN113729 performed the worst in both seed vigor and the growth rate among the 25

genotypes. CN113673 and CN11370 performed relatively better and had relatively longer root and shoot lengths compared with the rest. For the growth pouch method, the results were not that obvious compared to the former method. There was no significant effect of genotype and/or water potential on seed germination percentage (ranging from 90% to 100%). Genotype had a significant effect on root length and fresh weight of camelina seedlings, but water potential had no significant effect on root length and fresh weight.

Chapter 3: Effects of Nitrogen, Sulphur and Boron on the Growth, Yield and Seed Quality of *Camelina sativa* L. Crantz in Controlled Environmental Conditions

3.1 Introduction

More than 50% of the increase in yields of crops in the past 50 years is attributed to the use of fertilizers (FAO UN, 1984). As is true for all living beings, plants require nutrients for their growth and reproduction. N as one of the 3 primary nutrients (N, P and K) is used in large quantities. S is needed in smaller but appreciable quantities. B is one of trace elements and is applied in very small quantities. A productive soil for plant growth should have sufficient essential macro and micro nutrients with balanced proportions (FAO UN, 1984).

3.1.1 N

N is an important nutrient for high yield. N is an essential component of amino acids, amides, nucleotides and nucleoproteins and also plays an important role in cell division, expansion and growth, as well as leaf photosynthetic capacity and efficiency (Gardner, 1985). N is mobile in the plant, and moves to young tissues so N deficiency is first observed in older leaves. A deficiency results in plant stunting, yellowing and the reduction of crop dry matter, including the number of seeds per plant and the number of branches per plant (Gardner, 1985). For example, under N deficiency, camelina plants are thin and upright and the leaves are small and pale yellow-green. Ripening is premature and there are few pods and seed bearing branches.

3.1.2 S

S is necessary for plant growth and physiological functions including chlorophyll formation, protein and vitamin development, resistance to cold and water stress (Gardner

et al., 1985). S forms thiol bonds which is energetically analogous to the N peptide bonds. Sulfhydryl groups (SH) play a key role in the hardening of the protoplasm towards cold and drought stress. S also leads to the characteristic taste and smell of some crops including onions and garlic. S is available from the soil and the atmosphere. Approximately 90% of the soil S which is available for plants is organic S (Sharifi et al., 2010).

S is absorbed by plants primarily as SO_4^{2-}, and it is actively and passively translocated. Leaves can also take up relatively large amounts of SO_2 gas (Gardner et al., 1985). The increased application of S fertilizer enhances the seed oil content of several crops such as flax and soybeans (Gardner et al., 1985). The symptoms of S deficiency include stunting, general yellowing and thin stems, which are similar to those of N deficiency. S deficiency has a negative effect on the N use efficiency from fertilizers of plants (Tandon, 1992; Schnug et al., 1993) and also leads to N losses. Under S deficiency, the increasing levels of N can accelerate the deficiency symptoms and decrease production (Janzen and Bettany, 1984).

Plant performance responding to N application can reach optimum level only when S supply is sufficient; and that responding to S can be maximized only when N is sufficient (Hu and Sparks, 1992). The interaction of N (from 63 to 127 kg/ha) and S (75 and 135 kg/ha) was tested for *Camelina sativa* L. Crantz (Losak et al., 2011). The results showed that the high and medium levels of N with low S application enhanced the number of branches per plant. The seed yield increased with the enhancement of N rates, but the straw yield only increased with the highest supply of N. The N application had a negative effect on the seed oil content. It decreased from 39.8% to 37.1% when N

increased from 63 kg/ha to 127 kg/ha. The increasing N augmented the total content of oil and protein. In this experiment, the effects of S on the crop parameters including branches/plant, straw yield, 1000-seed weight, seed yield, oil content, oil yield, protein content and protein yield were not significant (Losak et al., 2011).

3.1.3 B

B is an essential micronutrient for plant growth and development, but high concentrations of B are toxic to plants (Banks, 1990). B is a unique micronutrient because deficiency and toxicity can occur (Yau and Ryan, 2008). B plays an important role in the synthesis of uracil (one of the RNA bases) and in cellular development such as cell division, differentiation, maturation and respiration (Jones, 1998). B affects cell development by regulating sugar transport and polysaccharide formation (Gardner et al., 1985). It develops a polyhydroxy complex with sugars that are more steadily translocated through cellular membranes, compared with the non-borate sugars (Sinha, 2000). B is crucial for pollen germination and growth, and it also promotes the stability of pollen tubes (Jones, 1998). B is relatively immobile in the phloem of plants and is mainly transported in the xylem. Therefore, young plant tissue uptake relies on current absorption (Gardner et al., 1985).

B deficiency in fenugreek caused anthesis and reproduction failure and yellowing (Gardner et al., 1985). B deficiency decreased the yield of mustard because of excessive accumulation of phenols and auxins, which resulted in early necrosis of the growing apex and inhibited plant growth (Sinha et al., 2000). B exists as the borate anion (BO_3^{3-}) in soil solution. The total B in the soil is from approximately 20 to 200 ppm, and the available amount for plant absorption ranges from 1 to 5 ppm (Jones, 1998). B availability is

affected by soil pH, and the optimum amount for maximum B availability is 5.5-7.0. Effects of different levels of B (0, 1.5, 3.0, and 4.5 kg N/ha) were tested on soybean (Seguin and Zheng, 2006).

A combination of S (0, 7.5, 15 and 30 kg/ha) and B (0, 2.5, 5.0, and 7.5 kg/ha) were evaluated on sesame. Gypsum (18% S) and borax (11% B) were used as S and B sources (Mathew and George, 2011). The highest yield was achieved with the highest application of 30 kg S/ha and 7.5 kg B/ha. It indicated both S and B are important in the production of oilseed crops (Mathew and George, 2011). S promotes primordial floral initiation, which leads to an increasing number of capsules per plant and seeds per capsule (Mathew and George, 2011).

3.1.4 Hypothesis

Independent and/or combined N, S and B would have significant effects on the growth, yield and seed quality of camelina.

3.1.5 Objectives

The objective of this study was to evaluate the independent and/or combined effects of N, S and B on plant growth, yield, and seed quality of camelina under controlled environment conditions.

3.2 Materials and Methods

3.2.1 Materials

Two advanced lines of camelina (CDI005 and CDI007) from Agriculture and Agri-Food Canada (AAFC), Saskatoon were tested. S, N and B sources were hydrated

magnesium sulfate ($MgSO_4.7H_2O$), ammonium nitrate (NH_4NO_3, 34-0-0) and bortrac (Yara, Vita, 10.9%w/w, 150g/L), respectively.

Soil medium Pro-mix "BX" was used as growing medium for plants. The results of nutrient analysis of the soil medium with Saturated Medium Extract show it contains 70-130ppm (mg/l) nitrate (NO_3-N), 5-40ppm phosphate (PO_4-P), 70-150ppm potassium (K), 130-210ppm calcium (Ca), 20-45ppm magnesium (Mg), 30-100ppm sulphate (S-SO_4), 1.0-2.5ppm iron (Fe), 0.1-1.0ppm zinc (Zn), less than 0.3ppm copper (Cu), 0.3-1.5ppm manganese (Mn) and less than 0.6ppm B (B). The volume of the pots used in these experiments is 1.7 L, and the surface size of pots is 177 cm^2. Therefore, the background nutrients for plants were 67.4-125.1 kg/ha nitrate (NO_3-N), 4.8-38.5 kg/ha phosphate (PO_4-P), 67.4-144.4 kg/ha potassium (K), 125.1-202.1 kg/ha calcium (Ca), 19.2-43.3 kg/ha magnesium (Mg), 28.9-96.2 kg/ha sulphate (S-SO_4), 1.0-2.4 kg/ha iron (Fe), 0.1-1.0 kg/ha zinc (Zn), less than 0.3 kg/ha copper (Cu), 0.3-1.4 kg/ha manganese (Mn) and less than 0.6 kg/ha B (B). The data were provided by the Company of Premier Horticulture.

3.2.2 Methods

3.2.2.1 Plant Cultivation

(1) N

This was a one factor design with four replications arranged in a Completely Randomized Design (CRD and one genotype, CDI007). N was supplied by dissolving ammonium nitrate in distilled water. Ammonium nitrate solution was applied with rates equivalent to 0, 25, 50, 100, 150 and 200 N/ha (0, 0.127, 0.253, 0.506, 0.759 and 1.012

g/pot). Higher N treatments including 100, 150 and 200 kg N/ha were split into two timings - one week within seeding and early flowering stage. Each pot received 100 ml of the N solution and the checks received 100 ml distilled water.

(2) S and B

This was a two factor factorial design with four replications arranged in a CRD and one genotype CDI007. The two factors were B and S. S and B sources were hydrated magnesium sulfate ($MgSO_4 \cdot 7H_2O$) and bortrac (Yara, Vita, 10.9%w/w, 150g/L), respectively. The three levels of S included 0 kg/ha, 25 kg/ha and 50 kg/ha; and the three levels of B were 0 kg/ha, 2.5 kg/ha and 5.0 kg/ha. S was supplied by dissolving hydrated magnesium sulfate in distilled water. Hydrated Magnesium sulfate ($MgSO_4.7H_2O$) was used two weeks after seeding with rates equivalent to 0, 25 and 50 (0, 0.340 and 0.680 g/pot). Each pot received 100 ml S solution and the checks received 200ml distilled water. B solution was applied at the (early) flowering stage with rates equivalent to 0, 2.5 and 5.0 (0, 0.4425 and 0.8850 ml bortrac solution into 1.5L water).

(3) N and S

This was a three factor factorial design with four replications arranged in a CRD. The three factors were the levels of S, N and genotype. CDI005 and CDI007 were tested in this experiment. N and S were supplied by dissolving ammonium nitrate and hydrated magnesium sulfate in distilled water. Ammonium nitrate solution was applied with rates equivalent to 0, 75 and 150 kg N/ha (0, 0.379, and 0.759 g/pot). N was split into two timings for the treatment of 150 kg N/ha - one week within after seeding and early flowering stages. Hydrated Magnesium sulfate ($MgSO_4 \cdot 7H_2O$) was used two weeks after seeding with rates equivalent to 0, 30 and 60 kg/ha (0, 0.408 and 0.816 g/pot). Each pot

received 100 ml of the N solution and 100 ml S solution and the checks received 200 ml distilled water.

For all these three experiments, ten seeds were sown per pot (15 cm diameter * 14 cm height) using soil medium (Pro-mix "BX"). Plants were thinned to 3 plants per pot after two weeks. The conditions of the growth chamber were 16-h photoperiod, with a mean day/night temperature of 23/18°C. Relative humidity was maintained at 70% ± 5%, and the light intensity was 1100-1200 $\mu E/m^2/sec$ photosynthetically active radiation (PAR).

3.2.2.2 Protein and oil analysis by NIR (Near-infrared spectroscopy)

For the N experiment and S & B experiment, a 0.5 gram seed sample from each pot was scanned twice by NIR, and total protein and oil content was determined.

3.2.2.3 Fatty acid composition analysis by gas chromatography

The method was based on the protocol developed by Agriculture and Agri-Food Canada, Saskatoon Research Centre quality laboratory: "Fatty acyl composition by gas chromatography". This method analyzes the fatty acid profile of esterified compounds of camelina seed oil by methylating the fatty acyl esters, and then these methyl esters are chromatographed on a gas chromatography. The standard component GLC 428 was used to identify retention time for components. One gram of seed was placed in 20 ml PET scintillation vials containing small steel rods (1.2cm diameter). Four ml hexane was added to every vial to extract oil from the seed. The second step was to put the vials in the Eberbach shaker and shake for 60 minutes at a high speed (270 revolutions per minute). The next procedure was to transfer the vials to a centrifuge (Thermo Scientific,

Sorvall ST 40R) at 2,300 rcf (3000 rpm) for 15 minutes. The fourth step was to open vials and transfer an aliquot (60 µl) to autosampler vials using a pipettor (Fisher brand, Finnpipette II, 2-20 µl). And then 0.05 ml hexane was dispensed and 0.1 ml of methylating solution was added into every autosampler vial; contents of vials were mixed and incubated at room temperature for 15 minutes for the methylating reaction. The next process was to add 0.05 ml of phosphate buffer to each vial to adjust the pH, and then evaporate off methanol under a stream of N for 2 minutes. Finally, 0.05 ml of hextane was added and vials were capped, and set up in the autosampler to inject 1 µl of the upper phase.

The conditions for gas chromatography were set as follows: (1) injection volume: 1µl; (2) split ratio: 1:50; (3) flow rate: 1.0 mL/min; (4) detector: flame ionization detector (FID) which has a wide range of liner response; (5) carrier gas: hydrogen, ultra high purity, 99.999%.

3.3 Data Collection:

(1) The surface area of the eighth leaf (full leaf) from the top of every plant (3 plants per pot) was measured by the leaf area meter (LI-3100 Area Meter, LI-Cor, inc. Lincoln, Nebraska, USA).

(2) The numbers of days that plants needed to reach the flowering stage were observed and recorded. The days to flowering were defined as two thirds of plants observed were in the flowering stage.

(3) Plant height (from the soil surface to the top of plant) at harvest.

(4) Yield components including branch number, pod number per plant were counted;

(5) The weight of 1000 seeds was weighed, which was done by counting 100 seeds, weighing and then multiplying by 10.

(6) Protein and oil analysis by NIRS (Near-infrared spectroscopy, Unity Scientific, Spectra Star, 2500x).

(7) Composition of fatty acids of seed was analyzed by gas chromatography (GC) using an Agilent 7890 GC.

3.4 Statistical Analysis

Minitab 16 statistical software (Minitab Inc., USA, 2012) was used to determine the three assumptions of normality, constant variance and independence; outliers were removed if they existed. Data were transformed (square, square root, cubic root, ln, or log10) if they were not normally distributed. Minitab 16 was used in all the regression analysis.

SAS 9.3 statistical software (SAS Institute Inc., Cary NC, USA, 2012-2013) was also used in the data analysis once the data were checked to be normally distributed in Minitab. Proc Mixed with Tukey Method ($p<0.05$) was used to examine whether there were significant effects of factors on the parameters tested.

Kruskal-Wallis non-parametric test was used to analyze days to maturity, because data points were not normally distributed. The non-parametric test uses the median and not the mean. There was significant difference between at least two of the median value using the Mann-Whitney test on all possible pairs of median values.

3.5 Results

3.5.1 Leaf Area

The interaction of genotype, N and S had a significant effect on the plant leaf area (the 8th leaf from the top) after 38 days of seeding (Table 3.1). CDI005 and CDI007 behaved differently with the combined N and S treatment (Table 3.2). The leaf area of CDI005 was positively correlated with the N application. With 60 kg S/ha application, the leaf area of CDI005 increased from 1.07 cm^2 without N to 7.06 cm^2 with 75 kg N/ha, and continued to increase to 12.52 cm^2 with the 150 kg N/ha application. The leaf area of CDI007 was largely unchanged when N increased from 75 kg/ha to 150 kg/ha with different levels of S. In general, the leaf area of CDI005 with 150 kg N/ha was much bigger than CDI007 (Table 3.2).

Table 3.1: ANOVA table of average leaf area (the 8th from top, 38 days after seeding) (N and S experiment)

Factors	F-value	P-value
Genotype (G)	59.95	<0.0001
N	988.03	<0.0001
S	4.58	0.0148
G*N	58.31	<0.0001
G*S	2.87	0.0657
N*S	3.1	0.0233
G*N*S	**2.79**	**0.0357**

(cubic root transformation)

Table 3.2: Effect of interaction of genotype, N and S on leaf area (N and S experiment)

N	CDI005			CDI007		
	0 kg S/ha	30 kg S/ha	60 kg S/ha	0 kg S/ha	30 kg S/ha	60 kg S/ha
0 kg N/ha	1.16 d	1.18 d	1.07 d	1.40 d	1.26 d	1.47 d
75 kg N/ha	6.57 bc	5.54 bc	7.06 b	6.85 b	5.33 bc	4.76 c
150 kg N/ha	10.56 a	10.28 a	12.52 a	6.08 bc	5.45bc	6.38bc

(Three-way interaction; means with a common letter are not significantly different at the 5% level)

3.5.2 Days to Flowering

Neither N nor S*B had a significant effect on the days to flowering (Table 3.3 and Table 3.4). Data from N and S*B experiments were not normally distributed, so Kruskal-Wallis non-parametric test was used to analyze the data. Genotype and N independently had significant effects on the days to flowering (Table 3.5). CDI007 grew significantly faster to reach the flowering stage than did CDI005. It took CDI007 and CDI005 30 days and 33 days to flowering, respectively. Plants with 75 kg N/ha application required less time to reach the flowering stage compared with the 0 and 150 kg N/ha applications. Other factors such as S and their interaction of N, S and genotype had no significant effect on the number of days to flower.

Table 3.3: Median of days to flowering (N experiment)

Factors	Rates (kg/ha)	Median of flowering days	P-value
N	0	39	0.352
	25	45	
	50	39	
	100	38	
	150	39	
	200	37	

Table 3.4: Median of days to flowering (S and B experiment)

S (ka/ha)	B (kg/ha)	Median of flowering days	P-value
0	0	37	0.857
	2.5	37	
	5	37	
25	0	37	
	2.5	37	
	5	37	
50	0	39	
	2.5	37	
	5	37	

Table 3.5: Effect of genotype*N*S on days to flowering (N and S experiment)

Factors	Levels	Mean (days)	F-value	P-value
Genotype (G)	CDI005	33 a	98.18	<0.0001
	CDI007	30 b		
N (kg/ha)	75	32 a	3.15	0.051
	150	31 b		
	0	31 b		
S			0.9	0.411
G*N			0.2	0.817
G*S			0.01	0.993
N*S			1.12	0.358
G*N*S			0.76	0.558

(Means with a common letter are not significantly different at the 5% level)

3.5.3 Days to Maturity

There was no significant effect of N on the days to maturity of CDI007 (Table 3.6). There was no significant effect of S and B on the days to maturity of CDI007 (Table 3.7). Data of S*B and N*S experiments were not normally distributed, so Kruskal-Wallis non-parametric test was used to analyze the data. CDI005 and CDI007, that received zero N, took approximately 62 days to reach maturity stage (Table 3.8). In general, days to mature were similar in the 0 and medium N applications for both entries. However,

CDI007 was later to mature than CDI005 with the high N application (150 kg N/ha).

Table 3.6: Effect of N on days to maturity (N experiment)

N (kg/ha)	Mean of maturity days	P-value
0	75	0.051
25	79	
50	78	
100	79	
150	79	
200	82	

Table 3.7: Median of days to maturity (S and B experiment)

S (ka/ha)	B (kg/ha)	Median of maturity days	P-value
0	0	78	0.713
	2.5	78	
	5	80	
25	0	81	
	2.5	78	
	5	78	
50	0	81	
	2.5	80	
	5	78	

Table 3.8: Median of days to maturity (N and S Experiment)

	CDI005			CDI007		
	0 kg S/ha	30 kg S/ha	60 kg S/ha	0 kg S/ha	30 kg S/ha	60 kg S/ha
0 kg N/ha	62 d	62 d	62 d	62 d	62 d	62 d
75 kg N/ha	70 c	70 cb	67 cd	68 bc	65 cd	91 a
150 kg N/ha	72 b	72 b	72 b	91 a	91 a	91 a

(Three-way interaction; means with a common letter are not significantly different at the 5% level)

3.5.4 Plant Height

N had a significant effect on plant height in the N experiment (Table 3.9). Plant height increased with an increase in N (Figure 3.1). S and/or B had no effect on plant height in the S and B experiment (Table 3.9); and genotypes behaved differently depending on the amount of N. Without N, CDI005 and CDI007 had similar plant height (Figure 3.2). With the medium and high N application, CDI005 was significantly taller than CDI007 while on CDI005 it had the opposite effect when compared to plants with the medium N application.

Table 3.9: Effect of fertilizers on canopy height

	Factors	F-value	P-value	R-Sq
1	**N**	**42.85**	**<.0001**	92.65%
	S	1.76	0.192	
2	B	2.09	0.144	38.53%
	S*B	2.31	0.084	
	Genotype (G)	26.17	<.0001	
	N	45.15	<.0001	
	S	0.27	0.768	
3	**G*N**	**5.92**	**0.005**	
	G*S	2.42	0.099	
	N*S	0.67	0.619	
	G*N*S	1.55	0.202	

(no transformation)

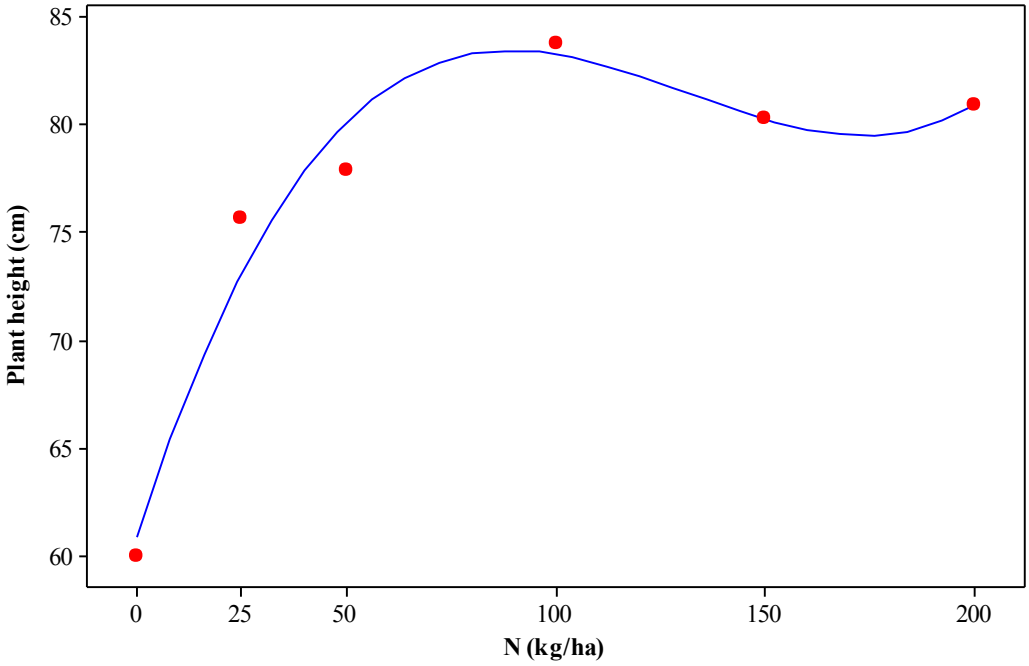


Figure 3.1: Regression analysis of N on plant height (N experiment)
(each dot represents mean of 4 samples)
Y=60.86+0.6123N-0.005200N**2+0.000013N**3 with R-Sq(adj)=91.3%

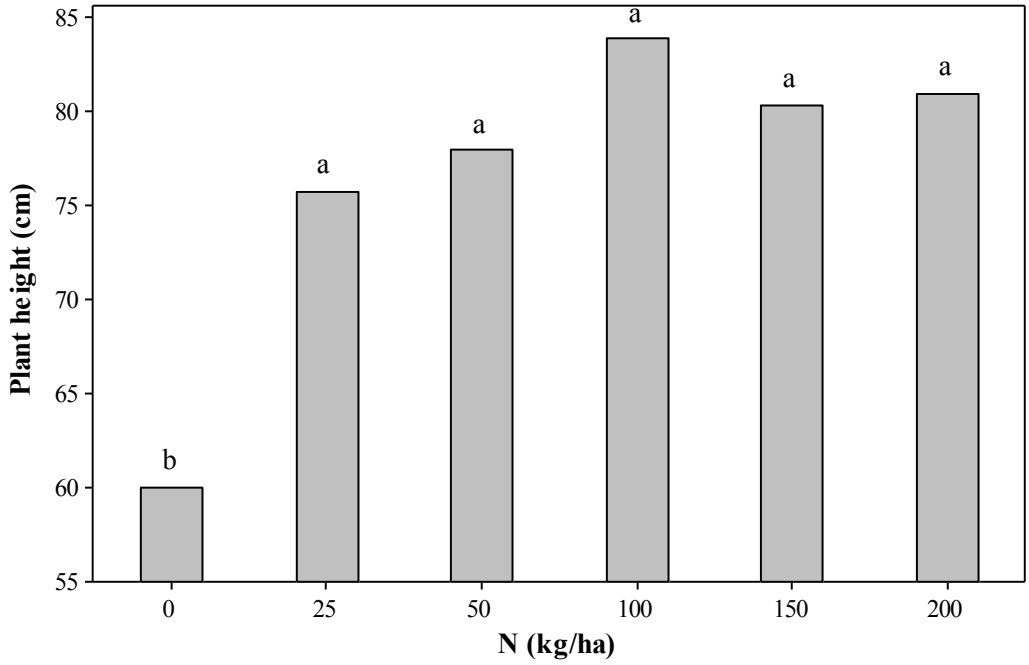


Figure 3.2: Effect of N on plant height (N experiment)
(Means with a common letter are not significantly different at the 5% level)

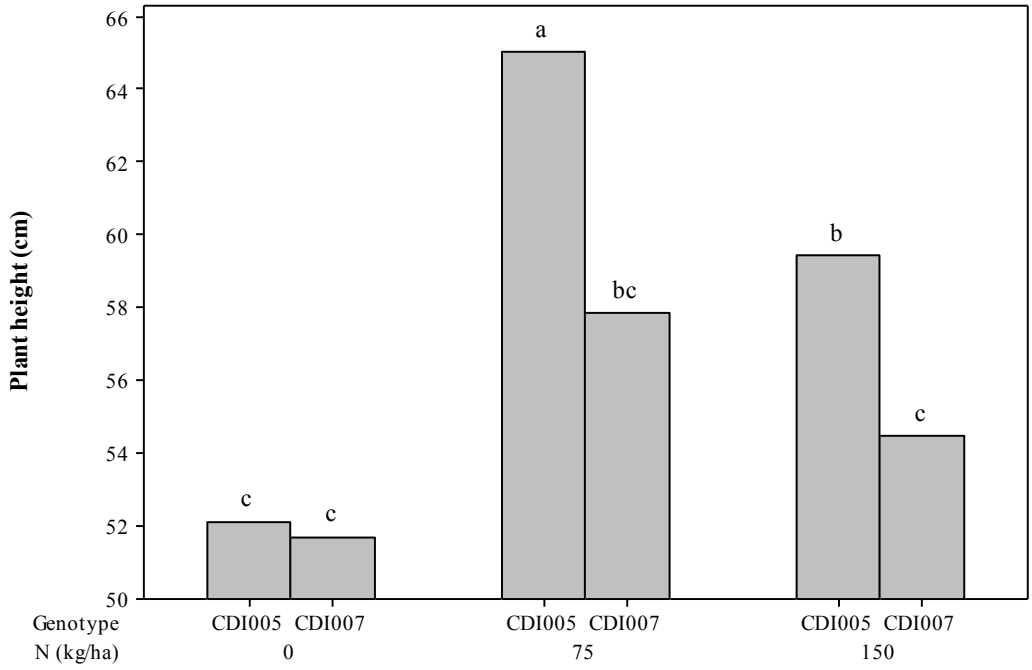


Figure 3.3: Interaction of genotype and N on plant height (N*S experiment)
(Means with a common letter are not significantly different at the 5% level)

3.5.5 Number of Branches per Plant

N had a significant effect on number of branches per plant in the N experiment (Table 3.10). The number of branches increased with an increase in N (Figure 3.4). S and/or B had no significant effect on the number of branches per plant in the S and B experiment (Table 3.10). In the N and S experiment, genotypes differed in the branching habits depending on the amount of S (Figure 3.7 and Table 3.10). Plants with the highest N application (150 kg/ha) produced the most branches, followed by those that received 75 kg/ha amounts of N, and plants without N application had fewer branches per plant (Figure 3.6). CDI005 and CDI007 behaved differently to the S application (Figure 3.7). S tended to increase the number of branches in CDI007, but not in CDI005 (Figure 3.7).

Table 3.10: Effect of genotype, N, S and B on number of branches per plant

	Factors	F-value	P-value	R-Sq
1	**N**	**33.57**	**<.0001**	91.80%
2	S	3.06	0.063	22.27%
	B	0.15	0.861	
	S*B	0.33	0.856	
3	Genotype (G)	110.01	<.0001	
	N	**275.6**	**<.0001**	
	S	0.61	0.5459	
	G*N	0.02	0.9839	
	G*S	**3.36**	**0.0422**	
	N*S	2.3	0.0708	
	G*N*S	0.26	0.9035	

(N: no transformation; S*B experiment: $\log_{10}$ transformation; G*N*S: square root transformation)

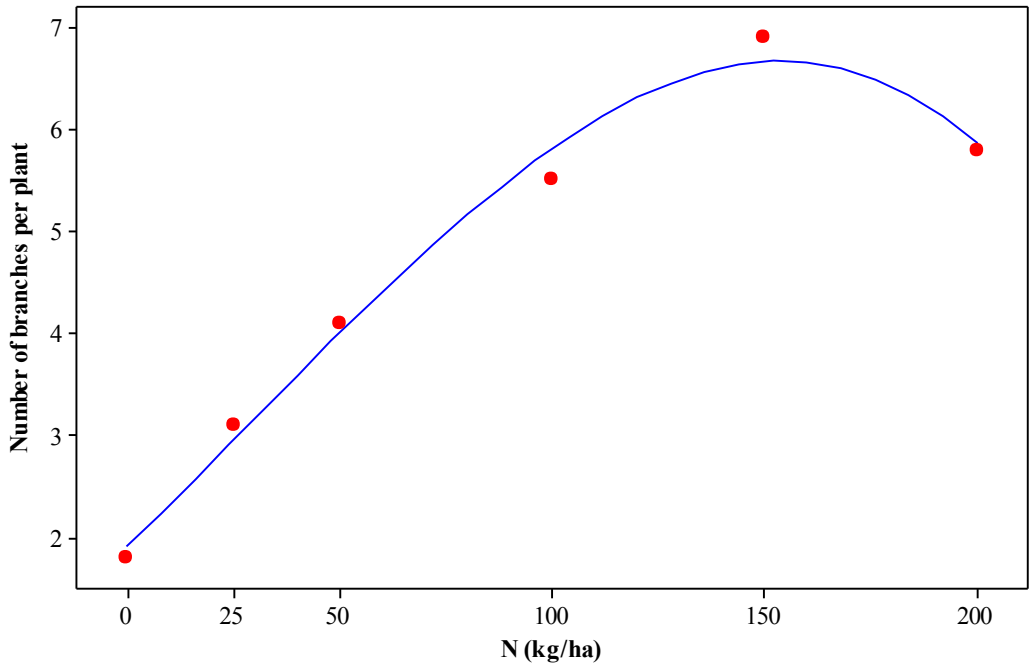


Figure 3.4: Regression analysis of N on branches/plant (N experiment)
(each dot represents mean of 4 samples)
Y(N)=1.909+0.04001N+0.000083N**2-0.000001N**3 with R-Sq(adj)=97.1%

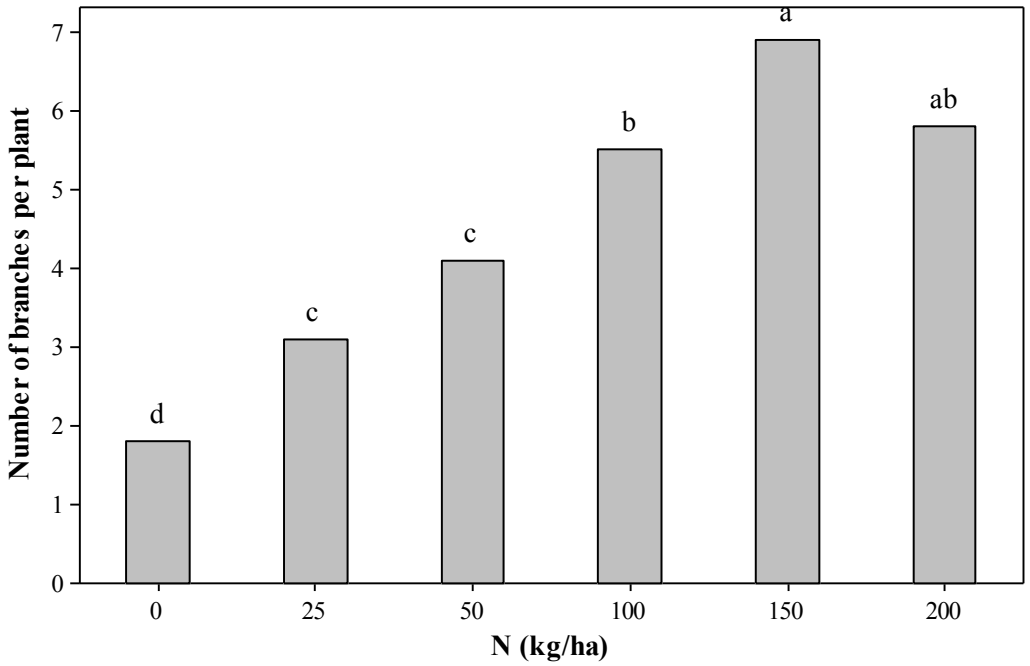


Figure 3.5: Effect of N on branches/plant (N experiment)
(Means with a common letter are not significantly different at the 5% level)

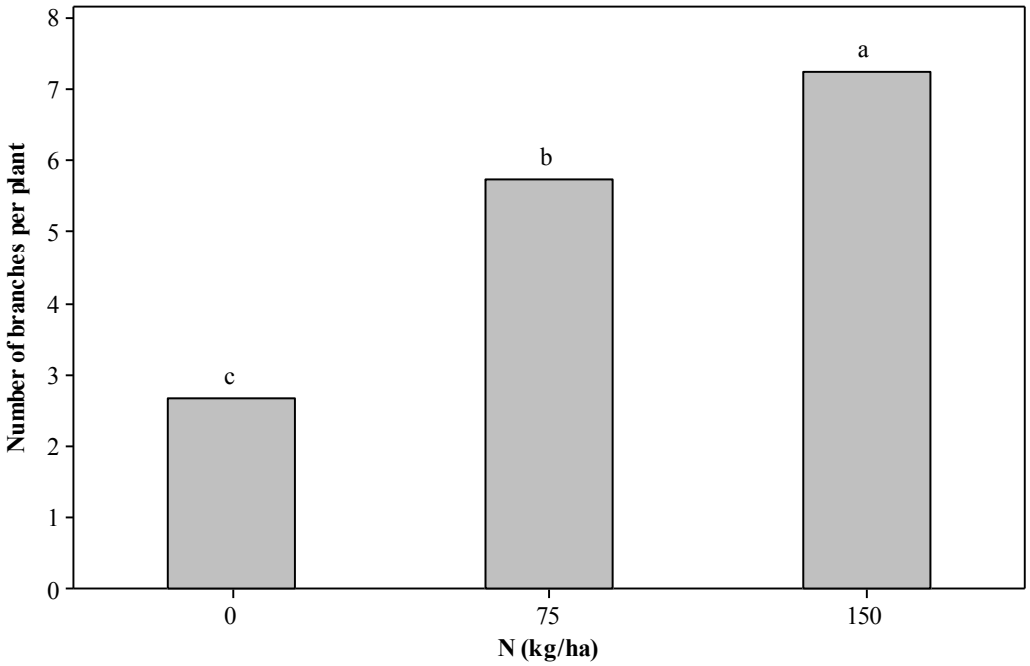


Figure 3.6: Effect of N on branches/plant (N and S experiment)
(Means with a common letter are not significantly different at the 5% level)

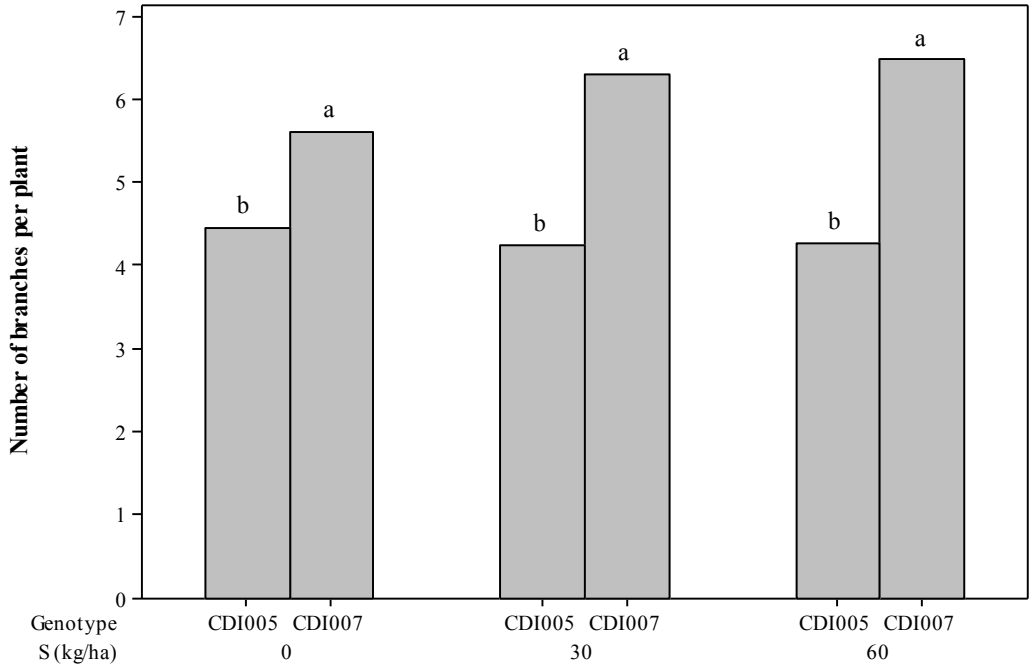


Figure 3.7: Effects of genotype and S on branches/plant (N and S experiment)
(Means with a common letter are not significantly different at the 5% level)

3.5.6 Number of Pods per Plant

N had a significant effect on number of pods per plant (Table 3.11). The number of pods increased with an increase of N (Figure 3.8 and Figure 3.9). S and/or B had no significant effect on the number of pods per plant (Table 3.11). The interaction of genotype and N was significant for the number of pods per plant. CDI007 had significantly more pods per plant than CDI005 when 75 kg N/ha application was applied (Figure 3.10). With the 0 and 150 kg/ha N application, CDI005 and CDI007 had similar numbers of pods per plant. In general, N application was positively correlated with the number of pods per plant (Figure 3.10).

Table 3.11: ANOVA table of effect of genotype, N, S and B on the number of pods per plant

	Factors	F-value	P-value	R-Sq
1	**N**	**157.18**	**<.0001**	98.13%
	S	**1.98**	**0.0158**	
2	B	1.18	0.325	35.88%
	S*B	1.85	0.15	
	Genotype (G)	**11.65**	**0.0013**	
	N	**637.8**	**<.0001**	
	S	0.27	0.766	
3	G*N	5.07	0.0097	
	G*S	0.93	0.4009	
	N*S	1.43	0.2358	
	G*N*S	1.23	0.311	

(S*B experiment: no transformation; G*N*S experiment: no transformation)

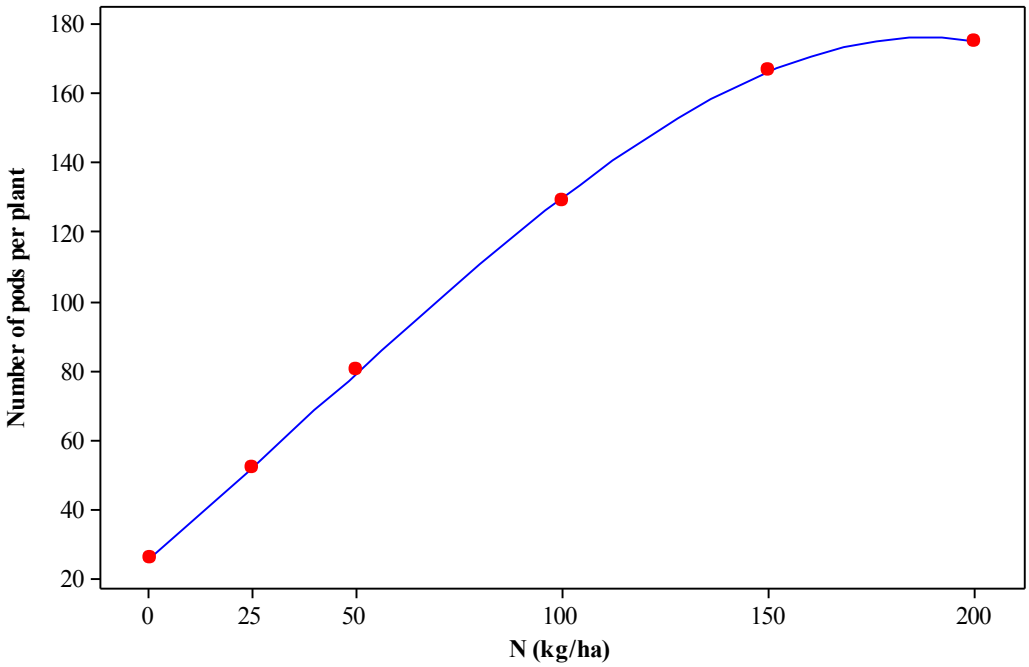


Figure 3.8: Regression analysis of N on pods/plant (N experiment)
(each dot represents mean of 4 samples)
Y(N)=26.13+0.9981N+0.002094N**2-0.000017N**3 with R-Sq(adj)=100.0%

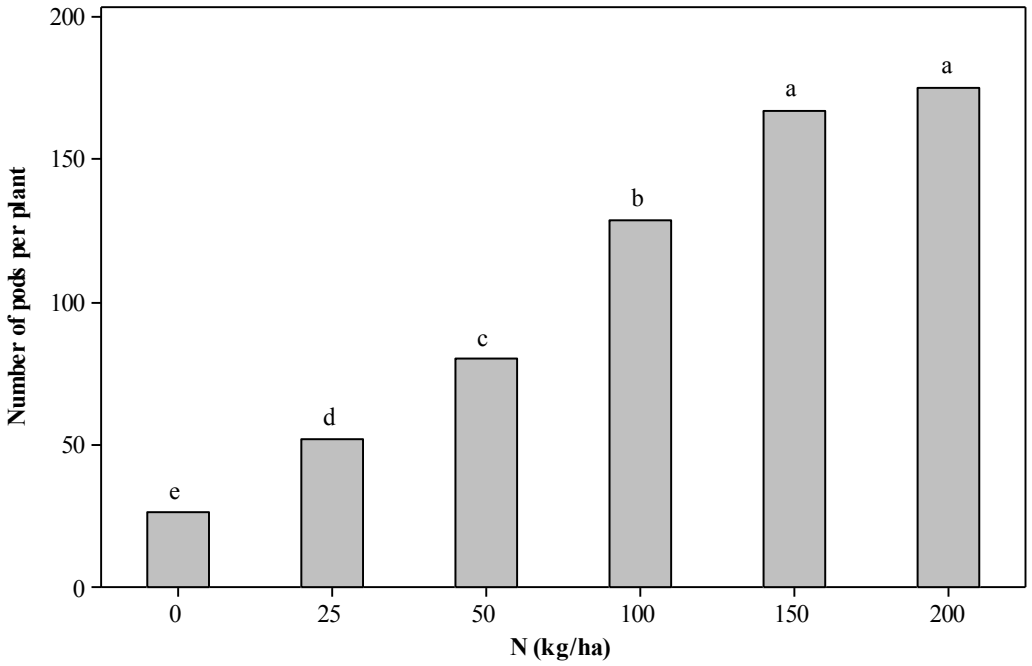


Figure 3.9: Effect of N on number of pods per plant (N experiment)
(Means with a common letter are not significantly different at the 5% level)

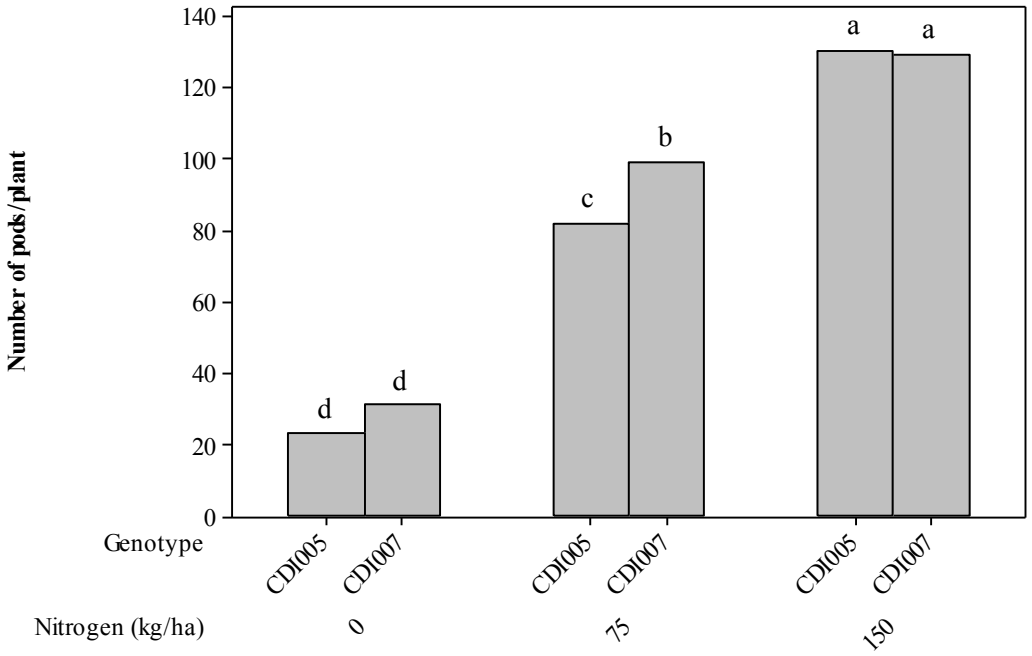


Figure 3.10: Interaction of genotype and N on pods/plant (N and S experiment)
(Means with a common letter are not significantly different at the 5% level)

3.5.7 Seed Yield

N had a significant effect on seed yield in the N experiment (Table 3.12). Seed yield increased with an increase in N (Figure 3.11 and Figure 3.12). S and/or B had no significant effect on seed yield (Table 3.12). In the N and S experiment, the interaction of genotype and N had a significant effect on seed yield. With the low and medium N application, the seed yield of CDI005 and CDI007 was very close (Figure 3.13). The seed yield of CDI005 was significantly higher than that of CDI007 with the high N application (150 kg/ha). N was positively correlated with the seed yield (Figure 3.13).

Table 3.12: ANOVA table of effect of genotype, N, S and B on seed yield per pot

	Factors	F-value	P-value	R-Sq
1	**N**	**295.74**	**<.0001**	99.20%
	S	2.71	0.088	
2	B	0.44	0.649	31.34%
	S*B	0.82	0.527	
	Genotype (G)	13.99	0.0004	
	N	996.43	<.0001	
	S	1.04	0.359	
3	**G*N**	**8.42**	**0.0007**	
	G*S	2.67	0.0786	
	N*S	1.2	0.3205	
	G*N*S	1.07	0.3791	

(N and S*B experiment: no transformation; G*N*S experiment: square root transformation)

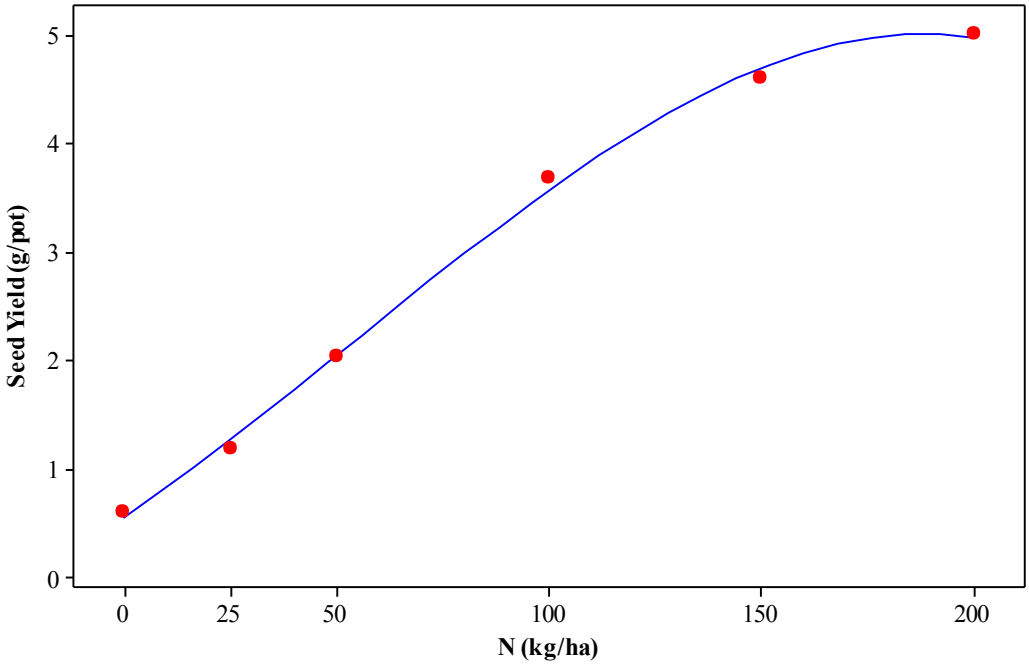


Figure 3.11: Regression analysis of N on seed yield (p/pot) (N experiment)
(each dot represents mean of 4 samples)
Y(N)=0.5530+0.02680N+0.000092N**2-0.000001N**3 with R-Sq(adj)=99.6%

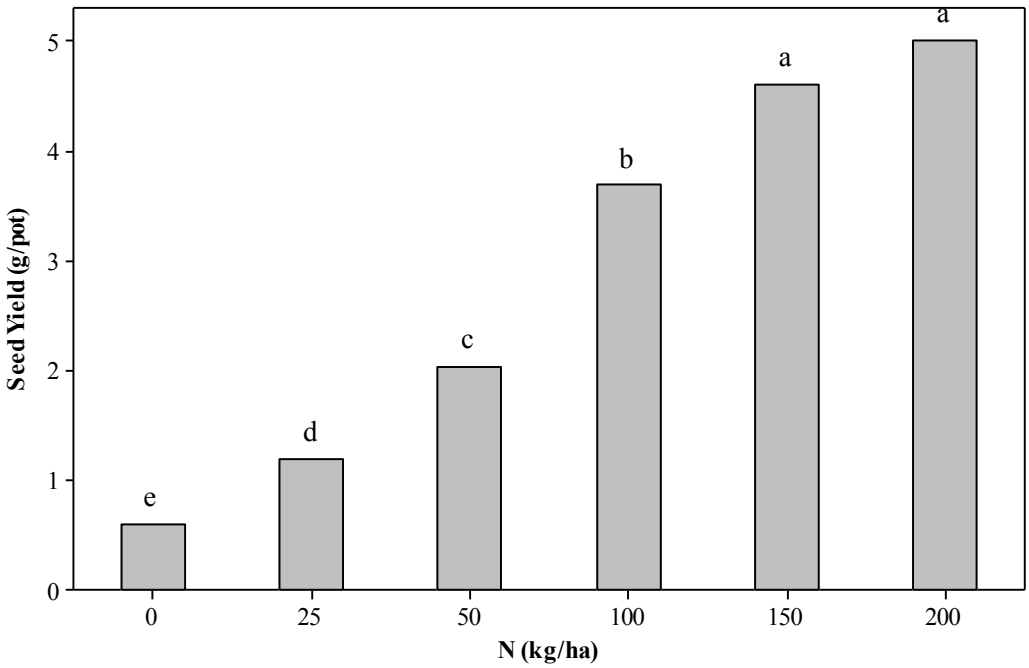


Figure 3.12: Effect of N on seed yield (N experiment)
(Means with a common letter are not significantly different at the 5% level)

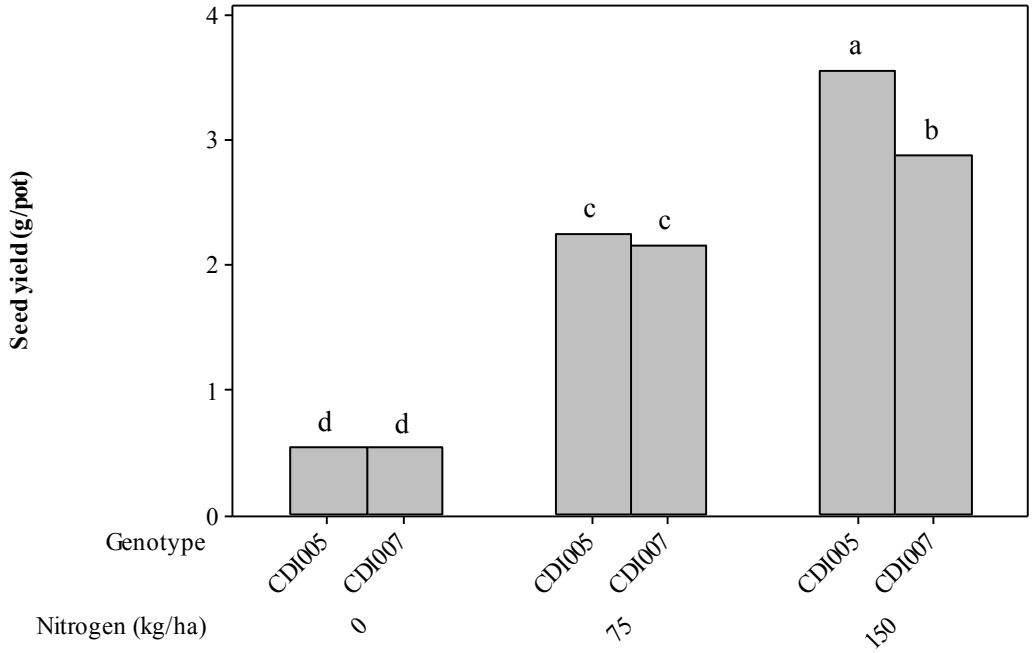


Figure 3.13: Effects of interaction of genotype and N on seed yield (N and S Experiment)
(Means with a common letter are not significantly different at the 5% level)

3.5.8 Thousand Seed Weight

N, S and/or B had no significant effect on the Thousand Seed Weight (Table 3.13). Genotypes behaved differently depending on the amounts of N and S. The 1000-seed weight of CDI007 was significantly higher than CDI005 (Figure 3.14). N was positively correlated with the 1000-seed weight of CDI007 and the 1000-seed weight of CDI005 with 150 kg N/ha application was significantly higher than that receiving 75 kg N/ha.

Table 3.13: ANOVA table of effect of genotype, N, S and B on 1000-seed weight

	Factors	F-value	P-value	R-Sq
1	N	1.79	0.168	34.55%
2	S	0.97	0.393	25.61%
	B	1.48	0.245	
	S*B	1.02	0.416	
3	Genotype (G)	136.91	<.0001	
	N	13.14	<.0001	
	S	0.47	0.6252	
	G*N	**3.39**	**0.0412**	
	G*S	**5.16**	**0.0089**	
	N*S	1.15	0.342	
	G*N*S	0.33	0.8539	

(N experiment: no transformation; S*B experiment: $\log_{10}$ transformation)

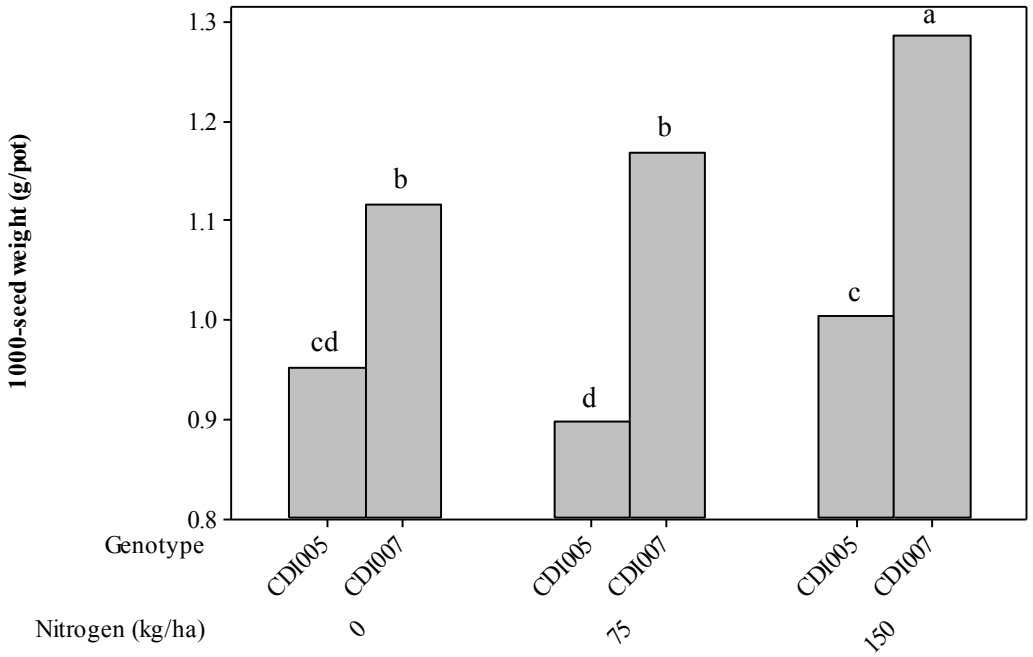


Figure 3.14: Interaction of genotype and N on the 1000-seed weight (N and S experiment)

(Means with a common letter are not significantly different at the 5% level)

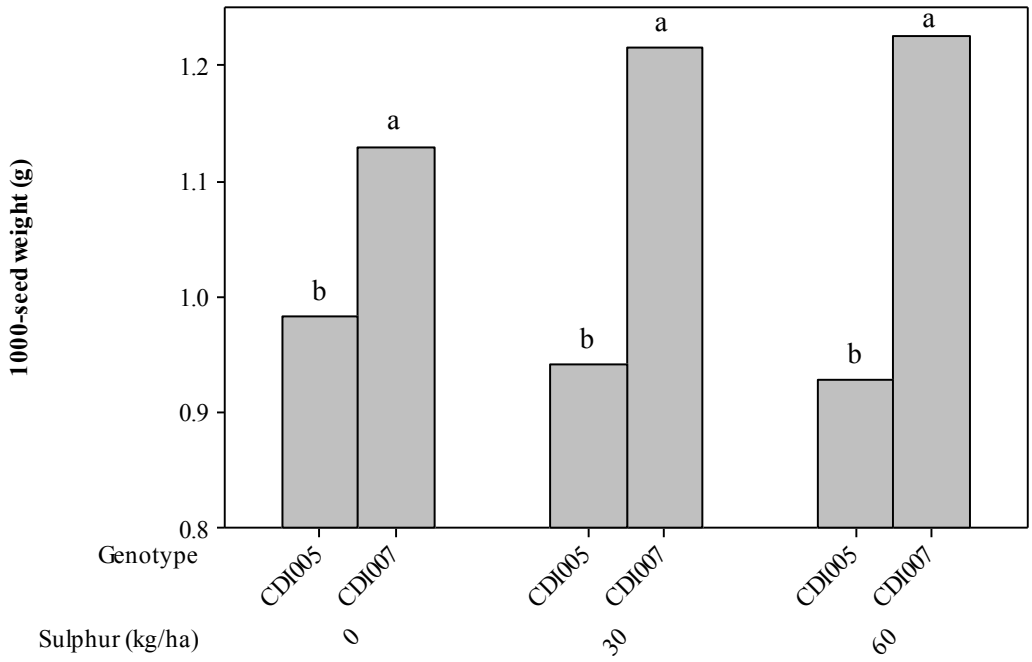


Figure 3.15: Interaction of genotype and S on the 1000-seed weight (N and S experiment)

(Means with a common letter are not significantly different at the 5% level)

3.5.9 Protein Content

N had a significant effect on the protein content (Table 3.14). The protein content increased with an increase in N (Figure 3.16 and Figure 3.17). The interaction of S and B had a significant effect on the protein content. With 0 and 25 kg/ha S application, B had no effects on the protein content (Figure 3.18). However, with 50 kg/ha S rate, the protein content from seeds with 2.5 kg B/ha application was significantly lower than that from seeds with 0 and 5.0 kg B/ha rate.

Table 3.14: ANOVA table of effect of fertilizer on the content of protein (%)

	Factors	F-value	P-value	R-Sq
1	**N**	**13.97**	**<0.0001**	80.42%
	S	0.84	0.441	
2	B	3.71	0.038	44.47%
	S*B	**2.87**	**0.043**	

(S*B experiment: no transformation)

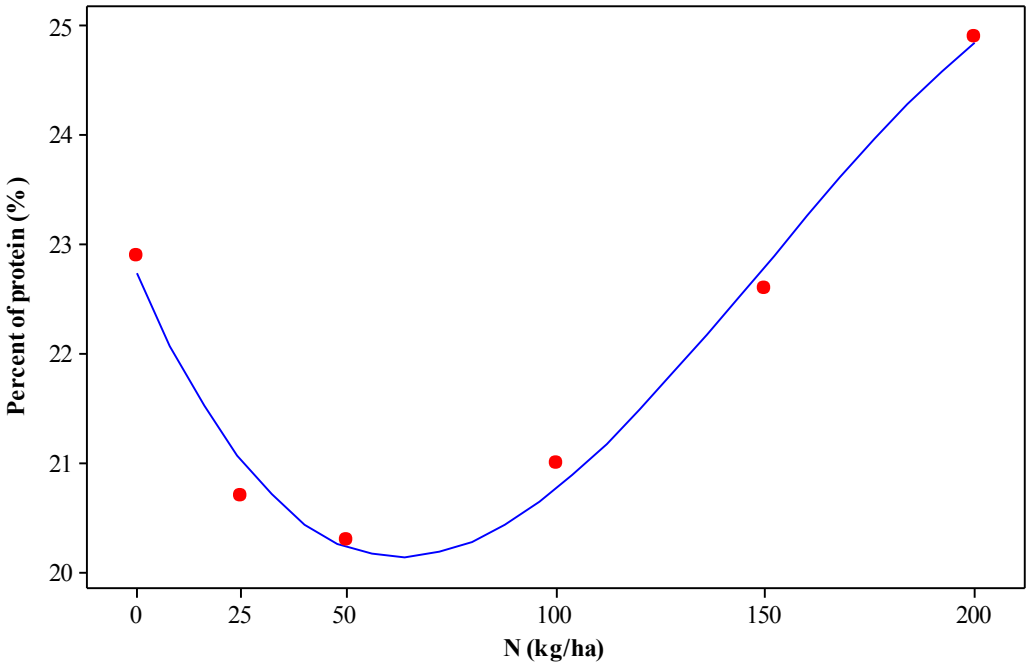


Figure 3.16: Regression analysis of N on protein content (N Experiment)
(each dot represents mean of 4 samples)
Y(N)=22.75-0.09087N+0.000914N**2-0.000002N**3 with R-Sq(adj)=96.2%

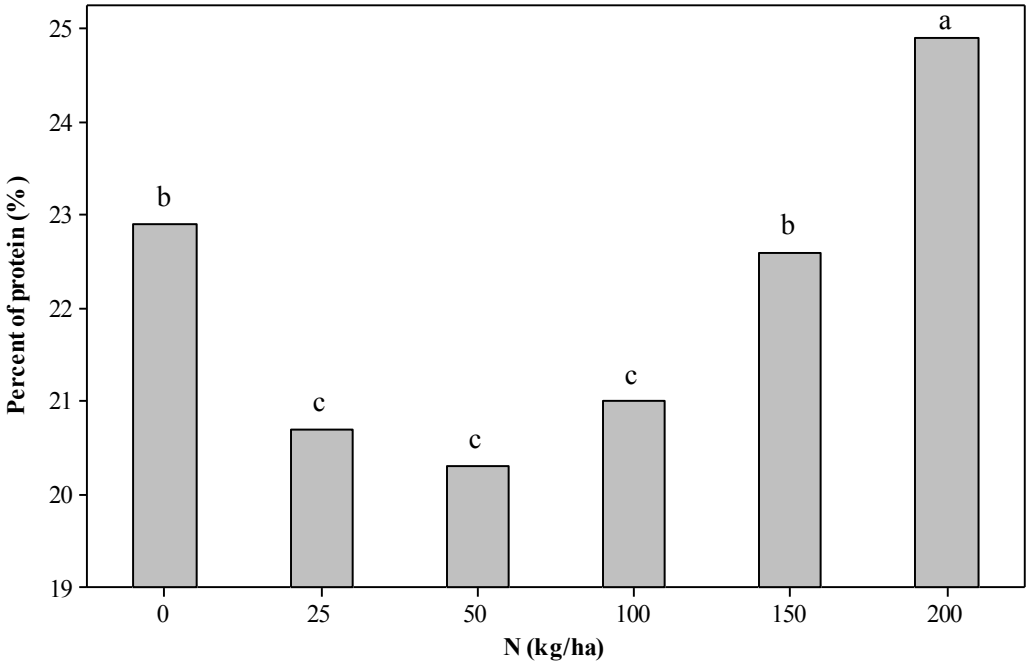


Figure 3.17: Effect of N on the percent of protein % (N experiment)
(Means with a common letter are not significantly different at the 5% level)

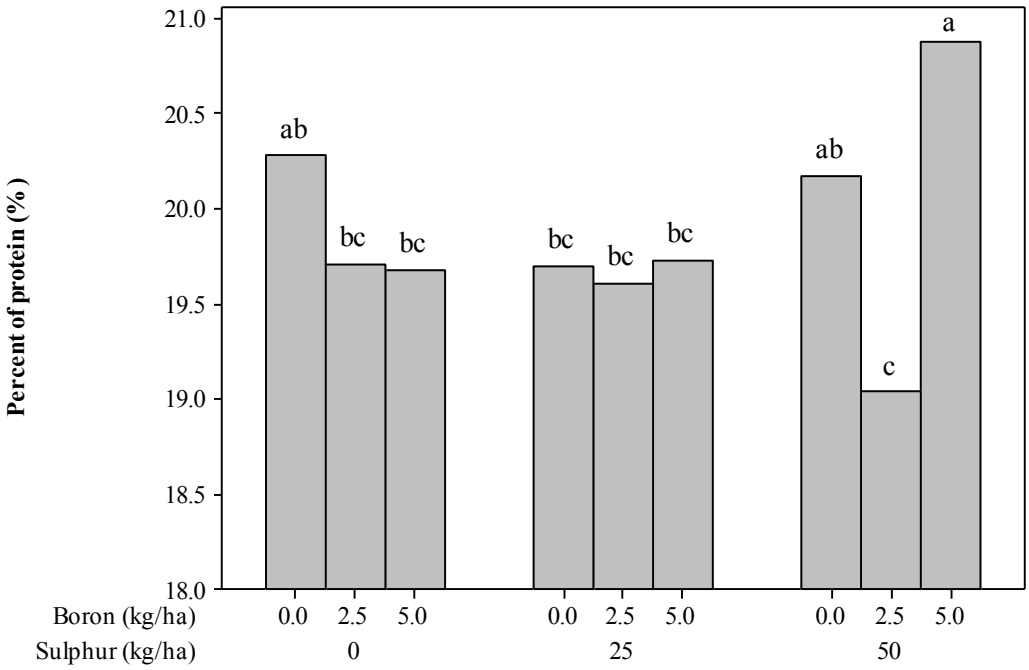


Figure 3.18: Effect of S and B on content of protein (S and B Experiment)
(Means with a common letter are not significantly different at the 5% level)

3.5.10 Oil Content

N had a significant effect on the oil content (Table 3.15). The oil content decreased with an increase in N (Figure 3.19 and Figure 3.20). The oil content of treatments with 200 kg N/ha application was significantly lower than those that received less N (Figure 3.20). S and/or B had no significant effect on the oil content (Table 3.15).

Table 3.15: ANOVA table of effect of fertilizer on the content of oil (%)

	Factors	F-value	P-value	R-Sq
1	**N**	**11.31**	**<0.0001**	79.03%
	S	0.01	0.993	
2	B	0.35	0.706	11.29%
	S*B	0.65	0.632	

(S*B experiment: no transformation)

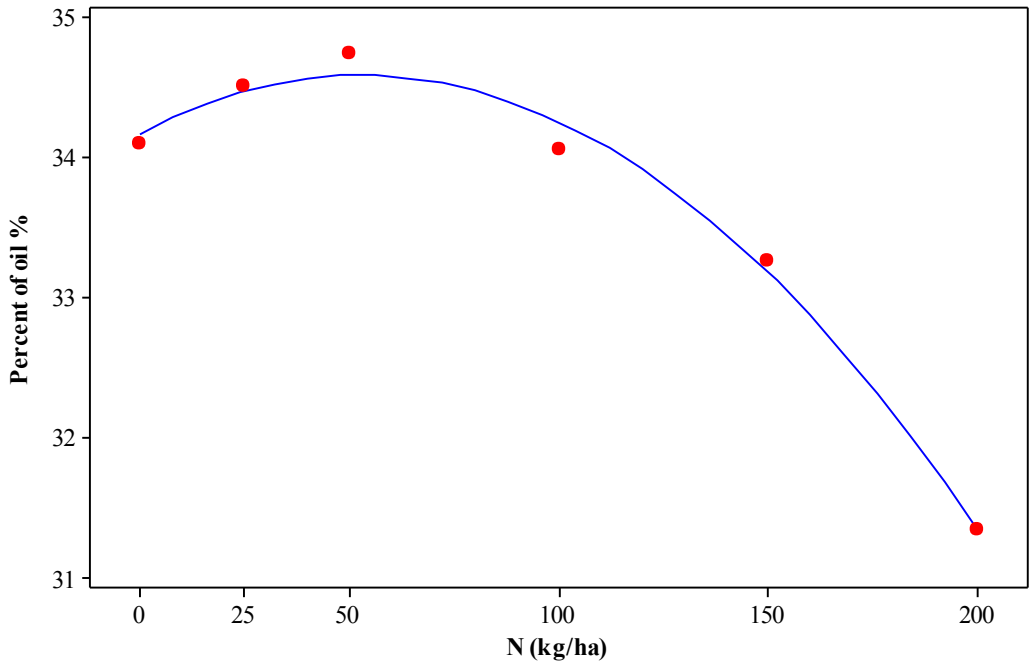


Figure 3.19: Regression analysis of effect of N on oil content (N Experiment)
(each dot represents mean of 4 samples)
Y(N)=34.17+0.01581N-0.000149N**2 with R-Sq(adj)=98.4%

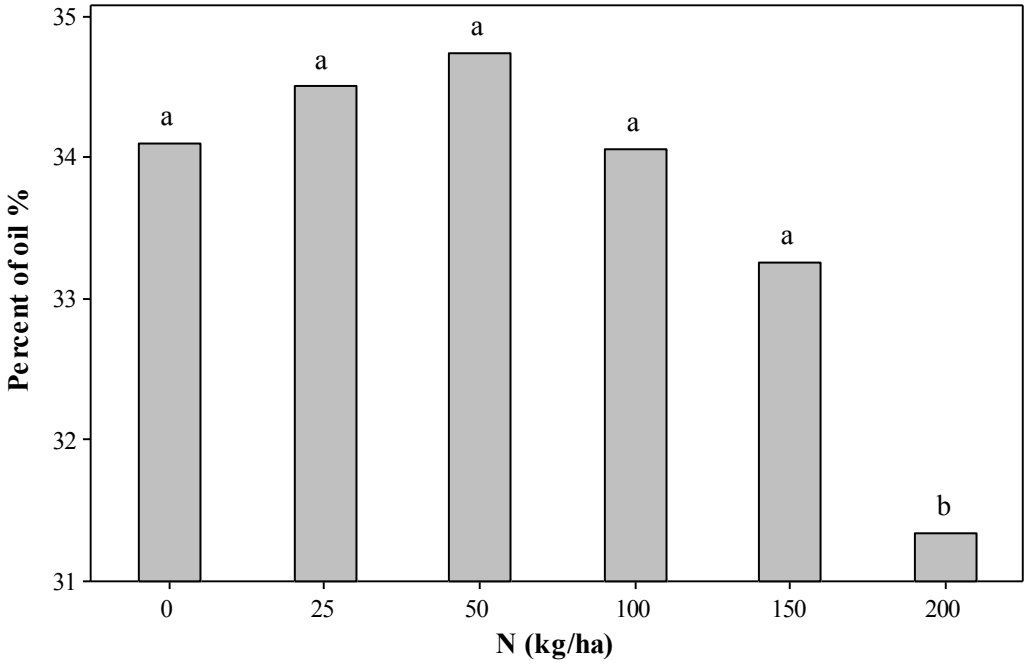


Figure 3.20: Effect of N on the percent of oil % (N experiment)
(Means with a common letter are not significantly different at the 5% level)

3.5.11 Fatty Acid Profile

Camelina seed consists of 10 main fatty acids – C16:0, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, C20:2, C20:3 and C22:1. They were grouped into 3 categories – saturated fatty acids (C16:0, C18:0 and C20:0), monounsaturated fatty acids (C18:1, C20:1 and C22:1), and polyunsaturated fatty acids (C18:2, C18:3, C20:2 and C20:3).

Genotypes differed in the percent of saturated fatty acids (Table 3.16). Genotypes differed in the percent of monounsaturated and polyunsaturated fatty acids depending on the amount of N and S (Table 3.16). S had a significant effect on the percent of polyunsaturated fatty acids in the S and B experiment (Table 3.16).

S increased the percent of polyunsaturated fatty acids (Figure 3.21). CDI005 had significantly higher content of saturated fatty acids than CDI007 (Table 3.17).

CDI005 had significantly more monounsaturated fatty acids than CDI007 (Figure 3.22). With 0 kg S/ha and 60 kg S/ha, the percent of monounsaturated fatty acids of CDI007 decreased with an increase in N, while with 30 kg S/ha, N had no effect on the percent of monounsaturated fatty acids. With 30 kg S/ha and 60 kg S/ha, N had no effect on the percent of monounsaturated fatty acids of CDI005 (Figure 3.22), while without S application, high N decreased the content of monounsaturated fatty acids of CDI005.

The percent of polyunsaturated fatty acids of CDI007 was significantly higher than CDI005 (Figure 3.23). With 0 and 60 kg S/ha application, the percent of polyunsaturated fatty acids of CDI007 increased with an increase in N, while with 30 kg S/ha, N had no effect on the percent of polyunsaturated fatty acids (Figure 3.23). Neither N nor S had effect on the percent of polyunsaturated fatty acids of CDI005 (Figure 3.23).

Table 3.16: ANOVA table of fertilizers on fatty acid composition

Effect	Saturated FA		Monounsaturated FA		Polyunsaturated FA	
	F value	P value	F value	P value	F value	P value
N	2.08	0.1153	1.6	0.2113	1.39	0.2739
S	0.86	0.4347	3.12	0.0609	**3.6**	**0.0417**
B	0.09	0.9098	0.07	0.9347	0.08	0.9252
S*B	0.71	0.5947	1.17	0.3456	1.48	0.238
Genotype (G)	**17.3**	**0.0001**	193.44	<.0001	171.51	<.0001
N	3.02	0.0573	18.22	<.0001	7.12	0.0018
G*N	0.36	0.6992	9.13	0.0004	6.54	0.0029
S	0.21	0.8141	0.01	0.9939	0.29	0.7507
G*S	0.55	0.58	2.59	0.0844	0.63	0.5344
N*S	1.65	0.1752	2.93	0.029	1.38	0.2537
G*N*S	0.71	0.5887	**2.63**	**0.0445**	**3.3**	**0.0174**

(no transformation)

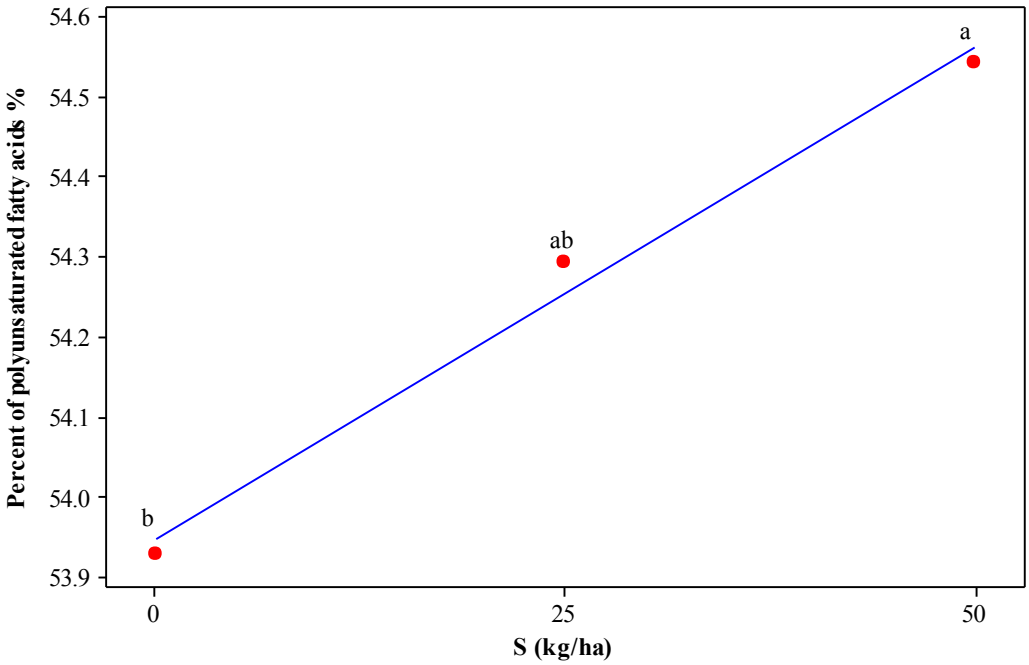


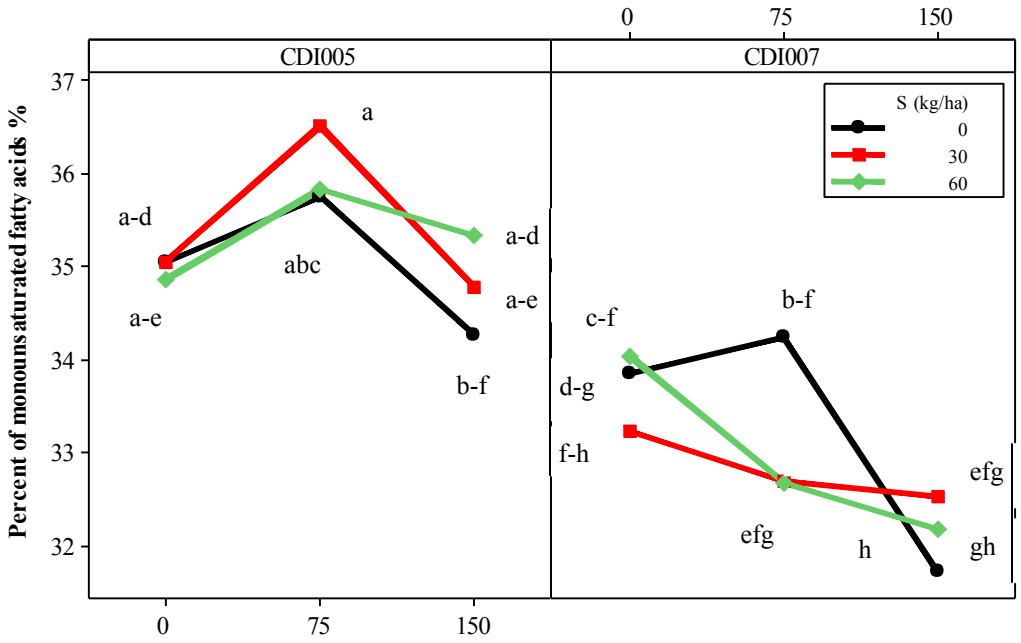
Figure 3.21: Effect of S on the percent of polyunsaturated fatty acids % (S and B experiment)

(Means with a common letter are not significantly different at the 5% level)

Table 3.17: Effect of genotype on percent of saturated fatty acids (N and S experiment)

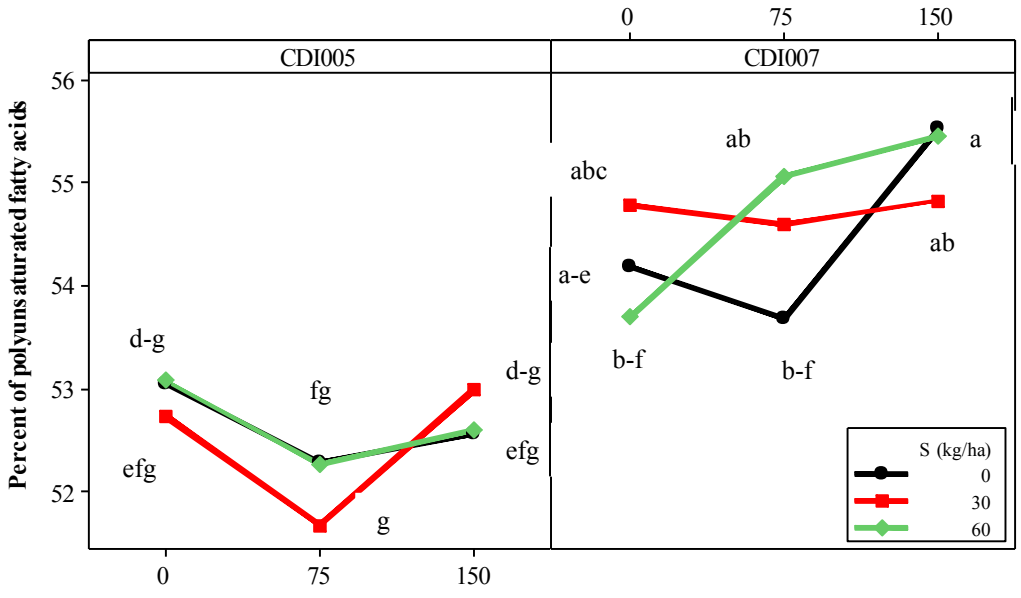
Genotype	Percent of saturated fatty acids %
CDI005	10.5 a
CDI007	10.3 b

(Means with a common letter are not significantly different at the 5% level)



Panel variable: Genotype

Figure 3.22: Effect of interaction of genotype, N and S on the percent of monounsaturated fatty acids (N and S experiment)
(Means with a common letter are not significantly different at the 5% level)



Panel variable: Genotype

Figure 3.23: Effect of interaction of genotype, N and S on the percent of polyunsaturated fatty acids (N and S Experiment)
(Means with a common letter are not significantly different at the 5% level)

3.6 Discussion

3.6.1 Comparison between CDI007 and CDI005

In the N*S*Genotype experiment, CDI005 had more favorable agronomic traits such as high seed yield when compared to CDI007. This is contradictory with the results from the previous greenhouse or field experiments (Chapter 4 – 2011 & 2012 N field trials and Chapter 5 – 2012 S & N field trials). In this growth chamber experiment, CDI005 had significantly higher seed yield than CDI007, and the canopy height of CDI005 was also higher than CDI007. However, CDI007 had many more branches and pods per plant than CDI005. This was probably due to CDI007 not having sufficient space for light and root growth in the growth chamber. Figure 3.24 and Figure 3.25 compare the plant canopy of CDI007 and CDI005 that both received the same N and S fertilizer. CDI007 had many more branches, but branches near the soil surface produced fewer pods. These branches also absorbed and used nutrients from soil, but failed to grow pods due to limited root system in the pots and the competition of light.

Figure 3.24: CDI007 at maturity
Figure 3.25: CDI005 at maturity

3.6.2 Thousand-seed Weight

In the N experiment and the S*B experiment, the fertilizers had no significant effect on the Thousand-seed weight. In the N*S*Genotype experiment, Thousand-seed weight of CDI007 was significantly higher than CDI005. Seed size of camelina is considered to be highly heritable (Marquard and Kuhlmann, 1986 in Losak et al., 2011; Vollmann et al., 1996). In N*S*genotype experiment, N was positively correlated with the seed size, which was consistent with the previous work by Losak et al. (2011). However, Marquard and Kuhlmann (1986) found N did not have an impact on camelina seed size. Similarly, the 1000-seed weight did not change when N increased from 0 to 120 kg/ha (Agegnehu and Honermeier, 1997). It has been reported that the 1000-seed weight ranged from 0.77 to 1.24g among 130 camelina accessions (Vollmann et al., 2005). Zubr (1997) found the 1000-seed weight averaged between 0.8 to 1.8g depending on genotype, growth conditions and nutrients.

3.6.3 Effects of S Application

In N*S*Genotype experiment, S had significant effects on the leaf area, the number of branches per plant, 1000-seed weight, and fatty acid profile, which was not consistent with the previous experiments by other researchers (Losak et al., 2011; Sipalova et al., 2011). It has been reported that effects of S on the main agronomic parameters of camelina were not significant, and these parameters included the seed yield, plant height, and the number of branches/pods per plant (Losak et al., 2011; Sipalova et al., 2011). The number of branches per plant increased from 10 to 16 with N application but with no effect of S, and plants had the highest 1000-seed weight at the highest N

application (Losak et al., 2011). Differences with different S treatments were not significant statistically (Losak et al., 2011). The only positive effect of S on the seed yield was found between treatments with no S and 75 kg S/ha with 95.5 kg N/ha (Losak et al., 2011). According to the experiment of combined N and S on the fatty acid composition of camelina, S had no significant effects on the total content of oil (Sipalova et al., 2011). However, S had significant effects on the specific fatty acids such as palmitic (C16:0), oleic (C18:1), arachidic (C20:0) and 11, 13 icosadienoic acid (C20:0) (Sipalova et al., 2011). For example, the amount of palmitic acid increased from 6.9% with low S to 11% with medium S application (Sipalova et al., 2011). With three levels of S application, camelina plants had similar growth performance (Figure 3.26). In the S*B experiment, the interaction of the two factors had a significant effect on the protein content; other than that, S and/or B had no significant effect on other agronomic parameters. It was interesting to note that the content of C18:3 increased with S rates in the S*B experiment, but decreased with S application in the N*S*Genotype experiment. Therefore, more samples with different S rates are recommended to analyze to confirm the relationship between C18:3 and S application.

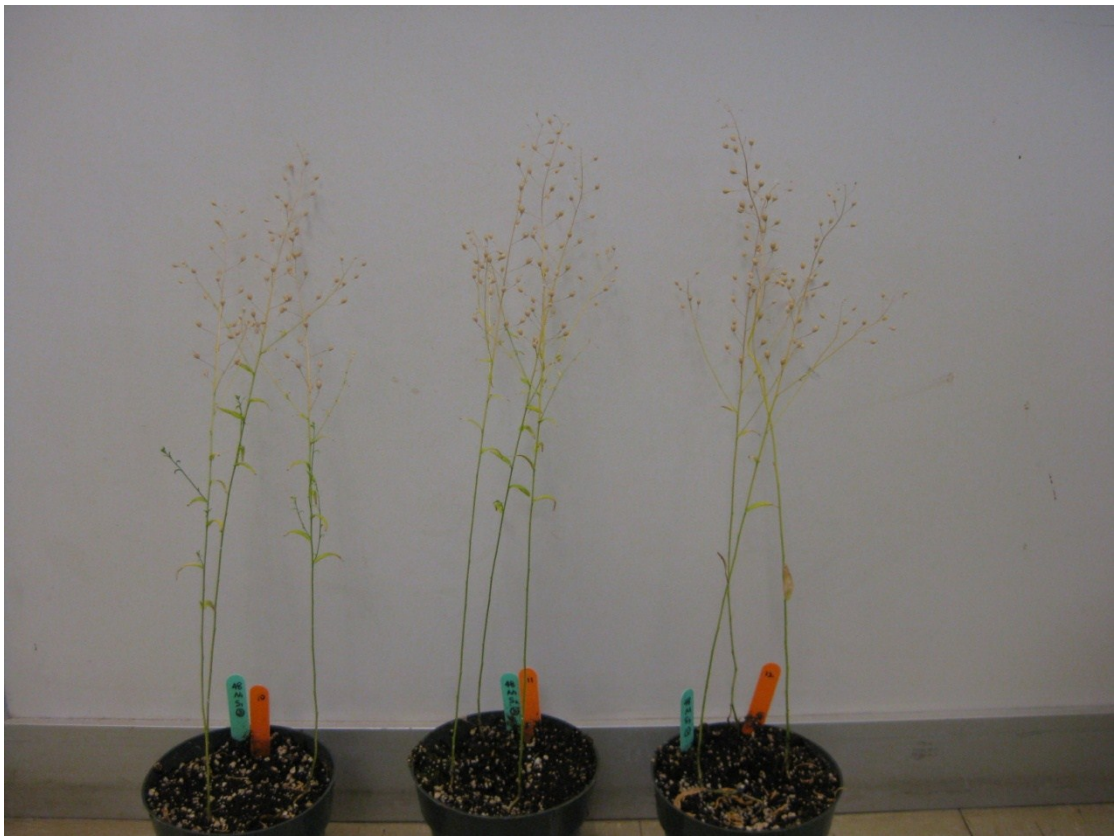


Figure 3.26: Camelina with three levels of S application (68 days after seeding)
(1) CDI007-N: 0kg/ha-S: 0kg/ha
(2) CDI007-N: 0kg/ha-S: 30kg/ha
(3) CDI007-N: 0kg/ha-S: 60kg/ha

3.6.4 Effects of N Application

In general, N increased the number of branches and pods per plant and seed yield per pot of both two genotypes of camelina. These results were consistent with the previous research work (Pan, 2009; Urbaniak et al., 2008a). From N*S*Genotype experiment, plants with medium N application (75 kg/ha) were taller than plants with high N application and without N application. Plants under N deficiency were slow growing and stunted, so plants without an N supply were shorter than plants that received N application. In terms of high N (150 kg/ha) application, plant height was lower than plants with 75 kg N/ha application, while plants had more branches when receiving 150 kg/ha N. N was used to develop more branches to compensate the lower plant height at

the high N application. From N experiment, N application had a positive correlation with protein content but a negative correlation with oil content. N had significant effects on the fatty acid composition.

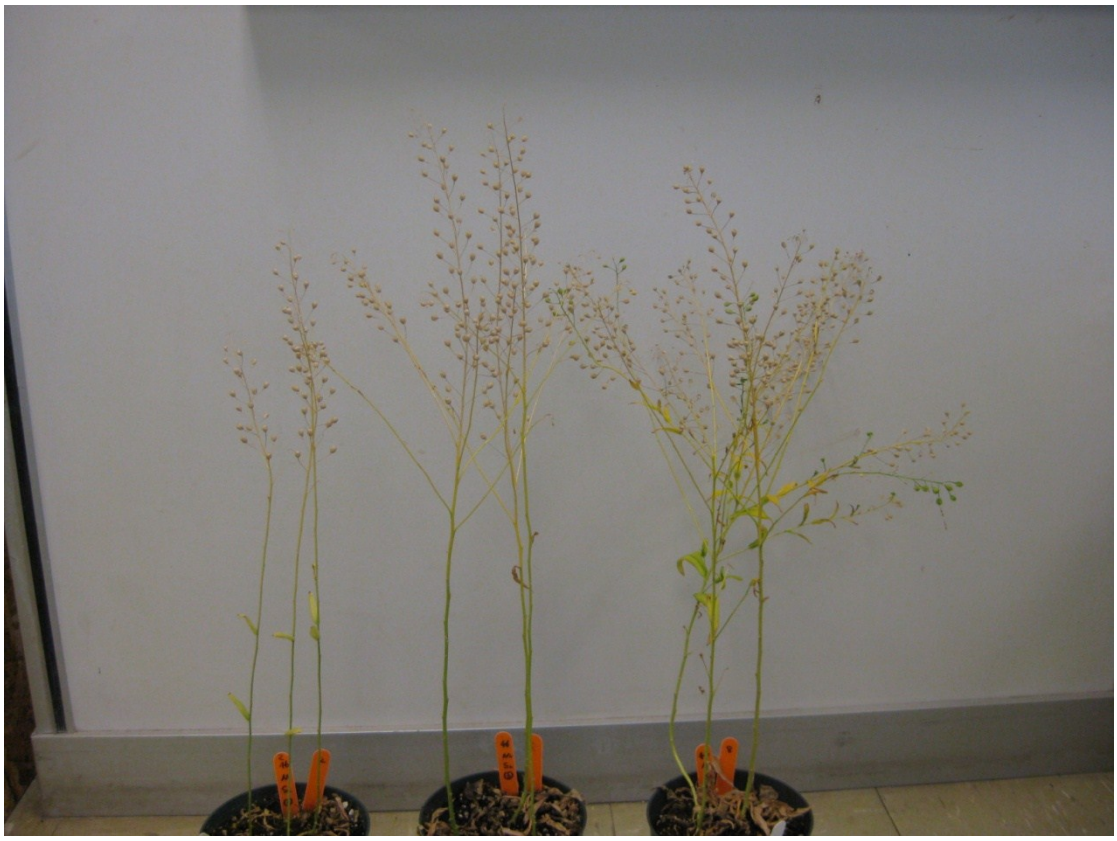


Figure 3.27: camelina with three levels of N application (68 days after seeding)
(1) CDI005-N: 0kg/ha-S: 30kg/ha
(2) CDI005-N: 75kg/ha-S: 30kg/ha
(3) CDI005-N: 150kg/ha-S: 30kg/ha

3.6.5 Fatty Acids

Differences between genotypes and fatty acids suggest camelina fatty acid profile was due to genetic makeup. CDI005 significantly had more saturated fatty acids but less polyunsaturated fatty acids than CDI007. CDI007 are more suitable for human consumption, because diets which are rich in α-linolenic acid (polyunsaturated) are beneficial for the prevention of coronary events and cardiac deaths (Zubr, 2003a). N effects on camelina fatty acid composition were not observed in the growth chamber

experiment. S increased the content of polyunsaturated fatty acids, which was also observed at all four tested field trials in 2012 (Chapter 5). Effect of N and S on camelina fatty acid profile was inconsistent and not obvious. Therefore, more fatty acid analyses of samples from field studies were recommended.

3.7 Conclusion

N, S and genotype proved to have the most important factors affecting plant height, seed yield and TKW (Thousand Seed Weight). All the experimental factors including N, S and genotype had significant effects on the fatty acid profile. The effects of B and S on the agronomic traits were negligible, but the interaction of S and B had a significant effect on the protein content and S had an effect on the fatty acid composition. It is difficult to determine whether the effect of S on the fatty acid profile of camelina is typical or not in this study. More studies of effects of S and N on the fatty acid profile are recommended. The N rate equivalent to 150 kg/ha is recommended for growing camelina in controlled environment conditions.

Chapter 4: Effects of Genotype and Nitrogen on Growth and Seed Quality of *Camelina sativa* L. Crantz at Five Canadian Locations in 2011 and 2012

4.1 Introduction

Previous studies have shown that the optimum N input for camelina is 100-125 kg N/ha with a yield that ranged from 800-2500 kg/ha in Nova Scotia (MacDonald and Li, 2010). Depending on the soil fertility, residual level of nutrients and weather conditions, the optimum N supply was found to be 100 kg/ha in Denmark (Zubr, 1997). According to Agegnehu and Honermeier (1997), the yield of camelina of all treatments ranged from 1160 kg/ha in 1994 to 1800 kg/ha in 1995 in Germany. The maximum yield of all treatments was 2280 kg/ha (120 kg N/ha, 400 seeds/m^2). Under N deficiency, camelina plants are thin, very upright and the leaves are small and pale yellow-green. Ripening is premature and there are few pods and seed bearing branches (Agegnehu and Honermeier, 1997).

According to Urbaniak et al. (2008a), the yield of camelina was enhanced by increasing N, but the increase was not obvious when the application of N exceeded 60 kg N/ha in NS and 80 kg N/ha in PEI. Also, oil content decreased with higher N application. Almost all the fatty acids, except for erucic acid in camelina, increased or decreased in response to the different levels of N. Plant height and total N content in plant tissue and seed protein content increased with the increase of N (Urbaniak et al., 2008). Urbaniak et al. (2008a) demonstrated camelina cultivar selection and applied N levels are important factors in obtaining optimum yield.

Crowley and Frohlich (1998) found that camelina yields reached the highest at 75 kg/ha N. However, the incidence of plant disease (Botrytis) increased with increased N rates but no obvious oil content changes were observed. It was found that N levels of 100

kg/ha led to yield increases of 58% of camelina compared to plants without N application, while slight decreases in oil content resulted from increases in N in Romania (Zubr, 2003). Research done by Agegnehu and Honermeier (1997) showed that increasing N levels from 60 to 130 kg N/ha resulted in a 30% yield increase and a dramatic decline in oil concentration.

These studies suggest that there are many inconclusive and somewhat contradictory results in references to N application in camelina production in different areas in the world. A location effect on camelina yield was reported by Pan et al. (2009) in Canada (NS, PEI and SK), Urbaniak (2006) in Canada (NS and PEI) and Putnam et al. (1991) in the United States. The objective of this study was to evaluate the effects of N on the growth, seed yield and seed quality of four advanced lines from the breeding program at AAFC Saskatoon and a standard check cultivar, Calena.

The hypothesis of this study was that different genotypes of camelina would differ in the growth and seed quality depending on the amount of N. In this study, the effects of individual and interactive effects of N and genotype were tested at Canning (NS) and Truro (NS) in 2011 and in five contrasting environments (Truro, Canning, Fredericton, New Glasgow and Saskatoon) in 2012. The evaluated parameters included plant stand, percent of plants with downy mildew, days to maturity, plant height, number of branches per plant, number of pods per plant, number of pods per m^2, number of plants per m^2 at harvest, seed yield, and seed oil and protein content.

4.2 Methods and Materials

Sites at Canning (lat. 45.16^{o}N; long. 64.43^{o}W), NS (Lyndhurst Farms) and Truro (lat. 45.36^{o}N; long. 63.28^{o}W), NS (Dal-AC) were selected for this study in 2011.

Whereas Canning, NS (Lyndhurst Farms), Truro, NS (Dal-AC), New Glasgow (lat. 46.41°N; long. 63.35°W), PEI (Technology Crops), Fredericton (lat. 45.96°N; long. 66.63°W), NB (AAFC) and Saskatoon (lat. 52.13°N; long. 106.64°W), SK (ICMS - Integrated Crop Management Services) were used in 2012. Five strains of camelina (CDI002, CDI005, CDI007, CDI008 and one check Calena) were sown with a seeding rate of 500 seeds/m^2. The four lines were numbered lines from the breeding program of Dr. Kevin Falk of AAFC Saskatoon (CDI002, CDI005, CDI007 and CDI008). Seeds were planted with row spacing 15 cm and seed depth 0.5-1.0 cm with a Hege plot drill (H and N Equipment Inc., Colwich, Kansas, USA) with press wheels and double disc openers at NS and PEI. ICMS (SK) used a double-disk press drill (made by ICMS, SK, Canada) with a belt driven cone. AAFC at Fredericton used a Brillion forage type of seeder. Crops were seeded in plots 5 m in length and 2.5 m wide at Canning and Truro in 2011 and 2012, at New Glasgow in 2012. Crops were seeded in plots 6 m in length and 1.25 m wide for one plot at Fredericton, 7 m in length and 2.5 m wide at Saskatoon in 2012.

Seeds were sown as a mixture of viable and dead seeds at a ratio of 2:3. The addition of dead seeds for each plot allowed for better distribution. Dead seeds were made by autoclaving and checked afterwards to ensure they were not viable. N inputs (Ammonium nitrate) were 0, 25, 50, 100, 150 and 200 kg N/ha. N application was split 50:50 for the high N treatments including 100, 150 and 200 kg N/ha. The first half of N fertilizer was applied one week within seed germination (N rates 25 and 50 applied all at first application) and the rest was applied at the beginning of flowering. In addition, 30-40 kg/ha P & K plus 20 kg/ha S was applied preplant; the S source was K-Mg (0-0-21-

22% S-10% Mg). The pre-plant herbicide trifluralin (active ingredient) was applied at a rate of 1.1 kg a.i./ha. There were four replications and thirty treatments arranged in a randomized complete block design (RCBD).

Table 4.1: Previous crops at these five sites

Year	2010	2011
Canning (NS)	spring wheat	winter wheat
Truro (NS)	soybean	soybean
New Glasgow (PEI)	barley	winter wheat
Fredericton (NB)		Timothy/redclover
Saskatoon (SK)	barley	Chem fallow

Table 4.2: Seeding, fertilizer and harvest dates in 2011 and 2012

Year	Location	Seeding date	First timing fertilizer	Second timing Fertilizer	Harvest date
2011	Canning (NS)	4-May-12	19-May-11	21-Jun-11	11-Aug-11
	Truro (NS)	14-May-12	26-May-11	29-Jun-11	29-Aug-11
2012	Canning (NS)	1-May-12	4-May-12	26-Jun-12	22-Aug-12
	Truro (NS)	7-May-12	14-May-12	3-Jul-12	24-Aug-12
	New Glasgow (PEI)	8-May-12	8-May-12	26-Jun-12	30-Aug-12
	Fredericton (NB)	6-Jun-12	18-Jun-12	11-Jul-12	4-Sep-12
	Saskatoon (SK)	16-May-12	18-May-12	11-Jul-12	13-Sep-12

Table 4.3: Soil characteristics of camelina N trials in 2011 and 2012

Year	Location	Organic Matter (%)	pH	CEC (meq/100g)	P_2O_5 (kg/ha)	K_2O (kg/ha)
2011	Canning	3	6.3	12.3	2507	355
	Truro	3.1	6	12.5	2245	500
2012	Canning	3.1	6.3	13.6	2455	336
	Fredericton					
	New Glasgow	3.1	5.9	9	211	153
	Truro	3	6.1	11.2	627	239

Year	Location	Ca (kg/ha)	Mg (kg/ha)	S (kg/ha)	B (ppm)	Cu (ppm)
2011	Canning	3711	205	31	<=0.50	25.64
	Truro	2897	288	26	<=0.50	2.11
2012	Canning	3734	263	29	<=0.50	16.92
	Fredericton					
	New Glasgow	680	68	18	0.4	0.6
	Truro	3098	449	23	<=0.50	0.7

Year	Location	Zn (ppm)	Mn (ppm)	Fe (ppm)	Na (kg/ha)	Al (ppm)
2011	Canning	6.2	41	215	27	1484.53
	Truro	9.2	103	211	24	1600.9
2012	Canning	8.6	41	176	28	1459.39
	Fredericton					
	New Glasgow	0.9	36	230	13	1485
	Truro	1.9	95	315	52	889.2

Table 4.4: Weather summary at the tested sites in 2011 and 2012

Year, Location	Month	Total Precip (mm)	Total GDD	Mean Temp (^{o}C)
2011, Canning, NS	May	159.6	213.6	11.9
	June	141.7	296.4	14.9
	July	124.0	443.5	19.3
	August	162.6	426.9	18.8
2011, Truro, NS	May	88.0	194.0	11.3
	June	92.0	260.0	13.7
	July	118.8	427.7	18.8
	August	139.6	428.2	18.8
2012, Canning, NS	May	38.3	242.7	13.0
	June	100.5	312.4	15.4
	July	36.8	483.6	20.3
	August	47.9	474.7	20.6
2012, Truro, NS	May	105.3	206.6	11.7
	June	124.7	281.5	14.4
	July	92.8	431.5	18.9
	August	262.0	468.6	20.1
2012, New Glasgow, PE	May	62.4	187.3	11.1
	June	61.5	276.0	14.2
	July	33.6	463.3	20.0
	August	69.6	496.8	21.0
2012, Fredericton, NB	June	91.5	319.7	16.1
	July	29.8	445.9	20.4
	August	63.8	446.5	20.2
	September	149.6	256.8	14.2

Data were from http://www.climate.weatheroffice.gc.ca

4.3 Data Collection

(1) Stand Counts and Downy Mildew Observation in June

Stand counts were done on June 6th, 2011 at Canning and June 8th, 2011 at Truro; May 30, 2012 at Canning, May 31 at Truro, June 13, 2012 at New Glasgow, and June 18, 2012 at Fredericton; data at Saskatoon were not collected. Plants reached 12-14 leaf

Stage on June 6th, 2011 at Canning and 8-10 leaf Stage on June 8th, 2011 at Truro. Two subsamples with three rows of plants (0.225m^2) from each plot were chosen randomly by using a quadrat (Square, 0.5m*0.5m), avoiding the outside rows. The number of plants in the specific area and the number of plants which were infected with downy mildew (caused by *Peronospora parasitica*) were counted at Truro and Canning in 2011. The infection with downy mildew was determined by visual observation of symptoms (Figure 4.1).

Figure 4.1: Camelina downy mildew at the vegatative stage (Canning, June 2011)

(2) Percent of Downy Mildew at the Reproductive Stage

The percentage of plants with downy mildew was determined on July 19, 2011 (77 days after seeding) and July 4, 2012 (61 days after seeding) at Canning and on July 20, 2011 (68 days after seeding) and July 6, 2012 (53 days after seeding) at Truro. Plants were at the reproductive stage at both locations. Two subsamples with two rows * 0.5 metre of plants from each plot were chosen randomly by using a metre stick, avoiding the

outside rows. Downy mildew infection was determined by visual observation (Figure 4.2). The number of plants in the specific area and the number of plants which were infected with downy mildew were counted. The ratio of the number of plants infected with downy mildew to plant stand was calculated.

Figure 4.2: Camelina downy mildew at the reproductive stage (New Glasgow, July 2012)

(3) Maturity Ratings at Truro

Maturity date was estimated visually as the date when approximately 90 % of the pods were brown at Truro from August 11 to August 21, 2011 and from July 31 to August 13, 2012.

(4) Plant Height at Canning and Truro

Plant height was measured on three plants per plot from the soil surface to the highest point on the erect plant at the time of maturity.

(5) Yield Components

Two subsamples of 0.5 m of two rows of plants from each plot were harvested and were calculated and converted to the values with plants/m^2. The number of branches and pods per plant were counted using a sub-sample of 10 plants from each plot.

(6) Seed Yield

Seeds were cleaned using a Clipper (Clipper Seed Cleaning Co., Bluffton, IN) seed cleaner. Clean seed was weighed (g) and g/plot values was converted to kg/ha based on plot areas for each location.

(7) Protein, Oil and Fatty Acid Analysis

Contents of protein, oil and fatty acids (2012 samples) were analyzed by Near-infrared spectroscopy (NIRS) (Unity Scientific, Spectra Star, 2500x).

(8) Fatty Acid Composition Analysis by Gas Chromatography (2011 samples)

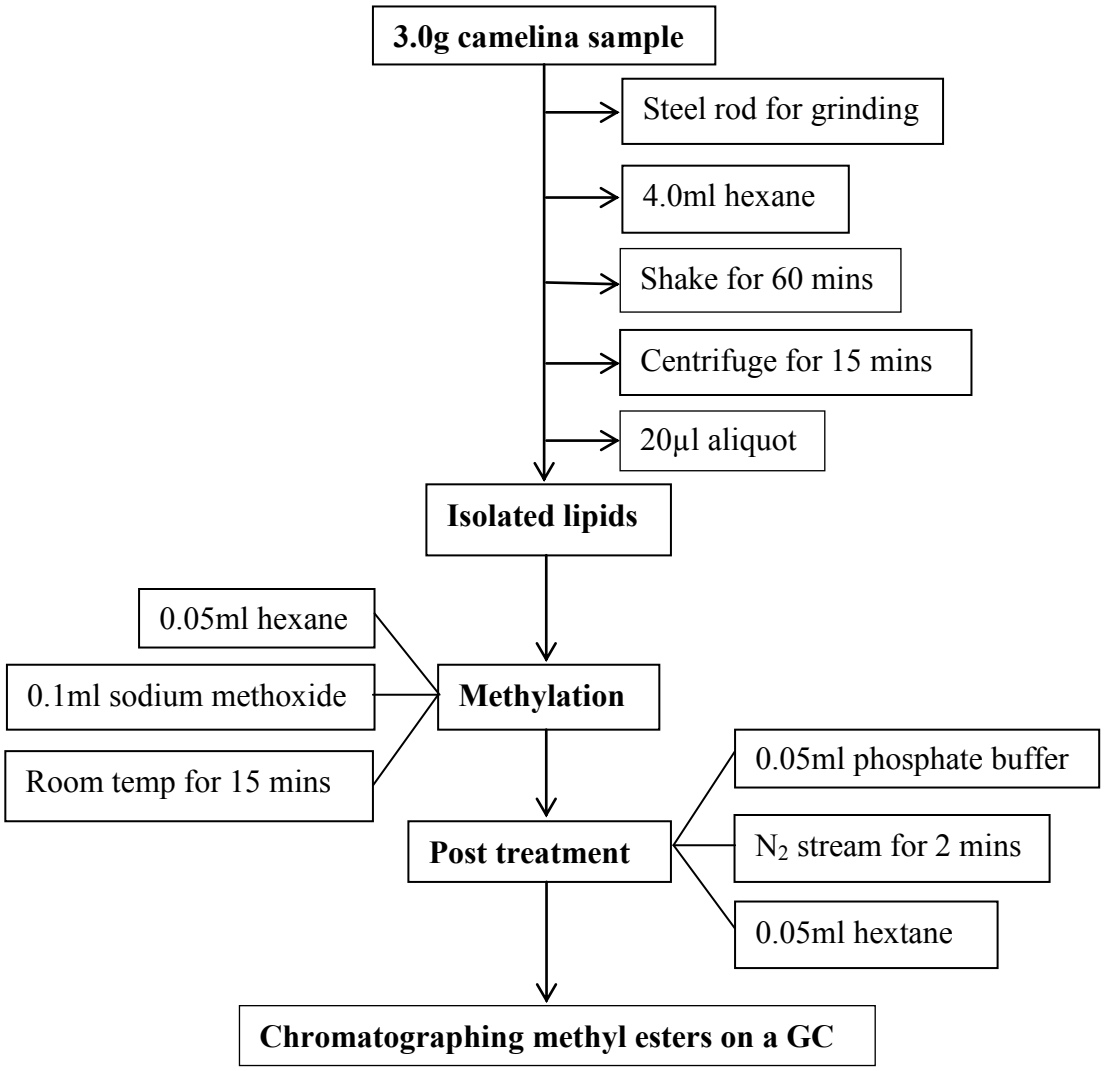


Figure 4.3: Sample preparation of camelina for GC analysis

The method was based on the protocol of the Quality Lab of Agriculture Agri-Food Canada, Saskatoon – "Fatty acyl composition by gas chromatography". This method analyzes the fatty acid profile of esterified compounds of camelina seed oil by methylating the fatty acyl esters, and then these methyl esters are chromatographed on a gas chromatography. Three gram camelina seed samples were placed in 20 ml PET scintillation vials containing small steel rods (1.2cm diameter). The vials were put in a

rack with 60, 28-mm holes. One series of samples consisted of 100 vials in two racks. No. 1 vial was GLC 428; No. 26 vial was R 500 check (canola R 500); No. 51 vial was Cutlass check; and No. 76 vial was Blank check (without adding samples). 4.0 ml hexane was added to every vial to extract oil from camelina seed. The second step was to put the vials in the Eberbach shaker and shake for 60 minutes at a high speed (270 revolutions per minute). It was ensured that seeds were crushed and no whole seeds were visible. The next procedure was to transfer the vials to a centrifuge (Thermo Scientific, Sorvall ST 40R) at 2,300 rcf (3000 rpm) for 15 minutes. The fourth step was to open vials and transfer an aliquot (20 µl) to autosampler vials using a pipettor (Brand: GILSON). And then 0.05 ml hexane was dispensed and 0.1 ml of methylating solution was added into every autosampler vial; contents of vials were mixed and incubated at room temperature for 15 minutes for the methylating reaction. The next process was to add 0.05 ml of phosphate buffer to each vial to adjust the pH and then evaporate off methanol under stream of N for 2 minutes. Finally, 0.05 ml of hextane was added and vials were capped and set up in the autosampler to inject 1 µl of the upper phase.

The conditions for gas chromatography were set as follows: (1) injection volume: 1 µl; (2) split ratio: 1:50; (3) flow rate: 1.0 mL/min; (4) detector: flame ionization detector (FID), which has a wide range of liner response; (5) carrier gas: hydrogen, ultra high purity, 99.999%. The standard component GLC 428 (NU-CHEK PREP, INC) was used to identify retention time for components.

4.4 Statistical Analysis

Minitab 16 statistical software (Minitab Inc., USA, 2012) was used to check three assumptions: normality, constant variance and independence; outliers were removed if

they existed. Data were transformed (square, square root, cubic root, ln or log base 10) if they were not normally distributed. Minitab was used in all the regression analyses.

SAS 9.3 statistical software (SAS Institute Inc., Cary NC, USA, 2012-2013) was also used in the data analyses once the data were checked to be normally distributed in Minitab. Proc Mixed with least significant differences (LSD) t-test method ($p<0.05$) was used to examine whether there were significant effects of factors on the targeted parameters.

Kruskal-Wallis non-parametric test was used to analyze the data of days to maturity at Truro, because the distribution was not normal and it could not be made normal by transformations. Non-parametric tests use the median and not the mean. If there was significant difference between at least two of the median values, Mann-Whitney tests of all the possible pairs of medians were analyzed to find out where the exact differences were.

Data from SK in 2012 were not used in any of the data analyses due to severe weed problems which produced greater effects than the test variables.

4.5 Results

4.5.1 Early Plant Stand (plants/m^2)

Neither genotype nor N had significant effect on the early plant stand (number of plant per m^2) at Truro in 2011 (Table 4.5). Different genotypes behaved differently depending on the levels of N at Canning in 2011. The interaction of location and genotype had a significant effect on the number of plants per m^2 at four locations in 2012 suggesting that the genotypes behaved differently in all the locations. Plant density of

Calena decreased with an increase of N (Table 4.6 and Figure 4.4), and 96.9% variability of Calena plant density could be explained by the change of N levels (Figure 4.4), while N had a weak relationship with plant density of other genotypes (Figure 4.4).

The number of plants per m^2 was highest at Fredericton, followed by Canning in 2012 (Figure 45). Plant density at Truro was significantly lower than that at Canning but significantly higher than that at New Glasgow. Genotype did not have a significant effect on the plant stand at New Glasgow. Plant density of Calena, CDI002, CDI007 and CDI008 was relatively higher at Canning; the density of Calena, CDI005 and CDI008 was higher than CDI007 and CDI002 at Fredericton; the density of Calena, CDI002, CDI007 and CDI008 was higher than CDI005 at Truro.

Table 4.5: ANOVA table of plant stand (plants/m^2) within one month of seeding (first timing of N)

Year	Location	Effect	F value	P value
2011	Truro	Genotype (G)	2.22	0.0726
		N	0.54	0.7490
		G*N	0.79	0.7144
2011	Canning	G	9.92	<.0001
		N	1.06	0.3876
		G*N	**1.77**	**0.037**
2012	Truro, Canning, New Glasgow, Fredericton	Rep	7.29	<.0001
		Location (L)	97.4	<.0001
		G	2.68	0.0315
		L*G	**2.02**	**0.0221**
		N	1.24	0.2878
		L*N	1.23	0.2483
		G*N	0.72	0.8073
		L*G*N	1.01	0.4650

(2011 Canning: no transformation; 2011 Truro & 2012: square root transformation)

Table 4.6: Effect of interaction of genotype and N on early plant stand (plants/m^2) at Canning in 2011 (first timing of N)

N (kg/ha)	Calena	CDI002	CDI005	CDI007	CDI008
0	217 a-c	131 hi	191 a-g	151 e-i	199 a-f
25	228 ab	113 i	111 i	198 a-f	212 a-d
50	177 a-h	138 g-i	144 f-i	138 g-i	168 c-i
50 (100)	209 a-e	142 f-i	192 a-g	129 hi	175 b-h
75 (150)	168 c-i	146 f-i	114 i	156 d-i	234 a
100 (200)	169 c-i	131 hi	139 g-i	179 a-h	161 c-i

(Two-way interaction; means with a common letter are not significantly different at the 5% level)

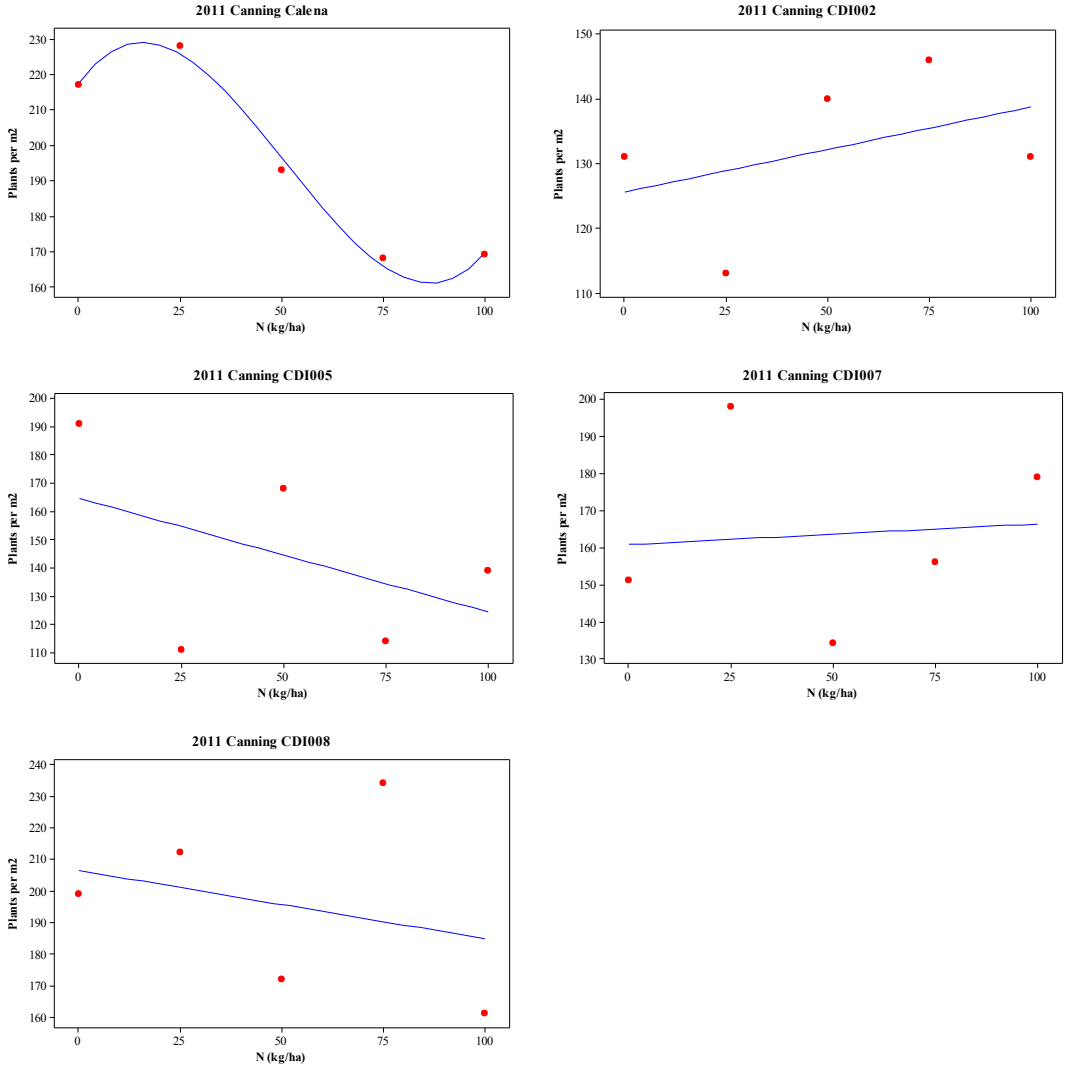


Figure 4.4: Regression analysis of N on early plant stand at Canning in 2011
(each dot represents mean of 4 samples)

(1) Y(2011, Canning, Calena)=217.6+0.1554N-0.05874N**2+0.000384N*3 with R-Sq (adj)=96.9%

(2) Y(2011, Canning, CDI002)=125.6+0.1320N with R-Sq=17.5%

(3) Y(2011, Canning, CDI005)=164.8-0.4040NN with R-Sq=21.3%

(4) Y(2011, Canning, CDI007)=160.8+0.0560N with R-Sq=0.8%
(5) Y(2011, Canning, CDI008)=206.4-0.2160N with R-Sq=8.3%

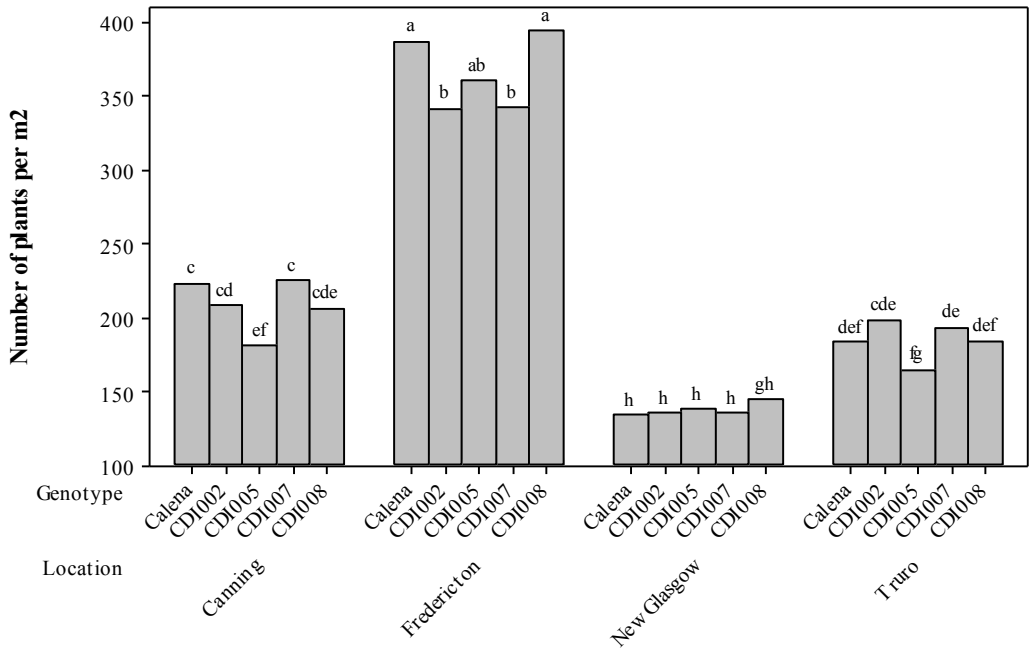


Figure 4.5: Effect of location and genotype on early plant density in 2012 (first timing of N)
(Means with a common letter are not significantly different at the 5% level)

4.5.2 Percent of Plants Infected with Downy Mildew (Before Flowering)

Genotype and N independently had significant effects on the percent of plants infected with downy mildew at the vegetative stage at Canning in 2011 (Table 4.7). Calena, CDI002 and CDI008 were less tolerant to downy mildew and CDI007 was the most tolerant (Table 4.8). The positive correlation between N and the occurrence of downy mildew was strong since the value of coefficient of determination was 95.6% (Figure 4.6).

Camelina plants had a healthier, more robust growth at Truro than Canning in 2011. Only three plants were found with downy mildew during stand counts at Truro in 2011 (data were not shown). In 2012, very few plants were found with downy mildew, and therefore disease data was not collected.

Table 4.7: ANOVA table of percent of plants infected with downy mildew before the flowering stage

Year	Location	Effect	F value	P value
		Genotype (G)	**25.74**	**<0.0001**
2011	Canning	**N**	**3.44**	**0.0069**
		G*N	0.82	0.6881

(square root transformation)

Table 4.8: Effect of genotype and N on percent of downy mildew (%) before the flowering stage at Canning in 2011

Genotype	N (kg/ha)	Percent of DM %
Calena		32.4 a
CDI002		30.1 a
CDI008		28.0 ab
CDI005		23.0 b
CDI007		10.2 c
	0	19.7 b
	25	19.5 b
	50	26.6 a
	100	24.3 ab
	150	29.6 a
	200	24.8 ab

(Means with a common letter are not significantly different at the 5% level)

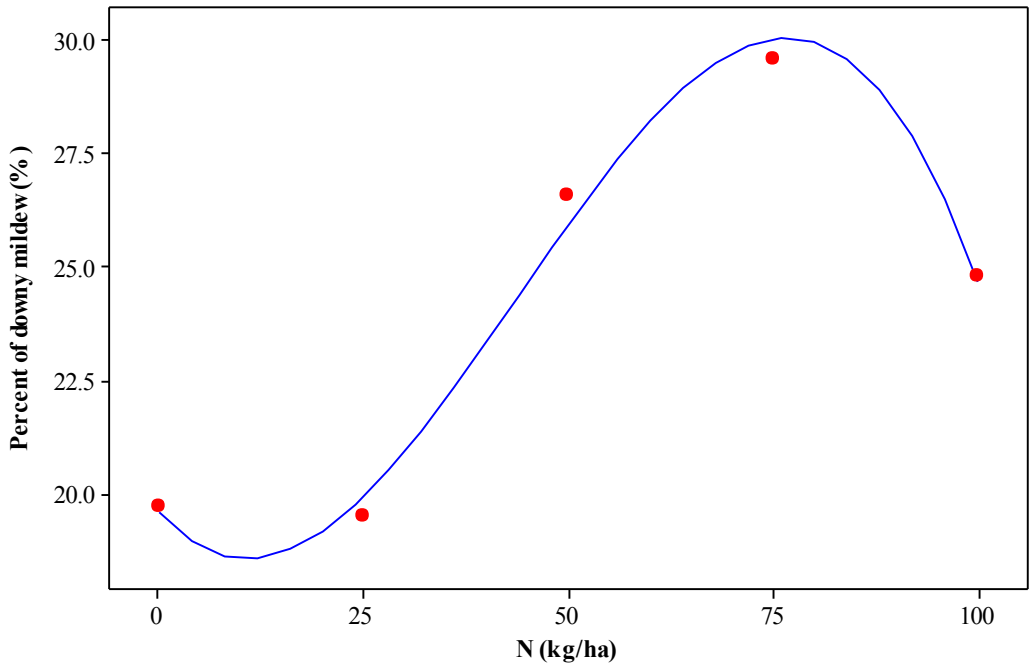


Figure 4.6: Regression analysis of N on percent of downy mildew at Canning in 2011 (each dot represents mean of 20 samples)
Y(Canning,2011)=0.1959-0.001997N+0.000106N**2-0.000001N**3 with R-Sq(adj)=95.6%

4.5.3 Percent of Plants Infected with Downy Mildew (At Flowering)

Genotype had a significant effect on the percent of plants infected with downy mildew at Truro in 2011; genotype and N independently had significant effects on the percent of plants with downy mildew at Canning in 2011; location, genotype and N independently had significant effects on the percent of plants with downy mildew at all 4 sites in 2012 (Table 4.9).

The percent of plants with downy mildew of CDI007 was the least among these five genotypes, therefore CDI007 was the most tolerant to downy mildew at all of the tested sites in 2011 and 2012 (Table 4.10).

The percent of plants infected with downy mildew was higher when plants received 100 and 150 kg N/ha than plants with 0 and 25 kg N/ha input but there was no significant difference from plants with 50 and 200 kg N/ha application (Table 4.11). In 2012, the occurrence of downy mildew at Canning was more severe than Truro, with 20.5% and 8.7% of plants infected at Canning and Truro, respectively (Table 4.12). Higher percent of plants infected with downy mildew when plants received 150 and 200 kg N/ha application at Truro and Canning in 2012 compared to plants with 0-100 kg N/ha application (Table 4.12). Increasing N levels increased the downy mildew infection at Canning only in 2011 (Figure 4.7) and at both Canning and Truro in 2012 (Figure 4.8).

Table 4.9: ANOVA table of percent of downy mildew at the reproductive stage

Year	Location	Effect	F value	P value
2011	Truro	**Genotype (G)**	**20.54**	**<.0001**
		N	1.76	0.1296
		G*N	0.44	0.9814
2011	Canning	**G**	**22.96**	**<.0001**
		N	**2.77**	**0.0240**
		G*N	0.82	0.6777
2012	Truro, Canning	Rep	0.16	0.8494
		Location (L)	**10.21**	**0.0017**
		G	**26.85**	**<.0001**
		L*G	0.44	0.7828
		N	**8.64**	**<.0001**
		L*N	0.86	0.5105
		G*N	0.73	0.7955
		L*G*N	1.14	0.3134

(square root transformation)

Table 4.10: Effect of genotype on percent of downy mildew in 2011 and 2012

Genotype	Truro, 2011	Canning, 2011	NS, 2012
	DM	DM	DM
Calena	40.8 a	19.0 a	24.3 a
CDI005	36.1 ab	20.3 a	15.8 bc
CDI002	30.5 bc	18.1 ab	18.6 ab
CDI008	27.2 c	12.9 b	12.9 c
CDI007	18.0 d	2.9 c	3.6 d

(Mean separation in a column with a common letter are not significantly different at the 5% level)

Table 4.11: Effect of N of percent of downy mildew at Canning in 2011

N (kg/ha)	Percent of DM %
0	9.9 b
25	10.9 b
50	13.1 ab
100	17.3 a
150	17.6 a
200	13.6 ab

(Means with a common letter are not significantly different at the 5% level)

Table 4.12: Effect location and N on percent of downy mildew in 2012

Location	N (kg/ha)	Percent of downy mildew
Canning		20.5 a
Truro		8.7 b
	0	11.4 b
	25	10.1 b
	50	11.3 b
	100	10.6 b
	150	19.9 a
	200	23.3 a

(Means with a common letter are not significantly different at the 5% level)

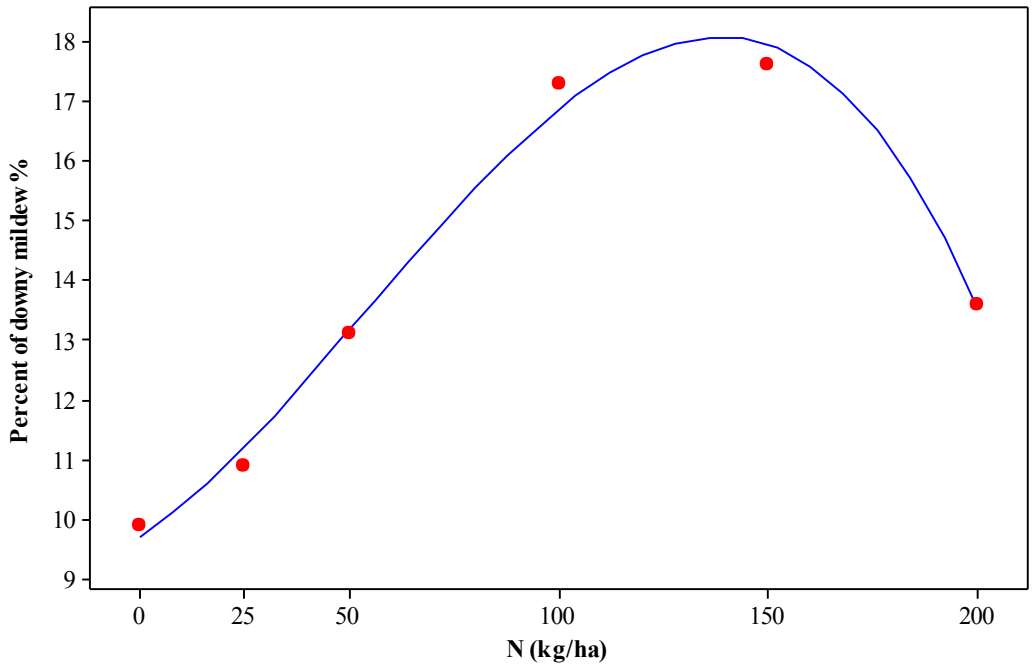


Figure 4.7: Regression analysis of N on percent of downy mildew at Canning in 2011 (Each dot represents the mean of 20 samples)

Y=0.09710+0.000472N+0.000006N**2-0.000000N**3 with R-Sq(adj)=97.7%

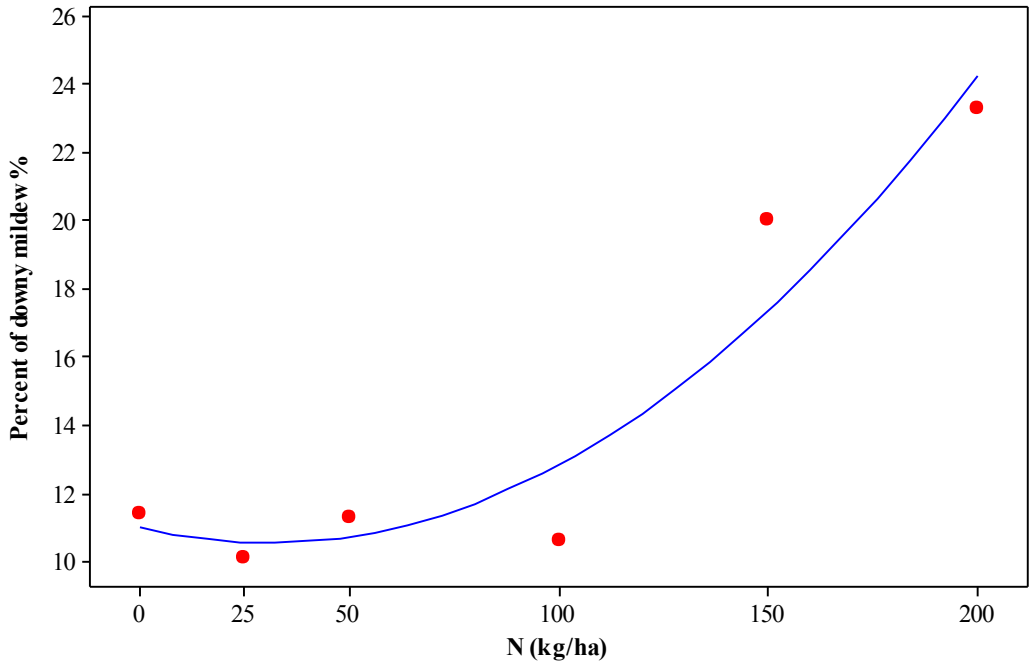


Figure 4.8: Regression analysis of N on percent of downy mildew at Canning and Truro in 2012

(Each dot represents the mean of 40 samples)

Y=0.1099-0.000296N+0.000005N**2 with R-Sq(adj)=86.0%

4.5.4 Days to Maturity

Effects of genotype and N on days to maturity at Truro in 2011 and 2012 was shown in Table 4.13. ANOVA table was not shown here because data of days to maturity were not normally distributed, so Kruskal-Wallis non parametric test was used with P value less than 0.0001, then Mann-Whitney was applied to test the significant difference. In 2011, CDI007 and Calena were the latest to mature, followed by CDI002 which took 104 days to reach maturity. CDI005 and CDI008 were the earliest to mature. In general it took longer for plants to mature with higher N treatments in 2012. Also, plants in 2011 took longer to mature than 2012.

Table 4.13: Median values of days to maturity at Truro in 2012

Year	N (kg/ha)	Calena	CDI002	CDI005	CDI007	CDI008	P value
2011	0	109 a	106 a	101 b	109 a	101 a	<.0001
	25	107 a	104 a	101 b	107 a	100 b	
	50	105 a	102 b	101 b	106 a	99 b	
	100	106 a	103 a	102 b	109 a	101 b	
	150	107 a	104 a	101 b	108 a	101 a	
	200	107 a	104 a	103 a	108 a	102 b	
2012	0	91 bc	91 bc	88 c	94 a	88 c	<.0001
	25	93 ab	91 bc	88 c	93 ab	88 c	
	50	91 bc	91 bc	88 c	94 a	88 c	
	100	94 a	91 bc	90 c	94 a	91 bc	
	150	94 a	94 a	93 ab	94 a	94 a	
	200	94 a	94 a	94 a	94 a	93 ab	

(Means with the same year – 2011 or 2012 with a common letter are not significantly different at the 5% level)

4.5.5 Plant Height

Genotype and N independently had significant effects on plant height at Truro in 2011; genotype had a significant effect on plant height at Canning in 2011; genotype, location and N independently had significant effects on the plant height at the four locations in 2012 (Table 4.14).

CDI005, CDI007 and CDI002 were relatively taller at Truro and Canning in 2011 and the four locations in 2012 (Table 4.15). At Truro in 2011 and four locations in 2012, CDI008 was the shortest entry while at Canning in 2011, Calena was the shortest (Table 4.15).

Plants at Canning were the tallest, followed by New Glasgow and Truro in 2012, while plants at Fredericton were the shortest (Table 4.16).

Plant height increased with an increase in N at Truro in 2011 (Table 4.17 and Figure 4.9). Plants with 25 kg/ha N application were taller than plants without N application, and plants with 50, 100, 150 and 200 kg N/ha were taller than plants with 0 and 25 kg N/ha at Truro in 2011 (Table 4.17). Plant height increased with an increase in N at Truro in 2011, and 80.6% variability in plant height could be explained by the change of N levels (Figure 4.9). Plant height increased with an increase in N at Canning, Fredericton, New Glasgow and Truro in 2012 (Table 4.17 and Figure 4.10), and 84.0% of the variability in plant height could be explained by the change of N rates (Figure 4.10).

Table 4.14: ANOVA table of plant height at harvest

Year	Location	Effect	F value	P value
2011	Truro	**Genotype (G)**	**7.4**	**<.0001**
		N	**18.07**	**<.0001**
		G*N	1.11	0.3522
2011	Canning	**G**	**16.17**	**<.0001**
		N	1.10	0.3703
		G*N	0.50	0.9560
2012	Truro, Canning, New Glasgow, Fredericton	Rep	7.96	<.0001
		Location (L)	**32.73**	**<.0001**
		G	**8.85**	**<.0001**
		L*G	0.86	0.5888
		N	**14.45**	**<.0001**
		L*N	1.54	0.0882
		G*N	0.85	0.6541
		L*G*N	0.63	0.9847

(no transformation)

Table 4.15: Effect of genotype on plant height (cm) in 2011 and 2012

Genotype	Truro, 2011 Height (cm)	Canning, 2011 Height (cm)	Four sites, 2012 Height (cm)
CDI005	85 a	67 a	79 a
CDI007	83 ab	67 a	79 ab
CDI002	83 ab	65 a	80 a
Calena	82 b	50 b	77 b
CDI008	78 c	67 a	75 c

(Means in each column with a common letter are not significantly different at the 5% level)

Table 4.16: Effect of location on plant height at 4 sites in 2012

Location	Plant height (cm)
Canning	85 a
New Glasgow	82 b
Truro	81 b
Fredericton	64 c

(Means with a common letter are not significantly different at the 5% level)

Table 4.17: Effect of N on plant height at Truro in 2011 and at 4 sites in 2012

N (kg/ha)	Truro, 2011	4 sites, 2012
0	75 c	74 e
25	79 b	77 d
50	85 a	79 bc
100	84 a	78 cd
150	86 a	80 ab
200	85 a	81 a

(Means in each column with a common letter are not significantly different at the 5% level)

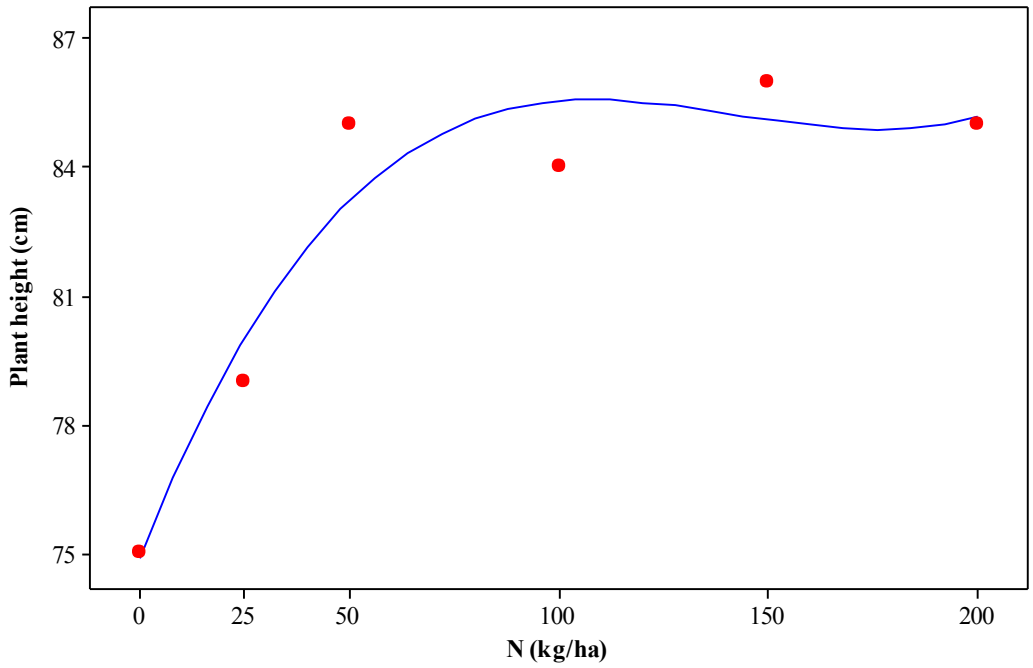


Figure 4.9: Regression of N on plant height at Truro in 2011
(each dot represents the mean of 20 samples)
Y(2011, Truro)=74.89+0.2488N-0.001858N**2+0.000004N**3 with R-Sq(adj)=80.6%

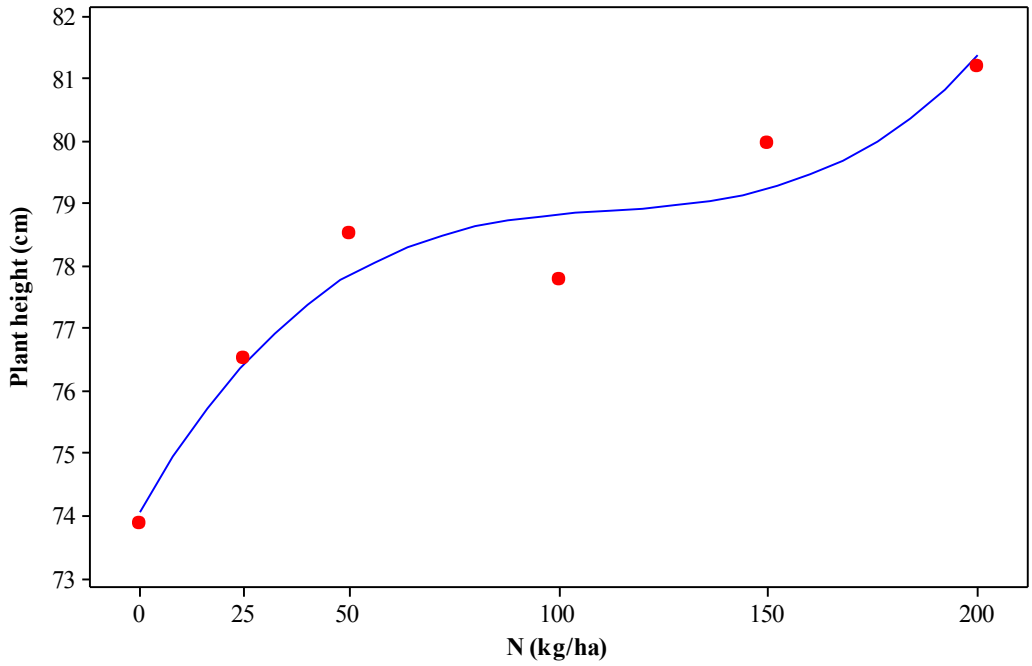


Figure 4.10: Regression analysis of N on plant height at all 4 sites in 2012
(each dot represents the mean of 80 samples)
Y(2012)=74.06+0.0090N-0.001015N**2+0.000003N**3 with R-Sq(adj)=84.0%

4.5.6 Final Plant Stand (plants/m^2)

N had a significant effect on the final plant stand (the number of plants per m^2) at Truro in 2011; N and the interaction of location and genotype had significant effects on the number of plants per m^2 at harvest at Canning, Truro, New Glasgow and Fredericton in 2012 (Table 4.18).

Plant density was significantly lower when plants received 200 kg N/ha compared to plants with 0-100 kg N/ha application at Truro at 2011 (Table 4.19). N was positively correlated with plant density at harvest with the value of coefficient of determination 71.2% (Figure 4.11).

The regression model in Figure 4.12 showed that N had a strong relationship with R-Sq(adj) 99.5% on the number of plants per m^2 at Canning, Truro, New Glasgow and

Fredericton in 2012. The number of plants per m^2 at harvest decreased with the increase in N in 2012 (Table 4.19 and Figure 4.12).

Locational effect was significant on final plant density in 2012 (Figure 4.13). At Canning, Calena, CDI007 and CDI008 had higher plant density; at Fredericton, genotype did not have a significant effect on the final plant density; at New Glasgow, CDI005 and CDI007 had higher plant density among these five genotypes; at Truro, CDI002, CDI007 and CDI008 had higher plant density. In general, the final plant density was relatively high at Canning and Fredericton compared with New Glasgow and Truro.

Table 4.18: ANOVA table of final plant stand (plants/m^2)

Year	Location	Effect	F value	P value
2011	Truro	Genotype (G)	0.38	0.8208
		N	**3.27**	**0.0093**
		G*N	0.7	0.8136
2012	Canning, Truro, New Glasgow, Fredericton	Rep	2.74	0.0288
		Location (L)	14.9	<.0001
		G	2.58	0.0369
		L*G	**2.6**	**0.0025**
		N	**5.51**	**<.0001**
		L*N	1.69	0.0515
		G*N	0.93	0.5519
		L*G*N	1.02	0.4502

(2011: square root transformation; 2012: no transformation)

Table 4.19: Effect of N on final plant stand (plants/m^2) at Truro in 2011 and at 4 sites in 2012

N (kg/ha)	Truro, 2011	4 sites, 2012
0	170 a	247 a
25	167 ab	299 ab
50	157 ab	216 bc
100	174 a	214 bc
150	135 bc	213 bc
200	124 c	197 c

(Means in each column with a common letter are not significantly different at the 5% level)

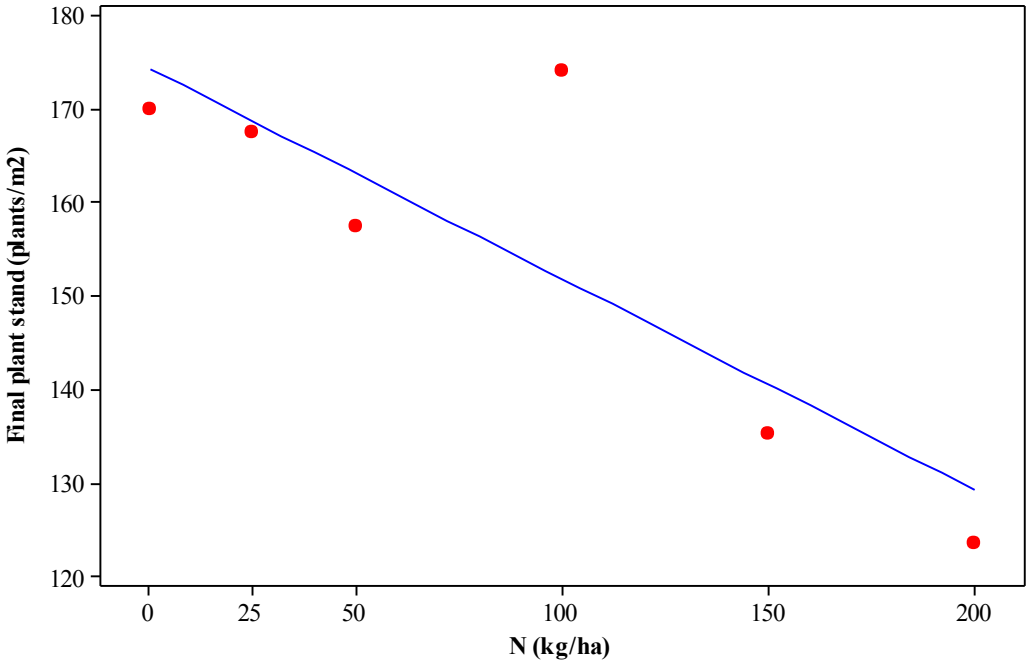


Figure 4.11: Regression analysis of N on final plant stand at Truro in 2011
(each dot was the mean of 20 samples)
Y(2011, Truro)=174.3-0.2255N with R-Sq=71.2%

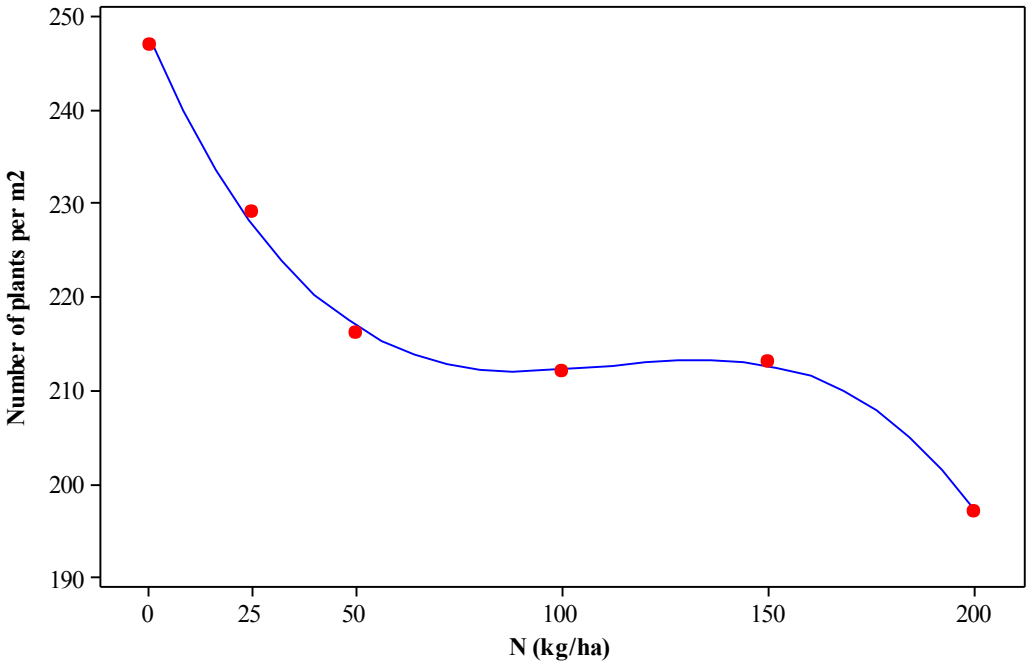


Figure 4.12: Regression analysis of N on final plant stand in 2012
(each dot was the mean of 80 samples)
Y(2012, NS, Fredericton and New Glasgow)=247.5-1.014N+0.009414N**2-0.000028N**3with R-Sq(adj)=99.5%

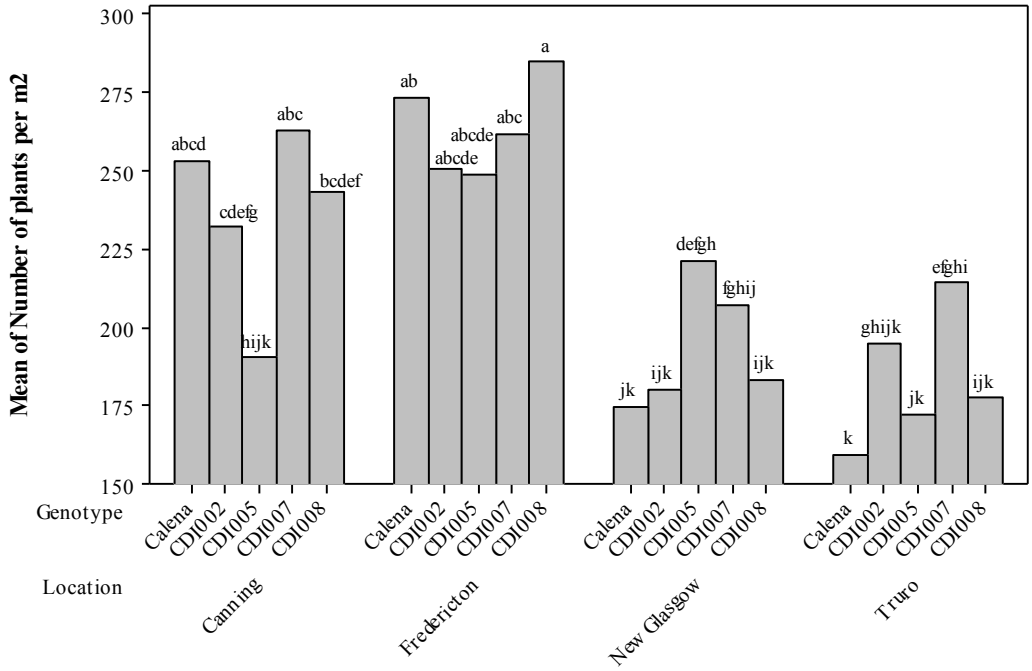


Figure 4.13: Effect of location and genotype on final plant stand in 2012
(Means with a common letter are not significantly different at the 5% level)

4.5.7 Number of Branches per plant

Genotype and N independently had significant effects on the number of branches per plant at Truro in 2011; the interaction of location and genotype and the interaction of genotype and N had significant effects on the number of branches per plant at Truro, Canning, New Glasgow and Fredericton in 2012 (Table 4.20).

CDI007 and CDI002 had the highest number of branches per plant at Truro in 2011, and CDI005 and Calena had the least number of branches (Table 4.21).

The number of branches per plant increased linearly with the increase of N input at Truro in 2011 (Table 4.21 and Figure 4.12). The relationship between N and branches/plant was strong with the value of coefficient determination 90.3% (Figure 4.12).

The branching habits of various genotypes differed significantly depending on location. CDI007 and CDI008 at Canning, CDI007 at Fredericton, CDI007 and CDI008 at New Glasgow had the most branches among these five genotypes. Genotype had no significant effect on the number of branches per plant at Truro in 2012 (Figure 4.15).

The branching habits differed significantly depending on genotype. The number of branches per plant of Calena, CDI002, CDI005 and CDI007 increased with an increase of N in 2012, while N had a very weak relationship with the number of branches per plants with the value coefficient of determination only 44.7% (Table 4.22 and Figure 4.16).

Table 4.20: ANOVA table of the number of branches per plant

Year	Location	Effect	F value	P value
2011	Truro	**Genotype (G)**	**6.56**	**<.0001**
		N	**7.16**	**<.0001**
		G*N	1.03	0.4346
2012	Truro, Canning, New Glasgow, Fredericton	Rep	5.37	0.0003
		Location (L)	11.02	<.0001
		G	13.87	<.0001
		L*G	**2.14**	**0.0141**
		N	14.72	<.0001
		L*N	0.97	0.4827
		G*N	**1.91**	**0.0111**
		L*G*N	0.99	0.4932

(2011Truro: no transformation; 2012: $\log_{10}$ transformation)

Table 4.21: Effect of genotype and N on number of branches per plant at Truro in 2011

Genotype	N (kg/ha)	Branches/plant
CDI007		8.3 a
CDI002		7.6 ab
CDI008		6.9 bc
CDI005		6.3 c
Calena		6.1 c
	0	5.5 d
	25	6.8 bc
	50	6.6 cd
	100	7.1 bc
	150	7.7 ab
	200	8.6 a

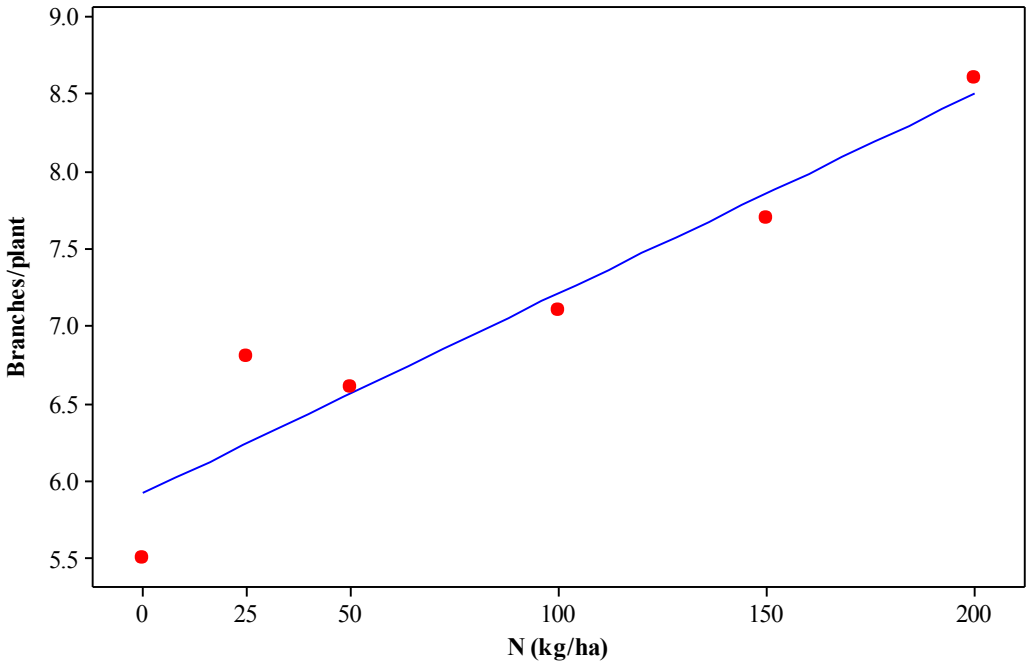


Figure 4.14: Regression analysis of N on branches/plant at Truro in 2011 (each dot represents the mean of 20 samples)

Y(2011,Truro)=5.919+0.01293N with R-Sq=90.3%

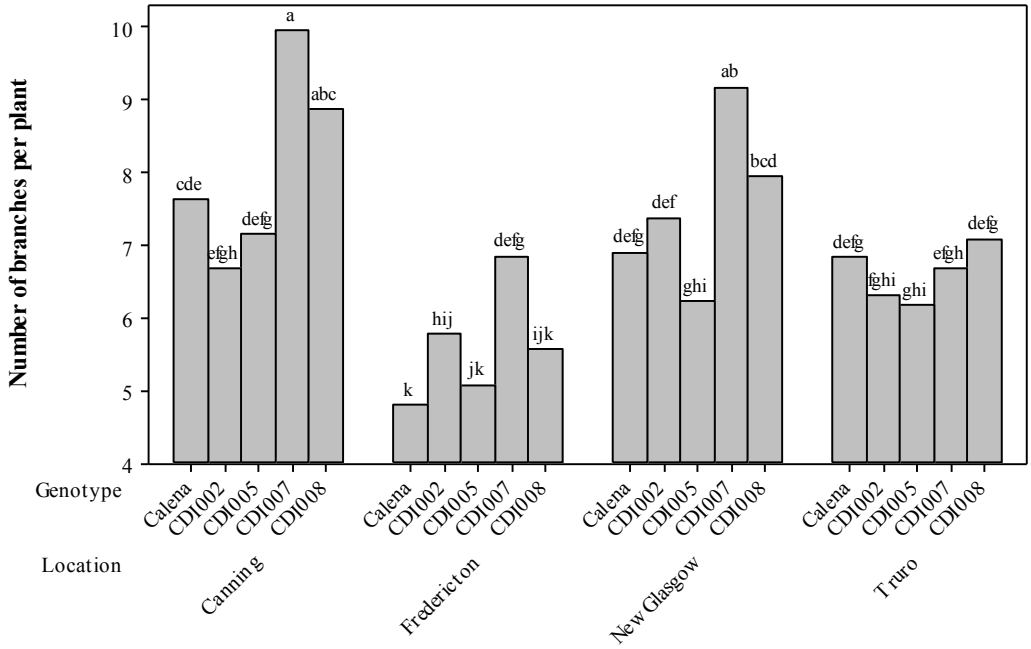


Figure 4.15: Effect of location and genotype on number of branches per plant in 2012

(Means with a common letter are not significantly different at the 5% level)

Table 4.22: Effect of interaction of genotype and N on branches/plant at 4 sites in 2012

N (kg/ha)	Calena	CDI002	CDI005	CDI007	CDI008
0	6 j-l	5 l	5 kl	6 g-k	7 e-j
25	6 i-l	6 f-k	6 f-k	7 e-j	8 c-e
50	6 g-k	7 c-h	6 j-l	10 ab	7 e-j
100	6 f-k	7 f-j	6 h-l	7 c-g	7 d-i
150	7 d-i	7 d-i	7 e-j	9 bc	8 b-d
200	8 b-d	7 c-f	7 c-g	10 a	7 c-g

(Two-way interaction; means with a common letter are not significantly different at the 5% level)

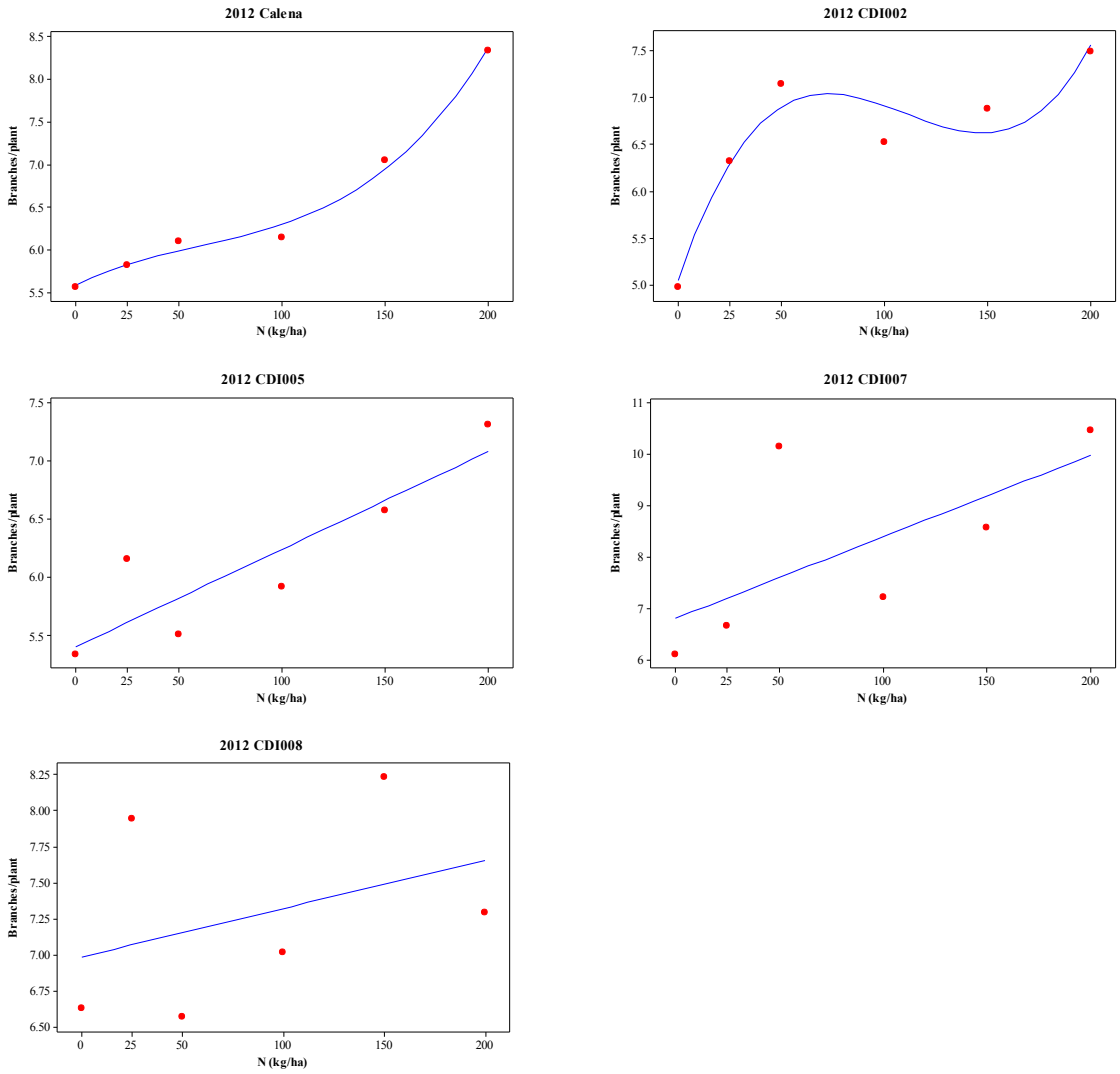


Figure 4.16: Regression analysis of N on branches/plant of 5 genotypes in 2012
(each dot represents the mean of 16 samples)

(1) Y(2012Calena)=5.588+0.01195N-0.000106N**2+0.000001N**3 with R-Sq(adj)=97.7%
(2) Y(2012CDI002)=5.053+0.06530N-0.000670N**2+0.000002N**3 with R-Sq(adj)=81.6%
(3) Y(2012CDI005)=5.403+0.008414N with R-Sq=78.8%
(4) Y(2012CDI007)=6.812+0.01591N with R-Sq=44.7%
(5) Y(2012CDI008)=6.987+0.003351N with R-Sq=14.2%

4.5.8 Number of Branches/m^2

Genotype had a significant effect on the number of branches per m^2 in 2011; the interaction of location and genotype and the interaction of location and N had significant

effects on the number of branches per m^2 at four locations in 2012 (Table 4.23).

CDI007 had the highest number of branches per m^2 at Truro in 2011, followed by CDI008. CDI002 was not significantly different than Calena nor was the check from CDI005 (Table 4.24).

CDI007 had the highest number of branches per m^2 in Canning and New Glasgow; CDI002, CDI007 and CDI008 had the highest number of branches per m^2 at Fredericton and Truro in 2012 (Figure 4.17).

The regression models of the relationship between N and the number of branches per m^2 at four locations in 2012 were shown in Figure 4.16. At Truro, 76.5% of variability of the number of branches per m^2 could be explained by the change in N rate. The number of branches per m^2 increased with N application from 0 kg/ha to 150 kg/ha, and the number of branches/m^2 tended to decrease when N continued to increase to 200 kg/ha (Figure 4.16 and Table 4.25). At Canning, Fredericton and New Glasgow, the relationship between N and number of branches/m^2 was weak (Figure 4.16 and Table 4.25).

Table 4.23: ANOVA table of branches per m^2 in 2011 and 2012

Year	Location	Effect	F value	P value
2011	Truro	Genotype (G)	3.02	**0.0224**
		N	1.48	0.2054
		G*N	1.01	0.4558
2012	Truro, Canning, New Glasgow, Fredericton	Rep	2.05	0.0867
		Location (L)	10.05	<.0001
		G	28.04	<.0001
		L*G	**4.51**	**<.0001**
		N	3.2	0.0077
		L*N	**1.98**	**0.0161**
		G*N	1.34	0.1534
		L*G*N	0.86	0.7556

(no transformation)

Table 4.24: Effect of genotype on branches/m^2 at Truro in 2011

Genotype	Branches/m^2
CDI007	1921 a
CDI008	1619 b
CDI002	1406 c
Calena	1340 cd
CDI005	1243 d

(Means with a common letter are not significantly different at the 5% level)

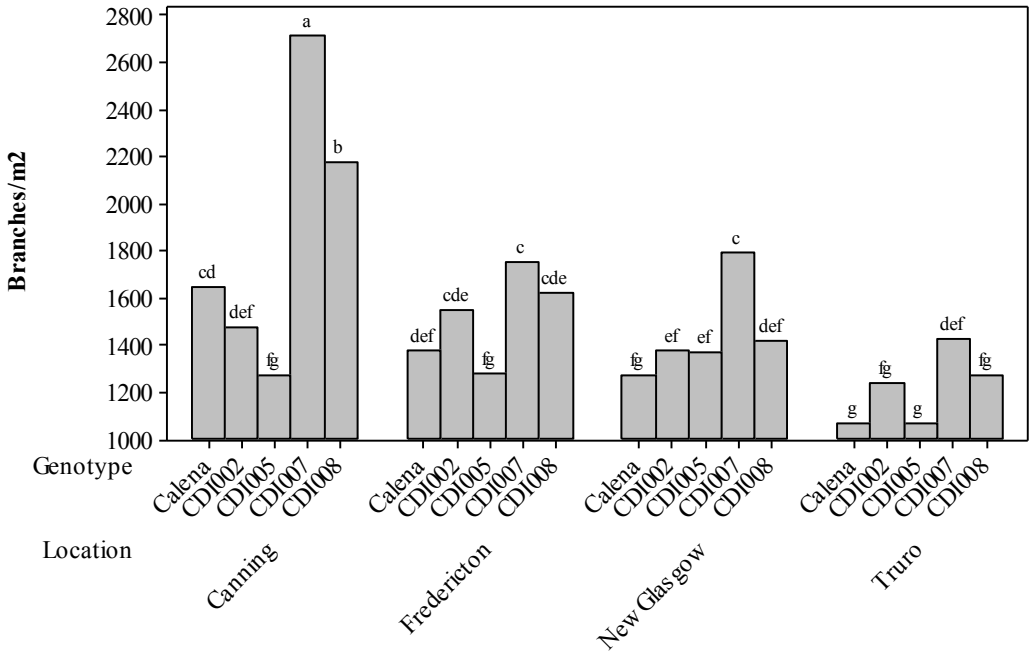


Figure 4.17: Effect of interaction of location and genotype on the number of branches per m^2 at four different locations 2012

(Means with a common letter are not significantly different at the 5% level)

Table 4.25: Effect of interaction of location and N on branches/m^2 in 2012

N (kg/ha)	Canning	Fredericton	New Glasgow	Truro
0	1599 c-f	1448 d-g	1439 d-h	984 j
25	1821 bc	1617 c-e	1443 d-g	1142 ij
50	2185 a	1344 e-i	1479 d-g	1145 h-j
100	1603 c-f	1373 e-i	1446 d-g	1273 g-j
150	1804 bc	1675 cd	1317 f-i	1469 d-g
200	2110 ab	1623 c-e	1537 c-g	1256 g-j

(Two-way interaction; means with a common letter are not significantly different at the 5% level)

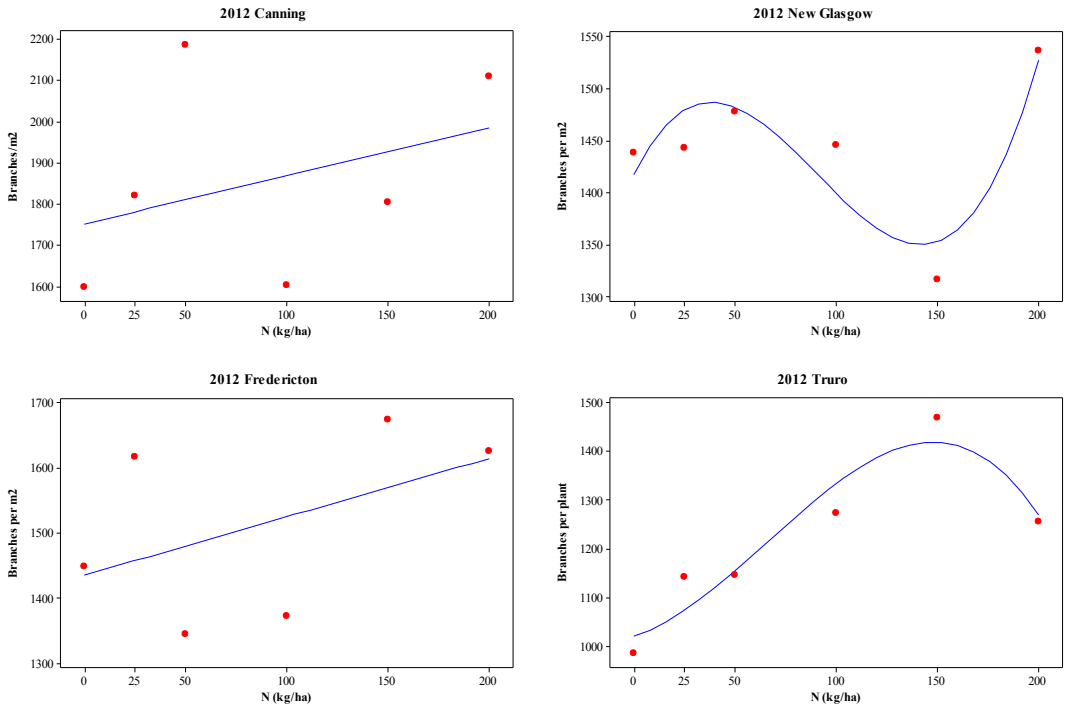


Figure 4.18: Regression analysis of N on branches/m^2 at four different locations in 2012
(each dot represents the mean of 20 samples)

(1) Y(2012,Canning)=1752+1.162N with R-Sq=13.1%

(2) Y(2012,Fredericton)=1435+0.8970N with R-Sq=23.45%

(3) Y(2012,New Glasgow)=1418+3.999N-0.06638N**2+0.000246N**3 with R-sq(adj)=48.4%

(4) Y(2012,Truro)=1020+1.325N+0.03660N**2-0.000185N**3 with R-Sq(adj)=76.5%

4.5.9 Number of Pods per Plant

Genotype and N independently had significant effects on number of pods per plant at Truro in 2011; location and the interaction of genotype and N had significant effects on number of pods per plant at four locations in 2012 (Table 4.26).

CDI007 had the most pods per plant, which was more than the pod number of CDI002, Calena and CDI005 at Truro in 2011 (Table 4.27). The number of pods per plant was enhanced with an increase in N (Table 4.27 and Figure 4.19), and the relationship

between N and pods/plant was strong with the value of coefficient of determination 90.3% (Figure 4.19).

Plants at Fredericton had the least number of pods per plant when compared to Canning, Truro and New Glasgow in 2012 (Table 4.28).

The regression models of N on the number of pods per plant for different genotypes in 2012 were shown in Figure 4.20. N did not have a significant effect on the number of pods per plant of CDI008. N had a positive effect on pods/plants of CDI002, CDI005, CDI007 and Calena with the coefficient of determination greater than 60.0% (Figure 4.20 and Table 4.29).

Table 4.26: ANOVA table of number of pods per plant

Year	Location	Effect	F value	P value
2011	Truro	**Genotype (G)**	**3.58**	**0.0093**
		N	**5.47**	**0.0002**
		G*N	0.84	0.6592
2012	Truro, Canning, New Glasgow, Fredericton	Rep	2.85	0.024
		Location (L)	**11**	**<.0001**
		G	9.65	<.0001
		L*G	1.5	0.1219
		N	20.67	<.0001
		L*N	1.01	0.4418
		G*N	**1.87**	**0.0139**
		L*G*N	1.09	0.3113

(2011 Truro: no transformation; 2012: $\log_{10}$ transformation)

Table 4.27: Effect of genotype and N on number of pods per plant at Truro in 2011

Genotype	N (kg/ha)	Pods/plant
CDI007		154 a
CDI008		139 ab
CDI002		121 b
Calena		119 b
CDI005		117 b
	0	98 c
	25	124 b
	50	119 bc
	100	134 b
	150	143 ab
	200	162 a

(Means with a common letter are not significantly different at the 5% level)

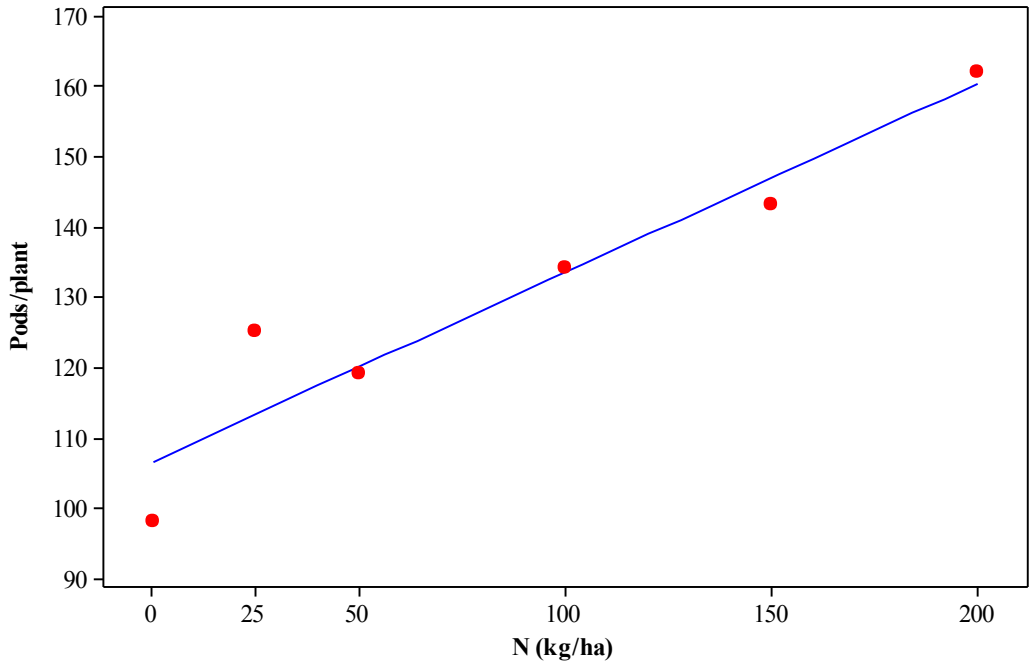


Figure 4.19: Regression analysis of N on pods/plant at Truro in 2011
(each dot represents the mean of 20 samples)
Y(2011,Truro)=106.6+0.2691N with R-Sq=90.3%

Table 4.28: Effect of location on number of pods per plant in 2012

Location	Pods/plant
Canning	127 a
Truro	121 a
New Glasgow	119 a
Fredericton	79 b

(Means with a common letter are not significantly different at the 5% level)

Table 4.29: Effect of interaction of genotype and N on pods/plant at 4 sites in 2012

N (kg/ha)	Calena	CDI002	CDI005	CDI007	CDI008
0	94 h-j	71 k	81 jk	91 h-j	100 f-j
25	92 h-j	91 h-j	95 h-j	101 f-j	130 b-d
50	98 g-j	108 d-i	89 ij	149 ab	105 d-i
100	102 e-i	105 d-i	94 h-j	111 c-i	114 c-h
150	127 b-e	104 e-i	121 b-g	135 bc	146 b
200	139 bc	121 b-g	127 b-e	183 a	123 b-f

(Two-way interaction; means with a common letter are not significantly different at the 5% level)

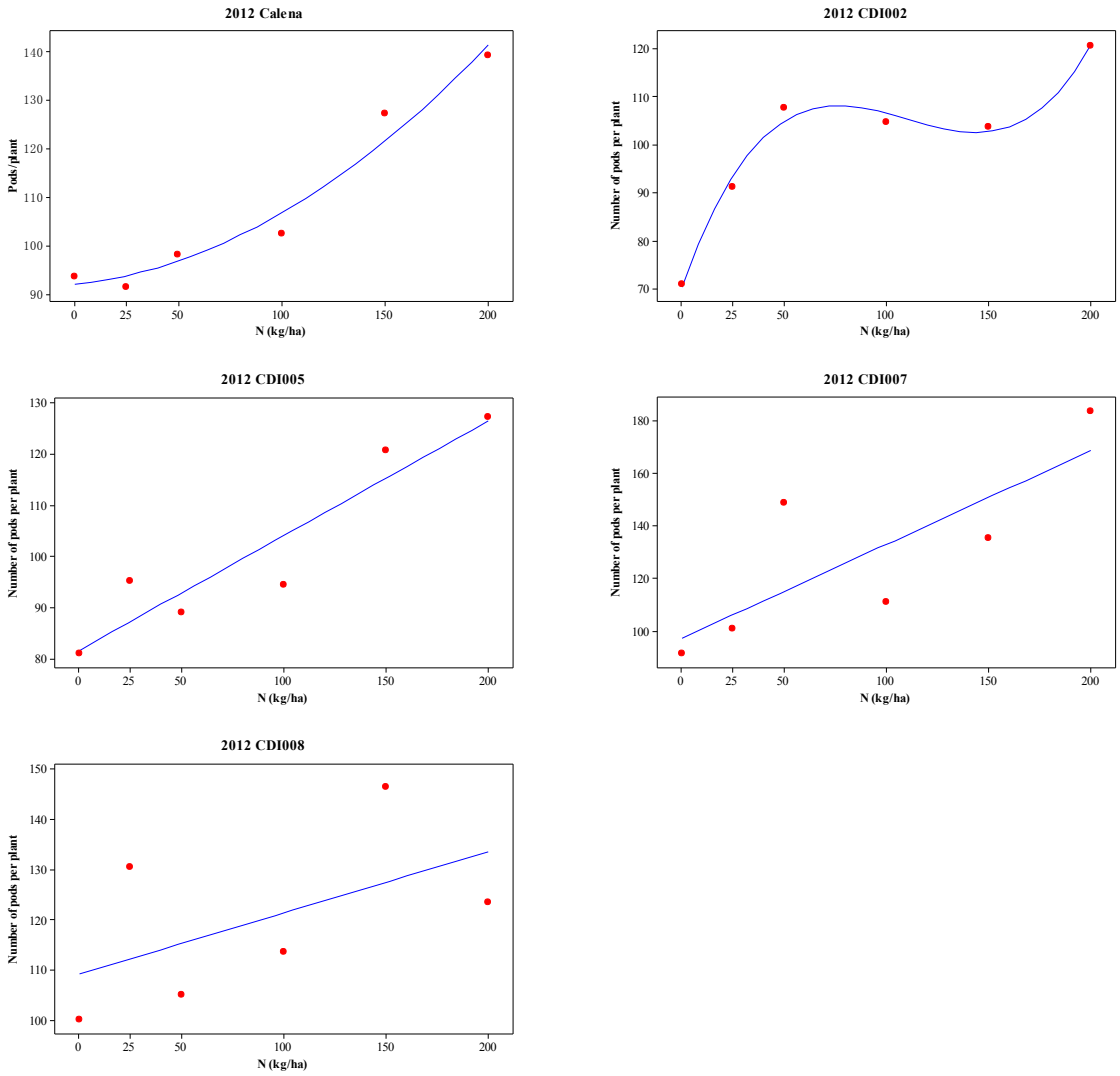


Figure 4.20: Regression analysis of N on pods/plant of 5 genotypes in 2012
(each dot represents the mean of 16 samples)

(1) Y(2012,Calena)=92.01+0.0484N+0.000992N**2 with R-Sq(adj)=94.4%

(2) Y(2012,CDI002)=70.39+1.200N-0.01202N**2+0.000036N**3 with R-Sq(adj)=97.0%

(3) Y(2012,CDI005)=81.68+0.2240N with R-Sq=88.2%

(4) Y(2012,CDI007)=97.22+0.3571N with R-Sq=63.8%

(5) Y(2012,CDI008)=109.1+0.1219N with R-Sq=29.7%

4.5.10 Number of Pods per m^2

N had a significant effect on the number of pods per m^2 at Truro in 2011; location,

genotype and N independently had significant effects on the number of pods per m^2 at Truro, Canning, New Glasgow and Fredericton in 2012 (Table 4.30).

The relationship between N on the number of pods per m^2 was weak (Figure 4.21 and Table 4.31), and 25.2% of variability in the number of pods per m^2 could be explained by the change of N levels (Figure 4.21).

The number of pods per m^2 at Canning was more than that at Fredericton, New Glasgow and Truro (Table 4.32). CDI007 and CDI008 had more pods per m^2 than Calena, CDI002 and CDI005 (Table 4.32). The number of pods per m^2 increased with the increase of N input (Table 4.32 and Figure 4.22), and 82.7% of variability in the number of pods/plant at all of the sites in 2012 could be explained by the change of N rates (Figure 4.22).

Table 4.30: ANOVA table of the number of pods per m^2 in 2011 and 2012

Year	Location	Effect	F value	P value
2011	Truro	Genotype (G)	2.4	0.0557
		N	**2.62**	**0.0293**
		G*N	1.12	0.3465
2012	Truro, Canning, New Glasgow, Fredericton	Rep	0.88	0.4743
		Location (L)	**3.38**	**0.0186**
		G	**14.23**	**<.0001**
		L*G	1.4	0.1655
		N	**6.21**	**<.0001**
		L*N	1.65	0.059
		G*N	0.96	0.5149
		L*G*N	0.62	0.9867

(2012: square root transformation)

Table 4.31: Effect of N on pods/m^2 at Truro in 2011

N (kg/ha)	Pods/m^2
0	16324 c
25	20323 ab
50	18651 bc
100	22587 a
150	18955 bc
200	18707 bc

(Means with a common letter are not significantly different at the 5% level)

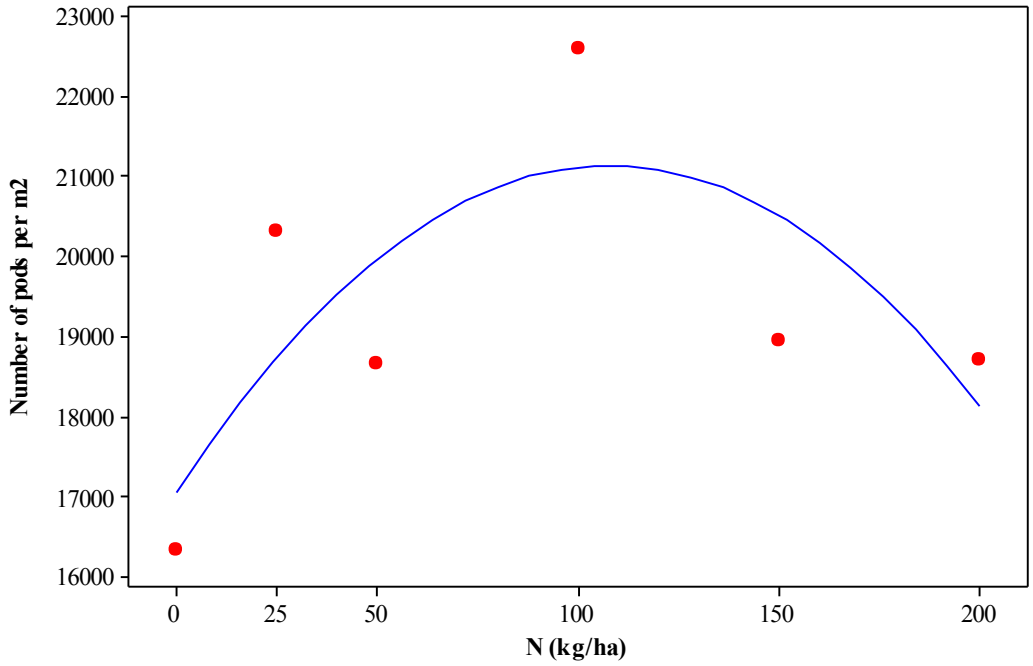


Figure 4.21: Regression analysis of N on pods/m^2 at Truro in 2011

(each dot represents the mean of 20 samples)

Y(2011,Truro)=17063+75.79N-0.3520N**2 with R-Sq(adj)=25.2%

Table 4.32: Effect of location genotype and N on number of pods per m^2 in 2012

Location	Genotype	N (kg/ha)	Pods/m^2
Canning			27403 a
Fredericton			21077 b
New Glasgow			22572 b
Truro			21054 b
	CDI007		27416 a
	CDI008		25462 a
	Calena		21565 b
	CDI002		20961 b
	CDI005		19824 b
		0	19921 c
		25	22521 b
		50	21919 bc
		100	22150 bc
		150	25776 a
		200	25747 a

(Means with a common letter are not significantly different at the 5% level)

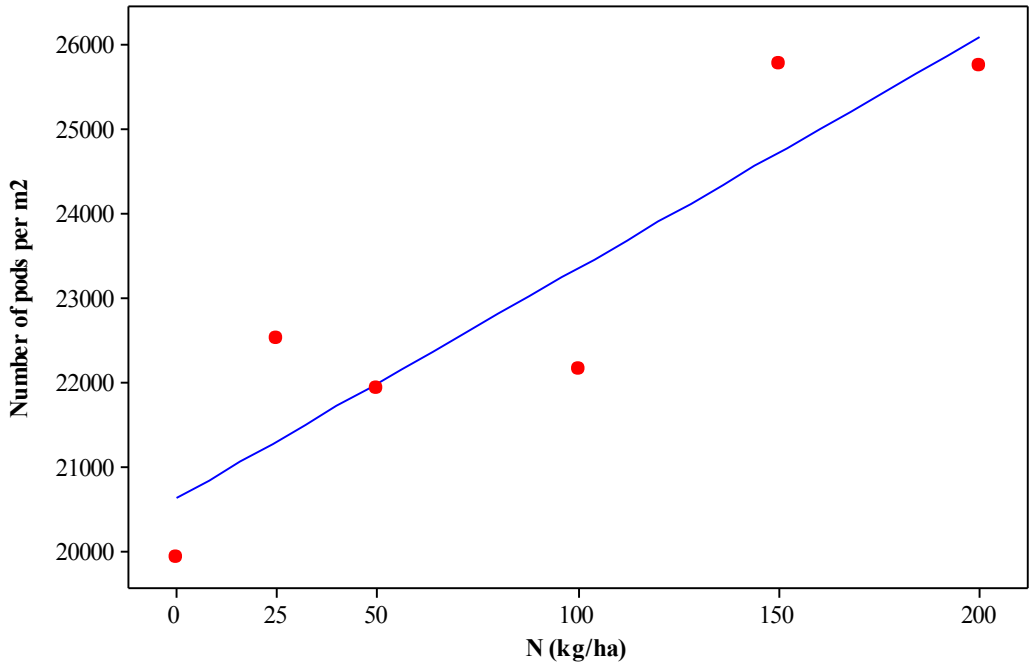


Figure 4.22: Regression analysis of N on pods/m^2 at all 4 sites in 2012
(each dot represents the mean of 80 samples)
Y(2012)=20613+27.35N with R-Sq=82.7%

4.5.11 Seed Yield

Genotype and N independently had significant effects on the yield at Truro and Canning in 2011. Genotype and the interaction of location and N had significant effects on the seed yield at Truro, Canning, New Glasgow and Fredericton in 2012 (Table 4.33).

CDI007 had the highest yield at all of the different sites in 2011 and 2012, but not significantly so at Canning in 2011 where CDI007 and CDI005 yielded the same (Table 4.34).

Yields increased with an increase in N input at both Truro and Canning in 2011 (Table 4.35 and Figure 4.23), and high ratios (74.1% and 98.4%) of variability of the yields could be explained by the change of N rates at Truro and Canning in 2011 (Figure 4.23).

Yields increased with an increase in N. In general, plants at Canning and New Glasgow yielded more than Fredericton, and the yield at Truro was the lowest among these four locations in 2012 (Figure 4.24). Yields at all the four sites in 2012 were enhanced with an increase in N (Figure 4.24 and Figure 4.25), and very high ratios (96.6%-99.4%) of variability of the yields could be explained by the change of N levels at all of the four sites in 2012 (Figure 4.25).

Table 4.33: ANOVA table of seed yield of N trials in 2011 and 2012

Year	Location	Effect	F value	P value
2011	Truro	**Genotype (G)**	**23.99**	**<.0001**
		N	**11.14**	**<.0001**
		G*N	0.43	0.9836
2011	Canning	**G**	**10.32**	**<.0001**
		N	**24.51**	**<.0001**
		G*N	1.12	0.3532
2012	Truro, Canning, New Glasgow, Fredericton	Rep	20.12	<.0001
		Location (L)	80.35	<.0001
		G	**13.48**	**<.0001**
		L*G	1.22	0.2673
		N	163.38	<.0001
		L*N	**3.25**	**<.0001**
		G*N	1.11	0.3333
		L*G*N	0.81	0.8361

(no transformation)

Table 4.34: Effect of genotype on seed yield (kg/ha) in 2011 and 2012

Genotype	Truro, 2011	Canning, 2011	NS, Fredericton & New Glasgow, 2012
	Yield (kg/ha)	Yield (kg/ha)	Yield (kg/ha)
CDI007	1435 a	1318 a	1616 a
Calena	1251 b	805 b	1512 b
CDI002	1167 bc	864 b	1452 c
CDI005	1108 cd	1182 a	1446 c
CDI008	1040 d	899 b	1420 c

(Means in each column with a common letter are not significantly different at the 5% level)

Table 4.35: Effect of N on seed yield (kg/ha) at Truro and Canning in 2011

N (kg/ha)	Truro yield (kg/ha)	Canning yield (kg/ha)
0	1064 d	450 d
25	1080 d	686 c
50	1184 c	941 b
100	1320 ab	1340 a
150	1230 bc	1355 a
200	1326 a	1308 a

(Means in each column with a common letter are not significantly different at the 5% level)

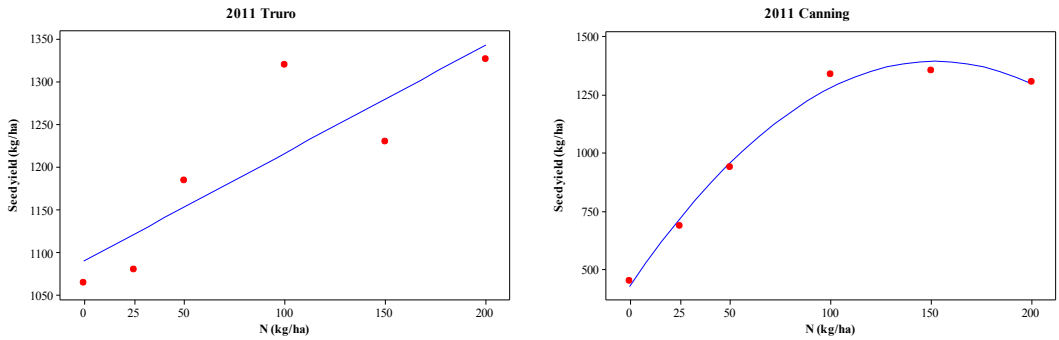


Figure 4.23: Regression analysis of N on yield at Canning and Truro in 2011 (each dot represents the mean of 20 samples)

(1) Y(2011, Truro)=1090+1.267N with R-Sq=74.1%

(2) Y(2011, Canning)=426.3+12.76N-0.04197N**2 with R-Sq(adj)=98.4%

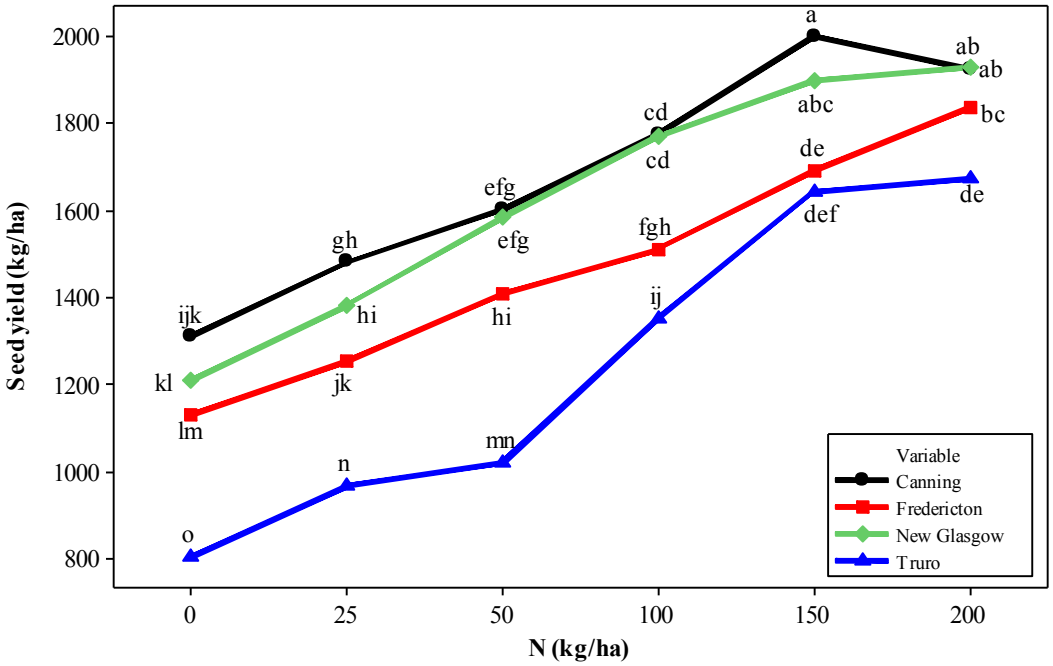


Figure 4.24: Effect of interaction of location and genotype on seed yields in 2012
(Means with a common letter are not significantly different at the 5% level)

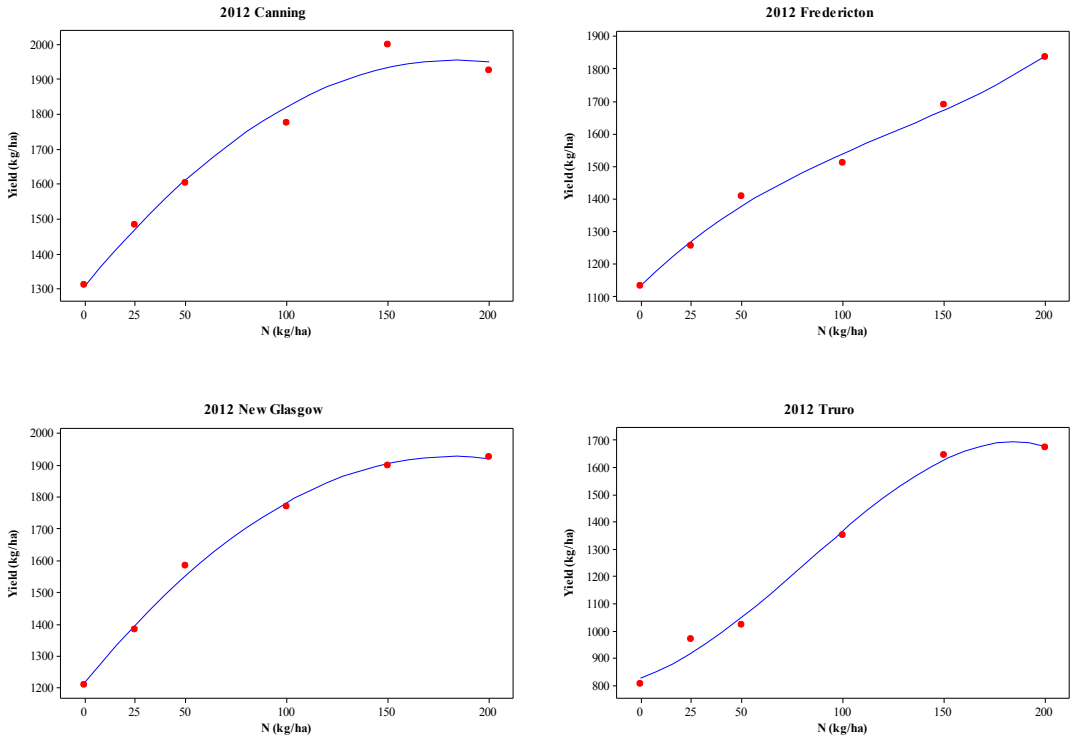


Figure 4.25: Regression analysis of N on yield at different locations in 2012 (each dot represents the mean of 20 samples)

(1) Y(2012,Canning)=1306+7.090N-0.01939N**2 with R-Sq(adj)=96.6%

(2) Y(2012,Fredericton)=1132+6.224N-0.02950N**2+0.000081N**3 with R-Sq(adj)=98.5%

(3) Y(2012,New Glasgow)=1214+7.851N-0.02156N**2 with R-Sq(adj)=99.4%

(4) Y(2012,Truro)=827.4+2.489N+0.04933N**2-0.000203N**3 with R-Sq(adj)=98.3%

4.5.12 Protein content

Genotype and N independently had significant effects on the seed protein content of camelina at Truro in 2011; the interaction of genotype and N had a significant effect on the protein content at Canning in 2011; genotype and the interaction of location and N had significant effects on the protein content at Canning, Truro, New Glasgow and Fredericton in 2012 (Table 4.36).

The seed protein content of CDI002 was significantly higher than the other four entries at Truro in 2011 (Table 4.37). CDI002 had the highest protein content at all sites

in 2012, but not significantly more than CDI008. CDI007 had the least protein content at Truro in 2011 and all of the sites in 2012 (Table 4.37).

Camelina seed protein content rose with an increase in N supply at Truro in 2011 (Table 4.38 and Figure 4.26), 93.8% of variability of protein content could be explained by the change of N at Truro in 2011 (Figure 4.26).

N was positively correlated with the protein content at Canning in 2011 (Figure 4.27) with the coefficient of determination ranging from 72.7% to 98.2% for different genotypes. CDI007 had the lowest content of protein among these 5 genotypes at Canning in 2011 (Figure 4.28). The optimum N rates for the highest protein content were 100 kg N/ha for Calena, CDI002, CDI005 and CDI008; 150 kg N/ha for CDI007 at Canning in 2011 (Figure 4.28).

The percent of protein increased with an increase in N at all of the sites in 2012 (Figure 4.29). In general, the percent of protein was the highest at Fredericton among these four locations (Figure 4.29). The regression models between N and protein content at Canning, Fredericton, New Glasgow and Truro in 2012 were shown in Figure 4.30. The content of protein increased with an increase in N input, and high ratios (97.2%-99.8%) of variability of the percent of protein could be explained by the change of N (Figure 4.30).

Table 4.36: ANOVA table of protein content in 2011 and 2012

Year	Location	Effect	F value	P value
2011	Truro	**Genotype (G)**	**3.50**	**0.0105**
		N	**20.25**	**<.0001**
		G*N	1.02	0.4445
2011	Canning	G	21.14	<.0001
		N	107.90	<.0001
		G*N	**2.55**	**0.0033**
2012	Truro, Canning, New Glasgow, Fredericton	Rep	11.45	<.0001
		Location (L)	97.80	<.0001
		G	**57.79**	**<.0001**
		L*G	1.74	0.0576
		N	415.12	<.0001
		L*N	**19.95**	**<.0001**
		G*N	1.15	0.3011
		L*G*N	0.84	0.7978

(no transformation)

Table 4.37: Effect of genotype on protein content in 2011 and 2012

Genotype	Truro, 2011	NS, Fredericton & New Glasgow, 2012
	Protein %	Protein %
CDI002	28.27 a	26.28 a
CDI008	27.40 b	26.22 ab
Calena	27.38 b	25.48 c
CDI005	27.35 b	26.07 b
CDI007	27.01 b	25.07 d

(Means in each column with a common letter are not significantly different at the 5% level)

Table 4.38: Effect of N on protein content at Truro in 2011

N (kg/ha)	Protein content %
0	26.0 c
25	26.2 c
50	27.1 b
100	28.3 a
150	29.0 a
200	28.3 a

(Means with a common letter are not significantly different at the 5% level)

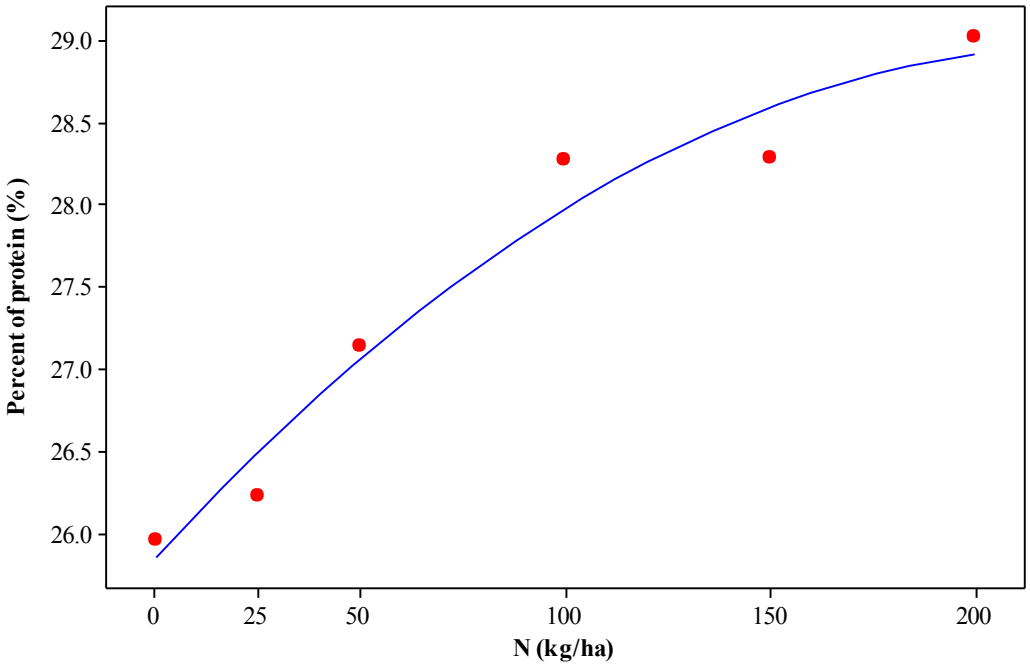


Figure 4.26: Regression analysis of N on protein content at Truro in 2011
(each dot represents the mean of 20 samples)
Y(2011, Truro)=25.85+0.02712N-0.000059N**2 with R-Sq(adj)=93.8%

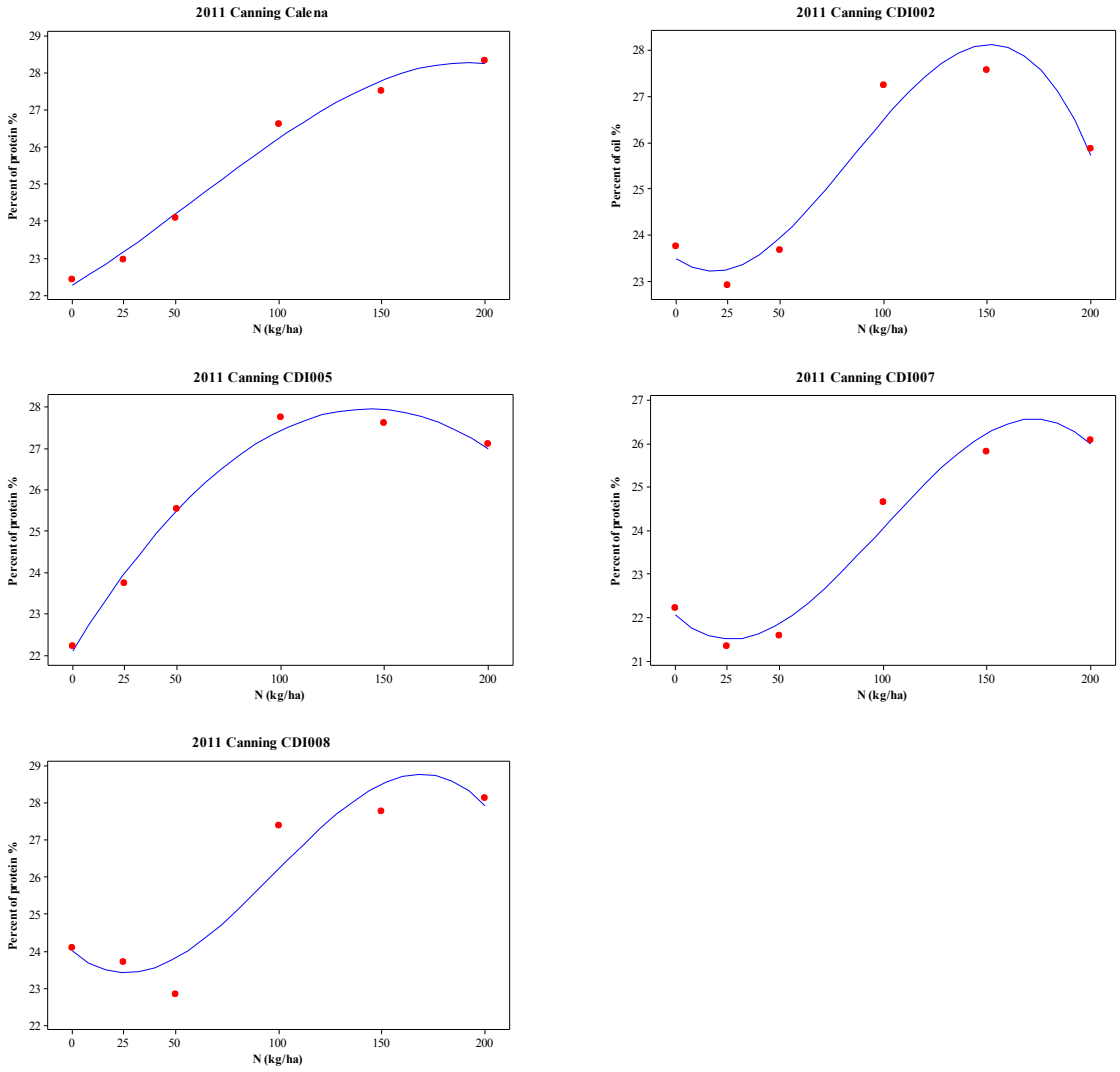


Figure 4.27: Regression analysis of N on protein content (%) at Canning in 2011
(each dot represents the mean of 4 samples)

(1) Y(2011,Canning,Calena)=22.27+0.03304N+0.000148N**2-0.000001N**3 with R-Sq(adj)=97.5%

(2) Y(2011,Canning,CDI002)=23.50-0.03310N+0.001041N**2-0.000004N**3 with R-Sq(adj)=85.9%

(3) Y(2011,Canning,CDI005)=22.10+0.08216N-0.000289N**2 with R-Sq(adj)=98.2%

(4) Y(2011,Canning,CDI007)=22.05-0.04425N+0.000967N**2-0.000003N**3 with R-Sq(adj)=93.0%

(5) Y(2011,Canning,CDI008)=24.00-0.04804N+0.001066N**2-0.000004N**3 with R-Sq(adj)=72.7%

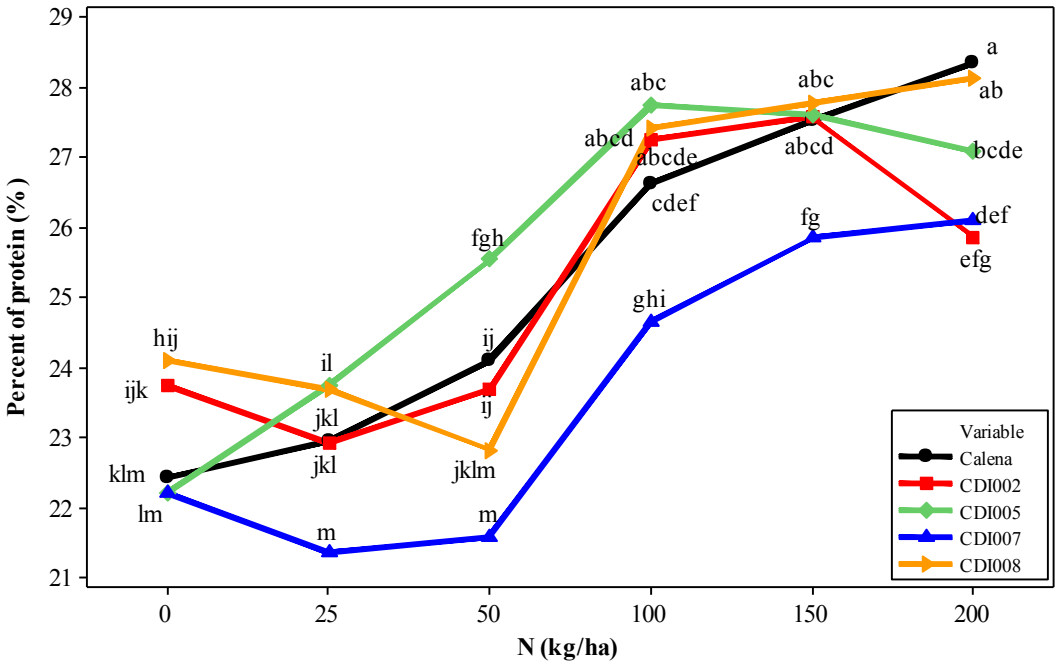


Figure 4.28: Effect of interaction of genotype and N on protein content (%) at Canning in 2011

(Means with a common letter are not significantly different at the 5% level)

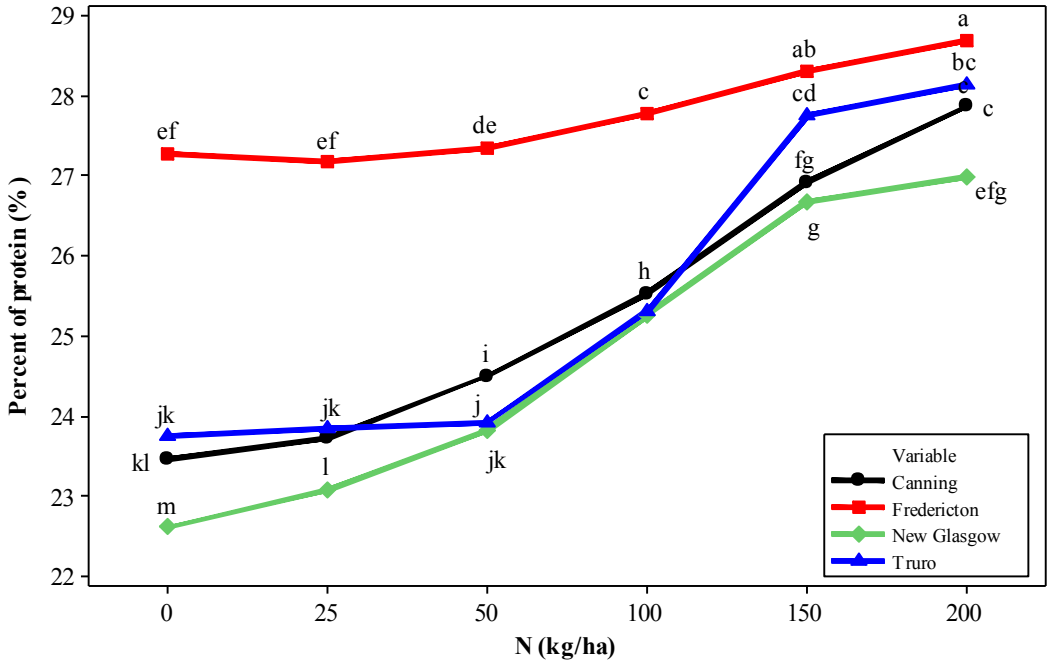


Figure 4.29: Effect of interaction of location and N in the percent of protein in 2012

(Means with a common letter are not significantly different at the 5% level)

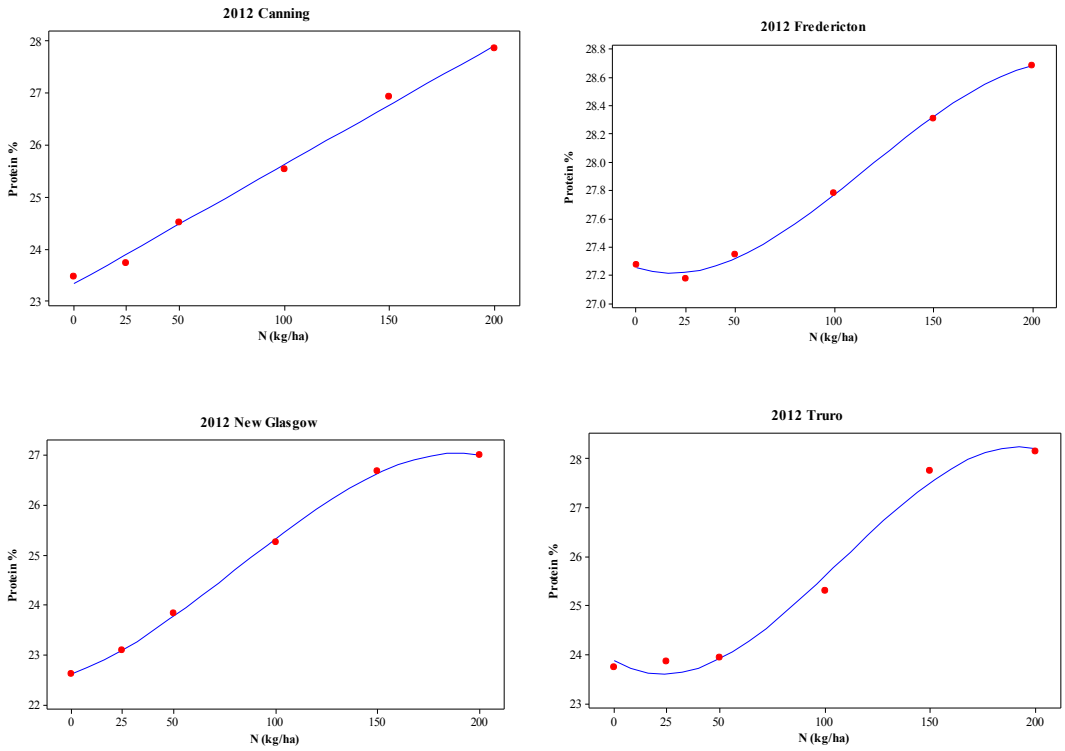


Figure 4.30: Regression analysis of N on protein % in 2012
(each dot represents the mean of 20 samples)

(1) Y(2012,Canning)=23.33+0.02294N with R-Sq=99.5%

(2) Y(2012,Fredericton)=27.25-0.004395N+0.000134N**2-0.000000N**3 with R-Sq(adj)=99.5%

(3) Y(2012,NewGlasgow)=22.62+0.01435N+0.000217N**2-0.000001N**3 with R-Sq(adj)=99.8%

(4) Y(2012,Truro)=23.88-0.02556N+0.000619N**2-0.000002N**3 with R-Sq(adj)=97.2%

4.5.13 Protein Yield

Genotype and N independently had significant effects on the protein yield at Truro in 2011; genotype and the interaction of location and N had significant effects on the protein yield at the four locations in 2012 (Table 4.39).

The protein yield of CDI007 was the highest at Truro in 2011, which was consistent at the four sites in 2012 (Table 4.40). At Truro in 2011, the protein yield of Calena and CDI002 was significantly higher than CDI005 and CDI008. At the four sites in 2012, however, there was no significant difference in the protein yield of Calena, CDI002, CDI005 and CDI008 (Table 4.40).

The protein yield increased with an increase in N at Truro in 2011 (Table 4.41 and Figure 4.31), and 86.0% of variability of the protein yield could be explained by the change of N rate (Figure 4.31).

The protein yield increased with the increase of N at all of the four sites in 2012. In general, the protein yield at Canning, Fredericton and New Glasgow were similar, which were significantly higher than Truro (Figure 4.32). High ratios (97.7%-99.6%) of variability of the protein yield could be explained by the change of N rate at four different locations in 2012 (Figure 4.33).

Table 4.39: ANOVA table of protein yield in 2011 and 2012

Year	Location	Effect	F value	P value
2011	Truro	**Genotype (G)**	**19.20**	**<.0001**
		N	**19.78**	**<.0001**
		G*N	0.67	0.8406
2012	Truro, Canning, New Glasgow, Fredericton	Rep	19.85	<.0001
		Location (L)	71.73	<.0001
		G	**5.43**	**0.0003**
		L*G	1.02	0.4299
		N	268.61	<.0001
		L*N	**4.30**	**<.0001**
		G*N	1.06	0.3921
		L*G*N	0.85	0.7842

(2012: square root transformation)

Table 4.40: Effect of genotype on protein yield in 2011 and 2012

Year	Location	Genotype	Protein Yield (kg/ha)
2011	Truro	CDI007	389 a
		Calena	344 b
		CDI002	331 b
		CDI005	304 c
		CDI008	286 c
2012	Canning, Fredericton, New Glasgow, Truro	CDI007	407 a
		Calena	387 b
		CDI002	381 b
		CDI005	381 b
		CDI008	372 b

(Means with the same year – 2011 or 2012 with a common letter are not significantly different at the 5% level)

Table 4.41: Effect of N on protein yield at Truro in 2011

N (kg/ha)	Protein yield (kg/ha)
0	276 c
25	283 c
50	320 b
100	373 a
150	357 a
200	375 a

(Means with a common letter are not significantly different at the 5% level)

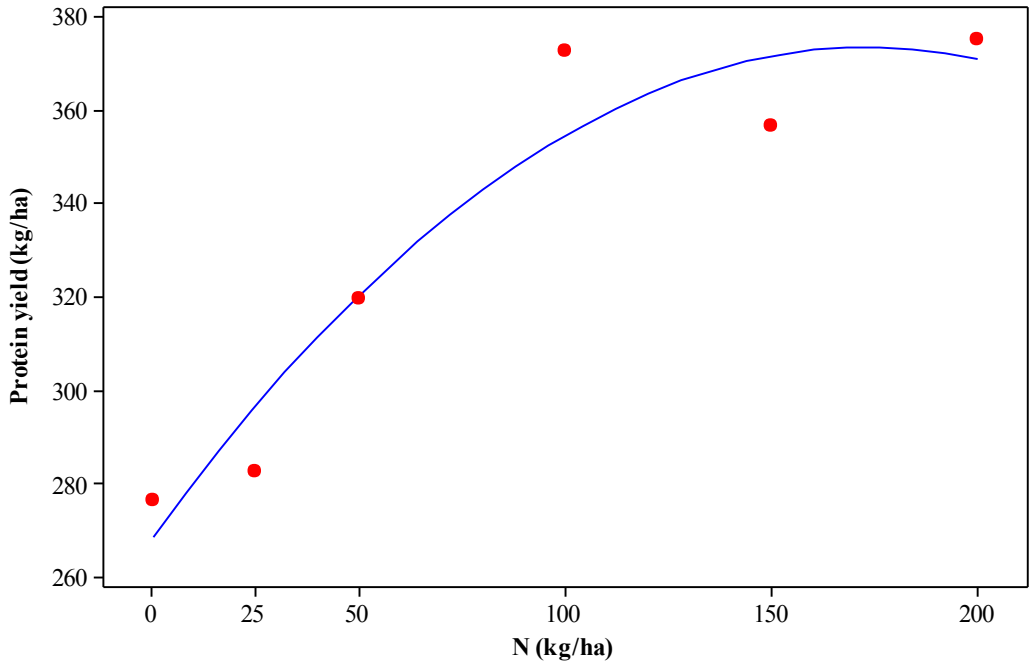


Figure 4.31: Regression analysis of N on protein yield at Truro in 2011
(each dot represents the mean of 20 samples)
Y(2011,Truro)=268.8+1.209N-0.003488N**2 with R-Sq(adj)=86.0%

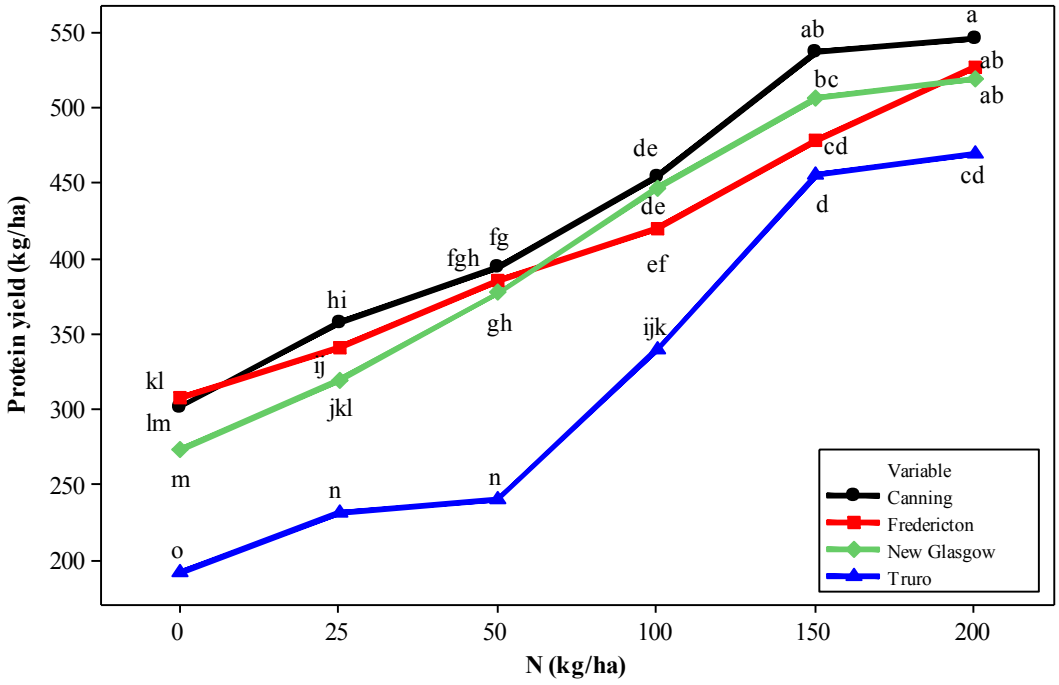


Figure 4.32: Effect of interaction of location and N on protein yield in 2012

(Means with a common letter are not significantly different at the 5% level)

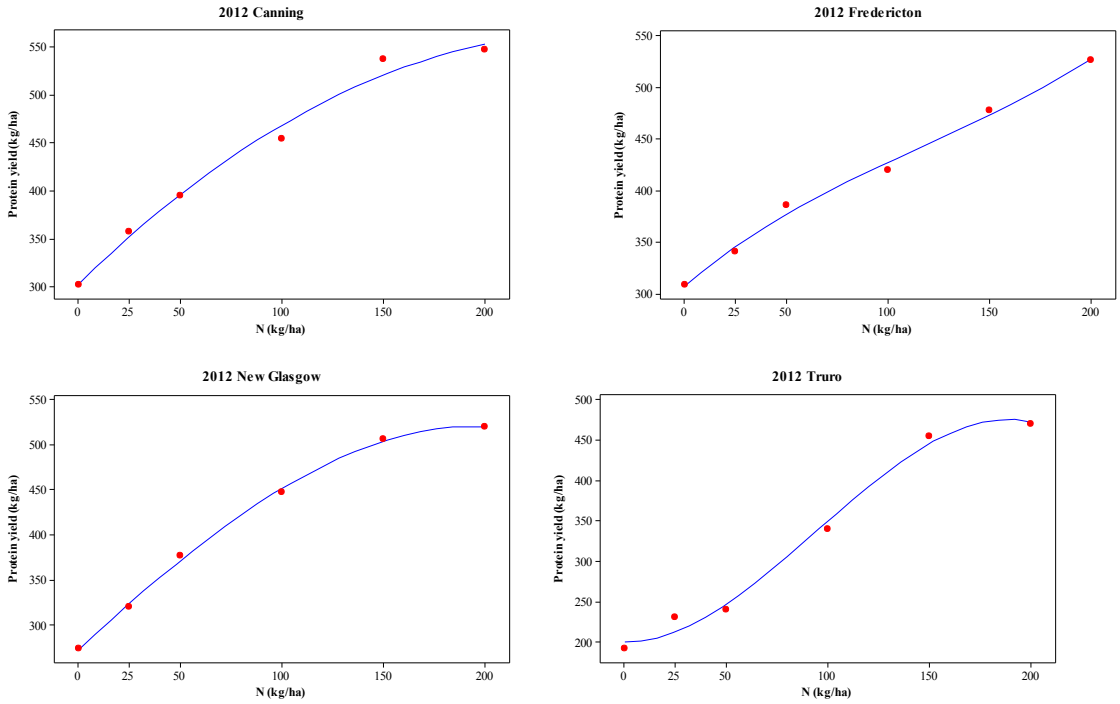


Figure 4.33: Regression analysis of N on protein yield at four different locations in 2012

(each dot represents the mean of 20 samples)

(1) Y(Canning)=303.0+2.058N-0.004050N**2 with R-Sq(adj)=98.2%

(2) Y(Fredericton)=307.9+1.649N-0.006282N**2+0.000018N**3 with R-Sq(adj)=98.7%

(3) Y(New Glasgow)=272.9+2.096N-0.001912N**2-0.000012N**3 with R-Sq(adj)=99.6%

(4) Y(Truro)=200.0-0.0240N+0.02354N**2-0.000083N**3 with R-Sq(adj)=97.7%

4.5.14 Oil Content

Genotype and N independently had significant effects on the seed oil content of camelina at Truro in 2011; the interaction of genotype and N had significant effects on the content of oil at Canning in 2011; the interaction of location and genotype and the interaction of location and genotype had significant effects on the content of oil at Truro, Canning, New Glasgow and Fredericton in 2012 (Table 4.42).

The seed oil content of CDI007, CDI002, Calena and CDI005 was similar, which was significantly higher than that of CDI008 at Truro in 2011 (Table 4.43).

Camelina seed oil content decreased with an increase in N at Truro in 2011 (Table 4.43 and Figure 4.34), and 97.5% of variability of the percent of oil could be explained by the change of N rate (Figure 4.34).

The oil content decreased with an increase in N at Canning in 2011 (Figure 4.35 and Figure 4.36), and high ratios of variability of the percent of oil could be explained by the change of N (Figure 4.36). CDI007 and CDI002 had higher oil content among these 5 genotypes at Canning in 2011 (Figure 4.35).

Different genotypes behaved differently at different locations. CDI007 at Canning, New Glasgow and Truro produced the highest content of oil among these five genotypes. At Fredericton, CDI007 was the richest in oil, which had no significant difference from CDI002 and CDI005 in 2012 (Figure 4.37).

The percent of oil decreased with an increase in N at four different sites in 2012 (Figure 4.38). The percent of oil at New Glasgow and Truro was significantly higher than Canning and Fredericton. N was negatively correlated with the oil content at all 4 sites in 2012 (Figure 4.39) with the coefficient of determination ranging from 93.7% to 98.8%.

Table 4.42: ANOVA table of the content of oil in 2011 and 2012

Year	Location	Effect	F value	P value
2011	Truro	**Genotype (G)**	**3.50**	**0.0105**
		N	**20.25**	**<.0001**
		G*N	1.02	0.4445
2011	Canning	G	21.14	<.0001
		N	107.90	<.0001
		G*N	**2.55**	**0.0033**
2012	Truro, Canning, New Glasgow, Fredericton	Rep	3.07	0.0166
		Location (L)	36.24	<.0001
		G	57.66	<.0001
		L*G	**2.82**	**0.0011**
		N	144.65	<.0001
		L*N	**4.52**	**<.0001**
		G*N	1.48	0.0848
		L*G*N	0.95	0.5813

(no transformation)

Table 4.43: Effect of genotype and N on the oil content at Truro in 2011

Genotype	N (kg/ha)	Oil percent (%)
CDI007		39.6 a
CDI002		39.5 a
Calena		39.4 a
CDI005		39.3 a
CDI008		38.5 b
	0	40.6 a
	25	40.3 a
	50	39.5 b
	100	38.6 c
	150	38.5 c
	200	38.0 c

(Means with a common letter are not significantly different at the 5% level)

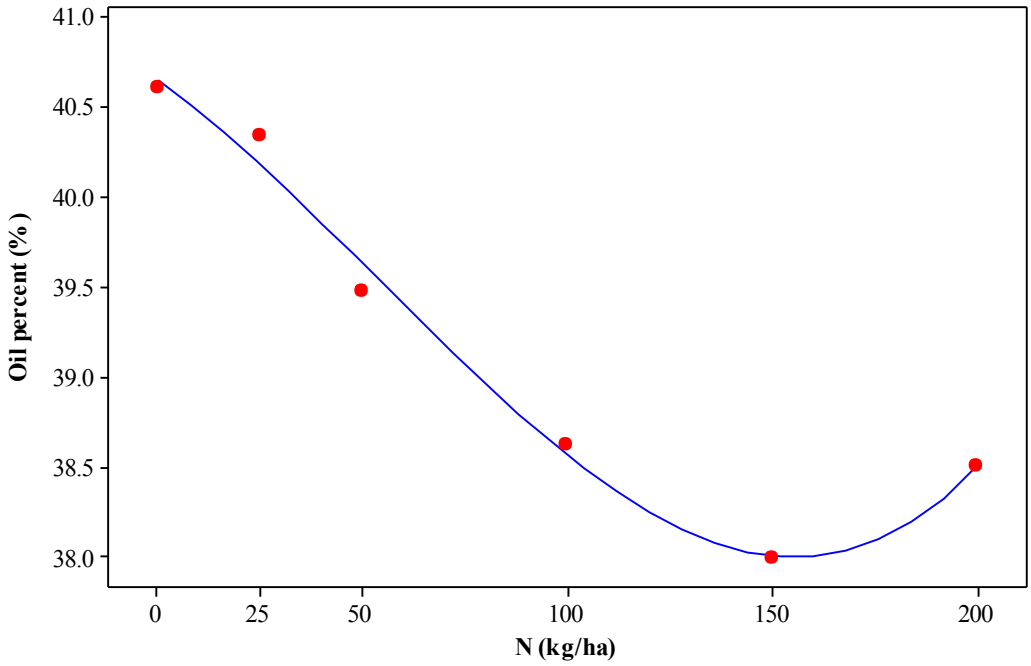


Figure 4.34: Regression analysis of N on oil content at Truro in 2011
(each dot represents the mean of 20 samples)

Y(2011, Truro)=40.65-0.01640N-0.000118N**2+0.000001N**3 with R-Sq(adj)=97.5%

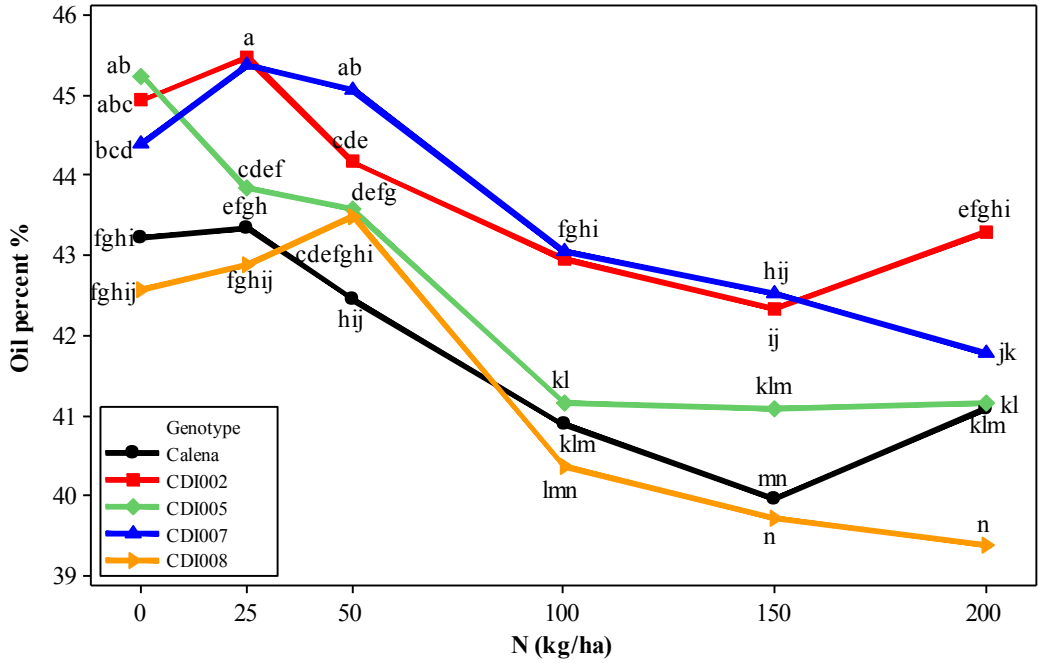


Figure 4.35: Effect of genotype and N on oil content at Canning in 2011
(Means with a common letter are not significantly different at the 5% level)

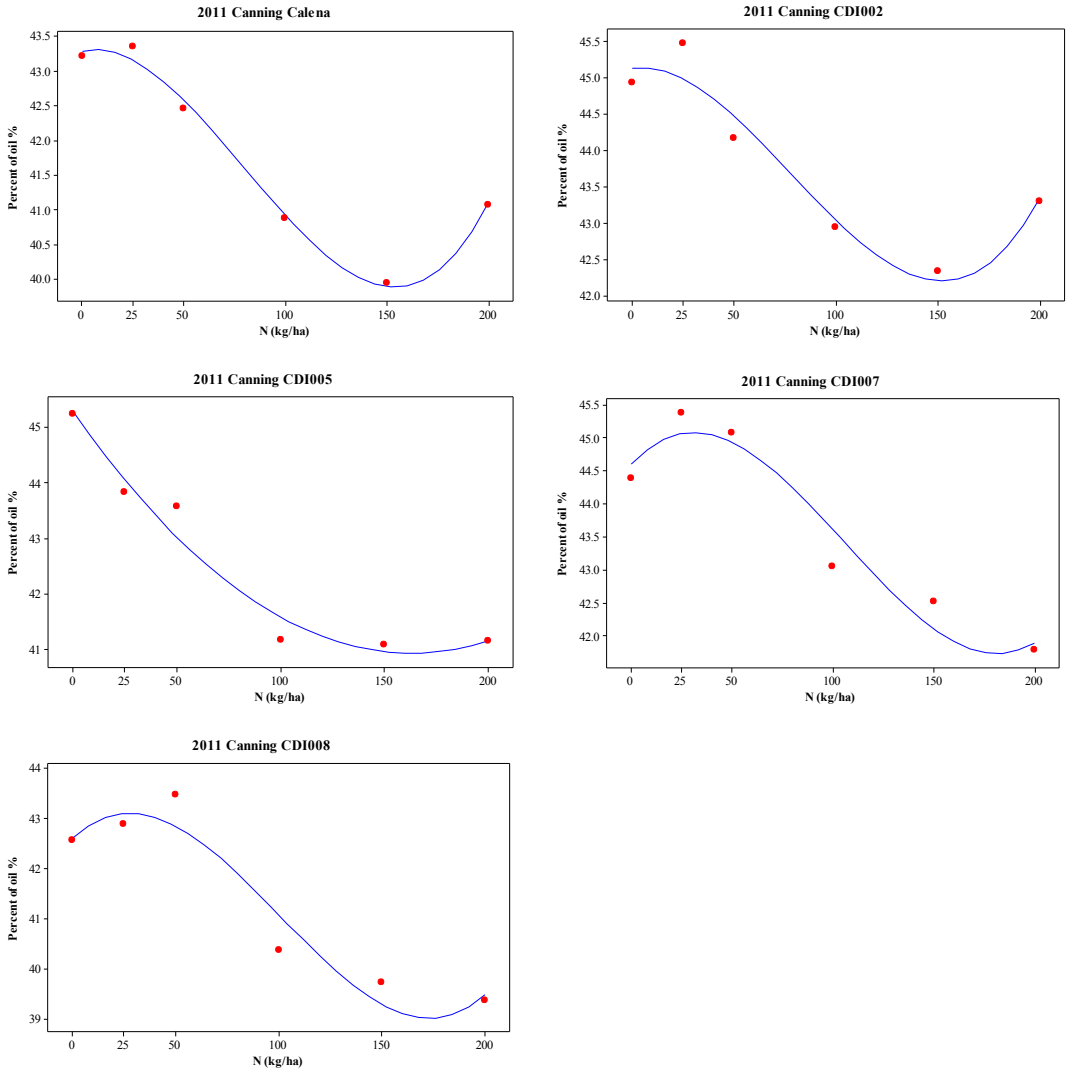


Figure 4.36: Regression analysis of N on oil % of 5 genotypes at Canning in 2011
(each dot represents the mean of 4 samples)

(1) Y(2011,Canning,Calena)=43.29+0.005859N-0.000506N**2+0.000002N**3 with R-Sq(adj)=98.4%

(2) Y(2011,Canning,CDI002)=45.13+0.00342N-0.000424N**2+0.000002N**3 with R-Sq(adj)=86.4%

(3) Y(2011,Canning,CDI005)=45.29-0.05346N+0.000164N**2 with R-Sq(adj)=94.3%

(4) Y(2011,Canning,CDI007)=44.60+0.03291N-0.000624N**2+0.000002N**3 with R-Sq(adj)=84.6%

(5) Y(2011,Canning,CDI008)=42.60+0.03876N-0.000808N**2+0.000003N**3 with R-Sq(adj)=81.8%

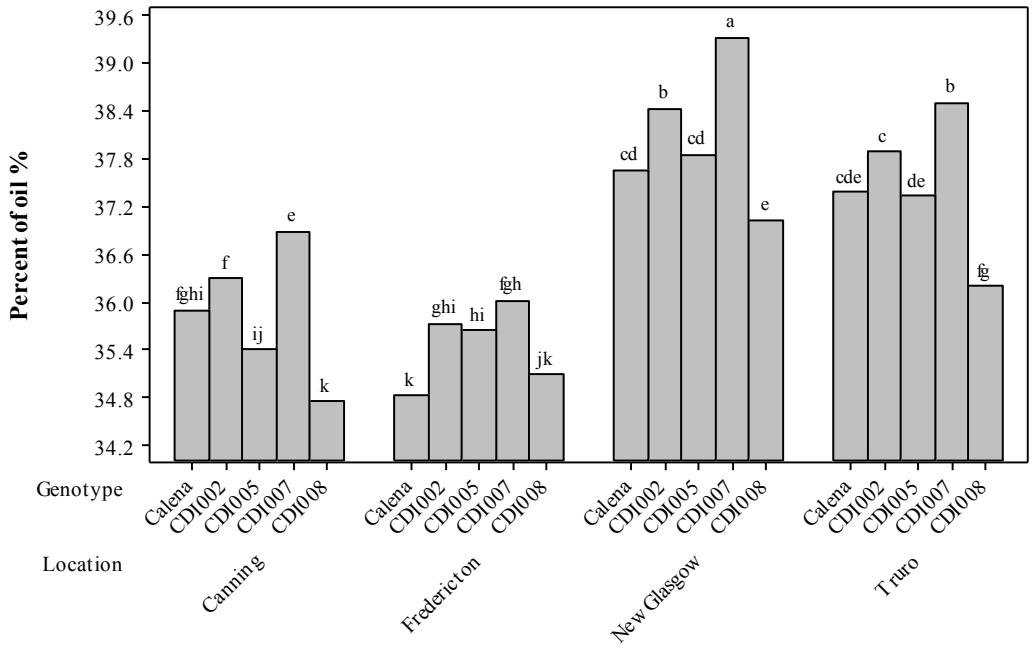


Figure 4.37: Effect of location and genotype on the percent of oil in 2012
(Means with a common letter are not significantly different at the 5% level)

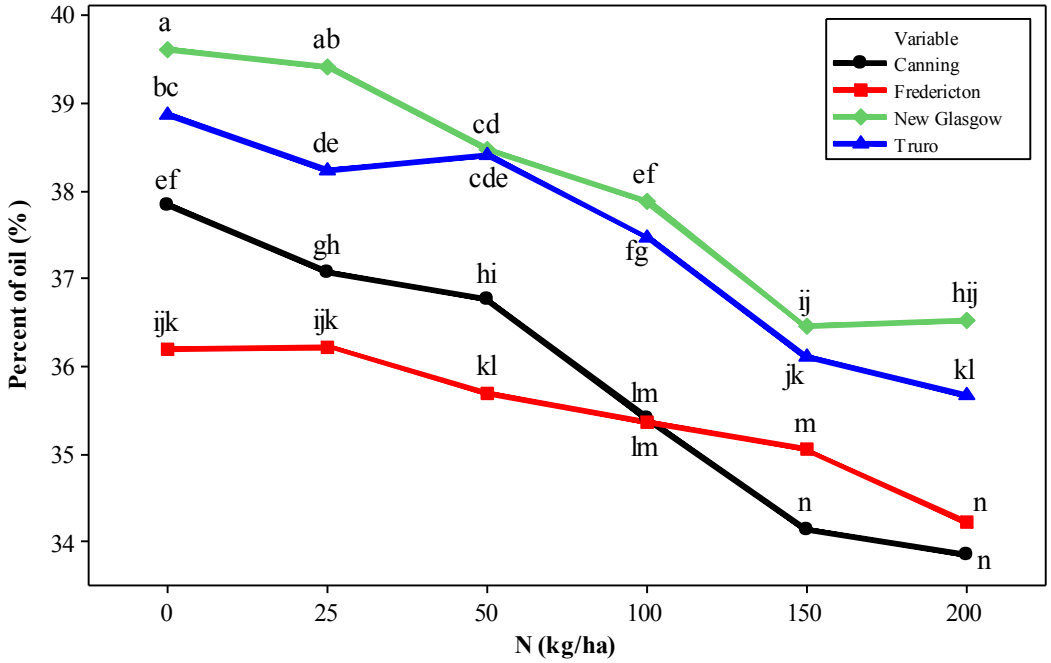


Figure 4.38: Effect of location and N on the percent of oil at four different sites in 2012

(Means with a common letter are not significantly different at the 5% level)

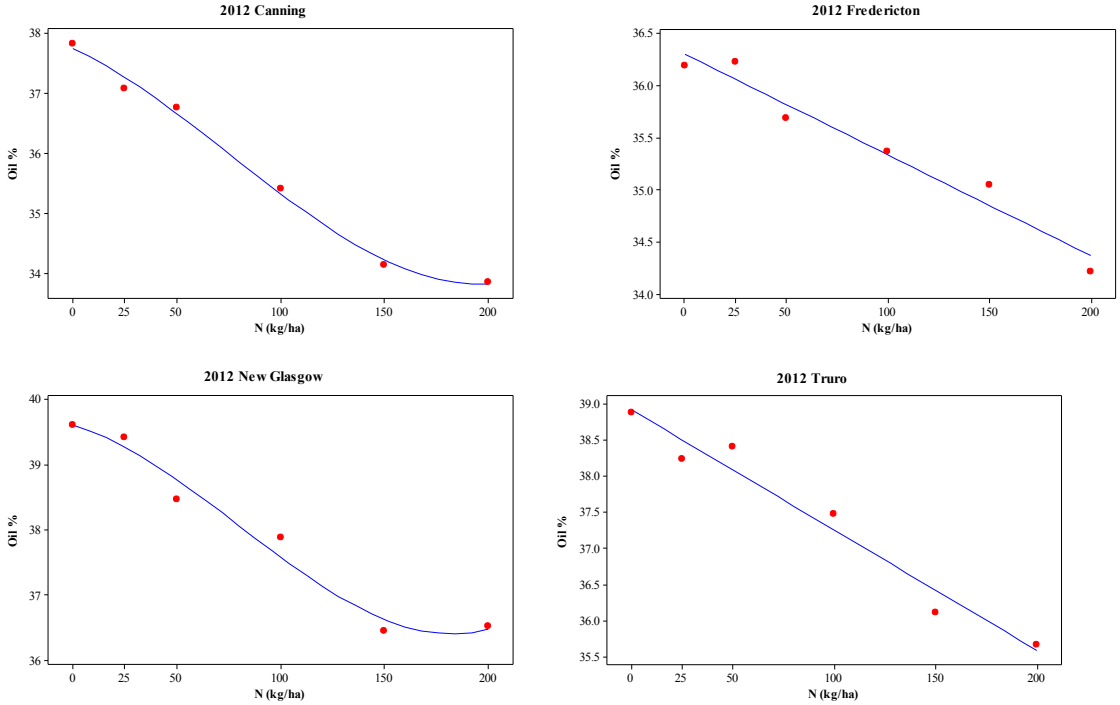


Figure 4.39: Regression analysis of N on oil content % in 2012

(each dot represents the mean of 20 samples)

(1) Y(2012,Canning)=37.75-0.01590N-0.000148N**2+0.000001N**3 with R-Sq(adj)=98.8%

(2) Y(2012,Fredericton)=36.31-0.009690N with R-Sq=95.9%

(3) Y(2012,NewGlasgow)=39.61-0.00950N-0.000185N**2+0.000001N**3 with R-Sq(adj)=93.7%

(4) Y(2012,Truro)=38.91-0.01662N with R-Sq=96.1%

4.5.15 Oil Yield

Genotype and N independently had significant effects on the oil yield at Truro in 2011; genotype and the interaction of location and N had significant effects on the oil yield at the four locations in 2012 (Table 4.44).

CDI007 had the highest oil yield in Truro in 2011 and in the four locations in 2012. Calena had the second highest oil yield in 2011 and 2012, which had no significant difference from CDI002. CDI005 and CDI008 in Truro in 2011 and CDI008 in 2012 had the lowest oil yield in all of the four sites in 2012 (Table 4.45).

The oil yield at Truro in 2011 increased with an increase in N, and 63.5% of variability of oil yield could be explained by the change of N rate (Figure 4.40).

Oil yields were positively correlated with the N input at all of the four locations in 2012 (Figure 4.41). The oil yield at Canning and New Glasgow were similar, which were significantly higher than the oil yield at Fredericton and Truro. The oil yield at Truro was lower than Fredericton with N rates ranging from 0 kg N/ha to 50 kg N/ha (Figure 4.41). High ratios (97.8%-99.7%) of variability of oil yield could be explained by the change of N rate in the four locations in 2012 (Figure 4.42).

Table 4.44: ANOVA table of oil yield in 2011 and 2012

Year	Location	Effect	F value	P value
2011	Truro	**Genotype (G)**	**23.36**	**<.0001**
		N	**5.8**	**0.0001**
		G*N	0.35	0.995
2012	Truro, Canning, New Glasgow, Fredericton	Rep	20.52	<.0001
		Location (L)	79.62	<.0001
		G	**26.03**	**<.0001**
		L*G	1.32	0.2021
		N	117.87	<.0001
		L*N	**2.61**	**0.001**
		G*N	1.53	0.0689
		L*G*N	0.87	0.7464

(no transformation)

Table 4.45: Effect of N on oil yield in 2011 and 2012

Year	Location	Genotype	Oil yield (kg/ha)
2011	Truro	CDI007	567 a
		Calena	492 b
		CDI002	461 bc
		CDI005	435 cd
		CDI008	400 d
2012	Canning, Fredericton, New Glasgow, Truro	CDI007	616 a
		Calena	558 b
		CDI002	543 bc
		CDI005	536 c
		CDI008	514 d

(Means with the same year – 2011 or 2012 with a common letter are not significantly different at the 5% level)

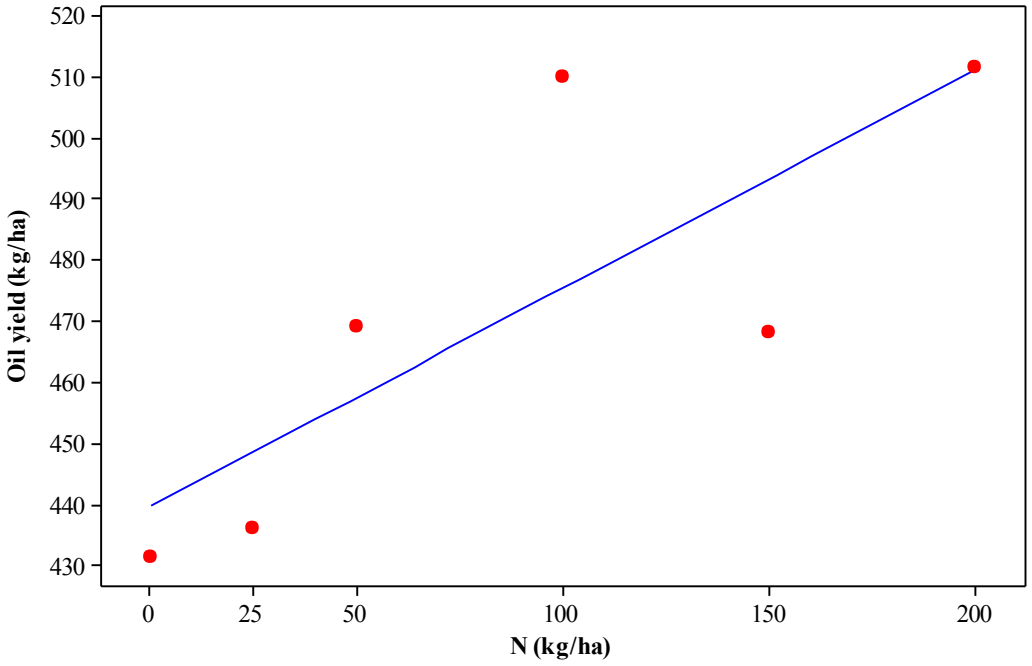


Figure 4.40: Regression analysis of N on oil yield at Truro in 2011
(each dot represents the mean of 20 samples)
Y(2011,Truro)=439.7+0.3577N with R-Sq=63.5%

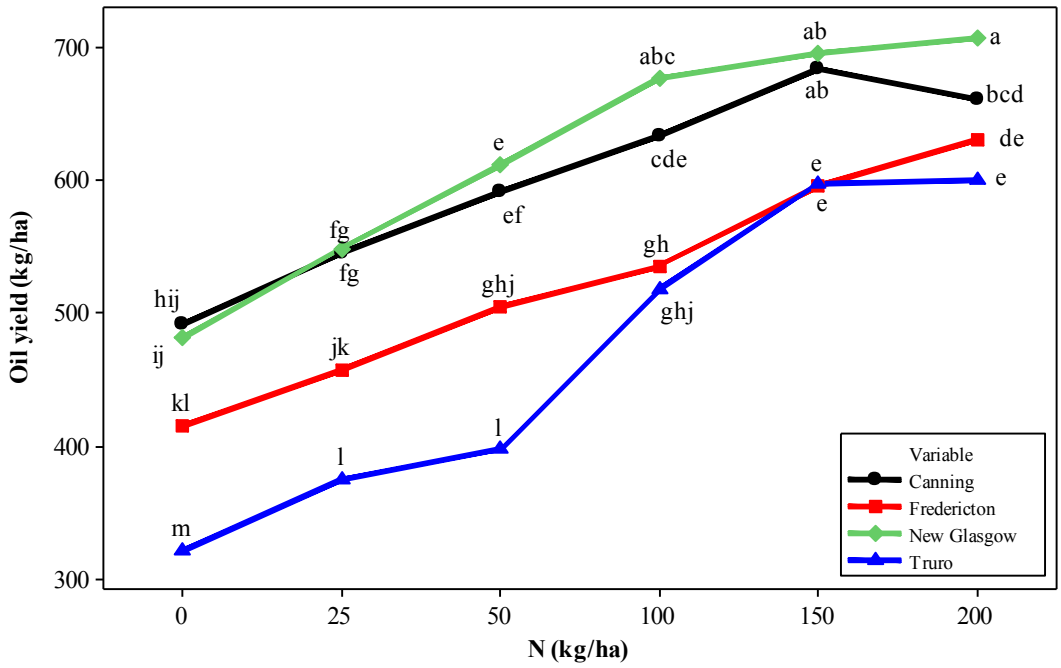


Figure 4.41: Effect of interaction of location and N on the oil yield in 2012
(Means with a common letter are not significantly different at the 5% level)

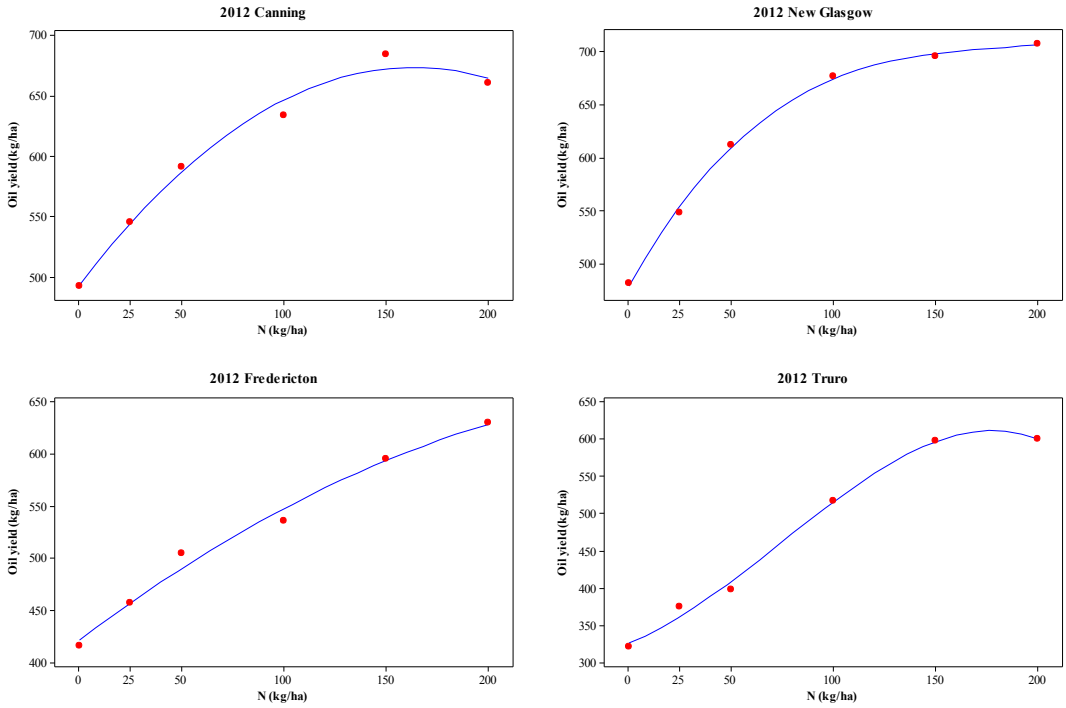


Figure 4.42: Regression analysis of N on oil yield at four different locations in 2012
(each dot represents the mean of 20 samples)

(1) Y(Canning)=493.1+2.209N-0.006769N**2 with R-Sq(adj)=97.8%

(2) Y(Fredericton)=421.2+1.493N-0.002269N**2 with R-Sq(adj)=98.0%

(3) Y(New Glasgow)=497.4+3.424N-0.01810N**2+0.000033N**3 with R-Sq(adj)=99.7%

(4) Y(Truro)=326.2+1.064N+0.01509N**2-0.000068N**3 with R-Sq(adj)=98.8%

4.5.16 Fatty acids

Camelina seed consists of 10 main fatty acids – C16:0, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, C20:2, C20:3 and C22:1. They were grouped into 3 categories – saturated fatty acids (C16:0, C18:0 and C20:0), monounsaturated fatty acids (C18:1, C20:1 and C22:1), and polyunsaturated fatty acids (C18:2, C18:3, C20:2 and C20:3).

(1) 2011 Truro N Trial

Genotypes differed in the percent of saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids depending on the amount of N at Truro in 2011 (Table 4.46).

CDI008 had the highest content of saturated fatty acids among these 5 genotypes, while CDI002 and CDI007 had the lowest percent of saturated fatty acids at Truro in 2011 (Figure 4.43). N was positively correlated with the percent of saturated fatty acids for Calena, CDI002, CDI005 and CDI007. The percent of saturated fatty acids of CDI008 increased when N increased from 0 kg N/ha to 100 kg N/ha, but it decreased when N was 150 kg N/ha, then it increased again when N was 200 kg N/ha (Figure 4.43).

The percent of monounsaturated fatty acids decreased with an increase in N at Truro in 2011 (Figure 4.44). CDI005 had the highest content of monounsaturated fatty acids

among these five genotypes (Figure 4.44).

N was positively correlated with the percent of polyunsaturated fatty acids depending on genotypes at Truro in 2011, which was true for Calena, CDI005, CDI007 and CDI008 (Figure 4.45). The content of polyunsaturated fatty acids of CDI002 kept almost unchanged when N increased from 0 kg N/ha to 100 kg N/ha, but it decreased when N was 150 kg N/ha, then it increased again when N was 200 kg N/ha (Figure 4.45).

Table 4.46: ANOVA table of fatty acid composition at Truro in 2011

Factors	Saturated FA		Monounsaturated FA		Polyunsaturated FA	
	F value	P value	F value	P value	F value	P value
Genotype (G)	95.32	<.0001	23.97	<.0001	29.06	<.0001
N	34.31	<.0001	55.09	<.0001	13.44	<.0001
G*N	**1.91**	**0.0207**	**2.52**	**0.0016**	**2.21**	**0.006**

(MUFA & PUFA: no transformation; Saturated FA: $\log_{10}$ transformation)

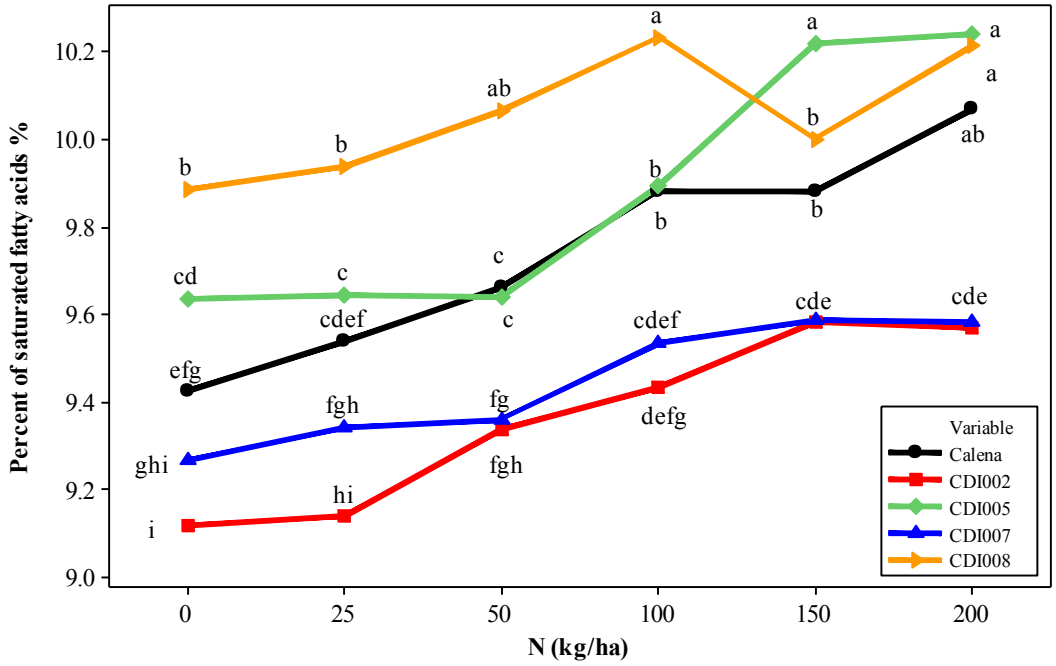


Figure 4.43: Effect of interaction of genotype and N on percent of saturated fatty acids at Truro in 2011

(Means with a common letter are not significantly different at the 5% level)

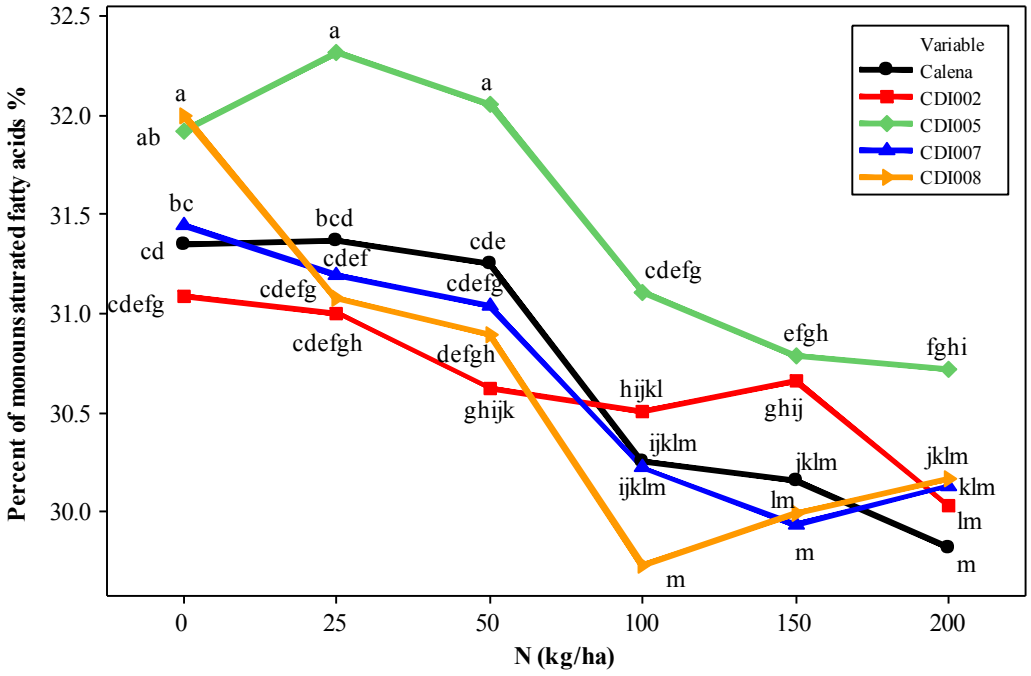


Figure 4.44: Effect of interaction of genotype and N on percent of monounsaturated fatty acids at Truro in 2011

(Means with a common letter are not significantly different at the 5% level)

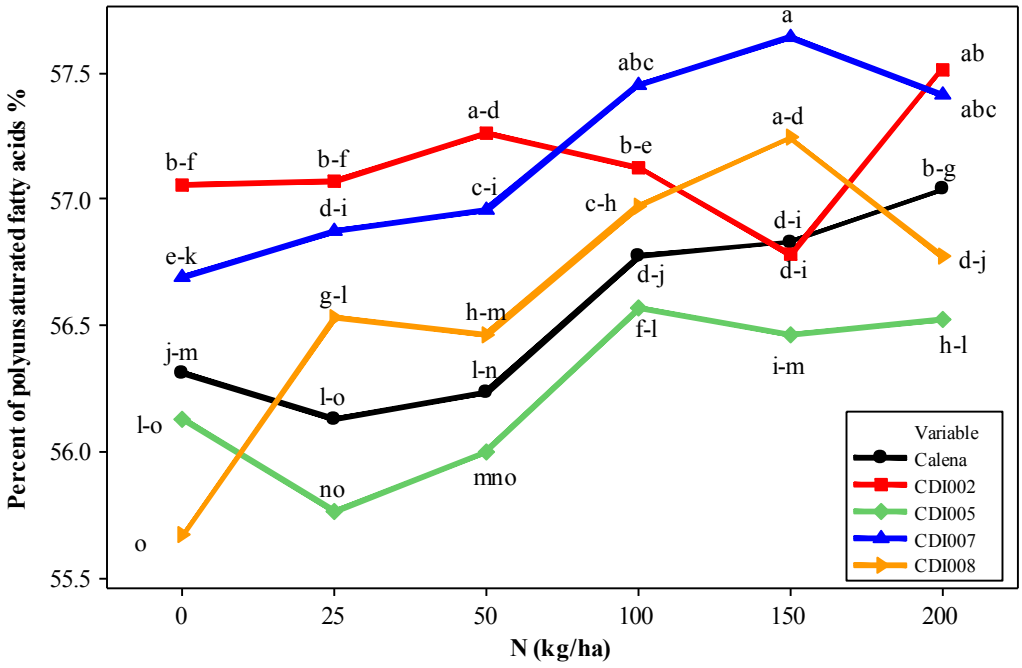


Figure 4.45: Effect of interaction of genotype and N on percent of polyunsaturated fatty acids at Truro in 2011

(Means with a common letter are not significantly different at the 5% level)

(2) 2012 N Trials at 4 Sites

Genotypes differed in the percent of saturated fatty acids depending on the amount of N and locations in 2012 (Table 4.47). Genotypes differed in the percent of monounsaturated fatty acids and polyunsaturated fatty acids depending on locations in 2012 (Table 4.47). Effect of N on the percent of monounsaturated fatty acids and polyunsaturated fatty acids differed depending on locations in 2012 (Table 4.47).

CDI008 had the highest amount of saturated fatty acids at Canning, New Glasgow and Truro with all N treatments in 2012, also at Fredericton when N ranged from 0 to 100 kg N/ha (Table 4.46). The percent of saturated fatty acids fluctuated when an increase in N for different genotypes at different locations in 2012 (Table 4.46).

CDI008, CDI005 and CDI002 at Canning, CDI008 and CDI005 at Fredericton, CDI005 at New Glasgow and CDI005 and CDI002 at Truro had the highest amount of monounsaturated fatty acids in 2012 (Figure 4.47) CDI005 had the highest content of monounsaturated fatty acids at all 4 sites in 2012, which was consistent with the results at Truro in 2011 (Figure 4.44).

The percent of monounsaturated fatty acids decreased with an increase in N at all 4 sites in 2012 (Figure 4.48). At Fredericton, the content of monounsaturated fatty acids decreased mildly when N increased from 0 kg N/ha to 200 kg N/ha, while at other locations, the content of monounsaturated fatty acids decreased greatly when N ranged from 0 kg N/ha to 200 kg N/ha (Figure 4.48).

CDI007, CDI002 and Calena at Canning and Fredericton, CDI007 and CDI002 at

New Glasgow and Truro had the highest amount of polyunsaturated fatty acids in 2012 (Figure 4.49). Plants at Truro produced the most content of polyunsaturated fatty acids compared to other locations in 2012 (Figure 4.49 and Figure 4.50).

N was positively correlated with the percent of polyunsaturated fatty acids at all 4 sites in 2012 (Figure 4.50). The percent of polyunsaturated fatty acids kept unchanged at Truro, Fredericton and Canning when N ranged from 0 kg N/ha to 50 kg N/ha. However, at Truro it increased greatly when N increased from 50 kg N/ha to 200 kg N/ha. At Fredericton and Canning, the percent of polyunsaturated fatty acids increased slightly when N increased from 50 kg N/ha to 200 kg N/ha. The content of polyunsaturated fatty acids kept unchanged with N ranging 0 to 25 kg N/ha, then it started to increase when N increased from 25 kg N/ha to 200 kg N/ha (Figure 4.50).

Table 4.47: ANOVA table of fatty acid composition at 4 sites in 2012

Effect	Saturated FA		Monounsaturated FA		Polyunsaturated FA	
	F value	P value	F value	P value	F value	P value
Rep	1.41	0.2316	6.71	<.0001	7.99	<.0001
Location (L)	65.02	<.0001	10.77	<.0001	39.9	<.0001
Genotype (G)	279.02	<.0001	111.38	<.0001	86.72	<.0001
L*G	13.22	<.0001	3.98	<.0001	1.98	0.0255
N	33.47	<.0001	251.27	<.0001	116.13	<.0001
L*N	7.88	<.0001	10.81	<.0001	9.41	<.0001
G*N	2.96	<.0001	1.06	0.3901	0.83	0.6726
L*G*N	1.91	0.0002	0.88	0.7199	0.76	0.9008

(no transformation)

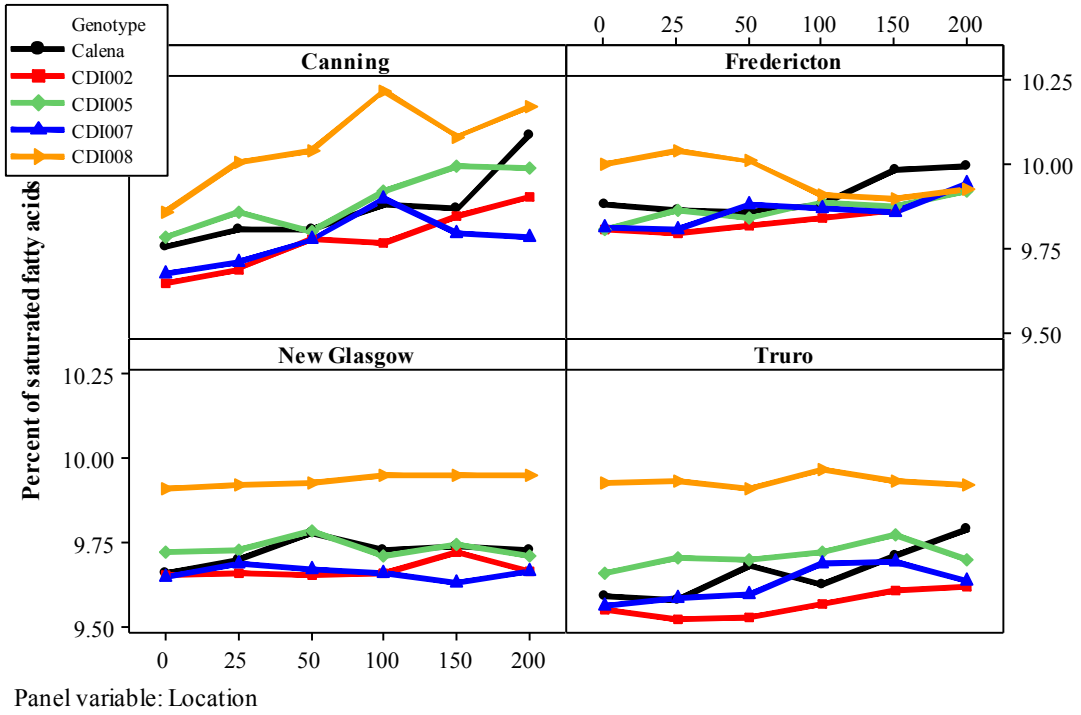


Figure 4.46: Effect of location, genotype and N on percent of saturated fatty acids % in 2012

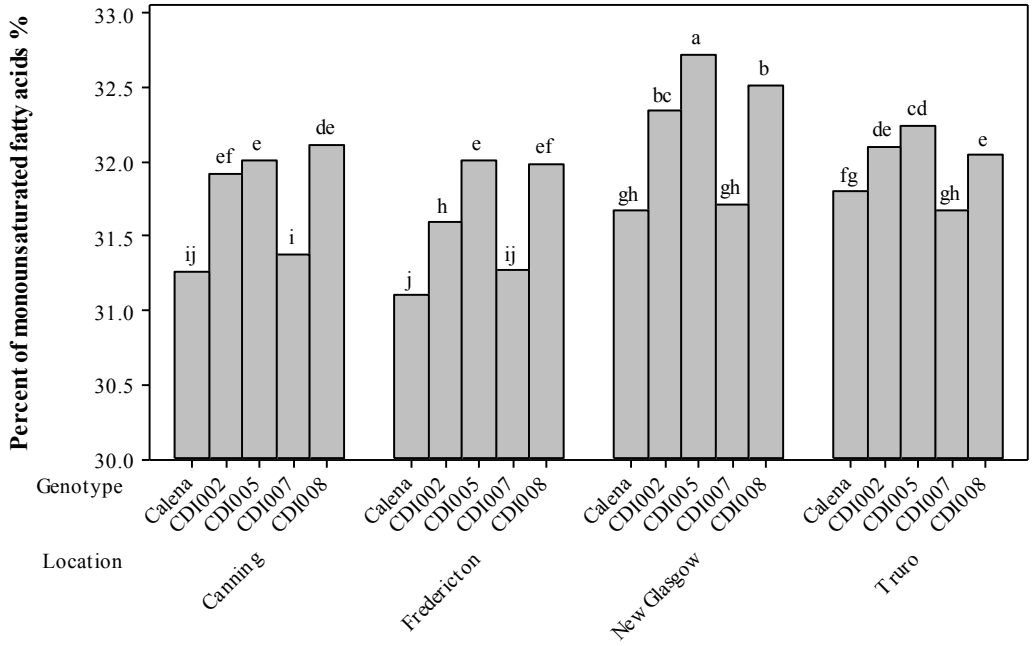


Figure 4.47: Effect of interaction of genotype and location on percent of monounsaturated fatty acids % in 2012
(Means with a common letter are not significantly different at the 5% level)

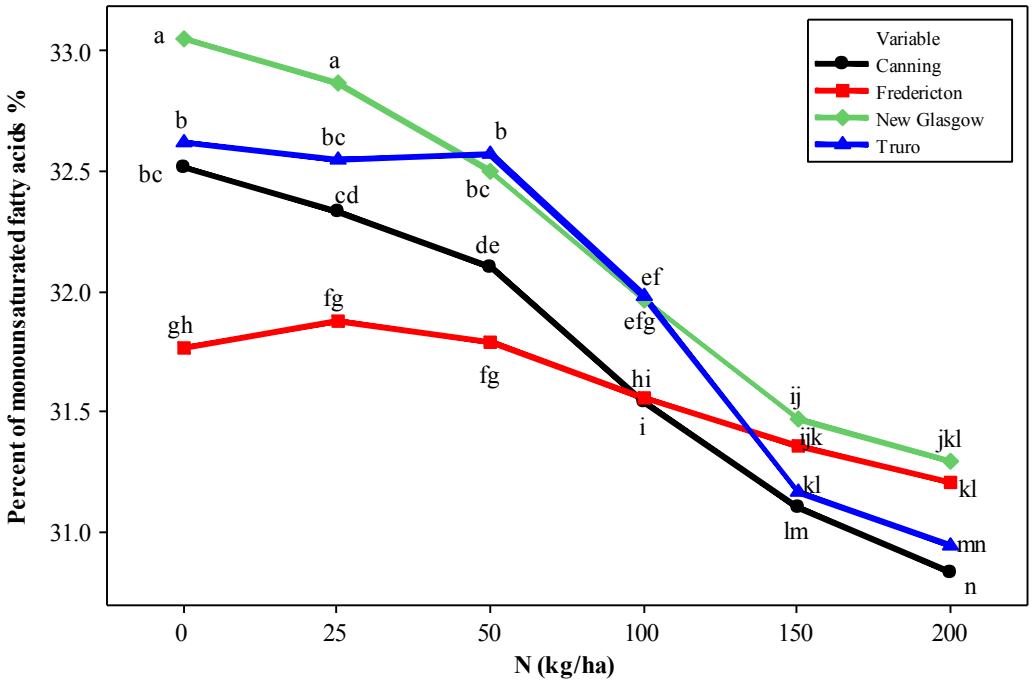


Figure 4.48: Effect of interaction of N and location on percent of monounsaturated fatty acids % in 2012
(Means with a common letter are not significantly different at the 5% level)

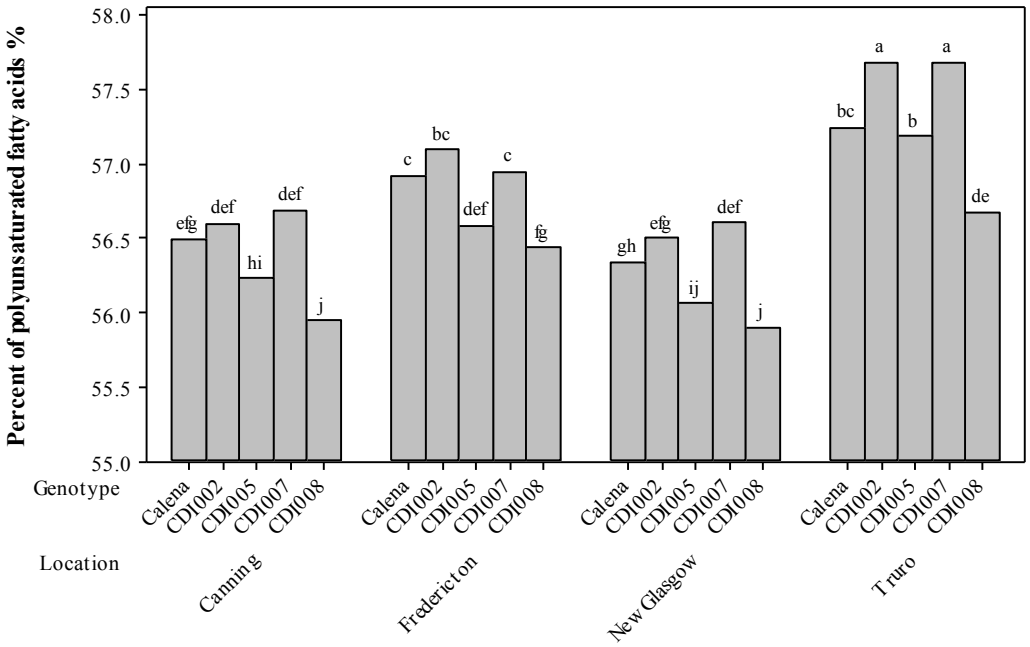


Figure 4.49: Effect of interaction of location and genotype on percent of polyunsaturated fatty acids % in 2012
(Means with a common letter are not significantly different at the 5% level)

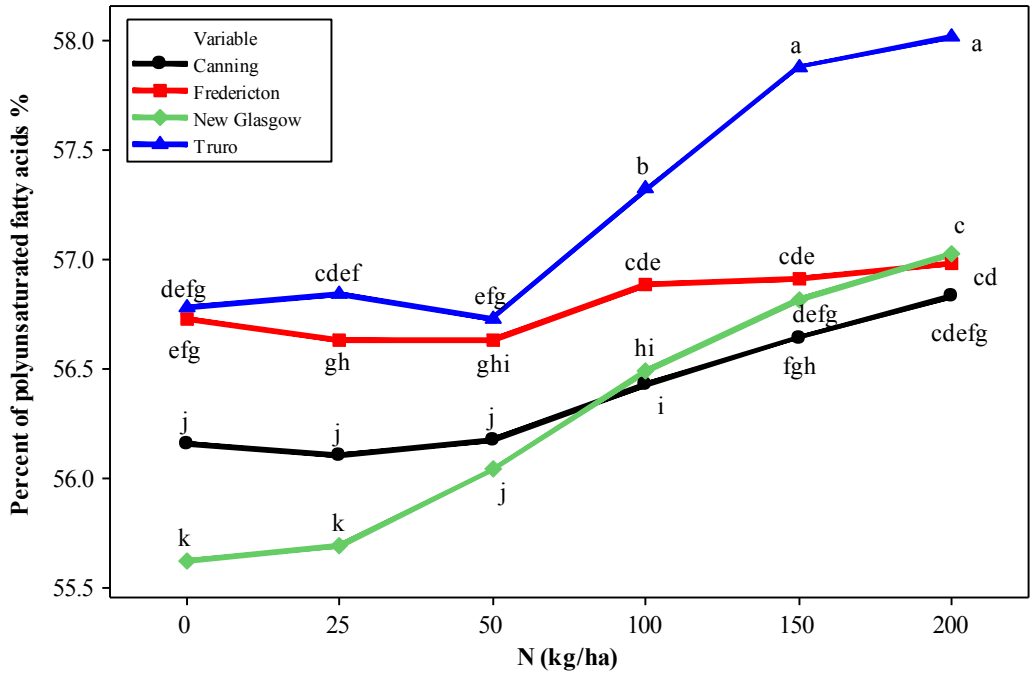


Figure 4.50: Effect of interaction of interaction of N and location on percent of polyunsaturated fatty acids in 2012
(Means with a common letter are not significantly different at the 5% level)

4.6 Discussion

4.6.1 Plant Elasticity

Camelina yielded the most at Canning and New Glasgow, while the yield was the lowest at Truro in 2012. It should be pointed out that seeds were lost during the seed cleaning of samples from Truro. The seeds were stuck together in clumps because of improper drying and could not pass through the sieve completely.

At Fredericton in 2012, plants had smaller canopy compared to other locations. The numbers of branches and pods per plant at Fredericton were the lowest, and plants were the shortest among these four locations. This is due to the fact that at Fredericton, plots were seeded late, which decreased the time for good crop establishment. However, plant density at harvest at Fredericton was the highest. Furthermore, plants at Fredericton produced larger pods based on visual observation, compared to pods from Canning, Truro and New Glasgow. This was perhaps a potentially crucial factor in the higher yield at Fredericton in 2012. The number of seeds per pod was not counted in the present study due to time constraints. The determination of seeds per pod and thousand seed weight would be highly recommended in future studies of yield components of camelina.

At Canning in 2012, yield components including the number of branches and pods per plant and per m^2 and plant density at harvest appeared to be potential key factors leading to high yield, because plant density at Canning was the second highest and the number of branches and pods per plant were the highest among these four locations. CDI007 and CDI008 at Canning had significantly more branches per m^2 than other

genotypes at Canning and all of the genotypes at Fredericton, New Glasgow and Truro. Plants at Canning had the most pods per m^2 among these four locations. Both CDI007 and CDI008 had the most pods per m^2, but CDI007 had the highest yield and CDI008 had the lowest yield at all of the sites, which indicated the number of seeds per pod and thousand seed weight might be crucial factors for yield determination.

At New Glasgow in 2012, although branches/plant was the highest, plant density at harvest was the lowest. At Truro in 2012, plant density at harvest was the lowest, and the number of branches/plant was the second to last. The number of plants per unit area at harvest and the number of pods per plant at New Glasgow and Truro were fewer than Canning, producing lower yields at New Glasgow and Truro relative to Canning. This observation suggests that both plants/m^2, branches/plant and pods/plant were important factors in determining yield potential.

4.6.2 N Response

Camelina yield increased with an increase in N resulting from a positive correlation between N and pods/m^2, which was consistent with previous studies in camelina (Johnson and Gesch, 2013; Losak et al., 2011; Urbaniak et al., 2008a; Pan, 2009). Both protein yield and oil yield increased with an increase in N input. Yield components including the number of branches and pods per plant increased with the increase of N in the present study. The positive correlation between N and yield was probably due to the acceleration of photosynthesis and the production of more carbohydrates with N input (Joshi et al. 1998).

The seed protein content of camelina increased with an increase in N, but the oil content decreased. This observation is also consistent with the findings of other

researchers (Johnson and Gesch, 2013; Losak et al., 2011; Urbaniak et al., 2008a; Pan et al., 2009). It was generally accepted that biosynthesis of fatty acids and amino acids compete for carbon skeletons and energy (Gehringer et al., 2006) and the availability of carbohydrates reduced oil synthesis with N input (Rathke et al., 2005). According to Rathke et al. (2005), levels of carbohydrates in proteins are fewer than oils; increased N input resulted in increased protein synthesis and corresponding decrease in fatty acid synthesis due to their competition for carbon skeletons during carbohydrate metabolism.

The optimum N rate for the highest yield potential at Truro and Canning in 2011 was 100 kg N/ha. In 2012, the optimum N rates for achieving the highest yield varied from one location to another. The optimum N rate for the highest yield was 150 kg N/ha at Canning, New Glasgow and Truro, and 200 kg N/ha was the optimum rate at Fredericton. This indicated that N application was affected by background nutrients in the soil, soil types, climatic factors and other environmental conditions.

4.6.3 Plant Stand

The seeding rate was 500 seeds/m^2 at all sites in 2011 and 2012. However, the plant densities during the maturity stage were 264 plants/m^2 at Fredericton, 236 plants/m^2 at Canning, 184 plants/m^2 at Truro and 194 plants at New Glasgow in 2012. Due to plant natural self-thinning ability, populations of camelina declined when plant size increased. Plant stand at harvest at Fredericton was the highest, perhaps because of the use of a different seeder compared with the seeder used at Canning, Truro and New Glasgow. It was suggested that a forage type seeder was able to achieve a better plant establishment. With the same seeding rates and using the same seeder, the plant densities were different

at Canning, Truro and New Glasgow, which was probably due to the different soil type and various environment conditions at different locations.

4.6.4 Downy Mildew

Downy mildew observation at Canning and Truro in 2011 and 2012 showed that CDI007 was the most tolerant to the disease and Calena, CDI002 and CDI008 were less tolerant in 2011. When plants reached the reproductive stage, CDI008 became more tolerant to downy mildew; but CDI005 become more sensitive compared with CDI005, at the vegetative stage. This suggests that CDI008 was sensitive to downy mildew when the plantlets were small, and it became more tolerant to the disease with time.

Downy mildew infection at Canning was more severe than that at Truro in 2012 due to different environmental factors. Humid and warm conditions were reported to result in the increased incidence of downy mildew. The regression models between N and the percent of downy mildew with downy mildew showed that N had a positive correlation with the incidence of downy mildew, which was consistent with other research on this disease by Pan (2009) and Chapter Five in this study. It is generally understood that N input promotes plant growth, and larger canopies with high shoot densities facilitate spore transfer and pathogen infection (Walters, 2009). Further, N promotes the development of young and succulent tissues, and N also prolongs the vegetative stage and delays plant maturity, which make plants susceptible to pathogens for a longer period (Agrios, 1988 in Zarafi et al., 2005).

4.6.5 Fatty Acids

Differences between genotypes and fatty acids suggest camelina fatty acid profile is under genetic control. In the present study, CDI008 had the highest content of saturated

fatty acids, while CDI002 and CDI007 had the highest content of polyunsaturated fatty acids but the lowest saturated fatty acids, which indicates that CDI002 and CDI007 are more suitable for human and animal consumption, because polyunsaturated fatty acids are considered to be beneficial for lowering cholesterol in human body (Cunnae, 1995 in Manaf et al., 2006).

Saturated and polyunsaturated fatty acids increased with an increase in N, while monounsaturated fatty acids did the opposite. Similar results were observed in Chapter 5. The inverse relationship between C18:1 (monounsaturated fatty acids) and C18:2 (polyunsaturated fatty acids) was reported in camelina by Urbaniak (2006), in canola by Manaf et al. (2006) and in sunflower by Flagella et al. (2002), because oleic acid (C18:1) is converted to linoleic acid (C18:2) and linolenic acid (C18:3) in angiosperm plants (Wallis et al., 2002). According to Wallis (2002), C16:0 and C18:0 are produced when acyl carrier protein (ACP) thioesters by a fatty acid synthase (FAS). This FAS is found in the chloroplasts (or plastids of the cell). With a soluble stearoyl-ACP desaturase, the 18:0-ACP is converted to 18:1-ACP in the chloroplast stroma. The C18:1 is integrated with glycerolipids in the chloroplasts and endoplasmic reticulum (ER). In the chloroplasts and ER, membrane-bound desaturases put a second double bond at $\Delta 12$ to produce C18:2, and then a third double bond at the n3 position to develop C18:3 (Wallis et al., 2002).

Fatty acid profile of camelina was different from one location to another. This was probably due to the different climate conditions at different sites. Plants at Truro produced the highest content of polyunsaturated fatty acids among these four locations, which was consistent with the results in Chapter 5. This was probably due to the positive

relationship between precipitation during crop growing season and biosynthesis of polyunsaturated fatty acids.

4.7 Conclusion

In General, CDI007 proved to be the most promising genotype among these five genotypes at all the sites in 2011 and 2012. This is in part because it was most tolerant to downy mildew at Truro and Canning in both years; it had the highest yield at all tested locations in 2011 and 2012; it was the richest in oil content although the content of protein was not significantly different from most of the tested genotypes. The optimum N rate varied by year and location. For example, it was 100 kg N/ha at Truro and Canning in 2011. In 2012, the optimum N rates in term of the highest yield were 150 kg N/ha at Canning, Truro, New Glasgow; and 200 kg N/ha at Fredericton.

Chapter 5: Effects of Sulphur, Nitrogen and Genotype on Growth and Seed Quality of *Camelina sativa* L. Crantz at Five Canadian Locations in 2012

5.1 Introduction

According to the Nova Scotia Department of Agriculture, a soil S level with over 40 kg/ha is sufficient for normal plant growth. Soils in Nova Scotia historically have contained sufficient levels of S for crop development, and most of the S is available from mineral breakdown and acid rain ("Understanding the soil test report", 2011). However, S deficiency has recently become severe due to a reduction in air pollution in Atlantic Canada (Sharifi et al., 2010). The S concentration in the soil has also decreased with the reduction of acid rain. Another reason leading to S deficiency is the increasing use of high-analysis inorganic fertilizers with lower than 1% S, such as the application of triple superphosphate. Furthermore, the supply of pesticides and fungicides with S has declined. An S survey was conducted on 17 farms between Windsor and Annapolis Royal in Nova Scotia in 2010. The results indicated that S levels of 71% of the tested soil samples are below the critical level of 40 kg/ha. This phenomenon may limit plant growth of crops such as canola and cabbage, which require higher amounts of S (Sharifi et al., 2010).

It has been reported that S deficiency reduced oil content in canola seed (Ridley, 1972 in Grant et al., 2003; Jackson, 2000; Ahmad et al., 2000b). S deficiency may limit acetyl-CoA carboxylase activity, leading to the reduction of oil biosynthesis due to lack of acetyl-CoA carboxylation (Ahmad et al., 2000b). In some studies, S has been associated with increased protein content in canola (Nuttall et al., 1987; Ahmad and Abdin, 2000a), while other studies showed that S had no impact on protein content (Asare and Scarisbuick, 1995). Protein content may be affected by the N:S ratio (Ahmad

and Abdin, 2000a). Combined S and N with a balanced ratio rather than N alone is capable of increasing activities of ATP-sulphurylase and nitrate reductase which are related to sulphate and nitrate assimilation (Ahmad et al., 1999). Research on canola by Malhi and Leach (2002) showed that crops yielded the highest with 90 kg N/ha and 18 kg S/ha application; 90 or 135 kg N/ha and 27 kg S/ha application (N:S ratios of 3.3:1 and 5:1).

The hypothesis of this study was that genotypes would differ in the growth and seed quality depending on the amount of N and S. In this study, the effects on camelina of S, N and genotype were tested in five contrasting environments (Truro and Canning in NS; Fredericton, NB; New Glasgow, PEI; and Saskatoon, SK) in 2012. The evaluated parameters included plant stand, percent of plants with downy mildew, days to maturity, plant height, number of branches per plant, number of pods per plants, number of pods per m^2, number of plants per m^2 at harvest, seed yield, seed oil content, protein content, oil yield, protein yield and fatty acids.

5.2 Methods and materials

Sites at Canning (lat. 45.16^{o}N; long. 64.43^{o}W), NS (Lyndhurst Farms), Truro (lat. 45.36^{o}N; long. 63.28^{o}W), NS, (Dal-AC), New Glasgow (lat. 46.41^{o}N; long. 63.35^{o}W), PEI (Technology Crops), Fredericton (lat. 45.96^{o}N; long. 66.63^{o}W), NB (AAFC) and Saskatoon (lat. 52.13^{o}N; long. 106.64^{o}W), SK (ICMS- Integrated Crop Management Services) were used in 2012. The seeding rate for all the trials of the five sites was 500 seeds/m^2. Camelina was seeded with a Hege plot drill (H and N Equipment Inc., Colwich, Kansas, USA) with press wheels and double disc openers at NS and New Glasgow. ICMS (SK) used a double-disk press drill (made by ICMS) with a belt driven cone. AAFC at

Fredericton used a Brillion forage type of seeder. Crops were seeded in plots 5m in length and 2.5m wide at Canning, Truro and New Glasgow, 6m in length and 1.25m wide at Fredericton, 7m in length and 2.5m wide at Saskatoon.

Two genotypes, CDI005 and CDI007 from the breeding program of Dr. Kevin Falk of AAFC Saskatoon were tested in this study. Seeds were sown with a mixture of viable and dead seeds at a ratio of 2:3. The addition of dead seeds for each plot allowed for better distribution. Dead seeds were made by autoclaving and checked afterwards to ensure they were not viable. N inputs (Ammonium nitrate) were 20, 40, 80, 120, 160 and 200 kg N/ha. S applications (Magnesium sulfate) were 0 and 25 kg S/ha. N application was split 50:50 for the higher N treatments including 120, 160 and 200 kg N/ha. The first half of N fertilizer was applied within one week after seed germination (N rates 20, 40 and 80 applied all at first application) and the rest was applied at the beginning of flowering. In addition, 30-40 kg/ha P & K was applied preplant. The pre-plant herbicide trifluralin (active ingredient) was applied at a rate of 1.1 kg a.i./ha. There were four replications and thirty treatments arranged in a randomized complete block design (RCBD). Data of soil characteristics and weather summary at all of the sites were listed in Table 4.3 and Table 4.4 in Chapter 4.

Table 5.1: Previous crops at these five sites

Location	2010	2011
Canning (NS)	Spring wheat	Winter wheat
Truro (NS)	Soybean	Soybean
New Glasgow (PEI)	Barley	Winter wheat
Fredericton (NB)		Timothy/red clover
Saskatoon (SK)	Barley	Chem fallow

Table 5.2: Seeding and fertilizer application dates

Location	Seeding date	First timing fertilizer	Second timing Fertilizer	Harvest date
Canning (NS)	1-May-12	4-May-12	26-Jun-12	21-Aug-12
Truro (NS)	7-May-12	14-May-12	3-Jul-12	24-Aug-12
New Glasgow (PEI)	8-May-12	8-May-12	26-Jun-12	30-Aug-12
Fredericton (NB)	6-Jun-12	18-Jun-12	11-Jul-12	4-Sep-12
Saskatoon (SK)	16-May-12	18-May-12	11-Jul-12	13-Sep-12

5.3 Data Collection

(1) Stand Counts within One Month of Seeding

Stand counts were done on May 29 (29 days after seeding) at Canning, May 31 (25 days after seeding) at Truro, June 13 (36 days after seeding) at New Glasgow and June 18 (6 days after seeding) at Fredericton, respectively. Two subsamples with three rows of plants ($0.225m^2$) from each plot were chosen randomly by using a quadrat (Square, 0.5m*0.5m), avoiding the outside rows. The number of plants in the specific area was counted.

(2) Percent of Downy Mildew (caused by *Peronospora parasitica*)

The percentage of plants with downy mildew was determined on July 4 (65 days after seeding) at Canning and July 6 (60 days after seeding) at Truro. Plants were in the reproductive stage at both locations. Two subsamples (1 subsample = two rows * 0.5m) from each plot were collected randomly by using a meter stick, avoiding the outside rows. The infection with downy mildew was determined by visual observation when the white mould was found on leaves, stem, or developing pods. The number of plants in the specific area and the number of plants which were infected with downy mildew were counted. The ratio of the number of plants infected with downy mildew to plant stand was calculated.

(3) Maturity Ratings at Truro

Maturity date was estimated visually as the date when approximately 90 % of the pods were brown at Truro from July 31 to August 13, 2012.

(4) Plant Height

Plant height was measured on three randomly selected plants per plot from the soil surface to the highest point on the erect plant at the time of maturity.

(5) Yield Components

Two subsamples of 0.5m of one row of plants from each plot were harvested and were calculated and converted to the values with plants/m^2. The number of branches and pods per plant were counted using a sub-sample of 10 plants from each plot.

(6) Seed Yield

Seeds were cleaned by using a Clipper (Clipper Seed Cleaning Co., Bluffton, IN) seed cleaner. Clean seed was weighed (g) and g/plot values was converted to kg/ha based on plot areas for each location.

(7) Seed Protein, Oil and Fatty acid Analyses

Content of protein, content of oil, and the percent of fatty acid profile were analyzed by Near-infrared spectroscopy (NIRS) (Unity Scientific, Spectra Star, 2500x).

5.4 Statistical Analysis

Minitab 16 statistical software (Minitab Inc., USA, 2012) was used to check the three assumptions - normality, constant variance and independence; outliers were removed if they existed. Data were transformed (square, square root, cubic root, ln, or log

base 10) if they were not normally distributed. Minitab was also used in all the regression analysis.

SAS 9.3 statistical software (SAS Institute Inc., Cary NC, USA, 2012-2013) was also used in the data analysis once the data were checked to be normally distributed in Minitab. Proc Mixed with least significant differences (LSD) t-test method ($p<0.05$) was used to examine whether there were significant effects of factors on the targeted parameters.

Kruskal-Wallis non-parametric test was used to analyze the data of days to maturity at Truro, because the distribution was not normal and it could not be made normal by transformations. Non-parametric tests used the median and not the mean. There was significant difference between at least two of the median values; Mann-Whitney tests of all the possible pairs of medians were analyzed to find out where the exact differences were.

Data from SK were not used in the data analyses due to severe weed problem which produced greater effects than the test variables.

5.5 Results

5.5.1 Early Plant Stand (plants/m^2)

Location, genotype and N independently had significant effects on the number of plants per square meter within one month of seeding at Canning, Fredericton, New Glasgow and Truro in 2012 (Table 5.3). The plant density at Fredericton was the highest, followed by Truro; Canning and New Glasgow had the fewest plants in each unit of area (Table 5.4). The plant density of CDI007 was significantly higher than CDI005 with 207 and 196 plants/m^2 (seeding rate of 500 seeds/m^2) for CDI007 and CDI005, respectively

(Table 5.4). The plant density with the treatment of 20 kg N/ha was significantly higher than that with 40 kg N/ha and higher N rates (Table 5.4). In general, N input decreased the plant density (the cubic regression in Figure 5.1) due to plant self-thinning ability, and plant density leveled out as N increased to 60 kg/ha and more. At this time, only the first half of N for higher N rates including 120, 160, and 200 kg N/ha was applied. Plants received 20, 40, 80, 60, 80, 100 kg N/ha for the N treatments of 20, 40, 80, 120, 160 and 200 kg N/ha.

Table 5.3: ANOVA table of early plant establishment at Canning, Fredericton, New Glasgow and Truro in 2012

Effect	F value	P value
Rep	1.48	0.2082
Location (L)	**38.47**	**<.0001**
Genotype (G)	**6.16**	**0.0136**
L*G	0.22	0.8795
N	**3.05**	**0.0106**
L*N	1.03	0.4199
G*N	1.15	0.3324
L*G*N	1.08	0.3736
S	0.28	0.5993
L*S	0.38	0.7689
G*S	1.03	0.3114
L*G*S	0.29	0.8342
N*S	1.84	0.1053
L*N*S	0.81	0.6679
G*N*S	0.24	0.9448
L*G*N*S	1.24	0.2383

(no transformation)

Table 5.4: Effect of location, genotype and N on number of plants per m^2 in 2012

Location	Genotype	N (kg/ha)	Plants/m^2
Fredericton			309 a
Truro			206 b
Canning			148 c
New Glasgow			144 c
	CDI007		207 a
	CDI005		196 b
		20	221 a
		40	204 b
		80	195 b
		60 (120)	196 b
		80 (160)	195 b
		100 (200)	198 b

(First timing of N; means with a common letter are not significantly different at the 5% level)

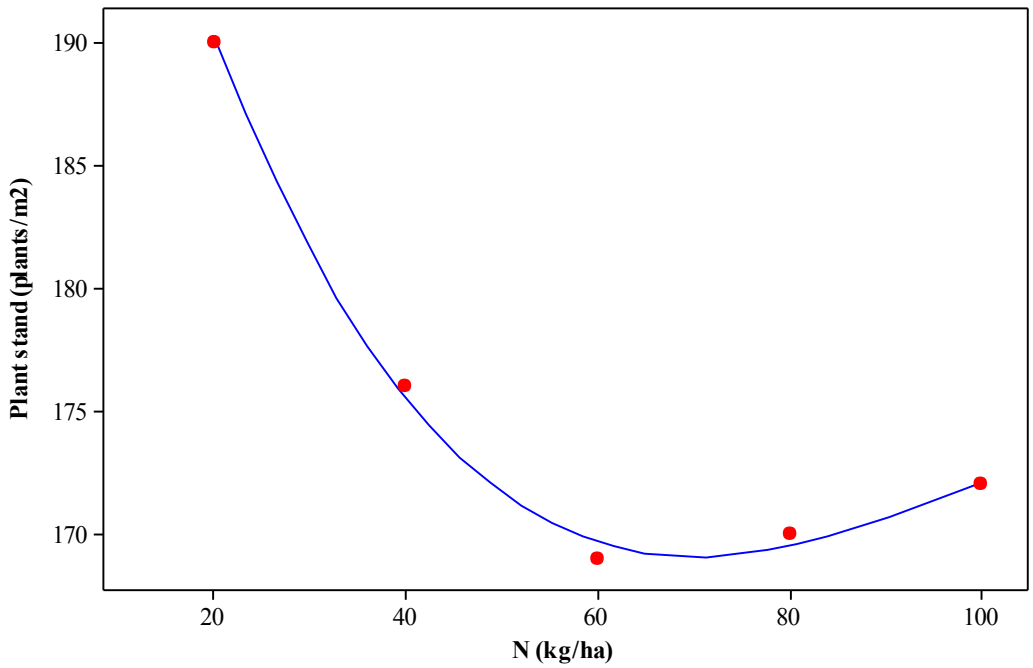


Figure 5.1: Regression analysis of N effect on plant stand at 4 sites in 2012 (first timing of N)

(the first three dots represent 16 samples/dot; the other two dots represents 32 samples/dot)

Y=216.4-1.657N+0.01839N**2-0.000063N**3 with R-Sq(adj)=98.8%

5.5.2 Percent of Plants Infected with Downy Mildew (at Flowering)

Location, genotype and N independently had significant effects on percent of plants infected with downy mildew at Canning and Truro in 2012 (Table 5.5). Data were collected when plants were at the reproductive stage on July 4th, 2012 (65 days after seeding; 10 days after second timing of N application) at Canning and July 6th, 2012 (61 days after seeding; 3 days after second timing of N application) at Truro, and both timing of N were applied at that time. The percent of plants with downy mildew at Canning (18.5%) was significantly higher than that at Truro (7.8%) (Table 5.6). CDI007 was more tolerant to downy mildew than CDI005 with 6.5% and 19.9% of plants infected with the disease, respectively (Table 5.6). N was positively correlated with downy mildew infection (Figure 5.2). The ratio of 63.7% of variability on the percent of plants infected by downy mildew could be explained by the change of N levels (Figure 5.2).

Table 5.5: ANOVA table of downy mildew at Truro and Canning

Effect	F Value	P value
Rep	2.09	0.1270
Location (L)	**4.28**	**0.0403**
Genotype (G)	**74.24**	**<.0001**
L*G	1.80	0.1813
N	**2.36**	**0.0435**
L*N	1.32	0.2606
G*N	0.77	0.5739
L*G*N	0.51	0.7675
S	0.01	0.9108
L*S	1.70	0.1940
G*S	1.15	0.2852
L*G*S	0.01	0.9238
N*S	0.29	0.9178
L*N*S	0.35	0.8826
G*N*S	0.38	0.8589
L*G*N*S	0.72	0.6116

(cubic root transformation)

Table 5.6: Effect of location and genotype on percent of downy mildew

Location	Genotype	N (kg/ha)	DM%
Canning			18.5 a
Truro			7.8 b
	CDI005		19.9 a
	CDI007		6.5 b
		20	11.4 bc
		40	9.8 c
		80	13.3 abc
		120	11.3 bc
		160	17.5 a
		200	15.7 ab

(Means with a common letter are not significantly different at the 5% level)

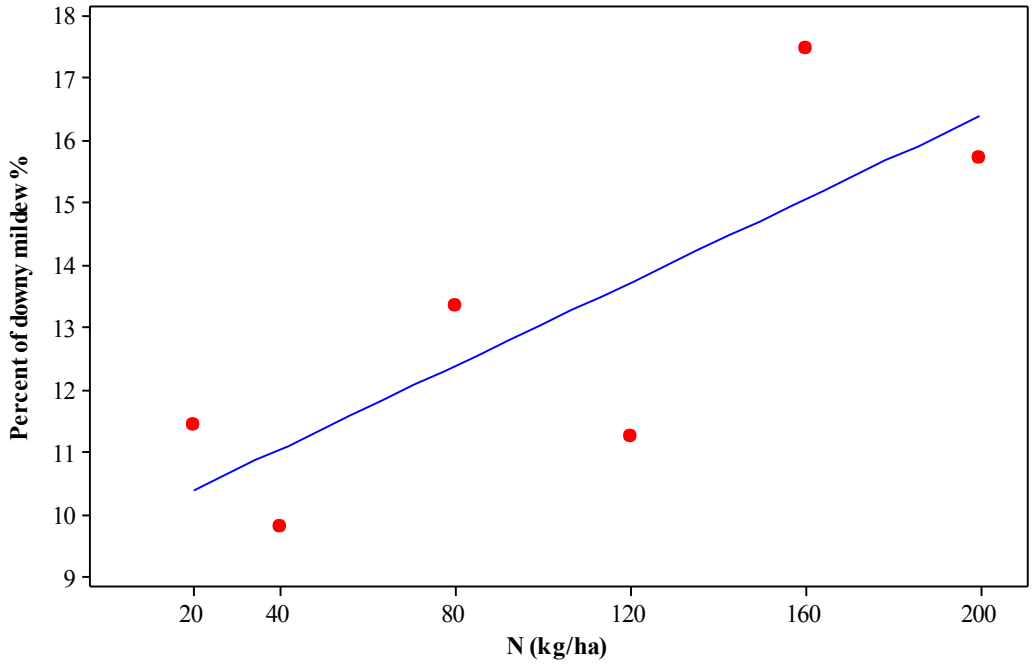


Figure 5.2: Regression analysis of effect of N on percent of downy mildew at the reproductive stage in 2012

(each dot represents 32 samples)

DM%=0.09718+0.00033N with R-Sq=63.1%

5.5.3 Days to Maturity

Data of days to maturity at Truro were not normally distributed, so the Kruskal-

Wallis non-parametric test was used to analyze the data. Genotype and N had a significant effect on the days to maturity. In general, CDI005 matured earlier than CDI007. Also, N delayed plant maturity, and S had no significant effect on days to maturity (Table 5.7).

Table 5.7: ANOVA table of days to maturity at Truro

N(kg/ha)	CDI005		CDI007	
	0 S	25 S	0 S	25 S
20	88 b	88 b	93 ab	91 b
40	91 b	90 b	94 a	94 a
80	91 b	90 b	94 a	94 a
120	93 ab	91 b	94 a	94 a
160	91 b	93 ab	94 a	94 a
200	94 a	94 a	94 a	94 a
P value	<0.0001			

(Means with a common letter are not significantly different at the 5% level)

5.5.4 Plant Height

Genotype had a significant effect on plant height, as well as the interaction of location, N and S (Table 5.8). CDI007 was significantly taller than CDI005 but only by 1cm (Table 5.9). N had an effect on the canopy height of plants depending on locations and S rates. It was true for plants without S application at Fredericton and plants with and without S application at Truro (Figure 5.3). The regression models show height increased with N input until a maximum value was reached and then plant height tended to decrease when N continued to increase at Fredericton and Truro (Figure 5.3). Low ratio of variability in plant height could be explained by the change of N levels at Canning and New Glasgow (Figure 5.3).

Table 5.8: ANOVA table of plant height at Canning, Fredericton, New Glasgow and Truro

Effect	F Value	P value
Rep	6.64	<.0001
Location (L)	4.03	0.0079
Genotype (G)	**8.16**	**0.0046**
L*G	0.93	0.4263
N	5.69	<.0001
L*N	1.26	0.2282
G*N	0.98	0.4298
L*G*N	0.65	0.8274
S	3.97	0.0474
L*S	0.86	0.4619
G*S	0.22	0.6424
L*G*S	0.39	0.7617
N*S	0.61	0.6924
L*N*S	**1.80**	**0.0344**
G*N*S	0.90	0.4837
L*G*N*S	1.18	0.2867

(square transformation)

Table 5.9: Effect of genotype on plant height (cm)

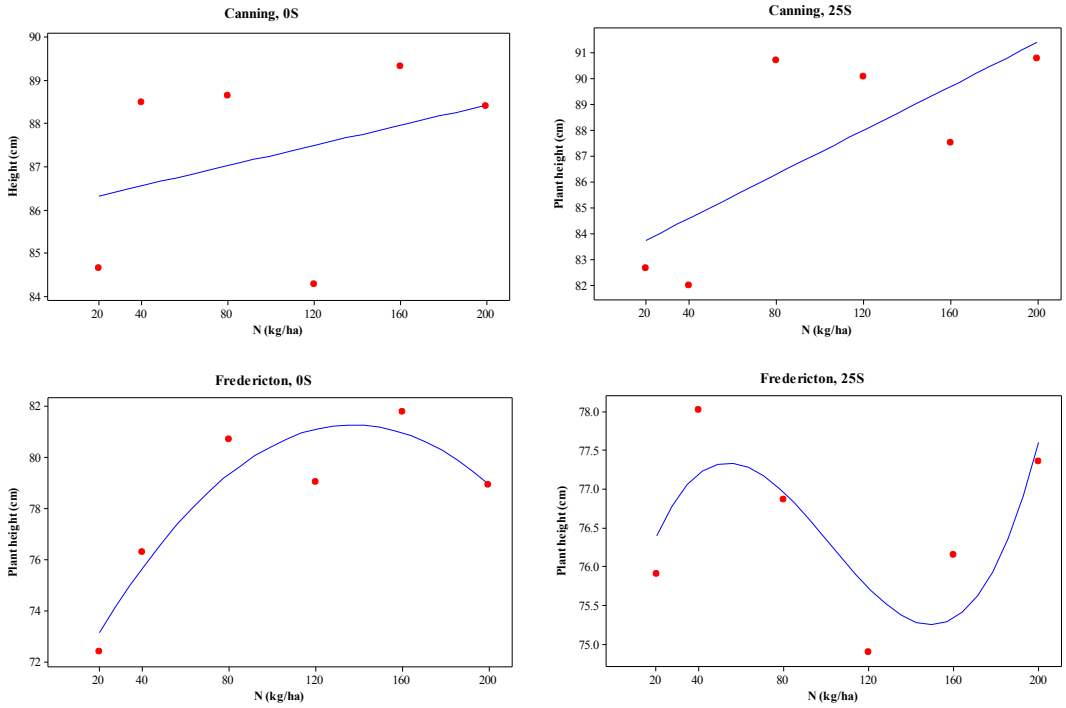
Genotype	Plant height
CDI007	82 a
CDI005	81 b

(Means with a common letter are not significantly different at the 5% level)

Table 5.10: Effect of interaction of location, N and S on plant height (cm)

S (kg/ha)	N (kg/ha)	Canning	Fredericton	New Glasgow	Truro
0	20	85 e-l	72 u	87 a-h	79 m-t
	40	89 a-f	76 s-u	88 a-g	82 i-r
	80	89 a-f	81 k-s	86 b-i	85 d-l
	120	84 f-m	79 o-t	89 a-e	85 f-l
	160	89 a-d	82 i-r	88 a-f	83 h-p
	200	88 a-f	79 o-t	87 a-h	84 f-n
25	20	83 h-p	76 s-u	86 c-j	79 n-t
	40	82 i-q	78 p-t	85 d-l	82 j-r
	80	91 ab	77 r-u	86 c-k	85 d-l
	120	90 abc	75 tu	88 a-g	84 g-o
	160	87 a-g	76 s-u	85 e-l	85 e-l
	200	91 a	77 q-u	85 d-l	81 l-s

(Two-way interaction; means with a common letter are not significantly different at the 5% level)



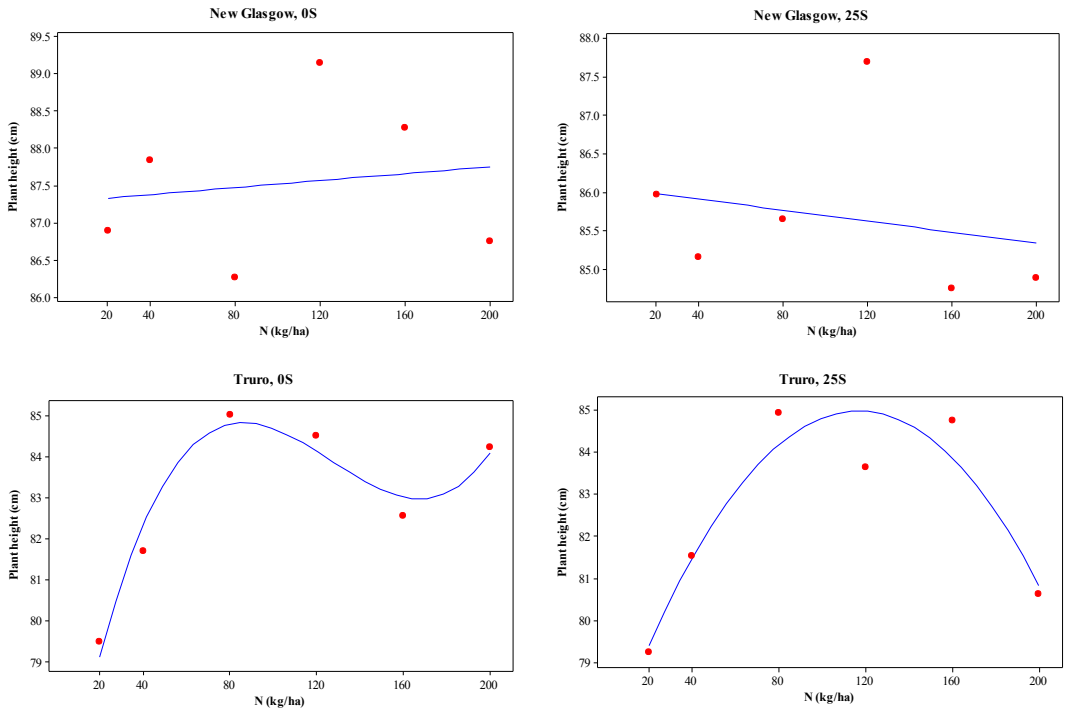


Figure 5.3: Regression analysis of N on height with two S rates at four locations in 2012

(each dot represents 8 samples)

(1) Y(Canning, 0S) =86.09+0.01171N with R-Sq=13.5%

(2) Y(Canning, 25S) =82.87+0.04250N with R-Sq=54.6%

(3) Y(Fredericton, 0S) =70.13+0.1623N-0.000591N**2 with R-Sq(adj)=77.9%

(4) Y(Fredericton,25S)=74.65+0.1139N-0.001443N**2+0.000005N**3 with R-Sq(adj)=6.7%

(5) Y(New Glasgow, 0S) =87.29+0.002346N with R-Sq=2.3%

(6) Y(New Glasgow, 25S) =86.06-0.003597N with R-Sq=5.3%

(7) Y(Truro,0S)=74.14+0.2992N-0.002627N**2+0.000007N**3 with R-Sq(adj)=88.9%

(8) Y(Truro,25S)=76.86+0.1389N-0.000595N**2 with R-Sq(adj)=80.4%

5.5.5 Number of Branches per Plant

The interaction of location, genotype, N and S had significant effects on the number of branches per plant at all 4 sites in 2012 (Table 5.10). The mean values of branches/plant were shown in Table 5.12. N had an effect on the number of branches per

plant depending on S levels, location and genotype (Figure 5.4). N affected the number of branches per plant on CDI005 with 25 kg S/ha at Canning, Fredericton & Truro and CDI007 without S application at New Glasgow (Figure 5.4).

Table 5.11: ANOVA table of number of branches per plant at four locations

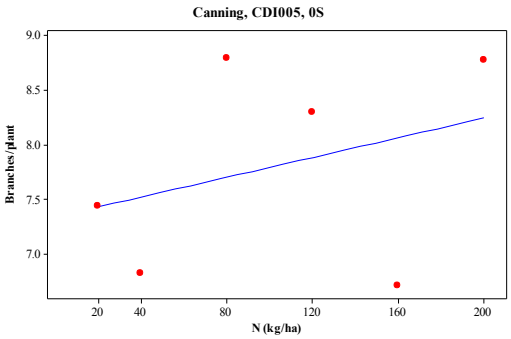
Effect	F value	P value
Rep	5.26	0.0004
Location (L)	9.82	<.0001
Genotype (G)	33.8	<.0001
L*G	1.19	0.3157
N	8.92	<.0001
L*N	0.92	0.5394
G*N	2.77	0.0184
L*G*N	1.35	0.174
S	18.18	<.0001
L*S	5.32	0.0014
G*S	0.38	0.5396
L*G*S	0.39	0.7625
N*S	0.69	0.6324
L*N*S	1.33	0.1815
G*N*S	1.05	0.3866
L*G*N*S	**2.01**	**0.0147**

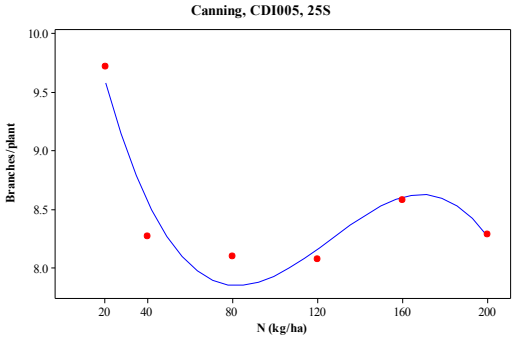
(log_{10} transformation)

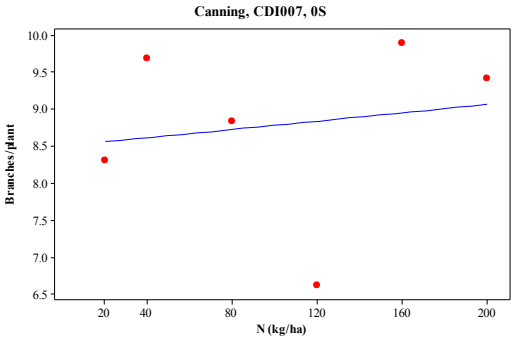
Table 5.12: Effect of interaction of location, genotype, N and S on branches/plant in 2012

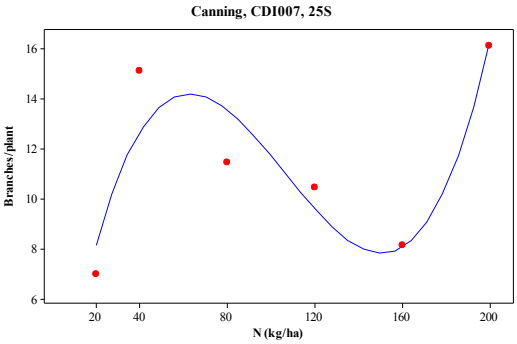
Genotype	S (kg/ha)	N (kg/ha)	Canning	Fredericton	New Glasgow	Truro
CDI005	0	20	7 e-s	5 p-x	4 wx	6 k-x
		40	7 f-t	7 f-s	4 v-x	4 u-x
		80	9 c-l	6 h-v	7 g-u	6 j-w
		120	8 d-p	6 i-w	7 d-s	6 n-x
		160	7 g-t	7 g-u	5 q-x	6 n-x
		200	9 c-l	8 d-q	6 g-v	6 j-x
	25	20	10 c-g	4 x	6 j-w	6 k-x
		40	8 d-q	5 p-x	7 g-u	6 i-v
		80	8 d-r	5 s-x	7 e-s	7 e-s
		120	8 c-s	9 c-n	8 d-s	7 f-s
		160	9 c-n	7 g-t	5 o-x	7 f-t
		200	8 d-p	9 d-q	8 d-s	10 c-g
CDI007	0	20	8 d-p	5 p-x	4 t-x	6 o-x
		40	10 c-g	8 d-s	7 g-u	8 d-s
		80	9 c-k	7 f-t	9 c-j	6 l-x
		120	7 g-u	8 d-p	7 e-s	8 c-o
		160	10 b-g	8 d-s	7 g-t	7 e-s
		200	9 c-i	8 d-s	9 c-m	7 f-t
	25	20	7 f-t	5 r-x	10 c-g	6 j-w
		40	15 ab	7 f-t	7 f-u	9 c-k
		80	11 a-d	8 d-s	8 d-s	11 a-e
		120	10 b-f	5 s-x	6 m-x	8 d-s
		160	8 d-r	8 d-s	15 ab	9 c-m
		200	16 a	8 d-s	13 a-c	10 c-h

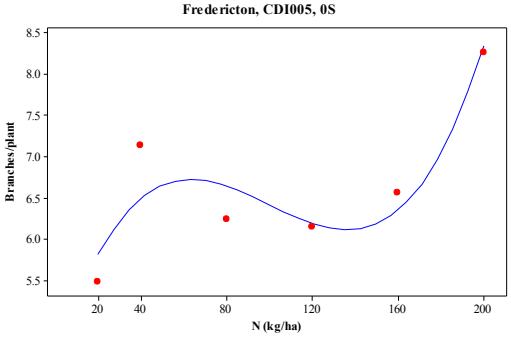
(Means with a common letter are not significantly different at the 5% level)

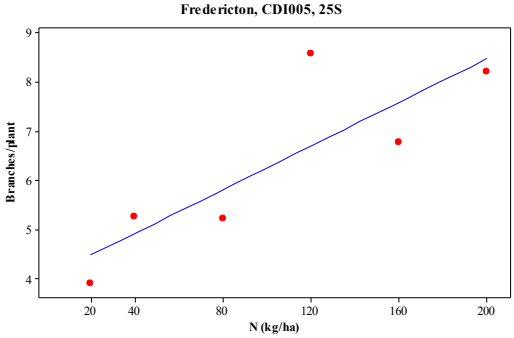

Canning, CDI005, 0S
Branches/plant
9.0
8.5
8.0
7.5
7.0
20
40
80
120
160
200
N (kg/ha)

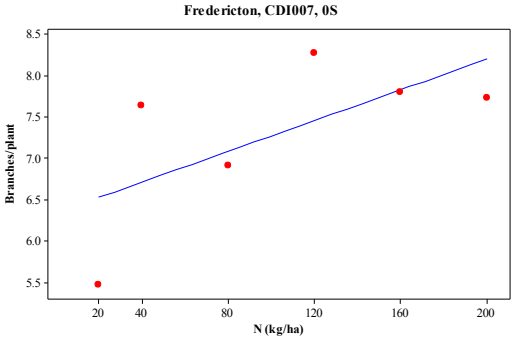

Canning, CDI005, 25S
Branches/plant
10.0
9.5
9.0
8.5
8.0
20
40
80
120
160
200
N (kg/ha)

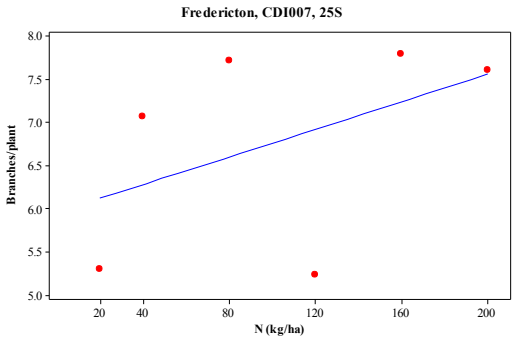

Canning, CDI007, 0S
Branches/plant
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
20
40
80
120
160
200
N (kg/ha)


Canning, CDI007, 25S
Branches/plant
16
14
12
10
8
6
20
40
80
120
160
200
N (kg/ha)


Fredericton, CDI005, 0S
Branches/plant
8.5
8.0
7.5
7.0
6.5
6.0
5.5
20
40
80
120
160
200
N (kg/ha)


Fredericton, CDI005, 25S
Branches/plant
9
8
7
6
5
4
20
40
80
120
160
200
N (kg/ha)


Fredericton, CDI007, 0S
Branches/plant
8.5
8.0
7.5
7.0
6.5
6.0
5.5
20
40
80
120
160
200
N (kg/ha)


Fredericton, CDI007, 25S
Branches/plant
8.0
7.5
7.0
6.5
6.0
5.5
5.0
20
40
80
120
160
200
N (kg/ha)

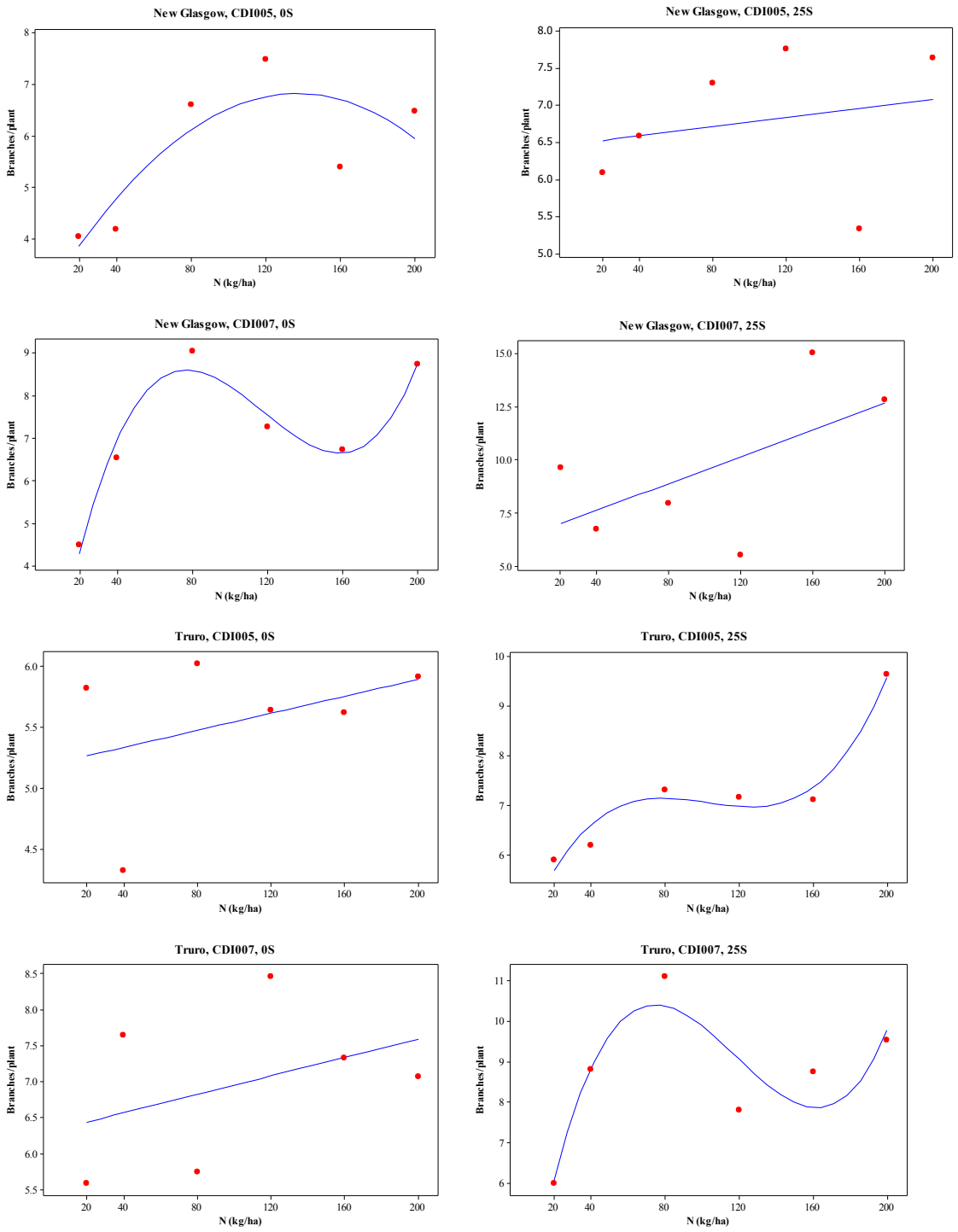


Figure 5.4: Regression analysis of N response to branches/plant in 2012
(each dot represents 4 samples)

(1) Y(Canning,CDI005,0S) =7.342+0.004533N with R-Sq(adj)=11.2%

(2) Y(Canning,CDI005,25S)=11.19-0.09728N-0.000881N**2-0.000002N**3 with R-Sq(adj)=77.4%

(3) Y(Canning, CDI007, 0S) =8.500+0.002800N with R-Sq=2.6%

(4) Y(Canning,CDI007,25S)=-0.319+0.5362N-0.006050N**2+0.000019N**3 with R-Sq=53.9%

(5) Y(Fredericton,CDI005,0S)=4.525+0.08198N-0.000947N**2+0.000003N**3 with R-Sq(adj)=59.7%

(6) Y(Fredericton, CDI005, 25S)=4.035+0.02224N with R-Sq=70.2%

(7) Y(Fredericton, CDI007, 0S)=6.339+0.009280N with R-Sq=42.0%

(8) Y(Fredericton, CDI007, 25S)=5.960+0.008018N with R-Sq=21.5%

(9) Y(New Glasgow,CDI005,0S)=2.749+0.05967N-0.000218N**2 with R-Sq(adj)=46.3%

(10) Y(New Glasgow, CDI005, 25S)=6.465+0.003109N with R-Sq=5.2%

(11) Y(NewGlasgow,CDI007,0S)=-0.116+0.2696N-0.002598N**2+0.000007N**3 with R-Sq=90.5%

(12) Y(New Glasgow,CDI007,25S)=6.374+0.03141N with R-Sq=35.9%

(13) Y(Truro,CDI005,0S)=5.196+0.003486N with R-Sq=15.3%

(14) Y(Truro,CDI005,25S)=4.094+0.09814N-0.001013N**2+0.000003N**3 with R-Sq=90.1%

(15) Y(Truro,CDI007,0S)=6.309+0.006409N with R-Sq=16.1%

(16) Y(Truro,CDI007,25S)=1.484+0.2815N-0.002750N**2+0.000008N**3 with R-Sq(adj)=49.9%

5.5.6 Number of Pods per Plant

Genotype and N independently had significant effects on the number of pods per plant, as well as the interaction of location and S (Table 5.13). CDI007 significantly had more pods than CDI005 (Table 5.14). N had a positive correlation with the number of pods per plant (Figure 5.5 and Table 5.14). Plants with 120, 160 and 200 kg N/ha significantly had more pods per plant than plants with 20 and 40 kg N/ha application (Table 5.14). The interaction of location and S on the number of pods per plant was shown in Figure 5.6. S did not affect the number of pods per plant at Canning, Fredericton and New Glasgow. At Truro, plants with 25 kg S/ha application had

significantly more pods per plant compared with plants without S application (Figure 5.6).

Table 5.13: ANOVA table of number of pods per plant at Canning, Fredericton, New Glasgow and Truro in 2012

Effect	F value	P value
Rep	1.7	0.1497
Location (L)	5.49	0.0011
Genotype (G)	**8.68**	**0.0035**
L*G	0.38	0.7649
N	**11.19**	**<.0001**
L*N	0.79	0.6837
G*N	1.28	0.2706
L*G*N	0.67	0.8168
S	7.09	0.0082
L*S	**2.83**	**0.0389**
G*S	0	0.9496
L*G*S	1.04	0.3754
N*S	0.51	0.7713
L*N*S	0.9	0.5682
G*N*S	0.72	0.6058
L*G*N*S	1.54	0.0897

($\log_{10}$ transformation)

Table 5.14: Effect of genotype and N on number of pods per plant

Genotype	N (kg/ha)	Pods/plant
CDI007		137 a
CDI005		123 b
	20	101 d
	40	119 c
	80	131 bc
	120	140 ab
	160	139 ab
	200	157 a

(Means with a common letter are not significantly different at the 5% level)

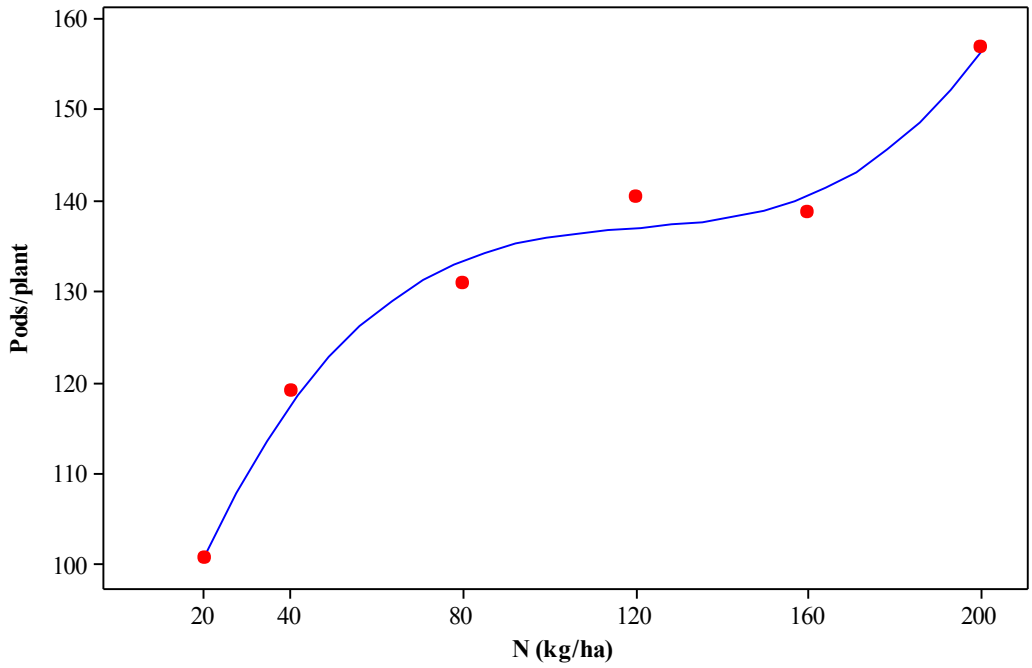


Figure 5.5: Regression analysis of N on number of pods per plant at all 4 sites with 2 levels of S

(each dot represents 64 samples)

Y=76.80+1.433N-0.01164N**2+0.000032N**3 with R-Sq(adj)=96.8%

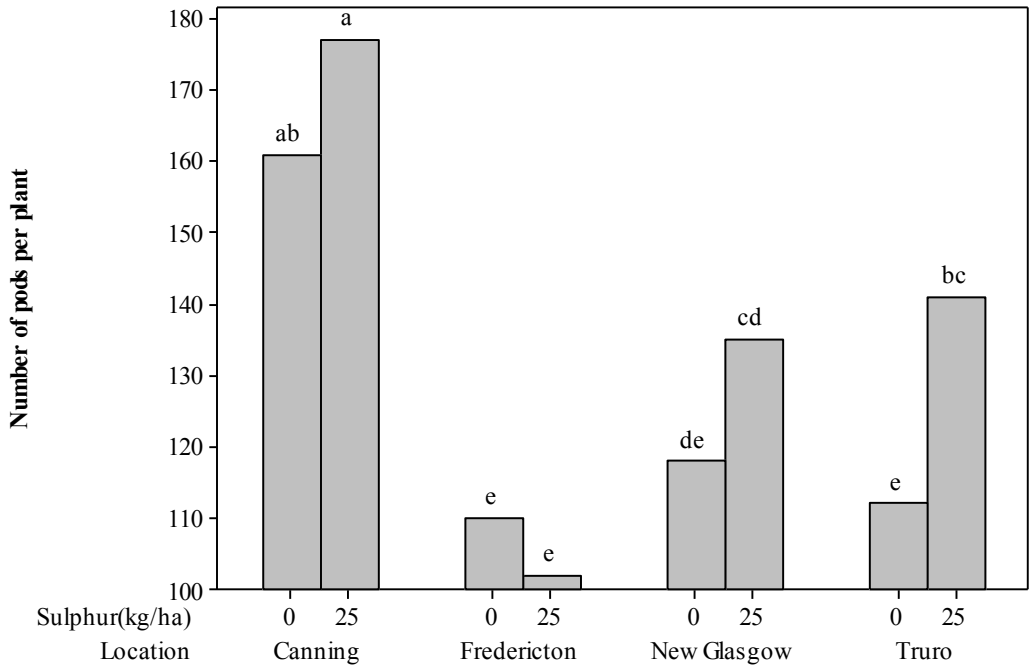


Figure 5.6: Effect of location and S on number of pods per plant in 2012

(Means with a common letter are not significantly different at the 5% level)

5.5.7 Number of Plants per m^2

Location, genotype and N independently had significant effects on the number of plants per m^2 at Canning, Truro, New Glasgow and Fredericton (Table 5.15). Plant density at Fredericton was the highest, followed by Truro and New Glasgow (Table 5.16). Plant density at Canning was the lowest, but it was not significantly different from New Glasgow. The number of CDI007 per m^2 was significantly higher than the number of CDI005 per m^2 (Table 5.16). Generally the number of plants per m^2 decreased with the increase of N application (Figure 5.7 and Table 5.16). With 200 kg/ha N application, the plant density was the lowest (Table 5.16).

Table 5.15: ANOVA table of number of plants/m^2 at harvest at Canning, Fredericton, New Glasgow and Truro in 2012

Effect	F Value	P value
Rep	1.76	0.1367
Location (L)	**3.48**	**0.0164**
Genotype (G)	**16.52**	**<.0001**
L*G	0.15	0.9275
N	**3.56**	**0.0038**
L*N	1.03	0.4271
G*N	0.34	0.8884
L*G*N	1.32	0.1864
S	3.16	0.0763
L*S	0.30	0.8287
G*S	0.16	0.6891
L*G*S	0.13	0.9412
N*S	0.62	0.6839
L*N*S	0.40	0.9789
G*N*S	0.31	0.9087
L*G*N*S	0.56	0.9058

(no transformation)

Table 5.16: Effect of location, genotype and N on number of plants per m^2

Location	Genotype	N (kg/ha)	Plants/m^2
Fredericton			208 a
Truro			179 b
New Glasgow			166 bc
Canning			156 c
	CDI007		192 a
	CDI005		163 b
		20	211 a
		40	201 ab
		80	190 ab
		120	188 ab
		160	186 b
		200	162 c

(Means with a common letter are not significantly different at the 5% level)

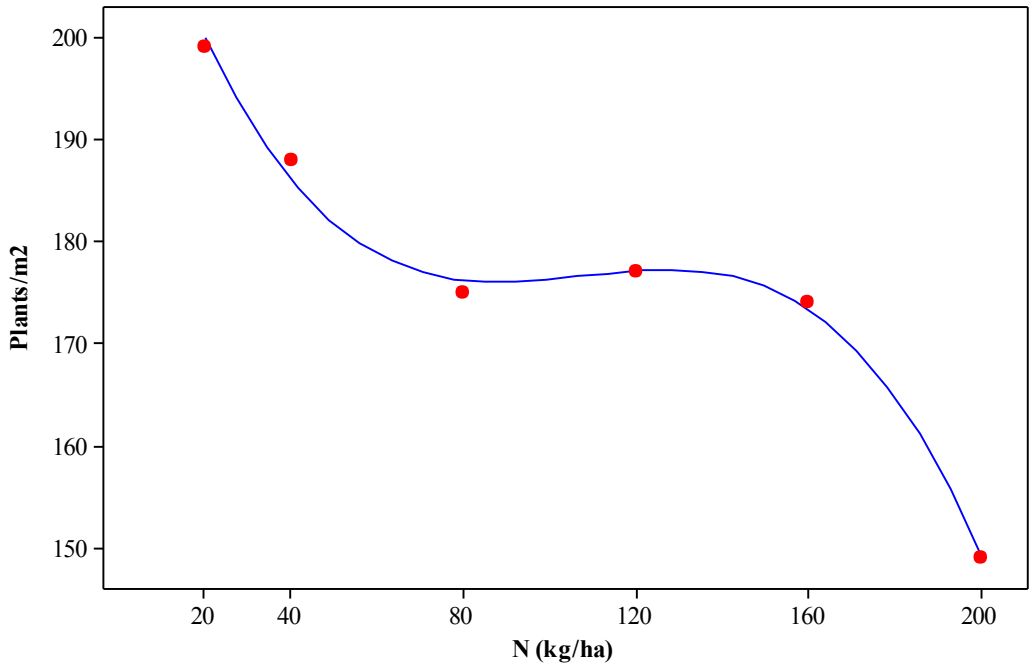


Figure 5.7: Regression analysis of effect of N on number of plants/m^2 at all 4 sites in 2012

(each dot represents 64 samples)

Y=222.4-1.369N+0.01313N**2-0.000041N**3 with R-Sq(adj)=98.8%

5.5.8 Number of Branches per m^2

Location and genotype independently had significant effects on the number of branches per m^2 at 4 sites in 2012 (Table 5.17). Plants at Fredericton and Canning had the most number of branches per m^2, followed by Truro, and plants at New Glasgow had the fewest branches per m^2 (Figure 5.8). The number of branches per m^2 of CDI007 was significantly higher than CDI005 (Table 5.18).

Table 5.17: ANOVA table of number of branches per m^2 at 4 sites in 2012

Effect	F Value	P value
Rep	5.50	0.0003
Location (L)	**5.24**	**0.0016**
Genotype (G)	**68.92**	**<.0001**
L*G	1.43	0.2344
N	0.92	0.4657
L*N	1.35	0.1713
G*N	1.85	0.1042
L*G*N	1.32	0.1903
S	2.52	0.1138
L*S	1.39	0.2473
G*S	0.09	0.7625
L*G*S	0.32	0.8097
N*S	0.39	0.8535
L*N*S	0.67	0.8105
G*N*S	0.72	0.6082
L*G*N*S	1.29	0.2066

(log_{10} transformation)

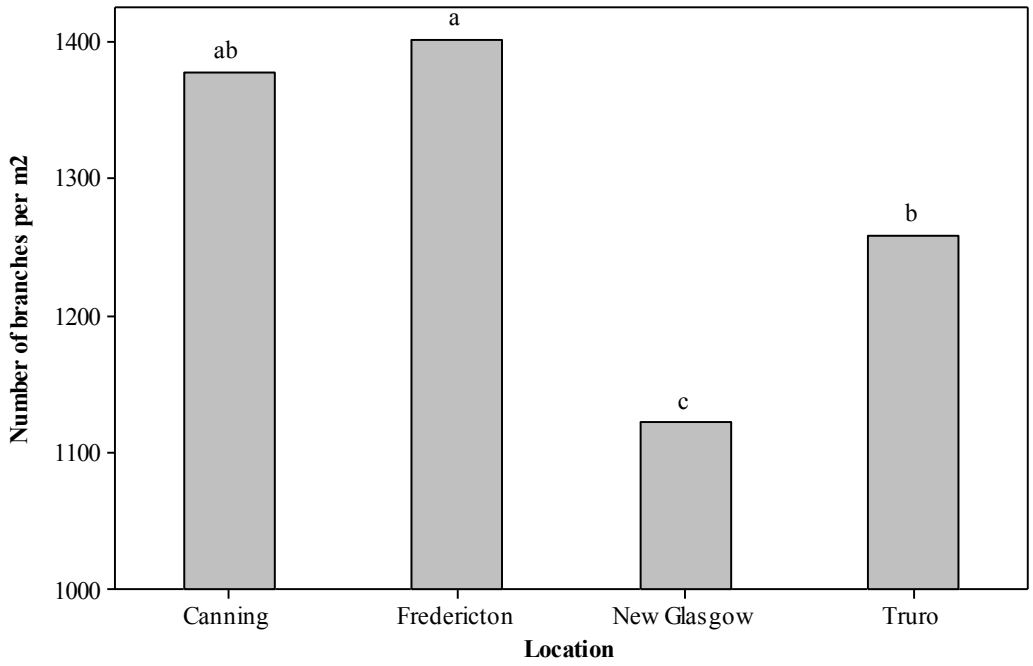


Figure 5.8: Effect of location on the number of branches per m^2 in 2012
(Means with a common letter are not significantly different at the 5% level)

Table 5.18: Effect of genotype on the number of branches per m^2

Genotype	Branches/m^2
CDI007	1504 a
CDI005	1098 b

(Means with a common letter are not significantly different at the 5% level)

5.5.9 Number of Pods per m^2

Genotype and N independently had significant effects on the number of pods per m^2 at 4 sites in 2012 (Table 5.19). CDI007 had more pods per m^2 than CDI005 (Table 5.20). The number of pods per m^2 was the highest when plants received 20 kg N/ha (Table 5.20). The number of pods per m^2 increased with N input, and the pods/m^2 was maximized when N was 120 kg N/ha (Figure 5.9).

Table 5.19: ANOVA table of number of pods per m^2 at 4 sites in 2012

Effect	F Value	P value
Rep	3.86	0.0045
Location (L)	1.92	0.1270
Genotype (G)	**41.71**	**<.0001**
L*G	0.41	0.7462
N	**2.77**	**0.0186**
L*N	1.17	0.2946
G*N	1.09	0.3660
L*G*N	0.81	0.6698
S	0.4	0.5274
L*S	1.81	0.1464
G*S	0.29	0.5909
L*G*S	0.72	0.5399
N*S	1.1	0.3632
L*N*S	0.69	0.7897
G*N*S	0.41	0.8424
L*G*N*S	1.35	0.1719

(log_{10} transformation)

Table 5.20: Effect of genotype and N on number of pods per m^2

Genotype	N (kg/ha)	Pods/m^2
CDI007		25888 a
CDI005		19966 b
	20	19490 b
	40	22511 a
	80	22867 a
	120	24457 a
	160	24116 a
	200	23340 a

(Means with a common letter are not significantly different at the 5% level)

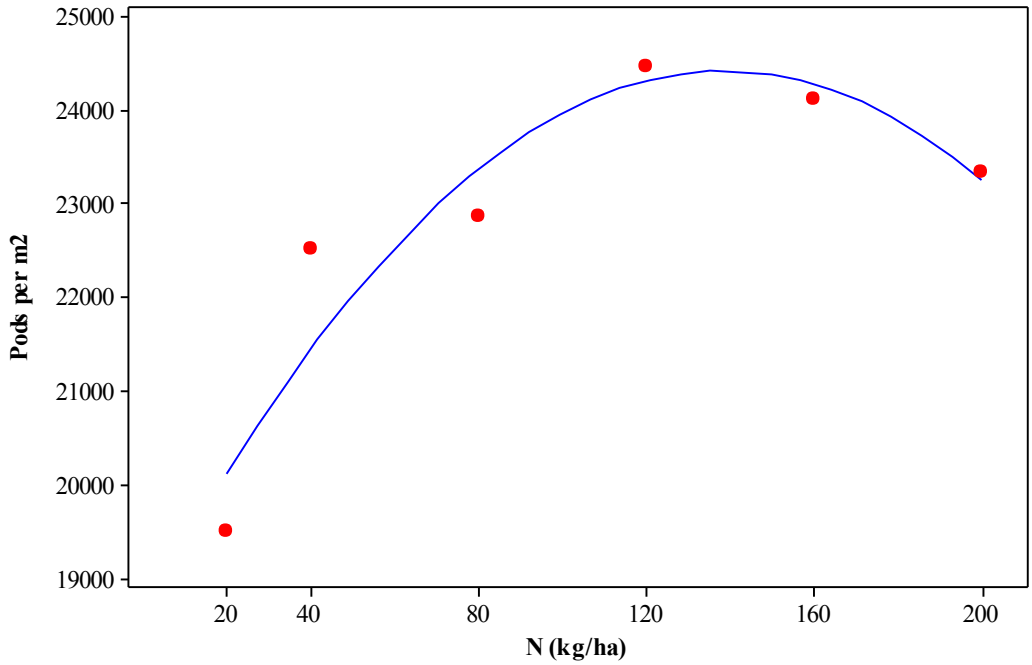


Figure 5.9: Regression analysis of N response to pods/m^2 with two S levels at four sites in 2012

(each dot represents 64 samples)

Y=18546+84.93N-0.3071N**2 with R-Sq(adj)=80.7%

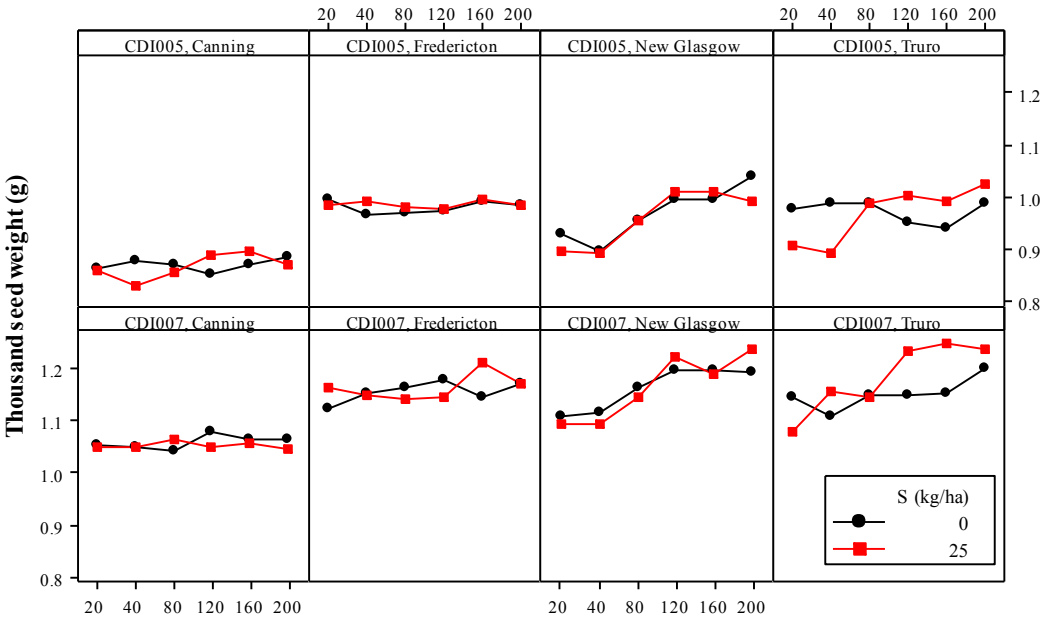
5.5.10 Thousand Seed Weight

Genotype differed in thousand seed weight depending on the amounts of N and S at different locations (Table 5.21). CDI007 (1.05-1.3g) had significantly higher thousand seed weight than CDI005 (approximately 0.8-1.05g) (Figure 5.10). N was positively correlated with thousand seed weight depending on location and S, which was true at New Glasgow for both CDI005 and CDI007 with two levels of S and Truro for both CDI005 and CDI007 with 25 kg S/ha application. S had no effect on thousand seed weight at Canning, Fredericton and New Glasgow. At Truro when plants received 25 kg S/ha, thousand seed weight increased with an increase in N, while when plants did not receive an S application, thousand seed weight fluctuated with an increase in N (Figure 5.10).

Table 5.21: ANOVA table of thousand seed weight in 2012

Effect	F Value	P value
Rep	16.43	<.0001
Location (L)	49.47	<.0001
Genotype (G)	2609.78	<.0001
L*G	3.24	0.0226
N	25.34	<.0001
L*N	5.74	<.0001
G*N	0.64	0.6728
L*G*N	0.82	0.6526
S	0.27	0.6027
L*S	1.22	0.3015
G*S	2.04	0.1542
L*G*S	1.08	0.3562
N*S	4.33	0.0008
L*N*S	2.24	0.0055
G*N*S	1.33	0.2522
L*G*N*S	**1.79**	**0.0362**

(log_{10} transformation)



Panel variables: Genotype, Location

Figure 5.10: Effect of interaction of genotype, location, N and S on thousand seed weight in 2012

Table 5.22: Mean values of thousand seed weight for CDI005 and CDI007 with different N and S treatments at 4 sites in 2012

Genotype	S (kg/ha)	N (kg/ha)	Canning	Fredericton	New Glasgow	Truro
CDI005	0	20	0.8609 (2)g-j	0.9937 u-z	0.9265 (2)b-e	0.9747 w-(2)a
		40	0.8771 (2)f-i	0.9657 z-(2)b	0.8962 (2)d-g	0.9880 v-(2)a
		80	0.8684 (2)f-j	0.9702 z-(2)b	0.9551 y-(2)b	0.9861 w-z
		120	0.8489 (2)ij	0.9725 z-(2)b	0.9943 u-z	0.9493 z-(2)c
		160	0.8683 (2)f-j	0.9910 v-z	0.9950 u-z	0.9379 (2) a-d
		200	0.8840 (2)e-i	0.9819 w-(2)a	1.0403 r-v	0.9866 w-z
	25	20	0.8595 (2)g-j	0.9846 w-(2)a	0.8939 (2)d-i	0.9077 (2)c-f
		40	0.8296 (2)j	0.9905 w-z	0.8909 (2)e-i	0.8925 (2)e-h
		80	0.8529 (2)h-j	0.9789 w-(2)a	0.9525 z-(2)c	0.9856 w-z
		120	0.8863 (2)e-i	0.9747 w-(2)a	1.0106 s-x	1.0030 t-y
		160	0.8949 (2)d-h	0.9929 u-z	1.0086s-x	0.9896 w-z
		200	0.8700 (2)f-j	0.9846 w-(2)a	0.9896 w-z	1.0231 r-w
CDI007	0	20	1.0525 o-t	1.1224 g-l	1.1108 h-m	1.1460 e-k
		40	1.0500 o-t	1.1542 d-j	1.1176 g-m	1.1075 i-n
		80	1.0418 r-u	1.1639 d-h	1.1647 d-h	1.1508 e-i
		120	1.0780 l-q	1.1803 b-f	1.1981 a-f	1.1480 e-j
		160	1.0663 m-r	1.1477 e-k	1.1986 a-f	1.1537 e-i
		200	1.0632 m-r	1.1709 c-g	1.1935 a-f	1.2020 a-e
	25	20	1.0518 q-t	1.1646 d-h	1.0929 k-p	1.0794 l-q
		40	1.0517 q-t	1.1504 e-i	1.0946 j-o	1.1567 d-i
		80	1.0640 m-r	1.1419 f-k	1.1474 e-k	1.1459 e-k
		120	1.0488 o-t	1.1457 e-k	1.2234 a-c	1.2356 ab
		160	1.0569 n-s	1.2136 a-d	1.1888 a-f	1.2480 a
		200	1.0478 o-t	1.1712 c-g	1.2367 ab	1.2389 ab

(Means with a common letter are not significantly different at the 5% level)

5.5.11 Seed Yield

Genotype, the interaction of location and N, the interaction of location and S and the interaction of N and S had significant effects on seed yields at 4 sites in 2012 (Table 5.23).

The yield of CDI007 was significantly higher than CDI005 with 1911 kg/ha and 1638 kg/ha for CDI007 and CDI005, respectively (Table 5.24).

The yields at Canning, Fredericton and New Glasgow were significantly higher than the yield at Truro (Figure 5.11). The optimum N rates for the highest yield were 120 kg N/ha at Canning, New Glasgow and Truro and 160 kg N/ha at Fredericton. Yields increased with the increase of N generally, but the regression models were different from one location to another (Figure 5.12).

S did not have significant effects at Canning, Fredericton, and New Glasgow, but at Truro, S application led to higher yield (Figure 5.13).

S increased seed yields only when N increased and reached 120 kg N/ha and more (Figure 5.14). With 120-200 kg N/ha rates, plants with 25 kg S/ha S had significantly higher yields than plants without S application (Figure 5.14). Yields increased with the increase of N under both two rates of S application (Figure 5.15).

Table 5.23: ANOVA table of seed yields at Canning, Truro, New Glasgow and Fredericton in 2012

Effect	F Value	P value
Rep	8.06	<.0001
Location (L)	47.61	<.0001
Genotype (G)	**177.61**	**<.0001**
L*G	0.5	0.6836
N	87.32	<.0001
L*N	**2.47**	**0.0021**
G*N	0.41	0.8440
L*G*N	0.43	0.9706
S	5.87	0.0160
L*S	**3.2**	**0.0238**
G*S	2.65	0.1050
L*G*S	1.09	0.3524
N*S	**3.16**	**0.0087**
L*N*S	1.34	0.1803
G*N*S	0.83	0.5280
L*G*N*S	0.59	0.8821

(square root transformation)

Table 5.24: Effect of genotype on seed yields

Genotype	Yield (kg/ha)
CDI007	1911 a
CDI005	1638 b

(Means with a common letter are not significantly different at the 5% level)

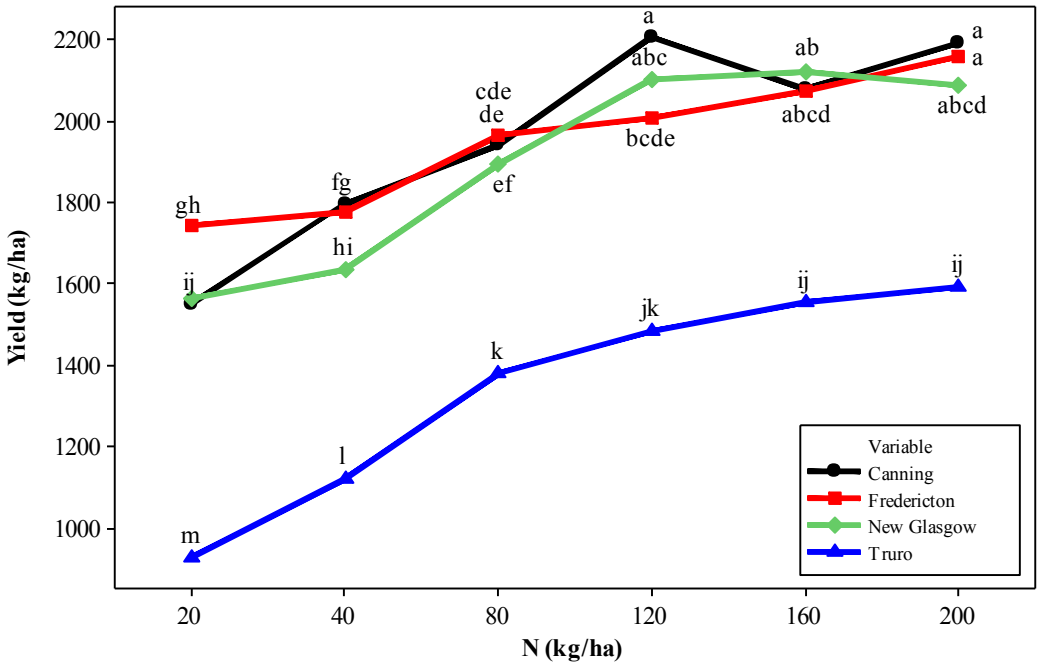
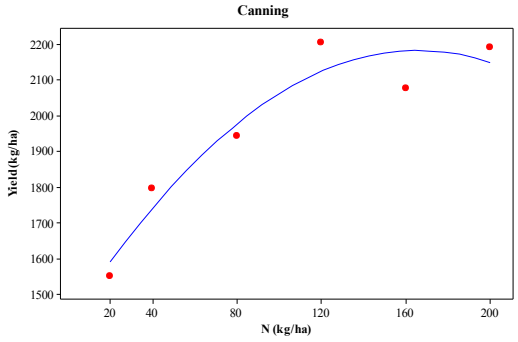
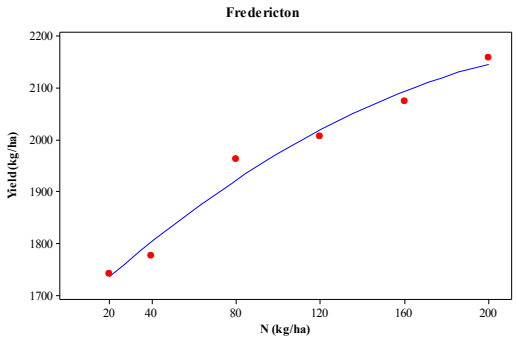


Figure 5.11: Effect of location and N on seed yield in 2012

(Means with a common letter are not significantly different at the 5% level)





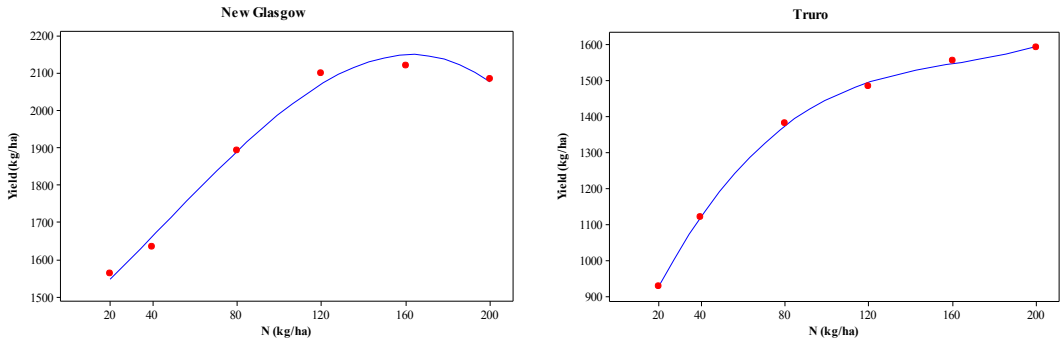


Figure 5.12: Regression analysis of N response to yields at different locations in 2012
(each dot represents 16 samples)

(1) Y(Canning)=1416+9.248N-0.02790N**2 with R-Sq(adj)=87.1%

(2) Y(Fredericton)=1661+3.817N-0.006946N**2 with R-Sq(adj)=96.1%

(3) Y(New Glasgow)=1436+5.204N+0.01694N**2-0.000135N**3 with R-Sq(adj)=97.5%

(4) Y(Truro)=677.0+14.06N-0.07995N**2+0.000163N**3 with R-Sq(adj)=99.8%

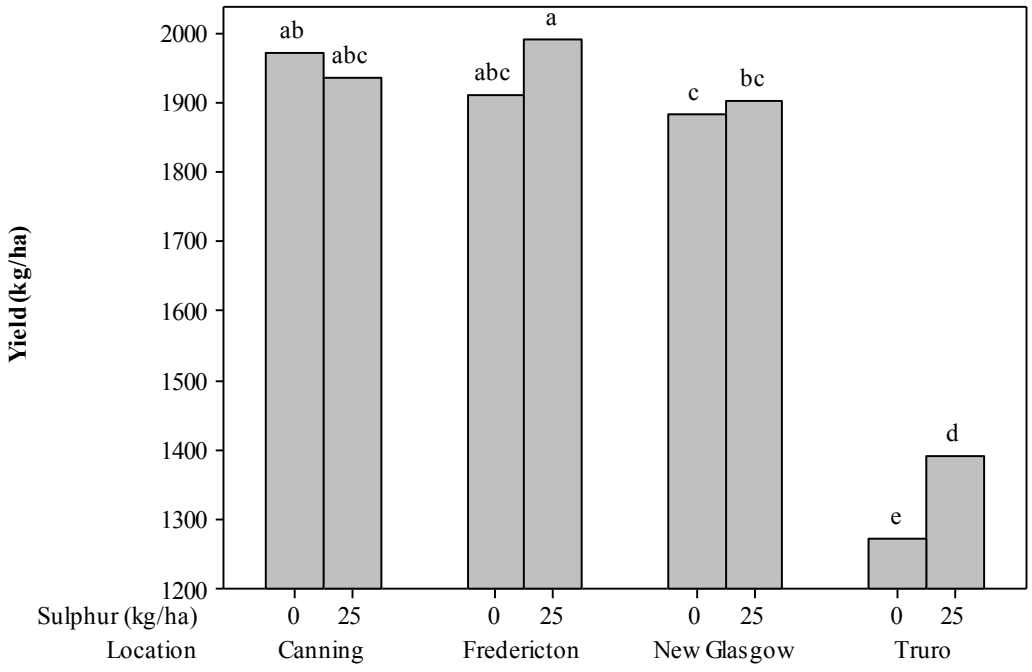


Figure 5.13: Effect of location and S on seed yields in 2012
(Means with a common letter are not significantly different at the 5% level)

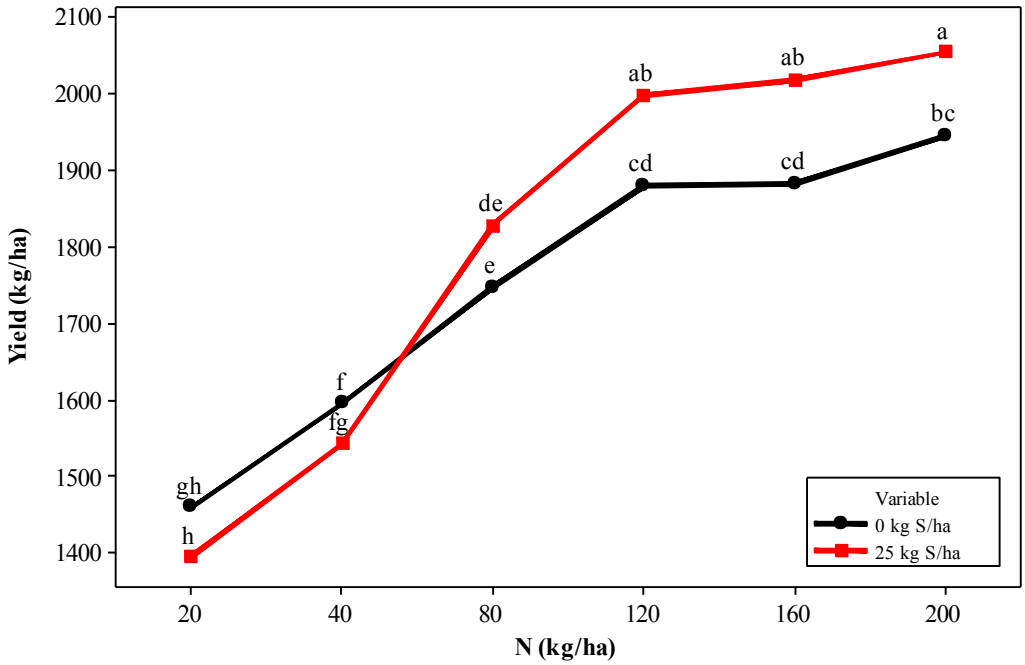


Figure 5.14: Effect of S and N on seed yields in 2012
(Means with a common letter are not significantly different at the 5% level)

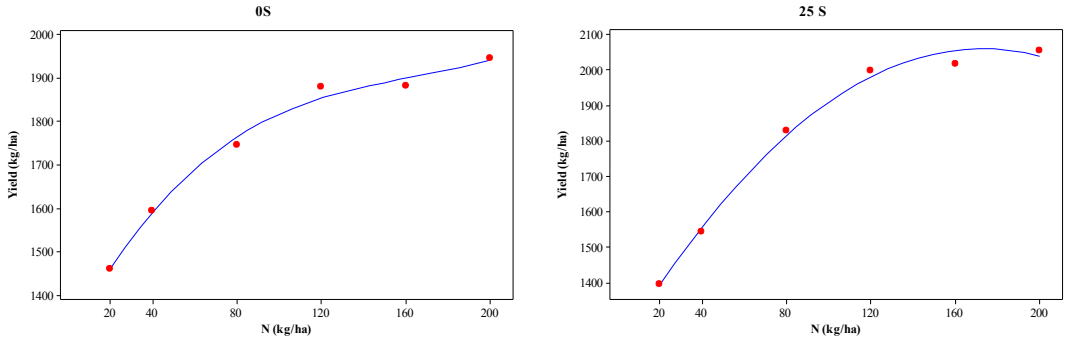


Figure 5.15: Regression analysis of N response to yield with two S rates in 2012
(each dot represents 32 samples)

(1) Y(0S)=1294+9.283N-0.05076N**2+0.000103N**3 with R-Sq(adj)=98.2%

(2) Y(25S)=1206+9.878N-0.02858N**2 with R-Sq(adj)=99.0%

5.5.12 Protein Content

The interaction of location and N, the interaction of location and S, the interaction of genotype and S and the interaction of N and S had significant effects on the percent of protein in camelina seeds (Table 5.25).

The protein content increased with an increase in N (Figure 5.16). The protein content increased with higher N until 120 kg N/ha at Fredericton and New Glasgow and 160 kg N/ha at Truro and Canning. In general, plants at Fredericton produced the highest content of protein, while plants at New Glasgow produced the lowest content of protein; the protein content at Canning and Truro were similar. The regression models of N effects on the content of protein at different locations were shown in Figure 5.17. High ratio (94.9%-99.6%) of variability of the content of protein could be explained by the N change.

S input increased the content of protein at all the four sites (Figure 5.18). With 25 kg S/ha application, plants at Canning and Truro produced similar protein contents. Plants with 25 kg S/ha at Fredericton had the highest content of protein, while plants without S application at New Glasgow produced the least protein content.

The content of protein increased with S input for both CDI007 and CDI005 (Figure 5.19).

Without S input, the content of protein increased until N reached 120 kg/ha; with 25 kg S/ha, the protein content increased until N reached 160 kg/ha (Figure 5.20). It also shows with the N treatments of 80, 120, 160, and 200 kg/ha, S input led to higher contents of protein (Figure 5.20). The percent of protein increased with the increase of N input, and higher ratio of variability in the protein increase could be explained by the increase of N application (Figure 5.21).

Table 5.25: ANOVA table of protein content of seeds at Canning, Fredericton, New Glasgow & Truro

Effect	F value	P value
Rep	4.32	0.0021
Location (L)	72.05	<.0001
Genotype (G)	272.66	<.0001
L*G	1.76	0.1546
N	275.11	<.0001
L*N	**6.56**	**<.0001**
G*N	0.87	0.4985
L*G*N	0.38	0.9827
S	101.6	<.0001
L*S	**6.36**	**0.0003**
G*S	**3.94**	**0.0481**
L*G*S	1.81	0.1464
N*S	**14.97**	**<.0001**
L*N*S	1.15	0.3077
G*N*S	0.62	0.6843
L*G*N*S	0.8	0.6809

(no transformation)

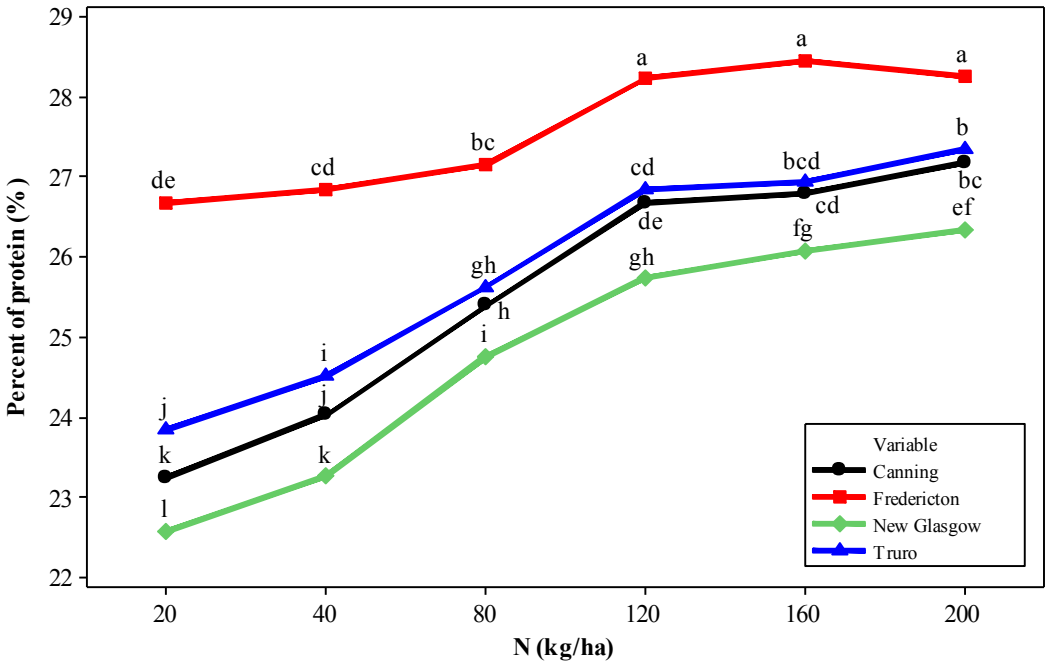


Figure 5.16: Effect of location and N on the percent of protein in 2012
(Means with a common letter are not significantly different at the 5% level)

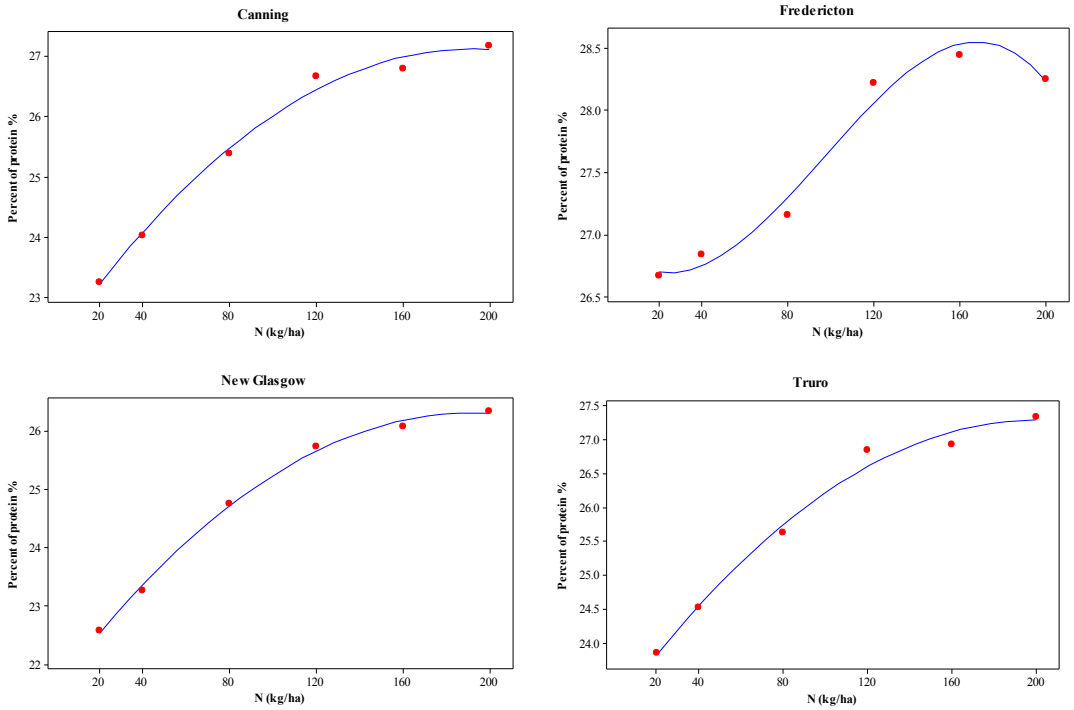


Figure 5.17: Regression analysis of N response to protein (%) at different locations in 2012

(each dot represents 16 samples)

(1) Y(Canning)=22.26+0.05081N-0.000133N**2 with R-Sq(adj)=98.6%

(2) Y(Fredericton)=26.89-0.01628N+0.000370N**2-0.000001N**3 with R-Sq(adj)=94.9%

(3) Y(New Glasgow)=21.59+0.04929N-0.000129N**2 without R-Sq(adj)=99.6%

(4) Y(Truro)=23.01+0.04266N-0.000106N**2 with R-Sq(adj)=98.1%

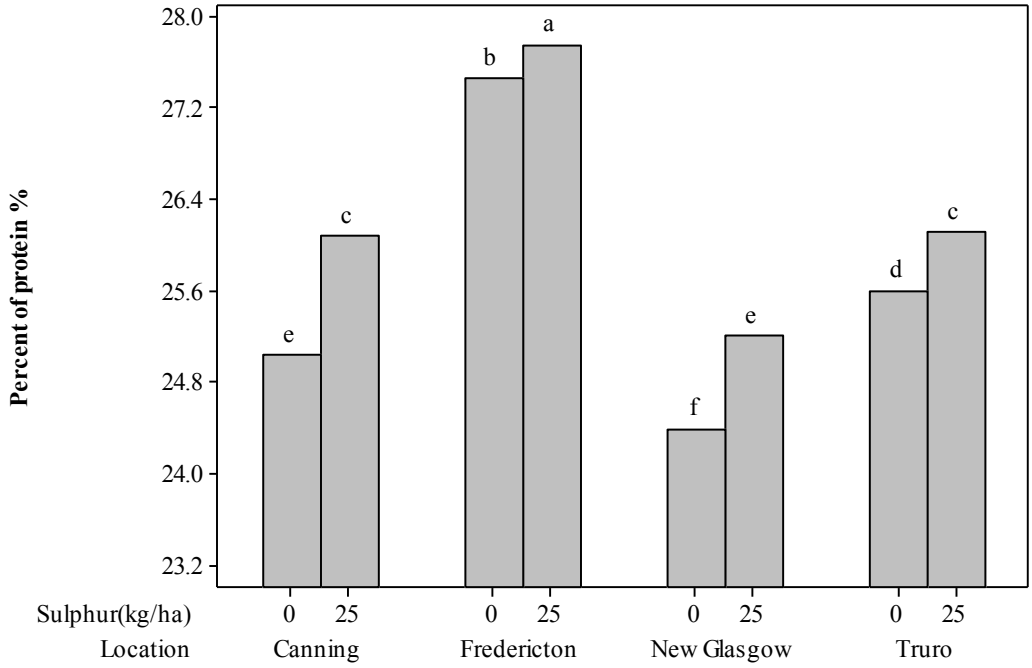


Figure 5.18: Effect of location and S on protein content % in 2012
(Means with a common letter are not significantly different at the 5% level)

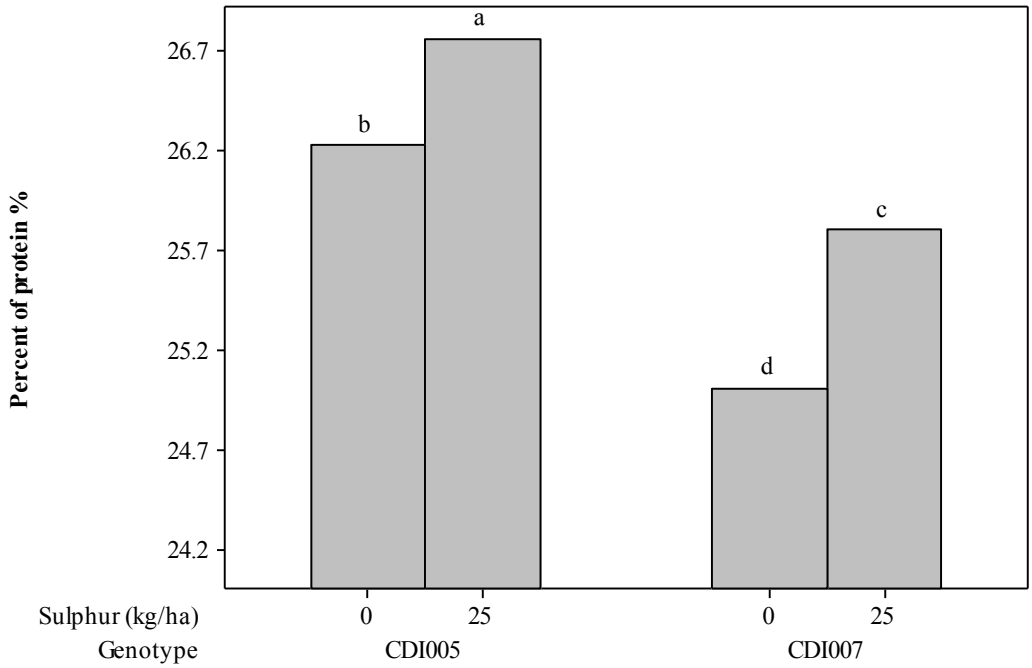


Figure 5.19: Effect of genotype and S on percent of protein % in 2012
(Means with a common letter are not significantly different at the 5% level)

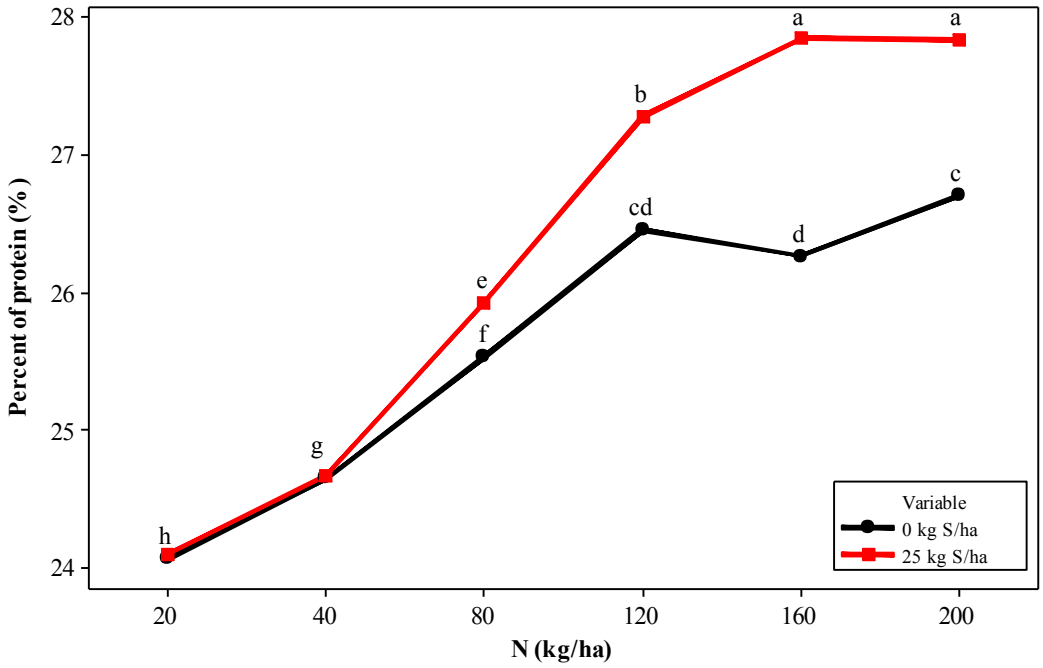


Figure 5.20: Effect of interaction of N and S on the content of protein in 2012
(Means with a common letter are not significantly different at the 5% level)

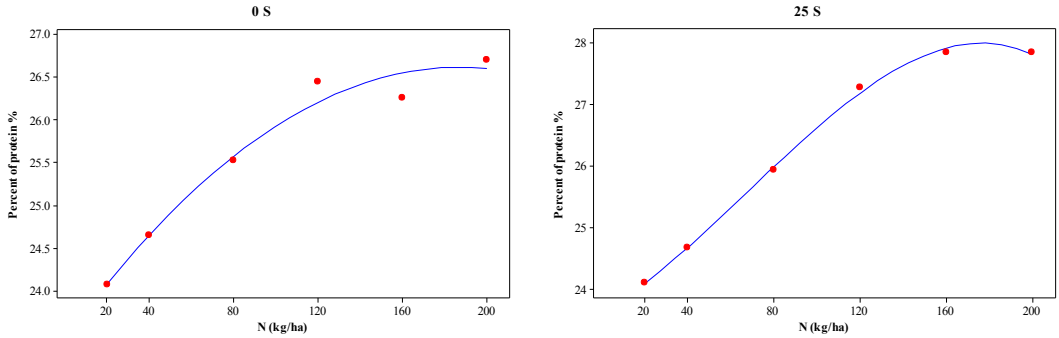


Figure 5.21: Regression analysis of effect of N on the percent of protein in 2012
(each dot represents 32 samples)

(1) Y(0S)=23.11+0.03396N-0.00009N**2 with R-Sq(adj)=95.5%

(2) Y(25S) =23.62+0.02029N+0.000190N**2-0.000001N**3 with R-Sq(adj)=99.7%

5.5.13 Protein Yield

The interaction of location and N, the interaction of location and S, and the interaction of N and S had significant effects on the protein yield (Table 5.26). The protein yield increased with the increase of N, and the protein yield at Truro was

significantly lower than Canning, Fredericton and New Glasgow (Figure 5.22). High ratios (91.3%-99.2%) of variability of protein yield could be explained by the change of N levels (Figure 5.23). S increased the protein yield at Fredericton, New Glasgow and Truro, but not at Canning (Figure 5.24). S increased the protein yield only when N (120-200 kg/ha) was sufficient in the soil (Figure 5.25). High ratios (94.4%-99.3%) of variability of protein yield could be explained by the change of N levels (Figure 5.26).

Table 5.26: ANOVA table of protein yield at Canning, Fredericton, New Glasgow and Truro in 2012

Effect	F Value	P value
Rep	8.10	<.0001
Location (L)	31.45	<.0001
Genotype (G)	67.14	<.0001
L*G	2.42	0.0666
N	98.08	<.0001
L*N	**3.13**	**<.0001**
G*N	0.49	0.7808
L*G*N	0.24	0.9986
S	26.40	<.0001
L*S	**2.80**	**0.0406**
G*S	0.06	0.8001
L*G*S	0.63	0.5977
N*S	**3.85**	**0.0022**
L*N*S	0.96	0.4947
G*N*S	0.90	0.4826
L*G*N*S	0.73	0.7500

(no transformation)

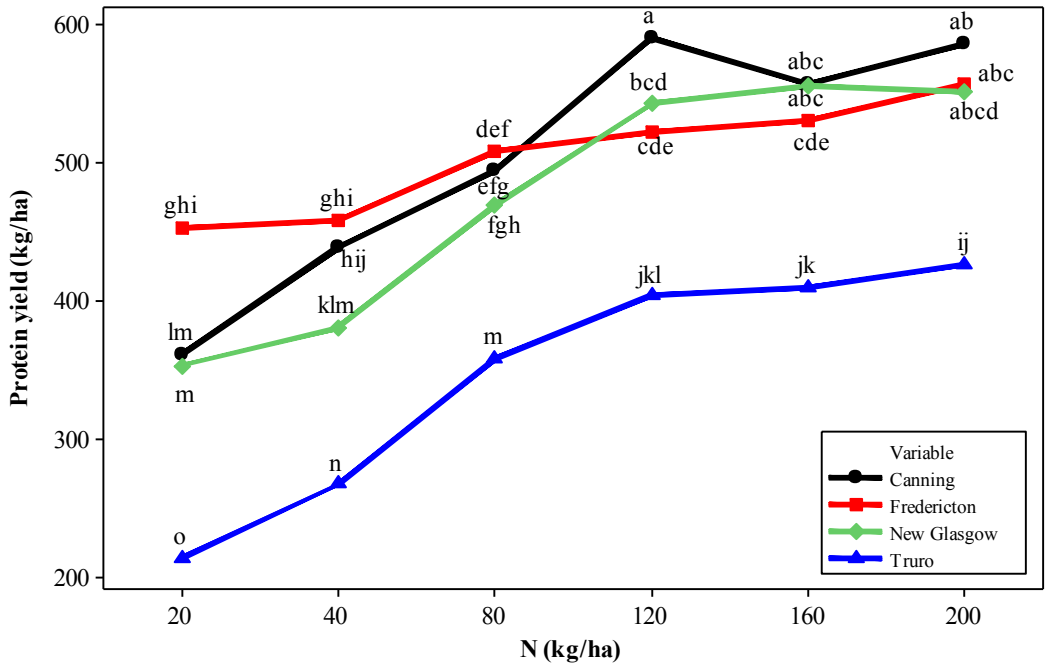


Figure 5.22: Effect of location and N on the protein yield in 2012
(Means with a common letter are not significantly different at the 5% level)

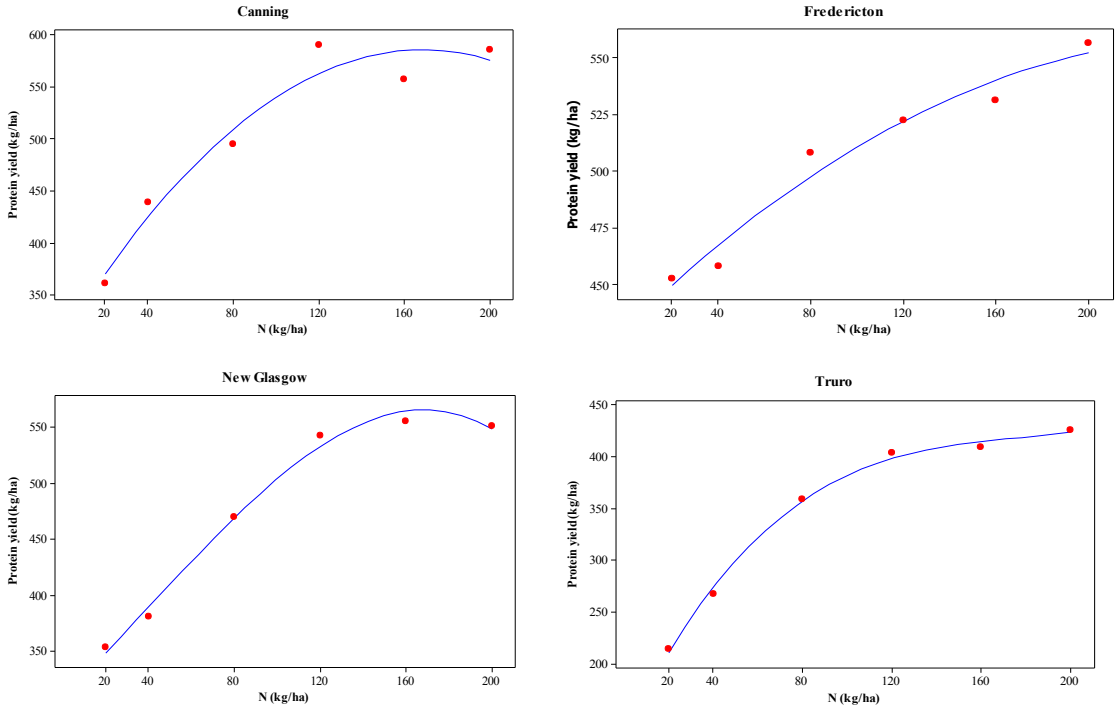


Figure 5.23: Regression analysis of N effect on the protein yield at four different locations in 2012
(each dot represents 16 samples)

(1) Y(Canning)=308.4+3.292N-0.009783N**2 with R-Sq(adj)=91.3%

(2) Y(Fredericton)=430.7+0.9894N-0.001914N**2 with R-Sq(adj)=94.0%

(3) Y(New Glasgow)=309.3+1.874N+0.00485N**2-0.000041N**3 with R-Sq(adj)=98.3%

(4) Y(Truro)=130.4+4.479N-0.02421N**2+0.000046N**3 with R-Sq(adj)=99.2%

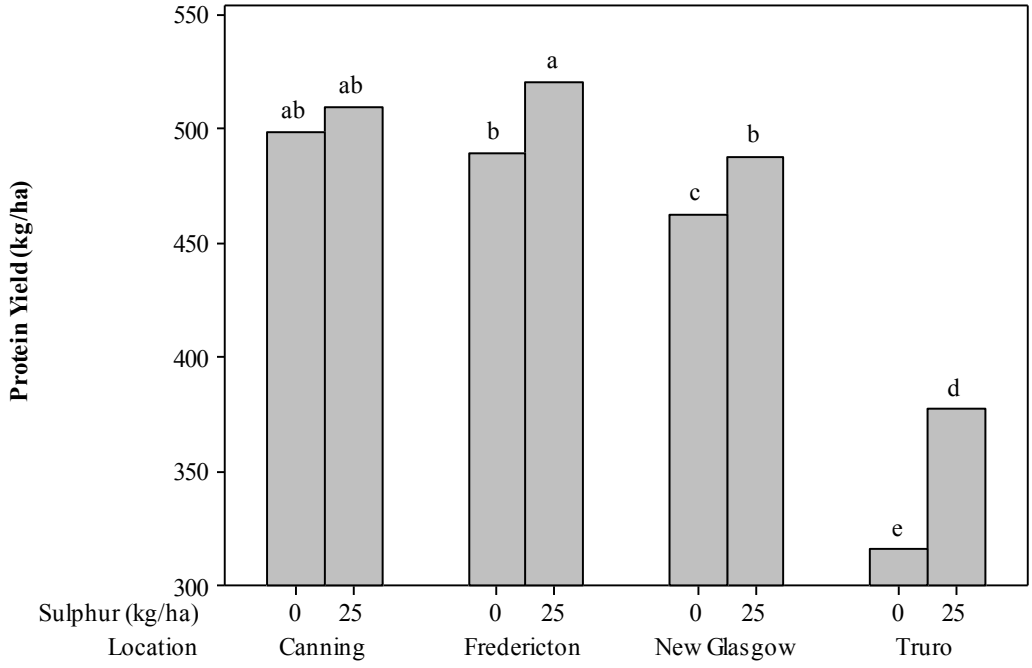


Figure 5.24: Effect of location and S on the protein yield in 2012
(Means with a common letter are not significantly different at the 5% level)

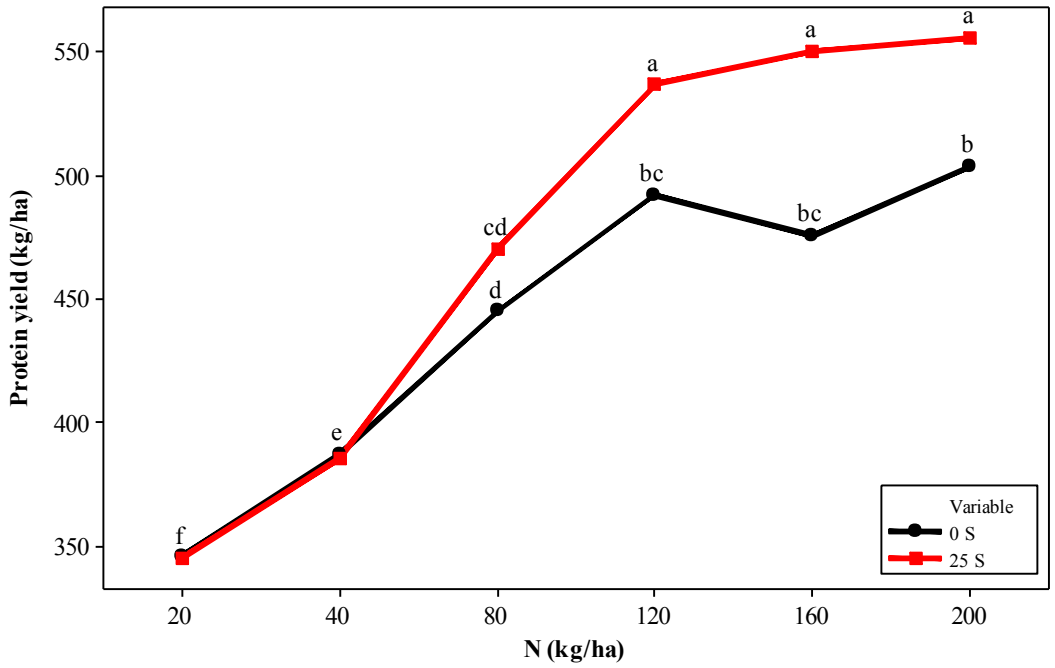


Figure 5.25: Effect of N and S on the protein yield in 2012
(Means with a common letter are not significantly different at the 5% level)

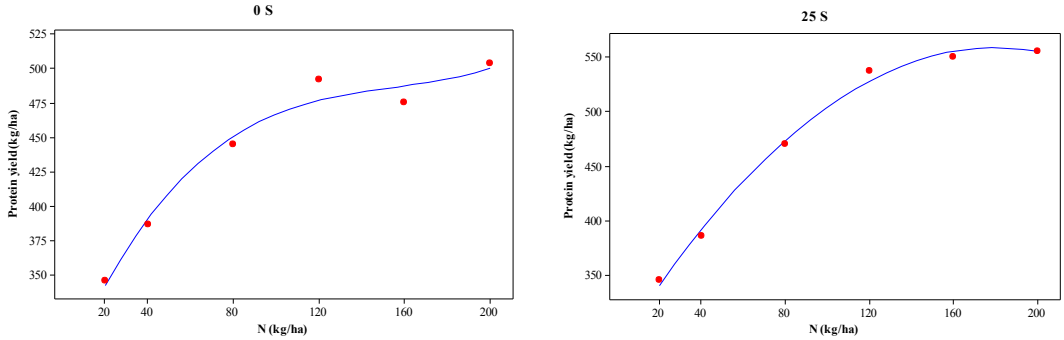


Figure 5.26: Regression analysis of N effect on protein yield with two levels of S in 2012
(each dot represents 32 samples)

(1) Y(0S)=278.7+3.607N-0.02197N**2+0.000047N**3 with R-Sq(adj)=94.4%

(2) Y(25S)=282.5+3.072N-0.008552N**2 with R-Sq(adj)=99.3%

5.5.14 Oil Content

The interaction of location and genotype, the interaction of location and N, the interaction of genotype and N, the interaction of location and S, and the interaction of N

and S had significant effects on the content of oil at Canning, Fredericton, New Glasgow and Truro (Table 5.27).

CDI007 was higher in oil content than CDI005 at all four sites (Figure 5.27). In general, seeds from New Glasgow had the highest oil content, followed by Truro, Canning and Fredericton (Figure 5.27).

The oil content decreased with the increase of N at all four locations (Figure 5.28). Plants at New Glasgow produced the highest content of oil; while plants at Fredericton produced the lowest content of oil. The oil content was the lowest with the 120 kg/ha N application at Canning, Fredericton and Truro and 160 kg/ha at New Glasgow. High ratios (85.0%-96.5%) of variability in the oil content could be explained by the change of N input (Figure 5.26).

CDI007 had the highest oil content when plants received 20 or 40 kg N/ha, and CDI005 had the highest oil content when plants received 20 kg N/ha (Figure 5.30). CDI007 had significantly higher oil content than CDI005 (Figure 5.30). High ratios (95.3%-96.5%) of variability on the oil content could be explained by the change of N levels (Figure 5.31).

S did not have an impact on the oil content at Fredericton, New Glasgow and Truro, but at Canning, S input decreased the oil content (Figure 5.32).

With lower N rates (20 kg N/ha to 80 kg N/ha), S did not have an effect on the oil content while with higher N rates (120, 160 and 200 kg N/ha), 25 kg/ha of S resulted in lower oil content (Figure 5.33). High ratios (89.2%-98.6%) of variability on the oil content could be explained by the change of N levels for both two levels of S application (Figure 5.34).

Table 5.27: ANOVA table of oil content of seeds at Canning, Fredericton, New Glasgow and Truro in 2012

Effect	F value	P value
Rep	2.62	0.0351
Location (L)	83.42	<.0001
Genotype (G)	251.39	<.0001
L*G	**3.44**	**0.0172**
N	101.19	<.0001
L*N	**3.9**	**<.0001**
G*N	**2.84**	**0.0162**
L*G*N	1.04	0.4183
S	15.48	0.0001
L*S	**7.15**	**0.0001**
G*S	0.29	0.5900
L*G*S	1.18	0.3164
N*S	**6.00**	**<.0001**
L*N*S	1.42	0.1377
G*N*S	1.45	0.2059
L*G*N*S	1.13	0.3312

(no transformation)

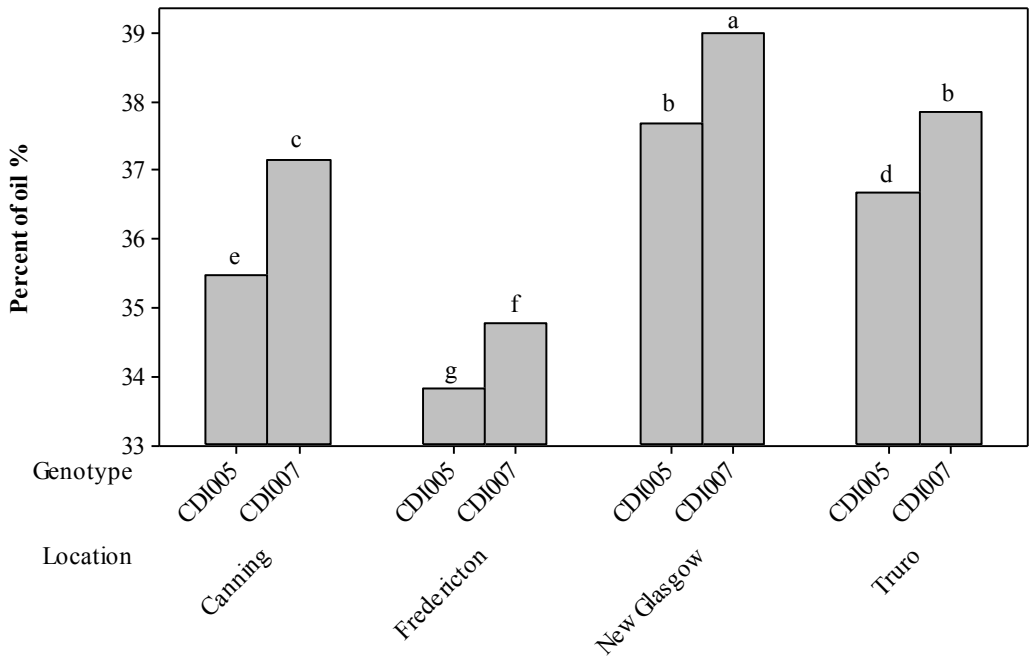


Figure 5.27: Effect of location and genotype on percent of oil in 2012
(Means with a common letter are not significantly different at the 5% level)

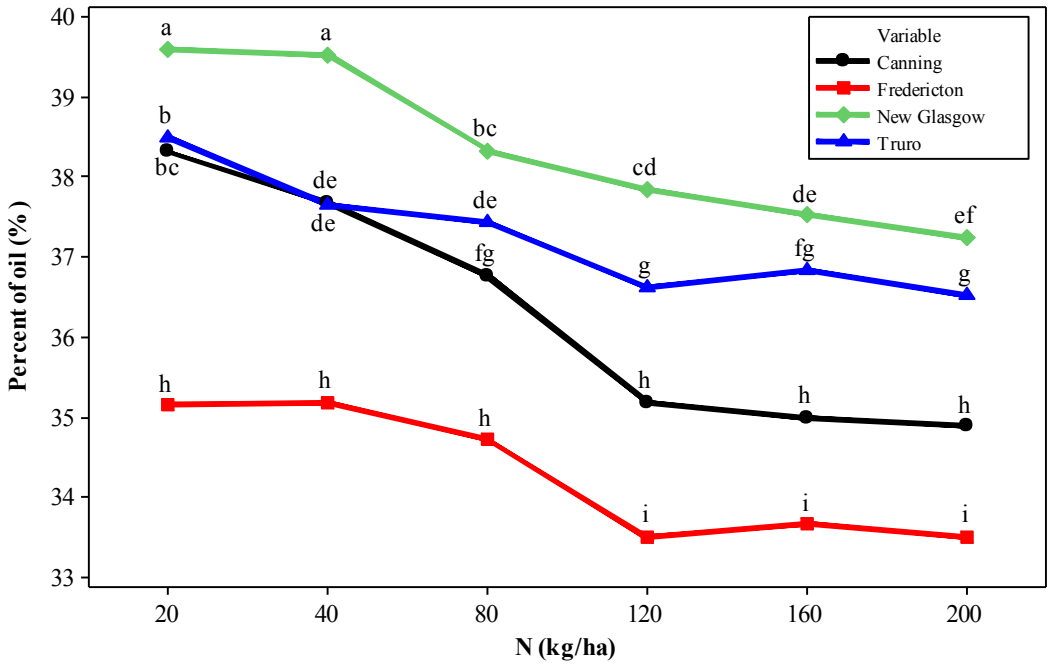


Figure 5.28: Effect of interaction of location and N on the oil content in 2012
(Means with a common letter are not significantly different at the 5% level)

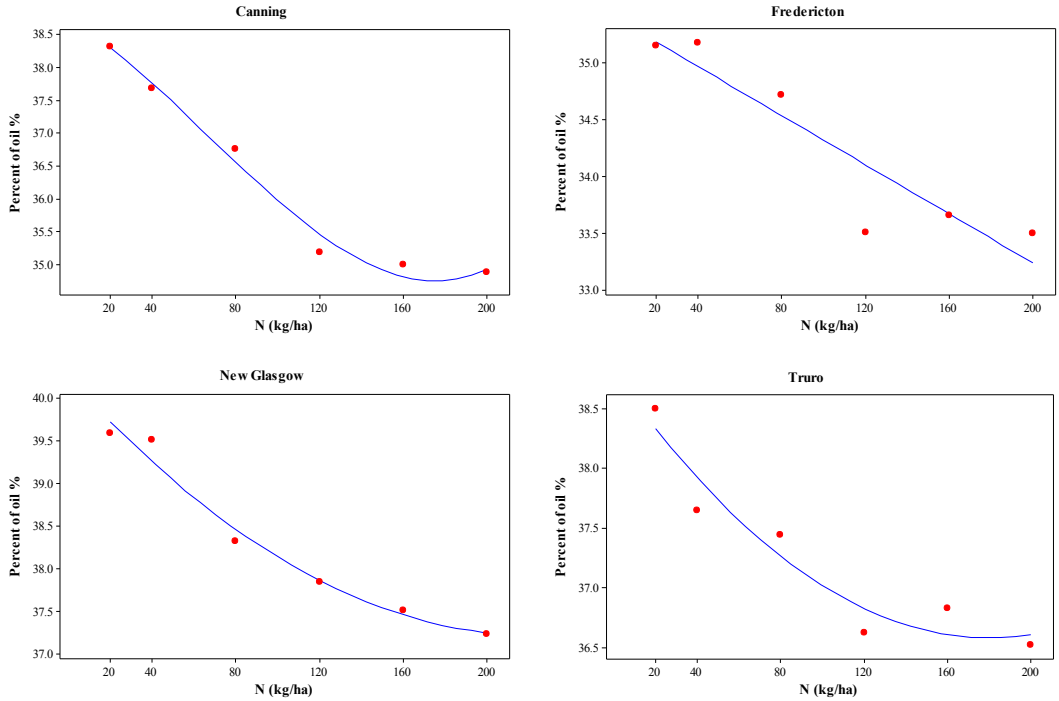


Figure 5.29: Regression analysis of N on oil at different locations
(each dot represents 16 samples)

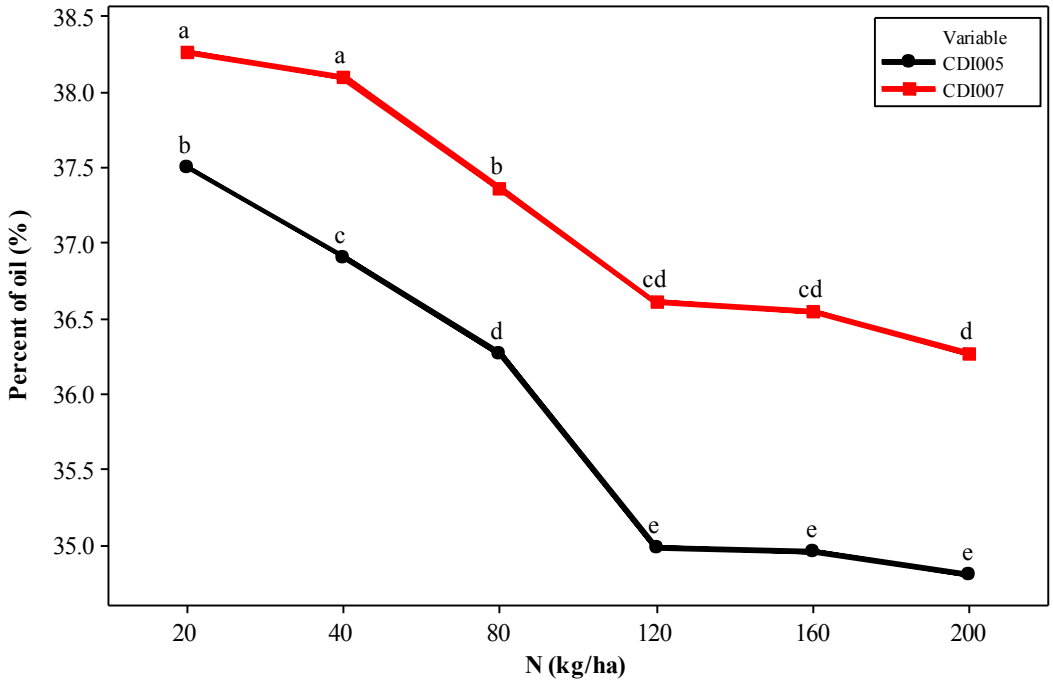
(1) Y(Canning)=38.74-0.01934N-0.000167N**2+0.0000001N**3 with R-

Sq(adj)=96.4%

(2) Y(Fredericton)=35.40-0.01079*N with R-Sq=85.0%

(3) Y(New Glasgow)=40.25-0.02716N+0.000061N**2 with R-Sq(adj)=96.5%

(4) Y(Truro)=38.79-0.02456N+0.000068N**2 with R-Sq(adj)=86.4%



(Means with a common letter are not significantly different at the 5% level)

Figure 5.30: Effect of interaction of genotype and N on the oil content %

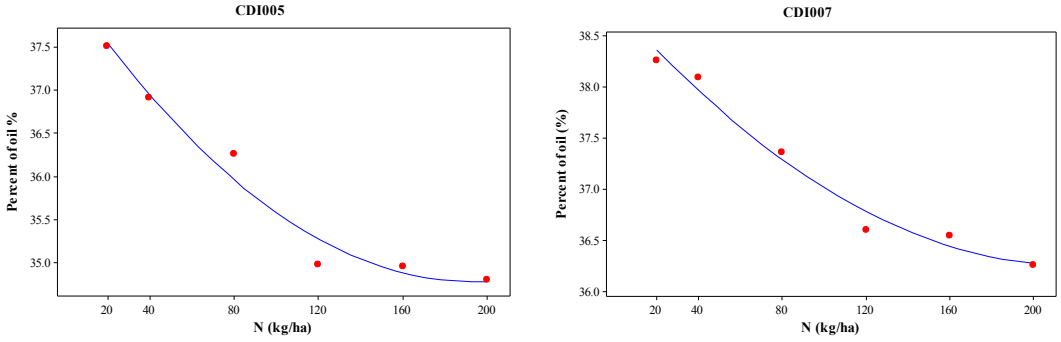


Figure 5.31: Regression of N on content of oil (%) of CDI005 and CDI007
(each dot represents 32 samples)

(1) Y(CDI005)=38.23-0.03557N+0.000092N**2 with R-Sq(adj)=95.3%

(2) Y(CDI007)=38.81-0.02319N+0.000053N**2 with R-Sq(adj)=96.5%

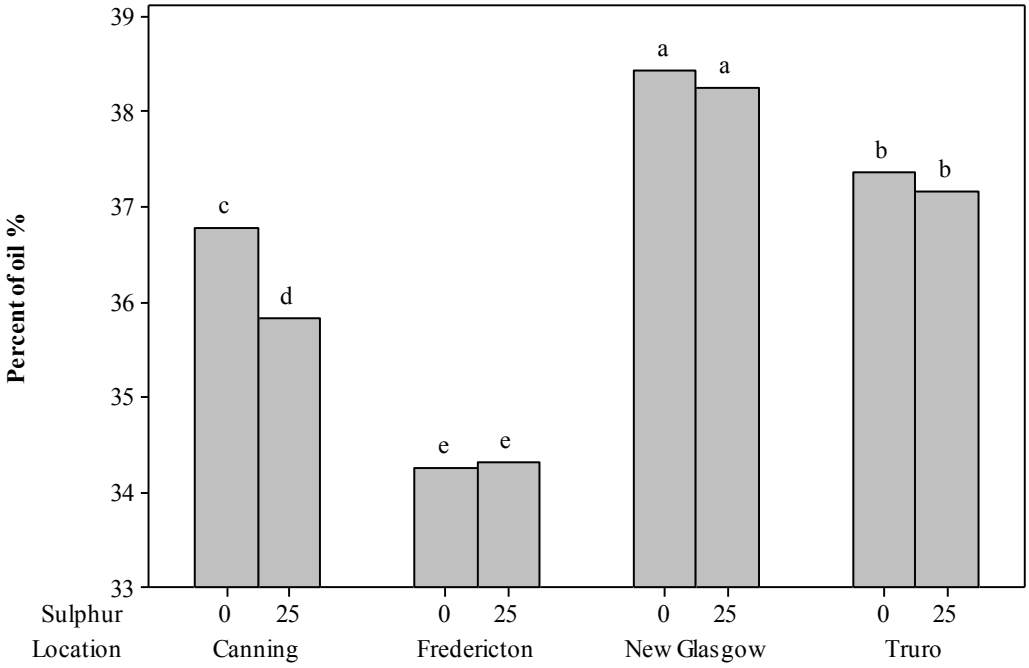


Figure 5.32: Effect of location and S on percent of oil (%)
(Means with a common letter are not significantly different at the 5% level)

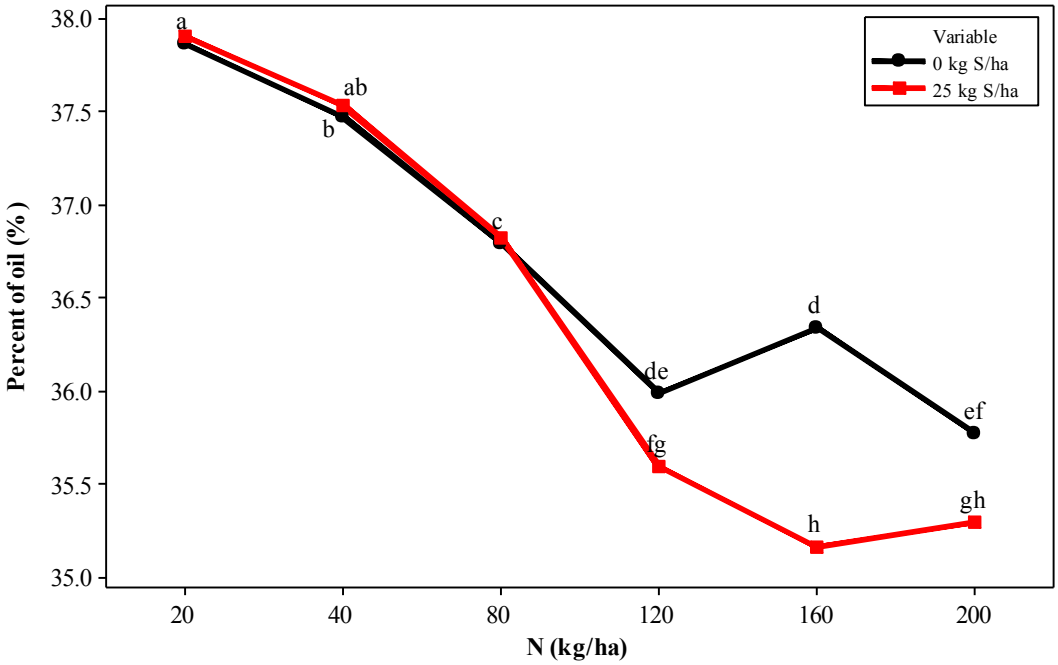


Figure 5.33: Effect of interaction of S and N on the oil content %
(Means with a common letter are not significantly different at the 5% level)

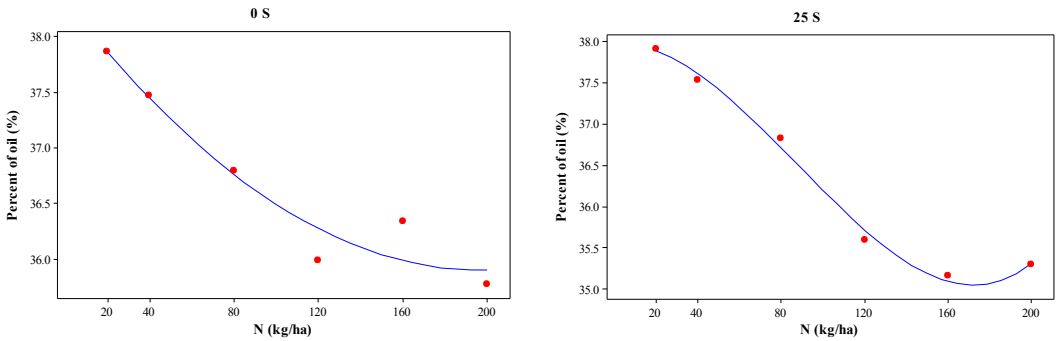


Figure 5.34: Regression analysis of N with different S levels on oil %
(each dot represents 32 samples)

(1) Y(0S)=38.33-0.02451N+0.000062N**2 with R-Sq(adj)=89.2%

(2) Y(25S)=37.95+0.00237N-0.000320N**2+0.000001N**3 with R-Sq(adj)=98.6%

5.5.15 Oil Yield

Genotype, the interaction of location and N, and the interaction of location and S had significant effects on the oil yield (Table 5.28). CDI007 (715 kg/ha) had significantly higher oil yield than CDI005 (591 kg/ha) (Table 5.29). The oil yield increased with the increase of N, and the oil yield at Truro was significantly lower than other locations (Figure 5.35). The optimum N rate for the highest oil yield was 160 kg N/ha at Truro and Fredericton; 120 kg/ha at Canning and New Glasgow. Oil yield was positively correlated with the N rates, and high ratios of variability of oil yield could be explained by the change of N levels (Figure 5.36). Effects of S on oil yield varied from one location to another (Figure 5.37). At Canning, S input decreased the oil yield; at Fredericton and Truro, S input increased the oil yield; at New Glasgow, S input had no impact on the oil yield.

Table 5.28: ANOVA table of oil yield at Canning, Fredericton, New Glasgow and Truro in 2012

Effect	F Value	P value
Rep	8.93	<.0001
Location (L)	32.42	<.0001
Genotype (G)	**280.40**	**<.0001**
L*G	0.61	0.6095
N	54.29	<.0001
L*N	**3.22**	**<.0001**
G*N	0.73	0.6042
L*G*N	0.59	0.8818
S	4.31	0.0388
L*S	**7.03**	**0.0001**
G*S	1.75	0.187
L*G*S	0.66	0.5755
N*S	1.18	0.3194
L*N*S	1.21	0.266
G*N*S	0.25	0.9411
L*G*N*S	0.73	0.7531

(no transformation)

Table 5.29: Effect of genotype on oil yield in 2012

Genotype	Oil yield (kg/ha)
CDI007	715 a
CDI005	591 b

(Means with a common letter are not significantly different at the 5% level)

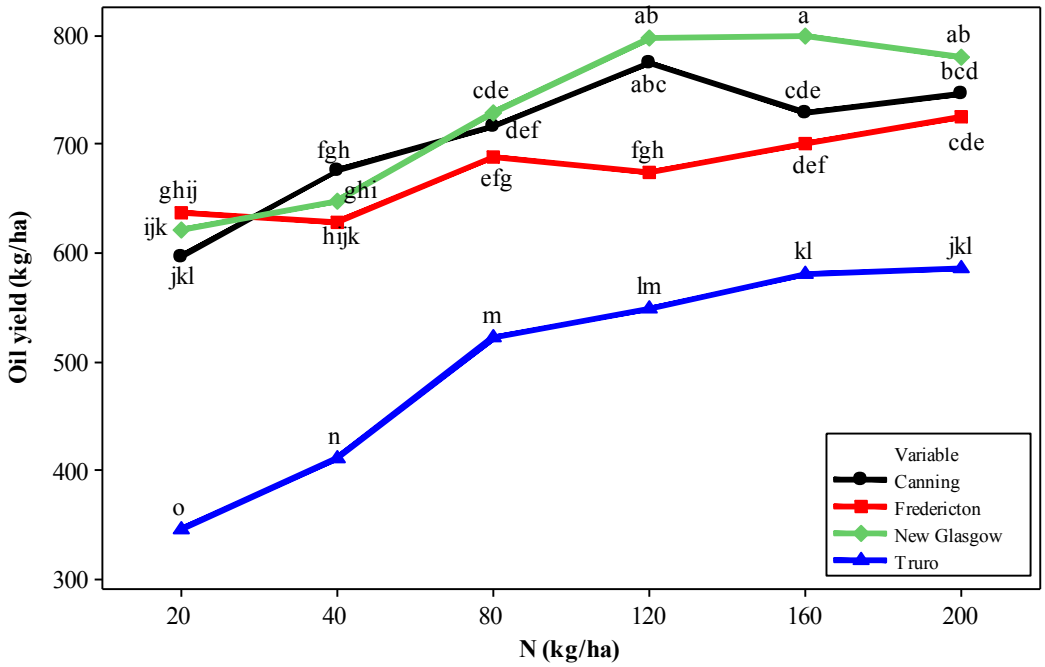


Figure 5.35: Effect of location and N on the oil yield in 2012
(Means with a common letter are not significantly different at the 5% level)

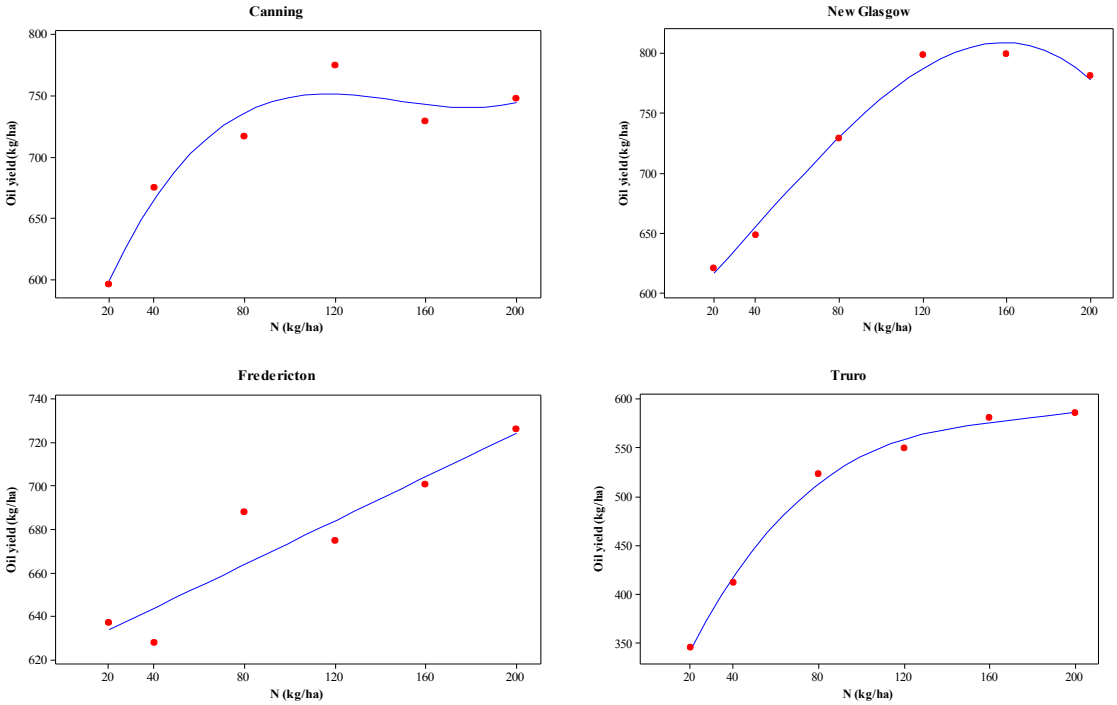


Figure 5.36: Regression analysis of N effect on oil yield at four different locations in 2012
(each dot represents 16 samples)

(1) Y(Canning)=506.1+5.365N-0.03787N**2+0.000082N**3 with R-Sq(adj)=85.0%

(2) Y(Fredericton)=623.6+0.5044N with R-Sq=86.9%

(3) Y(New Glasgow)=579.6+1.753N+0.00526N**2-0.000045N**3 with R-Sq(adj)=97.6%

(4) Y(Truro)=247.9+5.342N-0.02998N**2+0.000059N**3 with R-Sq(adj)=98.7%

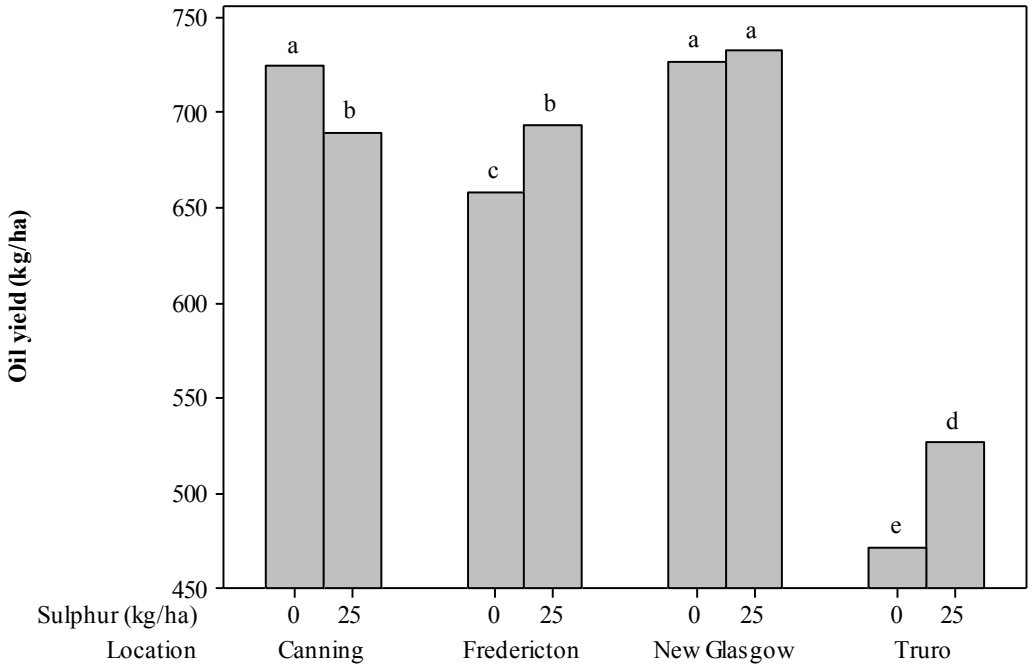


Figure 5.37: Effect of location and S on the oil yield at four different locations in 2012

(Means with a common letter are not significantly different at the 5% level)

5.5.16 Fatty Acid Composition

Camelina seed consists of 10 main fatty acids – C16:0, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, C20:2, C20:3 and C22:1. They were grouped into 3 categories – saturated fatty acids (C16:0, C18:0 and C20:0), monounsaturated fatty acids (C18:1, C20:1 and C22:1), and polyunsaturated fatty acids (C18:2, C18:3, C20:2 and C20:3).

The interaction of location and genotype had a significant effect on saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids. The interaction of location

and N had a significant effect on monounsaturated fatty acids and polyunsaturated fatty acids. Genotypes differed in fatty acid profile depending on the levels of N. S effect differed in fatty acid profile depending on location. The interaction of N and S had a significant effect on saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids (Table 5.30).

Genotype had a significant effect on the percent of saturated fatty acids depending on location. At Canning, New Glasgow and Truro, CDI005 had significantly higher contents of saturated fatty acids than CDI007, while at Fredericton, the amounts of saturated fatty acids of CDI005 and CDI007 were similar (Figure 5.38).

CDI005 had significantly higher content of monounsaturated fatty acids than CDI007 at all 4 locations in 2012 (Figure 5.39).

Genotype had a significant effect on the percent of polyunsaturated fatty acids depending on location. At Canning, New Glasgow and Truro, CDI007 had significantly higher content of polyunsaturated fatty acids than CDI005, while at Fredericton, genotype had no significant effect on the polyunsaturated fatty acids (Figure 5.40).

Plants at New Glasgow had the highest content of monounsaturated fatty acids compared to other locations (Figure 5.41). In contrast, plants at Fredericton had the lowest content of monounsaturated fatty acids (Figure 5.41). N was negatively correlated with the percent of monounsaturated fatty acids at all 4 sites in 2012 (Figure 5.41).

N was positively correlated with the percent of polyunsaturated fatty acids at all 4 sites in 2012 (Figure 5.42). Plants at Truro had the highest content of polyunsaturated fatty acids among these 4 locations, followed by Fredericton. Plants at Canning and New Glasgow had lower contents of polyunsaturated fatty acids (Figure 5.42).

N was positively correlated with the content of saturated fatty acids of CDI005, which was significantly higher than CDI007. The content of saturated fatty acids of CDI007 fluctuated with an increase in N. CDI007 with 200 kg N/ha input had significantly higher content of saturated fatty acids than CDI007 with 20, 40, 80 and 160 kg N/ha.

S had effects on the content of saturated fatty acids depending on location. At New Glasgow, S input led to higher content of saturated fatty acids; while S application had no effects on saturated fatty acids at Canning, Fredericton and Truro (Figure 5.44).

S had an effect on the percent of monounsaturated fatty acids depending on location. At Canning, Fredericton and New Glasgow, S input decreased the content of monounsaturated fatty acids. S had no significant effect on the content of monounsaturated fatty acids at Truro (Figure 5.45).

S increased the percent of polyunsaturated fatty acids at all 4 sites in 2012 (Figure 5.46).

With 25 kg S/ha application, the percent of saturated fatty acids increased with an increase in N (Figure 5.47). When plants did not receive S application, the percent of saturated fatty acids increased when N increased from 20 kg N/ha to 120 kg N/ha, then it decreased when N was 160 kg N/ha, and it rebounded when N was 200 kg N/ha (Figure 5.47).

The percent of monounsaturated fatty acids decreased with an increase in N for both 2 levels of S. Plants produced more monounsaturated fatty acids without S input compared to plants with 25 kg S/ha with N ranging from 80 to 200 kg N/ha (Figure 5.48).

The percent of polyunsaturated fatty acids increased with an increase in N for

both 2 levels of S. Plants produced more polyunsaturated fatty acids with 25 kg S/ha compared to plants without S application with N ranging from 80 to 200 kg N/ha (Figure 5.49).

Table 5.30: ANOVA table of fatty acid (FA) composition

	Saturated FA		Monounsaturated FA		Polyunsaturated FA	
	F value	P value	F value	P value	F value	P value
Rep	16.65	<.0001	5.53	0.0003	6.08	0.0001
Location (L)	94.12	<.0001	39.23	<.0001	58.74	<.0001
Genotype (G)	110.82	<.0001	401.42	<.0001	151.48	<.0001
L*G	19.09	<.0001	9.24	<.0001	8.96	<.0001
N	18.71	<.0001	180.44	<.0001	90.85	<.0001
L*N	8.93	<.0001	4.59	<.0001	5.02	<.0001
G*N	2.60	0.0255	2.06	0.0708	0.25	0.9383
L*G*N	0.56	0.9062	0.64	0.8380	0.53	0.9218
S	0.54	0.4644	78.70	<.0001	106.97	<.0001
L*S	4.00	0.0082	14.82	<.0001	4.01	0.0081
G*S	1.18	0.2778	3.41	0.0657	0.02	0.8996
L*G*S	0.79	0.5023	1.30	0.2750	0.80	0.4970
N*S	2.45	0.0340	10.02	<.0001	13.16	<.0001
L*N*S	1.16	0.3030	0.81	0.6647	0.88	0.5853
G*N*S	1.71	0.1329	0.40	0.8480	0.46	0.8094
L*G*N*S	1.56	0.0845	0.76	0.7239	0.42	0.9729

(no transformation)

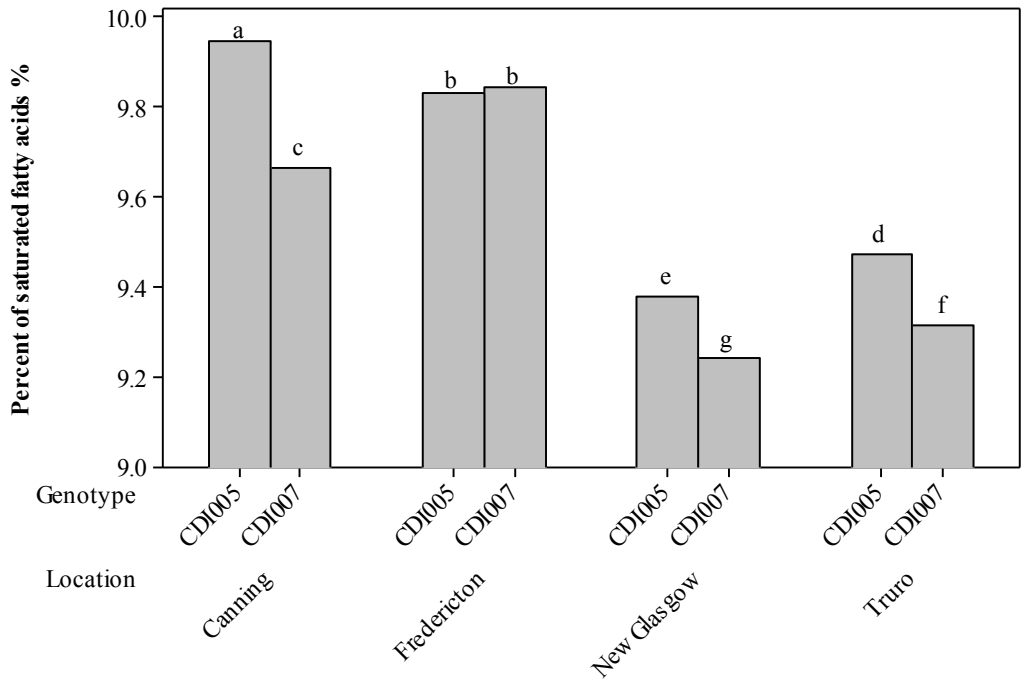


Figure 5.38: Effect of interaction of location and genotype on saturated fatty acids
(Means with a common letter are not significantly different at the 5% level)

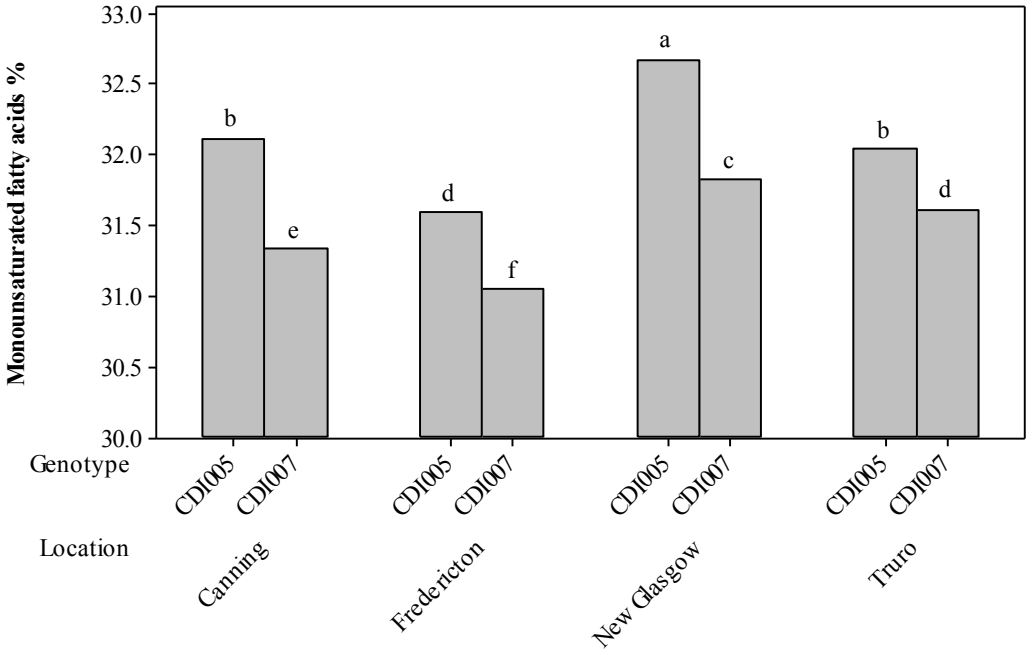


Figure 5.39: Effect of interaction of location and genotype on monounsaturated fatty acids
(Means with a common letter are not significantly different at the 5% level)

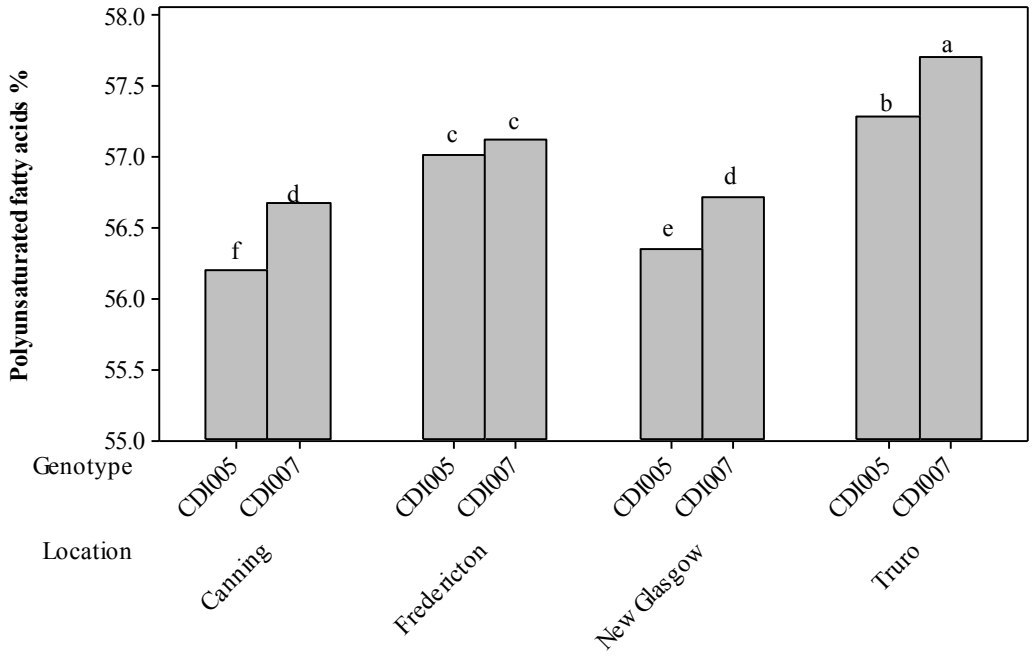


Figure 5.40: Effect of interaction of location and genotype on polyunsaturated fatty acids

(Means with a common letter are not significantly different at the 5% level)

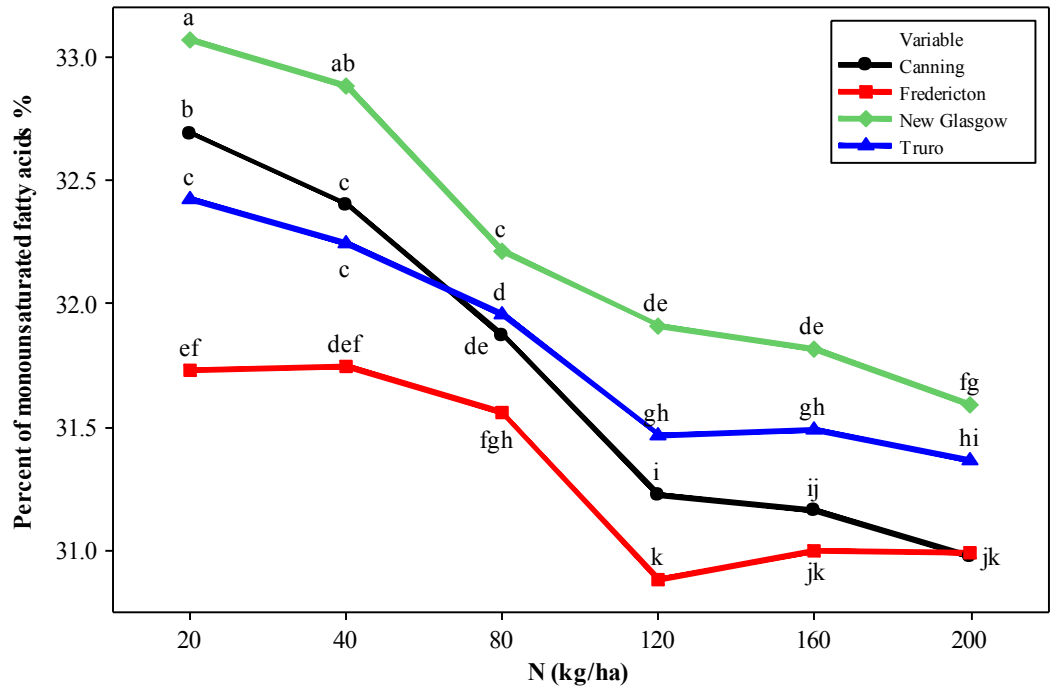


Figure 5.41: Effect of interaction of location and N on the percent of monounsaturated fatty acids in 2012

(Means with a common letter are not significantly different at the 5% level)

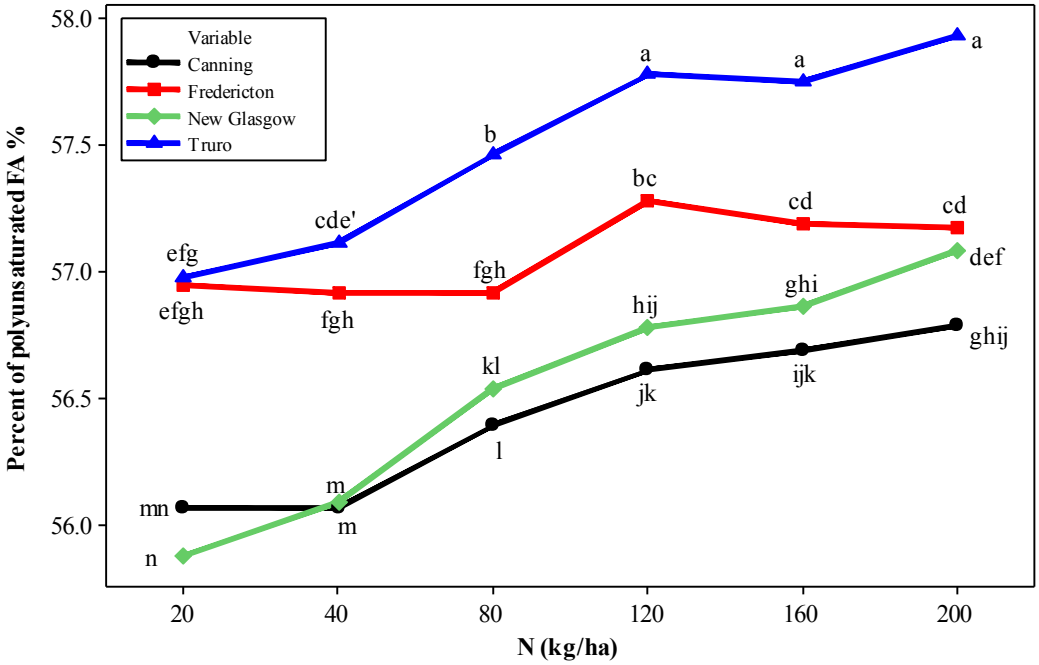


Figure 5.42: Effect of interaction of location and N on the percent of polyunsaturated fatty acids in 2012
(Means with a common letter are not significantly different at the 5% level)

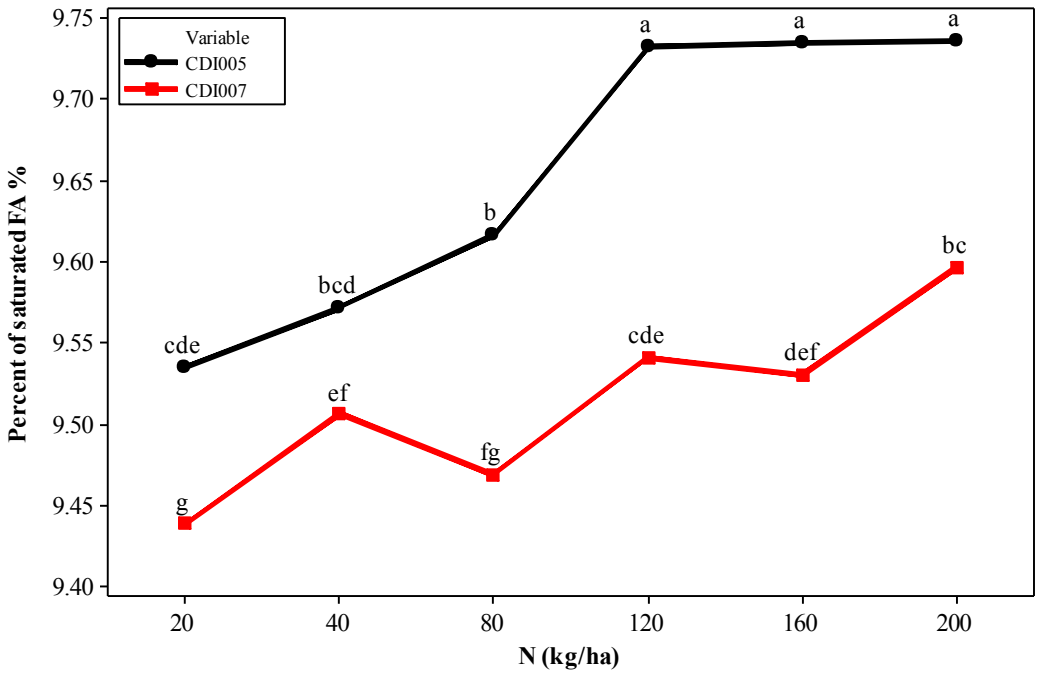


Figure 5.43: Effect of interaction of genotype and N on the percent of saturated fatty acids in 2012

(Means with a common letter are not significantly different at the 5% level)

Figure 5.44: Effect of interaction of location and S on the percent of saturated fatty acids in 2012

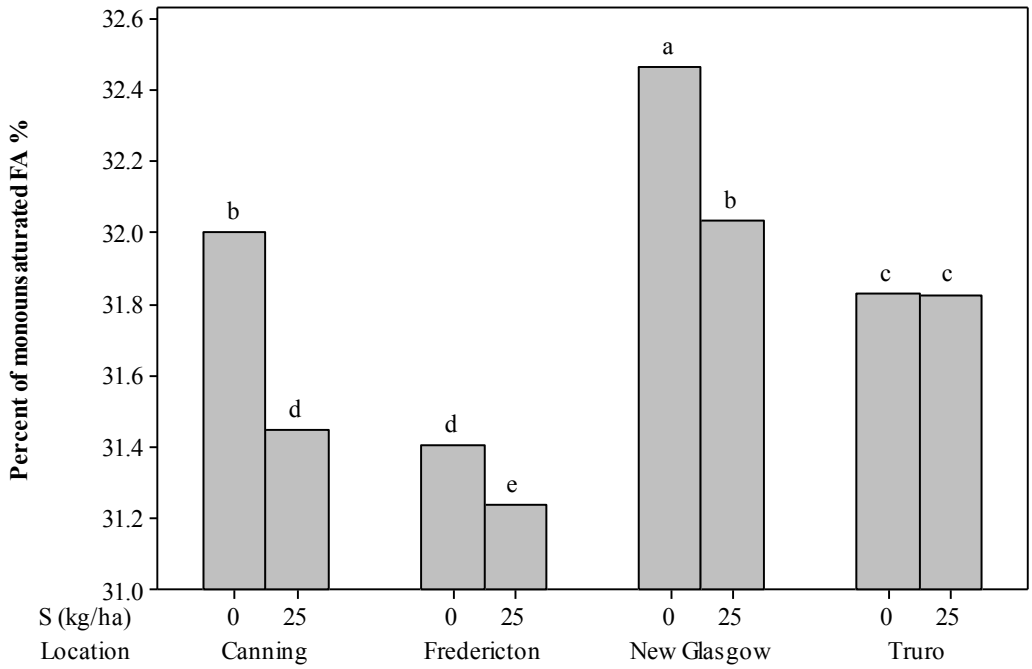


Figure 5.45: Effect of interaction of location and S on the percent of monounsaturated fatty acids in 2012

(Means with a common letter are not significantly different at the 5% level)

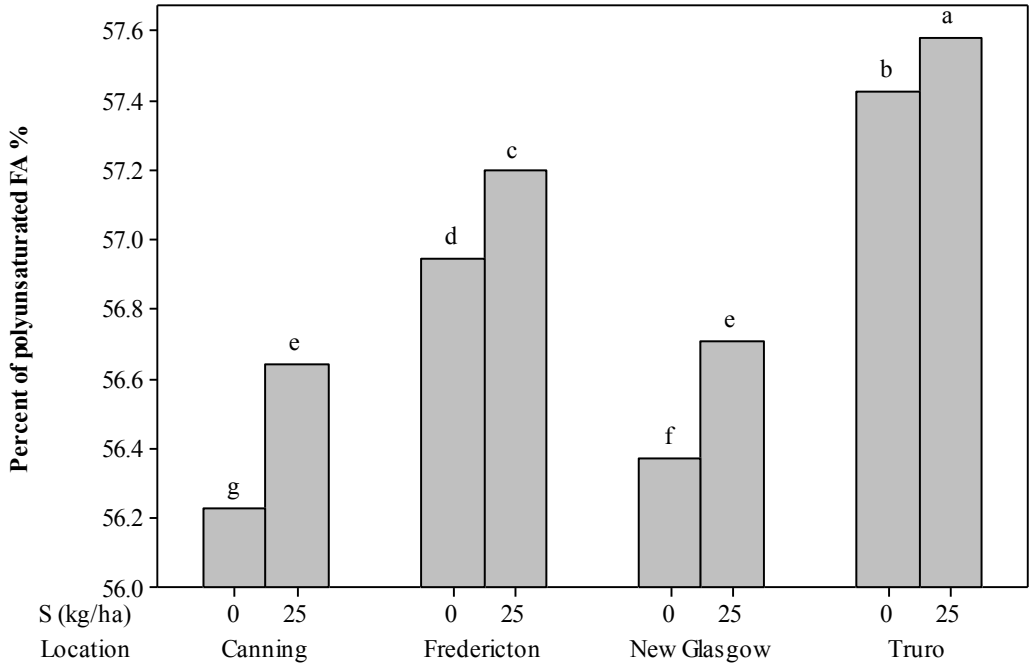


Figure 5.46: Effect of interaction of location and S on the percent of polyunsaturated fatty acids in 2012
(Means with a common letter are not significantly different at the 5% level)

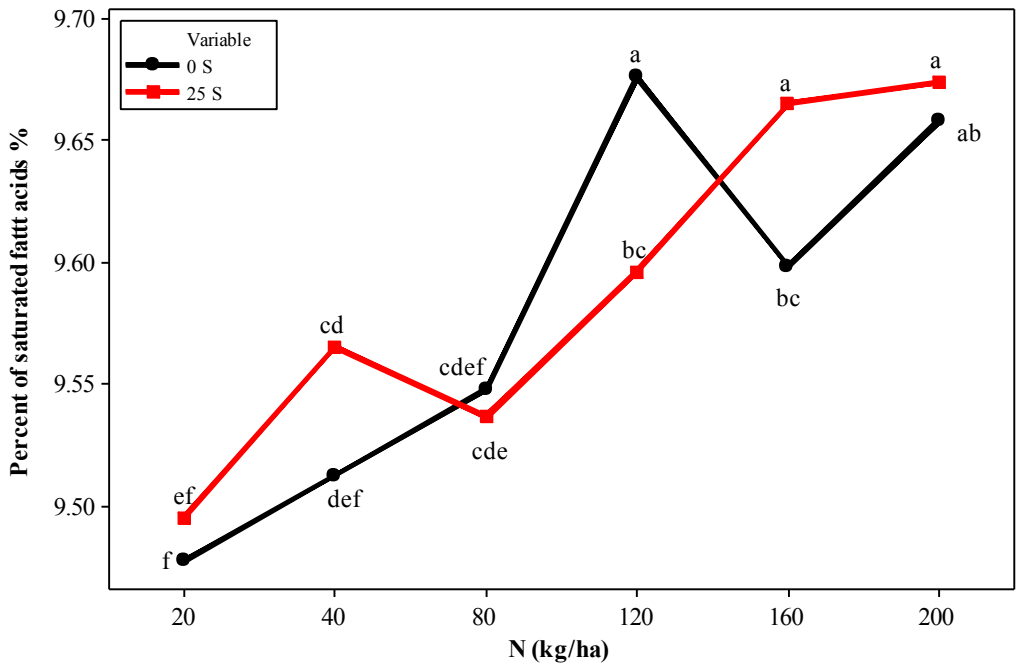


Figure 5.47: Effect of interaction of N and S on the percent of saturated fatty acids in 2012

(Means with a common letter are not significantly different at the 5% level)

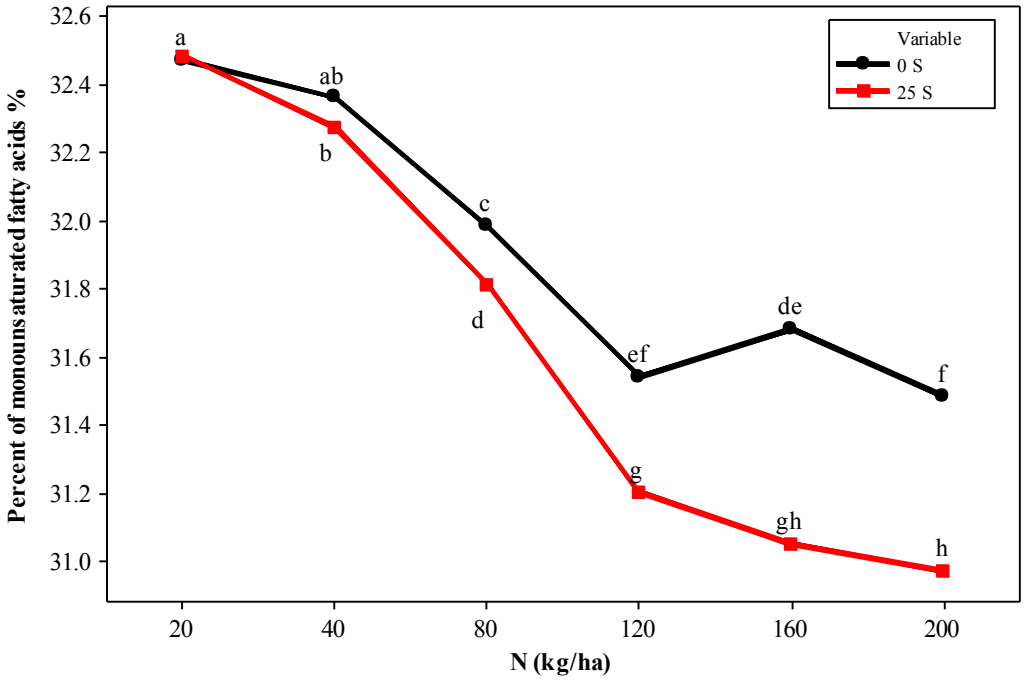


Figure 5.48: Effect of N and S on the percent of monounsaturated fatty acids % in 2012

(Means with a common letter are not significantly different at the 5% level)

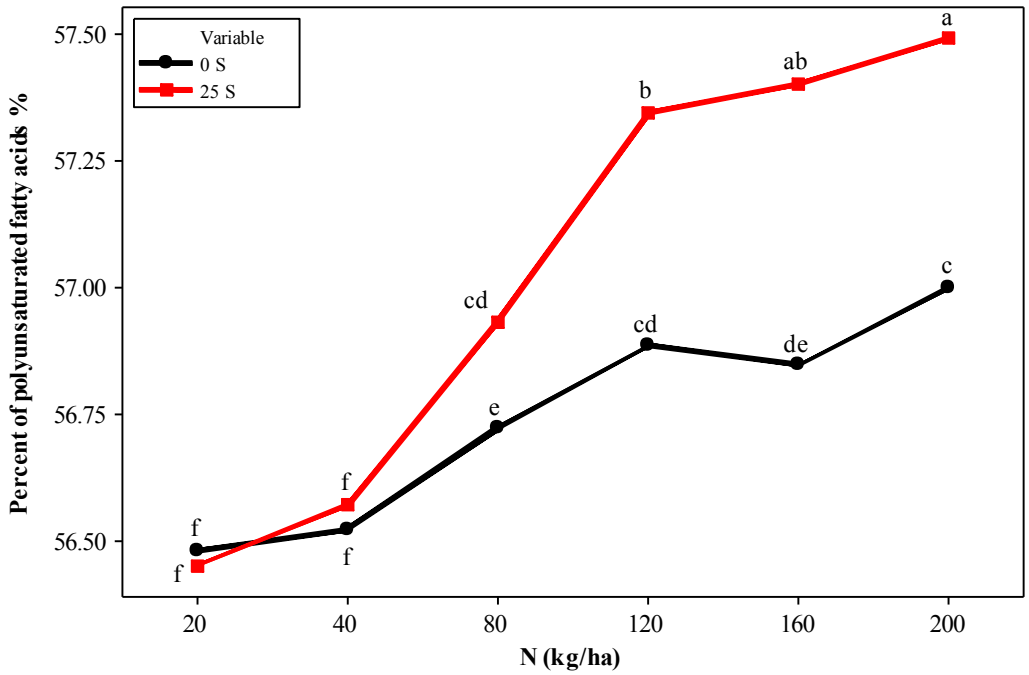


Figure 5.49: Effect of N and S on the percent of polyunsaturated fatty acids % in 2012

(Means with a common letter are not significantly different at the 5% level)

5.6 Discussion

5.6.1 Elasticity of Camelina

The yields of camelina at Canning, Fredericton and New Glasgow were similar, while the yield at Truro was the lowest. A location effect on camelina yield was reported by Pan (2009) in Canada (NS, PEI, SK), and Putnam et al. (1991) in the United States. It is worth mentioning that the apparent low yield at Truro was probably due to seeds being lost during the seed cleaning of samples from Truro, because the seeds were stuck together in clumps due to improper drying and could not pass through the sieve completely.

At Fredericton in 2012, although the plant density at harvest was the highest, pods/plant was the lowest among these 4 locations. Plants at Fredericton had smaller plant canopy (shortest, least pods/plant), which was due to the late seeding at Fredericton (one month later than other sites). Later seeding can decrease the time that the crop is able to grow at the vegetative stage before entering the reproductive stage (Berti, et al., 2011; Urbaniak et al., 2008b; Schillinger et al., 2012). However, plants at Fredericton produced larger pods and pods appeared to be filled (based on observation), which was potentially the key factors for the high yield at Fredericton. Unfortunately due to time constraints, the number of seeds per pod was not determined. Clearly, this should be done in the future for studies on yield components.

More pods per plant at Canning where plant stand was low suggested that camelina is able to compensate for the reduced plant number by developing more pods. This

observation is consistent with previous studies that showed that increased number of pods per plant produced at a lower plant stand is a yield compensatory factor (Agegnehu and Honermeier, 1997; Leach et al., 1999; Taylor and Smith, 1992; Urbaniak, 2006). Similar results were achieved by Blankman and Bunting (1954) and Albrechtsen and Dybing (1973) for flax.

5.6.2 Plant Stand

The seeding rate was 500 seeds/m^2 at all sites. However, plant stand at maturity was variable depending on location. Populations of camelina declined in density due to self-thinning, which is a natural process of plant competition - populations decrease as plant size increases. With the same seeding rate, plant densities varied from one location to another, because different soil types and environmental conditions contribute to differences. More importantly, camelina at Fredericton was seeded by a different seeder compared to Canning, Truro and New Glasgow. Plants seeded with a forage type seeder had better plant establishment than plants seeded with a seed drill (Urbaniak et al., 2008b).

5.6.3 Downy Mildew

CDI007 was more tolerant to downy mildew than CDI005, as observed in the N trials in 2011 and 2012 study summarized in Chapter Four. It is generally accepted that warm and humid mid-season growing conditions may lead to the increased disease incidence. Downy mildew is an obligate parasite which is only able to survive on living hosts (Birch et al., 2006; Turk, 2002). N input had a positive correlation with the occurrence of downy mildew in this study, which was consistent with the previous experiments by Pan (2009). N promotes the development of young and succulent tissues;

prolongs the vegetative stage and delays maturity, which makes plants susceptible to pathogens for a longer period (Agrios, 1988 in Zarafi, et al. 2005). N input increases the incidence and severity of downy mildew by affecting plant canopy development. Large canopies with high shoot densities promote spore transfer and infection (Walters, 2009). Previous studies have shown that N was positively correlated with the incidence of downy mildew of sugarcane, cucumber and pearl millet (Zarafi, et al. 2005), while others observed no correlation (Sivaparakasam et al., 1974; Anaso, 1985 in Zarafi, et al. 2005).

5.6.4 N effects

N is positively correlated with yield resulting from more pods per plant and per m^2 with high N, which is probably due to an increase in N, leading to the acceleration of photosynthesis and more production of carbohydrates (Joshi et al., 1998). Both protein and oil yield increased with an increase in N at all sites. Similar results on camelina were obtained by Johnson and Gesch (2013); Losak et al. (2011); Urbaniak et al. (2008a) and Pan (2009). The protein content increased with an increase in N input, and the oil percent decreased. It is generally accepted that biosynthesis of fatty acids and amino acids compete for carbon skeletons and energy (Gehringer et al., 2006). It was reported that availability of carbohydrates decreased for oil synthesis with higher N (Rathke et al., 2005). The level of carbohydrate in protein is less than that in oils. Increased N results in increased protein synthesis at the expense of fatty acid synthesis due to their competition for carbon skeletons during carbohydrate metabolism (Rathke et al., 2005).

5.6.5 S effects

So far, very limited studies have been done to evaluate the effects of S on the agronomic performance of camelina. Combined N and S were tested on camelina by

Losak et al. (2011). Losak et al. (2011) observed that differences in camelina agronomic parameters with different S treatments were not statistically significant, while S input tended to increase seed yield, as well as oil and protein content. In the present study, S had no impact on plant density and the incidence of downy mildew. However, S increased the number of pods per plant at Truro but not at Canning, New Glasgow and Fredericton. S increased yield depending on sites (at Truro but not at Canning, New Glasgow and Fredericton). Yield of canola (rapeseed) decreased when N was only applied to S deficient soils (Janzen and Bettany, 1984). S input increased yield and S uptake of oilseed rape (McNeill et al., 2005).

S also had a positive correlation on the content of protein at all of the sites and also for the different genotypes. This observation is consistent with the previous studies on canola in Saskatchewan and Manitoba where S was applied on S-deficient soils (Malhi and Gill, 2002; Grant et al., 2003). However, other studies showed that S had no impact on the content of protein of canola (Asare and Scarisbrick, 1995; Ali and Poshtmasari, 2010). It was reported that S is associated with the function of nitrate reductase linking to conversion of NO_3^--N into amino acids by the plant. S is also a component of the initiation amino acid methionine, which is a key in protein synthesis in eukaryotes (Ahmad and Abdin, 2000a).

S had a negative effect on oil content at Canning, but not the other sites. Losak et al. (2011) found that S had no effect on the oil content of camelina. The oil content of canola, however, was reported to increase when soil S increased (Malhi and Gill, 2002; Grant et al., 2003; Jackson, 2000; Jan et al. 2002; Saron and Giri, 1990; Chaudhary et al., 1992; and Ali and Poshtmasari, 2010). It is recommended that applying S to the soil and its

rates should be based on the available soil S and crop requirements.

5.6.6 Fatty Acids

CDI005 had more saturated and monounsaturated fatty acids, but less polyunsaturated fatty acids compared to CDI007. Higher content of polyunsaturated fatty acids is regarded to be beneficial for lowering cholesterol in humans (Cunnae, 1995 in Manaf et al., 2006). Differences between genotypes and fatty acids are due to genetic makeup. In order to increase desirable fatty acids or decrease undesirable fatty acids, selecting the right genotypes is important, while the fatty acid profile of camelina can be affected by management factors such as N and S to some extent. Saturated and polyunsaturated fatty acids increased with an increase in N, while monounsaturated fatty acids were negatively correlated with N. S increased the content of saturated fatty acids at only New Glasgow but no other locations, because the background S at New Glasgow was the lowest with only 18 kg S/ha compared to S ranging from 23-29 kg S/ha at other locations. S decreased the content of monounsaturated fatty acids at Canning, Fredericton and New Glasgow. The percent of monounsaturated fatty acids at New Glasgow were the highest because the background S was the lowest at New Glasgow.

The inverse relationship between monounsaturated fatty acids and polyunsaturated fatty acids was observed in the present study, which probably resulted from the inverse relationship between C18:1 (monounsaturated fatty acids) and C18:2 (polyunsaturated fatty acids). Oleic acid (C18:1) is converted to linoleic acid (C18:2) and linolenic acid (C18:3) in angiosperm plants (Wallis et al., 2002). The results were consistent with the previous study in camelina by Urbaniak (2006), in canola by Manaf et al. (2006) and in sunflower by Flagella et al. (2002), which usually takes place at low

temperatures during plant maturity (Manaf et al., 2006). Temperature is vital to the synthesis of plant fatty acid composition. In warmer climates, plants are higher in saturates and monounsaturates (such as C18:1 and C22:1), but lower in polyunsaturates such as C18:2 and C18:3 compared with cooler climates (McVetty, 2009). It was also reported that temperature had an influence on the desaturation of fatty acids, which indicated that enzyme activity during fatty acid synthesis is influenced by temperature changes (Urbaniak, 2006)

Fatty acid profile of camelina was different from one location to another. This was probably due to the different climate conditions at different sites. With the similar temperatures ranging from 20.1 to 21.0 ^{o}C at the reproductive stage at these four locations, the content of polyunsaturated fatty acids appeared to be positively correlated with the total precipitation. The total precipitations in August, 2012 were 262 mm at Truro, 69.6 mm at New Glasgow, 63.8 mm at Fredericton and 47.9 mm at Canning, and the percent of polyunsaturated fatty acids at Truro was the highest, while it was the lowest in Canning (Figure 5.42). A positive correlation between cumulative precipitation and linolenic acid (C18:3, polyunsaturated) in canola at southern Australia was observed by Pritchard et al (2000), which was consistent with previous studies by Gunasekera et al (2006) in B. *juncea* and B. *napus* in Mediterranean-type environments. Ample moisture availability leading to the high content of linolenic acid was also observed in Canola by Manaf et al. (2006).

5.7 Conclusion

Camelina has high yield potential and the ability to compensate for differing plant stands and is adaptable to different environmental and management conditions.

Reductions in plant stand and late seeding are overcome by the production of more pods per plant or physically larger pods. CDI007 had higher yield and more tolerance to downy mildew than CDI005. The addition of S is recommended, because S had a positive effect on yield, although this did depend on location. It also had a positive effect on protein content and a negative effect on oil content at one location. The optimum N rate ranged from 120-160 kg/ha depending on the site.

Chapter 6: Effects of Nitrogen and Genotype on Glucosinolates of Camelina Seed

6.1 Introduction

Glucosinolates (GS) are plant secondary metabolites commonly found in *Brassica* species such as broccoli, cabbage and oilseed rape (Yan and Chen, 2007). These rich sulfur-containing compounds comprise at least 120 anionic thioglycosides (Yan and Chen, 2007). The chemical structures of GS consist of a β-thioglucose moiety, a sulfonated oxime moiety and a variable side chain derived from one of eight amino acids (Wink, 1999). These amino acids are alanine, valine, leucine, isoleucine, phenylalanine, methionine, tyrosine and tryptophan (Hayes et al., 2008). GS can be categorized according to their precursor amino acids and the types and modification to their side chain. Compounds formed from alanine, leucine, isoleucine, methionine or valine are categorized into aliphatic GS, while those formed from phenylalanine or tyrosine are aromatic GS, while those derived from tryptophan are called indole GS (Halkier and Gershenzon, 2006). More than 115 different GS have been identified and described in the literature (Hayes et al., 2008). In general, GS concentration was 0.1% or less in fresh plant parts such as stems and leaves (Wink, 1999). These moderate levels of GS do not lead to health problems in animals and humans (Wink, 1999).

Based on the toxic properties and pungent taste of GS, they are often regarded as anti-nutritional factors (Wink, 1999). GS and their degradation products are precursors of compounds with goitrogenic action in animals and humans. These compounds block the uptake of iodine, which lead to modification of thyroid function. For example, one of the major GS in rapeseed, 2-hydroxy-3-butenyl GS, can be hydrolyzed to form an oxazolidine-2-thione which leads to goiter formation and affects animal nutrition

negatively (Wink, 1999). GS have also been reported to promote the activity of detoxification enzymes in normal cells and are able to limit tumor cell growth, which reduced the risk to the incidence of certain cancers (Martinez-Villaluengaet et al., 2008; Kestwal et al., 2011). Among the crucifers, cabbage (*Brassica oleracea*), broccoli (*Brassica capitata*) and radish (*Raphanus sativus*) sprouts have been reported to possess anti-proliferative potential against different cancer cells (Boivin et al., 2009).

Camelina contains three major GS: Glucoarabin (9-(methylsulfinyl) nonylglucosinolate-GS9), glucocamelinin (10-(methylsulfinyl) decylglucosinolate-GS10), and 11- (methylsulfinyl) undecylglucosinolate (GS11) (Schuster and Friedt 1998; Berhow et al., 2013). The GS of camelina are stored in its seeds (Berhow et al., 2013) and restrict the widespread use of this crop. The average amount of GS in camelina is about 24 μmol/g, and ranges from 13.2 to 36.2 μmol/g dry seed depending on genotypes (Schuster and Friedt, 1998).

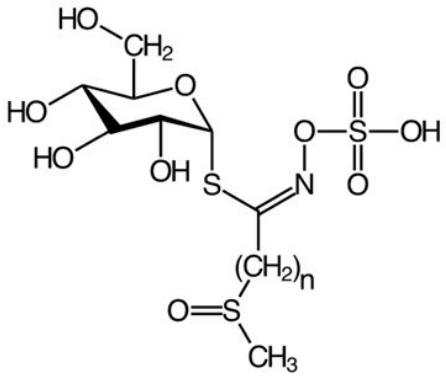


Figure 6.1: Basic structure of camelina GS
GS9, n=9; GS10, n=10; GS11, n=11. (Berhow et al., 2013)

The hypothesis of this study was that different genotypes of camelina would produce different amounts of glucosinolates and also that N would affect the glucosinolate content.

In this study, gas chromatography (GC) was used to analyze glucosinolate content and profile. The objectives of this study were to evaluate the effects of genotype and N on the amount and type of GS in camelina.

6.2 Materials and methods

6.2.1 Materials

Camelina seeds used in this analysis were obtained from the 2011 Truro N trial. There were 120 samples with 30 treatments – 6 levels of N (0, 25, 50, 100, 150 and 200 kg N/ha) and 5 genotypes (CDI002, CDI005, CDI007, CDI008 and Calena).

6.2.2 Methods

Gas chromatography of the trimethylsilyl (TMS) derivatives of desulphated GS was used in this study. The method was based on the protocol developed by Agriculture and Agri-Food Canada, Saskatoon Research Centre quality laboratory: "Gas chromatography of trimethylsilyl derivatives of GS of rapeseed and mustard". This method was also used by the Canadian Grain Commission (Daun and McGregor, 1983).

One gram (1.0000g-1.0100g) of camelina seed samples were placed into 20 ml PET scintillation vials containing small steel rods (1.2cm diameter). The vials were placed in a rack that contained 60, 28-mm holes. Five ml methanol was added to every vial to extract GS (GS can be easily dissolved in methanol because methanol penetrates cell wall and thereby extracts the GS). The next step was to add 0.4 ml of Lead-Barium acetate to denature the protein in the seeds and 2 ml of 1mM internal standard solution (241.3 mg Benzyl-glucosinolate, made up to 500 ml with distilled water). These vials were then capped and placed on a vortex immediately. Vials were put into an Eberbach shaker for 60 minutes at a high speed (270 r/min) to make sure no whole seeds were visible. The

next process was to centrifuge (Beckman Coulter Allegra X-15 R Centrifuge) the samples at 2,300 rcf for 10 minutes, and then transfer 0.2 ml of supernatant to 0.3 ml of pre-swollen DEAE-Sephadex in the Bio-Rad mini-columns. The columns were washed with 1.8 ml "Methanol: water: 0.6 M Lead-Barium Acetate" (500:200:40, v/v/v), 1.5 ml 6% acetic acid (weak acid), 1.8 ml distilled water (elute the solutions and make pH around 5.2) and 1 ml 0.02 M pyridine-acetate. The next step was to place columns (Bio-Rad micro-columns) on top of vials and add 0.05 ml of purified sulfatase to the top of the resin of columns, and then seal the column tops with tape and incubate columns overnight at room temperature. The next process was to elute desulphoglucosinolates with 1.3 ml water into 1.8 ml autosampler vials, and then to place vials in a dry block heater and dry down at 60 ^{o}C under a stream of N_2 provided by the manifolds and the compressed air source for 2-3 hours. The next step was to mix pyridine, BSA, TMCS and 1-methylimidazole (4:10:1:1, v/v/v/v), and then to add 50 µl of the mix to each vial and cap immediately (Teflon-lined caps), inverting vials to make sure the reagents rinse the inner walls of the vials and incubate at 80 ^{o}C for 60 minutes. Finally, those vials were placed in the autosampler and autoinjector was set to inject 1 µl.

The conditions for GC were set as follows: (a) column: DB-1, -60 ^{o}C to 325 ^{o}C; (b) injection volume: 1µl; (c) split ratio: 1:50; (d) flow rate: 1.0 mL/min; (e) detector: flame ionization detector (FID), which has a wide range of liner response; (f) carrier gas: hydrogen, ultra high purity, 99.999%.

6.3 Statistical analysis

Minitab 16 statistical software (Minitab Inc., USA, 2012) was used to check three assumptions - normality, constant variance and independence; outliers were removed if they existed. Minitab was also used in all the regression analyses.

SAS 9.3 statistical software (SAS Institute Inc., Cary NC, USA, 2012-2013) was also used in the data analysis once the data were checked to be normally distributed in Minitab. Proc Mixed with least significant differences (LSD) t-test method ($p<0.05$) was used to examine whether there were significant effects of factors on the targeted parameters.

6.4 Results

6.4.1 Typical Chromatogram of Camelina GS

A chromatogram of camelina (CDI002 with 150 kg N/ha) GS is presented in Figure 6.2. The peaks between 0 and 1 minute were produced by the solvents. The peak with the retention time 2.940 minutes was the internal standard – Benzyl-glucosinolate, and those peaks which were formed at the retention time of 3.179 minutes, 3.435 minutes and 3.727 minutes were GS9 (Glucoarabin), GS10 (glucocamelinin) and GS11 (11- (methylsulfinyl) undecylglucosinolate), respectively.

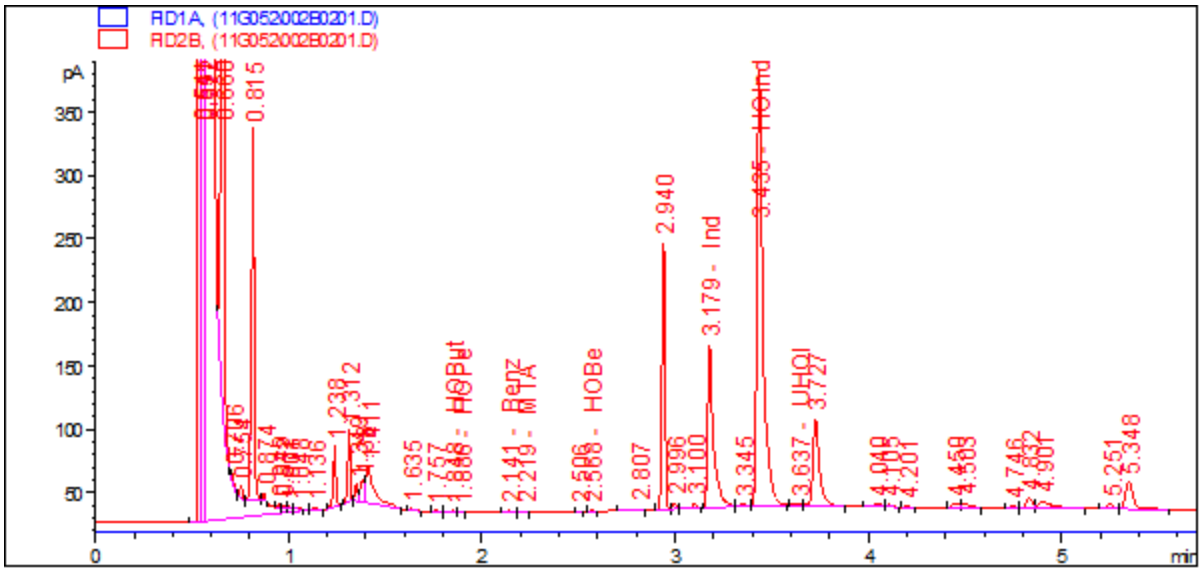


Figure 6.2: Typical chromatogram of camelina GS

6.4.2 Total and Individual of GS

The interaction of genotype and N had significant effects on the amounts of GS9, GS10, GS11 and the total amounts of GS (Table 6.1), which means different genotypes of camelina behaved differently depending on the amounts of N.

Table 6.1: ANOVA table of N and genotype on the GS of camelina seed (2011 Truro N trial)

Effect	Total amount of GS (µmol/g)		Amount of GS9 (µmol/g)		Amount of GS10 (µmol/g)		Amount of GS11 (µmol/g)	
	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
Genotype	9.98	<.0001	32.93	<.0001	8.85	<.0001	114.39	<.0001
N	8.95	<.0001	16.79	<.0001	6.55	<.0001	0.60	0.7037
G*N	**1.86**	**0.0258**	**2.00**	**0.0143**	**1.71**	**0.0467**	**2.35**	**0.0035**

(no transformation)

(1) Total GS

N was negatively correlated with the total amount of GS in general (Figure 6.3). The total GS of Calena was unchanged when N increased from 0 kg N/ha to 100 kg N/ha, then decreased when N increased to 200 kg N/ha. The total GS of CDI002 decreased

when N increased from 0 kg N/ha to 100 kg N/ha, then leveled off from 100 kg N/ha to 200 kg N/ha. The total GS of CDI005 declined dramatically when N increased from 0 kg N/ha to 100 kg N/ha, then rebounded and increased slightly when N increased from 100 kg N/ha to 200 kg N/ha. The total GS of CDI007 increased when N inclined from 0 kg N/ha to 50 kg N/ha, then decreased when N continued to increase to 150 kg N/ha, and it increased slightly when N increased from 150 kg N/ha to 200 kg N/ha. The total GS of CDI008 decreased when N increased from 0 kg N/ha to 200 kg N/ha. High ratio (all >70%) of the variability in the total GS could be explained by the change of N (Figure 6.3). CDI007 had lower content of total GS among these five genotypes (Figure 6.4).

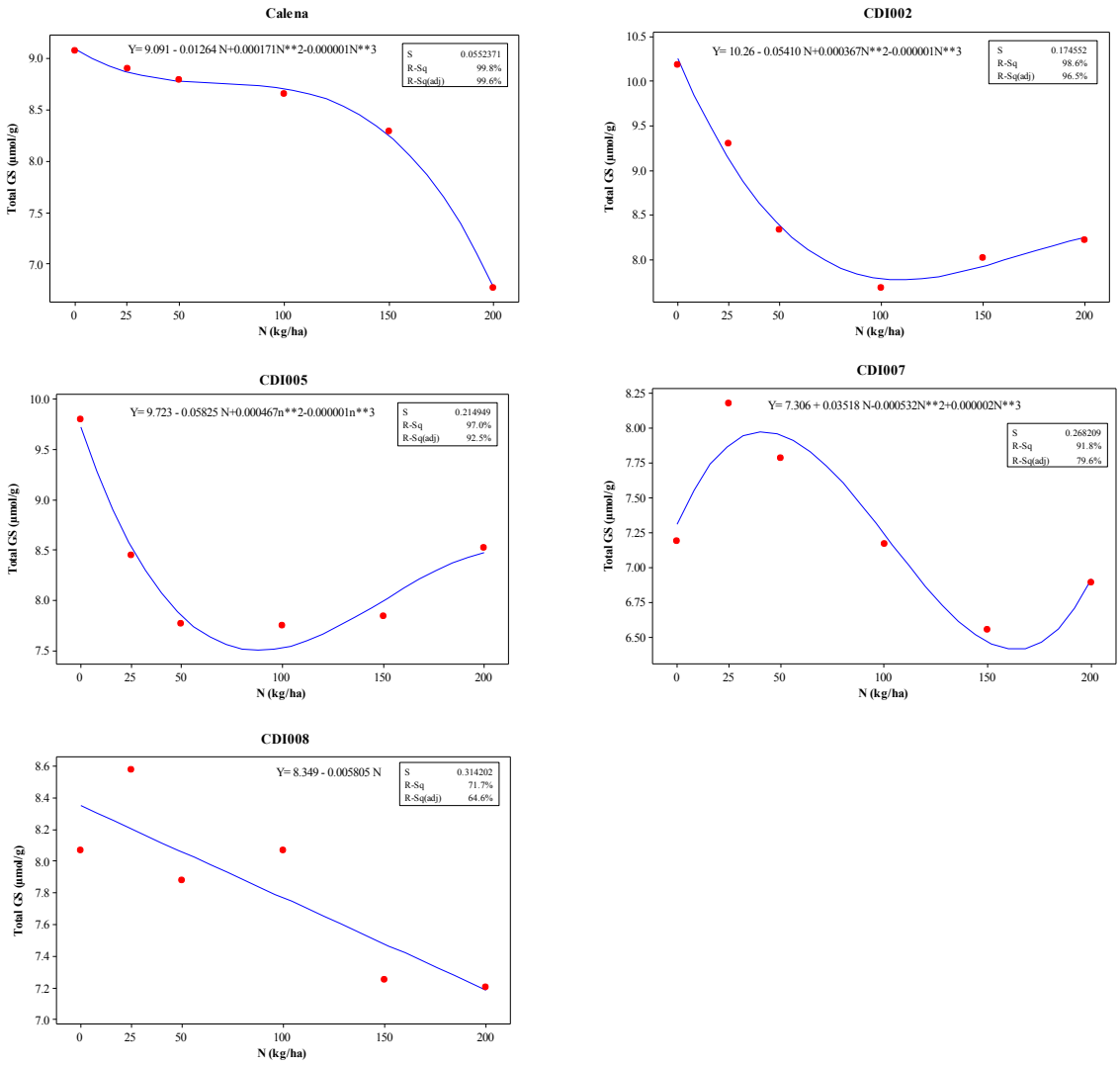


Figure 6.3: Regression analysis of N on the total amount of GS at Truro in 2011 (each dot represents the mean of 4 samples)

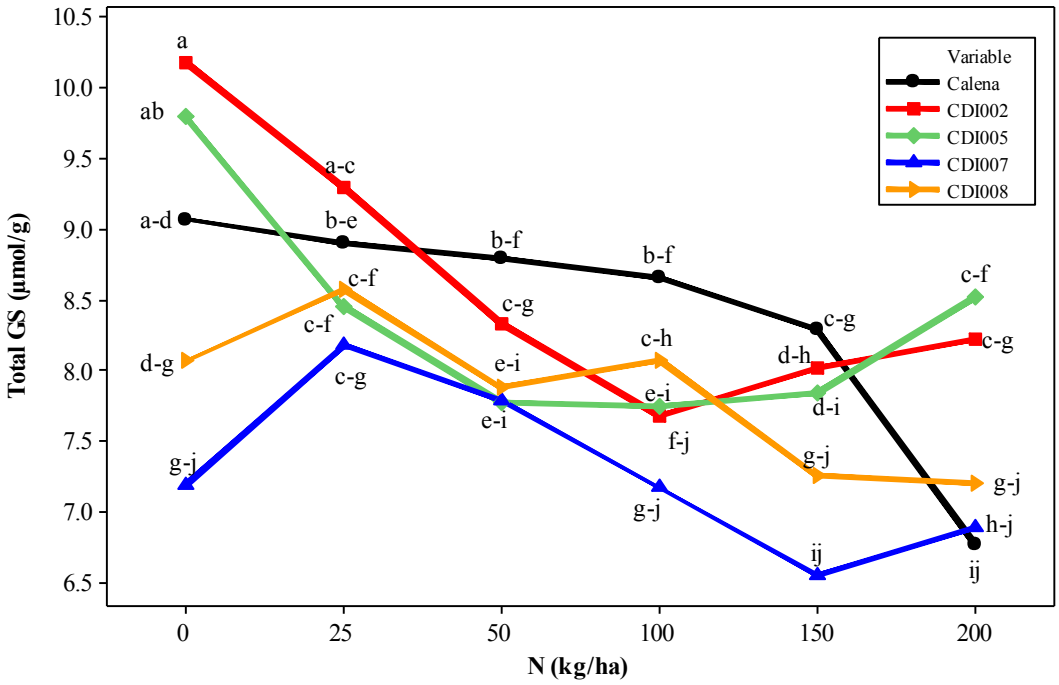


Figure 6.4: Effect of N and genotype on the total amount of GS at Truro in 2011
(Means with a common letter are not significantly different at the 5% level)

(2) **GS9**

N was positively correlated with the amount of GS9 in general (Figure 6.5). The content of GS9 of Calena and CDI008 decreased when N increased from 0 kg N/ha to 200 kg N/ha. The amount of GS9 of CDI002 declined when N increased from 0 kg N/ha to 100 kg N/ha, and started to level out when N continued to increase to 200 kg N/ha. The amount of GS9 of CDI005 decreased greatly when N increased from 0 kg N/ha to 100 kg N/ha, but started to increase slightly when N increased to 200 kg N/ha. The amount of GS9 of CDI007 increased slightly when N increased from 0 kg N/ha to 25 kg N/ha, then decreased when N increased from 25 kg N/ha to 150 kg N/ha, but increased slightly again when N continued to increase to 200 kg N/ha. High ratio (all >70%) of the variability in the amounts of GS9 could be explained by the change of N (Figure 6.5). CDI002 had the

highest amount of GS9 while CDI007 had the lowest amount of GS9 among these five genotypes (Figure 6.6).

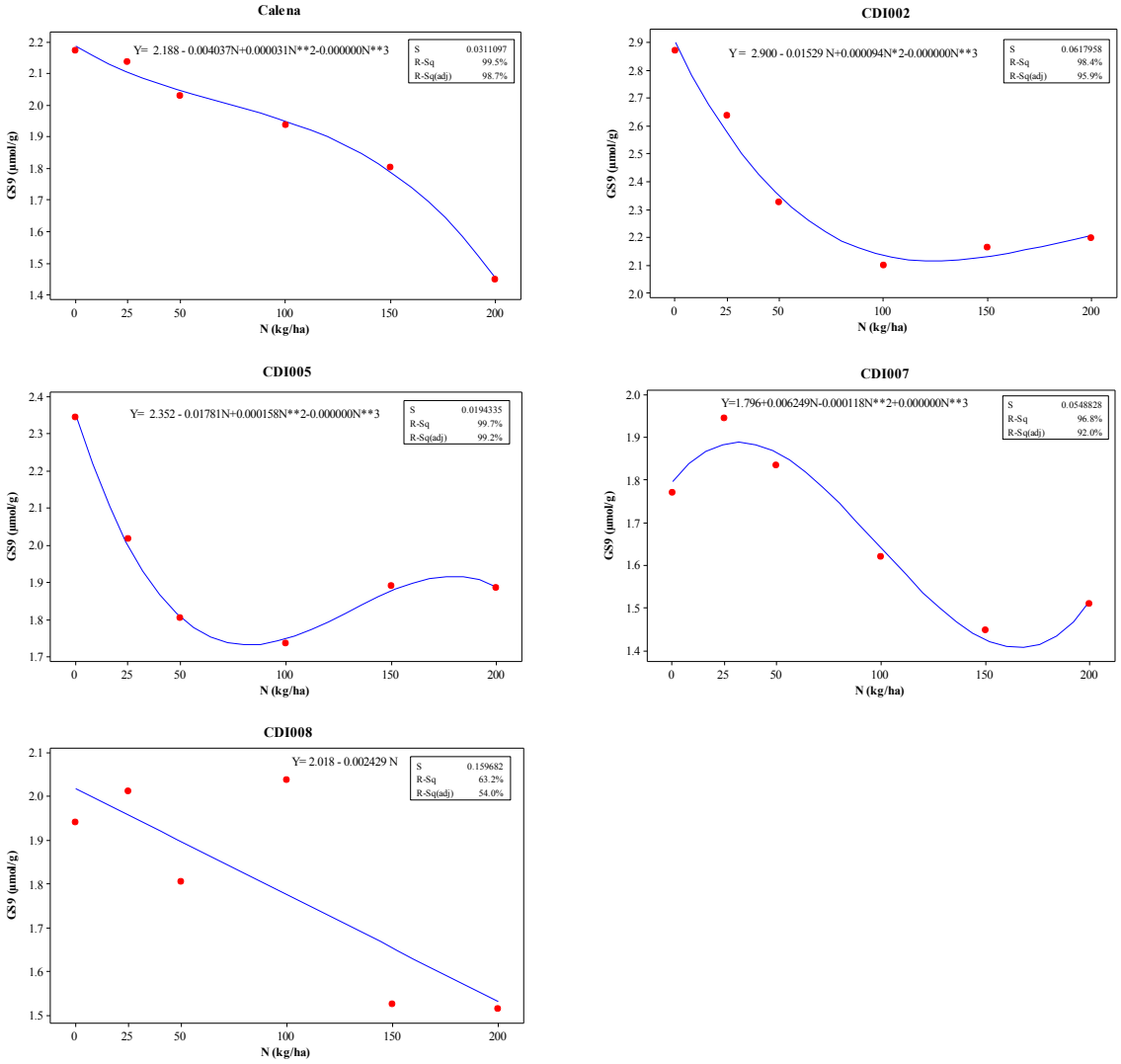


Figure 6.5: Regression analysis of N on the amount of GS9 at Truro in 2011
(each dot represents the mean of 4 samples)

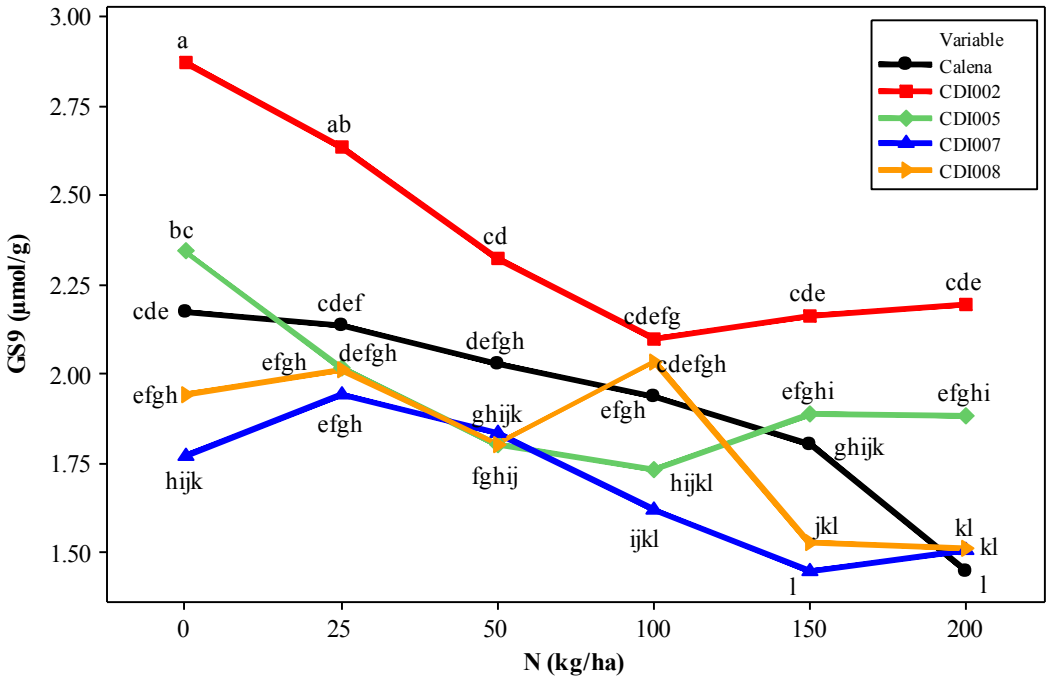


Figure 6.6: Effect of N and genotype on the amount of GS9 at Truro in 2011
(Means with a common letter are not significantly different at the 5% level)

(3) GS10

Different genotypes of camelina behaved differently on the amounts of GS10 depending on N (Figure 6.7). The amount of GS10 of Calena was unchanged when N increased from 0 kg N/ha to 100 kg N/ha, then decreased to 200 kg N/ha. The amount of GS10 of CDI002 decreased when N increased from 0 kg N/ha 100 kg N/ha, then increased slightly when N continued to incline to 200 kg N/ha. The content of GS10 of CDI005 decreased greatly when N increased from 0 kg N/ha to approximately 75 kg N/ha, while it started to increase when N increased from 75 kg N/ha to 200 kg N/ha. The amount of GS10 of CDI007 increased when N increased from 0 kg N/ha to 50 kg N/ha, then decreased when N increased from 50 kg N/ha to 170 kg N/ha, while it increased slightly again when N reached 200 kg N/ha. The amount of GS10 of CDI008 reduced

with N ranging from 0 kg N/ha to 200 kg N/ha. High ratios (all > 70%) of the variability in the amounts of GS10 could be explained by the change of N (Figure 6.7). CDI007 had the lowest content of GS10 among these five genotypes (Figure 6.8).

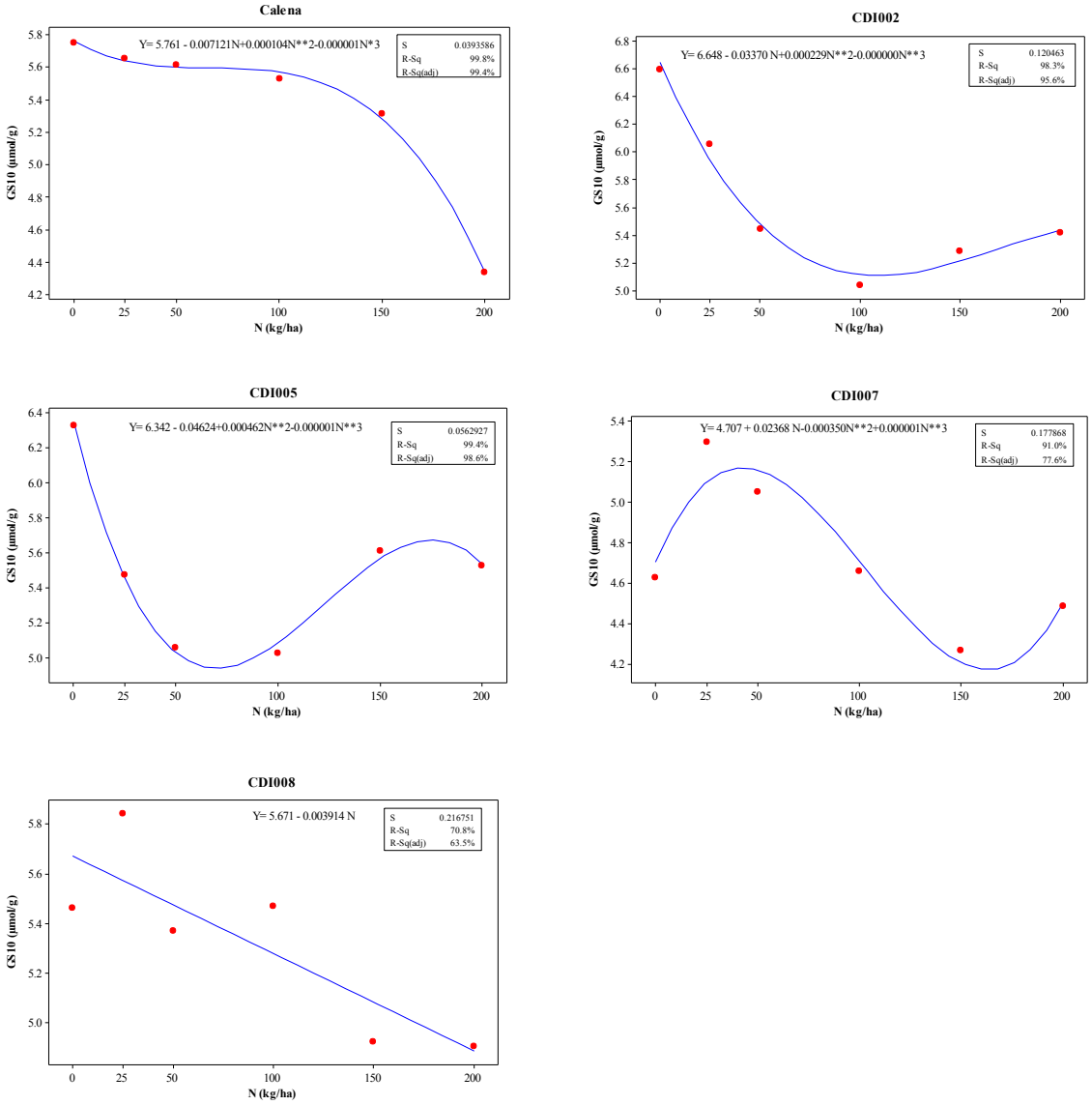


Figure 6.7: Regression analysis of N on the amount of GS10 at Truro in 2011
(each dot represents the mean of 4 samples)

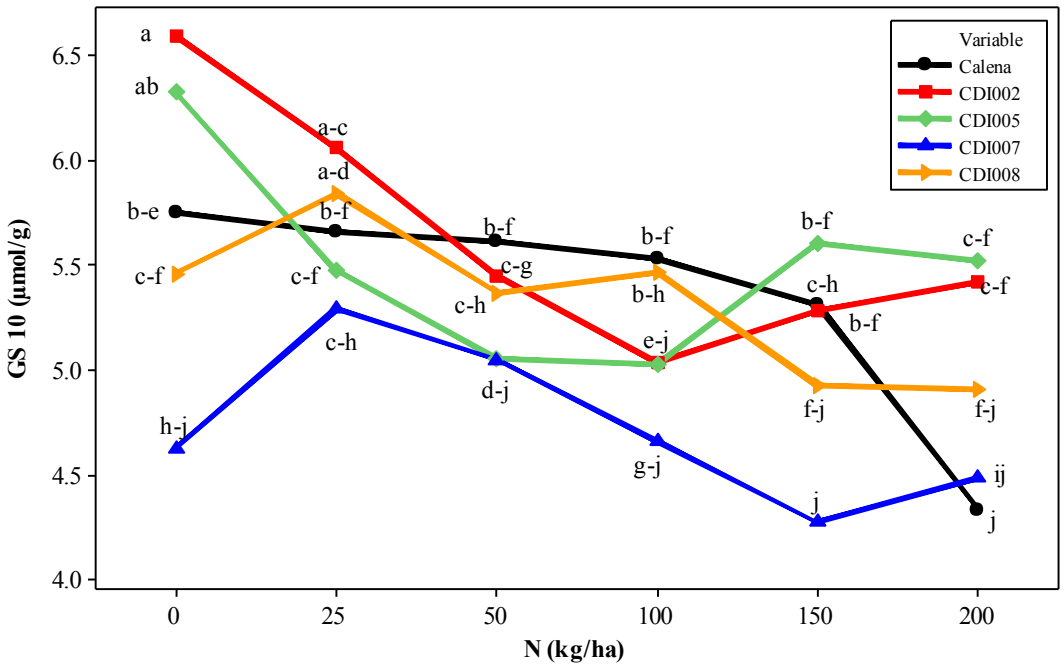


Figure 6.8: Effect of N and genotype on the amount of GS10 at Truro in 2011
(Means with a common letter are not significantly different at the 5% level)

(4) GS11

Different genotypes of camelina behaved differently on the amounts of GS11 responding to N application (Figure 6.9). The amount of GS11 of Calena kept almost unchanged when N increased from 0 kg N/ha to approximately 120 kg N/ha, while it started to decrease greatly when N continued to increase to 200 kg N/ha. The amount of GS11 of CDI002 decreased greatly when N increased from 0 kg N/ha to approximately 80 kg N/ha, while it increased when N increased slightly from 80 kg N/ha to 200 kg N/ha. The amount of GS11 of CDI005 decreased when N increased from 0 kg N/ha to 50 kg N/ha, then rebounded and increased when N increased from 50 kg N/ha to 200 kg N/ha. The amount of GS11 of CDI007 increased when N increased from 0 kg N/ha to 60 kg N/ha, while it increased when N continued to 150 kg N/ha, and then increased again

when N increased from 150 kg N/ha to 200 kg N/ha. N was positively correlated with the amount of GS11 of CDI008. Calena had the highest amount of GS11, while CDI002 had the lowest amount of GS11 among these five tested genotypes (Figure 6.10)

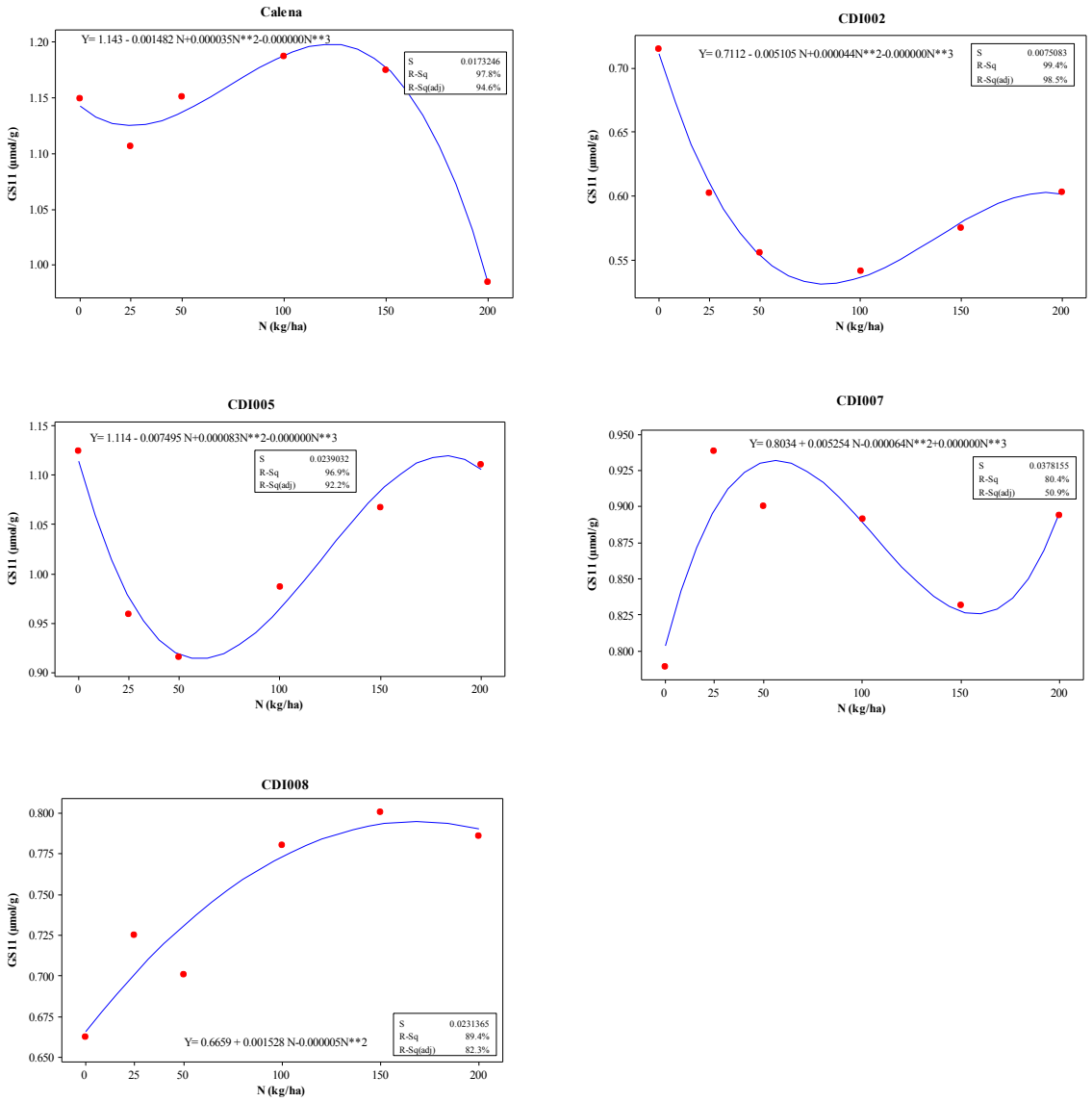


Figure 6.9: Regression analysis of N on the amount of GS11 at Truro in 2011
(each dot represents the mean of 4 samples)

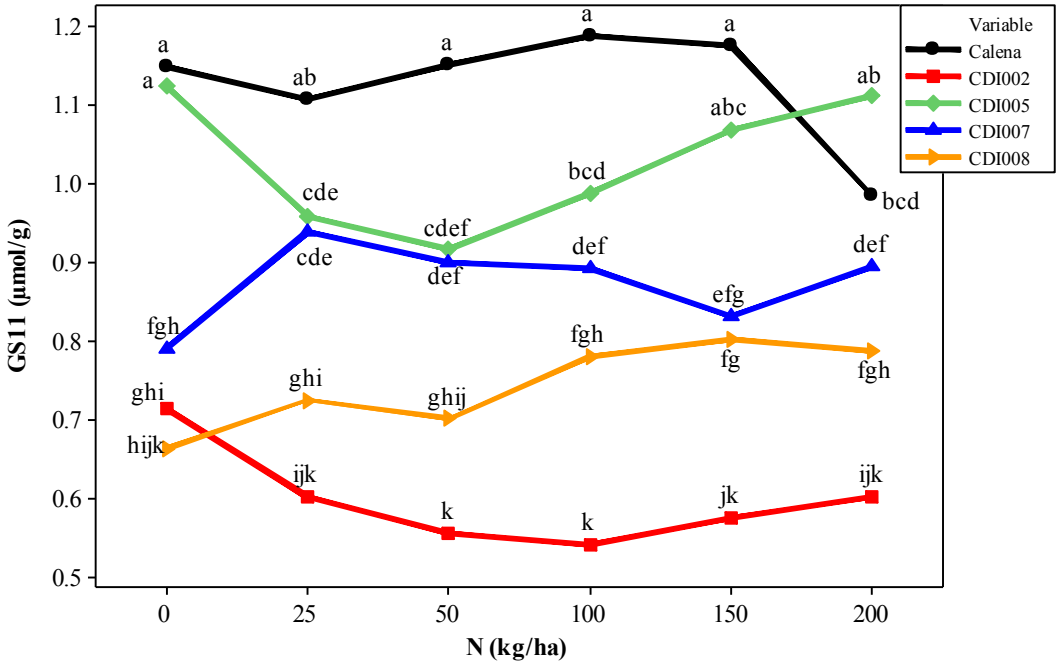


Figure 6.10: Effect of N and genotype on the amount of GS11 at Truro in 2011
(Means with a common letter are not significantly different at the 5% level)

6.5 Discussion

N was negatively correlated with the total amount of GS in this study. Reduced GS with the increase of N on oilseed rape (*Brussica napus* L.) has been reported by Josefsson (1970). In Josefsson's view, protein synthesis accelerated with the increase of N rates, which reduced the availability of carbohydrates for GS synthesis (Josefsson et al., 1970). Holmes (1980) attributed this lower GS content in oilseed rape to a dilution effect which was caused by the increase of dry matter production (Holmes, 1980 in Asare and Scarisbrik, 1995). The possible reason of decreased GS with increased N in the present study was camelina plant dry matter production increased with N rates, and the availability of S was decreased for plant tissue, leading to the reduction of GS synthesis since S affected GS formation. In contrast, increased GS with increased N in 1991 oilseed

rape trials have been reported by Asare and Scarisbrik (1995), which was also supported by similar results in spring turnip rape by Augustinussen et al. (1984) (Asare and Scarisbrik, 1995).

According to previous HPLC research results, glucocamelinin (10-methyl-sulfinyl-decyl-glucosinolate, GS10) was the main GS of camelina, which accounted for approximately 65% of the total GS (Schuster and Friedt, 1998). There are three major camelina GS: 9-methylsulfinylnonyl-glucosinolate (GS9), 10-methylsulfinyldecyl-glucosinolate (glucocamelinin, GS10) and 11-methylsulfinylundeyl-glucosinolate (GS11). The content of 9-methylsulfinylnonyl-glucosinolate was higher than that of 11-methylsulfinylundeyl-glucosinolate for most of the analyzed genotypes of camelina (Schuster and Friedt, 1998). Similar results were obtained in this study that GS10 accounted for 65% of the total GS which ranged from 4.3 to 6.6 μmol/g across all of the 30 treatments (5 genotypes * 6 N rates). The total amount of GS ranged from 6.6 to 10.2 μmol/g; the amounts of GS9 and GS11 were 1.4-2.9 μmol/g and 0.5-1.2 μmol/g in this study.

Genotype had a significant effect on the total GS of camelina. The total amount of GS of CDI002 (8.6 μmol/g), Calena (8.4 μmol/g) and CDI005 (8.4 μmol/g) were higher than CDI008 (7.8 μmol/g), and CDI007 (7.3 μmol/g) had the lowest content of GS among these five genotypes. Effect of genotype on the amount of GS had been reported by Schuster and Friedt (1998), the total GS in camelina ranged from 13.2 to 36.2 μmol/g dry seed for different genotypes.

6.6 Conclusion

Different genotypes of camelina have different contents of GS, so it can be recommended to reduce GS content by plant breeding. CDI007 had the lowest amount of total GS among these five genotypes. It has also been indicated the increase of N input decreased the content of GS. The amount of GS of camelina with 100 kg N/ha had no significant difference from plants with 150 and 200 kg N/ha.

Chapter 7: Conclusion

7.1 N Effects

The application of N has significant effects on the agronomic parameters of camelina. Those parameters include the incidence of downy mildew, plants/m^2, the number of branches per plant, the number of branches/m^2, the number of pods per plant, the number of pods/m^2, seed yield, seed protein content, protein yield, seed oil content, oil yield and fatty acid profile. N had a negative correlation with plants/m^2. N was positively correlated with the number of branches/plant, the number of pods/plant depending on genotype, the number of branches/m^2 depending on location and the number of pods/m^2. Higher protein content was negatively correlated with lower oil content. Seed yield, protein yield and oil yield increased with N until an optimum N value was attained, and then leveled out, as N continued to increase. N had a significant effect on fatty acid profile, but genotype was the predominant factor affecting fatty acid composition. The optimum N rate varied among locations, which indicated different soil types and environmental conditions were also important. To achieve maximum seed yield, the optimum N rate was 120-150 kg/ha at Canning, New Glasgow and Truro; 160-200 kg/ha at Fredericton in 2012 (Chapter 4 and 5). Results from the 2011 N trials indicated that 100 kg N/ha was the optimum rate for the highest yield and the highest content of protein and oil at Truro and Canning.

7.2 S Effects

Applied S increased the yield of camelina depending on the site (at Truro in 2012, but not at Canning, New Glasgow and Fredericton). Response of camelina to S was maximized when N was sufficient. S increased seed yield and protein yield only when N

application was sufficient or in excess. When N supply was 80 kg/ha and more, 25 kg/ha S input increased the protein content. S was positively associated with the content of protein at all the tested sites, although an inverse relationship between S and the oil content was observed at Canning.

7.3 Genotype Evaluation

One of the main objectives of this study was to evaluate advanced lines of camelina from the breeding program of AAFC Saskatoon (CDI002, CDI005, CDI007 and CDI008) and one check Calena in order to support registration of elite lines to cultivars. Overall, CDI007 had the best performance among the five genotypes at all the growing areas. CDI007 was the most tolerant to downy mildew; it had the highest seed yield, oil content, protein yield, and oil yield; but the protein content was approximately 1.2% lower than CDI002. CDI008 had the lowest seed yield, protein yield and oil yield at all of the tested locations in 2011 and 2012. Calena was the most sensitive to downy mildew. The yield of CDI005 varied greatly depending on the location and year; CDI005 had the highest yield at Canning in 2011, but it had the lowest yield at Truro in 2011 and all of the tested sites in 2012. The selection of genotype is a key determinant for the potential success or failure of camelina as a viable field crop.

7.4 Water Stress on Seed Germination and Early Growth of Camelina

In the seed germination test, the root length and the total length of root and shoot of camelina seedlings were reduced with decreasing water potential. CN113673 and CN11710 had the fastest root and shoot growth among the 25 lines. In the 4 elite line germination test, CDI007 showed the fastest root growth but CDI008 did the opposite through the water stress range. All 29 lines/genotypes had high germination percentage

(over 90%) under water stress. Camelina germinates even under high moisture deficit.

7.5 Environmentally Controlled Experiments

The effects of B and S on the agronomic performance of camelina were negligible in the controlled environment conditions perhaps due to sufficient B and S in the soil medium. If one were to base N recommendations on growth chamber experiment results, an N treatment of 150 kg/ha would appear to be optimum for the seed yield; however, pot experiments tend to over-estimate N values due to leaching under ideal watering conditions and low soil volume. Relative crop performance responding to N was consistent with the results in the field study (Chapter 7, 7.2 N effects).

7.6 Plant Plasticity

Camelina has a great potential for yield compensation. It adapts to various environmental and management conditions. Reductions in plant stand due to different seeder type, seeder malfunction and late seeding may be overcome by developing more pods per plant (Chapter 5, 2012 Canning), more branches per m^2 (Chapter 5, 2012 Fredericton) or producing physically bigger pods based on visual observation at Fredericton in 2012.

7.7 Overall Assessment

This study provides information about effects of the environment and management factors on the growth and seed quality of selected genotypes of camelina in Canada in 2011 and 2012. In the future, data of the number of seeds per pod and Thousand Seed Weight are recommended to be collected for further evaluation of the yield compensation of camelina. It would also be interesting to compare Water Use Efficiency and N Use Efficiency of camelina and other major oilseed crops (such as canola and mustard).

References

Abramovic, H., Butinar, B., & Nikolic, V. (2007). Changes occurring in phenolic content, tocopherol composition and oxidative stability of *Camelina sativa* oil during storage. *Food Chemistry, 104,* 903–909.

Agarwal, A., Pant, T., & Ahmed, Z. (2010). *Camelina sativa*: A new crop with biofuel potential introduced in India. *Current Science, 99,* 9, 1194-1195.

Agegnehu, M. & Honermeier, B. (1997). Effects of seeding rates and N fertilization on seed yield, seed quality and yield components of false flax (*Camelina sativa* L. Crantz). *Die Bodenkultur. 48:* 15-20.

Ahmad, A., & Abdin, M. Z. (2000a). Interactive effect of sulphur and nitrogen on the oil and protein contents and on the fatty acid profiles of oil in the seeds of rapeseed (*Brassica campestris* L.) and mustard (*Brassica juncea* L. Czern. and Coss.). *Journal of Agronomy and Crop Science, 185,* 1, 49-54.

Ahmad, A., Khan, I., & Abdin, M. Z. (2000b). Effect of sulfur fertilization on oil accumulation, acetyl-CoA concentration, and acetyl-CoA carboxylase activity in the developing seeds of rapeseed (*Brassica campestris* L.). *Australian Journal of Agricultural Research*, *51*, 1023-1030.

Ahmad, A., Abraham, G., & Abdin, M. Z. (1999). Physiological investigation of the impact of nitrogen and sulphur application on seed and oil yield of rapeseed (*Brassica campestris* L.) and mustard (*Brassica juncea* L. Czern. and Coss.) genotypes. *Journal of Agronomy and Crop Science, 183,* 1, 19-25.

Albrechtsen R. S. & Dybing C. D. (1973). Influence of seeding rate upon seed and oil yield and their components in Flax. *Crop Science, 13,* 277-280.

Ali, B. M., & Poshtmasari, H. K. (2010). Influence of nitrogen and sulphur on yield and seed quality of three canola cultivars. *Journal of Plant Nutrition*, *33,* 7, 953-965.

Antonious, G. F., Bomford, M. & Vincelli, P. (2009). Screening *Brassica* species for glucosinolate content. *Journal of Environmental Science and Health*. Part. B, Pesticides, Food Contaminants, and Agricultural Wastes. *44* (3): 311-6.

Asare, E., and Scarisbrick, D. H. (1995). Rate of nitrogen and sulphur fertilizers on yield, yield components and seed quality of oilseed rape (*Brassica napus* L.). *Field Crops Research, 44,* 1, 41-46.

Banks, A. (1990). Boron. *Journal of Chemical Education*, 67, 1.

Berhow, M. A., Polat, U., Glinski, J. A., Glensk, M., Vaughn, S. F., Isbell, T., … Gardner, C. (2013). Optimized analysis and quantification of glucosinolates from *Camelina sativa* seeds by reverse-phase liquid chromatography. *Industrial Crops and Products, 43,* 119-125.

Berti, M., Wilckens, R., Fischer, S., Solis, A., & Johnson, B. (2011). Seeding date influence on camelina seed yield, yield components, and oil content in Chile. *Industrial Crops and Products, 34,* 2, 1358-1365.

Birch, P. R. J., Rehmany, A. P., Pritchard, L., Kamoun, S., & Beynon, J. L. (2006). Trafficking arms: oomycete effectors enter host plant cells. *Trends in Microbiology*, *14, 1*, 8-11.

Blankman, G. E. & Bunting E. S. (1954). An assessment of the interrelationships between plant development and seed production in linseed (*Linum usitatissimum*), *Journal of Agricultural Science, 45,* 3-9.

Boivin, D., Lamy, S., Lord-Dufour, S., Jackson, J., Beaulieu, E., Cote, M., Moghrabi, A., ... Beliveau, R. (2009). Antiproliferative and antioxidant activities of common vegetables: A comparative study. *Food Chemistry, 112, 2,* 374-380.

British Pharmacopoeia Commission. "Ph Eur monograph 1371". British Pharmacopoeia. Norwich, England: The Stationery Office. ISBN 011-322682-9.

Budin, J. T., Breene, W. M., & Putnam, D. H. (1995). Some compositional properties of camelina (*Camelina sativa* L. Crantz) seeds and oils. *Journal of the American Oil Chemists' Society, 72, 3*, 309-315.

Camelina Plant Guide, Natural Resources Conservation Service, USDA, 2011. Taken Oct. 15, 2012 from http://plants.usda.gov/plantguide/pdf/pg_casa2.pdf

Canadian Food Inspection Agency, (2012). Camelina products, Chapter 3 – Specific registration information by feed type. Modified on Feb. 15, 2012. Taken from http://www.inspection.gc.ca/animals/feeds/regulatory-guidance/rg-1/chapter 3/eng/1329319549692/1329439126197?26

Cartea M.E., Velasco P., Padilla G., Obregon S., & de Haro A. (2008). Seasonal variation in glucosinolate content in *Brassica oleracea* crops grown in northwestern Spain. *Phytochemistry.* 69 (2): 403-410.

Chaudhary, S. K., N. M. Gogulwar, & A. K. Singh. (1992). Effect of sulfur and nitrogen on seed yield and oil content of mustard (*Brassica juncea*). *Indian Journal of Agronomy, 37*: 839–840.

Church, B. (2010). Currently allowed practices for the use of *Camelina sativa* meal as a commercial feed in Montana. Retrieved from http://agr.mt.gov/camelina/default.asp

Crowley, J. G., Fröhlich, A., & Teagasc (Organization). (1998). Factors affecting the composition and use of camelina. Dublin: Teagasc.

Daun, J. K., & McGregor, D. I. (1983). Glucosinolate analysis rapeseed (canola). Method of the Canadian Grain Commision Grain Research Laboratory. Winnipeg, MB. 1981 (revised 1983).

Ehrensing, D. T., & Guy, S. O. (2008).Camelina, Oilseed Crops. Oregon State University Extension Service. Taken Oct. 15, 2012 from http://extension.oregonstate.edu/catalog/pdf/em/em8953-e.pdf

Eidhin, D. N., Burke, J., & O'Beirne, D. (2003). Oxidative stability of w-3-rich camelina oil and camelina oil-based spread compared with plant and fish oils and sunflower spread. *Journal of Food Science, 68,* 345–353.

Eidhin, D. N., & O'Beirne, D. (2010a). Oxidative stability of camelina oil in salad dressings, mayonnaises and during frying. *International Journal of Food Science & Technology, 45* (3), 444-452.

Eidhin, D. N., & O'Beirne, D. (2010b). Oxidative stability and acceptability of camelina oil blended with selected fish oils. *European Journal of Lipid Science and Technology, 112* (8), 878-886.

Erucic acid in food: a toxicological review and risk assessment, technical report series no.21. Food standards Australia New Zealand, June 2003. Taken Aug. 10, 2012 from http://www.foodstandards.gov.au/_srcfiles/Erucic%20acid%20monograph.pdf

Eva Feldman, M.D. (2008). Thiobarbituric acid reactive substances (TBATS) assay. *Animal models of diabetic complications consortium, 1,* 1-3. Taken from http://www.amdcc.org/shared/showFile.aspx?doctypeid=3&docid=33

Falk, K. C. and Klein-Gebbinck, H. Camelina breeding at AAFC [Conference presentation]. Camelina Day, November 4, 2009.

Flagella, Z., Rotunno, T., Tarantino, E., Di, C. R., & De, C. A. (2002). Changes in seed yield and oil fatty acid composition of high oleic sunflower (*Helianthus annuus* L.) hybrids in relation to the sowing date and the water regime. *European Journal of Agronomy, 17,* 3, 221-230.

Food and Agriculture Organization of the United Nations. (1984). *Fertilizer and plant nutrition guide*. Rome: Food and Agriculture Organization of the United Nations.

French, A. N., Hunsaker, D., Thorp, K., & Clarke, T. (2009). Evapotranspiration over a camelina crop at Maricopa, Arizona. *Industrial Crops and Products, 29,* 289-300.

Gao, Y. P., Young, L., Bonham-Smith, P. & Gusta, L. V. (1999). Characterization and expression of plasma and tonoplast membrane aquaporins in primed seed of *Brassica napus* during germination under stress conditions. *Plant Molecular Biology*, *40*, 635-644.

Gardner, F. P., Pearce, R. B., & Mitchell, R. L. (1985). Physiology of crop plants. Ames: Iowa State University Press.

Grant, C., Clayton, G., & Johnston, A. (2003). Sulphur fertilizer and tillage effects on canola seed quality in the black soil zone of western Canada. *Canadian Journal of Plant Science*, 83, 745-758.

Grant, C., Johnston, A., & Clayton, G. (2004). Sulphur fertilizer and tillage management of canola and wheat in western Canada. *Canadian Journal of Plant Science*, *84*, 453-462.

Gehringer, A., Friedt, W., Lühs, W., & Snowdon, R. J. (2006). Genetic mapping of agronomic traits in false flax (*Camelina sativa* subsp. sativa). *Genome / National Research Council Canada = Génome / Conseil National De Recherches Canada*, *49,* 12, 1555-63.

Gugel, R. K., & Falk, K. C. (2006). Agronomic and seed quality evaluation of *Camelina sativa* in western Canada, *Canadian Journal of Plant Science, 86,* 1047-1058.

Gunasekera, C. P., Martin, L. D., Siddique, K. H. M., and Walton, G. H. (2006). Genotype by environment interactions of Indian mustard (*Brassica juncea* L.) and canola (*B. napus* L.) in Mediterranean-type environments. *European Journal of Agronomy*, 25, 1, 1-12.

Halkier, B. A., & Gershenzon, J. (2006). Biology and biochemistry of glucosinolates. *Annual Review of Plant Biology*, 57, 303-33.

Hayes, J. D., Kelleher, M. O., & Eggleston, I. M. (2008). The cancer chemopreventive actions of phytochemicals derived from glucosinolates. *European Journal of Nutrition, 47,* 73-88.

Hergert, G. W., Margheim, J., Pavlista A., Burgener, P., Lyon D., & Hazen A. (2011). Yields and ET of deficit to fully irrigated canola and camelina. Proceeding of the 23rd Annual Central Irrigation Conference, Burlington, CO., Feb 22-23, 2011. Available from CPIA, 760 N. Thompson, Colby, Kansas. Taken from http://www.ksre.ksu.edu/irrigate/OOW/P11/Hergert11.pdf

Hu, H. & Sparks, D. (1992). Nitrogen and sulphur interaction influences net photosynthesis and vegetative growth of pecan. *Journal of the American Society for Horticultural Science*, *117*(1): 59-64, 1992.

Imbrea, F., Botos, L., Jurcoane, S., Halmajan, H. V., & Duda, M. (2011). *Camelina sativa*: A new source of vegetal oils. *Romanian Biotechnological Letters*, 16, 3, 6263-6270.

Imura, K., & Okada, A., (1998). Amino acid metabolism in pediatric patients. *Journal of Nutrition, 14* (1): 143–8.

Jackson, G. D. (2000). Effects of nitrogen and sulfur on canola yield and nutrient uptake. *Agronomy Journa*l, 92, 4.

Jan, A., N. Khan, N. Khan, I. A. Khan, & B. Khattak. (2002). Chemical composition of canola as affected by nitrogen and sulfur. *Asian Journal of Plant Sciences*, 1: 521–521.

Janzen, H. H., & Bettany, J. R. (1984). Sulfur nutrition of rapeseed: I. Influence of fertilizer nitrogen and sulfur rates. *Soil Science Society of America Journal*, 48, 100-107.

Johnson, J. M. F., & Gesch, R. W. (2013). Calendula and camelina response to nitrogen fertility. *Industrial Crops and Products*, *43*, 684-691.

Jones, J. B. (1998). Plant nutrition manual. Boca Raton: CRC Press.

Josefsson, E. (1970). Glucosinolate content and amino acid composition of rapeseed (*Brassica napus*) meal as affected by sulphur and nitrogen nutrition. *Journal of the Science of Food and Agriculture, 21,* 2, 98-103.

Joshi, N. L., Mali, P. C., & Saxena, A. (1998). Effect of nitrogen and sulphur application on yield and fatty acid composition of mustard (*Brassica juncea* L) Oil. *Journal of Agronomy and Crop Science, 180,* 1, 59-63.

Karvonen, H. M., Aro, A., Tapola, N. S., Salminen, I., Uusitupa, M. I., & Sarkkinen, E. S. (2002). Effect of α-linolenic acid rich *Camelina sativa* oil on serum fatty acid composition and serum lipis in hyper cholesrolemic subjects. *Metabolism, 51* (10), 1253-1260.

Kestwal, R. M., Lin, J. C., Bagal-Kestwal, D., & Chiang, B. H. (2011). Glucosinolates fortification of cruciferous sprouts by sulphur supplementation during cultivation to enhance anti-cancer activity. *Food Chemistry, 126,* 3, 1164-1171.

Khan, M. A. M., Ulrichs, C., & Mewis, I. (2010). Influence of water stress on the glucosinolate profile of *Brassica oleracea* var. italica and the performance of *Brevicoryne brassicae* and *Myzus persicae*. *Entomologia Experimentalis Et Applicata*, *137*, 3, 229-236.

Leach, J. E., Stevenson, H. J., Rainbow, A. J., & Mullen, L. A. (1999). Effects of high plant populations on the growth and yield of winter oilseed rape (*Brassica napus*). *The Journal of Agricultural Science*, 132, 2, 173-180.

Losak, T., Hlusek, J., Martinec, J., Vollmann, J., Peterka, J., Filipcik, R., … Martensson, A. (2011). Effect of combined nitrogen and sulphur fertilization on yield and qualitative parameters of *Camelina sativa* L. Crtz. (false flax). *Acta Agriculturae Scandinavica*, *Section B - Soil & Plant Science, 61,* 4, 313-321.

MacDonald, D., & Li, J. L. (2010). *Camelina sativa* factsheet [personal communication]. Crop Development Institute at Dalhousie Faculty of Agriculture.

Malhi, S. S. & Leach, D. (2002). Optimizing yield and quality of canola seed with balanced fertilization in the parkland zone of western Canada. *In Proc. Soils and Crops 2002*. 17th WCSS, 14-21 August 2002, Thailand.

Malhi, S. S., & K. S. Gill. (2002). Effectiveness of sulfate–S fertilization at different growth stages for yield, seed quality and S uptake of canola. C*anadian Journal of Plant Science,* 82: 665–674.

Manaf, A., and Hassan, F. (2006). Effects of sulphur on fatty acid accumulation in Brassica cultivars. *International Journal of Agriculture and Biology, 8*(5), 588-592.

Martinez-Villaluenga, C., Frias, J., Gulewicz, P., Gulewicz, K., & Vidal-Valverde, C. (2008). Food safety evaluation of broccoli and radish sprouts. *Food and Chemical Toxicology*, *46,* 5, 1635-1644.

Matthaus, B., & Zubr, J. (2000). Variability of specific components in *Camelina sativa* oilseed cakes. *Industrial Crops and Products, 12,* 9-18.

Mathew, J., & George, S. (2011). Sulphur and boron improves yields of oilseed sesame in sandy loam soil of Onattukara. *Better Crops - South Asian*, p14-15.

McNeill, A. M, J. Eriksen, L. Bergstrom, K. A. Smith, H. Marstrop, H. Kirchmann, & I. Nilsson. (2005). Nitrogen and sulfur management: Challenges for organic sources in temperate agricultural systems. *Soil Use and Management, 21*: 82–93.

McVay, K. A. & Lamb, P. F. (2008). Camelina Production in Montana, Montana State University - Research on all fronts, from how to best grow camelina to developing end use products of the oil and meal of this unique seed, is currently underway in Montana. Taken from http://msuextension.org/publications/AgandNaturalResources/MT200701Ag.pdf

McVay, K. A., & Khan, Q. A. (2011). Camelina yield response to different plant populations under dryland conditions. *Agronomy Journal*, *103*, 4, 1265.

McVetty, P. B. E. (2009). Plant composition and climate change influence of climate and climate change on plant oil composition [presentation slides]. University of Manitoba. Taken from http://www.greencropnetwork.com/PDF/Peter%20Mcvetty.pdf

Mulligan, G. A. (2002). Weedy introduced mustards (*Brassicaceae*) of Canada. *The Canadian Field-Naturalist*, *116, 4*, 623.

Nettleton, J. A. (1991). Omega-3 fatty acids: comparison of plant and seafood sources in human nutrition. *Journal of the American Dietetic Association*, 91, 3, 331-7.

Niki, E., & Nakano, M. (1990). Estrogens and antioxidants. *Methods in Enzymology, 186,* 330-333.

Nuttall, W. F., H. Ukrainetz, J. W. G. Stewart & D. T. Spurr. (1987). The effect of nitrogen, sulphur and boron on yield and quality of rapeseed (*Brassica napus* L. and *B. campestris* L.). *Canadian Journal of Soil Science,* 67:545–559.

Pan, X. (2009). A two year agronomic evaluation of *Camelina sativa* and *Brassica carinata* in NS, PEI and SK. Halifax, N.S: Dalhousie University.

Parkin, I., Singh, R., Higgins, E., Clark, W., & Bollina, V. (2012). Uncovering the ancestral blocks reveals the hexaploid genome of the emerging biofuel crop, Camelina sativa. Conference 2012 – Plant and Animal Genome. [conference abstract]. Taken from https://pag.confex.com/pag/xx/webprogram/Paper1693.html

Paulsen, H. M., Wichmann, V., Schuemann, U., & Richter, B. (2011). Use of straight vegetable oil mixtures of rape and camelina as on farm fuels in agriculture. *Biomass and Bioenergy*, 35, 9, 4015-4024.

Pavlista, A. D., Baltensperger, D. D., Isbell, T. A., & Hergert, G. W. (2012). Comparative growth of spring-planted canola, brown mustard and camelina. *Industrial Crops and Products*, 36, 1, 9-13.

Peiretti, P.G., Mussa, P. P., Prola, L., & Meineri, G. (2007). Use of different levels of false flax (*Camelina sativa* L.) seed in diets for fattening rabbits. *Livestock Science, 107*(2-3), 192-198.

Pilgeram, A. (2008). The Scoop on *Camelina Sativa*. Retrieved October 5, 2010 from Montana State University website http://www.montana.edu/biobased/projects/CamelinaInfo.html

Plessers, A. G., McGregor, W. G., Carson, R. B., & Nakoneshny, W. (1962). Species trials with oilseed plants II. camelina. *Canadian Journal of Plant Science*, 42, 3, 452-459.

Pritchard, F. M., H. A. Eagles, R. M. Norton, P. A. Salisbury, and M. Nicolas, (2000). Environmental effects on seed composition of Victorian canola. *Australian Journal of Experimental Agriculture*, 40, 679-685.

Putnam, D. H., Budin, J. T, Field, L., & Breene, W. M. (1991). Camelina: a promising low-input oilseed. In: Janick, J., Simon, J. (Eds.), New Crops, Exploration, Research and Commercialization, Proceedings of the Second National Symposium. Indianapolis, October 6–9, 1991. John Wiley & Sons, Inc., New York, 314–322.

Raney, J. P. Gas chromatography of trimethylsiyl derivatives of glucosinolates of rape and mustard. Method of Agriculture Agri-food Canada Quality Laboratory. Saskatoon, SK.

Rathke, G. W., Christen, O., & Diepenbrock, W. (2005). Effects of nitrogen source and rate on productivity and quality of winter oilseed rape (*Brassica napus* L.) grown in different crop rotations. *Field Crops Research, 94,* 103-113.

Rizzo, W. B., Watkins, P. A., Phillips, M. W., Cranin, D., Campbell, B., & Avigan, J. (1986). Adrenoleukodystrophy: oleic acid lowers fibroblast saturated C22-26 fatty acids. *Neurology, 36* (3): 357-361.

Rode, J. (2002). Study of Autochthon *Camelina sativa* (L.) Crantz in Slovenia. *Journal of Herbs, Spices & Medicinal Plants*, *9,* 4, 313-318.

Rono. K. J. (1994). Comparative Eco-physiological seed adaptation of three barnyardgrass ecotypes [Doctoral dissertation]. Dalhousie University.

Ryhänen, E. L., Perttilä, S., Tupasela, T., Valaja, J., Eriksson, C., & Larkka, K. (2007). Effect of *Camelina sativa* expeller cake on performance and meat quality of broilers, *Journal of the Science of Food and Agriculture, 87* (8), 1489-1494.

Sampath, A. (2009). Chemical characterization of camelina seed oil [Master thesis]. The State University of New Jersey, New Brunswick.

Saron, G., & G. Giri. (1990). Influence of nitrogen, phosphorus and sulfur on mustard under semi-arid rainfall conditions of North-West India. *Indian Journal of Agronomy, 35:* 131–136.

Schillinger, W. F., Wysocki, D. J., Chastain, T. G., Guy, S. O., & Karow, R. S. (2012).Camelina: Planting date and method effects on stand establishment and seed yield. *Field Crops Research, 130,* 138-144.

Schnell, J., & Davis, S. (2011). Plant biology document of "The biology of *Camelina sativa* (L.) Crantz".Plant and Biotechnology Risk Assessment Unit, Plant Health Science Division, Canadian Food Inspection Agency, Ottawa, Ontario.

Schnug, E., Haneklaus, S., & Murphy, D. (1993). Impact of sulphur fertilisation on fertiliser nitrogen efficiency. *Sulphur in Agriculture*, *17,* 8, 12.

Schuster, A., & Friedt, W. (1998). Glucosinolate content and composition as parameters of quality of camelina seed. *Industrial Crops and Products, 7*(2-3), 297-302.

Schwartz, H., Ollilainen, V., Piironen, V., & Lampi, A. (2008).Tocopherol, tocotrienol and plant sterol contents of vegetable oils and industrial fats, *Journal of Food Composition and Analysis, 21*(2), 152-161.

Seguin, P., & Zheng, W. (2006). Potassium, phosphorus, sulfur, and boron fertilization effects on soybean isoflavone content and other seed characteristics. *Journal of Plant Nutrition*, 29, 4, 681-698.

Seguin-Swartz, G., Eynck, C., Gugel, R. K., Strelkov, S. E.,Olivier, C. Y., Li, J., Borhan, H., Falk, K. C., Caldwell, C. D., and Klein-Gebbinck, H. (2009). Diseases of *Camelina sativa* (false flax). *Canadian Journal of Plant Pathology*, 31, 4, 375-386.

Shao, H. B., Jaleel, C. A., Chu, L. Y., & Zhao, C. X. (2008). Water-deficit stress-induced anatomical changes in higher plants. *Comptes Rendus - Biologies, 331,* 3, 215-225.

Sharifi, M., Roestel, J. V., & Mahoney, K. (2010). Survey of sulphur levels in Nova Scotia Soils. Dalhousie Faculty of Agriculture. Taken Jan. 10, 2012 from http://www.scians.org/documents/factsheets/713f66ad2998cefa2d9f9a8529cba8b7cc429245.pdf

Sinha, P., Jain, R., & Chatterjee, C. (2000). Interactive effect of boron and zinc on growth and metabolism of mustard. *Communications in Soil Science and Plant Analysis*, *31*, 1-2.

Sipalova, M., Macek, M., Kracmar, S., Losak, T., Hlusek, J., Vollmann, J., … Filipcik, R. (2011). Fatty acid composition of *Camelina sativa* as affected by combined nitrogen and sulphur fertilization. *African Journal of Agricultural Research*, *6,* 16, 3919-3923.

Sivaprakasam, K., Pillayarsamy, K., & Soumini, R. C. K. (1974). Influence of nitrogen on the incidence of downy mildew disease of pearl millet (*Pennisetum typhoides* Stapf & Hubb.). *Plant and Soil, 41,* 3, 677-679.

Sy, A., Grouzis, M., & Danthu, P. (2001). Seed germination of seven Sahelian legume species. *Journal of Arid Environments, 49,* 4, 875-882.

Tandon, H. L. S. (1992). Sulphur in Indian Agriculture. *Sulphur in Agriculture, 16,* 20.

Taylor, A. J., & Smith, C. J. (1992). Effect of sowing date and seeding rate on yield and yield components of irrigated canola (*Brassica napus* L.) grown on a red-brown earth in south-eastern Australia. *Australian Journal of Agricultural Research*, 43, 7.

Trifluralin. (2009). Ottawa: Pest Management Regulatory Agency. Take Mar. 15, 2012 from http://www.hc-sc.gc.ca/cps-spc/alt_formats/pdf/pubs/pest/decisions/rvd-drv/rvd2009-09-eng.pdf

Turk, F. M. (2002). Microscopic evaluation of interactions between varieties of *Arabidopsis thaliana* challenged by *Peronospora parasitica*. *Turkish Journal of Agriculture and Forestry*, *26,* 125-132.

Understanding the soil test report (revised in November, 2011). Nova Scotia Agriculture, Government of Nova Scotia. Taken from www.gov.ns.ca/agri/qe/factsheets/understand-soil.pdf

Urbaniak, S. D. (2006). An agronomic evaluation of camelina and solin for the Maritime Provinces of Canada [Masters thesis]. Halifax, N.S: Dalhousie University.

Urbaniak, S. D., Caldwell, C. D., Zheljazkov, V. D., Lada, R., & Luan, L. (2008a). The effect of cultivar and applied nitrogen on the performance of *Camelina sativa* L. in the maritime provinces of Canada. *Canadian Journal of Plant Science, 88,* 111-119.

Urbaniak, S. D., Caldwell, C. D., Zheljazkov, V. D., Lada, R., & Luan, L. (2008b). The effect of seeding rate, seeding date and seeder type on the performance of *Camelina sativa* L. in the maritime provinces of Canada. *Canadian Journal of Plant Science, 88,* 501-508.

Vollmann, J., Damboeck, A., Eckl, A., Schrems, H. & Ruckenbauer, P. (1996). Improvement of *Camelina sativa*, an underexploited oilseed. *Progress in new crops,* 357-362.

Vollmann, J., Grausgruber, H., Stift, G., Dryzhyruk, V., & Lelley, T. (2005). Genetic diversity in camelina germplasm as revealed by seed quality characteristics and RAPD polymorphism. *Plant Breeding, 124, 5,* 446-453.

Wallis, J. G., Watts, J. L., & Browse, J. (2002). Polyunsaturated fatty acid synthesis: what will they think of next?. *Trends in Biochemical Sciences*, 27, 9.

Walters, D. (2009). Managing crop disease through cultural practices. P. 7-26. In: D. Walters (ed.), Disease Control in Crops. Biological and environmentally friendly approaches. Wiley-Blackwell, Oxford, UK.

What is cold pressed oil (last modified date: Sep. 24, 2012). Taken from http://www.wisegeek.com/what-is-cold-pressed-oil.htm

Wink, M. (1999). Biochemistry of plant secondary metabolism. Sheffield, England: Sheffield Academic Press.

Yan, X., & Chen, S. (2007). Regulation of plant glucosinolate metabolism. *Planta*, 226, 6, 1343-1352.

Yau, S. K., & Ryan, J. (2008). Boron Toxicity Tolerance in Crops: A Viable Alternative to Soil Amelioration. *Crop Science*, 48, 3.

Yang, Y., Wang, G. X., Wang, X. D., Guo, J. Y., & Liu, Q. (2010). Germination, osmotic adjustment, and antioxidant enzyme activities of gibberellin-pretreated Piceaasperata seeds under water stress. *New Forests*, 39, 2, 231-243.

Zarafi, A., Emechebe, A. M., Akpa, A. D., & Alabi, O. (2005). Effect of fertilizer levels on grain yield, incidence and severity of downy mildew in pearl millet. *Archives of Phytopathology and Plant Protection*, 38, 1, 11-17.

Zheng, Y., Xie, Z., Gao, Y., Jiang, L., Shimizu, H., & Tobe, K. (2004). Germination responses of *Caragana korshinskii* Kom. to light, temperature and water stress. *Ecological Research*, 19, 5, 553-558.

Zheng, Y., Xie, Z., Gao, Y, M., Jiang, L & Xing, X. (2005). Effects of light, temperature and water stress on germination of Artemisia sphaerocephala. *Annals of Applied Biology*, 146, 3, 327-335.

Zubr, J. (1997). Oil-seed crop: *Camelina sativa*. *Industrial Crops and Products, 6,* 113-119.

Zubr, J. (2003a). Dietary fatty acids and amino acids of *Camelina sativa* seed. *Journal of Food Quality*, 26, 6, 451-462.

Zubr, J. (2003b). Qualitative variation of *Camelina sativa* seed from different locations. *Industrial Crops Products, 17 (3),* 161-169.

Zubr, J. (2009). Unique dietary oil from *Camelina sativa* seed. *Agro Food industry hi-tech, 20 (2),* 42-46.