A TWO YEAR AGRONOMIC EVALUATION OF *CAMELINA SATIVA* AND *BRASSICA CARINATA* IN NS, PEI AND SK

by

Xue Pan

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Dated: December 10, 2009

Supervisor: _____

Readers: _____

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Abstract

This study assessed oilseed crops *Camelina sativa* (L.) Crantz and *Brassica carinata* A. Braun in NS, PEI and SK. Evaluations of basic agronomy were performed to determine optimum management practices. The *C. sativa* genotype "CN30479" performed best; all 10 *B. carinata* accessions had satisfactory performance. 125 kg N/ha in NS and PEI and 100 kg N/ha in SK gave maximum *C. sativa* seed yield while optimum plant density was 170, 280 and 150 plants/m^2 in NS, PEI and SK, respectively. *Brassica carinata* required 125 kg N/ha in NS and PEI, and 75 kg N/ha in SK for optimum seed yield. 200 seeds/m^2 (80 plants/m^2) was the optimum seeding rate for *B. carinata* in all sites. Controlled environment studies showed that seed yield response to N depended on the water regime. *C. sativa* and *B. carinata* have good agronomic suitability across Canada.

List of Abbreviations and Symbols Used

NS	Nova Scotia
PEI	Prince Edward Island
SK	Saskatchewan
PAR	photosynthetically active radiation
P_N	net photosynthesis
XPP	xylem pressure potential
E	transpiration rate
WUE_i	instantaneous water use efficiency
P_{max}	maximum rate of net photosynthesis
R_d	dark respiration
α	photochemical efficiency of photosynthesis at low light
θ	ratio of physical to total resistance to diffusion of CO_2
Gs	stomatal conductance
C_i	intercellular CO_2 concentration
N_{area}	leaf N content per leaf area basis
PNUE	instantaneous N use efficiency
TKW	thousand kernel weight

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Chapter 1 Introduction

1.1 General Introduction

As the world population expands and fossil resources decline, the need for developing oilseed crops with valuable agronomic attributes and diverse food and non-food applications continues to grow. The unremitting growth of oilseed demand makes it necessary to explore new oilseed crops to meet this requirement. Currently, soybean [*Glycine max* (L.) Merr.], sunflower (*Helianthus annuus* L.) and canola (*Brassica napus* L. and *B. rapa* L.) are the most significant edible oil crop in the international oilseed market (Wittkop *et al.* 2009); however, these oilseeds have limitations. For example, sunflower and canola, especially canola, require high amounts of nitrogen fertilizer and are susceptible to many insects and diseases (Putnam *et al.* 1993); soybeans cannot be well established in regions north of the Corn Belt in North America (the Lake region-Michigan, Wisconsin and Minnesota), Europe and Asia (Putnam *et al.* 1993). In order to develop a new oilseed crop, it is necessary to determine the unique and the attractive properties of that crop which are not possessed by other existing crops. The unusual and positive agronomic attributes of the oilseed *Camelina sativa* (L.) Crantz include low-input requirement (Putnam *et al.* 1993; Schuster and Friedt 1998; Zubr 1997), adaptation to semi-arid regions, tolerance of low-fertility or saline soils and high disease and insect resistance (Conn *et al.* 1988). Furthermore, it is also rare for a non-traditional crop to contain as high as 40 % oil. It is also considered one of the renewable resources of industrial raw materials since the oil has a high proportion of linolenic acid (Schuster and Friedt 1995). A second non-traditional oilseed crop, *Brassica carinata* A. Braun or Ethiopian mustard, exhibits good resistance to drought, disease, and pests and seed shattering and could also be an important source of genes which are unusual in other oilseed brassicas. Furthermore, it has recently attracted a growing interest in non-food applications such as bio-diesel, bio-polymers, lubricants, soaps and surfactants due to the high erucic acid content (Becker *et al.* 1999).

A vast amount of research has been devoted over many years evaluating the effect of oil consumption on human health in developed countries (Simopoulos 2002). Various sources of information suggest that the ideal ratio of omega-6 to omega-3 essential fatty

acids (EFA) in the human diet should be around 1 whereas in western diets the ratio is 15/1–16.7/1. Excessive amounts of omega-6 fatty acids found in today's western diets promote the risk of a number of health problems including cardiovascular disease, cancer, inflammatory and autoimmune diseases (Simopoulos 2002).

Increasing interest in diversifying cropping systems and developing new oilseed crops with higher quality oils in recent years, led us to evaluate the viability of developing *C. sativa* and *B. carinata* as industrial oilseeds in Canada. A question that remains to be answered is whether or not *C. sativa* and *B. carinata* can be produced in a sustainable and economical manner in Canada.

1.2 Literature Review

1.2.1 Climate and Soil Preparation (NS, PEI and SK)

Three sites (NS, PEI and SK) were selected in this study. The weather and soil variability among sites contributed significant effect on the interpretation of the results; therefore, a brief description of each site is provided.

Tillage is the agricultural preparation of the soil by mechanical manipulation of the soil and plant residue to prepare a seedbed for crop seeds. Most of the labile soil quality attributes, such as soil nitrogen mineralization, soil carbon storage, soil water holding ability and soil living organism activities are closely related to the tillage system (Reicosky and Allmaras 2003). Proper tillage could increase the release of soil nutrients, suppress weeds, reduce pest infection, and modify the circulation of water and air within the soil; however, intensive tillage accelerates the soil carbon loss and leads to environmental problems. Choosing the most appropriate tillage system in a certain region requires matching the operations to the elements which include weather conditions, soil living organisms, soil parent material, topography and crop sequence (Reicosky and Allmaras 2003).

The dominant climate in PEI and NS is a modified continental climate which is influenced by the Great Lakes and the Atlantic Ocean. The soil parent material is from glacial deposition and the main limitation of this kind of soil is imperfect or poor internal drainage. The poor subsoil structure with the humid climate results in excessive soil moisture content and makes the soil susceptible to soil compaction (Reicosky and

Allmaras 2003). A wide range of crops can be cultivated in eastern Canada, including corn, soybeans, potatoes, cereal crops, hay and pasture. In eastern Canada, the soil preparation for seeding field crops conventionally is the combination of fall moldboard plowing with spring secondary tillage. However, Sijtsma *et al.* (1998) noted that this conventional tillage system is the most costly system and suggested that adopting conservation tillage would be more economical. Furthermore, the frequent and intense rainfall in eastern Canada has the high potential of severe soil water erosion. A conservation tillage system could prevent severe water erosion by leaving residue on the surface.

In the western Canadian Prairie provinces, much of the area has a semiarid climate. The major threat to the long-term sustainability of farming is soil erosion by wind. Soil erosion is detrimental to soil quality and thus influences the long-term soil productivity. Keeping crop residues on the oil surface, also known as "trash cover farming" or stubble mulching technique, has been practiced since the land was brought into cultivation in western Canada (Reicosky and Allmaras 2003).

1.2.2 Background

1.2.2.1 *Camelina sativa*

Camelina sativa (L.) Crantz, also known by its common names "false flax" or "gold of pleasure" (2n = 40), is a member of the Brassicaceae family (Al-shaehbaz 1987). Archeological evidence suggests that its cultivation dates back to the Neolithic Age in southeast Europe and became well established during the Bronze Age (Bouby 1998). The crop was sporadically cultivated in the Middle Ages but it co-evolved as a weed with flax thus, also known as "false flax". Until the beginning of the 20th century, the crop was cultivated in many parts of the world, such as France, Belgium, Holland, Russia and Sweden (Zubr 1997). Although camelina has a long history of cultivation, it is currently underexploited. The recent focus on this crop was inspired by finding new sources of essential fatty acids, especially vegetable sources of omega-3 fatty acids (Karvonen *et al.* 2002) and its use as a source of biofuel (Cardone *et al.* 2003; Francis and Campbell 2003a).

1.2.2.2 *Brassica carinata*

Brassica carinata A. Braun, also known as Ethiopian or Abyssinian mustard, is considered to have originated in Ethiopia and its cultivation is believed to be traced back to 4 to 5 Millennia BC (Simmonds 1979). Present day evidence indicates that *B. carinata* is an amphidiploid species (2n = 34, BBCC) evolved from the hybridization of *B. nigra* (BB n = 8) and *B. oleracea* (CC n = 9) in the highlands of Ethiopia and the adjacent areas of East Africa and the Mediterranean coast where both the parental species exist (Gómez-Campo and Prakash 1999). The recent interest in this crop is due to its high resistance to biotic and abiotic stresses under semi-arid conditions (Teklewold and Becher 2006).

1.2.3 Morphology

1.2.3.1 *Camelina sativa*

Camelina as a cultural plant occurs in two different forms, the summer annual and winter hardy annual or biennial form. The major morphological differences between these two forms are the shape of the leaves, pods, and seeds and some other specific traits (Zubr 1997). *Camelina microcarpa* and *C. sativa* are two major species of camelina. Two subspecies of *C. sativa*, winter annual C. *sativa* subsp. *pilosa* and summer annual *Camelina sativa* are the most important species grown in agricultural systems (Plessers *et al.* 1962). *Camelina sativa* is a short-season crop, generally requiring 85 to 100 days to mature. During the initial growth stage, the major development parts, consisting of the underground conical roots and above ground a rosette of leaves develop. Later the rosette becomes the starting point of the erect stem with numerous leaves. The plants, with smooth branches and hairy stems, usually reach 70 to 100 cm in height. Leaves are 5 to 8 cm long, arrow-shaped with smooth edges. During the reproductive stage, the flower buds and axial branches bearing flowers start from the apex of the plants. The flowers, which are highly self-pollinated, are prolific, small, pale yellow with 4 petals. The small pear-shaped pods contain approximately 15 seeds which are small (0.7 mm x 1.5 mm), pale yellow-brown, oval-shaped with a ridged surface. When the seeds' color changes to dark-brown or reddish, it indicates that the seeds have reached maturity (Zubr 1997).

1.2.3.2 *Brassica carinata*

Brassica carinata, is an annual crop that grows to a height of 90 to 120 cm. Its stems are reddish-green with an abundance of branches. Its leaves are alternate with long petioles. *Brassica carinata* flowers are about 1.5 cm across and light yellow in color with 4 free sepals in one series and mature into a silique which is usually less than 5 cm long. Compared with *C. sativa*, seeds of *B. carinata* are large (usually 2 mm thick) in size with a thousand kernel weight (TKW) of approximately 3.5 g. *Brassica carinata* is still at the experimental stage. The current research suggests that this crop may be well adapted to a semi-arid climate (Knowles *et al.* 1981; Fereres *et al.* 1983; Malik 1990).

1.2.4 Crop Production

The three most important production factors for achieving optimum yield are climate, soil and crop factors (Table 1.1). In this study, the effects of light intensity and water supply were evaluated in growth chambers since these factors are generally not controllable in a field situation; the soil and crop factors of seeding rate, N supply, and varietal adaptation were evaluated in the field in order to investigate the interactive effects of these factors on yield and quality for *C. sativa* and *B. carinata* in Canada.

Table 1.1 Climate, soil and crop factors affect crop production

Climate factors	Soil factors	Crop factors
Air temperature	Texture	Crop species/variety
Relative humidity	Organic mater	Planting date
Altitude/latitude	Structure	Seeding rate and
Light	Slope and topography	geometry
Light Quantity	Soil temperature	Evapotranspiration
Light Intensity	Cation exchange capacity	Water availability
Light Duration	Base saturation	Nutrition
Precipitation	Soil management factors	Pests
Wind	Depth (root zone)	Harvest efficiency
CO_2 concentration		

Adapted from Havlin *et al.* 1999.

1.2.4.1 Nitrogen

Soil fertilizer management plays a critical role in any cropping system designed to achieve optimum yield. Excessive fertilizer application can have a negative influence on water quality and emission of trace gases into the air. Therefore, reducing the dependence

on chemical fertilizers as well as improving crop productivity is a pressing issue for the future farming system. Lal (2003) suggested three ways to reduce the fertilizer application: i) enhancing the fertilizer use efficiency through properly chosen fertilizer application rate and time; ii) reducing fertilizer loss from the soil caused by erosion, leaching and volatilization and iii) reinforcing nutrient recycling mechanisms.

Among all the required plant nutrients, nitrogen is the most common limiting factor in crop performance. Lincoln (2002) indicated that nitrogen, which serves as a constituent of many plant cell components including amino acids and nucleic acids, plays an important role in plant growth. Slight nitrogen deficiencies can cause malfunctions in cell processes and inhibition of plant growth (Lincoln 2002). N fertilizer has the strongest influence on productivity in most natural and agricultural ecosystems (Rathke *et al.* 2006). It is essential to use N- fertilizer more efficiently not only for improving the economic output of the farm but also for promoting environment stewardship. Therefore, the study of optimum N supply to maximize N use efficiency in novel crops such as camelina and *B. carinata* is particularly pertinent.

1.2.4.1.1 *Camelina sativa*

Previous nutrient evaluation tests have indicated that the nutrient requirement for camelina is moderate to low. It is necessary to provide approximately 30 kg/ha phosphorous and 50 kg/ha potassium to the field before sowing (Zubr 1997). However, the conclusions on nitrogen requirements from previous studies are conflicting (Urbaniak *et al.* 2008a). Some studies suggested that the basic nitrogen requirement for camelina is relatively low compared with other oilseed crops (Putnam *et al.* 1993; Vollmann *et al.* 1996). Generally, the maximum seed yield can be achieved when nitrogen application is approximately 75 kg/ha. However, other studies have shown a linear response of seed yield to N rates up to 120 kg/ha (Urbaniak *et al.* 2008a). Agegnehu and Honermeier (1997) found that the amount of nitrogen supplied greatly influenced the formation of camelina yield components. The number of branches, pods, seeds per pod and seed weight have all increased as the nitrogen increased. Agegnehu and Honermeier (1997) also reported that, when the level of nitrogen increased, the oil content is also dramatically decreased. The highest yield has been obtained by supplying 120 kg/ha

nitrogen with a seeding rate of 400 seeds/m^2. Therefore, more nitrogen evaluation studies are required.

Generally, camelina can be successfully grown in a range of different environmental and soil conditions except heavy-clay and organic soil (Zubr 1997). One of the biggest challenges for small seed to achieving optimum yield is successful stand establishment. Zubr (1997) reported that careful seedbed preparation before sowing, such as repeated harrowing, is a necessary precaution for promoting improved emergence rates. However, a yield study was conducted at the University of Minnesota, and the result has shown that the practice of surface seeding on frozen ground in the late fall, winter or early spring is feasible for camelina production (Putnam *et al.* 1993). They also reported that winter-sown camelina is highly compatible with cover crops and seeded with a fall-sown cover crop can increase seed yield by approximately 9 %. This unique trait could substantially reduce the requirement of tillage and protect the soil from erosion. On a large scale, the ordinary seeder and combine harvester used for canola or rapeseed can be used for camelina. Generally, the water content of seeds should be less than 11 % during harvesting. Avoiding harvest in humid weather is an advisable management practice for protecting the seeds in capsules. The optimum moisture content for seed storage is below 8 %; therefore, post harvest drying is a common process for maintaining seed viability (Zubr 1997).

1.2.4.1.2 *Brassica carinata*

In recent years, numerous attempts have been made to evaluate the optimum nitrogen level for successful growing *B. carinata*. Kaur and Sidhu (2004) reported that plant height, dry matter accumulation and seed yield of *B. carinata* were positively correlated with nitrogen application. Punia *et al.* (2001) observed that the plant height, siliques per plant, and seed yield significantly responded to N rates up to 90 kg/ha. Parmanik *et al.* (1996) noted that the growth, seed and oil yields of *B. carinata* all significantly improved when nitrogen was applied at rates up to 100 kg N/ha . Sharma *et al.* (2007) suggested that the number of secondary branches per plant, number of siliques per plant, seed and straw yield all increase significantly as nitrogen level is increased to 90 kg N/ha.

In conclusion, almost all investigations indicated that increasing N rate could increase seed yield substantially; however, nitrogen requirement can vary over a wide range depending on growing conditions. It is critical to investigate the nitrogen requirement of *C. sativa* and *B. carinata* under the prevailing production zone.

1.2.4.2. Seeding Rate

Seeding rate is one of the important variables involved in crop production, which if managed optimally can significantly enhance crop yield. Therefore, one of the first decisions in growing *C. sativa* or *B. carinata* is the seeding rate, which is dependent on a number of factors, such as spatial arrangement of the plants, varieties, available moisture, soil fertility, germination, cultural practices, suitability of the growing season, disease and insect considerations, emergence and crop establishment and/or seedbed preparation (Granberry *et al.* 2008). With the introduction of these two new crops, it is important to investigate the optimum seeding rate in different growing conditions.

1.2.4.2.1 *Camelina sativa*

Seeding rate influences not only seed yield but also yield components of *C. sativa* (Agegnehu and Honermeier1997). Agegnehu and Honermeier (1997) evaluated the effect of seeding rates on yield and its components of summer *C. sativa* in Germany and reported that increasing the seeding rate to 800 seeds/m^2 decreased the number of branches. They also found that the seeding rate of 400 seeds/m^2 was the most efficient rate to obtain the highest yield. The effect of seeding rate and seeding date has been explored by Crowley and Fröhlich (1998) in Ireland. However, they found that the seeding rate had no significant effect on the growth and yield of camelina and recommended that the lower rate of 500 seeds/m^2 and a seeding date ranging from mid-March to mid-April would be the most suitable. Urbaniak *et al.* (2008b) suggested that the optimum seed yield can be achieved at 400-600 seeds/m^2. In conclusion, seeding rate recommendation varied among locations, so further research needs to be carried out.

1.2.4.2.2 *Brassica carinata*

There is little information about the effect of seeding rate on the yield of Ethiopian mustard. Singh and Dhingra (2004) examined the effect of four sowing dates (Oct. 10, Oct. 30, Nov. 20 and Dec.10) and six plant densities (333, 222, 167, 167, 111 and 83 thousand plants/ha) on the phenology and growth of *B. carinata in Ludhian,* Punjab, India

during 1998-99 on loamy sand soil. They found that plant density had no significant effect on phenology and growth of *B. carinata*. Askew (2004) reported that seeding date influenced the yield of *B. carinata* to a much greater extent than seeding rate and also suggested that with more branches than rapeseed, *B. carinata* had a lower seeding rate requirement.

1.2.4.3 Water Use Efficiency

Water shortages, due to the increasing world population, increased irrigated agriculture and other water uses, result in less water availability to support sustainable crop production (Wallace *et al.* 2003). The sensitivity of seed yield and seed composition of rapeseed to drought has been indicated by Bouchereau *et al.* (1996). Sinaki (2007) showed that the silique numbers per plant and seed weight of canola have substantively decreased due to severe water stress. Gan *et al.* (2004) found that insufficient plant turgor and lack of assimilates lead to significantly lower seed yield of canola and mustard. Many researchers have also found that oil quality and quantity are affected by irrigation. Water stress modifies fatty acid profiles (Tognetti1 *et al.* 2007), decreases seed oil and protein content (Sinaki *et al.* 2007; Bouchereau *et al.* 1996), and total oil production (Pramanik *et al.* 1996). Therefore, an optimal amount of irrigation water should be determined with an objective to get good quality oil. In conclusion, the yield of an oilseed crop is closely related to the soil water availability; long periods of water shortage would negatively affect the growth and yield of an oil crop by decreasing plant productivity. Therefore, evaluating the effect of moisture deficit on biomass and seed yield of *C. sativa* and *B. carinata* is necessary for introducing them to Canada.

1.2.4.3.1 *Camelina sativa*

Previous studies have reported that plant response to water stress varies in different developmental stages. Vollmann evaluated the agronomic performance of 32 *C. sativa* genotypes in Austria during 1993-94. They reported that water stress during the flowering stage decreased plant yield while sufficient irrigation during the seed filling period increased oil content. Putnam *et al.* (1993) reported that *C. sativa* can more easily compensate for early water deficits than either flax or poppy, which are better suited to drier regions. In summary, there is little information available on the response of camelina to water stress and therefore, more detailed studies are necessary.

1.2.4.3.2 *Brassica carinata*

The effects of sowing date and irrigation on *B. carinata* were studied by Pramanik *et al.* (1996) at the Indian Agricultural Research Institute, New Delhi. They designed four treatments for sowing date (19 or 30 Oct. or 10 or 21 Nov.) and three treatments for irrigation (water: cumulative pan evaporation ratios of 0.4 or 0.6 and no post-sowing irrigation). The experimental results showed that water use efficiency was highest under non-irrigated controls and was significantly decreased when sowing seed later than 30 October (Pramanik *et al.* 1996). A similar investigation was conducted by Gill and Bains (2004) in Ludhiana, Punjab, India. In this research, four sowing dates (5 October, 25 October, 14 November and 4 December) were conducted. They found that the highest water use efficiency was achieved in the earliest sowing date and observed a linear relationship between water use and dry matter production (Gill and Bains 2004). Velasco *et al.* (1999) also pointed out that the advantage of *B. carinata* over other oilseeds is that it is highly drought tolerant.

1.2.4.4 Light

Light is one of the major factors involved in plant growth. Photosynthesis, the plant's most basic metabolic process, converts light energy to chemical energy to maintain plant growth and development. Therefore, the amount of light a plant receives can affect the rate of growth and development. Three major areas should be considered to determine the effect of light on the growth of plants: light intensity, light duration and quality (Havlin *et al.* 1999). It is well documented that red and blue light are the two most efficient wave lengths for plant growth. Since the study was conducted in the growth chamber, the artificial light was the only source of light for plant growth. Generally, incandescent lights provide mostly red and some infrared light while fluorescent light produce largely blue wavelengths. Therefore, the combination of incandescent and fluorescent light sources was used in the growth chamber for *C. sativa* and *B. carinata* study to provide high quality light. It is well known that the leaf anatomy and mesophyll structure are significantly affected by irradiance (Parkhurst 1994; Monti *et al.* 2006) and thus irradiance can be envisaged to affect the gas conductance, photosynthesis and transpiration rate. Both *C. sativa* and *B. carinata* are plants possessing the C_3 photosynthetic pathway. The response curve of net photosynthesis to

photosynthetically active radiance (PAR) for C_3 plants has been extensively studied and most of these experiments indicate that a non-rectangular hyperbola model provides good fits to this response (Marshall and Biscoe 1980; Kyei-boahen *et al.* 2003). Very low light intensity reduces the photosynthetic rate and thus limits the plant development while very high light intensity imbalances the photosynthesis-respiration system and retards the growth of plants. Investigating the optimum light intensity is a crucial step to enhance crop yield. Generally, light saturation point varies among species. Hand *et al.* (1993) for aubergine, Wilson *et al.* (1992) for tomato did not reach light saturation at 900 μmol $m^{-2}s^{-1}$and Kyei-boahen *et al.* (2003) reported that none of the carrot varieties reached saturation at 1000 μmol $m^{-2}s^{-1}$. However, little is known about the effects of irradiance on photosynthetic capacity of *C. sativa* or *B. carinata*. Therefore, the study which follows is an attempt to investigate the effect of light on the photosynthetic parameters, with particular emphasis upon the effect of light intensity.

1.2.4.5 Pest Management

1.2.4.5.1 *Camelina sativa*

Camelina is noted in literature to be a very competitive crop but still can have weed control problems. The information about the compatibility of *C. sativa* with commonly used herbicides is limited. There is no available herbicide recommendation for *C. sativa* production. However, many studies have noted that use of pre-emergence herbicide might not be necessary if the field has suffered low weed infestation (Putnam *et al.* 1993). Individual *C. sativa* seed is non-competitive; however, dense seeding could create considerable competition pressure on many annual weeds. The competition pressure of crops on weeds can be enhanced by selecting a variety with broad leaves. Furthermore, increasing the plant density or sowing plants in narrow rows could also improve competition. If a field is highly infested by weeds, it is feasible to use pre-emergence herbicides such as trifluralin for relieving weed impact (Putnam *et al.* 1993). Francis and Campbell (2003b) also reported that *C. sativa* performed well with some other pre-emergence herbicides such as Stomp (Pendimethalin) and Devrinol (Napromide) and post-emergence herbicides Butisam-S (Metazachlor) and Stratos (Cycoxydim).

Downy mildew [*Peronospora parasitica* (Pers. Ex Fr.) Fr.], the seed-borne fungal disease was observed in many commercial trials and there is no fungicide approved for *C. sativa* at this time (McVay and Lamb 2007). Crowley and Fröhlich (1998) also found some other disease infection in Ireland such as *Sclerotinia, Botrytris* and *Ustilago*. Conn *et al.* (1998) and Browne *et al.* (1991) reported that camelina with two special phytoalexins which are camalexin and methoxycamalexin, has high resistance to some plant diseases such as *Alternaria brassicae* (Berk.) Sacc and *Leptosphaeria maculans* (Desm.) Ces. Et de Not. and insects such as *Phyllotreta nemorum* (L.) Coleoptera: Chrysomelidae: Alticinae).

Camelina sativa, as a new crop, requires more research on insect and disease monitoring to be carried out.

1.2.4.5.2 *Brassica carinata*

Weed-suppressive effects of *Brassica* species have been noted in numerous reports (Oleszek, 1987; Vera *et al.* 1987; Krishnan *et al.* 1998). Al-khatib and Boydston (1999) reported that there are many advantages to using brassicas as cover crops, such as suppressing nematodes, diseases and insects. Other studies have also shown that high glucosinolate-containing Brassica species can efficiently control not only weeds (Al-khatib and Boydston 1999) but also other soil-borne pests (Brown and Morra 1997). Furthermore, *B. carinata* is noted to be highly resistant to blackleg, *L. maculans* (Gugel *et al.* 1990), white rust, *Albugo candida* (Singh and Singh 1988), and *Sclerotinia* and *Phyllotreta cruciferae* (Askew 2004).

1.2.5 Germplasm Development

1.2.5.1 *Camelina sativa*

Little breeding work has been carried out in Canada on *C. sativa* in the past and the availability of germplasm is limited (Gugel and Falk 2006). Breeding research and genetic improvement of camelina started in Germany and resulted in two cultivars, Lindo and Soledo (summer-forms), with high oil and seed yield as well as with an enhanced oil and seed quality, and both varieties have been certified (Agegnehu and Honermeier 1997). Seehuber *et al.* (1987) developed transgressions over parental lines in many yield traits for *C. sativa* through using the single-seed descent method. China also developed a new

oil crop, Camelina NO.1 by hybridizing *C. sativa* which originated from France and *C. macrocarpa f. longistipata,* which originated from China. The content of unsaturated fatty acids in these new crops is extremely high, reaching 90 %, among which the poly-unsaturated fatty acid is about 71.1 % including a linolenic acid content of 34.5 % (Huang *et al.* 2005). The small seed size of *C. sativa* is a major disadvantage for production. The correlation between seed size and seed yield has been studied by many researchers. For example, Vollmann *et al.* (1996) reported that the larger seed of *C. sativa* corresponds to the decline in seed yield and oil content but Gugel and Falk (2006) reported that large seed size strains did not necessarily have the lowest oil content. Vollmann *et al.* (2005) reported that oil content and seed weight varied in genotypes, such as the *C. sativa* accessions, BGRC 51562 and land race Poland have the lowest oil content but highest protein content. The accessions 304270 Sweden and BGRC 28347 have the highest oil content but lowest seed weight. The positive correlation between seed yield and oil content has also been found by Vollmann *et al.* (1996), which makes it possible to enhance both characteristics at the same time.

1.2.5.2 *Brassica carinata*

The diverse ecotypes of *B. carinata* with high variation of morphological and agronomic traits among the accessions have been reported by Abebe *et al.* (1992). Generally, *B. carinata* oil with high amounts of erucic acid has undesirable side effects for human consumption but is highly applicable for industry usage. Therefore, the major breeding objectives in *B. carinata* include developing both varieties free of erucic acid and with high amount of erucic acid (Velasco *et al.* 1998). In 1994, Getinet *et al.* developed a *B. carinata* strain with essentially no erucic acid through an interspecific cross with zero erucic acid *B. juncea* followed by four backcrosses to *B. carinata*. However, it seems like the desaturation process of C18 fatty acid is very strong in zero erucic acid *B. carinata*, which resulted in a significantly high linoleic (37 %) and linolenic acid (21 %) contents and low oleic acid (33 %). In 1998, Rakow and Getinet tried to use the B genome chromosomes of *B. juncea* and C genome chromosomes of *B. napus* to replace the carrying genes for allyl glucosinolate synthesis (B and C genome chromosomes) in *B. carinata*. The result showed that this interspecific cross strategy significantly reduced the glucosinolate content approximate 85 % but did not achieve

complete removal. By exploring erucic acid in germplasm collections, Alemayehu and Becker (2002) found out that the erucic acid level could be fixed to zero without interspecific crossing since the existence of a multiple allelic series of erucic acid in *B. carinata*. However, seed oil of current zero erucic acid germplasm consists of high concentrations of polyunsaturated fatty acids which are susceptible to the process of autoxidation. Research based on this problem was conducted by Velasco *et al.* (2003) and resulted in the development of new germplasm of *B. carinata* with high-oleic and low-linoleic acid.

Furthermore, high glucosinolate level (GSL) of *B. carinata* seed greatly impaired the feed value of the meal. Six *B. carinata* lines with glucosinolate content which is from 20 to 30 μmol g^{-1} lower than the wild line C-101 have been developed by an intraspecific breeding method (Velasco *et al.* 1999). Márquez-Lema *et al.* (2006) reported that the crosses involving line S2-1241 produced F_2 phenotypes with a transgressive GSL content lower than the parents.

1.2.6 Oil Quality

Lipids, as one of the major components constituting plants, play several roles in plant development. Their major functions include acting as an important energy storage material, a principle component in plant membranes, and involvement in some biological activities (Murphy *et al.* 2001). Generally, triacylglycerols are a favorite form for energy storage whereas glycosylglycerides or phosphoglycerides are the main membrane lipids in the vast majority of the commercial oilseed crops. In order to maintain the fluidity of membranes, plants must contain a large amount of unsaturated fatty acids since introducing a *cis*-double bond in an acyl chain of plant membrane lipid has a dramatic effect on the membrane melting properties (Lehninger *et al.* 1993). Seeds are the most common site in plants for accumulating reserve lipids and it is likely that all seeds, even from the non-oilseed plants, contain some amount of cytoplasmic lipid. The amount of major storage lipid bodies in oilseed crops varies among species and ranges from 20 % seed weight in soybean to as high as 76 % in some larger seeded nuts (Murphy *et al.* 2001).

The primary purpose of growing oilseed crops is for the oil contained in the seeds. Oil quality features are important characteristics for marketing and processing of the oilseed crop. In general, *C. sativa* seeds have 38 – 43 % oil and 27 – 32 % protein content. Camelina oil contains approximately 90 % unsaturated fatty acids, the unusual fatty acid pattern made up by the abundant amount of oleic (12.8 – 14.7 %), linoleic (16.3 – 17.2 %), linolenic (36.2 – 39.4 %) and eicosenoic (14.0 – 15.5 %) (Vollman *et al.* 2007). The oil of *C. sativa* with the high proportion of unsaturated fatty acids and a high ratio of omega-3 to omega-6 fatty acids has been identified as high quality human edible oils (Crowley and Fröhlich 1998).

Table 1.2 Fatty acid composition of selected oilseed crops

	Fatty acid content (% of oil)					
Fatty acid	Canola[a]	Soybean[a]	Sunflower[a]	Flax[a]	Camelina[a]	*B.carinata*[b]
Palmitic (16:0)	6.19	10.44	6.05	5.12	7.80	3.1
Stearic (18:0)	0	3.95	3.83	4.56	2.96	1.0
Oleic (18:1)	61.33	27.17	17.36	24.27	16.77	9.7
Linoleic (18:2)	21.55	45.49	69.26	16.25	23.08	16.8
Linolenic (18:3)	6.55	7.16	0	45.12	31.20	16.6
Arachidic (20:0)	0	0	0	0	0	0.7
Eicosenoic((20:1)	0	0	0	0	11.99	0
Erucic (22:1)	0	0	0	0.88	2.80	42.5
Other FA	4.38	5.79	3.5	3.80	3.40	9.6

[a]Putnam *et al.* 1993
[b]Cardone *et al.* 2003

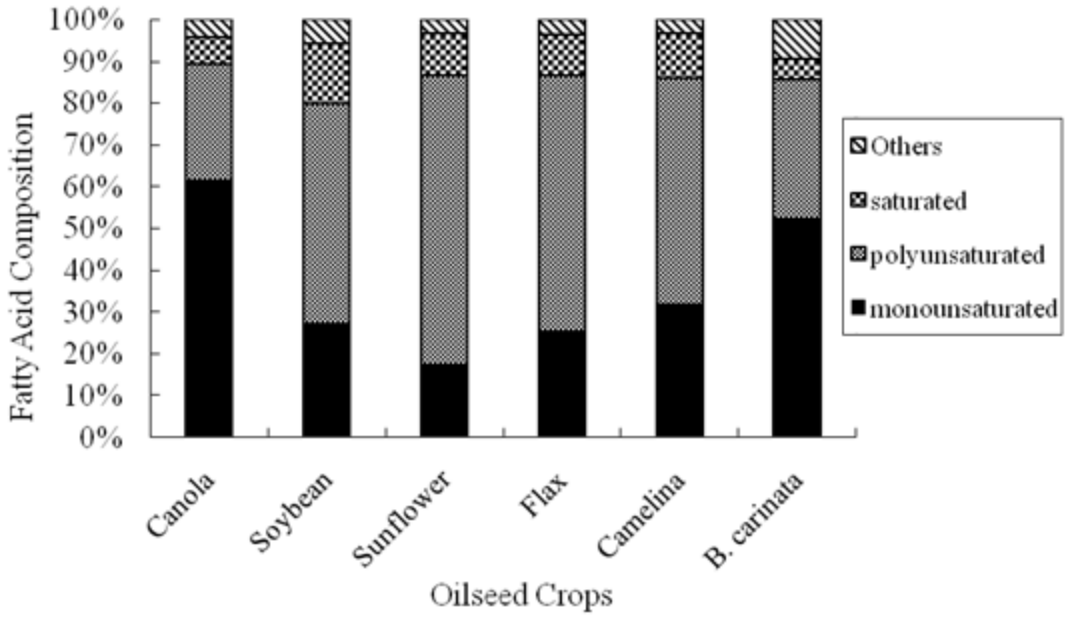

Figure 1.1 Percent saturated and unsaturated fatty acid in selected oilseed crops (Putnam *et al.* 1993; Cardone *et al.* 2003)

Compared with other oilseed crops, camelina oil is more highly saturated than canola, sunflower and flax, while more unsaturated than soybean (Figure1.1 and Table 1.2). The balance of polyunsaturated vs. monounsaturated fatty acid is almost the same as that of soybean, more polyunsaturated than canola, and less polyunsaturated than sunflower. Even if *C. sativa* oil contains comparatively low content of erucic acid (approximately 2 % to 4 % among different lines), this content is still higher than the maximum limits for canola-quality edible oil. It is known that erucic acid in feeding diets is detrimental to animals by inducing changes in various organs which then inhibit growth (Kramer and Sauer 1983). However, based on preliminary germplasm screening, the discovery of camelina accessions with absence of erucic acid provides some potential to totally eliminate this content through plant breeding as it has been for canola (Putnam *et al.* 1993). Camelina oil is the only oil among these six oilseeds which contains eicosenoic acid, and in a relatively high content (around 12 %). However, the potential value of this fatty acid is not currently understood. One of the possible disadvantages of highly unsaturated oil is that this kind of oil might be unsuitable for food applications since the high degree of unsaturated fats are highly prone to autoxidation. However, the significantly higher natural antioxidant agent tocopherol in Camelina oil than canola,

crambe, linseed, soybean and sunflower oil, has been identified in the prevention of camelina oil oxidization (Budin *et al.* 1995).

Due to all these unique seed quality features, serious consideration should be given to camelina as a potential oilseed crop for Canada. First of all, deodorized camelina oil can be used directly for human diet oil. The specific dermatological effect of polyunsaturated fatty acid makes camelina an applicable ingredient in cosmetic products. Other potential applications include use as a biofuel, cover crop, ornamental, or in dried flower arrangements (Robinson 1987).

In comparison to *C. sativa*, the seed oil profile of *B. carinata* is characterized by a significant amount of erucic acid (mono-unsaturated), which is 42.5 % but the linoleic (16.8 %) and linolenic acids (16.6 %) content is lower than *C. sativa* (Velasco *et al.* 2003, Zubr and Matthaus 2002). However, this profile can vary considerably among strains with some at 25 % or lower erucic acid. For example, strains with 25 % erucic (C22:1) typically have 8 % C18:1, 17 % C18:2 and 16 % C18:3 whereas low erucic acid forms have 19 %, 35 % and 37 %, respectively (Falk, personal communication 2009). Falk (personal communication 2009) has developed both low and high erucic acid forms of *B. carinata*. Becker *et al.* (1999) reported that *B. carinata* oil is well suited for use in non-food applications such as bio-diesel, bio-polymers, lubricants, soaps and surfactants.

1.2.7 Seed Meal

1.2.7.1 *Camelina sativa*

The primary purpose of growing oilseed crops is for the oil contained in the seed; however, the economical usage of oilseeds would include proper utility of the meal (Schuster and Friedt 1998). The by-product (camelina meal) from oil extraction, which contains approximately 45 % protein, 13 % fiber, and 5 % mineral, is similar to rapeseed meal (Acamovic *et al.* 1999). Using seed meal as animal feed is the popular project relating to efficient application for shred. Seed meal is a good protein source for animals, but the glucosinolate content in Brassica oilseeds limits this application. Compared with other crucifers, *C. sativa* contains a relatively low amount of glucosinolates in seed meal (Schuster and Friedt 1998). Therefore, camelina meal could be a promising protein supplement for animal feed.

1.2.7.2 *Brassica carinata*

Brassica carinata meal which remains after oil extraction has a significantly high amount of allyl glucosinolate (approx. 100 μmol g^{-1} seed). Both erucic acid and glucosinolate are detrimental to animal growth; therefore, the *B. carinata* meal is always discarded as waste even though it could be a potentially valuable plant protein source (Rakow and Getinet 1998). However, this biologically active compound (glucosinolate) could be used as soil amendments for plant defense (Anon 2000). Rakow and Getinet (1998) suggested that the recent discovery of the low erucic and glucosinolate traits indicated that it is possible to develop canola quality *B. carinata* to stabilize and diversify the canola production in Canada. However, Canola Council of Canada decided that three canola species (*B. napus* L. canola, *B. rapa* L. canola and *B. juncea* var. *juncea* canola) was plenty (Falk, personal communication 2009). Therefore, using *B. carinata* as an industrial platform was the best choice (Falk, personal communication 2009).

1.3 Objectives

The overall goal of this study was to investigate the environmental and agronomic adaptability of *Camelina sativa* (L.) Crantz and *Brassica carinata* A. Braun as high value oilseeds in Canada.

This was achieved by the following objective:

1. To evaluate and compare the location effects on the performance and agronomic suitability of selected genotypes of *C. sativa* and *B. carinata*
2. To evaluate the nitrogen effects on seed yield, oil content and oil quality of *C. sativa* and *B. carinata.*
3. To assess the effects of seeding rate on agronomic performance of *C. sativa* and *B. carinata.*
4. To assess the interactive effect of nitrogen and water supply on growth and yield of *C. sativa* and *B. carinata* and
5. To establish the response curve of net photosynthesis to the PAR levels for both *C. sativa* and *B. carinata* under varied nitrogen and water regimes.

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Chapter 2 Photosynthetic and Growth Responses of *Camelina sativa* to Varying Nitrogen and Soil Water Status

2.1 Introduction

Introducing a new oilseed crop requires not only an important agronomic benefit to the cropping system but also an enhancement of the economic position of the farmers. The growth, yield and oil quality of oilseed crops is affected by their tolerance to abiotic stresses such as heat and drought and to agronomic management practices such as irrigation and nitrogen fertilization (Kaffka and Kearney 1998; Weiss 2000).

In many areas of the world, nitrogen and water availability are two major constraints for enhancing agricultural productivity. Drought induces a decrease in plant tissue water potential, lowering leaf net photosynthesis, altering nitrogen metabolism, reducing protein synthesis and cell membrane stability, resulting in a reduction in crop yield (Shangguan *et al.* 2000; Saneoka *et al.* 2004). Plants tolerate water stress through their physiological adaptations that include osmotic adjustment, leaf desiccation, increased leaf abscisic acid concentration, and anatomical modifications including development of a thicker cuticle (Reddy *et al.* 2003). While this is so, a plant's drought tolerance depends upon interactions with several environmental factors, one of the most important being N supply (Reddy *et al.* 2003).

Nitrogen is one of the most important nutrients for crop development. It directly or indirectly influences plant dry matter production by affecting leaf area expansion and duration as well as leaf photosynthetic capacity and efficiency (Steer and Harrigan 1986). Nitrogen deficiency reduces leaf area index, radiation interception, protein content of the plant, and dry matter partitioning to reproductive organs (Steer and Harrigan 1986; Jones and Tucker 1986). In addition, nitrogen deficiency postpones both vegetative and reproductive phenological stage, inhibits plant growth, reduces yield and yield components such as the number of seeds per plant and number of branches per plant, and seed weight (Steer and Harrigan 1986; Jones and Tucker 1986). Alternatively, increasing N rate could hasten the leaf area development and thus, directly enhance the transpiration rate, which can lower plant water status, if soil moisture is limiting. On the other hand, water shortage can decrease the rate of N-mineralization leading to reduced effectiveness

of applied N-fertilizers thus, weakening of N-transport to the root indirectly causing N-deficiency in plants (Rathke 2006).

The effect of nitrogen level or water supply on the plant physiological response has been well documented (Chen *et al.* 1986; Taiz and Zeiger 1998; Shangguan *et al.* 2000); however, there are no reports on the interaction of N and water supply on photosynthesis and growth of C. *sativa*. Hence, this study was designed to assess the response of camelina to various nitrogen and moisture regimes. The main objectives of the study were to: (1) determine the interactive effects of nitrogen and moisture regimes on yield and yield components, (2) evaluate the effect of applied nitrogen and water on photosynthetic rate, transpiration rate and water use efficiency; (3) model the relationship between photosynthetically active radiation (PAR) and leaf net photosynthesis (P_N) using the non-rectangular hyperbola model (Marshall and Biscoe 1980) and (4) to compare photosynthetic characteristics of camelina under various combinations of nitrogen and water regimes using nested non-rectangular models. In order to achieve these objectives, camelina was grown under different combinations of rates of applied nitrogen and exposed to various soil water potential regimes, in a controlled environment.

2.2 Materials and Methods

2.2.1 Plant Material and Growth Conditions

In a controlled environment study, *C. sativa* cv. Calena was used as the experimental material to study the effect of different N rates (0, 50, 100 and 150 kg N/ha, which corresponded to 0, 0.088, 0.176 and 0.264 g/pot of ammonium nitrate) and soil water potential (0, -65 and -130 cbars) on its growth, root development and yield components. Ten seeds were sown in 15-cm diameter plastic pots filled with equal quantities of Pro-mix BX (*Premier Horticulture, Canada*) and field soil. Soil mixture characteristics are shown in Table 2.1. Seedlings were thinned to two plants/pot at the first true leaf stage, approximately seven days following emergence. A combination of incandescent and cool white fluorescent lights provided 350 $\pm$10 µmol $m^{-2}s^{-1}$ photosynthetically active radiation (PAR) at the top of the plant canopy. Growth chamber was maintained at a 16h photoperiod, with a mean temperature of 25 °C during the day and 15 °C during the night. Relative humidity was maintained at 70 % $\pm$ 5 %. In order to minimize the variation in

micro-environment, the plants were rotated randomly within and between blocks three times during the experiment.

Table 2.1 Soil mixture characteristics of growth chamber study

PH	P_2O_5 (kg/ha)	K_2O (kg/ha)	Ca (kg/ha)	Mg (kg/ha)	Sulfur (kg/ha)	Nitrate (ppm)	CEC (meq/100gm)	N (%)
5.9	551	460	3653	445	88	26.2	15.2	0.25

2.2.2 Water Stress Imposition

Three water deficit treatments: control (no water stress - soil moisture potential near 0 cbars), moderate (soil moisture potential at -65 cbars) and high water deficit (soil moisture potential at -130 cbars) were evaluated in this study. Water was withheld 21 days after seedling emergence to impose water deficit. To simulate more realistic responses to drought, a cyclical water stress method was imposed. Moisture deficit was gradually imposed by withholding irrigation. Withholding water allowed a decline in soil moisture potentials to -65 and -130 cbars, respectively. After the soil moisture potential dropped to the desired level, water was added to the soil until field capacity was reached. Control pots were irrigated daily to maintain field capacity. Soil moisture potential was measured daily by using *Watermark* soil moisture sensors (Spectrum Technologies, IL, USA).

2.2.3 Nitrogen Application

Four N rates 0, 0.088, 0.176, 0.264 N g/pot (0, 50, 100 and 150 kg N/ha) of ammonium nitrate were evaluated in this experiment. Nitrogen was supplied by dissolving ammonium nitrate in distilled water. Each pot received 100 ml of the nitrogen solution after 7 days of seeding.

2.2.4 Experimental Time Course

Pots with lower N treatments (0 and 50 kg N/ha) had smaller plants and required a longer drying cycle to achieve equivalent soil water potential due perhaps to lower transpiration rates. Generally, it took 2-3 days in the 100 and 150 kg N/ha treatment, and after 4-5 days in the 50 and 0 kg N/ha treatments to achieve the first moderate drought phase. To achieve severe water stress, it took 5-7 days in plants under 100 and 150 kg N/ha treatments but approximately 9 days for 0 and 50 kg N/ha treatments. The experiment was terminated after 60 days from first exposure to water stress.

2.2.5 Measurements

Growth parameters: All of the aboveground portions of two plants per pot were collected at the time of harvest. The plant height was measured after harvest. Two plants were used to measure the xylem pressure potential (XPP) with a Scholander Pressure Bomb (PMS Instrument Co. Corvallis, Oregon, USA), as an indicator of plant physiological status (e.g., Mason 1969) and the same plants were used to determine yield components (number of branches and pods per plant) and dry matter following XPP measurement. Xylem pressure potentials were measured by cutting a stem from a plant and placing it in a pressure bomb apparatus. The pressure inside the chamber was slowly increased in a ratio of 0.01 MPa by using the control valve on the pressure bomb. The xylem pressure data was recorded as soon as the xylem fluid appeared on the cut surface. Roots were washed by hand after harvest to evaluate the root dry matter and root: shoot ratio. Two Shoot samples were ground and analyzed for N by combustion (method 968.06; AOAC, 1990) using a Leco protein/nitrogen determinator (*Model FP-528, Leco Corp., St. Joseph, MI*). Total shoot nitrogen was calculated by the products of shoot dry matter and shoot N content.

Photosynthetic parameters: A portable gas analyzer (*LCA-4, Analytical Development Company, Hoddesdon, UK*) associated leaf chamber was used to measure net photosynthesis (P_N) and transpiration rate (E). The sixth fully expanded leaf from a branch tip of two plants of each treatment was selected for photosynthetic measurements. All the measurements were taken between 10: 00 am to 1: 00 pm over two consecutive days. Before the photosynthetic measurements, the print of the leaf was used to determine the leaf areas by using leaf area meter (*Li-3000, Licor, Lincoln, NE, USA)* at the start of flowering stage. The instantaneous water use efficiency (WUE_i) was derived by the ratio of P_N to E.

Pots with the lowest and the highest N rates and soil water potential (150 kg N/ha * well watered, 150 kg N/ha * severe water stress, 0 kg N/ha * well watered, and 0 kg N/ha * severe water stress) were selected to study the photosynthesis-light response curve. Net photosynthesis was measured at 90 days after seedling emergence on the ninth fully expanded intact leaf from a branch tip by using a portable gas analyzer (*LCA-4, Analytical Development Company, Hoddesdon, UK*) associated leaf chamber together

with a portable leaf microclimate control system (*Analytical Development Company, Hoddesdon, UK*). All the measurements were taken under 8 levels of PAR (150, 300, 450, 600, 750, 900, 1050 and 1200 µmol $m^{-2}s^{-1}$) at 350 $\pm$ 20 µmol $mol^{-1}(CO_2)$, leaf temperature of 20 °C, and relative humidity of 70 %. Five minutes were allowed to reach the stable condition for each PAR level prior to recording the data. The analyzer was operated at an airflow rate of 400 µmol s^{-1}. Two measurements were taken for each pot on the same day.

2.2.6 Experimental Design and Statistical Analysis

The experiment followed a two factor factorial randomized complete block design with 3 replications for each treatment. The first factor was soil water potential at three levels (0, -65 and -130 cbars) and the second factor was nitrogen at four levels (0, 50, 100 and 150 kg N/ha). The response variables plant height, xylem pressure potential, yield component (branches and pods per plant), root and shoot dry matter, root: shoot ratio, shoot N content, net photosynthesis, transpiration rate and WUE_i were collected and subjected to the PROC MIXED procedure in SAS. Tukey's test was used to compare the differences among treatments at 5 % significant level.

The study on the effect of PAR on photosynthesis followed a three factor factorial design. These three factors were N rate with two levels, soil water potential with two levels, and eight levels of PAR. The non-rectangular hyperbola proposed by Marshall and Biscoe (1980) was used to model leaf photosynthesis as a function of PAR. Following this, a nested nonlinear regression with incremental parameters model was used for comparing pairs of treatment combination (N rate * soil water potential) in terms of the model parameters. The analysis was completed by using the NLIN procedure of SAS (SAS Institute 1999). The general form of the non-rectangular model is:

$$\theta P_N^2 - (P_{max} + aI - \theta R_d) P_N + aI [P_{max} - (1 - \theta) R_d] - P_{max} R_d = 0$$

where P_{max} is maximum rate of net photosynthesis, R_d is rate of dark respiration, a is photochemical efficiency of photosynthesis at low light, and θ is ratio of physical to total resistance to diffusion of CO_2.

To facilitate easier nonlinear regression estimation, we fitted the following re-parameterization of the non-rectangular hyperbola model:

$$P_N = \frac{P_{max} R_d - aI[P_{max} - (1-\theta) R_d]}{\theta P_N - (P_{max} + aI - \theta R_d)} + \varepsilon$$

P_{max} - Estimated values of maximum rate of net photosynthetic

α - Photochemical efficiency at low light

θ - Ratio of physical to total resistance to diffusion of CO_2

R_d - Dark respiration rate

Where ε is the error term assumed to be normally distributed with zero mean, constant variance and independence of one another.

2.3 Results

2.3.1 Shoot Nitrogen Content and Total Nitrogen

Shoot nitrogen content ranged from 2.42 % to 2.73 % and was not significantly affected by soil moisture deficit under supra-optimal levels of N (Table 2.2 and Figure 2.1). However, shoot nitrogen content of camelina plants supplied with relatively low N supply (0, 50, and 100 kg N/ha) showed a linear increase with increasing soil water deficit (1.46 % to 2.59 %, 1.61 % to 2.81 % and 1.53 % to 2.85 % in 0, 50 and 100 kg N/ha treatments, respectively (Table 2.2 and Figure 2.1). Soil moisture deficit adversely affected total shoot nitrogen at all nitrogen levels and the degree of reduction was highly related to N supply. The greatest decrease by 61 % was observed in 150 kg N/ha treatment while the lowest reduction (28 %) was showed in 0 kg N/ha treatment (Table 2.2 and Figure2.2).

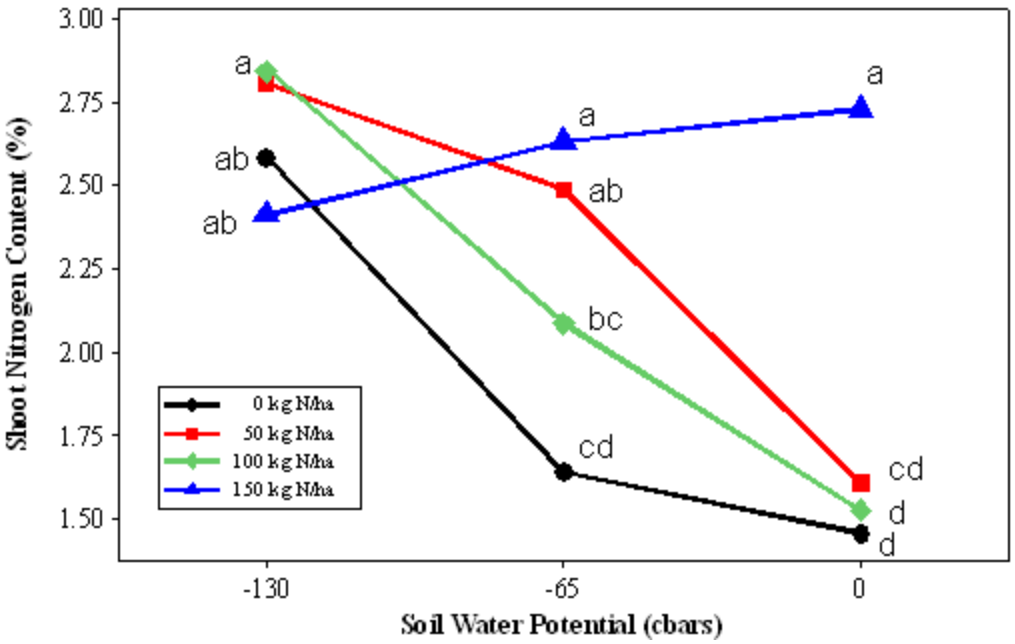


Figure 2.1 Relationship between soil water potential and shoot nitrogen content (Figures followed by different letters are significantly different $p < 0.05$)

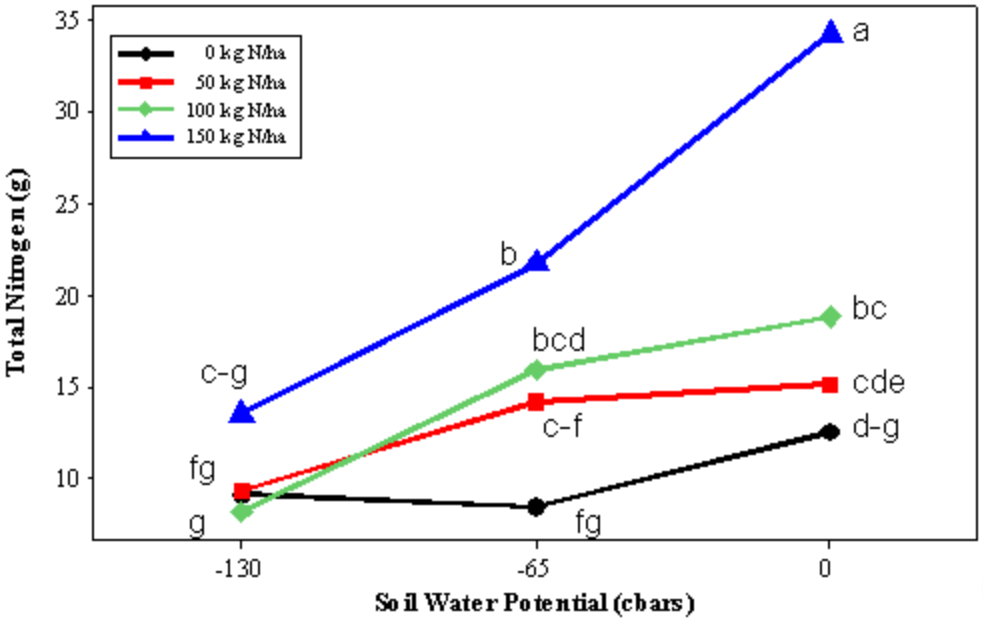


Figure 2.2 Relationship between soil water potential and shoot total nitrogen amount (Figures followed by different letters are significantly different $p < 0.05$)

2.3.2 Root and Shoot Dry Matter

Root and shoot dry matter and root: shoot ratio changed dramatically with soil nitrogen and water availability. The response of root dry matter to water deficit varied in relation to the N supply. Without N supply, root dry matter increased significantly from 3.55 to 5.05 g with the increasing water deficit. There was however, an inverse relationship between root dry matter and water deficit for all plants that received nitrogen fertilizer. The plants that received highest N supply produced the highest root dry matter (4.1 g to 7.8 g) irrespective of the soil water status (Figure 2.4 and Table 2.2). Although shoot dry matter was not affected by the interactive effect of nitrogen and water supply, it was significantly influenced by nitrogen and water levels independently (Figure 2.5). Table 2.3 shows that shoot dry matter increased 53 % (5.73 - 8.78 g) as N rate increased from 0 to 150 kg N/ha and decreased 64 % (10.7 - 3.81g) with the decreasing soil water potential from 0 to -130 cbars. The root: shoot ratio increased significantly as the increased water deficit in all N rates but the relative magnitude of these increases varied with the level of nitrogen. Figure 2.3 shows that at the 0 N level the root: shoot increased 2.5 times (0.41 to 1.44) in response to water deficit, which was significantly greater compared with the plants received nitrogen application. The increases of root: shoot were only 26 % (0.73 - 0.92), 57 % (0.56 to 0.88) and 19 % (0.62 to 0.74) in 50, 100 and 150 N kg/ha treatments, respectively.

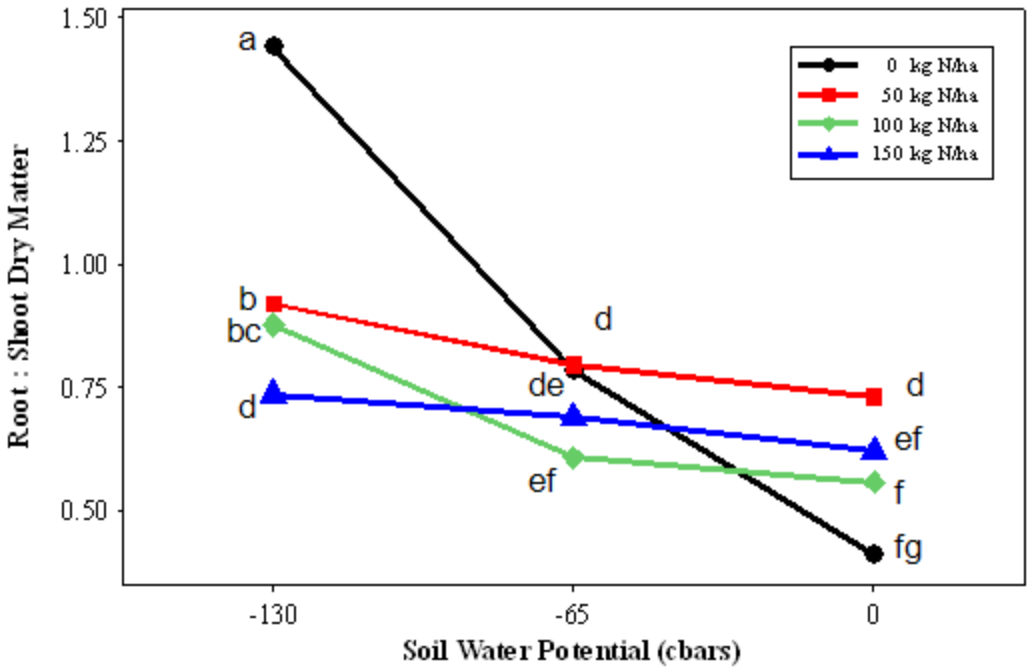


Figure 2.3 Relationship between soil water potential and root: shoot ratio (Figures followed by different letters are significantly different $p < 0.05$)

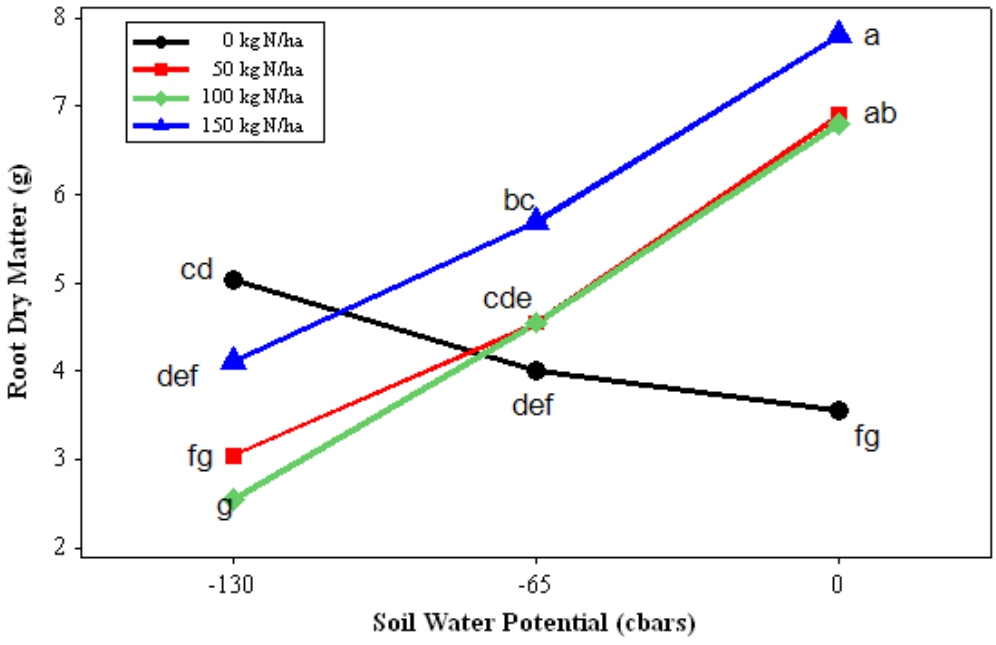


Figure 2.4 Relationship between soil water potential and root dry matter (Figures followed by different letters are significantly different $p < 0.05$)

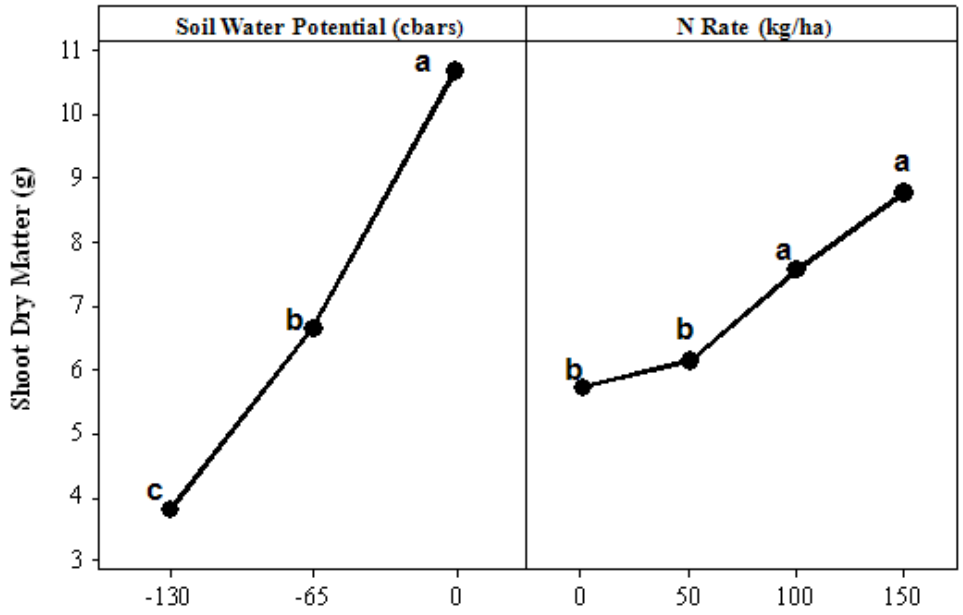


Figure 2.5 Effect of soil water potential (left) and N rate (right) on shoot dry matter (Figures followed by different letters are significantly different p < 0.05)

Table 2.2 Interactive effects of nitrogen and soil moisture regimes on xylem pressure potential, root dry matter, root: shoot dry matter, nitrogen content, total nitrogen amount and plant height

Nitrogen (kg/ha)	Soil water potential (cbars)	XPP (-MPa)	Root dry matter (g)	Root: shoot	N (%)	Total N amount (g)	Plant height (cm)
0	0	0.81b	3.55fg	0.41g	1.46d	12.53d-g	73bc
0	-65	1.14de	4def	0.79cd	1.65cd	8.41fg	61f
0	-130	1.53g	5.05cd	1.44a	2.59ab	9.07fg	52h
50	0	0.76a	6.9ab	0.73d	1.61cd	15.14c-e	71cd
50	-65	1.35fg	4.55cde	0.80cd	2.49ab	14.20c-f	57g
50	-130	1.84h	3.05fg	0.92b	2.81a	9.33e-g	49i
100	0	0.83c	6.8ab	0.56f	1.53d	18.83bc	75b
100	-65	1.13de	4.55cde	0.61ef	2.09bc	15.92b-d	66e
100	-130	1.38fg	2.55g	0.88bc	2.85a	8.16g	61f
150	0	0.95cd	7.8a	0.62ef	2.73a	34.22	81a
150	-65	1.01cde	5.7bc	0.69de	2.63a	21.70b	69d
150	-130	1.20ef	4.1def	0.74d	2.42ab	13.44c-g	63f
P-value		0.001	0.002	0.001	0.011	0.003	0.001

Means within a column followed by different letters are significantly different (p < 0.05)

2.3.3 Xylem Pressure Potential and Yield Component

Water deficit significantly decreased the plant xylem pressure potential in plants under all N rates. Nitrogen application in combination under declining soil water potential dramatically affected the development of branches and pods. Increasing N rate up to 100 kg N/ha had significantly increased the number of branches/plant (8-9) and pods/plant (75-95). The differences between the plants supplied with 100 kg N/ha and 150 kg N/ha on branches/plant and pods/plant were not significant statistically. The number of branches/plant and pods/plant decreased from 10 to 7 and 124 to 62 as the soil water potential decreased from 0 to -130 cbars (Table 2.4). In contrast, water deficit reduced the pods/plant by 50 % while nitrogen deficit decreased pods/plant only by 20 % (Table 2.3 and 2.4) suggesting that water deficit can be more detrimental to yield than N supply.

Table 2.3 Nitrogen effect on yield components (branches/plant and pods/plant) and shoot dry matter

N rate (kg/ha)	Branches/plant	Pods/plant	Shoot dry matter (g)
0	8b	75b	5.73b
50	8b	82b	6.13b
100	9a	95a	7.58a
150	9a	99a	8.78a
P-value	0.023	0.031	0.015

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 2.4 Water deficit effect on yield components (branches/plant and pods/plant) and shoot dry matter

Soil water potential (cbars)	Branches/plant	Pods/plant	Shoot dry matter (g)
0	10a	124a	10.70a
-65	7b	80b	6.66b
-130	7b	62c	3.81c
P-value	0.038	0.003	0.029

Means within a column followed by different letters are significantly different ($p < 0.05$)

2.3.4 Effect of Irradiance on Photosynthesis

The relationship between P_N and PAR was modeled and the residual check indicated that the assumptions used in the model are valid. The differences among all treatment combinations could be attributed to the interaction effect of N rate and water deficit on P_N. All photosynthetic parameters except for ratio of physical to total resistance to diffusion of CO_2 (θ), were significantly affected by PAR levels (Table 2.4). The results indicated

that P_N for all treatment combinations increased hyperbolically with increasing PAR (Figure 2.6). The predicted P_N at saturation (P_{max}) was the highest (6.27 μmol $m^{-2}s^{-1}$) in plants under high N * well-watered treatment and lowest (0.71 μmol $m^{-2}s^{-1}$) in plants under low N* water stress treatment. Photochemical efficiency ranged between 0.0007-0.0061 and decreased dramatically with the water stress. Dark respiration varied from 0.16 μmol $m^{-2}s^{-1}$ in plants under low N * water stress treatment to 1.31 μmol $m^{-2}s^{-1}$ in plants under high N* well-watered treatment. Increasing PAR enhanced P_N only under adequate N and moisture supply and in contrast, moisture deficit can over rid the benefits of N application under increasing PAR. The highest P_{max} value (6.27 μmol $m^{-2}s^{-1}$) and the highest photochemical efficiency (0.0061) were achieved in the plants under 150 kg N/ha * well-watered treatment. Generally, under well-watered conditions, high N supply leads high photosynthetic rate. In contrast, P_{max} in plants under from water deficient was 1.88 μmol $m^{-2}s^{-1}$, which was lower than the plants that received low or no N (2.39 μmol $m^{-2}s^{-1}$). This result suggested that P_{max} of camelina is significantly inhibited by the water deficit rather than nitrogen deficiency. Plants which suffered from water or nitrogen deficit had the similar photochemical efficiency (0.0021). Plants that suffered from both water and nitrogen stress had the lowest value for all photosynthetic parameters (Table 2.5). Increasing PAR from 150 to 900 μmol $m^{-2}s^{-1}$, increased P_N around 3-folds for all treatment combinations and this increasing trend seemed to level off after 900 μmol $m^{-2}s^{-1}$.

Table 2.5 Estimated values of photosynthetic parameters for irradiance response curves

Treatments	P_{max} (μmol $m^{-2}s^{-1}$)	α	θ	R_d (μmol $m^{-2}s^{-1}$)
N0W0	2.39b	0.0021b	1.01a	-0.69b
N0W-130	0.71d	0.0007c	0.98a	-0.16c
N150W0	6.27a	0.0061a	1.00a	-1.31a
N150W-130	1.88c	0.0021b	1.02a	-0.26c

N0W0 = 0 kg N/ha * 0 cbars soil water potential
N0W-130 = 0 kg N/ha * -130 cbars soil water potential
N150W0 = 150 kg N/ha * 0 cbars soil water potential
N150W-130 =150 kg N/ha * -130 cbars soil water potential
Means within a column followed by different letters are significantly different ($p < 0.05$)

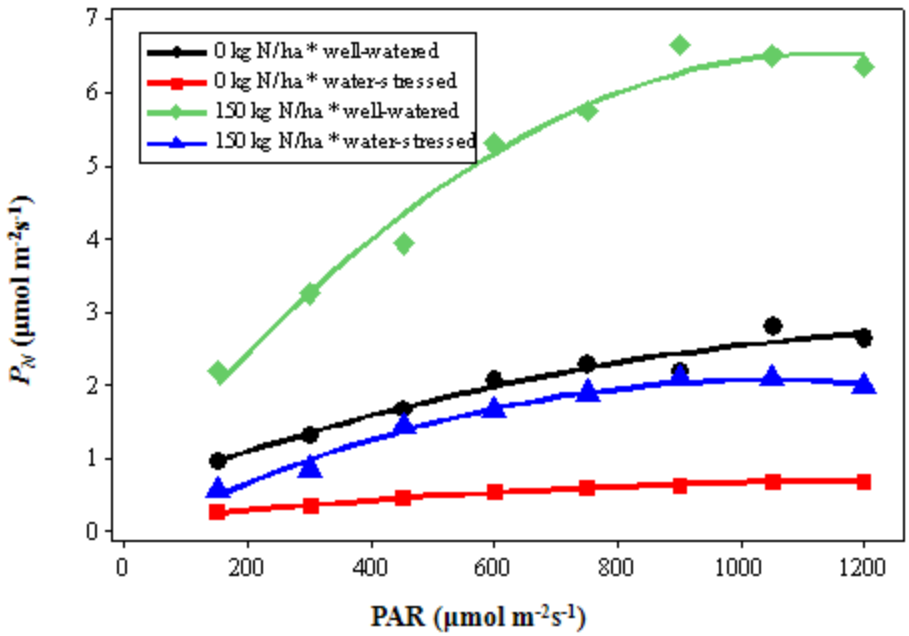


Figure 2.6 Net photosynthesis-light response curve for a fully expanded leaf of *Camelina sativa* (Figures followed by different letters are significantly different $p < 0.05$)

2.3.5 Leaf Net Photosynthesis and WUE_i

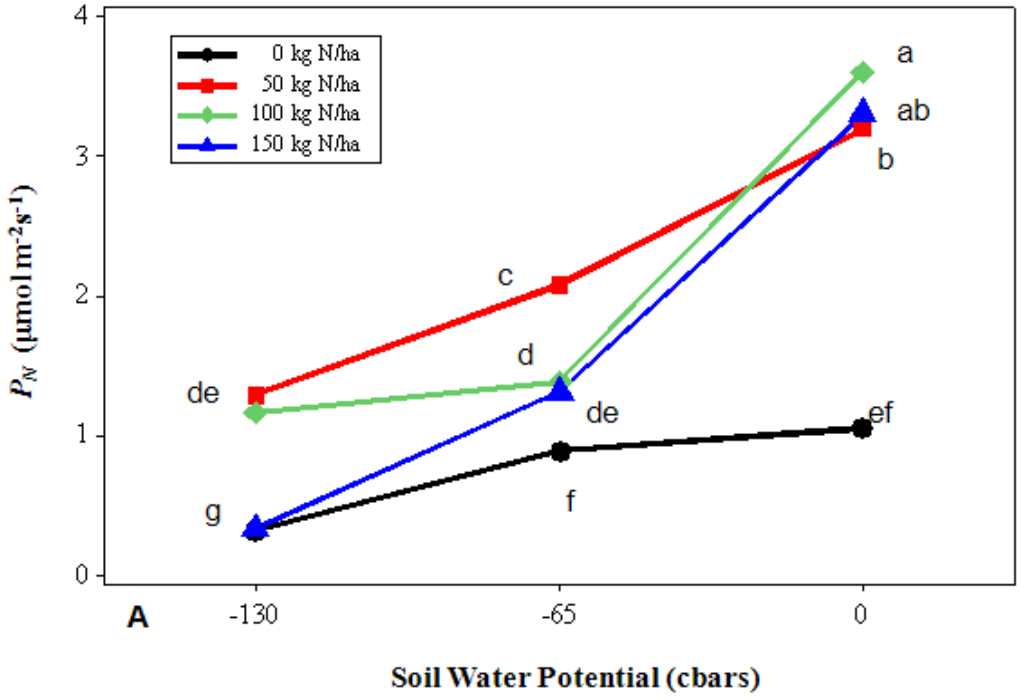
The results in this study showed that the stomatal conductance, internal CO_2 concentration, net photosynthesis, transpiration rate and instantaneous water use efficiency were all significantly affected by the interaction of N rate and soil water deficit (Figure 2.7 and Table 2.6). P_N dropped dramatically as soil water potential decreased in all N treatments. Pots with 50 and 100 kg N/ha had significantly higher P_N compared with controlled pots for all three soil water conditions. Under well-watered conditions, a positive relation between N supply and net photosynthesis was found until nitrogen application reached 100 kg N/ha. WUE_i was significantly higher in the plants that received nitrogen than the ones in the control treatment. Under moisture deficit conditions, plants that received 50 kg N/ha had the highest P_N while at 150 kg N/ha, P_N was significantly inhibited. In contrast, P_N decreased more by water deficit than by nitrogen deficiency. Transpiration rate decreased with water deficit. WUE_i differed between high and low N rate treatments. WUE_i decreased 64 % (7.26 - 2.57 µmol m^{-2}s^{-1}) in 150 kg N/ha and 44 % (8.64 - 4.88 µmol m^{-2}s^{-1}) in 100 kg N/ha. This sharp decrease in WUE_i in plants

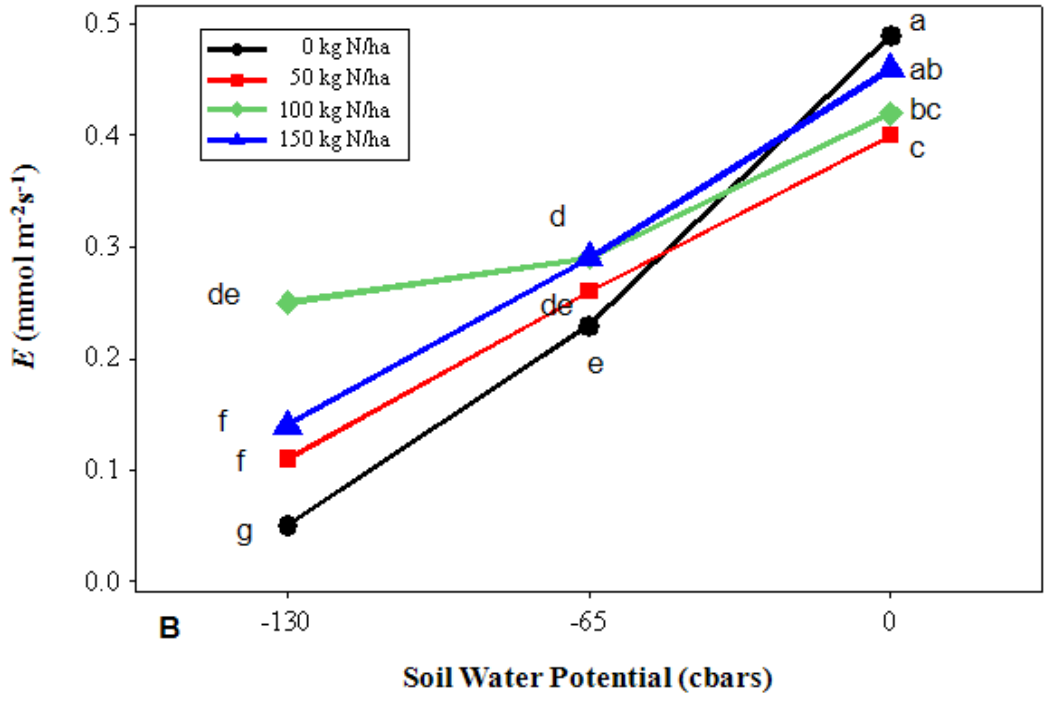
under high nitrogen treatment was due to a significant decrease in P_N than in transpiration rate. However, WUE_i in the low-N treatment increased substantially with water deficit, which was 1.6 times (2.12 to 5.62 μmol $m^{-2}s^{-1}$) in 0 N treatments and 39 % (8.14 to 11.34 μmol $m^{-2}s^{-1}$) in 50 kg N/ha treatments. WUE_i was always greater at 50 kg N/ha treatment for any level of water status (Table 2.6). Both *Gs* and *Ci* decreased substantially as the water deficit increased irrespective of the level of nitrogen application.

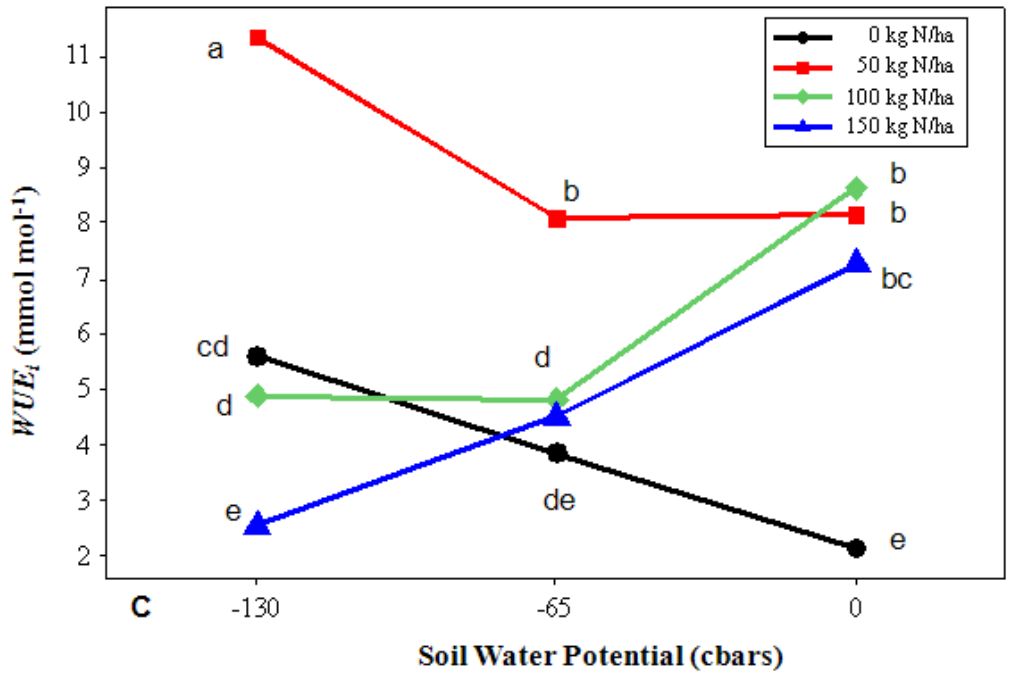
Table 2.6 Stomatal conductance (*Gs*), transpiration rate (*E*), rate of net photosynthesis (P_N), internal CO_2 concentration (C_i) and instantaneous water-use efficiency (WUE_i) of camelina leaves

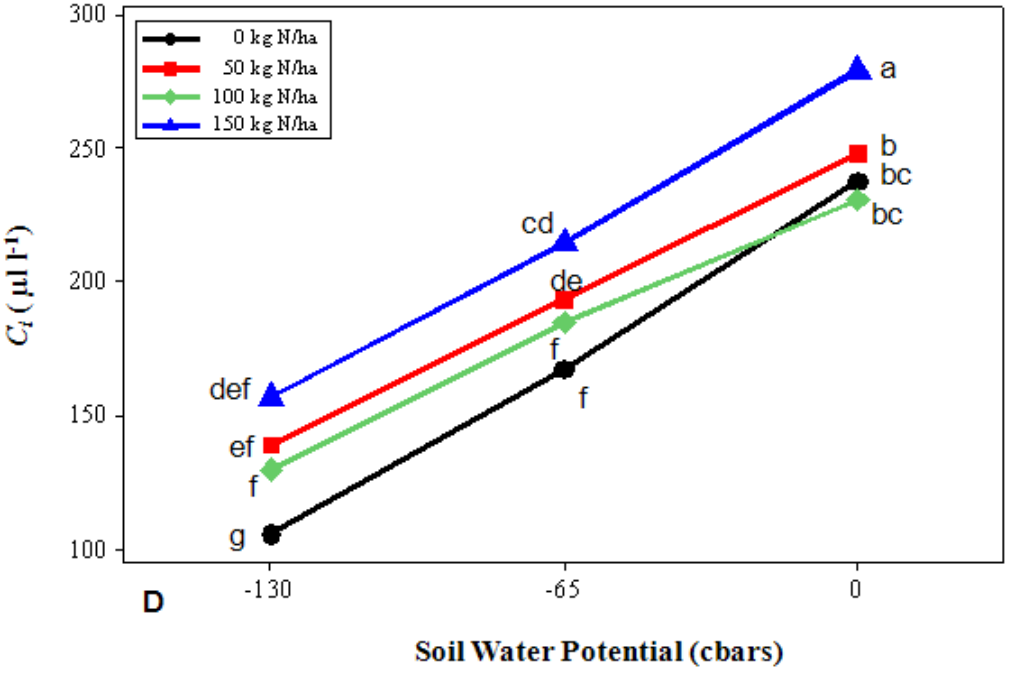
Treatment	Well-watered (0 cbars)				Moderate water stress (-65 cbars)				Severe water stress (-130 cbars)			
	N rate (kg/ha)				N rate (kg/ha)				N rate (kg/ha)			
	0	50	100	150	0	50	100	150	0	50	100	150
Gs (mol $m^{-2}s^{-1}$)	0.09c	0.18a	0.15ab	0.15ab	0.06cd	0.09c	0.08c	0.15ab	0.02e	0.04de	0.05de	0.14b
E (mmol $m^{-2}s^{-1}$)	0.49a	0.40c	0.42bc	0.46ab	0.23e	0.26de	0.29d	0.29d	0.05g	0.11f	0.25de	0.14f
P_N (µmol $m^{-2}s^{-1}$)	1.06ef	3.2b	3.60ab	3.3a	0.89f	2.08c	1.39d	1.31de	0.32g	1.29de	1.16de	0.34g
C_i (µmol $m^{-2}s^{-1}$)	238bc	248.1b	231bc	279a	167d-f	194cde	185c-f	215bcd	106g	139ef	130f	157def
WUE_i (µmol $m^{-2}s^{-1}$)	2.15e	8.14b	8.64b	7.26bc	3.85de	8.10b	4.83d	4.50d	5.62cd	11.34a	4.88d	2.57e

Means within a row followed by different letters are significantly different ($p < 0.05$)


0 kg N/ha
50 kg N/ha
100 kg N/ha
150 kg N/ha
P_N (µmol m^{-2}s^{-1})
a
ab
b
c
d
de
de
ef
g
f
0
1
2
3
4
A
-130
-65
0
Soil Water Potential (cbars)


0 kg N/ha
50 kg N/ha
100 kg N/ha
150 kg N/ha
E (mmol m^{-2}s^{-1})
a
ab
bc
c
d
de
de
e
f
f
g
0.0
0.1
0.2
0.3
0.4
0.5
B
-130
-65
0
Soil Water Potential (cbars)


0 kg N/ha
50 kg N/ha
100 kg N/ha
150 kg N/ha
WUE_i (mmol mol^{-1})
11
10
9
8
7
6
5
4
3
2
a
b
b
b
bc
cd
d
d
e
de
e
C
-130
-65
0
Soil Water Potential (cbars)


0 kg N/ha
50 kg N/ha
100 kg N/ha
150 kg N/ha
C_i (µl l^{-1})
300
250
200
150
100
a
b
bc
bc
cd
de
f
f
def
ef
f
g
D
-130
-65
0
Soil Water Potential (cbars)

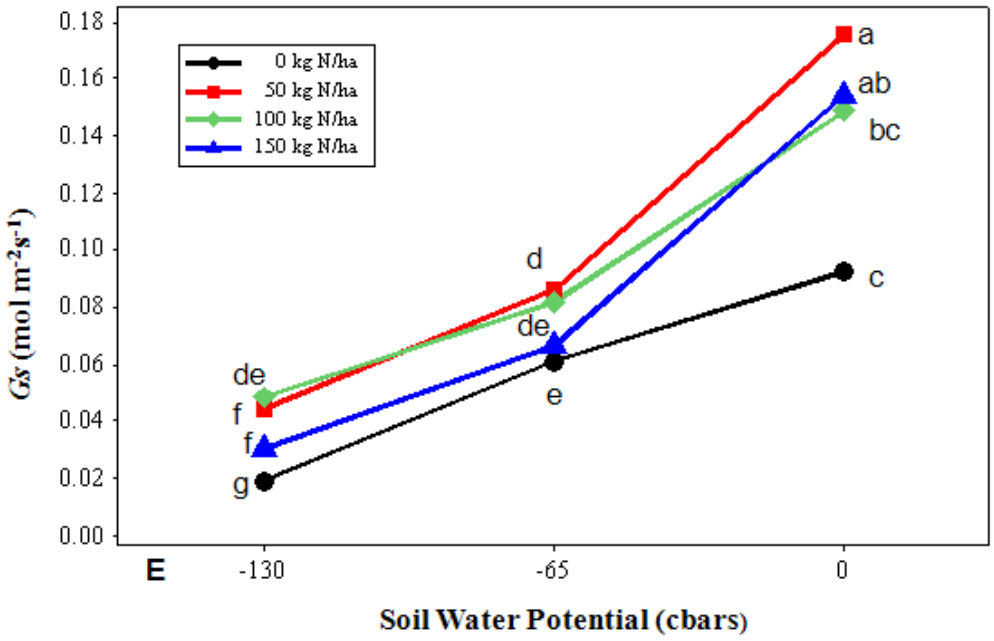


Figure 2.7 Response of leaf net photosynthetic rate, P_N (A), transpiration rate, E (B), instantaneous water use efficiency, WUE_i (C), intercellular CO_2 concentration, C_i (D), stomatal conductance, Gs (E), to the interaction effect of nitrogen and water supply (Figures followed by different letters are significantly different $p < 0.05$)

2.4 Discussion

2.4.1. Net Photosynthesis and Yield Components

Results of this study clearly demonstrated that limitation to water and nitrogen during growth of camelina can directly influence camelina growth in many different ways including a decline in xylem pressure potential, altered partitioning patterns, influence on net photosynthesis, transpiration rate and yield components. Under well-water condition, net photosynthesis and yield components increased significantly as the N supply increased up to 100 kg N/ha. Even though plants with 150 kg N/ha supply had the highest shoot total N, net photosynthesis and yield components were not significantly higher than 100 kg N/ha, which suggested that plants with 150 kg N/ha may absorb nitrogen but may not be utilized for photofunctions or for growth. It is well documented that increased N supply usually leads to larger leaf area, high leaf N, and generally results in increased leaf photosynthetic capacity and growth (Field and Mooney 1986). Relatively high amounts of internal CO_2 and stomatal conductance in well-watered condition suggested that the

difference of net photosynthesis among varied nitrogen treatments was more related to the biochemical limitations rather than stomatal limitations. The response of camelina to water deficit differed with N application rate. Increasing water deficit resulted in a greater relative decline in photosynthetic rates for 150 kg N/ha treatment plants than for the plants in other N treatments. However, the plants which suffered from nitrogen deficiency when encounters water deficit, the limitation to P_N was primarily due to enzymatic limitation rather than stomatal limitation as the *Gs* did not differ significantly. Greater photosynthetic sensitivity to water deficit was found in plants with high N level, which is similar to those reported for beans (Shimshi 1970) and wheat (Morgan 1986). Walters and Reich (1989) reported that this greater sensitivity might be related to the low hydraulic conductance or other aspect of water transport caused by lower root: shoot ratio in high nitrogen treatment. Even though the plants received N supply had significantly higher net photosynthesis and yield components than plants without N supply, only a small degree of variation was observed (between 50 and 100 kg N/ha). This small effect can be explained by several probably factors. First, significantly less total N content in plants under water deficit for all N treatments suggested that less amount of soil water availability may have reduced the N uptake. Rathke *et al.* (2006) suggested that water deficit could decrease the rate of N-mineralization leading to reduced effectiveness of N-fertilizers and weakening of N-transport to the root (Rathke *et al.* 2006). Secondly, the low soil water potential can affect the relationship between available N and photosynthesis by increasing stomatal resistance, or mesophyll resistance, and generally results in reduced photosynthetic capacity (Walters and Reich 1989). The decreased internal CO_2 concentration and stomatal conductance indicated that both stomatal and biochemical limitations appear to account for the decline in net photosynthesis. Overall, water stress had more negative effects on yield components than nitrogen stress, which suggested that camelina was more sensitive to water deficit than N-deficiency.

The effect of PAR on photosynthesis has been extensively studied. The response of camelina leaf photosynthesis to irradiance is similar to many horticultural crops such as cucumber, sweet pepper and carrot (Kyei-boahen *et al.* 2003). P_N for all treatment combination increased hyperbolically in response to increased irradiance. Plants with sufficient water and N supply had the highest P_N in all PAR levels. Magnitude of the

difference in P_N between stressed and unstressed plants did become progressively more marked at each PAR levels. P_N of the plants with high N rate reached saturation at 900 μmol $m^{-2}s^{-1}$ which was lower than the saturation point of plants without N supply since none of the plants under nitrogen deficit reached saturation in this study. This implies that camelina plants did not reach their P_{max} during most growth period in this study since the PAR of 350 ±20 μmol $m^{-2}s^{-1}$ in growth chamber is much lower than the saturation point. Plants under sufficient nitrogen and water resources had the highest $P_{max,}$ possibly due to the relatively high photochemical use efficiency and high stomatal conductance. Plants with insufficient nitrogen and water supply had the lowest P_{max} which may perhaps be due to low stomatal conductance but the possibility that these conditions can lead to low amount of chlorophyll and Rubisco (Reich *et al.* 1989).

2.4.2 Water Use Efficiency and Drought Tolerant Strategy

Collectively these results showed that camelina displays considerable plasticity in water use efficiency under varied soil nitrogen and water regimes. Several conclusions can be drawn from this study: 1) under well-watered conditions, camelina with nitrogen supplied had significantly higher WUE_i than those under nitrogen limitation and the difference of WUE_i between plants with high or low amount N supply was not significant. 2) under moderate water-stressed conditions, plants with 50 kg N/ha had the highest WUE_i followed by plants with 100 and 150 kg N/ha; 3) under severe water-stressed conditions, plants with P_N under 150 kg N/ha supply was significantly inhibited and WUE_i dropped dramatically.

Generally, plants respond to water stress by exhibiting significant changes in their physiological and biological mechanisms in order to adapt to multiple abiotic stresses. The strategy for adapting to moderate or severe water stress in camelina appears to be related to two mechanisms.

1) Root: shoot ratio

Extending roots to extract water from soil is the common way for plants to improve water uptake from soil. Most studies on root-shoot responses to nitrogen and water deficit showed that a decrease in applied nitrogen and water tends to change the patterns of resource allocations and gives rise to an increase in growth partitioning to the root (Wilson 1988). The result from this study is consistent with the earlier reports. In contrast,

plants which suffered from both nitrogen and water deficit increased root: shoot ratio more significantly than the plants suffered from only water stress. The significant increase in root: shoot ratio in water deficit condition indicated there is preferential allocation of carbon for root growth, which would have enabled increased hydraulic conductance as hydraulic conductance is directly related to root volume. Therefore, developing a massive root system is one of the important mechanisms for camelina to tolerate the water deficit.

2) Stomata control

The stomatal transpiration is another major contributing factor to water loss in the plant. Therefore, controlling stomata aperture is the most important mechanism to reduce water loss. In this study, stomata conductance decreased progressively with increasing water deficit. This result is similar to the early study reported by Reddy *et al.* (2003) and Tognetti *et al.* (2007). The advantage of efficient stomatal control is that, through reductions in transpiration, the water use efficiency would substantially increase, if the P_N is maintained.

2.5 Conclusion

The results of this study suggested that plants with sufficient water supply and 100 kg N/ha are critical for high yield. Moisture deficit singly or in combination with N deficiency could significantly affect yield components. Therefore, I conclude that, under low water availability, camelina has a nitrogen requirement of approximately 100 kg N/ha for achieve optimum performance. While under moisture non-limiting conditions, a N rate of 150 kg/ha would likely be the most appropriate option.

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Chapter 3 Photosynthetic and Growth Responses of *B. carinata* to Varying Nitrogen and Soil Water Status

3.1 Introduction

Brassica carinata A. Braun is an interesting C_3 oilseed crop with high erucic acid content which has attracted a growing interest in using it in non-food applications such as bio-diesel, bio-polymers, lubricants, soaps and surfactants (Becker *et al.* 1999). In addition, the residual defatted meal of *B. carinata* can be used as a soil amendment for plant defence due to the highly biologically active compounds (i.e. allyl glucosinolates) in the meal. Furthermore, with good drought resistance and low pod-shattering tendency (Andrea *et al.* 2009), *B. carinata* could be developed as an oilseed crop in Canada to diversify and stabilize oilseed production.

Since many oilseed crops are indeterminate plants, adaptation is affected by tolerance to environmental stress such as drought and light stress, and by crop management such as time and rate of nitrogen application to take advantage of optimum conditions for plant growth (Kaffka and Keaney 1988; Weiss 2000). Light, water and N deficiency are three major constraints limiting the yield and quality of many oilseed crops worldwide. An efficient use of resources reflects the ability of the plant to tolerate the variation of resource availability and therefore is a desirable trait for developing new oilseed crops. A vast amount of literature about physiological responses of plants to environmental stress has been published; however, little attention has been paid to the interaction between these two factors. Furthermore, the information about the effects of irradiance on photosynthetic capacity of *B. carinata* is limited.

This study was designed to assess the response of *B. carinata* to the availability of nitrogen, water and light resources. Two indices of intrinsic resource-use efficiency are 1) instantaneous N-use efficiency, defined as net CO_2 exchange per unit of leaf N, and 2) instantaneous water-use efficiency, defined as net CO_2 exchange per unit of water lost; these were used to investigate the effect of resource availability on photosynthetic capacity. The main objectives of the study were: (1) to determine the effect of N and water supply on yield and yield components, (2) to evaluate the instantaneous water use efficiency and N use efficiency, (3) to model the relationship between photosynthetically

active radiation (PAR) and leaf net photosynthesis by using the non-rectangular hyperbola model; (4) to compare photosynthetic characteristics of *B. carinata* under various combinations of N and water supply using nested non-rectangular models. In order to achieve the objectives, *B. carinata* was grown under different rates of applied N and soil water potential, in a controlled environment.

3.2 Materials and Methods

3.2.1 Plant Material and Growth Conditions

In a controlled environment study, *Brassica carinata* A. Braun line 070760EM was used as the experimental material to study the effect of different N rates (0, 50, 100 and 150 kg N/ha, which corresponded to 0, 0.088, 0.176 and 0.264 g/pot of ammonium nitrate) and soil water potential (0, -65 and -130 cbars) on its growth, root development and yield components. Ten seeds were sown in 15-cm diameter plastic pots filled with equal quantities of Pro-mix BX (*Premier Horticulture, Canada*) and field soil (Table 3.1). Seedlings were thinned to four plants/pot at the first true leaf stage, approximately seven days following emergence. A combination of incandescent and cool white fluorescent lights provided photosynthetically active radiation (PAR) of approximately 420 ± 20 µmol $m^{-2}s^{-1}$ at the top of the plant canopy. Growth chamber conditions were maintained at a 16-h photoperiod, with a mean day/night temperature of 25 °C/15°C. Relative humidity was maintained at 70 % ± 5 %. In order to minimize the variation in the micro-environment, plants were rotated randomly within and between blocks, three times during the experiment.

Table 3.1 Soil mixture characteristics of growth chamber study for *B. carinata*

pH	P_2O_5 (kg/ha)	K_2O (kg/ha)	Ca (kg/ha)	Mg (kg/ha)	Sulfur (kg/ha)	Nitrate-N (ppm)	CEC (meq/100gm)	N (%)
5.9	551	460	3653	445	88	26.2	15.2	0.25

3.2.2 Water Stress Imposition

Three water stress treatments: control (no water stress - soil moisture potential near 0 cbars), moderate (soil moisture potential at -65 cbars) and high water deficit (soil moisture potential at -130 cbars) were evaluated in this study. Water was withheld 21 days after seedling emergence to impose water deficit. To simulate more realistic

responses to drought, a cyclical water stress method was imposed. Moisture deficit was gradually imposed by withholding water and allowing a decline in soil moisture potentials to -65 and -130 cbars, respectively. After the soil moisture potential dropped to the required level, water was added to the soil until field capacity was reached. Control pots were irrigated daily to maintain field capacity. Soil moisture potential was measured daily by using *Watermark* soil moisture sensors (Spectrum Technologies, IL, USA).

3.2.3 N Application

Four N rates 0, 0.088, 0.176, 0.264 N g/pot (0, 50, 100 and 150 kg N/ha) of ammonium nitrate were evaluated in this experiment. Nitrogen was supplied by dissolving ammonium nitrate in distilled water. Each pot received 100 ml of the nitrogen solution after 7 days of seeding.

3.2.4 Measurements

Growth variables: All of the aboveground portions of four plants per pot were collected at the time of harvest. Two plants were used to measure the xylem pressure potential (XPP) with a Scholander Pressure Bomb (PMS Instrument Co. Corvallis, Oregon, USA), as an indicator of plant physiological status (e.g., Mason 1969) and the same plants were used to determine yield components (number of branches and pods per plant) and dry matter following XPP measurement. Xylem pressure potentials were measured by cutting a stem from a plant and placing it in a pressure bomb apparatus. The pressure inside the chamber was slowly increased in a ratio of 0.01 MPa by using the control valve on the pressure bomb. The xylem pressure data was recorded as soon as the xylem fluid appeared on the cut surface. Roots were washed by hand after harvest to evaluate the root dry matter and root: shoot ratio. The chlorophyll content was measured by SPAD-502 chlorophyll meter (Spectrum Technologies, In. USA)

Photosynthetic variables: A portable gas analyzer (LCA-4, Analytical Development Company, Hoddesdon, UK) associated leaf chamber was used to measure net photosynthesis (P_N) and transpiration rate (E). The sixth fully expanded leaf from a branch tip of two plants of each treatment was selected for photosynthetic measurements. All the measurements were taken between 10: 00 am to 1: 00 pm in two consecutive days at the beginning of the flowering stage. Before the photosynthetic measurements, two leaves of each treatment were collected to determine the leaf areas by using a leaf area

meter (Li-3000, Licor, Lincoln, NE, USA). The same leaves were used to analyze leaf N content (note: the leaves were all dried in 60 °C oven for 48 h before the analysis). The dry leave samples were ground and analyzed for N by combustion (method 968.06; AOAC, 1990) using a Leco protein/nitrogen determinator (*Model FP-528, Leco Corp., St. Joseph, MI*). The leaf N content per leaf area basis (N_{area}) was calculated by (leaf dry mass * leaf N content)/leaf area. The instantaneous water use efficiency (WUE_i) was measured by the ratio of P_N to E. The instantaneous N use efficiency (PNUE) was measured by the ratio of P_N to N_{area}.

Pots with the lowest and highest N rates and soil water potential (150 kg N/ha * well watered, 150 kg N/ha * severe water stress, 0 kg N/ha * well watered and 0 kg N/ha * severe water stress) were selected to study the photosynthesis-light response curve. Net photosynthesis was measured at the beginning of the flowering stage on the eighth fully expanded intact leaf from a branch tip by using a portable gas analyzer (LCA-4, Analytical Development Company, Hoddesdon, UK) associated leaf chamber together with a portable leaf microclimate control system (Analytical Development Company, Hoddesdon, UK). All measurements were taken under 9 levels of PAR (100, 200, 300, 400, 500, 600, 700, 800 and 900 μmol $m^{-2}s^{-1}$) at 350 ± 10 μmol $mol^{-1}(CO_2)$, leaf temperature of 20 °C, and relative humidity of 70 %. Five minutes were allowed to reach the stable condition for each PAR level prior to record the data. The analyzer was operated at an airflow rate of 400 μmol s^{-1}. Two measurements were taken for each pot on the same day.

3.2.5 Experimental Design and Statistical Analysis

The experiment followed a two factor factorial randomized complete block design with 3 replications for each treatment. The first factor was soil water potential at three levels (0, -65 and -130 cbars) and the second factor was N at four levels (0, 50, 100 and 150 kg N/ha). The response variables plant height, xylem pressure potential, yield components (branches and pods/plant), root and shoot dry matter, root: shoot ratio, specific leaf area, leaf N content, N_{area}, net photosynthesis, transpiration rate, WUE_i, PNUE, seed yield/pot were collected and subjected to the PROC MIXED procedure in SAS. Tukey's test was used to compare the differences among treatments at 5 % significant level.

The study on the effect of PAR on photosynthesis followed a three factor factorial design. These three factors were N rate with two levels, soil water potential with two levels, and eight levels of PAR. The non-rectangular hyperbola proposed by Marshall and Biscoe (1980) was used to model leaf photosynthesis as a function of PAR. Following this, a nested non-linear regression with incremental parameters model was used for comparing pairs of treatment combinations (N rate * soil water potential) in terms of the model parameters. The analysis was completed by using the NLIN procedure of SAS (SAS Institute 1999). The general form of the non-rectangular model is:

$$\theta P_N^2 - (P_{max} + aI - \theta R_d) P_N + aI [P_{max} - (1 - \theta) R_d] - P_{max} R_d = 0$$

where P_{max} is maximum rate of net photosynthesis, R_d is rate of dark respiration, a is photochemical efficiency of photosynthesis at low light, and θ is ratio of physical to total resistance to diffusion of CO_2.

To facilitate simpler non-linear regression estimation, we fitted the following re-parameterization of the non-rectangular hyperbola model:

$$P_N = \frac{P_{max} R_d - aI[P_{max} - (1-\theta) R_d]}{\theta P_N - (P_{max} + aI - \theta R_d)} + \varepsilon$$

P_{max} - Estimated values of maximum rate of net photosynthetic
α - Photochemical efficiency at low light
θ - Ratio of physical to total resistance to diffusion of CO_2
R_d - Dark respiration rate

Where ε is the error term assumed to be normally distributed with zero mean, constant variance and independence of one another.

3.3 Results

3.3.1 Effect of N and Water Availability on Leaf Characteristics

Growth and related characteristics among *B. carinata* seedlings were highly sensitive to the availability of water and N. Leaf area, leaf N content and N_{area} were all significantly affected by the interactive effect of N and watering treatments (Table 3.2). Leaf area and leaf N content were dramatically higher in plants supplied with N than plants without N under all watering treatments. Under the well-watered treatment, the leaf area of a plant supplied with 150 kg N/ha, which was approximately 15.3 cm^2, was approximately three times that of the plant without N supply, which was around 5.7 cm^2.

While the difference of leaf area between plants under the highest N supply and plants without N increased to five times under severe water stress. Figure 3.1 and 3.2 shows that the leaf area decreased while leaf N content increased with applied water stress in all N treatments. However, the relative magnitude of these changes varied. Plants with the 150 kg N/ha treatment reduced their leaf area from 15.3 to 7.8 cm^2 and increased their leaf N content from 2.07 to 3.64 %, with increasing water stress. Plants without N decreased the leaf area from 5.73 to 1.44 cm^2 and increased the leaf N content from 1.15 to 1.55 % as the soil water potential fell from 0 to -130 cbars. There was no interactive effect on the leaf chlorophyll but the main effect N rate and soil watering treatment significantly affected the leaf chlorophyll content (Table 3.6 and 3.7). Leaf chlorophyll increased from 50 to 57.5 µmol/m^2 as the N supply increased from 0 to 50 kg N/ha but the differences between the N treatments higher than 50 kg N/ha were not significant. Leaf chlorophyll also increased substantially as the increased water stress from 51 to 61 µmol/m^2.

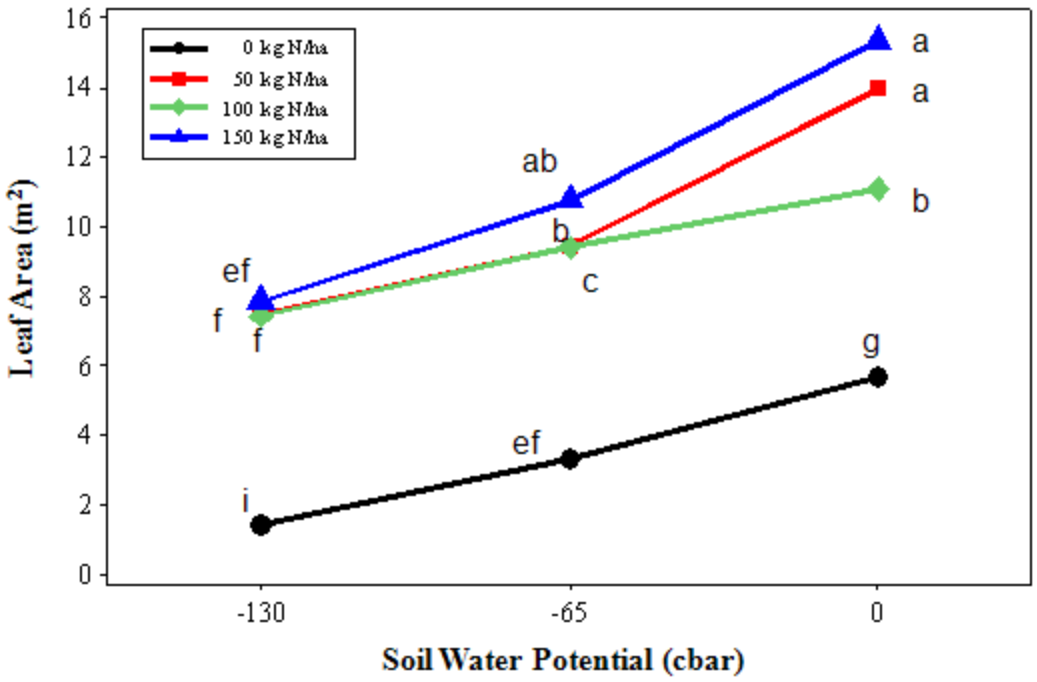

Figure 3.1 Interactive effect of N rate and soil water potential on leaf area (Figures followed by different letters are significantly different $p < 0.05$)

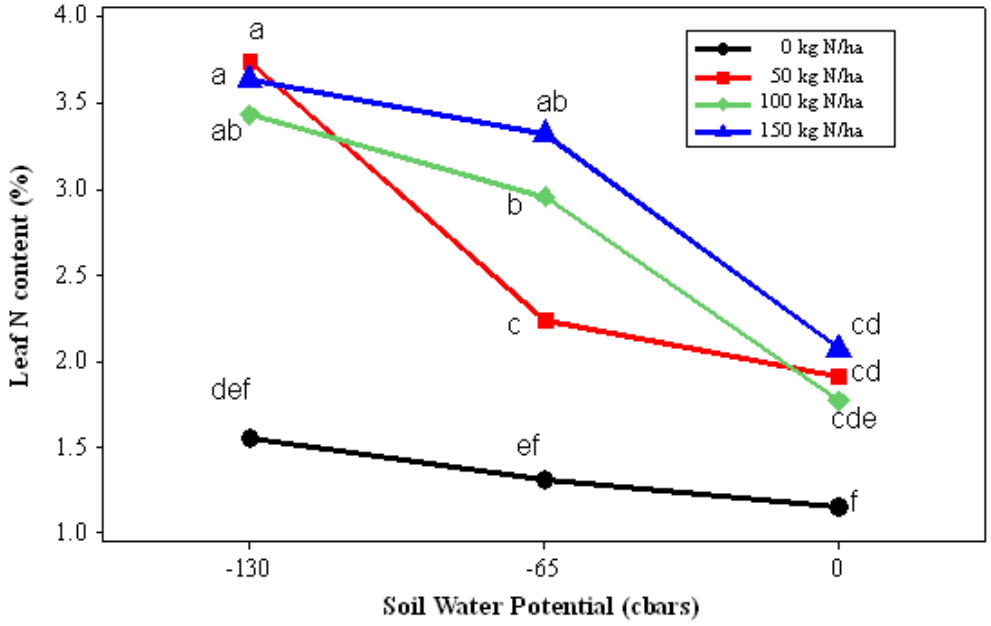

Figure 3.2 Interactive effect of N rate and soil water potential on leaf N content (Figures followed by different letters are significantly different $p < 0.05$)

Table 3.2 Interactive effect of N and water supply on leaf area, leaf N content, leaf N content per leaf area unit (N_{area})

Treatment	Severe water stress N rate (kg/ha)				Moderate water stress N rate (kg/ha)				Well-watered N rate (kg/ha)			
	0	50	100	150	0	50	100	150	0	50	100	150
Leaf Area (cm^2)	1.44i	7.23f	7.65f	7.84ef	3.35h	9.21de	9.38cd	10.78bc	5.73g	11.09b	13.97a	15.33a
Leaf N Content (%)	1.55def	3.745a	3.44ab	3.64a	1.31ef	2.23c	2.96b	3.32ab	1.145f	1.91cd	1.77cde	2.07cd
N_{area} (mmol m^{-2})	0.021a	0.019a	0.017ab	0.019a	0.010c	0.011c	0.015b	0.015b	0.008c	0.010c	0.008c	0.009c

Means within a row followed by different letters are significantly different ($p < 0.05$)

Table 3.3 Interactive effects of N and water supply on shoot and root dry matter, root: shoot ratio and total biomass

Treatment	Severe water stress N rate (kg/ha)				Moderate water stress N rate (kg/ha)				Well-watered N rate (kg/ha)			
	0	50	100	150	0	50	100	150	0	50	100	150
Shoot dry matter (g)	5.5f	7.5f	10.5ef	10ef	10ef	19.5c	19.5c	18cd	13.5de	27b	28b	35a
Root dry matter (g)	6efg	4h	5.5fgh	4.5gh	8.5d	7def	7.5de	8d	10.5c	11c	13.5b	15.5a
Root: shoot ratio	1.08a	0.54c	0.52cd	0.45cd	0.85b	0.36d	0.39cd	0.46cd	0.78b	0.41cd	0.49cd	0.44cd
Total biomass(g)	11.5e	11.5e	16de	14.5de	18.5d	26.5c	27c	26c	24c	38b	41.5b	50.5a

Means within a row followed by different letters are significantly different ($p < 0.05$)

3.3.2 Plant Height and Root and Shoot Dry Matter

Neither the N supply nor the interaction between soil water and N availability substantially affected plant height. However, the plant height was highly sensitive to water stress and it dropped from 115 to 70 cm as the water stress increased. Shoot dry matter, root dry matter, total biomass, and root: shoot ratio were all significantly affected by the interaction of N and water availability (Table 3.3 and Figure 3.3- 3.6). Shoot and root dry matter, as well as total biomass, all increased significantly as the increased N supply in well-watered plants. The 150 kg N/ha plants had two times the total biomass and half the root: shoot ratio of 0 N plants in well-watered treatments. In the moderate water stress treatment, plants with N had significantly higher shoot dry matter and total biomass than plants without N while the difference between N treatments was not significant. There was no significant difference of shoot and root dry matter, and total biomass in severely water-stressed plants, at any level of N. Increased water stress resulted in a greater relative decline in root and shoot dry matter for high-N than low-N plants. Total biomass in 150 kg N/ha plant decreased 71 % (50.5 to 14.5 g) while that of plants without N supply dropped only 52 % (24 to 11.5 g) as the water stress increased. However, the root: shoot ratio of plants in low N treatments (0 and 50 kg N/ha) increased dramatically under severely water-stressed conditions while that of plants with higher N kept statistically constant in all water regimes.

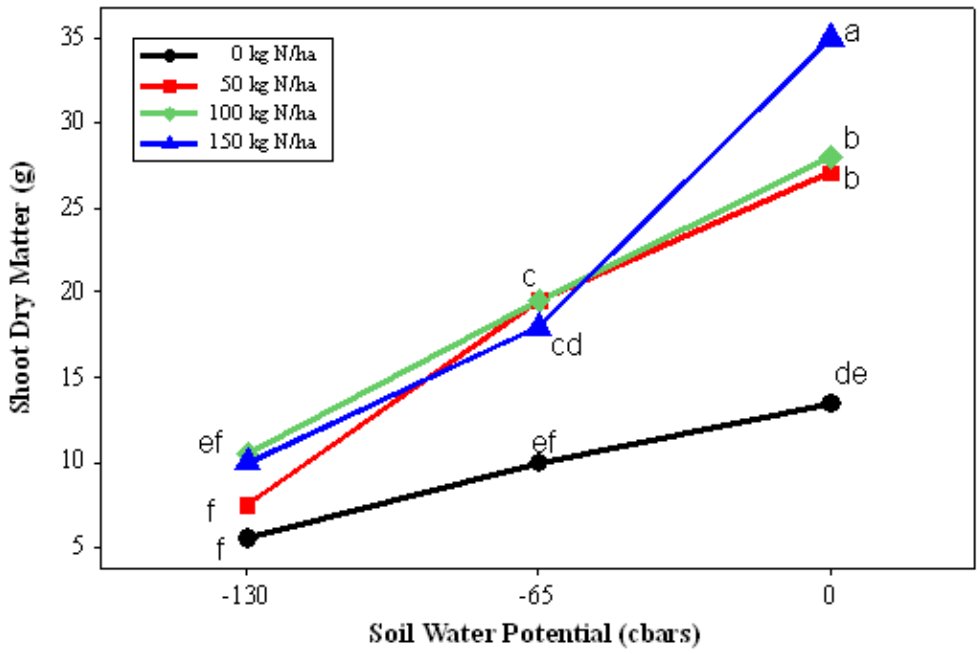

Figure 3.3 Interactive effect of N rate and soil water potential on shoot dry matter (Figures followed by different letters are significantly different $p < 0.05$)

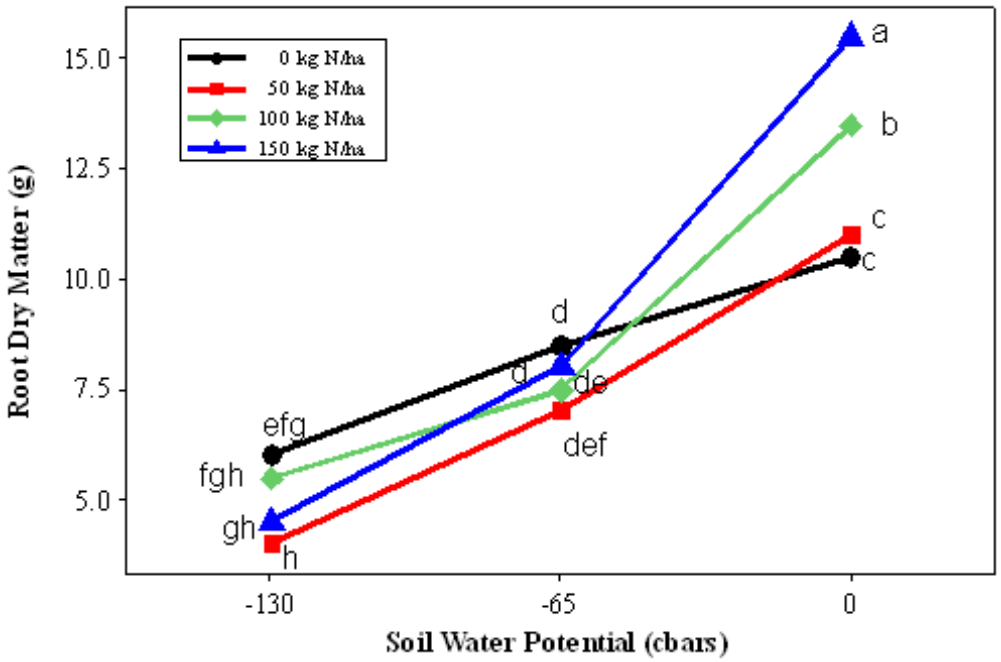

Figure 3.4 Interactive effect of N rate and soil water potential on root dry matter (Figures followed by different letters are significantly different $p < 0.05$)

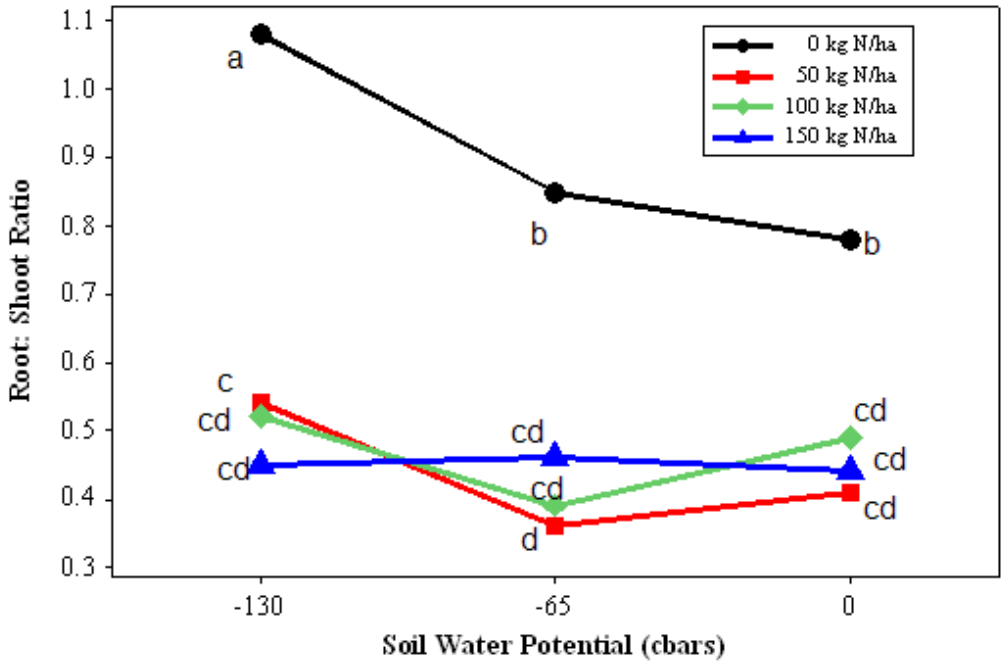

Figure 3.5 Interactive effect of N rate and soil water potential on root: shoot ratio (Figures followed by different letters are significantly different $p < 0.05$)

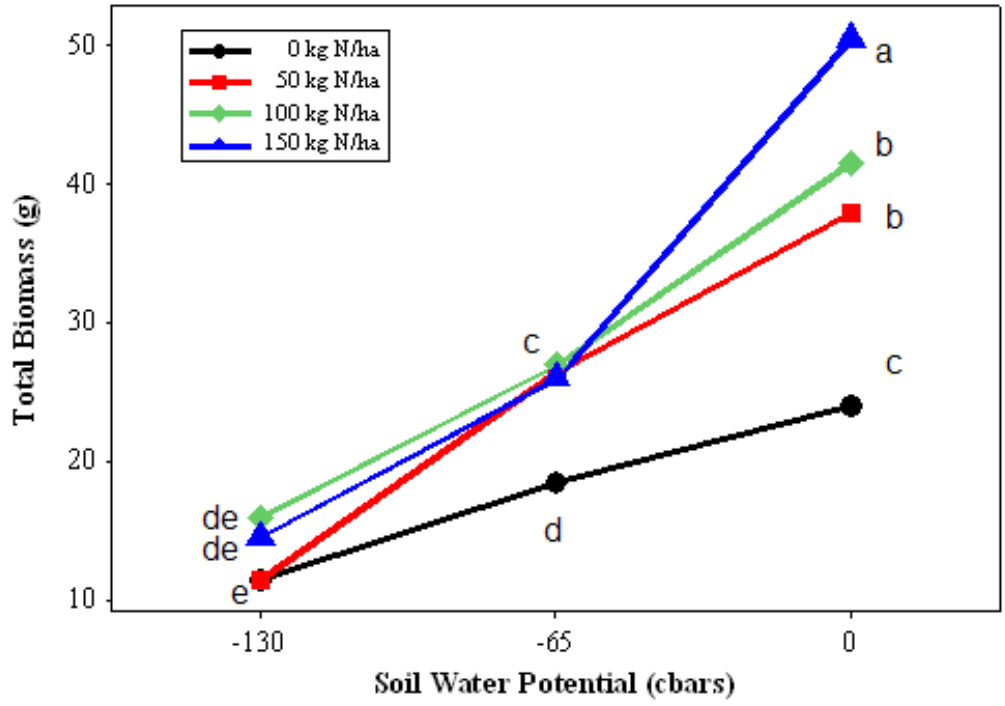

Figure 3.6 Interactive effect of N rate and soil water potential on total biomass (Figures followed by different letters are significantly different $p < 0.05$)

3.3.3 Intrinsic Water Use Efficiency

The effects of N availability on net photosynthesis, transpiration rate and intrinsic water use efficiency are not identical under different water status (Table 3.4 and Figure 3.7- 3.10). Net photosynthesis was negatively correlated with water stress and increased with N (Figure 3.7). The net photosynthesis of plants at 150 kg N/ha treatment decreased 50 % (3.52 to 1.73 μmol $m^{-2}s^{-1}$) while that of the plants at 0 N decreased 30 % (1.24 to 0.85 μmol $m^{-2}s^{-1}$). Transpiration rate was also negatively related to drought intensity and decreased with N (Figure 3.8). The highest transpiration rate (3.67 mmol $m^{-2}s^{-1}$) was achieved in the treatment combination of 0 N and well watered, while the lowest value (0.9 mmol $m^{-2}s^{-1}$) was observed in the plants suffering from severe water stress and supplied with 150 kg N/ha. The intercellular CO_2 concentration decreased dramatically as the water stress increased in all N treatments (Figure 3.9). But the magnitude of increases for each N level was different. The intercellular CO_2 concentration of plants with 150 kg N/ha dropped to the greatest extent from 448.7 to 395.4 μl l^{-1} while plants without N decreased their intercellular CO_2 from 431.8 to 421.1 μl l^{-1}. Intrinsic water use efficiency was remarkably improved as the drought intensity increased in all N treatments except for the plants supplied with 150 kg N/ha, which have the statistically consistent intrinsic water use efficiency at around 1.85 μmol mol^{-1} under all watering treatments (Figure 3.10). Plants at the higher-N nutrition had significantly larger value of intrinsic water use than that of the plants in the low-N treatment in all watering treatments. The intrinsic water use efficiency of the plants dropped 40 % (1.54 to 0.91 mmol mol^{-1}) in 100 kg N/ha treatment, 9 % (1.21 to 1.10 mmol mol^{-1}) in 50 kg N/ha treatment, and 47 % (0.64 to 0.34 mmol mol^{-1}) in 0 kg N/ha treatment.

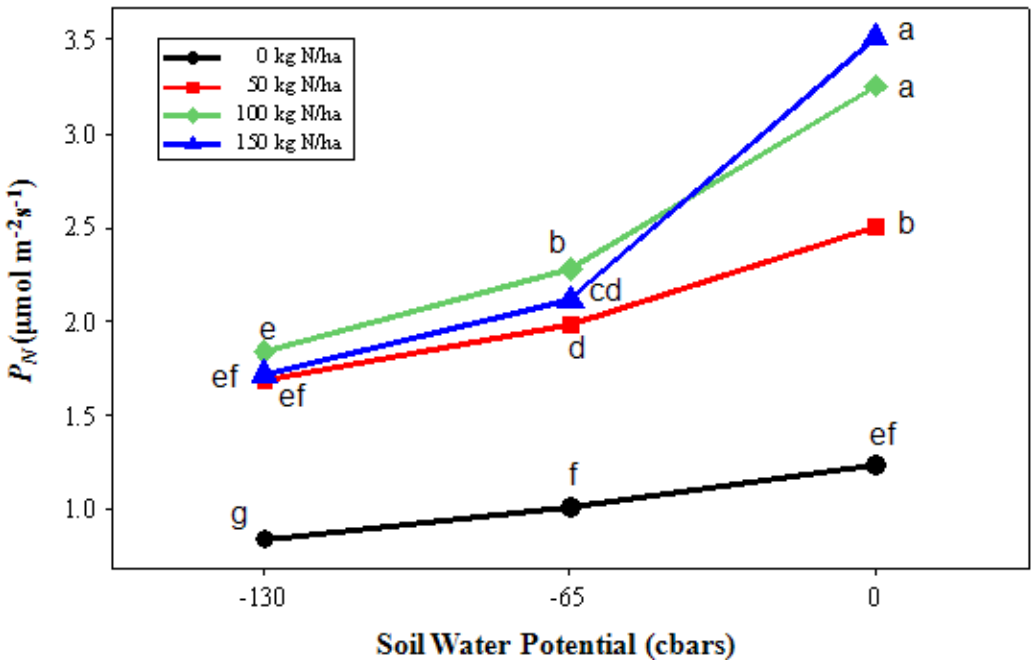

Figure 3.7 Interactive effect of N rate and soil water potential on net photosynthesis (Figures followed by different letters are significantly different p < 0.05)

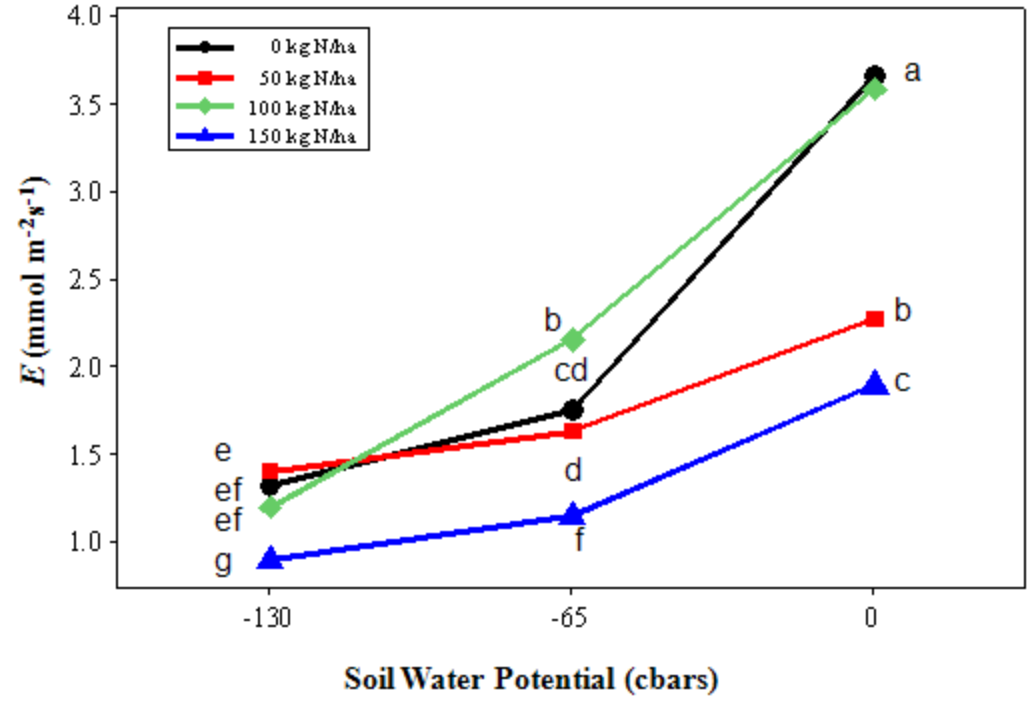

Figure 3.8 Interactive effect of N rate and soil water potential on transpiration rate (Figures followed by different letters are significantly different p < 0.05)

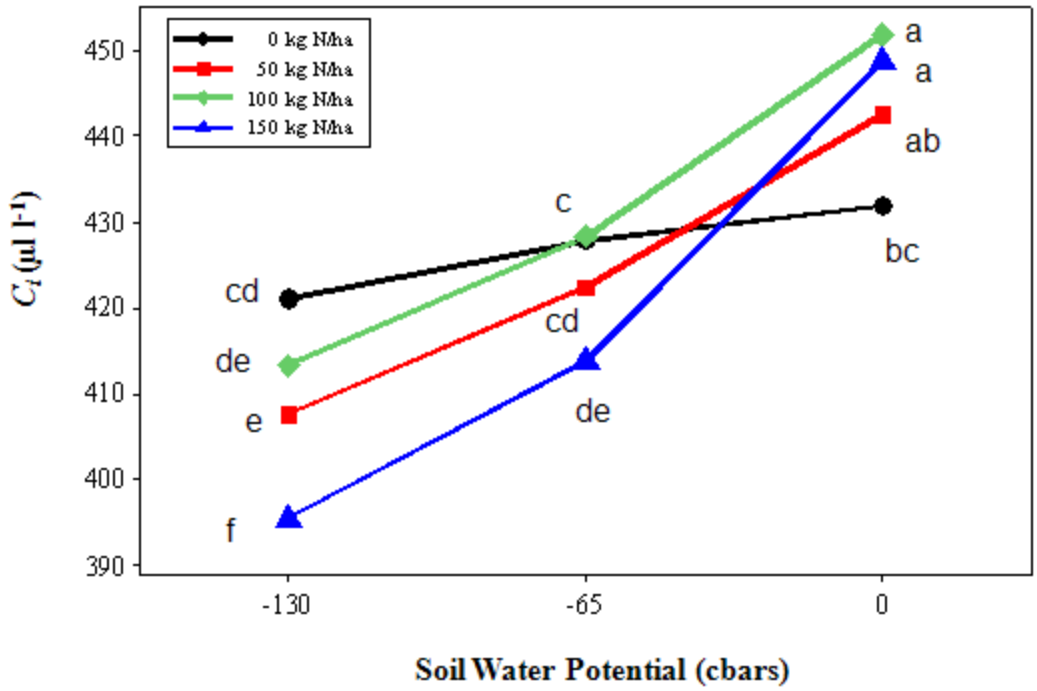

Figure 3.9 Interactive effect of N rate and soil water potential on intercellular CO_2 ($\mu l\, l^{-1}$) (Figures followed by different letters are significantly different $p < 0.05$)

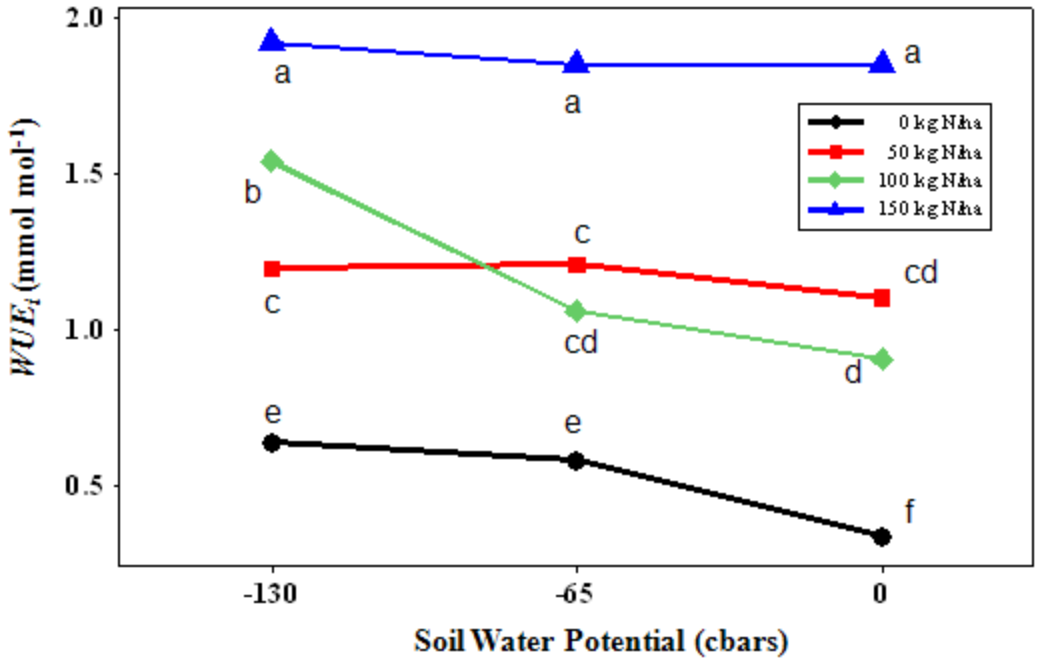

Figure 3.10 Interactive effect of N rate and soil water potential on *WUEi* (Figures followed by different letters are significantly different $p < 0.05$

Table 3.4 Interactive effect of N and water supply on net photosynthesis (P_N), transpiration rate (E), intrinsic water use efficiency (WUE_i), intercellular CO_2 (C_i), photosynthetic N use efficiency (PNUE) and seed yield

Treatment	Severe water stress				Moderate water stress				Well-watered			
	N rate (kg/ha)				N rate (kg/ha)				N rate (kg/ha)			
	0	50	100	150	0	50	100	150	0	50	100	150
P_N ($\mu mol\ m^{-2}s^{-1}$)	0.85f	1.70de	1.84cd	1.73de	1.02f	1.99cd	2.29bc	2.12bcd	1.24ef	2.52b	3.26a	3.52a
E ($mmol\ m^{-2}s^{-1}$)	1.32ef	1.41e	1.2ef	0.9g	1.75cd	1.64d	2.16b	1.15f	3.665a	2.28b	3.59a	1.9c
WUE_i ($mmol\ mol^{-1}$)	0.64e	1.20c	1.54b	1.92a	0.58e	1.21c	1.06cd	1.85a	0.34f	1.10cd	0.91d	1.85a
C_i ($\mu l\ l^{-1}$)	421.1cd	407.6e	413.3de	395.4f	427.9c	422.3cd	428.4c	413.7de	431.8bc	442.5ab	451.8a	448.7a
PNUE ($\mu molmmol^{-1}s^{-1}$)	41.23d	88.51cd	109.39cd	90.49cd	100.40cd	184.64bc	149.56c	141.1c	162.54bc	247.76b	411.48a	393.90a
Seed Yield (g)	0.36f	2.50d	2.23de	1.14f	1.31ef	3.05d	4.37c	4.12c	2.52d	4.63bc	5.54ab	5.81a

Means within a row followed by different letters are significantly different ($p < 0.05$)

3.3.4 Intrinsic N Use Efficiency

Photosynthetic N use efficiency decreased significantly with increasing N_{area} within all N treatments, for any level of water status (Figure 3.11-3.12 and Table 3.4). Photosynthetic N use efficiency also decreased with increasing leaf N content and declined with increased water stress. Plants with N had significantly higher photosynthetic N use efficiency than the plants with insufficient N, in all watering treatments. In well-watered plants, highest photosynthetic N use efficiency was achieved in plants with higher N (100 and 150 kg N/ha), which was approximately 400 µmol $mmol^{-1}s^{-1}$, followed by the plants with 50 kg N/ha (250 µmol $mmol^{-1}s^{-1}$), while the plants without the N had the lowest photosynthetic N use efficiency, which was 165 µmol $mmol^{-1}s^{-1}$. The difference of the photosynthetic N use efficiency between the N treatments declined dramatically in water-stressed plants. In moderate water-stressed plants, the photosynthetic N use efficiency ranged from 184 to 100 µmol $mmol^{-1}s^{-1}$ among N treatments; while that of plants suffered from sever water stress ranged from 109 to 41 µmol $mmol^{-1}s^{-1}$.

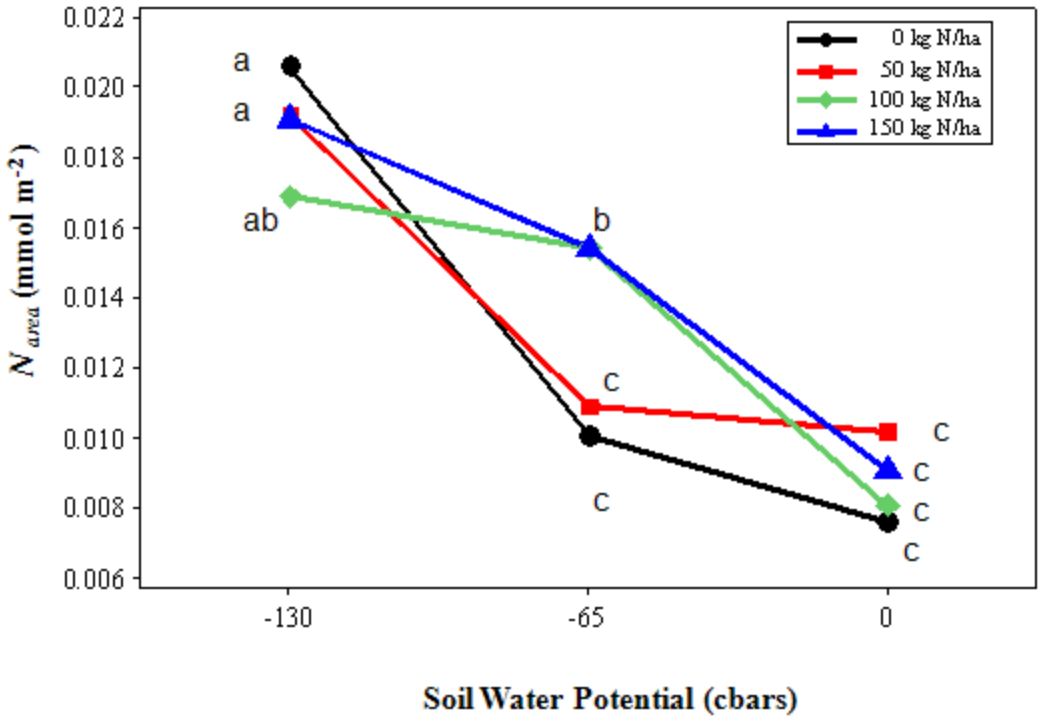

Figure 3.11 Interactive effect of N rate and soil water potential on N_{area} (Figures followed by different letters are significantly different $p < 0.05$)

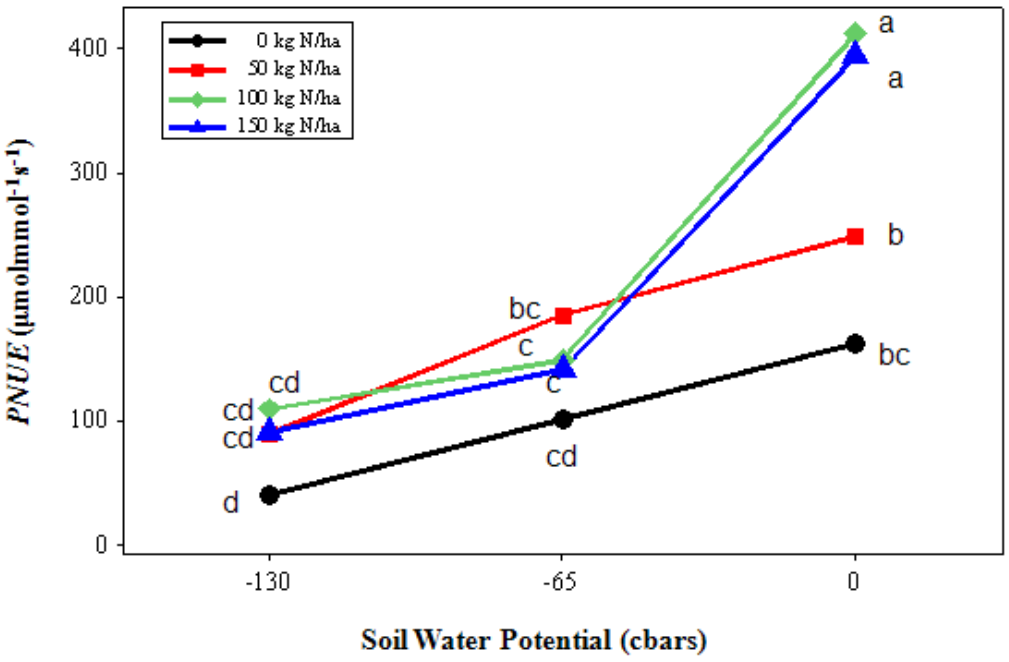

Figure 3.12 Interactive effect of N rate and soil water potential on *PNUE* (Figures followed by different letters are significantly different $p < 0.05$)

3.3.5 Effect of Irradiance on Photosynthesis

Figure 3.13 shows that P_N for all treatment combinations increased hyperbolically with increasing PAR. Plants with sufficient N and water supply had the highest net photosynthesis while plants in the 150 kg N/ha * severe water stress treatment had significantly lower photosynthetic activity in all PAR levels. For the well-watered treatment, the net photosynthetic rate was stimulated in the plants with high N as compared with plants with 0 N. As water stress progressed, the net photosynthetic rate was inhibited in the plant with high-N as compared with the low-N plants. Table 3.5 shows that control of soil water and N nutrition during growth of *B. carinata* produced substantial changes in the irradiance response of photosynthesis. Maximum net photosynthetic rate varied significantly among treatments. Plants with sufficient water and N had the highest P_{max} (8.46 μmol $m^{-2}s^{-1}$,) followed by the P_{max} of the well-watered plants without N (6.66 μmol $m^{-2}s^{-1)}$. The plants suffering from water stress had the lowest P_{max} (3.3 μmol $m^{-2}s^{-1}$) under both N treatments. A similar pattern was found in photochemical efficiency. The highest value of 0.0111 was achieved in the treatment combination of 150 kg N/ha * well-watered while the plants under water-stress had the lowest value of 0.004. No differences were observed among N treatments. Well-watered plants had the highest dark respiration rate of 2.40 μmol $m^{-2}s^{-1}$ for both N treatments. The value of dark respiration decreased significantly with increasing water stress. The lowest value of dark respiration of 0.22 μmol $m^{-2}s^{-1}$ was observed in treatment 150 kg N/ha * water stress. Higher N substantially inhibited the dark respiration when plants suffered from water stress.

Table 3.5 Estimated values of photosynthetic parameters for irradiance response curves

Treatments	P_{max} (µmol $m^{-2}s^{-1}$)	α	θ	R_d (µmol $m^{-2}s^{-1}$)
N0W-130	3.31c	0.00371c	0.99a	-1.83b
N0W0	6.66b	0.0057b	1.0016a	-2.42a
N150W0	8.46a	0.0111a	1.025a	-2.35a
N150W-130	3.0 6c	0.00477c	1.00a	-0.22c

N0W-130 = 0 kg N/ha * -130 cbars soil water potential
N0W0 = 0 kg N/ha * 0 cbars soil water potential
N150W0 = 150 kg N/ha * 0 cbars soil water potential
N150W-130 =150 kg N/ha * -130 cbars soil water potential
Means within a column followed by different letters are significantly different ($p < 0.05$)

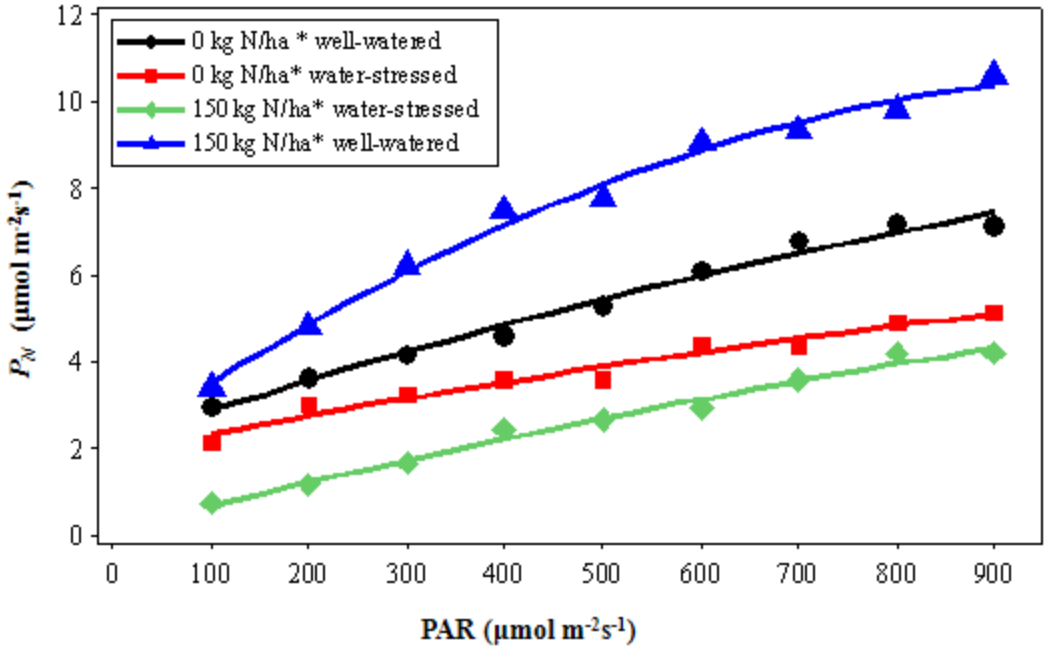

Figure 3.13 Net photosynthesis-light response curves for a fully expanded leaf of *B. carinata*

3.3.6 Branches/plant and Pods/Plant and Seed Yield

Branches/plant and pods/plant were significantly influenced by both main effects (N and water treatments) but not by the interaction effect (Table 3.6 and 3.7). Increasing N substantially increased the branch number from 4 to 9 and pod number from 19 to 40 per plants; increasing water stress negatively affected branch and pod number per plant. Branch number dropped from 9 to 5 and pod number decreased 42 to 17 per plant as the soil water potential dropped from 0 to -130 cbars. N fertilizer application significantly

increased seed yield, but the N effect was dependent on the availability of water (Figure 3.14 and Table 3.4). The seed yield of well-watered plants increased linearly from 2.5 to 5.8 g per pot as N increased. Water stress substantially decreased the seed yield in all N treatments, but the relative magnitude of these decreases varied. Plants with highest N dropped the seed yield approximately 5 times while plants with 50 and 100 kg N/ha reduced half seed yield as the increasing water stress. For plants without N, the seed yield decreased from 2.52 to 0.36 g as water stress increased.

Table 3.6 Soil water potential effect on plant height, branch and pod number per plant, chlorophyll content, and xylem pressure potential

Soil water potential (cbars)	Plant height (cm)	Branches /plant	Pods /plant	Chlorophyll content	Xylem pressure potential (MPa)
0	115a	9a	42a	53.5b	-0.62c
-65	105b	6b	31b	52.9b	-0.83b
-130	70c	5b	17c	61.0a	-1.03a
P-value	0.037	0.002	0.001	0.041	0.025

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 3.7 Nitrogen effect on Branch and pod number per plant as well as chlorophyll content

N(kg/ha)	Branches/plant	Pods/plant	Chlorophyll content
0	4c	19c	50.5b
50	6b	29b	58.0a
100	6b	31b	57.3a
150	9a	40a	57.5a
P-value	0.029	0.018	0.042

Means within a column followed by different letters are significantly different ($p < 0.05$)

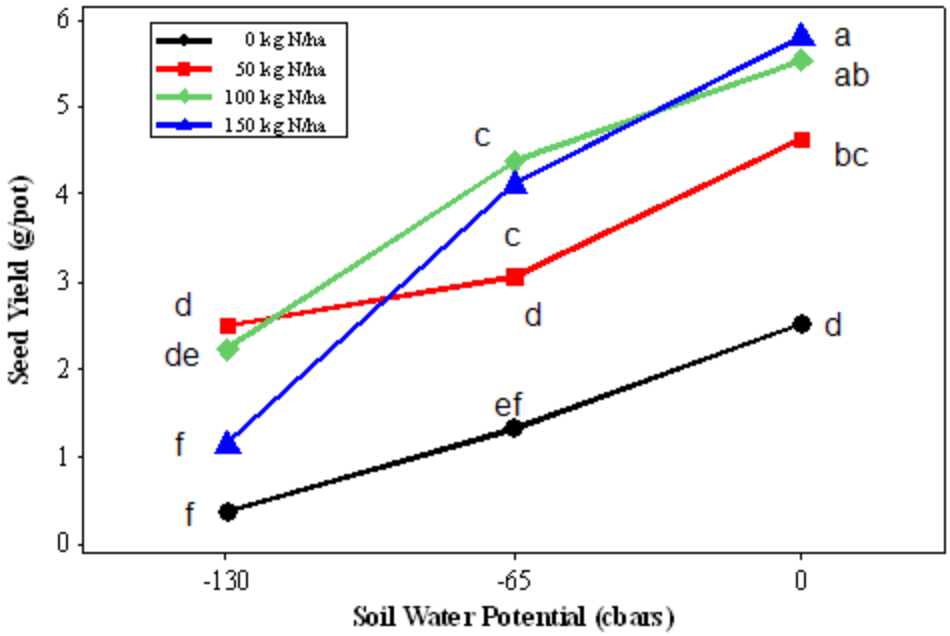

Figure 3.14 Interactive effect of N rate and soil water potential on seed yield (Figures followed by different letters are significantly different $p < 0.05$)

Table 3.8 Summary of Pearson correlation coefficients describing the relationship among leaf characteristics, and leaf water and nutrient status for *B. carinata* plants

	Leaf Area	N %	P_N	WUE	Leaf Mass	N area	PNUE
P_N	0.941***						
N%	NS		NS				
E	NS	-0.62***	NS				
WUE	0.542**	0.719***	0.489*				
Leaf mass	0.974***	NS	0.913***	0.421*			
Narea	-0.444**	0.676***	NS	NS	-0.494*		
PNUE	0.817***	NS	0.876***	NS	0.819***	-0.712***	
Seed wt.	0.910***	NS	0.885***	NS	0.919***	-0.548**	0.807***
Chlorophyll	NS	0.674***	NS	0.569**	NS	NS	NS
LM area	-0.769***	-0.445*	-0.655***	-0.552**	-0.712***	NS	-0.479*

* Significant at the 0.05 level of probability.
** Significant at the 0.01 level of probability.
*** Significant at the 0.001 level of probability.

3.4 Discussion

3.4.1 P_N and Leaf Characteristic and Seed Yield

Photosynthesis is the integrated operation of a series of biochemical and photobiological processes related to not only environmental factors but also leaf physiology and structure. The chlorophyll (Chl), as the important component of light-harvesting protein complexes (LHPC) and reaction centre proteins in the thylakoid membrane, is essential for photosynthesis process (Anand *et al.* 2007). Ellison *et al.* (1983) in wheat showed the existence of a significant positive relationship in wheat between leaf P_N and Chl contents. However, in our investigation of *B. carinata* no significant correlation was found between P_N and Chl, which was consistent with the results for *Brassica* species reported by Anand *et al.* (2007). In this study, water stress may strongly interfere with the relationship between P_N and Chl contents. Low transpiration rate in water-stressed plants suggested that stomatal limitation rather than the photosynthetic apparatus limitation (low chlorophyll content), was the dominant factor responsible for the low rate of photosynthesis.

The strong linear relationship between P_N and leaf N content has been reported in many previous studies (Yoshida and Coronell 1976; Bolton and Brown 1980,; Press *et al.* 1993; Lynch and Rodriguez 1994; Reddy *et al.* 1996; Grindlay 1997). Even though there was no significant correlation between pooled N content and P_N in this study, a strong relationship between N content and P_N was found in each water treatment; correlation coefficients were 62.1 %, 74.4 % and 91.5 % in well-watered, moderate water stressed, and sever water stressed treatments, respectively. The correlation between P_N and N_{area} was not significant in any watering treatments. However, there is a positive trend between P_N and N_{area} in well-watered and moderate water stress treatment and negative trend in sever water stress treatment. Higher N_{area} and lower P_N in the severe water stress treatment indicated that the drought-associated decrease in photosynthetic capacity may be caused by the downregulation of photosynthetic electron transport and to reallocation of leaf N content. In many studies it was found that there is a positive linear relationship between P_N and seed yield (Richards 2000). A similar relationship was observed in *B. carinata* plants in this study. This suggested that P_N can be used as a tool for selection of new genotype with high seed yield and high PNUE.

3.4.2 P_N and Photosynthetic Active Radiance

The effect of PAR on photosynthesis has been extensively studied. The response of *B. carinata* leaf photosynthesis to irradiance is similar to many horticultural crops, including cucumber, sweet pepper and carrot (Kyei-boahen *et al.* 2003). The values obtained in this study were fitted to the non-rectangular hyperbola model. P_N for all treatment combination increased hyperbolically in response to increased irradiance. Plants with sufficient water and N had the highest P_N while plants under sever water stress combined with highest N had the lowest P_N in all PAR levels. Magnitude of the difference in P_N between stressed and unstressed plants became progressively more marked at each PAR levels. Plants under sufficient N and water resources had highest $P_{max,}$ possibly due to the relatively high chemical use efficiency and high stomatal conductance. The studies on beans (Shimshi 1970), coffee (Tesha and Kumar 1978) and winter wheat (Shangguan 1997) revealed that increasing N nutrition significantly enhanced stomatal conductance (*Gs*) under well-watered conditions. However, plants with insufficient N and water had the lowest P_{max} might be related to not only low stomatal conductance but also low amount of photosynthetic apparatus, such as chlorophyll and Rubisco. Seemann *et al.* (1987) reported that Rubisco, which plays an important role in photosynthesis, was significantly influenced by N deficiency. None of the plants reached saturation at 900 μmol $m^{-2}s^{-1}$ in this study. This suggests that *B. carinata* plants did not reach their P_{max} during the growth period in this study since the PAR of 420 ± 20 μmol $m^{-2}s^{-1}$ in growth chamber is much lower than the saturation point.

3.4.3 WUE_i

Collectively these results showed that *B. carinata* displays considerable plasticity in WUE_i under varied soil N and water status. In well-watered *B. carinata* plants, biochemical rather than stomatal limitations appear to account for the difference in photosynthesis among N treatment, since the high amount of intercellular CO_2 in all N levels. Even though there was no difference of N_{area} among N levels, but significantly higher amount of leaf chlorophyll in plants with higher N suggested that plants invested a larger fraction of leaf N into photosynthetic apparatus in higher N treatments. Higher PNUE in higher N supplied plants provided further evidence that N allocation to the photosynthetic apparatus is a major factor for the difference in photosynthesis among N

treatments in well-watered conditions. Many previous studies suggested that species with lower PNUE had less ability to allocate the N to the photosynthetic apparatus (Warren and Adams 2000; Ripullone *et al.* 2003; Takashima*et al.* 2004). There were strong linear declines in C_i as the water stress increased. This indicated that the development of stomatal limitations to gaseous CO_2 exchange drives C_i lower, which is the dominant factor for the reduction in photosynthesis and increase in WUE_i.

Generally, plants respond to water stress by showing significant changes in their physiological and biological mechanisms in order to accommodate this abiotic stress. In this study, drought stress increased root: shoot ratio considerably indicating that extending the root proliferation in the soil is an essential drought-resistant strategy for *B. carinata*. Highest root: shoot ratio in plants with N combined with severe water stress indicated that root growth may last throughout the growing season, even when N fertilization is insufficient. In addition, the negative relationship between internal CO_2 concentration and water stress found in this study suggested that stomatal closure is another important strategy for *B. carinata* to control water use. Furthermore, the positive correlation between chlorophyll content and water stress in this study suggested that accumulating special fatty acid proline to increase the osmotic pressure is another mechanism for *B. carinata* to tolerant water stress.

3.4.4 Constraints on PNUE

The relationship between leaf N content and PNUE has been found in many previous studies. However, the results are varied. Sage and Pearcy (1987) observed that PNUE increased as a function of increasing leaf N. In this study, PNUE were positive correlated to leaf area while negative related to leaf N content per leaf unit, which suggests that *B. carinata* may prefer to produce large leaf size with lower N content instead of a smaller leaf area with higher N contents to optimize the utilization of leaf N in photosynthesis. The negative relationship between PNUE and leaf N content was also found by Reich and Schoettle (1988), Hirose and Werger (1994) and Anand *et al.* (2007). The negative correlation between PUNE and leaf N content can be explained by that, a higher amount of leaf N was used as structural or defensive compounds instead of photosynthetic apparatus. The significantly positive correlation between P_N and PNUE (Table 3.8) found in this study suggested that all the factors negatively affected the P_N also had the similar

effect on PNUE. Therefore, the carbon economy is closely related to the N economy within the whole plant. This positive relationship was also reported by Field and Mooney (1986) and Anand *et al.* (2007).

3.4 5 WUE_i versus PNUE

The negative correlation between WUE_i and PNUE has been reported in many studies (Reich *et al.* 1989). Even though mean values of WUE_i and PNUE for combinations of N treatments and soil water potentials were not significantly negatively correlated ($P > 0.05$), there was a trend of tradeoff between WUE_i and PNUE. In well-watered plants, PNUE was high, whereas WUE_i was low. In severely watered-stressed plants, these trends were reversed with PNUE ultimately decreasing 4-fold, and WUE_i increasing by a smaller extent. N use modified the water use in all water regimes, in the sense that the seed yield production dropped to a much larger extent as the water shortage increased at high N level.

The extreme variation in plant size (note: this can be reflected by the plant height) due to the different resource availability among treatments is often a problem in studies citing differences in WUE_i and PNUE between the treatments (Walters and Reich 1989). However, in this study, only soil watering treatments had a significant effect on plant height. Therefore, it can be concluded that there are certain relationships which seem to be independent of plant size in this study due to the consistency across the watering treatments. Firstly, WUE_i rose significant as the water stress increased. Plants with higher N always had higher WUE_i. Secondly, plants with N had higher PNUE than plants without N but the difference of PNUE between N treatments varied with different watering regimes. Thirdly, the differences in photosynthesis, WUE_i and PNUE in different treatments combination were all caused by both stomatal and non-stomatal components. In well-watered conditions, N-related biochemical limitation seems to be the predominant factor while in water-stressed conditions; stomatal limitation appears to become the most important component.

3.4.6 Seed Yield and Soil Water and N Availability

There are several possible explanations for the negative relationship between seed yield and drought stress in this study. These include: 1) water stress reduced the plant leaf area, thus decreased carbon gain throughout the growing period, which was reflected by

the negative correlation between leaf area and seed yield; 2) water stress could affect the cell size development and, thus, the length of the internodes which determine the capacity for storing assimilates. The negative relationship between net photosynthesis and water stress indicated that the latter limited photosynthesis and the primary carbohydrate source for seed filling would be the remobilization of stem reserves; 3) water stress changed the patterns of resource allocation and a higher amount of resource was used to support root development (Wilson 1988).

The positive correlation between seed yield and soil N availability in this study can also be explained by the following reasons: Sufficient N increased leaf area development and yield components. Furthermore, the seed yield can be increased by increasing photosynthesis in the high N level due to the increases in photosynthetic N components.

The significant watering treatment by N interactive effects on seed yield of *B. carinata* indicated that seed yield increases resulting from N depended on the water regime. The results from this study suggest that 150 kg N/ha was required for achieving maximum seed yield under adequate water or moderate drought environment. However, the application of 100 kg N/ha appears to be sufficient to achieve maximum yield under severe water stress.

3.5 References

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Chapter 4 Diversity and Adaptation of *Camelina sativa* (L.) Crantz Genotypes to Contrasting Environments

4.1 Introduction

Camelina sativa (L.) Crantz, also known as false flax or "gold of pleasure", is a member of Brassicaceae. The recent focus on this crop was inspired by the interest in finding new sources of essential fatty acids, especially from vegetable sources of omega-3 fatty acids. The oil from the seed contains about 90 % unsaturated fatty acids of total fatty acid and a high proportion of OMEGA-3 and OMEGA-6 type fatty acids; these fats have been identified as high quality human edible oils (Crowley and Fröhlich 1998). Camelina is also known to be highly drought tolerant, less susceptible to frost, have high disease and pest resistance, and low input requirements (Zubr 1997). Therefore, *C. sativa* could be a potential oilseed crop for Canada.

Since there has been no intensive breeding efforts in *Camelina sativa*, the availability of germplasm is limited (Gugel and Falk 2006). Some genetic improvement of *C. sativa* that was started in Germany produced two cultivars, Lindo and Soledo (spring annuals) with high oil and seed yield as well as with an enhanced oil and seed quality (Agegnehu and Honermeier 1997). Seehuber *et al.* (1987) developed transgressions over parental lines in many yield traits using the single-seed descent method. China also developed *C. sativa* NO.1 by hybridizing *C. sativa* which originated from France and *C. macrocarpa f. longistipata,* which originated from China (Liu *et al.* 2005). The content of unsaturated fatty acid in this new crop is extremely high, reaching 90 % of the total fatty acid, among which the poly-unsaturated fatty acid is about 71.1 % including a linolenic acid content of 34.5 % (Liu *et al.* 2005). Germplasm selection and genetic improvement research is presently underway. More research is needed to identify more attractive agronomic traits such as increased seed size, early maturity, high yield, high oil and protein content, low erucic acid and glucosinolate, through pedigree selection from large numbers of genetically diverse germplasm sources.

This study was undertaken to assess the diversity of genotypes of *C. sativa* to three contrasting environments (NS, PEI and SK), based on the analysis of genotype effects on plant stand, flowering date, plant height, maturity date, seed yield, TKW, seed oil content, seed protein content and oil quality.

4.2 Materials and Methods

Sites in Truro, NS, (NSAC); Harrington, PEI, (AAFC) and Saskatoon, SK (AAFC), were selected for the 2008 and 2009 field trials (Table 4.1). Truro, situated on the east coast of Canada near the Atlantic Ocean, has a long-term average rainfall during the main season which was about 687 mm (2008) and 403 mm (2009). The growing degree day (>5 °C) was approximately 1544 in 2008 and 1505 in 2009. Prince Edward Island, which is next to Nova Scotia, is located off the eastern coast of Canada. PEI has almost the same climate as NS; the total precipitation was 620 mm in 2008 and 596 mm in 2009. GDD was 1593 in 2008 and 1563 in 2009 during the main growing season. Saskatoon, located in the western part of Canada, in comparison with the other two locations, is fairly dry with a total precipitation during the main growing season for 2008 of 196 mm and around 272 mm in 2009. The GDD was approximately 1475 in 2008 and 1426 in 2009 (Appendix A). Fertilizer application schedule in three sites is listed in Table 4.2. The selected soil characteristics for each field are shown in Table 4.3 and 4.4. The pre-emergent herbicide Treflan was applied at a rate of 2.3 L/ha was applied in NS and PEI for both years. The crop previously grown at Truro, NS and Harrington, PEI sites were spring cereals in 2008. In 2009, the previous crops were flax in NS and red clover in PEI. In SK, the previous crops were oats and superb wheat in 2008 and 2009, respectively. Plots were seeded using a Hege plot drill (H and N Equipment Inc., Colwich, Kansas, USA) with double disc openers and press wheels in NS and PEI. In SK, plants were seeded with a 1.5 meter R-Tech hoe drill (AAFC, Saskatchewan, Canada) (packaged seed through a cone and splitter). All trials were sown at a constant seeding rate of 500 seeds/m^2 in both 2008 and 2009. In 2008, plots were seeded on May 14, May 26 and May 15 in NS, PEI and SK, respectively. The area of the plot seeded was 7.5 m^2 (8 rows @ 15 cm x 6 m in length) in NS and PEI site. In 2009, the trials were seeded on May 5, May 21 and 16 in NS, PEI and SK. In 2009, the seeded plot size was 6.25 m^2 in NS and 7.5 m^2 in PEI. The row spacing was 15 cm and the distance between plots and blocks were 25 cm and 2.5 m respectively at the NS and PEI sites. Plots at SK consisted of four rows spaced 30 cm apart. The row length was 6 m and the harvested plot area was 7.32 m^2.

This experiment was set up as a randomized complete block design (RCBD), with four replications. The eleven genotypes of *C. sativa* tested were CN101981, SRS933,

CN101985, CN101988, CN101989, CN101982, CN30475, CN30476, CN30478, CN30479 and Calena. The information for these genotypes is presented in Table 4.5. Plots were harvested with a Hege 125C plot combine (Hege USA, Colwich, Kansas, USA) and the plot harvest area was 5 m x 1.25 m in NS and PEI. In SK, plants were straight combined with a Wintersteiger plot combine (Wintersteiger AG, Austria).

Table 4.1 Longitude, latitude and elevation of three selected sites

Location	Longitude	Latitude	Elevation
Truro, NS, (NSAC)	63° 28.200' W	45° 25.200' N	37.50 m
Harrington, PEI, (AAFC)	63° 7.800' W	46° 17.400' N	48.80 m
Saskatoon, SK, (AAFC)	106° 43.200' W	52° 10.200' N	504.10 m

Table 4.2 Fertilizer application schedule for camelina genotype study in three sites

Site	Date	Form	Method
NS 2008	May 13	370 kg/ha 14N-14P-14 K-10.19S	Broadcast and incorporated
	June 28	140 kg/ha 34N- 0P-0K	Topdressing
PEI 2008	May 25	200 kg/ha 0N-20P-20K plus 240 kg/ha 21N-0P-0K- 21S	Broadcast and incorporated
	July 7	140 kg/ha 34N- 0P-0K	Topdressing
SK 2008	May 15	236 kg/ha 28.4N-14.2P-0K-11.8S	Incorporated
NS 2009	May 4	370 kg/ha 14N-14P-14 K-10.19S	Broadcast and incorporated
	June 26	140 kg/ha 34N- 0P-0K	Topdressing
PEI 2009	May 21	200 kg/ha 0N-20P-20K plus 240 kg/ha 21N-0P-0K-21S	Broadcast and incorporated
	July 15	150 kg/ha 34N- 0P-0K	Topdressing
SK 2009	May 16	114 19.5N-19.6P-0K-19.6S	Incorporated

Table 4.3 Soil characteristics of *C. sativa* genotype study in 2008

Location	pH	Organic Matter (%)	CEC (meq/100g)	P_2O_5 (kg/ha)	K_2O (kg/ha)	Ca (kg/ha)	Mg (kg/ha)	Sulfur (kg/ha)
NS	6.2	2.6	13.5	1033	266	2936	643	35
PEI	5.9	3.9	12.1	454	334	1866	228	48
SK	8.1	3.7	-	137	1229	-	-	19

Table 4.4 Soil characteristics of *C. sativa* genotype study in 2009

Location	pH	Organic Matter (%)	CEC (meq/100g)	P_2O_5 (kg/ha)	K_2O (kg/ha)	Ca (kg/ha)	Mg (kg/ha)	Nitrate-N(ppm)	N %	Sulfur (kg/ha)
NS	5.9	3.0	12.7	1562	348	2640	356	12.3	0.17	30
PEI	6.0	3.6	12.0	855	398	2612	148	33.1	0.24	30
SK	7.4	-	-	205	1301	-	-	24.8	-	30

Table 4.5 Genotype number, country of origin and source of seed for *C. sativa*

Genotype	Country of Origin	Source
CN101989	-	AAFC-SRC
SRS933	Former USSR	AAFC-SRC
CN101988	Denmark	AAFC-SRC
CN101982	Former Yugoslavis	AAFC-SRC
CN30475	Former USSR	AAFC-SRC
CN30476	Former USSR	AAFC-SRC
CN30478	Former USSR	AAFC-SRC
CN30479	Former USSR	AAFC-SRC
CN101981	Poland	AAFC-SRC
CN101985	-	AAFC-SRC
Calena	-	CDI- NSAC

AAFC- SRC: Agriculture and Agri-Food Canada in Saskatchewan

CDI- NSAC: Crop Development Institute in Nova Scotia Agricultural College

4.3 Measurements

4.3.1 Agronomic Traits

Plant emergence was measured approximately three weeks after planting. Two counts were completed in each plot by placing two 0.25 m^2 quadrants in the plot, avoiding the outside rows. 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. Days to the beginning of flowering was estimated visually as the date when approximately 10 % of plants had one flower open. Maturity date was also estimated visually as the date when approximately 95 % of the pods were brown (Urbaniak *et al.* 2008a). Lodging was visually evaluated at harvest using a scale of 1-5 with 1 being erect and 5 being flat on the ground. After drying to approximately 8 % moisture content seeds were cleaned using a Clipper (Clipper Seed Cleaning Co., Bluffton, IN) seed cleaner. Clean seed was weighed (g) and g/plot values were converted to kg/ha based on plot areas for each location. Climatic data (including weekly precipitation and temperature) at three sites (NS, PEI and SK) and two years (2008 and 2009) were obtained from Environment Canada on-line data (http:// www.climate.weatheroffice.ec.gc.ca/).

4.3.2 Downy Mildew [*Peronospora parasitica* (Pers. (ex Fr.) Fr.] Disease Rating

Two 0.5 m row segments were chosen specifically in a position of the plot which was the third row from the right side of each end of the plot. The total numbers of infected plants were counted in these two 0.5 m row sections in each plot. A plant was considered to be infected if it showed any level of infected symptom. The disease incidence was calculated by a percentage of infected plants in each plot. Since the variance of infected level between plots was significant, the visual ratings based on a 1-9 scale were taken for each plot (Appendix B). The final disease assessment for individual plot was calculated by the product of disease incidence and ratings number.

4.3.3 Oil and Protein Content

Total seed oil and protein content was determined on 5.0 g of whole seed using a near-infrared reflectance (NIR) spectroscopy (FOSS NIR Systems model 6500 spectrometer, spinning cup autosampler, WIN ISI II calibration software, Technicon Canada Inc., Mississauga, ON). Samples were analyzed in bulk by combining blocks 1 and 2, as well as 3 and 4. Oil and protein contents are reported on a dry matter basis.

4.3.4 Fatty Acid Analysis

The lipid extraction and methylation process were generated based on the protocol of Budge *et al.* (2006) with minor modification. Approximately 1.5 g of *C. sativa* seed was first soaked with 3 ml methanol in 50 ml tubes overnight. Then, 6 ml chloroform was added into each tube. This mixture was then placed in sonicator for 60 min followed by 20 min centrifuge. All the liquid was transferred into a 20 ml tube, followed by adding 3 ml of 7 % sodium chloride to the sample. This mixture was centrifuged (500 rpm) for 20 min. The top layer removed and discarded. The bottom layer was filter through glass funnel filled with sodium sulfate into another 20 ml test tube (chloroform was used in this step to filter the sample). The mixture was evaporated for approximately 20 min in nitrogen evaporator. After evaporation, the extracted oil was kept in 1.5 ml dichloromethane overnight.

The oil sample was methylated by adding 3 ml of Hilditch reagent (100ml methanol mixed with 1.5 ml sulfuric acid). Samples were incubated in a heat block set at 100 ^{o}C for 1 hour followed by cooling in the fridge for 20 min. Following cooling 3 ml hexane and 1.5 ml chloroform treated water were added and then vortex for 20 s. The upper layer was

transferred into a new 20 ml tube (Tube A). One ml was added to the original tube and vortexed for anther 20 s. The upper layer was again transferred into the new tube (Tube A). This step was repeated one more time and approximately 10 ml liquid was collected in tube A. Approximately 1ml chloroform treated water was added into the new tube (Tube A) followed by 3 min centrifuge (1000 rpm). The upper layer was transferred into another new tube filled with around 1/5 sodium sulfate. The samples were placed in a refrigerator (4 °C) for at least 20 min to encourage the water to be absorbed by sodium sulfate and then transferred the solvent (fatty acid methyl ester and hexane) into a pre-weighted new tube. Samples were evaporated in a nitrogen evaporator and a vacuum apparatus. Samples were weighed and the total FAME weight calculated. The final samples were kept in hexane at 50 mg/ml concentration. Before injection, the sample was diluted into 10 mg/ml. One µL of the sample was then injected into Gas Chromatograph with a run time of approximately 5 min. A HP INNOWAX fused silica column 7.5 m x 0.25 mm with a film thickness of 0.5 µL was used. The temperature program of 190-250 °C was selected to maximize sample throughput with a minimal loss in resolution. Flame ionization detection analysis was performed. Samples were analyzed as block 1 and 2 combined and block 3 and 4 combined.

4.3.5 Statistical Analysis

The experiment was designed as a (randomized complete block design) RCBD with four replications. The main factor in this study was 11 camelina genotypes. The response variables stand count, plant height, days to flowering, days to maturity, thousand kernel weight (TKW), seed yield, oil and protein content, fatty acid composition were collected and subjected to the PROC Mixed procedure in SAS version 9.1 (SAS Institute Inc., Cary, NC,USA, 2002-2003). Tukey test was used to compare the differences among treatments at 5 % significant level. PROC Mixed in SAS version 9.1 (SAS Institute Inc., Cary, NC, USA, 2002-2003) was also used for analyses of variance across environments, in which blocks were considered as random factors. Minitab statistical software version 14 (Minitab Inc., USA, 1972-2004) was used for all regression analysis.

4.4 Results

4.4.1 Plant Emergence (Stand Count/Seeding Rate)

The seedling emergence rates varied among three sites (Table 4.6). The seedling establishment differed among the field sites ranging from 23 % at NS in 2008 to a high of 62 % at PEI in 2009**.** Stand counts in NS were lower than at the PEI site in both years. In 2008, these values were not significantly different among genotypes and ranged from 98 to 139 plants/m^2 in NS and 160 to 266 plants/m^2 in PEI (Table 4.7). In 2009, a good emergence led to a uniform stand establishment in both NS and PEI site. The stand count ranged from 180 to 236 plants/m^2 in NS and 251 to 371 plants/m^2 in PEI (Table 4.8). No significant difference in stand count among genotypes was found in 2009. The stand count data in SK 2008 was not collected. Poor emergence and uneven stand establishment were observed in SK (9 - 15 %) in 2009 which may perhaps be due to a widespread drought and twice receiving frost at the seed germination stage. The difference of stand count among 11 genotypes in SK was not statistically significant due to the high variance within blocks (cv = 31.6 %).

Table 4.6 Overall percent emergence and plant height of *C. sativa* at sites in NS, PEI and SK (11 genotypes). 2008-09

Site	Year	Emergence (%)	Plant height (cm)
NS	2008	23	92
	2009	42	95
PEI	2008	44	83
	2009	62	99
SK	2008	-	82
	2009	12	83
Mean		27.2	84

4.4.2 Plant Height

In 2008, plant heights were significantly different among genotypes in NS and SK, which ranged from 86.5 to 99 cm in NS and 74.7 to 90 cm in SK; however, the differences in PEI were not significant (Table 4.7). The average plant height in PEI was 83 cm. In 2009, a significant genotype effect was found in PEI and SK sites (Table 4.8). The plant height ranged from 86 to 107 cm and 70 to 88 cm in PEI and SK sites

respectively. The average plant height in NS was 95 cm. Compared to the 2008 data, plants grew taller (16 cm) in PEI in 2009. The values in NS and SK sites were almost the same in two years, which was approximately 94 cm and 83 cm, respectively.

4.4.3 Thousand Kernel Weight (TKW)

TKW ranged from 0.77 to 1.38 g and was significantly different among all genotypes (Table 4.7 and 4.8). CN101981 had the highest TKW value while CN101982 and CN101989 had the lowest TKW at both PEI and NS sites in two years. The TKW for CN101981 was 1.08 g in 2008 and 1.38 g in 2009 in NS while 1.15 g in 2008 and 1.31 g in 2009 in PEI. Therefore, CN101981 may serve as a useful source of genes for improving the seed size.

4.4.4 Days to Flower and Days to Maturity

The genotype response on days to flower and days to maturity is listed in Table 4.9 and Table 4.10. Mean days to flower ranged from 45 to 49 days. CN101981 which required 45 days to flower was the earliest flowering variety while CN101982 had longest days to flower (49 days). The differences among genotypes in days to flower were not significant in 2008. However, there was a significant difference found in 2009 at all three sites. CN101981 and CN101985 required the shortest number of days to flower while CN30475 and CN30476 required the longest days to flower in all three sites. There was 7 days difference for mean maturity days among 11 varieties. CN101981 and CN101985 required 84 days to reach the maturity and were the earliest matured genotypes while CN30476 was the last variety to mature, requiring 91 days. This result suggests that CN101981 and CN101985 may be an important source of genes to improve earliness.

4.4.5 Seed Yield

The interactive effect of site, year and genotypes on seed yield of *C. sativa* was highly significant (Table 4.11), which indicated that seasonal conditions at each of the locations in a given year influenced seed yield. The significant higher yields of all *C. sativa* genotypes were observed at SK (2254 kg/ha), followed by PEI (1620 kg/ha); yields were generally low in NS (1036 kg/ha). The genotype effect on seed yield in all trials is shown in Table 4.12. Among sites, some genotypes did not maintain their high yields, confirming the significant presence of genotype-environment interaction. In NS, the genotype effect was not consistent across two years. CN101985 had the highest seed yield

of 1648 kg/ha in 2008, while CN30476 with 1720 kg/ha seed yield performed best in 2009. In PEI, all 11 genotypes achieved higher seed yield in 2008 than 2009. CN30479, with seed yield of 2044 kg/ha in 2008 and 1580 kg/ha in 2009, performed consistently best in two years. The seed yield of line CN101981 decreased in the largest magnitude from 2180 kg/ha to 780 kg/ha in 2008 and 2009, respectively. SRS933 had the lowest seed yield in both 2008 and 2009. In SK site, the difference in seed yield among 11 genotypes was not significant in 2008 and the average seed yield was 2188 kg/ha. In 2009, Calena and CN30479 had the highest seed yield while CN101985 had the lowest seed yield. Overall, genotype CN30479 maintained relatively significantly high yield values at five out of six sites, showing certain stability in the yield prediction. Therefore, CN30479 could be considered as a potential source for genes of high seed yield.

Table 4.7 *Camelina sativa* L. genotypic diversity in growth and yield responses in NS, PEI and SK in 2008

Genotype	Site								
	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK
	Plant stand (plants/m^2)			Plant height (cm)			TKW (g)		
CN101989	124 a	162a	N/A	90cd	78a	79cd	0.77b	0.78b	N/A
SRS933	122a	223a	N/A	92bcd	85a	85b	1.08a	1.18a	N/A
CN101988	110a	220a	N/A	97ab	87a	85b	0.98a	1.16a	N/A
CN101982	125a	266a	N/A	92bcd	81a	81bc	0.80b	0.88b	N/A
CN30475	103a	267a	N/A	89cd	83a	81bcd	1.03a	1.18a	N/A
CN30476	98a	212a	N/A	92bcd	84a	85b	0.97a	1.17a	N/A
CN30478	110a	190a	N/A	95abc	84a	85b	1.0a	1.15a	N/A
CN30479	117a	237a	N/A	99a	87a	90a	0.97a	1.12a	N/A
CN101981	139a	232a	N/A	86d	80a	75e	1.08a	1.15a	N/A
CN101985	129a	232a	N/A	93abcd	82a	77ed	0.78b	0.85b	N/A
Calena	99a	160a	N/A	88d	81a	81bc	0.97a	1.18a	N/A
P-value	0.726	0.244	N/A	0.013	0.05	0.000	0.001	0.000	N/A

Means within a column followed by different letters are significantly different ($p < 0.05$)
N/A- Data is not available

Table 4.8 *Camelina sativa* L. genotypic diversity in growth and yield responses in NS, PEI and SK in 2009

Genotype	Site								
	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK
	Plant stand (plants/m^2)			Plant height (cm)			TKW(g)		
CN101989	215a	317a	55a	97a	86c	80c	0.82d	0.84d	N/A
SRS933	180a	302a	61a	97a	105a	85ab	1.26a	1.12b	N/A
CN101988	236a	251a	53a	96a	100ab	85ab	1.09c	1.11b	N/A
CN101982	208a	371a	68a	94a	93bc	88a	0.83d	0.84d	N/A
CN30475	213a	309a	74a	98a	101ab	84ab	1.21b	1.17b	N/A
CN30476	215a	317a	57a	97a	98ab	83ab	1.25a	1.14b	N/A
CN30478	217a	326a	66a	96a	102ab	85ab	1.18bc	1.12b	N/A
CN30479	192a	281a	55a	98a	107a	87a	1.07c	1.07c	N/A
CN101981	198a	305a	69a	89a	100ab	70d	1.38a	1.31a	N/A
CN101985	218a	276a	51a	90a	96ab	81c	0.91d	0.83d	N/A
Calena	216a	329a	47a	96a	99ab	84ab	1.08c	1.11b	N/A
P-value	0.219	0.404	0.617	0.248	0.023	0.025	0.031	0.028	N/A

Means within a column followed by different letters are significantly different ($p < 0.05$)
N/A- Data is not available

Table 4.9 *Camelina sativa* L. genotypic diversity in developmental responses (days to flower) in three sites for two years

Genotype	Site and Year						
	NS 08	PEI 08	SK 08	NS 09	PEI 09	SK 09	Mean
CN101989	51a	N/A	47a	49a	43a	48ab	48
SRS933	52a	N/A	45a	49a	44a	48ab	48
CN101988	51a	N/A	46a	50a	44a	49a	48
CN101982	52a	N/A	48a	50a	43a	50a	49
CN30475	52a	N/A	47a	48ab	43a	49a	48
CN30476	52a	N/A	47a	50a	43a	49a	48
CN30478	51a	N/A	47a	47b	43a	48ab	47
CN30479	51a	N/A	45a	50a	43a	48ab	47
CN101981	51a	N/A	45a	44c	41b	45c	45
CN101985	51a	N/A	46a	43c	40b	45c	46
Calena	52a	N/A	47a	49a	44a	49a	48
P-value	0.645	N/A	0.579	0.021	0.034	0.027	

Means within a column followed by different letters are significantly different ($p < 0.05$)
N/A- Data is not available

Table 4.10 *Camelina sativa* L. genotypic diversity in developmental response (days to maturity) in three sites for two years

Genotype	Site and Year						
	NS 08	PEI 08	SK 08	NS 09	PEI 09	SK 09	Mean
CN101989	87d	81c	82d	90bc	N/A	89c	86
SRS933	89bc	82bc	84abc	96a	N/A	96ab	89
CN101988	89cd	83ab	83bcd	97a	N/A	94b	89
CN101982	90bc	81c	82cd	95ab	N/A	94b	88
CN30475	92a	84a	84abcd	96a	N/A	97ab	90
CN30476	91ab	84a	86a	97a	N/A	99a	91
CN30478	88cd	82bc	83bcd	92b	N/A	94b	88
CN30479	89bc	83ab	85ab	96a	N/A	96ab	90
CN101981	87d	79d	78e	87c	N/A	88c	84
CN101985	87d	78d	78e	88c	N/A	90c	84
Calena	90bc	84a	84abcd	94ab	N/A	93b	89
P-value	0.000	0.000	0.000	0.031	N/A	0.014	

Means within a column followed by different letters are significantly different ($p < 0.05$)
N/A- Data is not available

Table 4.11 ANOVA table for seed yield of 11 genotype *C. sativa* at NS, PEI, and SK in 2008 and 2009

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Genotype (G)	10	1096086	1096086	109609	3.81	0.000
Site	2	35767557	35767557	17883778	621.23	0.000
Year	1	3104940	3104940	3104940	107.86	0.000
G*Site	3	661330	661330	220443	7.66	0.000
G*Year	20	1414699	1414699	70735	2.46	0.001
Site*Year	10	1084089	1084089	108409	3.77	0.000
G*Site*Year	2	11001740	11001740	5500870	191.08	0.000
Block	20	2145441	2145441	107272	3.73	0.000
Error	195	5613583	5613583	28788		
Total	263	61889466				

Table 4.12 *Camelina sativa* L. genotypic diversity in yield response (kg/ha) in six site-years

Genotype	Site-year					
	NS08	PEI08	SK08	NS09	PEI09	SK09
CN101989	1380b	1964bc	2190a	1312bc	1230c	2268bc
SRS933	1292b	1716d	2348a	1476b	895ef	2229bc
CN101988	1364b	1964bc	2175a	1113c	1350bc	2245bc
CN101982	1348b	1900cd	2117a	1301bc	1461ab	2252bc
CN30475	1292b	2197a	2049a	1483b	1014de	2383bc
CN30476	1292b	2032abc	2328a	1720a	1375bc	2387bc
CN30478	1356b	2048abc	2235a	1446b	1059d	2315bc
CN30479	1428ab	2044abc	2088a	1329bc	1580a	2457ab
CN101981	1456ab	2180a	2045a	1291bc	780f	2207c
CN101985	1648a	2104ab	2251a	1338b	136bc2	2155c
Calena	1308b	2020abc	2242a	1432b	1357bc	2626a
P-value	0.025	0.022	0.699	0.0014	<0.0001	0.0171

Means within a column followed by different letters are significantly different ($p < 0.05$)

4.4.6 Oil and Protein Content

The result of analysis of variance (Table 4.13) indicated that the three-way interaction between genotype, site and year did not significantly affect oil content. The genotype x year effect was very small and was not significant, which suggests that the genotype effect is quite stable in each site. Significant genotype x site effect on oil content was observed. All 11 genotypes had significantly higher oil content in SK than NS and PEI. Genotype CN30476 showed significantly higher oil content in all sites. The genotype x site x year interaction was highly significant for protein content, which suggest that this trait was not stable across all three contrasting environments. Among 11 accessions, CN30476 had the lowest protein content in all sites. An overview of variation for oil and protein content across all 11 genotypes of *Camelina sativa* in three sites is shown in Tables 4.15 and 4.15. The results indicated a significant genetic diversity in *C. sativa* seed quality parameters (oil content and protein content) was present in *C. sativa* germplasm. When average oil and protein content across year-sites, the value ranged from 38.03 % (CN101981) to 40.72 % (CN30476) and 27.37 % (CN30476) to 29.89 % (CN30478), respectively.

4.4.7 Fatty Acid Compositions

The fatty acid composition of 11 genotypes in three sites is shown in Tables 4.16-4.20. *Camelina sativa* oil was primarily made up by poly- and mono-unsaturated fatty acids with linolenic (30.51-36.20 %), linoleic (15.09- 21.05 %), oleic (11.34- 17.26 %) and eicosenic acid (11.79-15.01 %), and less amounts of erucic acid (2.59-4.00 %). Saturated fatty acids constituted only 9.17-11.19 % of the total fatty acids which included mainly palmitic (4.62-6.54 %) and stearic acid (2.08-2.79 %). Data from five trials were pooled together to evaluate the effect of genotype and environment on fatty acid composition. The p-value of analysis of variance (Table 4.21) shows that GE (genotype x environment) interaction effects occurred for all major fatty acid components. However, the F-value (Table 4.22) indicated that the size of GE effect was always smaller than that of purely genotype and environment effects. For instance, the genotypic F-value was from 16-fold (linolenic acid) to 40-fold (palmitic acid) greater than the value of GE effect. Much larger extent of genotype effect suggests the high broad sense heritability of these traits. The mean value of fatty acid components across environments is listed in Table

4.23. CN101989 had the highest amount of linolenic (18:3) acid (35.12 %) in all sites, which suggests that this genotype could be an important source of genes for high linolenic acid content. CN30479 had the highest linoleic acid content (20.3 %) across the environment. CN101985 had the highest amount of stearic and oleic acid but contained the lowest amount of linoleic acid across all sites. CN101988 had the highest eicosenoic acid content (14.37 %) in all trials. Therefore, all these genotypes can be used as an important source of gene to modify the fatty acid profile. The correlation coefficient value for the main fatty acids is presented in Table 4.24. The results showed that oleic acid is highly and positively correlated with stearic acid content. The negative relationship between linoleic acid and linolenic acid content was also found in this study. An overview of variation in fatty acid concentrations for the three sites is shown in Table 4.25. The means of oleic and linolenic amounts differed by more than 2 % across the three environments. For the other fatty acids, the mean differed by less than 2 %. The means for 16:0, 18:2 relative amounts under the three environments were in the order: NS > PEI > SK, whereas for 18:3 relative amounts, the means from high to low were in the exact opposite order. Furthermore, the means for saturated fatty acid (16:0, 18:0 and 20:0) from high to low were in the order of 10.1 % NS, 9.6 % PEI, and 9.1% SK; while mean relative amounts of unsaturated in the three environments were in the exact opposite order which were 87.0 % in SK, 85.3 % in PEI and 85 % in NS in 2008.

Table 4.13 ANOVA table for oil and protein content of 11 genotype *C. sativa* at NS, PEI and SK in 2008 and 2009

Effect	Oil content			Protein content		
	Num DF	F-value	P-value	Num DF	F-value	P-value
Site	2	619.46	<.0001	2	234.23	<.0001
Year	1	100.49	<.0001	1	98.09	<.0001
Site*Year	2	74.05	<.0001	2	23.70	<.0001
Genotype (G)	10	17.00	<.0001	10	28.97	<.0001
G*Site	20	1.93	0.0246	20	2.02	0.0177
G*Year	10	1.45	0.1784	10	2.26	0.0244
G*Site*Year	20	1.52	0.1031	20	1.83	0.0346

Table 4.14 Diversity in oil and protein content of 11 *C. sativa* genotypes in 2008

Genotype	NS		PEI		SK	
	Oil (%)	Protein (%)	Oil (%)	Protein (%)	Oil (%)	Protein (%)
CN101989	37.09a	28.54abc	37.83cde	30.17ab	40.68defg	28.42bc
SRS933	38.55a	28.91abc	38.28bcde	30.0abc	41.38cde	29.76a
CN101988	37.27a	28.70abc	37.87bcde	29.87abc	40.49efg	27.99bcd
CN101982	37.35a	29.27a	37.6e	30.53a	41.55bcd	28.30bc
CN30475	38.31a	28.06bc	39.7a	28.23d	41.78bc	26.79de
CN30476	39.44a	26.95d	40.0a	27.73d	43.14a	26.31e
CN30478	37.39a	28.97ab	37.73ed	30.46a	39.84g	29.24ab
CN30479	36.94a	28.29abc	38.59b	29.35bc	42.50ab	26.62e
CN101981	37.40a	28.39abc	38.51bc	29.46bc	40.33fg	28.67abc
CN101985	38.73a	27.89cd	38.57bc	30.02ab	41.62bcd	28.08bcd
Calena	36.62a	28.10bc	38.38bcd	29.08c	40.85cdef	27.47cde
p-value	0.259	0.028	0.0003	0.0002	0.0003	0.002
Mean	37.69	28.37	38.46	29.54	41.29	27.97

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 4.15 Diversity in oil and protein content of 11 *C. sativa* genotypes in 2009

Genotype	NS		PEI		SK	
	Oil (%)	Protein (%)	Oil (%)	Protein (%)	Oil (%)	Protein (%)
CN101989	37.27bc	28.9ab	35.39cd	31.94b	41.54def	28.92ab
SRS933	37.90b	29.09ab	36.32bc	31.81bc	42.62b	29.25ab
CN101988	36.95bc	28.29bc	35.60cd	30.38d	40.86fg	28.72bc
CN101982	36.96bc	28.88ab	35.72cd	31.81bc	40.93efg	29.27ab
CN30475	37.30bc	28.61ab	36.34bc	31.07cd	42.21bcd	27.92d
CN30476	39.60a	27.43c	38.51a	28.74e	43.60a	27.06e
CN30478	37.99b	29.21a	35.05d	32.03b	41.05efg	29.45ab
CN30479	37.63bc	28.37ab	37.06b	30.31d	42.42bc	27.82de
CN101981	37.60bc	29.02ab	33.62d	33.34a	40.73g	29.69a
CN101985	37.98b	28.96ab	36.33bc	31.30bcd	41.62de	29.00ab
Calena	36.38c	28.57ab	36.35bc	30.70d	41.83cd	28.09cd
p-value	0.0469	0.0351	<.0001	<.0001	<.0001	<.0001
Mean	37.60	28.67	36.03	31.22	41.76	28.65

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 4.16 Diversity in fatty acid composition of *C. sativa* genotypes in 2008 in NS

Genotype	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3	Eicosenoic 20:1	Erucic 22:1	Others	Saturated
CN101989	5.90bcd	2.70a	13.81bc	18.06d	33.31ab	13.57cde	3.05efg	2.67a	10.89abc
SRS933	5.41e	2.43c	13.55c	18.56cd	33.41a	13.35e	3.51bc	2.52a	10.25e
CN101988	5.75cd	2.52bc	14.30abc	17.50de	32.21b-e	14.18a	3.62ab	2.89a	10.75cd
CN101982	6.05b	2.68ab	14.78ab	18.41d	32.54a-d	13.79bcd	2.83g	2.54a	10.88bc
CN30475	6.10b	2.39c	13.70c	19.83ab	31.51def	13.39e	3.18def	2.98a	10.66cd
CN30476	5.87bcd	2.38c	13.44cd	20.10ab	31.22ef	13.78bcd	3.33cd	2.67a	10.49de
CN30478	5.98bc	2.45c	13.82bc	19.67bc	32.77abc	12.48g	3.00fg	2.84a	10.61cd
CN30479	5.99bc	2.68ab	13.59c	20.92a	30.68f	12.96	3.25de	2.63a	11.10ab
CN101981	6.42a	2.42c	12.47d	19.79b	31.65c-f	13.48de	3.29cd	2.86a	11.19a
CN101985	5.66de	2.84a	15.25a	16.64e	33.59a	13.87abc	3.02fg	2.50a	10.87bc
Calena	5.95bc	2.41c	12.53d	18.25d	32.54a-d	13.96ab	3.79a	2.97a	10.85bc
p-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.805	0.0016

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 4.17 Diversity in fatty acid composition of *C. sativa* genotypes in 2008 in PEI

Genotype	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3	Eicosenoic 20:1	Erucic 22:1	others	saturated
CN101989	5.66ef	2.39c	13.14d	16.45e	36.10a	13.44bc	3.04g	2.80a	10.15de
SRS933	5.18h	2.34cd	12.34e	17.92cd	34.44cde	13.61b	3.73b	3.05a	9.90fg
CN101988	5.67ef	2.32de	13.6c	16.29ef	34.16def	14.15a	3.64c	3.01a	10.32bcd
CN101982	5.88c	2.52ab	14.36b	17.52d	34.23cde	13.60b	2.79i	2.68a	10.41ab
CN30475	6.02b	2.29de	13.69c	18.75ab	33.23fg	13.53b	3.10f	2.40a	10.35abc
CN30476	5.48g	2.21fg	13.03d	18.07cd	33.63efg	13.92a	3.46d	2.69a	9.82g
CN30478	5.74de	2.26ef	13.50c	18.13cd	34.71cd	13.09d	3.14f	2.22a	10.09ef
CN30479	5.79cd	2.46b	13.05d	19.01a	32.98g	12.79e	3.26e	3.22a	10.55a
CN101981	6.23a	2.16g	12.30e	18.29bc	33.92defg	13.25cd	3.28e	3.02a	10.51ab
CN101985	5.49g	2.54a	14.96a	15.83f	35.11bc	13.58b	2.98h	2.93a	10.17cde
Calena	5.58fg	2.17g	11.83f	16.02ef	35.68ab	13.98a	3.84a	3.06a	10.02ef
P-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.59	<.0001

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 4.18 Diversity in fatty acid composition of *C. sativa* genotypes in 2008 in SK

Genotype	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3	Eicosenoic 20:1	Erucic 22:1	others	saturated
CN101989	5.12d	2.53cd	15.41de	15.87d	36.18a	13.98d	2.61de	2.04	9.63c
SRS933	4.62f	2.27ef	13.66f	17.28b	35.93a	13.61f	3.38a	2.07	9.17e
CN101988	5.14d	2.48d	16.27bc	15.86d	33.72d	15.01a	3.30a	1.75	9.86b
CN101982	5.33b	2.55bc	16.85ab	16.61c	34.89c	13.78e	2.35f	1.98	9.68c
CN30475	5.60a	2.49cd	15.70cd	19.07a	32.25f	13.97d	2.70cd	1.96	10.07a
CN30476	5.18cd	2.27ef	15.21de	18.89a	32.91e	14.20c	2.90b	1.90	9.38d
CN30478	5.12d	2.32e	15.05e	17.51b	35.60ab	13.03h	2.76c	1.97	9.39d
CN30479	5.38b	2.61b	16.17c	19.17a	32.24f	13.29g	2.64de	2.07	10.05a
CN101981	5.56a	2.21f	13.63f	17.56b	34.98bc	13.63ef	2.98b	2.28	9.85b
CN101985	4.91e	2.79a	17.26a	15.09e	35.10bc	14.14c	2.59e	2.09	9.87b
Calena	5.28bc	2.33e	14.23f	16.62c	34.74c	14.48b	3.32a	2.07	9.78bc
p-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.5116	<.0001

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 4.19 Diversity in fatty acid composition of *C. sativa* genotypes in 2009 in NS

Genotype	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3	Eicosenoic 20:1	Erucic 22:1	others	Saturated
CN101989	5.76b	2.61bc	12.81d	17.51f	36.20a	14.33bc	3.20c	1.08	10.71bc
SRS933	5.27c	2.46d	12.87d	19.18c	34.05c	13.72e	3.64a	1.08	10.32de
CN101988	6.00a	2.55c	13.22c	18.34de	32.91d	14.62a	3.78a	1.24	11.15a
CN101982	6.05a	2.70a	13.73b	18.42d	34.12c	14.17cd	2.95cd	1.12	10.95ab
CN30475	6.19a	2.48d	13.20c	20.46b	32.38ef	13.86e	3.18c	1.07	10.94ab
CN30476	5.66b	2.28e	13.24c	20.01b	32.77de	14.06d	3.44b	1.09	10.17e
CN30478	5.79b	2.44d	13.75b	19.04c	35.57b	12.45g	2.76d	1.13	10.34de
CN30479	6.07a	2.66ab	12.87d	20.93a	32.22f	13.42f	3.29b	1.14	11.16a
CN101981	6.13a	2.08f	11.34e	17.92e	35.82ab	14.26bc	3.43b	1.12	10.29de
CN101985	5.64b	2.69ab	14.17a	16.07g	36.20a	14.40b	2.98cd	1.09	10.58cd
Calena	6.12a	2.41d	11.56e	18.18de	35.43c	14.37b	4.00a	1.18	11.13a
p-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.2545	0.0001

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 4.20 Diversity in fatty acid composition of *C. sativa* genotypes in 2009 in PEI

Genotype	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3	Eicosenoic 20:1	Erucic 22:1	others	Saturated
CN101989	5.96d	2.49b	13.70d	18.57de	34.59a	13.15c	3.09de	1.64a	10.72b
SRS933	5.64f	2.38cd	13.43d	21.04ab	31.83e	12.96cd	3.69b	1.56a	10.67b
CN101988	5.93de	2.40c	14.40c	18.04e	32.84d	13.93a	3.71b	1.78a	10.80b
CN101982	6.00d	2.60a	14.91b	19.17cd	32.90d	13.42b	2.94f	1.65a	10.75b
CN30475	6.26b	2.32de	14.34c	21.18ab	31.29e	13.02c	3.12de	1.62a	10.76b
CN30476	5.77ef	2.29e	14.14c	21.61a	30.51f	13.57b	3.41c	1.54a	10.31c
CN30478	6.23bc	2.41c	14.27c	20.67b	33.43cd	11.79f	2.77g	1.64a	10.77b
CN30479	5.99d	2.50b	14.12c	21.50a	31.37e	12.57e	3.19d	1.66a	10.84b
CN101981	6.54a	2.12f	11.71f	19.77c	33.92bc	12.71de	3.51c	1.78a	11.09a
CN101985	5.74f	2.60a	15.29a	17.19f	34.44a	13.55b	3.07e	1.52a	10.64b
Calena	6.08cd	2.28e	12.39e	18.61de	33.88bc	13.62b	3.84a	1.68a	10.82b
p-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.7793	0.0045

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 4.21 P-value of analysis of variance for *Camelina sativa* fatty acid components

Effect	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3	Eicosenoic 20:1	Erucic 22:1	Others	Saturated
Genotype	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.2663	<.0001
Environment	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
G*E	0.0004	<.0001	0.0109	<.0001	<.0001	<.0001	0.0014	0.9036	<.0001

Table 4.22 F-value of analysis of variance for *Camelina sativa* fatty acid components

Effect	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3	Eicosenoic 20:1	Erucic 22:1	Others	Saturated
Genotype	88.66	107.07	60.49	159.03	84.93	150.73	140.36	1.28	25.15
Environment	305.37	58.09	126.51	299.34	143.22	158.65	104.00	130.49	237.48
G*E	2.67	3.64	1.95	3.75	5.34	5.23	2.41	0.67	3.34

Table 4.23 Mean value of fatty acid components across environments

Genotype	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3	Eicosenoic 20:1	Erucic 22:1	Others	Saturated
CN101989	5.68ef	2.54b	13.77d	17.29e	35.12a	13.69de	3.00e	2.05a	10.42bc
SRS933	5.23h	2.38d	13.17e	18.78c	33.94cd	13.44f	3.59b	2.00a	10.06d
CN101988	5.7def	2.45c	14.36bc	17.21e	33.17e	14.37a	3.61b	2.13a	10.58ab
CN101982	5.86c	2.61b	14.92ab	18.03d	33.74de	13.75cd	2.77f	1.99a	10.53ab
CN30475	6.03b	2.39cd	14.12cd	19.86ab	32.13f	13.55ef	3.06d	2.01a	10.56ab
CN30476	5.59fg	2.29f	13.81cd	19.74b	32.21f	13.91bc	3.31c	1.98a	10.03d
CN30478	5.77cde	2.37de	14.08cd	19.00c	34.41bc	12.57h	2.89ef	1.96a	10.24cd
CN30479	5.84c	2.58b	13.96cd	20.30a	31.90f	13.01g	3.13d	2.14a	10.74a
CN101981	6.18a	2.2g	12.29f	18.66c	34.06cd	13.47f	3.3c	2.21a	10.59ab
CN101985	5.49g	2.69a	15.39a	16.16f	34.89ab	13.91bc	2.93ef	2.03a	10.42abc
Calena	5.82cd	2.31e	12.36f	17.51e	34.21cd	14.06b	3.79a	2.26a	10.54ab

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 4.24 Summary of Pearson correlation coefficient describing the relationship among the essential fatty acid components in *C. sativa* oil

	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2
Oleic (18:1)	-0.488***	0.586***		
Linoleic (18:2)	0.573***	-	-0.347**	
Linolenic (18:3)	-0.613***	-	-	-0.815***
Eicosenoic (20:1)	-0.378**	-	-	-0.555***
Erucic (22:1)	-	-0.455**	-0.766***	-

*Significant at the 0.05 level of probability.
** Significant at the 0.01 level of probability.
*** Significant at the 0.001 level of probability.

Table 4.25 Overview of variation in individual fatty acid concentration in three different macro-environments (n =11 genotypes in 2008 and 2009)

Parameter	Fatty acid concentration (%)										
	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C20:3	C22:0	C22:1
NS2008											
Minimum	5.38	2.33	11.92	16.21	30.58	1.49	12.46	1.50	1.16	0.33	2.80
Maximum	6.58	2.86	15.64	20.96	34.00	1.82	14.35	2.28	1.39	0.39	3.84
Mean	5.91	2.54	13.75	18.88	32.31	1.66	13.53	1.92	1.28	0.36	3.26
CV (%)	4.55	6.46	6.39	6.89	3.11	6.40	3.58	10.35	5.87	5.66	9.01
PEI 2008											
Minimum	5.18	2.14	11.72	15.78	32.87	1.41	12.79	1.53	1.30	0.32	2.79
Maximum	6.28	2.57	15.19	19.08	36.62	1.74	14.15	2.2	1.69	0.39	3.85
Mean	5.70	2.33	13.25	17.48	34.38	1.54	13.54	1.93	1.45	0.35	3.30
CV (%)	4.9	5.68	6.78	6.45	2.91	6.48	2.92	10.72	7.49	6.73	9.93
SK 2008											
Minimum	4.62	2.21	13.50	15.07	31.90	1.27	12.96	1.38	1.15	0.28	2.32
Maximum	5.63	2.82	17.35	19.32	36.5	1.63	15.09	2.07	1.54	0.36	3.39
Mean	5.20	2.44	15.40	17.23	34.41	1.47	13.92	1.76	1.34	0.32	2.86
CV (%)	5.26	7.19	7.79	7.89	4.07	7.72	3.85	11.81	9.24	6.99	11.72
NS 2009											
Minimum	5.25	2.07	11.26	15.97	32.05	1.38	12.39	1.63	1.23	0.33	2.75
Maximum	6.35	2.71	14.19	20.96	36.26	1.92	14.65	2.37	1.73	0.43	4.02
Mean	5.88	2.49	12.98	18.73	34.15	1.66	13.97	2.01	1.42	0.37	3.33
CV (%)	4.82	7.45	6.56	7.33	4.15	9.74	4.28	10.35	9.69	8.12	11.05
PEI 2009											
Minimum	5.62	2.11	11.60	17.15	30.10	1.38	11.70	1.51	1.09	0.34	2.69
Maximum	6.55	2.61	15.33	21.93	34.63	1.83	13.98	2.19	1.43	0.43	3.90
Mean	6.01	2.40	13.88	19.76	32.82	1.62	13.12	1.84	1.21	0.38	3.30
CV (%)	4.31	5.97	7.4	7.65	4.17	7.14	4.55	10.78	8.44	6.84	10.38

4.5 Discussion

Successful plant establishment is a crucial step for achieving the optimum yield performance for a small seeded crop. Gugel and Falk (2006) noted that seeding equipment used to sow canola and mustard posed no problems to sow *C. sativa*. Zubr (1997) also reported that the ordinary combine harvester which is used for harvesting rapeseed needed no modification to harvest *C. sativa,* other than adjustment in sieves and wind. Good emergence and uniform stand establishment observed in NS and PEI 2009 suggested that the ordinary seeder used for canola provided satisfactory establishment.

The significant diversity of agronomic and seed quality traits were found in this study among the selected genotypes, which suggests that *C. sativa* has a high potential to be further improved to become a more attractive oilseed crop. The seed yield of *C. sativa* was significantly influenced by the genotype and environment effect. The ANOVA analysis results (Table 4.7) indicated that location had a dominant effect on seed yield. Similar location effect on seed yield of *C. sativa* was reported by Vollmann *et al.* (1996) in Austria, Francis and Campbell (2003b) in Australia and Putnam *et al.* (1993) in the United Sates. Yield rankings of the 11 genotypes were different at each location (Table 4.8). The mean seed yield of 11 genotypes was highest in SK, followed by PEI, and then NS. The high seed yield achieved in SK site can be explained by several reasons. First, different branching architecture responses to SK environment conditions might be one crucial factor contributed to the high seed yield. Seed yield components such as the number of branches/plant plays an essential role in the seed yield. The heat and drought shock during the branching stage at the SK site might also depress the apical dominance. The poor stand count and relatively high yield in 2009 in SK suggested that *C. sativa* may change its phenotypic behavior to be more an indeterminate type under a less competitive environment. Plants with weaker apical dominance produce intensive branching (number of branch/plant in chapter 5), which could have contributed to the higher seed yield. Secondly, much higher amount of precipitation (Appendix A) occurred in the seed filling stage than the vegetative stage, which would have provided a suitable environment for seed and oil development. Although the actual amount of precipitation in SK is lower than NS and PEI, the soil with higher amount of clay in SK has the better water holding ability and thus may provide more available water during the seed-filling stage. The year

effect on seed yield in PEI was dramatically obvious. The seed yield of all 11 lines was significantly higher in 2008. There are many possible reasons for the lower seed yield at PEI in 2009. First, high amount of soil available nitrogen led to an excessive vegetative growth, which was reflected by the plant height. The plants in PEI site were approximately 20 cm higher than in 2008. Secondly, severe lodging problems (Figure 4.5) caused by high soil nitrogen residual from the previous red clover crop(*Trifolium pratense* L.) and intense rainfall, which occurred on June 14 (approximately 61.2mm) led to a large amount of axillary branches during the reproductive stage. Plants partitioned the energy for branching rather than seed filling, and then resulted in the extensive yield loss. Thirdly, the most susceptible stages of plant growth to downy mildew for *C. sativa* are from flowering to seed mature period. The relatively cooler weather and less amount of rainfall in July (Appendix A) could have decreased the downy mildew incidence in PEI (no difference was found among varieties and all infected plots were visually rated as level 1 infection), however, it may also reduce the pollination and then contribute to the depressing yield. Last but not least, seeds were lost in the seed cleaning process since the seeds tended to stick together in clumps and could not pass through the sieve. It is worth noting that warm and humid mid-season conditions in 2008 also caused the high disease incidence at PEI sites (Figure 4.1). There was a strong genotype effect on disease rate in PEI site, CN101982 and Calena had the highest disease incidence while CN30475 and CN101981 were more resistant to the downy mildew (Figure 4.1). CN101982, which had some severely lodged plots (Figure 4.2), this created the much more moist conditions under the lodged canopies and more plant-to-plant contact resulting in significantly higher levels of disease. The comparatively higher seed yield at PEI in 2008 suggested that the downy mildew might only have slight negative effect on seed yield. Genotypes responded differently in NS in 2008 and 2009, therefore, it is difficult to recommend particular lines in NS according to seed yield. Low stand count in 2008 may be a major reason for the significantly low seed yield. It is worthwhile to noting that even though the lab test germination rate for all the 11 genotypes was approximately 95 %, the actual plant stand achieved in the field was only from 19.7 % to 28.7 % in NS in 2008. This result was lower than the findings reported by Agegnehu and Honermeier (1997) who indicated that the emergence rate was from 38 % to 45 %. High amount of rainfall

followed by dry weather, there may have been some soil crusting, which prevented emergence of some seedlings in 2008. Generally, the actual emergence rate is likely dependent on the environmental conditions and will differ from year to year. Overall, CN30479 had significantly higher seed yield in five out of six trials, which suggested that this genotype could be an important source of gene for seed yield improvement.

The oil content from 36.62 % to 43.14 % in 11 genotypes is consistent with the previous studies reported by Vollmann *et al.* (2005) and Budin *et al.* (1995). The results of analysis of variance indicated that the effect of sites was predominant for both oil and protein content. Generally, seed quality characteristics, mainly oil and protein content are strongly affected by the temperature and precipitation during seed fill. Higher temperature in the seed filling stage tends to enhance protein content while simultaneously decreasing the oil content (Vollmann *et al.* 2000). Furthermore, nitrogen availability during the seed-filling stage is another crucial factor influencing the seed protein content. The seeds from SK tended to be high in oil content and seed from PEI tended to be high in protein; this is support for this observation. High rates of precipitation and low temperature in SK (Appendix A) during the seed-filling period may result in high oil and low seed protein content. In PEI, high temperatures during the seed-filling period clearly favored protein instead of oil synthesis. Compared with PEI site, the lower protein content in NS was probably due to insufficient soil nitrogen since the applied N may be leached from the soil due to heavy rainfall after the second N application in 2008. In 2009, high amount of soil available N could be the major reason for high protein content in PEI. Overall, CN30476 had significantly higher oil content and lower protein content in all trials. Significant effect of genotype and environment on fatty acid composition was observed in this study. The similar result was found by Budin *et al.* (1995), Zubr and Matthäus (2002) and Gugel and Falk (2006). The greater environment effect on fatty acid composition was found by Zubr and Matthäus (2002) while the greater genotype effect was also observed by Francis and Campbell (2003b) and Gugel and Falk (2006). The GE interaction effect was fairly small in comparison with genotype and environment effects in this study, which suggested genotype effect on fatty acid composition was consistently across environments. Genotypes with high amount of specific fatty acid found in this study can be useful sources of germplasm for altering camelina oil quality.

The correlation between seed size and the seed yield has been studied by many researchers. Vollmann *et al.* (1996) reported that larger seeded *C. sativa* corresponds to a decline in seed yield and oil content but Gugel and Falk (2006) reported that large seed size strains did not necessarily have the lowest oil content. Vollmann *et al.* (2005) reported that oil content and seed weight varied in genotypes. The positive correlation between seed yield and oil content was found by Vollmann *et al.* (1996) which makes it possible to enhance both characteristics at the same time. However, there was no correlation between TKW, seed oil content and seed yield ($p > 0.05$) in this study, which suggested that increasing seed size may not necessarily lead to high oil and seed yield. Improving the seed oil content and other seed quality characteristics such as fatty acid composition would undoubtedly enhance the attractiveness of *C. sativa* crops as a field crop and thus increase the competitiveness of *C. sativa* in the market of oilseed crops. The recent genetic study on this new oilseed crop is fairly limited and more research is needed.

In conclusion, the overall result showed that useful genetic variation in agronomic and seed quality characteristics was found among 11 genotypes. For example, CN30479 with the high yield potential and high yield stability across the environments can be an important source for yield improvement; CN30476 had the consistently higher oil content in all trials can be used as gene source for enhancing oil content; CN101989 with highest amount of linolenic acid content and CN30479 with highest amount of linolenic acid can be the potential sources of gene for modifying the fatty acid profile; SRS933 with high protein content can be developed into a desirable seed meal for poultry, swine and ruminants; CN101981 and CN101985 with the earliest flowering and maturity date could be a useful source for earliness gene. The other positive agronomic traits found among these 11 genotypes included highly downy mildew resistant lines CN101981 and CN30475. This result indicates a high potential of developing improved *C. sativa* germplasms for Maritime and western environments in Canada.

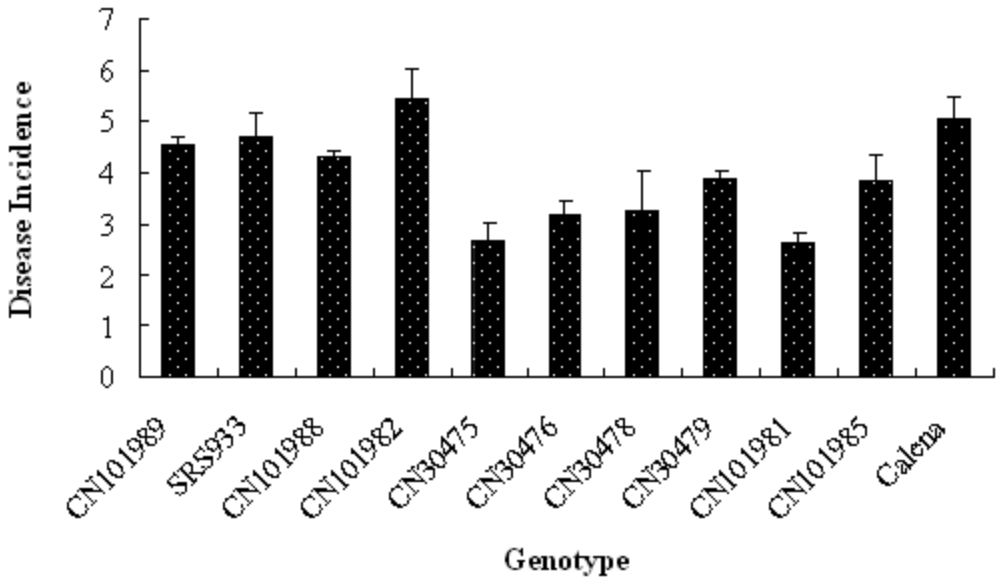

Figure 4.1 Downy mildew infection data in PEI (2008)
(Note: data was transformed in power ¼ in order to achieve normality)

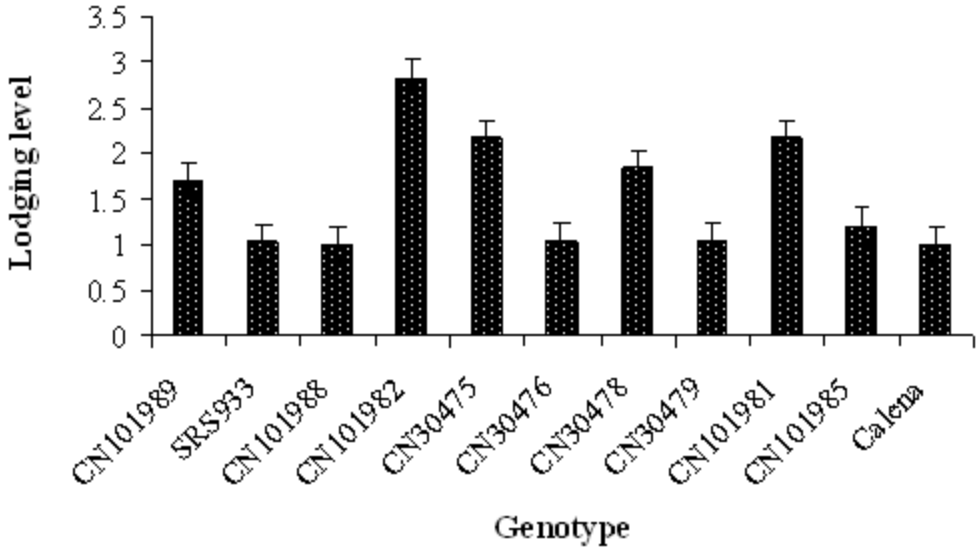

Figure 4.2 Lodging level of 11 genotypes in PEI (2008)

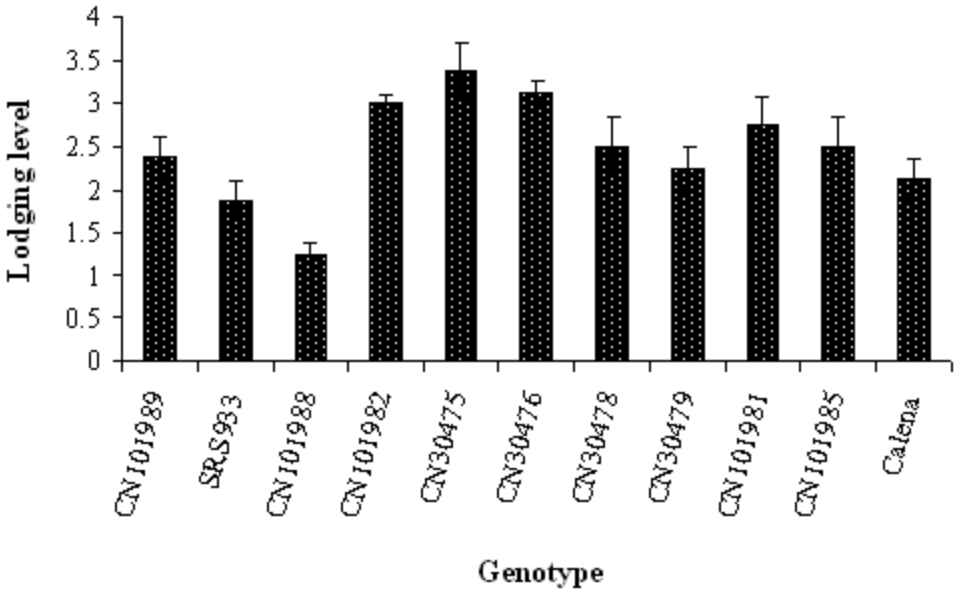

Figure 4.3 Lodging level of 11 genotypes in PEI (2009)

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Chapter 5 Seeding Rate and Nitrogen Management Effects on *Camelina sativa* (L.) Crantz Seed Yield and Seed Quality

5.1 Introduction

When introducing new crops, it is necessary to assess the appropriate production management for different environments. Among the many agronomic factors, seeding rate and N application are considered to be key management factors to achieve maximum economic returns with acceptable quality (Otteson *et al.* 2007).

Plant stand has significant effects on plant competition, branching, pod set and on the microclimate beneath the canopy. Increasing plant stands by increasing the seeding rate does modify the microclimate and might be one of the major factors contributing to an increase in plant-to-plant disease spread. Results of previous research exploring the optimum seeding rate of successfully growing *C. sativa* are variable. Agegnehu and Honermeier (1997) reported that increasing the seeding rate to 800 seeds/m^2 decreased the number of branches/plant and found that the seeding rate of 400 seeds/m^2 was the most efficient method to achieve the greatest yield in *C. sativa*. Researchers in Ireland recommended that the lower rate of 500 seeds/m^2 was most suitable (Crowley and Fröhlich 1998). Other researchers, including Zubr (2003), reported that *C. sativa* can be successfully grown under higher seeding rates of 600 seeds/m^2. The study conducted by Urbaniak *et al.* (2008b) suggested that seeding rates from 400 to 600 seeds/m^2 would be the most appropriate option. Current seeding rate recommendations for *C. sativa* are variable and highly dependent on seedbed conditions and environment.

Nitrogen, which serves as a constituent of many plant cell components, including amino acids and nucleic acids, plays an important role in plant growth (Lincoln 2002). With the increasing concern over nitrogen contamination of soil and nitrate leaching problems, combined with the increased cost, nitrogen fertility management has become a popular research topic (Blanc *et al.* 1979; Matsumoto *et al.* 2000). In order to decrease input costs while maintaining optimal crop productivity, a close assessment of the nitrogen requirements of *C. sativa* is needed. However, nitrogen recommendations are difficult to make because of the dynamic changes in soil-available N. The soil-available nitrogen concentration, uptake, transport and utilization are related to the environmental factors such as soil temperatures and rainfall. The results of previous research on nitrogen

requirement in *C. sativa* are somewhat variable. According to studies conducted in Europe by Zubr (2003), the nitrogen requirement in *C. sativa* is moderate to low and the optimum N supply is about 100 kg/ha. Researchers in Ireland found that the optimum economic response to nitrogen application for *C. sativa* is 75 kg/ha (Crowley and Fröhlich 1998). Bugnarug and Borcean (2000) reported that yield increased up to 58 % at 100 kg N/ha in Romania. Urbaniak *et al.* (2008a) in Truro, NS and Hartland, NB, found that *C. sativa* did not appear to be a low input crop; seed yield was positively correlated to N rates as high as 120 kg/ha. In response to nitrogen input, increased nitrogen application can cause higher levels of lodging (Strasil and Skala 1995; Crowley 1999) and higher disease infection (Crowley 1999), which contribute to poor yield performance (Murray 2000). Agegnehu and Honermeier (1997) found that the amount of nitrogen supplied greatly influenced the formation of *C. sativa* yield components. The number of branches, pods, seeds per pod, and seed weight increased as the nitrogen application rate increased. He also reported that, when the level of nitrogen increased, the oil content is dramatically decreased. The highest yield was obtained by supplying 120 kg N/ha with a seeding rate of 400 seeds/m^2. Zubr (1997) suggested that the nitrogen fertilizer should be supplied at the four to six leaf stage for spring annuals but early in the spring for the winter annuals in order to avoid the loss of nitrogen leaching.

The results of nitrogen requirement and optimum seeding rate of *C. sativa* were inconclusive and varied among the different regions of the world; further evaluation of nitrogen and seeding rate effect on *C. sativa* is needed. The objective of this study was to determine the optimum nitrogen and seeding rate for the production of *C. sativa* in NS, PEI and SK. This was achieved by evaluating the effect of nitrogen and seeding rate on plant stand, flowering date, plant height, maturity date, seed yield, TKW, seed oil content, seed protein content and oil quality.

5.2 Materials and Methods

5.2.1 Experiment 1 (Seeding Rate Effect)

Three fields in Truro, NS, NSAC; AAFC Harrington, PEI and AAFC, Saskatoon, Saskatchewan, were selected for the 2008 and 2009 field trials. The effect of five seeding rates (100, 200, 400, 800 and 1600 seeds/m^2) was determined for one cultivar of *C. sativa*

Calena and one breeding strain, CS0005. Fertilizer application schedule in three sites is listed in Table 5.1. The selected soil characteristics for each field are shown in Table 5.2 and 5.3. Pre-emergence herbicide Treflan 2.3 L ha^{-1} was applied in NS and PEI in both years. The crop previously grown at Truro, NS and Harrington was a variety of cereal crops and in PEI sites was barley in 2008. In 2009, the previous crop was flax in NS and red clover in PEI. In SK, the previous crop was oats and superb wheat in 2008 and 2009, respectively. Plots were seeded using a Hege plot drill (H and N Equipment Inc., Colwich, Kansas, USA) with double disc openers and press wheels in NS and PEI. In SK, plants were seeded with a 1.5 meter R-Tech hoe drill (AAFC, Saskatchewan, Canada) (packaged seed through a cone and splitter). In 2008, *Camelina sativa* was seeded on May 14, May 26 and May 15 in NS, PEI and SK, respectively. The seeded plot area was 7.5 m^2 (8 rows @ 15 cm x 6 m in length) which were trimmed to 5 m in length after emergence. In 2009, the plants were seeded on May 5, May 21 and May 16 in NS, PEI and SK. The seeded plot size was 6.25 m^2 in NS and 7.5 m^2 in PEI. The row spacing was 15 cm and the distance between plots and blocks were 25 cm and 2.5 m in NS and PEI site for both 2008 and 2009. Fields were harvested with a Hege 125C plot combine (Hege USA, Colwich, Kansas, USA). Plots at SK consisted of four rows spaced 30 cm apart. The row length was 6 m and the harvested plot area was 7.32 m^2. In SK, plants were straight combined with a Wintersteiger plot combine (Wintersteiger, Austria).

Table 5.1 Fertilizer application schedule for *C. sativa* seeding rate study in three sites

Site	Date	Form	Method
NS 2008	May 13	370 kg/ha 14N-14P-14K-10.19S	Broadcast and incorporated
	June 28	140 kg/ha 34N-0P-0K	Topdressing
PEI 2008	May 25	200 kg/ha 0N-20P-20K + 240S kg/ha 21N-0P-0K-21S	Broadcast and incorporated
	July 7	140 kg/ha 34N-0P-0K	Topdressing
SK 2008	May 15	236 kg/ha 28.4N-14.2P-0K-11.8S	Incorporated
NS 2009	May 4	370 kg/ha 14N-14P-14K-10.19S	Broadcast and incorporated
	June 26	190 kg/ha 27N-0P-0K	Topdressing
PEI 2009	May 20	240 kg/ha 21N-0P-0K + 200 kg/ha 0N-20P-20K	Broadcast and incorporated
	July 15	150 kg/ha 34-0-0	Topdressing
SK 2009	May 15	114 19.5N-19.6P-0K-19.6S	Incorporated

Table 5.2 Soil characteristics of camelina seeding rate study in 2008

Location	pH	Organic Matter (%)	CEC (meq/100g)	P_2O_5 (kg/ha)	K_2O (kg/ha)	Ca (kg/ha)	Mg (kg/ha)	Sulfur (kg/ha)
NS	6.2	2.6	13.5	1033	266	2936	643	35
PEI	5.9	3.9	12.1	454	334	1866	228	48
SK	8.1	3.7	-	137	1229	-	-	19

Table 5.3 Soil characteristics of camelina seeding rate study in 2009

Location	pH	Organic Matter (%)	CEC (meq/100g)	P_2O_5 (kg/ha)	K_2O (kg/ha)	Ca (kg/ha)	Mg (kg/ha)	Nitrate-N(ppm)	% N	Sulfur (kg/ha)
NS	5.9	3.0	12.7	1562	348	2640	356	12.3	0.17	30
PEI	6.0	3.6	12.0	855	398	2612	148	33.1	0.24	30
SK	7.4	-	-	205	1300	-	-	24.8	-	30

5.2.2 Experiment 2 (Nitrogen Effect)

Three fields in Truro, NS, NSAC; AAFC Harrington, PEI and AAFC, Scott, Saskatchewan, were selected for the 2008 and 2009 field trials. Data in Scott is only available in 2008. Fertilizer application schedule in three sites is listed in Table 5.4. The selected soil characteristics for each field are shown in Table 5.5 and 5.6. The effect of N fertilizer rate was determined for one cultivar Calena in all three sites. In 2008, seven N rates (0, 25, 50, 75, 100, 125 and 150 kg/ha) were evaluated. Fields were seeded using a Hege plot drill (H and N Equipment Inc., Colwich, Kansas, USA) with double disc openers and press wheels in NS and PEI. In SK, plants were seeded with a 1.5 meter R-Tech hoe drill (AAFC, Saskatchewan, Canada) (packaged seed through a cone and splitter). The crop previously grown in NS, PEI and SK sites were spring cereals, barley and winter wheat, respectively. *Camelina sativa* was seeded on May 13, May 26 and May 15 in NS, PEI and SK, respectively. The seeding rate for this trial was 500 seeds/m^2 for all three sites. The plot size was 15 m^2 (16 rows @ 15 cm x 5 m in length) in NS and PEI. In SK, the plot size was 7.5 m^2 (16 rows @ 10cm x 5m). Fields were harvested with a Hege 125C plot combine (Hege USA, Colwich, Kansas, USA) with a harvest area of 6.25 m^2 for all trials in NS and PEI. In SK site, the whole plot was harvested by a Wintersteiger plot combine (Wintersteiger, Austria). In 2009, one more N rate (200 kg N/ha) was added to the study. The crops previously grown at the NS and PEI sites were flax and red clover, respectively. *Camelina sativa* was seeded on May 5 and May 21 in NS and PEI. The seeding rate for this trial was 500 seeds/m^2 and plot size was 12.5 m^2 in NS and 15 m^2 in PEI. Fields were harvested with a Hege 125C plot combine (Hege USA, Colwich, Kansas, USA) with a harvest area of 5 m^2 in NS and 6.25 m^2 in PEI. In both years, nitrogen application was split with half the amount applied one week after seed germination and a further half applied at the start of flowering at the NS and PEI sites. In 2008, the nitrogen fertilizer was mid-row banded at the time of seeding in Scott, SK. N put on in the spring was applied all at once since farmers do not split applications in western Canada. The nitrogen source was ammonium nitrate (34-0-0) in NS and PEI. In Scott, the nitrogen source was urea (46-0-0).

Table 5.4 Fertilizer application schedule for *C. sativa* N study in three sites

Site	Date	Form	Method
NS 2008	May 12	200 kg/ha 0N-20P-20K	Broadcast and incorporated
PEI 2008	May 25	200 kg/ha 0N-20P-20K	Broadcast and incorporated
SK 2008	May 15	225 kg/ha of 23P-25K-17S	Incorporated
NS 2009	May 4	220 kg/ha 0N-20P-20K	Broadcast and incorporated
PEI 2009	May 20	200 kg/ha 0N-20P-20K	Broadcast and incorporated

Table 5.5 Soil characteristics of *C. sativa* N study in 2008

Location	pH	Organic Matter (%)	CEC (meq/100g)	P_2O_5 (kg/ha)	K_2O (kg/ha)	Ca (kg/ha)	Mg (kg/ha)	Sulfur (kg/ha)
NS	6.2	2.6	13.5	1033	266	2936	643	35
PEI	5.9	3.9	12.1	454	334	1866	228	48
SK	7.2	-	-	137	1530	-	-	20

Table 5.6 Soil characteristics of *C. sativa* N study in 2009

Location	pH	Organic Matter (%)	CEC (meq/100g)	P_2O_5 (kg/ha)	K_2O (kg/ha)	Ca (kg/ha)	Mg (kg/ha)	Nitrate-N(ppm)	% Nitrogen	Sulfur (kg/ha)
NS	5.9	3.0	12.7	1562	348	2640	356	12.3	0.17	30
PEI	6.0	3.6	12.0	855	398	2612	148	33.1	0.24	30

5.3 Data collection

5.3.1 Measurements

The plant emergence counts were measured approximately three weeks after planting. Two values were collected in each plot by randomly placing two 0.25 m^2 quadrats in the plot, avoiding the outside rows. Plant height was measured from the soil surface to highest point on the erect plant at the time of maturity. Three data points (front, middle and back part of plot) were collected from each plot. The flowering date was estimated visually as the date when approximately 10 % of plants have one flower open. The number of days from seeding to flowering was calculated. Maturity date was also estimated visually as the date when approximately 95 % of the pods had changed from green to brown (Urbaniak *et al.* 2008a). Lodging was visually evaluated using a scale of 1-5; with 1 being erect and 5 being flat on the ground. To determine the seed yield, plots were harvested with a Hege 125C small plot combine (Hege USA, Colwich, KS) in NS and PEI, but with a Hege 140 plot combine in SK. All seeds were then dried to approximately 8 % moisture content and cleaned using a Clipper seed cleaner (Clipper Seed Cleaning Co., Bluffton, IN), weighed (g) and g/plot values were converted to kg/ha based on plot areas for each location. Climatic data (including weekly precipitation, temperature and sunshine hours) at three sites (NS, PEI and SK) and for two years (2008 and 2009) were obtained from Environment Canada on-line data (http:// www.climate.weatheroffice.ec.gc.ca/).

5.3.2 Biomass and Harvest Index

Two whole plant samples were cut just above ground level, one from 0.25 m^2 at the front of the plot and one from the back of the plot (different rows). The two samples per plot were combined to get a 0.5 m^2 sample. This was then dried and weighed. Biomass was then converted to kg/ha. The harvest index was calculated as the ratio of seed yield to the total aboveground biomass and was expressed as a percentage.

5.3.3 Downy Mildew [*Peronospora parasitica* (Pers. (ex Fr.) Fr.] Disease Rating

Two 0.5 m rows, the third row from the right side of the front and the end of the plot, were chosen specifically. The total number of infected plants was counted in these two 0.5 m sections. A plant was considered to be infected if it showed any level of disease. The incidence was calculated by a percentage of infected plants and the visual ratings

based on a 1-9 scale were taken for each plot (Appendix B). The final disease assessment for an individual plot was determined by the product of disease incidence and ratings number.

5.3.4 Oil and Protein Content

Total seed oil and protein content was analyzed on 5.0 g whole seed using near-infrared reflectance (NIR) spectroscopy (FOSS NIR Systems model 6500 spectrometer, spinning cup autosampler, WIN ISI II calibration software, Technicon Canada Inc., Mississauga, ON). Samples were analyzed in bulk by combining block1 and 2 as well as 3 and 4. Oil and protein contents are reported on a dry matter basis.

5.3.5 Fatty Acid Analysis

The lipid extraction and methylation process were generated based on the protocol of Budge *et al.* (2006) with minor modification. For detailed information, see chapter 4.

5.3.6 Statistical Analysis

Experiment 1 was designed as a RCBD (randomized complete block design) factorial with genotype and seeding rate as main factors. Each treatment was replicated 4 times. Experiment 2 was designed as a RCBD with four replications. The main factor in this study was N rate. The response variables from both experiments, including stand count, plant height, days to flowering, days to maturity, thousand kernel weight (TKW), seed yield, oil and protein content and fatty acid composition were collected and subjected to the PROC Mixed procedure in SAS version 9.1 (SAS Institute Inc., Cary, NC,USA, 2002-2003). Tukey test was used to compare the differences among treatments at the 5 % significant level. PROC Mixed procedure in SAS version 9.1 was also used in analyzing the site and year effects. Minitab statistical software version 14 (Minitab Inc., USA, 1972-2004) was used for all regression analysis.

5.4 Results

5.4.1 Experiment 1 (Seeding Rate Effect)

Table 5.7 is a summary of the seeding rate effects in 2008 and 2009 at three sites: NS, PEI and SK. Plant stand increased dramatically from 64 to 295 with the increasing seeding rates. The effect of seeding rate on the number of branches and pods per plant was consistent in both years. The number of branches and pods per plant fell from 33-5

and 435-58 as the plant population increased. Mean yield over six site-years was 1685 kg/ha and increased significantly with higher seeding rates and leveled at 400 seeds/m^2. The oil and protein content was not significantly affected by seeding rate and the mean value was 40.3 % and 27.3 %. Results from the analysis of variance (Table 5.8) for seed yield indicated that the interaction effect between site, year and seeding rate significantly affected the seed yield ($P<0.0001$). Therefore, the relationship between seeding rate (plant stand) and seed yield was analyzed separately for each site. Seed yield was strongly correlated with the seeding rate (r^2 = 97.4 % in 2008 and 94.8 % in 2009) and the plant stand (r^2 = 90.4 % in 2008 and 97.6 % in 2009) in two years in NS (Figure 5.1). At the NS site for both 2008 and 2009, the 100 and 200 seeds/m^2 rate had the lowest seed yield; however, no significant yield differences were found between the 400, 800 and 1600 seeds/m^2 (Table 5.10 and 5.13). Figure 5.4 suggests that the lower seed yield in 2008 is likely due to the lower plant stand and the optimum plant stand; the optimum plant stand in NS is around 170 plants/m^2. At the PEI site, seed yield increased dramatically as seeding rate increased and leveled off at approximately 800 seeds/m^2, which corresponded with a plant stand of 280 plants/m^2. The regression analysis showed that the seed yield in 2008 was strongly related to seeding rate (r^2 = 96.4 %) and plant stand (r^2 = 97.8 %). However, in 2009, seed yield was not correlated to either seeding rate (56.9 %) or plant stand (r^2 = 35.3 %) (Figure 5.2 and 5.5). Many axillary branches were produced during the reproductive stage and resulted in low seed yield. Therefore, the seeding rate effect was confounded with axillary branches. In comparison with NS and PEI site, seeding rate affected seed yield in a lesser degree in SK. In 2008, seed yield was not significantly affected by the seeding rate except at the seeding rate of 1600 seeds/m^2 which had a significantly lower seed yield than all other seeding rates. In 2009, no differences have been found among different seeding rates from 200 to 1600 seeds/m^2. The relationship between seed yield and seeding rate was not significant due to the extremely poor stand establishment in 2009. The significantly relationship (r^2 = 99.3%) between seed yield and plant density observed in 2008 (Figure 5.6) revealed a plant population of approximately 150 plants/m^2 was sufficient for maximizing seed yield. The oil and protein contents did not differ with different seeding rates save for two genotypes. Generally, Calena seed had 2 % more protein content and 2 % less oil content than

CS0005. None of the measured variables were significantly influenced by the interaction effect of genotype and seeding rate.

Table 5.7 Mean value of *C. sativa* agronomic traits across three sites and two years

Seeding Rate (seeds/m^2)	Plant stand (plants/m^2)	Percent Emergence (%)	Seed yield (kg/ha)	Branches/ plant	Pods/plant	Plant height (cm)	Protein content (%)	Oil content (%)	TKW (g)
100	64d	64a	1441b	33a	435a	85a	27.59a	39.97a	1.11a
200	85cd	43b	1608b	19b	305b	85a	27.07a	40.54a	1.12a
400	126c	32bc	1744a	11bc	196c	88a	27.21a	40.41a	1.11a
800	194b	24c	1853a	9c	116cd	86a	27.32a	40.3a	1.12a
1600	295a	18c	1777a	5c	58d	86a	27.32a	40.29a	1.13a
P-value	0.012	0.001	0.075	0.001	0.0023	0.073	0.081	0.091	0.123
Mean	130	36.2	1684.6	15.4	222	86	27.30	40.30	1.11a

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 5.8 Analysis of variance for seed yield in seeding rate study in three sites (NS, PEI and SK) and two years (2008-09)

Effect	Num DF	Den DF	F Value	Pr > F
Seeding Rate(SR)	4	177	30.16	<.0001
Site	2	177	595.88	<.0001
SR*Site	8	177	4.74	<.0001
Year	1	177	1.53	0.2173
SR*Year	4	177	2.94	0.0220
Year*Site	2	177	147.7	<.0001
SR*Year*Site	8	177	10.94	<.0001
Genotype (G)	1	177	0.01	0.9423
SR*G	4	177	1.96	0.1021
G*Site	2	177	30.10	<.0001
SR*G*Site	8	177	1.56	0.1395
G*Year	1	177	9.27	0.0027
SR*G*Year	4	177	1.11	0.3515
G*Year*Site	2	177	21.15	<.0001
SR*G*Year*Site	8	177	1.54	0.1452

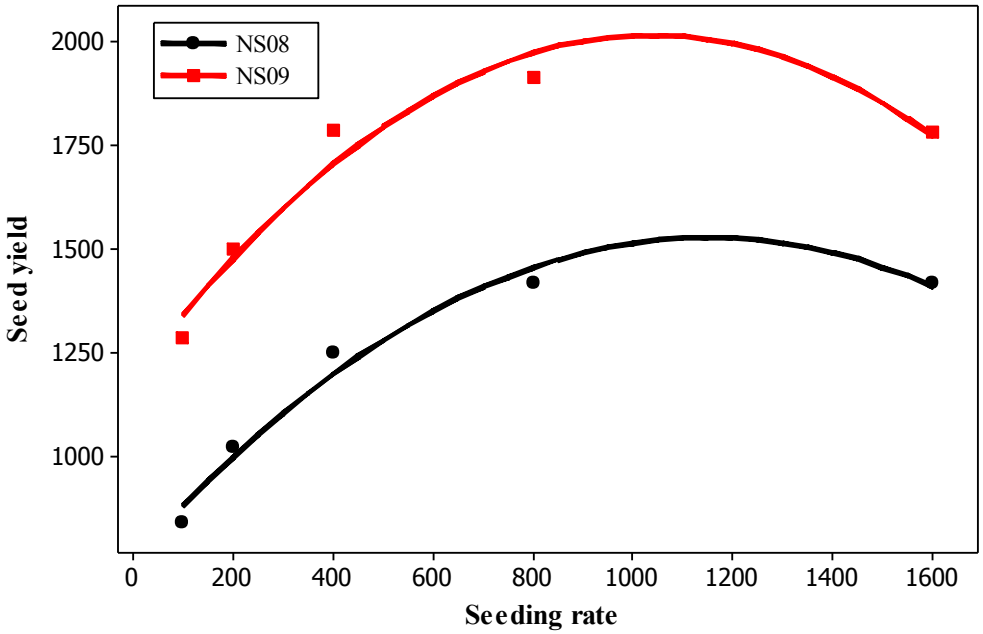

Figure 5.1 Relationship between seeding rate (seeds/m^2) and *C. sativa* seed yield (kg/ha) in NS

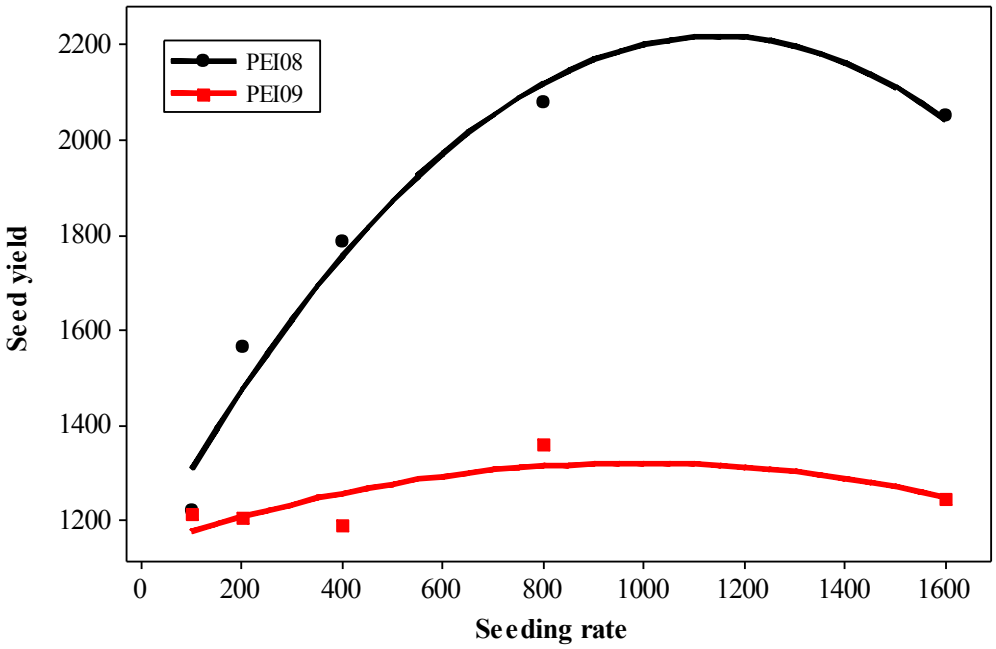

Figure 5.2 Relationship between seeding rate (seeds/m^2) and *C. sativa* seed yield (kg/ha) in PEI

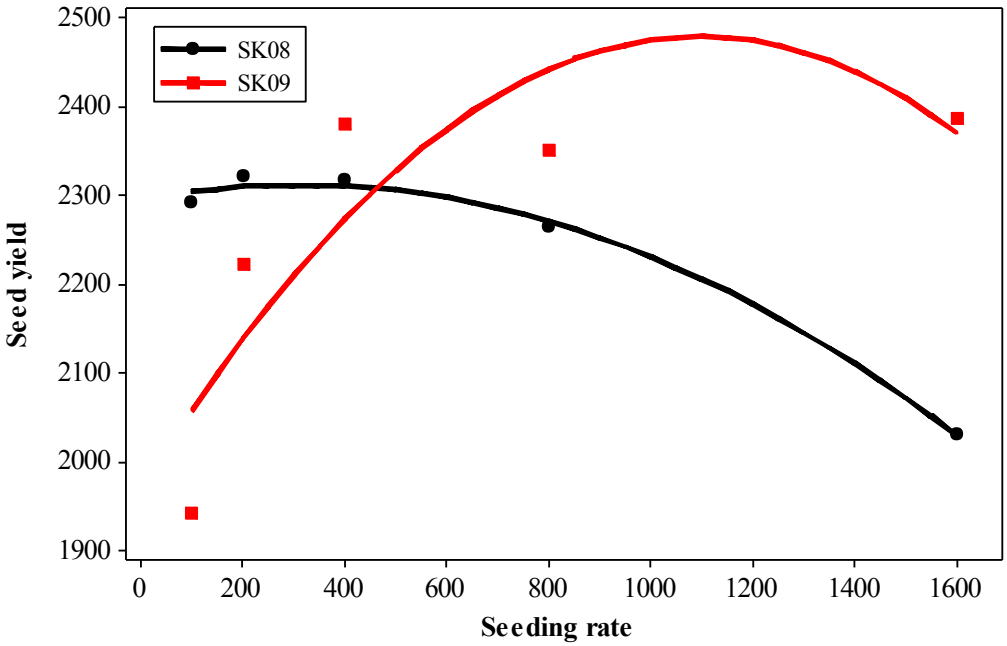

Figure 5.3 Relationship between seeding rate (seeds/m^2) and *C. sativa* seed yield (kg/ha) in SK

The regression equation for seed yield and seeding rate (SR):

y = 753.0 + 1.350 SR - 0.000586 SR**2 (r^2 = 97.4 %) ---------- NS08;

y = 1191 + 1.594 SR - 0.000768 SR**2 (r^2 = 94.8 %) ---------- NS09;

y = 1126 + 1.911 SR - 0.000835 SR**2 (r^2 = 96.4 %) ----------PEI08;

y = 1142 + 0.3639 SR - 0.000185 SR**2 (r^2 = 56.9 %) ----------PEI09;

y = 2296 + 0.1046 SR - 0.000169 SR**2 (r^2 = 99.3 %) ---------- SK08;

y = 1969 + 0.9308 SR - 0.000425 SR**2 (r^2 = 71.5 %) ---------- SK09

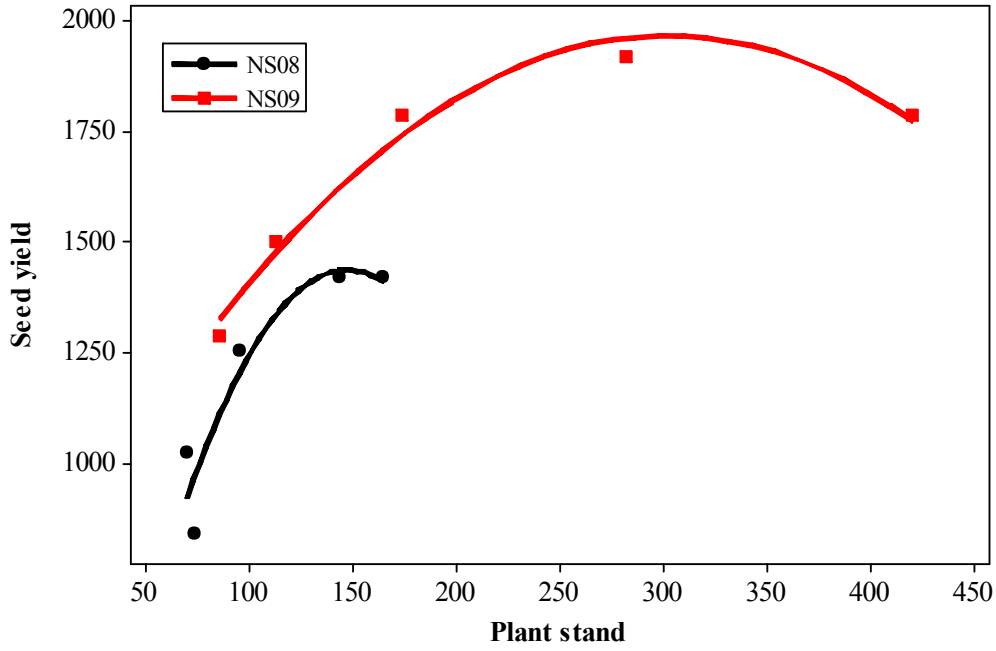

Figure 5.4 Relationship between plant stand (plants/m^2) and *C. sativa* seed yield (kg/ha) (NS)

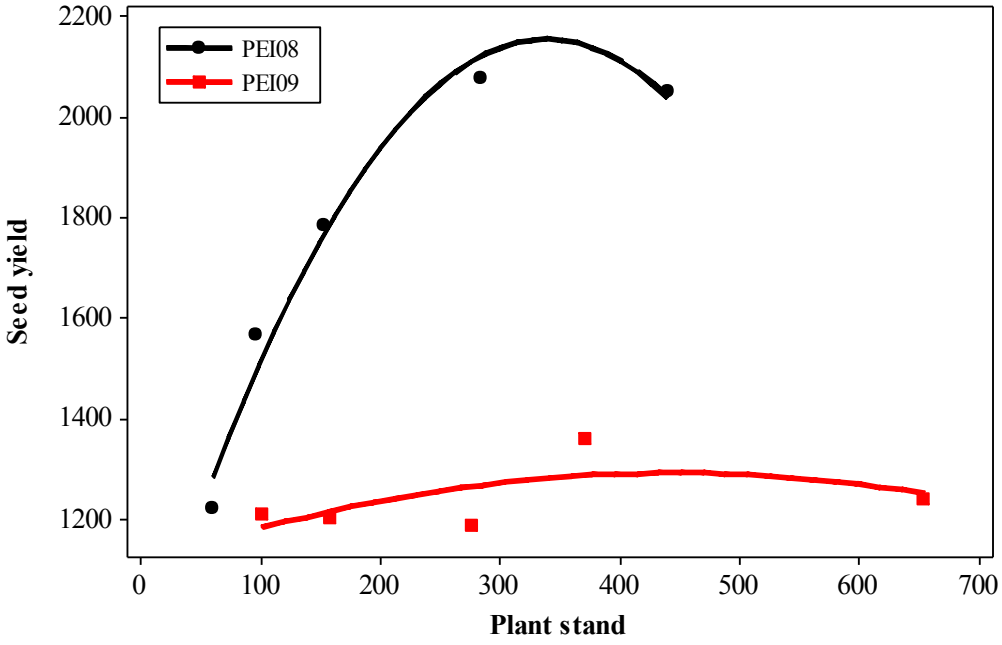

Figure 5.5 Relationship between plant stand (plants/m^2) and *C. sativa* seed yield (kg/ha) (PEI)

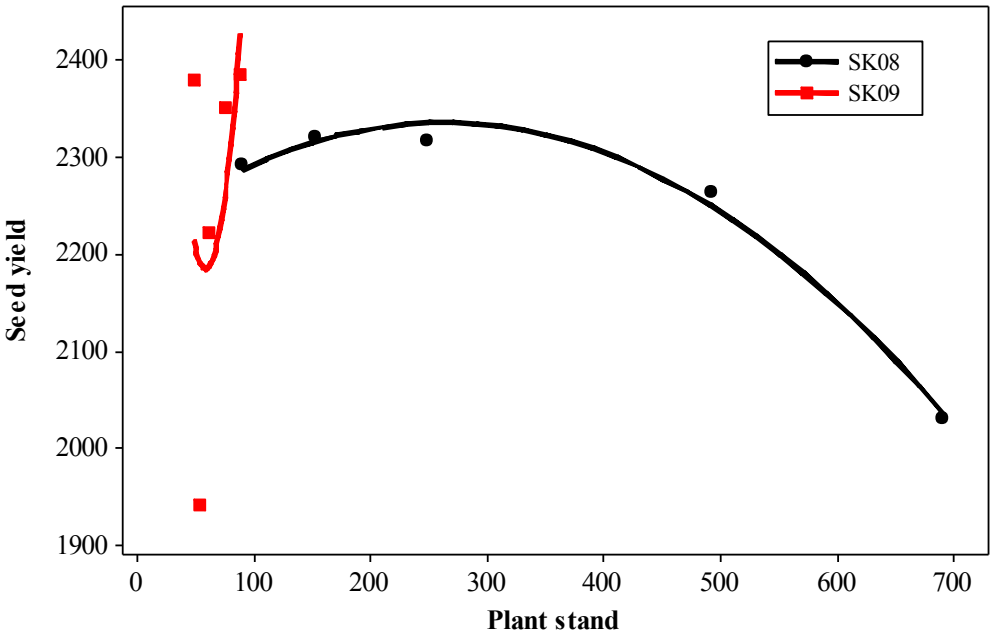

Figure 5.6 Relationship between plant stand (plants/m^2) and *C. sativa* seed yield (kg/ha) (SK)

The regression equation for seed yield and plant stand (PS)

y = - 433.1 + 25.37 PS - 0.08608 PS**2 (r^2 = 90.4 %) ---------- NS08;

y = 713.6 + 8.308 PS - 0.01377 PS**2 (r^2 = 97.6 %) ---------- NS09;

y = 871.1 + 7.576 PS - 0.01118 PS**2 (r^2 = 97.8 %) ---------- PEI08;

y = 1110 + 0.8225 PS - 0.000926 PS**2 (r^2 = 35.3 %) ---------- PEI09;

y = 2224 + 0.8555 PS - 0.001631 PS**2 (r^2 = 99 %) ----------SK08;

y = 3116 - 31.94 PS + 0.2739 PS**2 (r^2 = 21.8 %) ---------- SK09

5.4.1.1 Plant Emergence

All the measured variables are listed in Tables 5.9-5.13. Seeding rate had a significant effect on plant stand across all environments except in SK in 2009. The extremely dry weather in SK (2009) resulted in a poor and uneven germination. The variance within treatments covered the seeding rate effect. Generally, plant stand rose significantly with the increased seeding rate. The actual plant population (plants/m^2) in each seeding rate differed from year to year. In NS, the actual establishment of the plant populations ranged from 70 to 165 plants/m^2 in 2008 and 86 to 420 plants/m^2 in 2009. In PEI, the actual plant populations ranged from 60 to 440 plants/m^2 in 2008 and from 91 to 654 plants/m^2 in 2009. In SK, the actual plant population was from 90 to 691 plants/m^2 in SK in 2008 and from 53 to 88 plants/m^2 in 2009.

Table 5.9 *Camelina sativa* seeding rate effects on plant stand, branches/plant, and pods/plant in 2008 in NS, PEI and SK

Seeding Rate					Site				
	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK
seeds/m^2	Plant stand (plants/m^2)			Branches/plant			Pods/plant		
100	74b	60e	90e	28a	17a	15a	561a	327a	N/A
200	70b	96d	153d	20b	12b	13b	397a	214a	N/A
400	96ab	152c	248c	14bc	8bc	12bc	260b	133b	N/A
800	144a	283b	492b	10c	4c	11bc	165b	67b	N/A
1600	165a	440a	691a	5d	3d	10c	79c	38c	N/A
P-value	0.010	0.000	0.000	0.00	0.000	0.000	0.000	0.000	N/A

Means within a column followed by different letters are significantly different ($p < 0.05$)
(N/A – Data is not available)

Table 5.10 *Camelina sativa* seeding rate effects on plant height, TKW and seed yield in 2008 in NS, PEI and SK

Seeding Rate	Site								
	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK
seeds/m^2	Plant height (cm)			TKW (g)			Seed yield (kg/ha)		
100	82b	78b	77a	1.07a	1.15a	N/A	840b	1222d	2292a
200	82b	83a	75a	1.06a	1.18a	N/A	1022b	1566c	2322a
400	91a	81a	74a	1.06a	1.16a	N/A	1252a	1786bc	2317a
800	90a	81ab	74a	1.05a	1.19a	N/A	1418a	2078ab	2264a
1600	89a	78b	72a	1.08a	1.19a	N/A	1418a	2052a	2031b
P-value	0.030	0.026	0.467	0.576	0.322	N/A	0.031	0.016	0.021

Means within a column followed by different letters are significantly different ($p < 0.05$)
(N/A – Data is not available)

Table 5.11 *Camelina sativa* seeding rate effect on stand count, plant height and days to flower in 2009 in NS, PEI and SK

Seeding rate	Sites								
	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK
seeds/m^2	Plant stand (plants/m^2)			Plant height (cm)			Days to flower		
100	86d	91c	53a	88.7b	89b	79a	48a	43a	50a
200	113d	157c	61a	93.4a	100a	80a	47a	42a	49a
400	174c	276b	48a	95.4a	103a	80a	48a	43a	49a
800	282b	371b	75a	94.1a	97a	79a	48a	44a	49a
1600	420a	654a	88a	91.5ab	95ab	78a	48a	43a	50a
P-value	0.006	0.002	0.142	0.043	0.047	0.063	0.089	0.23	0.421

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 5.12 *Camelina sativa* seeding rate effect on branches/plant, pods/plant and days to maturity in 2009 in NS, PEI and SK

Seeding rate	Sites								
	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK
seeds/m^2	Branches/plant			Pods/plant			Days to Maturity		
100	15a	N/A	84a	290a	N/A	N/A	98a	N/A	102a
200	9b	N/A	46b	167b	N/A	N/A	98a	N/A	101a
400	6c	N/A	17c	108bc	N/A	N/A	97a	N/A	98b
800	3cd	N/A	17c	64cd	N/A	N/A	96a	N/A	98b
1600	2d	N/A	4d	33d	N/A	N/A	96a	N/A	99b
P-value	0.007	N/A	0.012	0.005	N/A	N/A	0.067	N/A	0.023

Means within a column followed by different letters are significantly different ($p < 0.05$)
(N/A – Data is not available)

Table 5.13 *Camelina sativa* seeding rate effect on seed yield in 2009 in NS, PEI and SK

Seeding rate seeds/m^2	Sites		
	NS	PEI	SK
	Seed yield (kg/ha)		
100	1287c	1212b	1942b
200	1503b	1204b	2222a
400	1787a	1188b	2381a
800	1917a	1361a	2352a
1600	1785a	1243ab	2386a
P-value	0.035	0.028	0.047

Means within a column followed by different letters are significantly different ($p < 0.05$)

5.4.1.2 Yield Components

Variations in seeding rate not only affected plant populations, but also had an impact on seed yield and therefore its components. The formation of yield components (branches/plant and pods/plant) was dependent on seeding rates as given in Table 5.9 and 5.12. The number of branches/plant and pods/plant were negatively correlated to seeding rate at all three sites. Increasing the seeding rate from 100 to 1600 seeds/m^2 decreased the number of branches produced on each plant from 28 to 5 in NS, 17 to 3 in PEI and 15 to 10 in SK in 2008. In 2009, the branches/plant data was only taken at the NS and SK sites, since the branch number in PEI was highly confounded with the axillary branches which emerged during the reproductive stage. The branches/plant decreased from 15 to 2 in NS and 84 to 4 in SK as the plant density increased in 2009. The changes in the number of pods/plant were also negatively related to the seeding rate in the three sites. The number of pods rose from 79-561 in NS 2008, 33-290 in NS 2009, and 38-327 in PEI 2008 as the plant population decreased. Therefore, there was a tendency to produce more branches and pods at the lower seeding rates. The effect of genotype on the number of branches and pods per plant varied among environments. In 2008, CS0005 had significantly higher stand counts but fewer pods compared with Calena in PEI. In SK, genotypes significantly affected the branch number; CS0005 (13) had a higher average number of branches/plant than that of Calena (11). In 2009, Calena (8) had two more branches/plant than CS0005 (6) in NS, but no significant difference was found in SK. There was no interaction effect in this study at any of the three sites.

5.4.1.3 Plant Height

Plant height increased significantly as the seeding rate rose in both NS and PEI. In SK, the difference in the plant height was not significant among the seeding rates. The mean value of plant height increased from 86-93 cm, 80-97 cm, and 74-79 cm in NS, PEI and SK, respectively.

5.4.1.4 Oil and Protein Content

The p-value of the analysis of variance results for oil and protein content in different seeding rate trials are presented in Table 5.14. Seeding rates had no significant effect on the oil and protein content at all sites. When the data of the three sites was averaged, the variation of the oil content was between 38 % and 43 % and protein content ranged from 26 % to 29 %. Line CS0005 had a significantly higher amount of oil than Calena at all sites (Table 5.15).

Table 5.14 P-value of Analysis of variance for oil and protein content in three sites (SR = Seeding rate; G = Genotype)

Source	NS (08)		PEI (08)		SK(08)		NS (09)		PEI (09)	
	Oil	Protein	Oil	Protein	Oil	Protein	Oil	Protein	Oil	Protein
Genotype	0.0012	0.033	<.0001	<.0001	0.0003	0.0037	<.0001	0.0001	0.0002	<.0001
SR	0.2758	0.464	0.8261	0.9948	0.1218	0.583	0.519	0.425	0.383	0.474
G*SR	0.8189	0.929	0.56	0.4961	0.3511	0.109	0.283	0.251	0.289	0.251

Table 5.15 Genotype effect on oil and protein content (%) in three sites and two years

Genotype	NS (08)		PEI (08)		SK (08)		NS (09)		PEI (09)	
	Oil	Protein	Oil	Protein	Oil	Protein	Oil	Protein	Oil	Protein
Calena	39.09b	26.94a	38.40b	29.02a	40.71b	27.68a	37.77b	28.40a	36.31b	31.19a
CS0005	40.77a	26.04b	40.24a	27.60b	42.63a	26.53b	40.06a	27.03b	37.79a	29.68b

5.4.2 Experiment 2 (Nitrogen Effect)

5.4.2.1 Seed Yield

The analysis of variance (Table 5.16) showed that the interaction effect among N rate, year and site was highly significant. Therefore, the nitrogen recommendations should reflect specific environments. The averaged overall trials (without the trial in SK 2009) indicated that the lowest and highest yields lay among 886 to 2391 kg/ha (Table 5.17). The mean yield over all years and all N levels was approximately 1634 kg/ha. However, the highest seed yield at each site was 1808 kg/ha, 2099 kg/ha and 2964 kg/ha in NS, PEI and SK, respectively. This suggested that *C. sativa* had a high yield capacity. The significant N rate effect was found at all sites but the maximum N requirement differed among sites. In NS, the seed yield reached a plateau at N rates of 125 kg/ha in two years. The same observations were seen in PEI in 2008. No difference in seed yield was found among N treatments in PEI in 2009 due to the high amount of soil-available N from previous red clover residues. In SK, the nitrogen requirement to achieve maximum yield was 100 kg N/ha. Figure 5.7- 5.9 shown that the seed yield was the strongly correlated with the N rate in all trials (with the $r^2 > 95$ %) except for the trial in PEI in 2009. No significant difference in seed yield was found among nitrogen treatments since the nitrogen effect was confounded with the high soil N residue from the previous crop.

Table 5.16 Analysis of variance results for *C. sativa* nitrogen response study across three sites in NS, PEI and SK (7 N rates) 2008-09

Effect	Num DF	Den DF	F Value	Pr > F
N rate (N)	6	102	33.37	<.0001
Year	1	102	68.21	<.0001
N*Year	6	102	4.23	0.0008
Site	2	102	380.72	<.0001
N*Site	12	102	4.52	<.0001
Year*Site	2	102	111.01	<.0001
N*Year*Site	12	102	2.81	0.0141

Table 5.17 Nitrogen effect on seed yield of *C. sativa* in across three sites in NS, PEI and SK (7 N rates) 2008-09

N rate	Seed yield (kg/ha)				
(kg/ha)	NS 08	PEI 08	SK 08	NS 09	PEI 09
0	414e	1019c	1470c	1085d	2099a
25	645d	1207c	1768c	1360c	1959a
50	846c	1351b	2368b	1540bc	1818a
75	984bc	1482b	2413b	1539bc	1821a
100	979bc	1515b	2753ab	1628abc	1917a
125	1144ab	1807a	2998a	1735ab	1862a
150	1188a	1827a	2964a	1808a	1869a
Mean	886	1458	2391	1528	1906
P-value	0.0032	0.005	0.021	0.007	0.087

Means within a column followed by different letters are significantly different ($p < 0.05$)

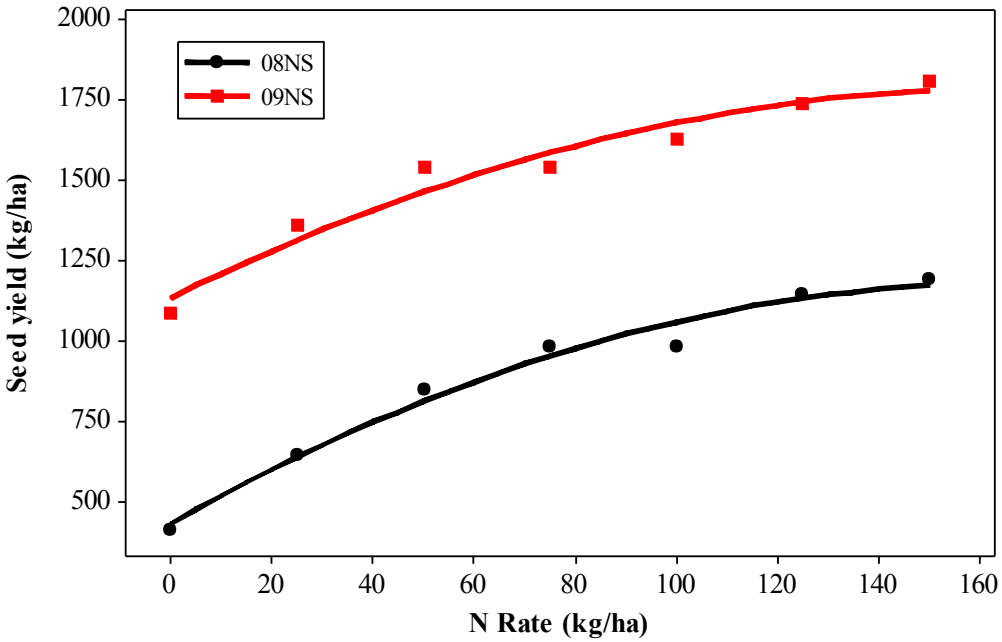

Figure 5.7 Nitrogen effect on *C. sativa* seed yield in NS. 2008-09

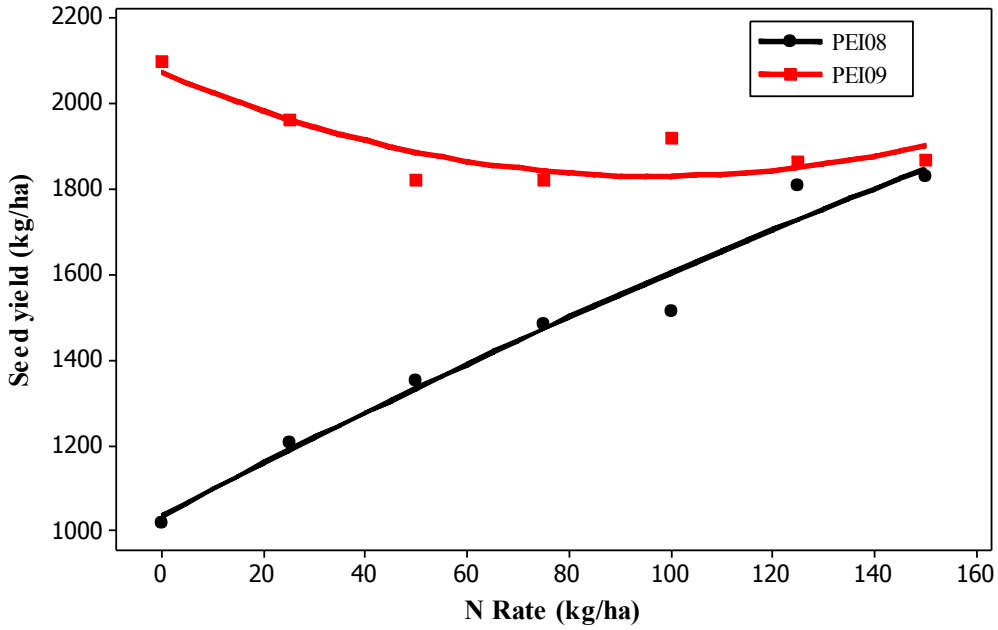

Figure 5.8 Nitrogen effect on *C. sativa* seed yield in PEI. 2008-09

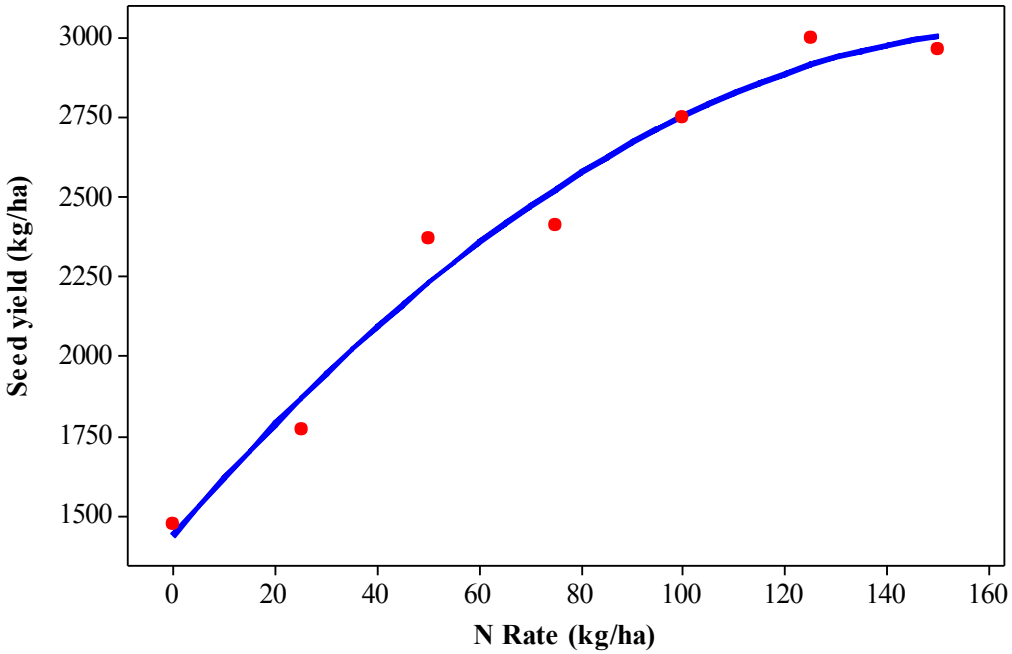

Figure 5.9 Nitrogen effect on *C. sativa* seed yield in SK. 2008-09

The regression equation between seed yield and N rate:

$y = 432.4 + 8.925\ N - 0.02661\ N^{**}2$ ($r^2 = 98\ \%$) ---------- NS08;

$y = 1135 + 7.700\ N - 0.02269\ N^{**}2$ ($r^2 = 95.5\ \%$) ---------- NS09;

$y = 1035 + 6.257N - 0.00564\ N^{**}2$ ($r^2 = 97.1\ \%$) ---------- PEI08;

$y = 2072 - 4.996N + 0.02579\ N^{**}2$ ($r^2 = 60\ \%$) ---------- PEI09;

$y = 1436 + 18.60\ N - 0.05420\ N^{**}2$ ($r^2 = 97.5\ \%$) ---------- SK08

5.4.2.2 Plant Stand

Tables 5.21 and 5.24 showed that the differences in plant stand among the N treatments were not statistically significant in either NS or PEI sites across two years (data was not available in SK). In NS, the plant stand ranged from 138 to 170 plants/m^2 in 2008 and was significantly lower than that in 2009, which ranged from 221 to 281 plants/m^2. The actual plant stand in PEI was also higher in 2009 (313-426 plants/m^2) than in 2008(164-246 plants/m^2).

5.4.2.3 Plant Height, Branches/Plant, Pods/Plant and HI

In all trials, plant height increased linearly with the increasing N applications. In 2008, plant height increased from 69 to 79 cm, 71 to 82 cm, and 76 to 86 cm in NS, PEI and SK respectively. In 2009, the plant height was significantly higher at both the NS and PEI sites. The effect of the N rate on yield components (branches/plant and pods/plant) is given in Table 5.21. Due to the timing problem, data was only taken in NS and PEI in 2008. The data showed that the number of branches/plant was not affected by the N treatment. However, the number of pods/plant increased significantly (46 to 108 in NS and 71 to 122 in PEI) as the N supply increased. The same trend was found in the number of pods/branch which increased significantly from 9-22 in NS and 14-24 in PEI as the N rate rose. This suggested that *C. sativa* tended to produce few pods and seed bearing branches at the low N level. The harvest index ranged from 16.2 % to 19.7 % in SK (2008), 25.9 % to 43.9 % in NS (2009) and 20.1 % to 33.9 % in PEI (2009) and there were no significant differences among N treatments.

5.4.2.4 Oil and Protein Analysis

Tables 5.18 and 5.19 showed that oil content and protein content were significantly affected by N rates at the all three sites. Protein content grew with increasing rates of N application, with concomitant decrease in oil content in all trials (Figure 5.10 and 5.11).

The negative correlation between oil and protein content was observed across the environments (Figure 5.12). The oil yield was enhanced significantly as the rate of N application increased. The response of fatty acids was not consistent over five year-sites (Table 5.20). In 2008, N effect had a significantly negative influence on oleic acid and eicosenoic acid but a positive effect on erucic acid at both the NS and PEI sites but not at the SK site. Palmitic acid was negatively correlated with an increased N supply at both the PEI and SK sites but not at the NS site. In 2009, the N rate was negatively correlated to palmitic and stearic acid but positively correlated to erucic acid in NS. In PEI, the only significant difference was found in stearic acid.

Table 5.18 Nitrogen effect on the oil and protein content and oil yield of *C. sativa* in 2008

N rate	NS			PEI			SK		
(kg/ha)	Oil (%)	Protein (%)	Oil Wt. (kg/ha)	Oil (%)	Protein (%)	Oil wt. (kg/ha)	Oil (%)	Protein (%)	Oil wt. (kg/ha)
0	41.24a	24.55e	171d	42.05a	24.15e	428d	42.39b	24.98de	623e
25	40.31ab	25.73ed	260c	41.87a	24.78e	505c	43.56a	24.51e	770d
50	40.55a	25.80d	343bc	41.21a	25.7d	557c	42.78ab	25.26cd	1013bc
75	39.00bc	27.48c	384b	40.29b	27.04c	597b	41.93bcd	25.77bc	1012bc
100	38.25cd	28.12bc	374b	39.57bc	27.82bc	599b	41.4cd	26.43a	1140b
125	37.59d	28.84ab	430a	39.34c	28.25ab	711a	41.29d	26.26ab	1238a
150	37.00d	29.56a	440a	39.02c	28.83a	713a	42.19bc	25.47cd	1251a
P-value	0.0011	0.0002	0.001	0.0003	<.0001	0.003	0.0043	0.0011	0.0002

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 5.19 Nitrogen effect on the oil and protein content (%) of *C. sativa* seeds in 2009

N rate	NS			PEI		
(kg/ha)	Oil (%)	Protein (%)	Oil Wt. (Kg/ha)	Oil (%)	Protein (%)	Oil wt. (Kg/ha)
0	41.54a	25.77d	451d	40.45a	27.02a	849a
25	41.29a	25.56d	562c	39.27ab	28.20a	769bc
50	39.85b	27.02c	614b	39.02b	27.93a	710c
75	39.20bc	27.51bc	603bc	38.91b	28.41a	708c
100	39.28bc	27.37bc	639b	38.74b	27.82a	743c
125	38.62bc	27.81bc	670a	38.65b	28.54a	720c
150	38.39cd	28.24ab	694a	38.72b	28.30a	724c
200	37.18d	28.87a	610b	38.82b	28.02a	680d
P-value	0.0016	0.0005	0.0001	0.011	0.1647	0.0342

Means within a column followed by different letters are significantly different ($p < 0.05$)

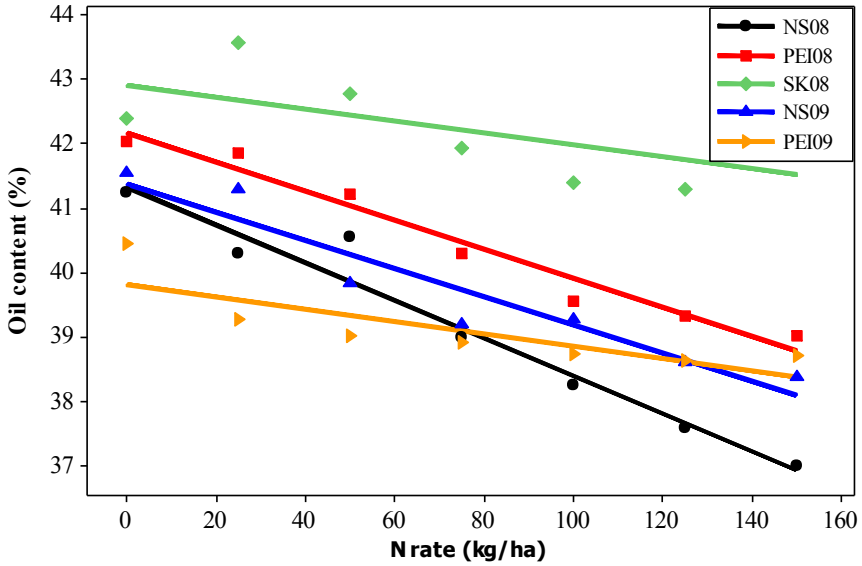

Figure 5.10 Correlation between *C. sativa* oil content and N supply

The regression equation between oil content and N supply:

$y = 41.33 - 0.02923\ N$ ($r^2 = 96\ \%$) ---------- NS08;

$y = 42.17 - 0.02256\ N$ ($r^2 = 97\ \%$) ---------- PEI08;

$y = 42.92 - 0.009314\ N$ ($r^2 = 61\ \%$) ---------- SK08;

$y = 41.38 - 0.02194\ N$ ($r^2 = 91\ \%$) ---------- NS09;

$y = 39.83 - 0.009586\ N$ ($r^2 = 67\ \%$) ---------- PEI09;

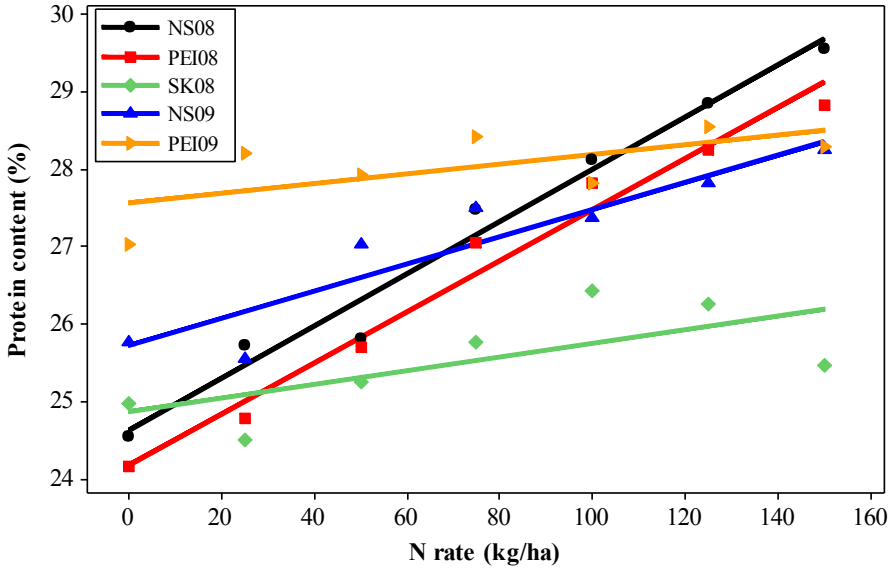

Figure 5.11 Correlation between *C. sativa* protein content and N supply

The regression equation between protein content and N supply

$y = 24.63 + 0.03367\ N$ ($r^2 = 97\ \%$) ---------- NS08;

$y = 24.18 + 0.03300\ N$ ($r^2 = 98\ \%$) ---------- PEI08;

$y = 24.87 + 0.008771\ N$ ($r^2 = 63\ \%$) ---------- SK08;

$y = 25.73 + 0.01751\ N$ ($r^2 = 90\ \%$) ---------- NS09;

$y = 27.56 + 0.006300\ N$ ($r^2 = 50\ \%$) ---------- PEI09

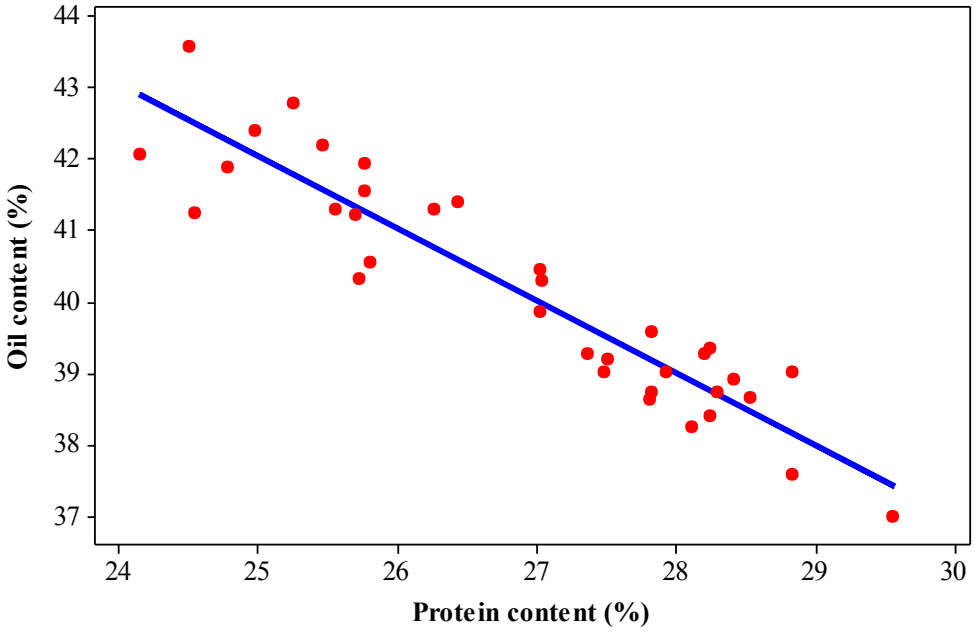

Figure 5.12 Correlation between *C. sativa* oil and protein content in nitrogen study (r^2 = 87%)

Table 5.20 Nitrogen effect on the fatty acid composition in three sites in 2008

N rate	16:0	18:0	18:1	18:2	18:3	20:1	22:1
N0(NS08)	5.90a	2.32a	13.74a	18.08a	32.65a	14.67a	3.63b
N7(NS08)	5.95a	2.34a	12.12b	18.12a	33.56a	14.13b	4.02a
P-value	0.06	0.15	0.0003	0.354	0.0561	0.0163	0.0001
N0(NS09)	5.67b	2.23a	13.12a	16.85a	34.83a	14.68a	3.61b
N7(NS09)	6.00a	2.49a	12.38a	18.11a	33.82a	14.18b	3.66a
P-value	0.07	0.104	0.055	0.178	0.305	0.011	0.028
N0(PEI08)	5.73a	2.30a	13.42a	17.51a	33.22a	14.75a	3.48b
N7(PEI08)	5.60b	2.12a	11.68b	15.86b	35.77a	14.09b	3.90a
P-value	0.0007	0.052	0.0088	0.0319	0.065	0.0174	0.0031
N0(PEI09)	6.07a	2.34b	14.04a	18.67a	33.37a	13.84a	3.31a
N7(PEI09)	6.25a	2.44a	14.44a	20.7a	31.63a	13.18a	2.93a
P-value	0.202	0.012	0.483	0.108	0.165	0.143	0.246
N0(SK08)	5.38a	2.33a	14.74a	17.12a	33.92a	14.62a	3.13a
N7 (SK08)	5.25b	2.26a	14.79a	16.43a	34.59a	14.91a	3.09a
P-value	0.5253	0.1625	0.413	0.4385	0.5725	0.3458	0.6978

N0 = 0 kg N/ha; N7 = 150 kg N/ha

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 5.21 Nitrogen effect on plant stand, branches/plant and pods/plant for *C. sativa* in 2008 in NS, PEI and SK

N rate	Site								
(kg/ha)	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK
	Plant stand (plants/m^2)			Braches/plant			Pods/plant		
0	138a	164a	N/A	5a	6a	N/A	46c	71b	N/A
25	148a	236a	N/A	5a	5a	N/A	53b	73b	N/A
50	148a	188a	N/A	5a	5a	N/A	76b	89b	N/A
75	165a	178a	N/A	5a	6a	N/A	83ab	88b	N/A
100	170a	212a	N/A	6a	6a	N/A	96a	103a	N/A
125	142a	246a	N/A	5a	5a	N/A	99a	107a	N/A
150	160a	178a	N/A	5a	6a	N/A	108a	122a	N/A
P-value	0.78	0.871	N/A	0.867	0.966	N/A	N/A	0.013	N/A

Table 5.22 Nitrogen effect on plant height, TKW and days to flower for *C. sativa* in 2008 in NS, PEI and SK

N rate	Site								
(kg/ha)	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK
	Plant height (cm)			TKW(g)			Days to flower		
0	69b	72bc	76.3d	1.05a	1.06a	1.275a	54a	N/A	50a
25	75a	74bc	82.8abc	1.06a	1.05a	1.245a	54a	N/A	50a
50	75a	71c	85.5a	1.04a	1.04a	1.203a	54a	N/A	50a
75	75a	78ab	86.0a	1.04a	1.05a	1.183a	54a	N/A	50a
100	77a	76abc	84.5ab	1.04a	1.06a	1.200a	53a	N/A	49a
125	78a	82a	79.0cd	1.13a	1.10a	1.253a	53a	N/A	50a
150	79a	82a	79.5bcd	1.08a	1.07a	1.203a	54a	N/A	50a
P-value	0.046	0.02	0.006	0.276	0.342	0.339	0.893	N/A	0.812

Means within a column followed by different letters are significantly different ($p < 0.05$)
(N/A – Data is not available)

Table 5.23 Nitrogen effect on seed yield, biomass and harvest index for *C. sativa* in 2008 in NS, PEI and SK

N rate	Site								
(kg/ha)	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK
	Seed yield (kg/ha)			Biomass(kg/ha)			Harvest index		
0	414e	1019c	1470c	N/A	N/A	6875a	N/A	N/A	0.197a
25	645d	1207c	1768c	N/A	N/A	8100a	N/A	N/A	0.183a
50	846c	1351b	2368b	N/A	N/A	9250a	N/A	N/A	0.162a
75	984bc	1482b	2413b	N/A	N/A	9575a	N/A	N/A	0.188a
100	979bc	1515b	2753ab	N/A	N/A	11650a	N/A	N/A	0.165a
125	1144ab	1807a	2998a	N/A	N/A	10425a	N/A	N/A	0.184a
150	1188a	1827a	2964a	N/A	N/A	10525a	N/A	N/A	0.179a
P-value	0.012	0.004	0.024	N/A	N/A	0.436	N/A	N/A	0.764

Means within a column followed by different letters are significantly different ($p < 0.05$)
(N/A – Data is not available)

Table 5.24 Nitrogen effect on plant stand, plant height and lodging level for *C. sativa* in 2009 in NS, PEI and SK

N rate	Site								
(kg/ha)	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK
	Plant stand (plants/m^2)			Plant height (cm)			Lodging		
0	223a	386a	N/A	71e	96a	N/A	1c	1.5a	N/A
25	235a	426a	N/A	87d	100a	N/A	1c	1a	N/A
50	244a	356a	N/A	88cd	97a	N/A	1.2c	1.5a	N/A
75	221a	316a	N/A	93c	104a	N/A	2.3b	1.5a	N/A
100	281a	350a	N/A	92c	99a	N/A	2.3b	1.5a	N/A
125	223a	313a	N/A	92c	99a	N/A	2.8ab	1a	N/A
150	231a	333a	N/A	97ab	101a	N/A	3.3a	1a	N/A
200	229a	322a	N/A	99a	102a	N/A	3.5a	1a	N/A
P-value	0.536	0.624	N/A	0.012	0.324	N/A	0.021	0.524	N/A

Means within a column followed by different letters are significantly different ($p < 0.05$)
(N/A – Data is not available)

Table 5.25 Nitrogen effect on seed yield, biomass and harvest index for *C. sativa* in 2009 in NS, PEI and SK

N rate	Site								
(kg/ha)	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK
	Seed yield (kg/ha)			Biomass(kg/ha)			Harvest index		
0	1085d	2099a	N/A	3467b	5467a	N/A	0.317a	0.3261a	N/A
25	1360c	1959a	N/A	5134ab	5867a	N/A	0.276a	0.2766a	N/A
50	1540bc	1818a	N/A	4867ab	5133a	N/A	0.340a	0.2953a	N/A
75	1539bc	1821a	N/A	4733ab	5200a	N/A	0.439a	0.2873a	N/A
100	1628abc	1917a	N/A	6000ab	5133a	N/A	0.277a	0.3120a	N/A
125	1735ab	1862a	N/A	5867ab	4600a	N/A	0.272a	0.3386a	N/A
150	1808a	1869a	N/A	6533a	5800a	N/A	0.262a	0.2627a	N/A
200	1641ab	1751a	N/A	6000ab	7133a	N/A	0.259a	0.2080a	N/A
P-value	0.016	0.076	N/A	0.034	0.081	N/A	0.462	0.524	N/A

Means within a column followed by different letters are significantly different ($p < 0.05$)
(N/A – Data is not available)

5.5 Discussion

5.5.1 Seed Establishment

Seeding rate, seeding date, seeder type and seeding depth can greatly influence the *C. sativa* establishment (Urbaniak *et al.* 2008b). Francis and Campbell (2003b) suggested that the optimum seeding depth for *C. sativa* is from 1 to 1.5 cm and also reported that press wheels could improve establishment. McVay and Lamb (2008) reported that *C. sativa* is a cool climate crop and needs to be seeded early to achieve maximum yield; they also recommended that a suitable method for establishing *C. sativa* is to drill the seeds very shallow by utilizing packer wheels and the seeding depth should not be more than 0.5 cm.

Plant stand differed from year to year at each site. The lower plant stand in 2008 in NS could be possibly caused by several factors. Firstly, relatively higher amount of precipitation in NS in 2008 might have washed the seeds into variable (both too shallow and too deep) seeding depths, and this could have developed a competitive hierarchy among seedlings producing a detrimental influence on the emergence of small seeds of *C. sativa.* The other reason for the lower plant stand is likely due to uneven seed distribution at the time of seeding due to seeder malfunctions. The much lower plant emergence in SK 2009 was due to the abnormal dry conditions after seeding. The significantly lower emergence at the higher seeding rate might be caused by the increased competition for water and nutrients between plants, especially in the case of uneven distribution. McVay and Lamb (2008) indicated that small seeds of *C. sativa* planted shallow in the soil, and drilled through high residue levels can result in a low germination rate and poor plant density. There are many factors such as soil moisture, soil texture, soil temperature, seeding depth, disease, insects, and other climatic factors which could affect seeding establishment. It has also been recognized that one of the biggest challenges for growing small seeded crops such as canola is successful stand establishment since the small seed size leads to more variable and higher seedling mortality in the field (Hanson *et al.* 2008). Therefore, determining the seeding rate to achieve the optimum plant densities is crucial to the successful growth of *C. sativa*.

5.5.2 Seeding Rate Effect

Although at all three sites the higher seeding rate significantly increased the plant stand, the actual percent emergence declined dramatically from 64 % to 18 %. The variation in seeding rate is reflected not only in the change of the plant stand, but also in the differences in the yield components. Increasing seeding rates had an adverse effect on the number of branches/plant and number of seeds/plant. In our investigation, the number of branches/plant ranged from 5 to 33 and pods/plant ranged from 58 to 435. Our mean number of 15 branches was significantly lower than the reported figures of 54 branches/plant by Seehuber and Dambroth (1983); but higher than the 6 branches/plant reported by Agegnehu and Honermeier (1997). Our mean number of 222 pods/plant surpassed the figure of 99 pods per plant reported by Seehuber and Dambroth (1983) and 185 pods/plant found by Agegnehu and Honermeier (1997). The changes in the seed yield components were also important factors in determining the relationship between seed yield and plant density. The regression analysis revealed that the response of *C. sativa* seed yield to plant density could be best expressed by second-order polynomial curves, which was consistent with the relationship found by Holliday (1960). The optimum plant density depended upon the sites, which were approximately 170, 280 and 150 plants/m^2 in NS, PEI and SK, respectively. The different seeding rate requirement in the three sites was primarily due to variable weather conditions. In SK, the drought period in the early growing stage released the lateral buds from apical dominance. More branches were produced to compensate for the plant numbers. However, because of the humid conditions in NS and PEI, plants tended to maintain their apical dominance. Therefore, the seed yield could not be fully compensated by producing more branches or pods when the plant density was too low in NS and PEI. Since the emergence rate varied across the sites, it is important for farmers to find seeding rates which maintain the optimum plant population to achieve maximum yield for their location.

5.5.3 Nitrogen Effect

Increasing the N rate significantly increased the seed yield in all three sites. In NS, the seed yield rose from 414 to1182 kg/ha in NS (2008), 1085 to 1808 kg/ha in NS (2009), 1019 to 1827 kg/ha in PEI (2008), and 1470 to 2998 kg/ha in SK (2008) with the increasing N rate. Wright *et al.* (1998) reported that a high N application could escalate

leaf area development, improve leaf area duration after flowering, and enhance crop photosynthetic rate, contributing to increased seed yield. Plants with small pale yellow-green leaves as well as thin and very upright stems in low N treatment suggested that N plays a vital role in achieving optimum yield performance. Allen and Morgan (1972) also indicated that N increased yield by affecting a number of growth parameters such as the number of branches/plant, the number of pods/plant, the total plant weight, and the leaf area index. Even though the differences of the number of branches/plant were not significant in this study, the pods/branch and pods/plant increased significantly with the N rate increased. This result is consistent with many previous studies. Nielson (1997) reported that increasing the N or irrigation application significantly enhanced the number of pods/plant. Similar results were reported by many other workers, who also observed that the number of pods/plant was linearly related to N rate (Bishnoi and Singh 1979; Mudholkar and Ahlawat 1981; Basak *et al.* 1990). Even though the similar yield response to N rate was found in all trials, the optimum N supply differed among sites. To achieve potential yield, *C. sativa* required 125 kg N/ha in NS and PEI, but 100 kg N/ha in SK. Variations in soil-available N at the three sites may have been an important factor in causing the different nitrogen requirements. In NS and PEI, the humid environment resulted in the loss of residual mineral N over winter and the in-season soil N mineralization is the major soil N supply for crops (Sharifi *et al.* 2007). However, higher nitrogen mineralization from soil organic matter and crop residues and residual mineral N from the previous growing season is a significant component of soil N supply in SK (Hergert 1987). Therefore, higher soil N availability and less N leaching in SK may contribute to lower N requirements.

5.5.4 Oil and Protein Content

The oil and protein contents were not significantly affected by seeding rates. This result is consistent with the study on *C. sativa* reported by Urbaniak *et al.* (2008b) and supported by other research on oilseed crops (Albrechtsen and Dybing 1973; Leach *et al.* 1999). However, a significant N effect on oil and protein content was found in this study. It is widely accepted that biosynthetic pathways of fatty acid and amino acid compete for carbon skeletons and energy (Gehringer *et al.* 2006). In *C. sativa* culture, chemical fertilizers, especially N, are one of the most important production inputs. N and carbon

skeletons are not only the essential components in building the protein structure, but also the important substrates for synthesis of fatty acids. In this study, protein accumulation increased while oil accumulation decreased with the increasing N rate. This inverse relationship between protein and oil accumulation could simply reflect the competition between these two synthesis pathways for C-skeletons which are derived from sucrose (Gehringer *et al.* 2006). This inverse relationship between oil and protein content has been reported by a number of studies (Gehringer *et al.* 2006). The significant N effect on fatty acid composition was found in all sites but the change pattern of each fatty acid was not consistent. Since the study on the effect of N on fatty acid composition of *C. sativa* oil was very limited, the result found in this study might not be typical.

5.5.5 Seed Yield

According to previous results found by Seehuber and Dambroth (1983), Schuster and Friedt (1995) and Merrien and Chaternet (1996), *C. sativa* has the potential to achieve 2500 and 3000 kg/ha. Even though the mean value of all sites was only 1685 kg/ha, we did record yields up to 2000 kg/ha within each experimental site. The variation of seed yield between three sites for both N and seeding rate study might be due to genetic, environmental and agronomic factors as well as the interaction between them. The plants had the ability to produce more branches to compensate for the seed yield due to the typical weather conditions in SK, especially the dry period at the beginning of the branching stage. The different soil type, climate and disease could also be the critical factors which resulted in the different yields at different sites.

5.5.6 Downy Mildew Infection

Although *C. sativa* is a crop with good disease resistance, it can be susceptible to downy mildew. Severe downy mildew infection was found in the N response trial in NS and all trials in PEI in 2008. The significant genotype difference was found between CS0005 and Calena. CS0005 was highly resistant to downy mildew at all seeding rates with an average disease rate of 1. A significant negative seeding rate effect on disease incidence in Calena was found in this study (Figure 5.12).The higher disease rate in low seeding rate in this study may be caused by the severe lodging problems which occurred in the low seeding rate. Due to space between plants in the lower seeding rate plots, plants could not find support from adjacent plants and tended to lean and fall over. The results

from N trial were consistent with the early report found by Crowley (1999) which indicated that the disease incidence was positively correlated with N rate (Figure 5.13). In 2009, due to the relatively dry growing conditions, mild downy mildew infection (disease rating of 1) was found in NS and PEI. The difference was not significant among the seeding rates and N treatments. It is worth noting that downy mildew is a seed-borne fungal disease so seeds harvested from the infected fields should not be used for planting. Planting pathogen-free seeds, dryland production, and limited irrigation, greater air circulation and good crop rotation practice may help to minimize the disease problems (McVay and Lamb 2008).

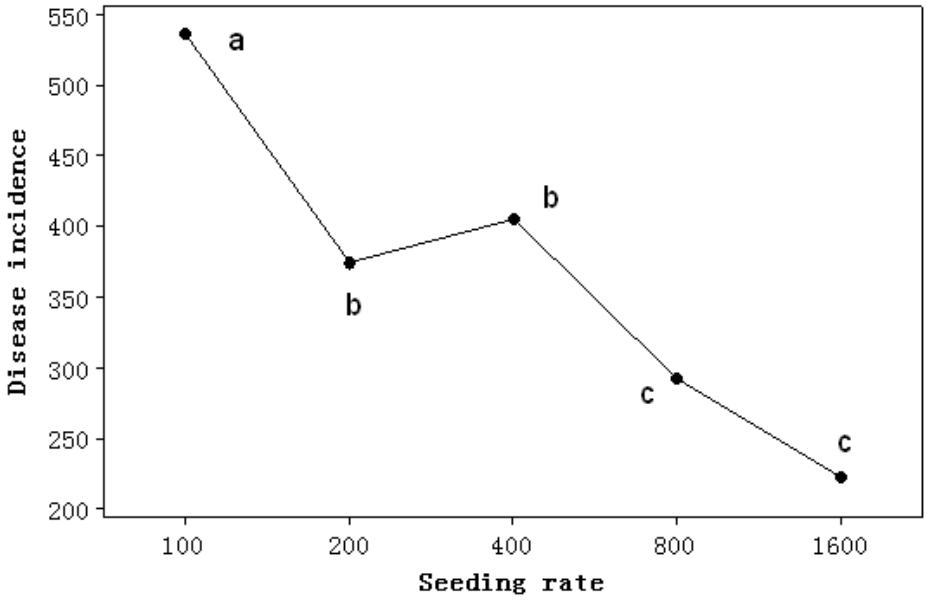

Figure 5.13 Seeding rate effect (seeds/m^2) on disease incidence for Calena (PEI 2008)

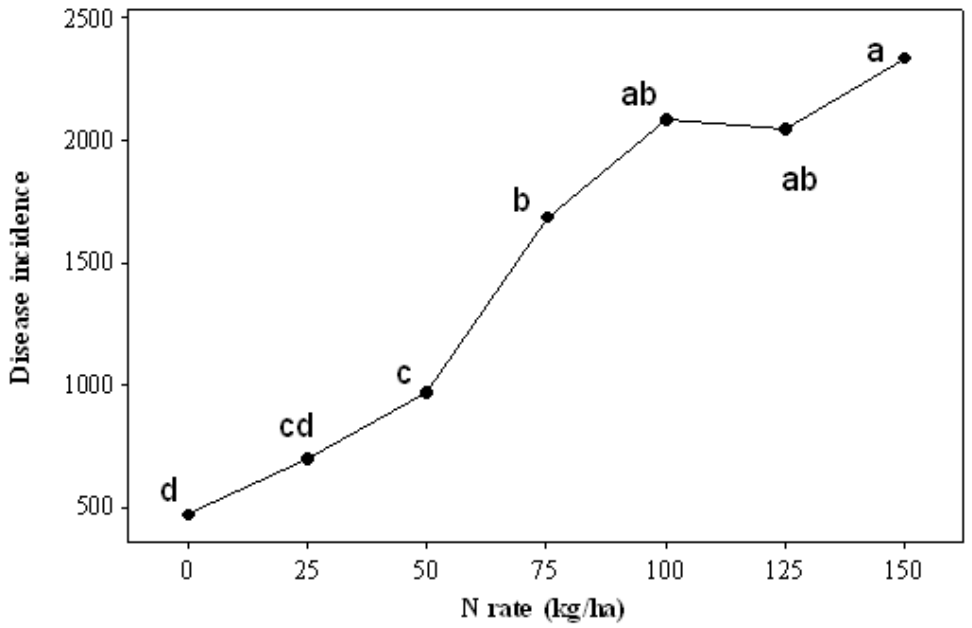

Figure 5.14 Nitrogen effect on disease incidence for Calena (PEI 2008)

5.6 Conclusion

The results showed that seeding and N rates significantly influenced the seed yield but this depended on the soil type and specific environmental condition. To achieve maximum yield, *C. sativa* required 125 kg N/ha at both the NS and PEI sites but only 100 kg N/ha in SK. The optimum plant density for growing *C. sativa* is approximately 170, 280 and 150 plant/m^2 in NS, PEI and SK, respectively.

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Chapter 6 Diversity and Adaptation of *Brassica carinata* Genotypes to Contrasting Environments

6.1 Introduction

With the increasing demand for vegetable-based oil around the world, developing new oilseed crops will be a major task facing researchers and agronomists. *Brassica carinata* A. Braun, also known as Ethiopian mustard or Abyssinian mustard, has the potential to increase the oilseed production area in Canada. *Brassica carinata* originated from the natural cross between the diploid species *B. nigra* (L.) Koch and *B. oleracea* (L.) (Warwick *et al.* 2006). Compared with *B. juncea*, the genetic diversity of *B. carinata* is relatively low due to the limited areas of cultivation, primarily in Ethiopia and its surrounding area. Sanhu and Gupta (1996) indicated that the intraspecific diversity in morphological and quality traits of *B. carinata* is greatly less than in *B. juncea* and *B. napus*. However, a diversity of ecotypes of *B. carinata,* with high variation of morphological and agronomic traits among the accessions, has been reported by Demissie *et al.* (1992).

Preliminary agronomic evaluation of *B. carinata* suggested that its relatively large seed contains about 26 % to 40 % oil, and varied among the cultivars and genotypes (Getinet *et al.* 1996). Alemayehu and Becker (2002) have identified the *B. carinata* accessions with potential genes for early maturity, higher yield components and oil and protein content. Bansal *et al.* (1990) noted that disease resistance also differed among cultivars; some accessions are more resistant to blackleg and white rust than other *Brassica* crop species.

Knowles *et al.* (1981) suggested that the *B. carinata* has the potential to become an important new oilseed crop for western Canada because of its high drought and heat tolerance. *Brassica carinata,* with its many agronomic advantages, could be an important source of genes which are rare in other oilseed *brassicas* and could enhance the agronomic traits of other *Brassica* crops by interspecific hybridization. In order to make use of such important genes, the basic study on the geographic pattern of the variability should be a prime task. This study was undertaken to assess the agronomic diversity

among a total of 10 accessions of *B. carinata* in NS, PEI and SK, based on the analysis of the genotype effect on plant stand, flowering date, plant height, maturity date, seed yield, TKW, seed oil content, seed protein content and oil quality.

6.2 Materials and Methods

6.2.1 Plant Material

Ten accessions of *B. carinata*, tested were 070742EM, 070756EM, 070732EM, 070727EM, 040758EM, 070714EM, 050488EM, 050334EM, 070768EM and 070760EM. *Brassica juncea* cv. AC Vulcan was used as a check for comparison in both years. All accessions were obtained from the Agriculture and Agri-Food Canada, Saskatoon Research Centre (AAFC-SRC) breeding program.

Line 070742EM is self-pollinated F_5 from original accession with selection for earliness and general good agronomic performance. Line 070756EM is derived from multi-line bulk of two dihaploid S_2 selections for earliness and good agronomic performance from Ethiopian variety S71. 040758EM is from an elite breeding line bulk, which was developed from 21156Y for high seed oil content, earliness and good agronomic performance. Both lines 050488EM and 070760EM are derived from S67. Line 050488EM is F_5 line while line 070760EM is F_4 line derived line using the single seed descent breeding method form the Ethiopian cultivar S67. Line 050334EM is an elite AAFC breeding line derived from the Ethiopian cultivar Dodolla. The remaining lines 070732EM, 070727EM, 070714EM and 070768EM are elite AAFC germplasm selections. AC Vulcan [*Brassica juncea* (L.) Czern.] resulted from a single plant selection from Cutlass (Rakow and Rode 2008).

6.2.2 Field Evaluation Trial

The experiment was conducted at three locations (Truro, NS, NSAC; AAFC Harrington, PEI and AAFC, Saskatoon, Saskatchewan) in Canada during the main cropping season of 2008-09 (detailed information see chapter 4).

At each location, the experiment was designed as a randomized completely block design (RCBD) with four replications. Fertilizer application schedule in three sites is listed in Table 6.1. The selected soil characteristics for each field are shown in Table 6.2 and 6.3. The pre-emergent herbicide Treflan at a rate of 2.3 L/ha was applied in NS and PEI in both years. In NS, the seeds were planted previously fallowed land. The previous

crop in PEI was barley. In SK, the previous crops were oats in 2008. In 2009, the previous crop in NS, PEI and SK were flax, red clover and superb wheat, respectively. Plots were seeded using a Hege plot drill (H and N Equipment Inc., Colwich, Kansas, USA) with double disc openers and press wheels at a rate of 130 seeds/m^2 in NS and PEI. In SK, plants were seeded with a 1.5 meter R-Tech hoe drill (AAFC, Saskatchewan) (packaged seed through a cone and splitter). Plots were seeded on May 16, May 26 and May 15 in 2008 and May 13, May 21 and May 15 in 2009, at the NS, PEI and SK sites, respectively. The seeded plot size at the NS and PEI sites were 7.5 m^2 (8 rows @ 15 cm x 6 m in length) and were trimmed to 5 m after emergence in 2008. In 2009, the seeded plot size was 6.25 m^2 in NS and 7.5 m^2 in PEI. The row spacing was 15 cm and the distance between plots and blocks were 25 cm and 2.5 m respectively at the NS and PEI sites. Plots were harvested with a Hege 125C plot combine (Hege USA, Colwich, Kansas, USA) with a harvest area of 5 m x 1.25 m for the NS and PEI sites. In SK, plants were also straight combined with a Hege combine (Hege, Germany).

Table 6.1 Fertilizer Application Schedule for *B. carinata* genotype study in three sites

Site	Date	Form	Method
NS 2008	May 15	200 kg/ha 0N-20P-20K + 240 kg/ha 21N-0P-0K-21S	Broadcast and incorporated
	June 28	140 kg/ha 34N-0P-0K	Topdressing
PEI 2008	May 25	200 kg/ha 0N-20P-20K + 240 kg/ha 21N-0P-0K-21S	Broadcast and incorporated
	July 7	140 kg/ha 34N-0P-0K	Topdressing
SK 2008	May 15	236 kg/ha 28.4N-14.2P-0K-11.8S	Incorporated
NS 2009	May 12	370 kg/ha 14N-14P-14K-10.19S	Broadcast and incorporated
	July 6	190 kg/ha 27N-0P-0K	Topdressing
PEI 2009	May 20	240 kg/ha 21N-0P-0K- 21S + 200 kg/ha 0N-20P-20K	Broadcast and incorporated
	July 15	150 kg/ha 34-0-0	Topdressing
SK 2009	May 15	114 19.5N-19.6P-0K-19.6S	Incorporated

Table 6.2 Soil characteristics of *B. carinata* genotype study in 2008

Location	pH	Organic Matter (%)	CEC (meq/100g)	P_2O_5 (kg/ha)	K_2O (kg/ha)	Ca (kg/ha)	Mg (kg/ha)	Sulfur (kg/ha)
NS	6.2	2.6	13.5	1033	266	2936	643	35
PEI	5.9	3.9	12.1	454	334	1866	228	48
SK	8.1	3.7	-	137	1229	-	-	19

Table 6.3 Soil characteristics of *B. carinata* genotype study in 2009

Location	pH	Organic Matter (%)	CEC (meq/100g)	P_2O_5 (kg/ha)	K_2O (kg/ha)	Ca (kg/ha)	Mg (kg/ha)	Nitrate-N(ppm)	% N	Sulfur (kg/ha)
NS	5.9	3.0	12.7	1562	348	2640	356	12.3	0.17	30
PEI	6.0	3.6	12.0	855	398	2612	148	33.1	0.24	30
SK	7.4	-	-	205	1301	-	-	24.8	-	30

6.3 Data Collection

6.3.1 Measurements

The plant emergence was measured approximately three weeks after planting. Two counts were completed in each plot by randomly placing two 0.25 m^2 quadrats in the plot, avoiding the outside rows. Plant height was measured from the soil surface to highest point on the erect plant at the time of maturity. Three data points (front, middle and back part of plot) were collected from each plot. The flowering date was estimated visually as the date when approximately 10 % of plants had one flower open. The number of days from seeding to flowering was calculated. Maturity date was also estimated visually as the date when approximately 95 % of the pods were brown. Lodging was visually evaluated using a scale of 1-5; with 1 being erect and 5 being flat on the ground. After drying to approximately 8 % moisture content seeds were cleaned using a Clipper seed cleaner (Clipper Seed Cleaning Co., Bluffton, IN). Cleaned seeds were weighed (g) and g/plot values were converted to kg/ha based on plot areas for each location. Climatic data (including weekly precipitation, temperature and sunshine hours) at three sites (NS, PEI and SK) and for two years (2008 and 2009) were obtained from Environment Canada on-line data (http:// www.climate.weatheroffice.ec.gc.ca/).

6.3.2 Oil and Protein Content

Total seed oil and protein content was analyzed on 5.0 g of whole seed using a near-infrared reflectance (NIR) spectroscopy (FOSS NIR Systems model 6500 spectrometer, spinning cup autosampler, WIN ISI II calibration software, Technicon Canada Inc., Mississauga, ON Technicon Canada Inc., Mississauga, ON). Samples were analyzed in bulk by combining blocks 1 and 2, as well as 3 and 4. Oil and protein contents are reported on a dry matter basis.

6.3.3 Fatty Acid Analysis

The lipid extraction and methylation process were generated based on the protocol of Budge *et al.* (2006) with minor modification. For detailed information, see chapter 4.

6.3.4 Statistical Analysis

Experiment was designed as a RCBD with four replications. The main factor in this study was genotype. The response variables from both experiments, including stand count, plant height, days to flowering, days to maturity, thousand kernel weight (TKW), seed

yield, oil and protein content and fatty acid composition were collected and subjected to the PROC Mixed procedure in SAS version 9.1 (SAS Institute Inc., Cary, NC,USA, 2002-2003). Tukey test was used to compare the differences among treatments at the 5 % significant level. PROC Mixed procedure in SAS version 9.1 was also used in analyzing the site and year effects. Agronomic and seed quality data for 10 accessions of *B. carinata* was summarized by using the Minitab 14 statistic software (Minitab Inc., USA, 1972-2004), the information included means, standard deviations and ranges of each trait.

6.4 Results

6.4.1 Agronomic Trait

Mean values, stand deviations and the ranges of agronomic and seed quality traits of 10 *B. carinata* accessions are shown in Table 6.4. Large variation of agronomic and seed quality traits were observed among the 10 accessions in this study. For these 10 accessions, mean days to flowering and days to maturity ranged from 47-53 and 95-118, respectively. The plant height varied by more than 60 cm among 10 accessions. TKW ranged from 3.45 g to 5.30 g. Average seed oil and protein content of *B. carinata* ranged from 31.54 % to 47.07 % and from 25.5 % to 35.87 %, respectively, across all trials. On average, the oil of *B. carinata* typically contained from 2.72 % to 3.58 % palmitic, 6.67 % to 11.6 % oleic, 15.39 % to 20.91 % linoleic, 10.77 % to 17.09 % linolenic, 6.08 % to 9.45 % eicosenoic and 33.43 % to 42.92 % erucic acid.

Table 6.4 Mean values for agronomic and seed quality traits in 10 accessions of *B. carinata* at three sites: NS, PEI and SK. 2008-09

Trait	Mean	SD	Range
Days to flowering	50	1.64	47-53
Days to maturity	104	5.85	95-118
Plant height (cm)	121	18.41	96-164
TKW (g)	4.19	0.49	3.45-5.30
Seed yield (kg/ha)	2016	826.4	556-3493.0
Oil content (%, dry wt. basis)	37.56	3.54	31.54-47.07
Protein content (%, dry wt. basis)	30.65	2.68	25.5-35.87
Fatty acids (% of total)			
C16:1 – palmitic	3.16	0.208	2.72-3.58
C18:1- oleic	8.58	10108	6.67-11.6
C18:2- linoleic	17.17	0.933	15.39-20.91
C18:3- linolenic	13.42	1.369	10.77-17.09
C20:1- eicosenoic	7.51	0.718	6.08-9.45
C20:2 – eicosadienoic	1.13	0.104	0.89-1.38
C22:1-erucic	38.82	1.905	33.43-42.92
C22:2-docosadienoic	1.51	0.231	1.03-2.06
C24:1- nervonic	2.50	0.238	1.79-2.96
Other fatty acids	2.54	0.686	1.52-4.19
Total saturated fatty acids	5.99	0.233	5.43-6.51

6.4.2 Plant stand, Days to flower and Days to maturity

Plant stand, days to flower and days to maturity for the 10 *B. carinata* accessions and AC Vulcan are listed in Table 6.5. Data for plant stand in SK 2008 was not collected. In SK 2009 trial, no differences in plant stand were observed among the tested lines due to poor emergence and uneven plant stand establishment, due to both drought and frost. Based on the data from NS and PEI, plant stand for the 10 *B. carinata* accessions ranged from 75 to 109 plants/m^2 in 2008 and 103 to 164 plants/m^2 in 2009. AC Vulcan had the significantly lowest plant stand (39-90 plants/m^2) in all these four trials. Average value of days to flower and days to maturity for 10 *B. carinata* accessions and AC Vulcan across all the trials is shown in Table 6.6. Significant difference for days to flower and days to maturity was observed among the 10 accessions. As expected, the differences between the accessions and the check were greater than within accessions. Generally, all the *B. carinata* accessions were 7 to 8 days later flowering than AC Vulcan and required 4 to 15 days longer to reach maturity (Table 6.6). Among the 10 accessions, line 070414EM required the shortest number of days to mature, which was approximately 99 days.

Table 6.5 Plant stand, days to flower and days to maturity of *B. carinata* accessions and AC Vulcan grown at NS, PEI and SK. 2008-09

Entries	Site															
	Plant stand (plants/m^2)					Days to flower						Days to maturity				
	2008		2009			2008			2009			2008			2009	
	NS	PEI	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK	NS	SK
070742EM	77b	85b-e	128a	124a	32	52a	51ab	53a	47b	49ab	50ab	103b	108a	101cd	102b	112b
070756EM	90ab	87a-e	124ab	126a	29	49b	49b	52ab	48b	49ab	49b	98bc	96e	102bcd	104ab	111bc
070732 EM	85ab	86b-e	116bc	125a	21	51ab	51ab	53a	49ab	50ab	50ab	99bc	99d	104bc	105ab	112b
070727EM	101a	109ab	123ab	118b	25	51ab	51ab	50bc	48b	49ab	49b	97bc	95e	99ed	98bc	112b
040758EM	96ab	81cde	121ab	123a	49	51ab	51ab	53a	51a	52a	50ab	104ab	105b	102cd	108a	114ab
070714EM	102a	114a	124ab	123a	40	51ab	50b	51b	48b	50ab	49b	95c	96e	95e	97c	112b
050488EM	86ab	108abc	108c	104c	19	53a	52a	51b	47b	47b	49b	103b	103c	103bcd	104ab	113ab
050334EM	104a	80de	105c	103c	54	49b	48bc	53a	49ab	51a	50ab	105ab	106b	104bcd	105ab	115ab
070768EM	75b	81cde	121ab	124a	25	49b	48bc	53a	50a	51a	51a	110a	109a	109a	106a	118a
070760EM	90ab	104a-d	120ab	125a	31	50b	49b	50bc	47b	48ab	50ab	104ab	100d	106ab	101b	113ab
AC Vulcan	39c	61e	90d	76d	41	44c	43d	43d	41c	40c	43c	89d	91f	90f	92d	102d
P-value	0.000	0.011	0.000	0.000	0.9	0.001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.000	0.000	0.000	0.001

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 6.6 Average values of days to flower and days to maturity for 10 *B. carinata* accessions and AC Vulcan across all the trials

Entries	Days to flower	Days to maturity
070742EM	50	105
070756EM	49	102
070732 EM	51	104
070727EM	50	100
040758EM	51	107
070714EM	50	99
050488EM	50	105
050334EM	50	107
070768EM	50	110
070760EM	49	105
AC Vulcan	42	95

6.4.3 Seed Yield

The results of analysis of variance (Table 6.7) show that the seed yield varied not only among genotypes, but also among six year-sites (p-value for three-way interaction effect is 0.0008). The effect of year was predominant for seed yield (F = 1414), followed by the site effect (F = 507). In comparison with year and site effect, genotype effect on seed yield was relatively small. This indicated that the genotype performances were highly dependent on environmental factors. Yield data from each trial, which are reported in Table 6.8, were analyzed separately. The lowest yields (556-957 kg/ha) were observed in NS in 2008; yields were generally higher in PEI and SK in 2009. The significantly lower seed yield in NS 2008 was probably due to weed competition (wild radish, *Raphanus raphanistrum* L.). Good germination and uniform stand establishment was noted for plots in NS 2009, which might be the contributing factor for the higher seed yield in 2009. It is worth noting that in SK 2009, widespread drought and frost damage occurred in the seed establishment stage; even though this caused poor emergence and uneven stand establishment, all the accessions still achieved the highest seed yield (2333-3105 kg/ha). No significant difference in seed yield was found among lines in NS 2008

and SK 2008. In NS 2009, seed yield did not differ significantly among accessions except for 070714EM, which had significantly lower seed yield (1668 kg/ha). In PEI, seed yield varied dramatically among the entries and line 050488EM and 050334EM had the highest seed yields in both years. The significantly higher seed yield in 2009 PEI was probably due to good germination and high soil-available nitrogen. In SK 2009, all lines achieved a seed yield above 2700 kg/ha except for line 070714EM (2333 kg/ha) and AC Vulcan (2488 kg/ha). Furthermore, line 070756EM, 070732EM, 070727EM, 040758EM, 070768EM and 070760 had the highest seed yields which were approximately 3000 kg/ha in 2009 SK.

Table 6.7 Analysis of variance for seed yield of 10 accessions of *B. carinata* in field trials across three sites: NS, PEI and SK. 2008-09

Effect	Num DF	Den DF	F Value	Pr > F
Site	2	195	507.81	<.0001
Year	1	195	1414.40	<.0001
Site*Year	2	195	95.07	<.0001
Genotype (G)	9	195	6.60	<.0001
G*Site	18	195	3.08	<.0001
G*Year	9	195	4.94	<.0001
G*Site*Year	18	195	2.46	0.0008

Table 6.8 Seed yield of 10 *B. carinata* accessions and AC Vulcan in six site-years

Entries	Seed yield (kg/ha)					
	NS08	PEI08	SK08	NS09	PEI09	SK09
070742EM	872a	1456a-d	2202a	2134abc	2614de	2729cd
070756EM	895a	1348a-d	2272a	2464 a	2556de	3105a
070732 EM	739a	1308bcd	2209a	2094abc	2563de	3051ab
070727EM	872a	1192cd	2385a	1907cd	3493a	2979abc
040758EM	556a	1140d	2180a	2105abc	2495e	2850abc
070714EM	957a	1452a-d	2182a	1668d	2529e	2333e
050488EM	899a	1588ab	2324a	2480a	3341ab	2784bcd
050334EM	872a	1484abc	2303a	2082abc	3039abc	2783bcd
070768EM	788a	1136d	2457a	2110abc	2442e	3055ab
070760EM	863a	1656a	2399a	2353ab	2905bcd	3023abc
AC Vulcan	863a	1428a-d	2474a	2002bcd	2359e	2488de
P-value	0.516	0.045	0.498	0.0128	<.0001	<.0001
Mean	834	1381	2308	2127	2758	2834

Means within a column followed by different letters are significantly different ($p < 0.05$)

6.4.4 Plant Height and TKW

No obvious differences in plant height were noted among the lines in all trials except for the trial in SK 2009. The significantly shorter line 050488EM (96 cm) was observed in SK 2009. However, this value was only found in one site for a single year. The plant height was strongly affected by the environmental conditions. Plants in NS and PEI in 2009, with the mean value of approximately 156 cm, were significantly taller than the plants in NS 2008(113 cm), PEI 2008(124 cm), SK 2008 (111 cm) and SK 2009 (106 cm). The TKW of all 10 *B. carinata* accessions ranged from 3.45 g to 5.30 g and were significantly higher than that of AC Vulcan, which was approximately 2.6 g. Line 070714EM had the largest TKW (4.65 - 5.05 g) in all trials.

6.4.5 Oil and Protein Content

The oil and protein content of the 10 accessions of *B. carinata* and AC Vulcan are listed in Tables 6.9 and 6.10. The mean oil and protein content ranged from 30.16 to 48.75 % and from 24.55 to 36.85 %, respectively, across all trials. Oil contents of all

accessions were highest in SK 2009 and higher overall in 2009 than in 2008. Within a trial, 070768EM always had the highest oil content; while 070714EM and 050334EM had the lowest oil content of the 10 accessions. The negative correlation between oil and protein content was found in all trials (Figure 6.1). Therefore, the highest oil contents were associated with the lowest protein content across all trials. 070714EM had the highest protein content and 070768EM had the lowest protein content in all trials.

Table 6.9 Diversity in oil and protein content (%) of 10 *B. carinata* accessions and AC Vulcan in 2008

Entries	NS		PEI		SK	
	Oil	Protein	Oil	Protein	Oil	Protein
070742EM	33.53cd	34.08abc	34.04d	34.15ab	41.24b	27.24bc
070756EM	33.86bcd	33.13bc	35.8bcd	32.59abcd	39.56bcd	28.78ab
070732 EM	32.09d	35.87a	34.36d	34.44a	40.95bc	28.11b
070727EM	36.63ab	30.74ed	36.86abc	32.17cd	38.91de	29.19ab
040758EM	36.46ab	32.24cde	36.73abc	33.14abc	43.67a	25.50c
070714EM	31.84d	35.07ab	33.6d	34.32ab	37.39e	30.80a
050488EM	32.71d	32.77bcd	35.48bcd	31.05de	38.95de	27.48bc
050334EM	31.54d	35.70a	33.88d	33.96abc	37.47e	28.88ab
070768EM	38.39a	31.80cde	37.76ab	32.88abcd	44.8a	25.93c
070760EM	33.20d	32.91bcd	34.82cd	32.48bcd	39.45cd	27.37bc
AC Vulcan	37.87a	30.00e	38.49a	30.03e	40.65bcd	28.96ab
Mean	34.37	33.12	35.62	32.84	40.18	28.02
P-value	0.0026	0.0025	0.0066	0.0047	<.0001	0.0049

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 6.10 Diversity in oil and protein content (%) of 10 *B. carinata* accessions and AC Vulcan in 2009

Entries	NS		PEI		SK	
	Oil	Protein	Oil	Protein	Oil	Protein
070742EM	36.80cde	30.64bc	35.89de	31.67bc	41.76b	28.45bc
070756EM	36.29e	31.24b	36.639cde	30.60c	40.73bc	29.38ab
070732 EM	36.39de	31.08bc	34.689e	32.86ab	42.31b	27.75bcd
070727EM	38.57bcd	29.94bcd	38.77ab	29.86c	41.85b	28.52bc
040758EM	40.44ab	29.11cd	37.51bcd	31.21bc	45.93a	25.94e
070714EM	33.84f	33.30a	32.60f	34.68a	39.58c	30.53a
050488EM	36.35e	29.08cd	36.08cde	30.49c	41.12bc	26.63de
050334EM	35.88ef	30.75bc	35.36ef	31.98bc	40.55bc	27.48cde
070768EM	41.29a	28.57d	40.72a	29.88c	47.07a	25.85e
070760EM	36.41de	29.57bcd	35.72de	30.55c	40.81bc	26.70de
AC Vulcan	38.80bc	30.86bc	37.90bc	31.32bc	41.96b	29.14ab
Mean	37.37	30.38	36.53	31.37	42.15	27.85
P-value	0.0004	0.0124	0.0002	0.013	<0.0001	<0.0001

Means within a column followed by different letters are significantly different ($p < 0.05$)

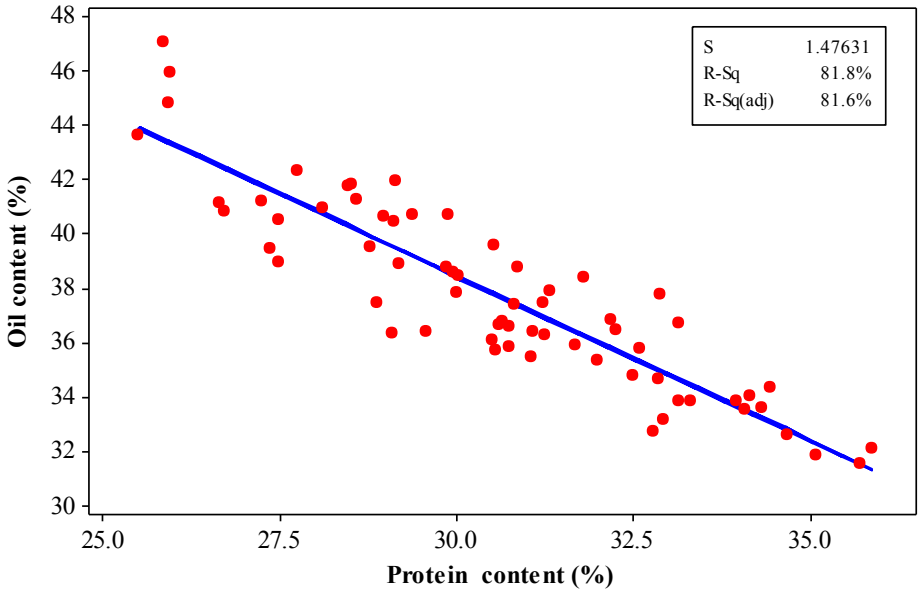

Figure 6.1 Correlation between oil and protein content in all trials

6.4.6 Fatty Acid Composition

The fatty acid composition of the 10 *B. carinata* genotypes and AC Vulcan in NS (08-09), PEI (08-09) and SK (08) was shown in Table 6.11- 6.15. *B. carinata* oil is mainly made up 2.76 % to 3.56 % palmitic, 6.68 % to 11.51 % oleic, 15.44 % to 20.01 % linoleic, 10.90 % to 17.07 % linolenic, 6.11 % to 9.23 % eicosenoic and 33.78 % to 42.77 % erucic acid. An overview of variation in fatty acid concentrations for the five site-year environments demonstrated that wider ranges of values for fatty acid composition of *B. carinata* seed oil were found among accessions within each trial than among sites in 2008 and 2009 (Table 6.16). Therefore, the genotype effect was predominant for the fatty acid composition. Line 070714EM had the highest erucic acid content but the lowest linolenic and eicosenoic acid in all trials. AC Vulcan contained the lowest amount of erucic acid and the highest amount of oleic, linoleic and eicosenoic acid in all studies.

Table 6.11 Diversity in fatty acid composition of 10 *B. carinata* accessions and AC Vulcan seed oil in NS 2008

Entries	C16:0	C18:1	C18:2	C18:3	C20:1	C20:2	C22:1	C22:2	C24:1	other
070742EM	2.76f	8.74c	17.51cd	13.05c	7.21c	1.14d	38.58bcd	1.54de	2.60cd	3.34a
070756EM	2.95de	9.29b	17.44c-e	12.60de	7.21c	1.05e	37.80d	1.43f	2.63c	4.14a
070732 EM	3.23bc	8.66c	17.03def	16.10a	7.78b	1.17cd	35.02e	1.46ef	2.45d	3.36a
070727EM	3.40a	6.68e	16.86ef	14.34b	6.70d	1.25b	38.92bc	1.79b	2.70bc	3.58a
040758EM	3.10bcd	8.11d	18.54b	12.69cd	7.09cd	1.28a	37.77d	1.67c	2.56cd	3.57a
070714EM	2.87ef	6.82e	17.89c	12.29e	6.13e	1.17cd	40.19a	2.06a	2.91a	3.78a
050488EM	3.08cd	8.35cd	16.93def	12.74cd	7.06cd	1.09e	39.33ab	1.58cd	2.68bc	3.67a
050334EM	3.24abc	7.11e	16.96def	14.54b	6.11e	1.16cd	39.15bc	1.78b	2.66bc	3.67a
070768EM	3.02de	8.28cd	16.55f	14.27b	7.47bc	1.19c	38.39cd	1.48def	2.79ab	2.86a
070760EM	3.26ab	8.49cd	17.04def	13.06c	7.18c	1.09e	38.80bc	1.58cd	2.70bc	3.27a
AC Vulcan	2.97de	17.87a	21.99a	12.75cd	11.22a	1.15d	23.37e	0.62g	1.58e	3.00a
Mean	3.08	8.95	17.70	13.49	7.38	1.16	37.02	1.54	2.57	3.48
P-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0725

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 6.12 Diversity in fatty acid composition of 10 *B. carinata* accessions and AC Vulcan seed oil in PEI 2008

Entries	C16:0	C18:1	C18:2	C18:3	C20:1	C20:2	C22:1	C22:2	C24:1	other
070742EM	2.75g	10.25cd	16.03e	11.61e	8.45cd	0.96e	40.31d	1.14fg	2.07d	2.88
070756EM	2.75g	11.51b	15.56f	11.30ef	8.66c	0.90g	39.79de	1.04h	2.16cd	2.89
070732 EM	3.29ab	10.55c	16.33de	14.48a	9.23b	1.01d	35.42f	1.08gh	1.84e	3.00
070727EM	3.28abc	7.44g	16.31de	13.13b	7.35e	1.14a	40.47cd	1.60b	2.26bc	3.08
040758EM	3.05de	9.69ef	18.03b	10.90g	8.31d	1.13a	39.18e	1.33c	2.05d	2.67
070714EM	2.87fg	7.57g	17.03c	11.08fg	6.62f	1.02cd	42.77a	1.74a	2.48a	2.83
050488EM	3.16bcd	9.90de	15.55f	11.93d	8.17d	0.93f	40.51cd	1.19e	2.12d	2.91
050334EM	3.31a	7.72g	16.69cd	12.84bc	6.64f	1.04c	41.25b	1.61b	2.27b	2.85
070768EM	3.00ef	9.48f	15.44f	12.82c	8.68c	1.02cd	39.81de	1.18ef	2.10d	2.73
070760EM	3.14cd	9.63ef	15.64f	11.53e	8.15d	0.95ef	41.02bc	1.26d	2.13d	2.90
AC Vulcan	2.90f	19.26a	20.96a	12.94bc	11.99a	1.10b	22.80g	0.47i	1.48f	2.61
Mean	3.05	10.27	16.69	12.23	8.39	1.02	38.48	1.24	2.09	2.85
P-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.896

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 6.13 Diversity in fatty acid composition of 10 *B. carinata* accessions and AC Vulcan seed oil in SK 2008

Entries	C16:0	C18:1	C18:2	C18:3	C20:1	C20:2	C22:1	C22:2	C24:1	other
070742EM	3.04e	8.91c	18.10c	13.57ef	7.78c	1.22d	37.40cd	1.47def	2.62de	2.41cd
070756EM	3.16cd	9.54b	17.61de	13.41f	8.1b	1.16f	37.27cd	1.36g	2.67cde	2.34d
070732 EM	3.45a	8.69cd	17.31ef	17.07a	8.31b	1.25c	33.78e	1.41fg	2.55e	2.47bcd
070727EM	3.46a	7.00f	16.87g	15.53b	7.30e	1.36a	37.95bc	1.72b	2.75abc	2.38cd
040758EM	3.13d	8.24de	18.63b	13.86d	7.47de	1.36a	36.92d	1.60c	2.63de	2.63a-d
070714EM	2.92f	7.34f	17.95cd	13.09g	6.91f	1.29b	39.17a	1.94a	2.87a	2.80ab
050488EM	3.39ab	8.57cd	17.32ef	13.63def	7.80c	1.19ef	37.66cd	1.49de	2.66cde	2.86a
050334EM	3.39ab	7.09f	16.66g	15.08c	6.80f	1.24cd	38.71ab	1.73b	2.71bcd	2.90a
070768EM	3.24c	8.04e	16.95fg	15.46b	7.83c	1.30b	36.88d	1.46ef	2.83ab	2.36d
070760EM	3.35b	8.31de	17.06fg	13.82de	7.64cd	1.18ef	38.20bc	1.52d	2.74bcd	2.71abc
AC Vulcan	2.99ef	16.55a	21.95a	13.53f	10.96a	1.20e	24.63f	0.61h	1.71f	2.47bcd
Mean	3.23	8.93	17.86	14.37	7.91	1.50	36.23	1.48	2.61	2.58
P-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0198

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 6.14 Diversity in fatty acid composition of 10 *B. carinata* accessions and AC Vulcan seed oil in NS 2009

Entries	C16:0	C18:1	C18:2	C18:3	C20:1	C20:2	C22:1	C22:2	C24:1	other
070742EM	2.86d	9.83bc	17.27cd	12.97e	7.84c	1.08e	39.38d	1.34ef	2.33ef	1.58e
070756EM	2.88d	9.88b	17.02cde	12.79ef	7.77c	1.03f	39.62cd	1.34ef	2.49bc	1.71de
070732 EM	3.30a	9.28c	16.68efg	16.19a	8.55b	1.12c	35.74e	1.30f	2.25f	1.80cd
070727EM	3.30a	7.13e	16.18gh	14.61b	6.91e	1.18b	40.71b	1.75b	2.57b	1.79cd
040758EM	3.11b	8.94d	18.42b	12.44fg	7.50d	1.22a	39.03d	1.58c	2.39de	1.72de
070714EM	2.84d	6.95e	17.62c	12.08g	6.28f	1.12c	42.50a	2.03a	2.73a	1.93bc
050488EM	3.21ab	8.81d	16.65e-h	12.85e	7.42d	1.04f	40.48bc	1.53cd	2.55bc	1.89bcd
050334EM	3.22ab	7.21e	16.24fgh	14.13c	6.24f	1.09de	41.76a	1.79b	2.55bc	2.04ab
070768EM	3.09bc	8.59d	16.06h	14.10c	7.87c	1.11cd	39.66cd	1.39e	2.50bc	1.89bcd
070760EM	3.30a	8.87d	16.83def	12.90e	7.42d	1.05f	39.93bcd	1.49d	2.48cd	2.16a
AC Vulcan	2.95cd	18.64a	22.45a	13.48d	11.56a	1.17b	22.53f	0.54g	1.51g	1.80cd
Mean	3.10	9.47	17.40	13.50	7.76	1.11	38.30	1.46	2.40	1.85
P-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0021

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 6.15 Diversity in fatty acid composition of 10 *B. carinata* accessions and AC Vulcan seed oil in PEI 2009

Entries	C16:0	C18:1	C18:2	C18:3	C20:1	C20:2	C22:1	C22:2	C24:1	other
070742EM	3.07e	9.52c	18.54c	12.56ef	7.81cd	1.12f	38.19d	1.37def	2.43de	1.79
070756EM	3.04e	10.35b	17.77de	12.41fg	7.93bcd	1.05h	38.43cd	1.28g	2.48bcd	1.79
070732 EM	3.56a	9.16d	17.84d	15.95a	8.23b	1.15e	34.53f	1.33fg	2.32e	2.11
070727EM	3.41b	7.57g	17.41de	14.22b	7.39e	1.24b	39.01bcd	1.59b	2.51bcd	1.95
040758EM	3.39bc	8.81ef	20.01b	12.83de	7.51e	1.26a	36.71e	1.50c	2.44de	1.91
070714EM	3.01ef	7.44g	18.97c	12.13g	6.46f	1.17d	40.37a	1.92a	2.78a	1.81
050488EM	3.32cd	9.14d	17.34ef	13.08cd	7.65de	1.08g	39.10bc	1.39de	2.47cd	1.87
050334EM	3.37bc	7.67g	17.37ef	14.13b	6.64f	1.14e	39.73ab	1.64b	2.59b	2.04
070768EM	3.25d	8.69f	16.96f	14.10b	8.02bc	1.16de	38.39cd	1.34ef	2.58bc	1.71
070760EM	3.32cd	9.07de	17.51de	12.80de	7.64de	1.08g	39.23bc	1.41d	2.51bcd	1.89
AC Vulcan	2.94f	17.89a	22.60a	13.37c	11.36a	1.19c	23.44g	0.57h	1.59f	1.50
Mean	3.24	9.57	18.39	13.42	7.88	1.15	37.01	1.32	2.43	1.85
P-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0021

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 6.16 Overview of variation individual fatty acid concentration in five year-sites (n=10 accessions in 2008 and 2009)

Parameter	Fatty acid concentration (%)										
	C16:0	C18:1	C18:2	C18:3	C20:1	C20:2	C22:1	C22:2	C24:1	Other	Saturated
NS 2008											
Minimum	2.72	6.67	16.39	12.24	6.08	1.036	34.94	1.35	2.34	2.73	5.423
Maximum	3.40	9.68	18.89	16.15	8.19	1.28	40.42	2.06	2.92	4.19	6.23
Mean	3.09	8.05	17.27	13.57	6.99	1.158	38.40	1.64	2.67	3.52	5.86
CV (%)	6.40	10.88	3.54	8.69	7.87	6.00	3.61	11.76	5.09	11.57	3.75
NS 2009											
Minimum	2.81	6.87	15.87	12.05	6.15	1.03	35.06	1.29	2.25	1.56	5.46
Maximum	3.39	10.21	18.58	16.39	8.66	1.23	42.56	2.05	2.79	2.20	6.19
Mean	3.11	8.55	16.90	13.51	7.38	1.10	39.88	1.55	2.48	1.85	5.93
CV (%)	6.06	12.61	4.36	9.02	9.58	5.43	4.52	14.91	5.46	9.44	3.48
PEI 2008											
Minimum	2.89	6.86	16.63	13.05	6.69	1.16	33.43	1.36	2.50	2.31	5.66
Maximum	3.49	9.67	18.69	17.09	8.39	1.38	39.65	1.98	2.96	3.05	6.24
Mean	3.25	8.17	17.45	14.45	7.60	1.25	37.40	1.57	2.70	2.59	5.94
CV (%)	5.68	10.01	3.64	8.60	6.39	5.52	3.97	11.15	3.94	9.33	3.13
PEI 2009											
Minimum	2.99	7.34	16.81	12.06	6.45	1.04	34.28	1.27	2.27	1.52	5.75
Maximum	3.58	10.59	20.10	15.96	8.46	1.28	40.88	1.92	2.82	2.33	6.52
Mean	3.28	8.74	17.97	13.42	7.53	1.14	38.37	1.48	2.51	1.89	6.14
CV (%)	5.46	10.51	5.13	8.50	7.52	5.82	4.31	12.84	4.97	9.88	3.11
SK 2008											
Minimum	2.72	7.36	15.39	10.77	6.54	0.89	35.28	1.03	1.79	2.51	5.50
Maximum	3.33	11.61	18.09	14.55	9.45	1.14	42.92	1.76	2.51	3.50	6.38
Mean	3.06	9.37	16.26	12.16	8.03	1.01	40.05	1.32	2.15	2.87	6.06
CV (%)	6.95	14.30	4.95	9.08	10.72	7.65	4.66	18.16	7.87	9.26	4.31

Table 6.17 Summary of Pearson correlation coefficient describing the relationship among the essential fatty acid components in *B. carinata* oil

	C16:0	C18:1	C18:2	C18:3	C20:1	C20:2	C22:1
C18:1	-0.363***						
C18:2	-0.211**	0.745***					
C18:3	0.621***	-	-				
C20:1	-0.236**	0.952***	0.639***	-			
C20:2	0.332***	-	0.37***	0.525***	-		
C22:1	-	-0.898***	-0.839***	-0.207*	-0.885***	-0.227*	
C22:2	0.245**	-0.932***	-0.535***	-	-0.962***	0.276**	0.801***
C24:1	0.25**	-0.868***	-0.511***	0.193*	-0.876***	0.342***	0.71***

6.5 Discussion

The results have generally indicated that genetic variability among the 10 accessions of *B. carinata* was large. The diversity of agronomic traits was also reported by many previous studies. A total of 66 accessions of *B. carinata* were evaluated by Warwick *et al.* in 1998 in Saskatoon, Saskatchewan. Based on the field evaluation data, 66 accessions were divided into five main clusters and some accessions with potential genes such as earliness and high oil content, were found. More variation among accessions was identified in this study than had been described in Warwick *et al.* (2006). The less genetically diverse accessions of *B. carinata* found by Warwick *et al.* (2006) were probably due to some cultivars being selected from similar populations and the field data being collected from a single year's data. Similar research was conducted by Alemayehu and Becker (2002), in which a total of 36 accessions were tested in three locations (Holetta, Debrezeit and Kulumsa) in Ethiopia in 1999. A comparable range of divergence has been noted in the length of time to reach flowering, maturity time, yield components and oil and protein contents among 36 accessions and accessions with potential genes of interest to improve earliness, yield components and oil and protein content was found in this study. The high level of diversity found by Alemayehu and Becker (2002) was likely because all the accessions were selected from diverse ecological regions of the mid- and high-altitude areas of Ethiopia. Similarly, field trials were conducted by Getinet *et al.* (1996), in which 11 accessions of *B. carinata* from the Plant Gene Resource Centre/Ethiopia were evaluated at Saskatoon, Canada in 1984 and 1985. A large amount

of variability for agronomic and seed quality traits was reported; however, none of these accessions can adapt to western Canadian growing conditions due to the late maturity and low yield. Other studies (Knowles *et al.* 1981; Katiyar *et al.* 1986; Raj *et al.* 1998; Mazzoncini *et al.* 1993) have also provided useful information about the genetic variation of *B. carinata* germplasm. Overall, the significant variation of agronomic and seed quality traits among *B. carinata* accessions revealed considerable potential for further improvement of this crop.

6.5.1 Seed Yield

Brassica carinata, which originated in Ethiopia, can be successfully grown in an environment which has a cool (14-18 °C), moist (600-900 mm) and long growing season (180 days) (Warwick *et al.* 2006). Furthermore, it could also be developed into an oilseed crop for dry areas due to its high tolerance to drought and heat stresses (Rakow and Getinet 1998; Warwick *et al.* 2006). The high seed yield found in this study for all three sites indicated a considerable yielding ability of *B. carinata* when grown in a wide range of environments. There were highly significant differences of seed yield among the genotypes, the sites, the years and their interactions. The largest part of the total variance was ascribed to the differences due to years followed by locations, and then the genotypes. The similar location effect on seed yield of oilseed species was also reported by Plessers *et al.* (1962), Vollmann *et al.* (1996) and Gugel and Falk (2006). A substantial variation in yield in two years had been noted in NS and PEI. The seed yield of all accessions in 2009 was more than two-fold the yield in 2008 at both the NS and PEI sites. The severe wild radish (*Raphanus raphanistrum* L.) infestation in the 2008 NS field was probably the dominant seed yield depressing factor. Good emergence and uniform seed establishment in NS and PEI 2009 could be the main contributing factors for the higher seed yield. Flea beetle (*Phyllotreta cruciferae* Goeze) damage at the early seedling stage was found in PEI 2009. The relatively higher seed yield in 2009 PEI indicated that the flea beetle damage on the early leaves, if not really severe, does not result in yield losses. Compared with the year and site effects, the genotype effect was small. The difference of seed yield among lines within each trial was low. The seed yield of all 10 accessions was comparable to AC Vulcan. Rakow and Getinet (1998) indicated that *B. carinata* can achieve a yield as high

as 3000 kg/ha with oil content of 40 % to 42 % on a dry seed basis under favorable long-season growing conditions in Ethiopia (average 900 mm rainfall and temperatures from 12 to 18 ^{o}C during the growing season from late June to early December). Punia (2001) also reported that yield of Ethiopian mustard was up to 4000 kg/ha under irrigated conditions and 3600 kg/ha under rain fed conditions. In this study, all the lines achieved approximately 2500 kg/ha. Line 070727EM, 050488EM and 050334EM had the record of approximately 3000 kg/ha seed yield in PEI 2009 and line 070756EM, 070732EM, 070768EM and 070760EM produced more than 3000 kg/ha seed yield in SK 2009. Therefore, these lines can serve as a useful source of genes for improvement of seed yield.

One of the biggest challenges of developing *B. carinata* in Canada is that some of the accessions are very late maturing (Getinet *et al.* 1996). As compared with AC Vulcan, the earliest-maturing line 070714EM was 4 days later, while the latest-maturing line 070768EM was approximately 15 days later maturing than AC Vulcan. This later maturation would be the major limiting factor for cultivating in western Canada. The earlier maturing genotype must be developed in order to become a significant crop on the prairies.

The other limiting factor of cultivating small seed crops is successful stand establishment, since the small seed size leads to more variable and higher seedling mortality in the field (Hanson *et al.* 2008). Getinet *et al.* (1996) indicated that a positive correlation exists between seed weight and seedling vigor/stand establishment. The findings in this study supported this result. Line 070714EM, with the highest seed weight, had the highest plant stand in all trials. However, the larger seed weight was not correlated to the higher seed yield. 070714EM had the lowest seed yield in all three sites in 2009, which was 1688 kg/ha in NS, 2529 kg/ha in PEI and 2333 kg/ha in SK. Furthermore, the oil content of 070714EM was significantly lower than all the other lines. Therefore, 070714EM could be the potential source for genes for improving seed establishment by virtue of its larger seed size, but it is not suitable for improved oil and seed yield.

Early studies on *B. carinata* noted that the yellow seeded lines with higher seed weights, higher oil content and lower fiber contents had superior seed quality over the brown seeded lines (Getinet *et al.* 1996). The similar experiments on *B. rapa*, *B. napus*

and *B. juncea* have the same associations between seed color and oil and fiber content (Stringam *et al.* 1974; Xiao 1982; Woods 1980). The result found in this study is inconsistent with the early report. The yellow line 050334EM and AC Vulcan had relatively lower seed weight and lower plant stand. Consequently, selection for increased seed weight for both yellow and brown seeded lines could be another important selection criterion for developing *B. carinata* in Canada.

6.5.2 Seed Quality

The oil content, which ranged from 30.16 % to 48.75 %, found in this study agreed with that reported earlier whereby *B. carinata* has the potential to produce 42 % to 44 % oil under favorable long-season growing conditions (Getinet *et al.* 1996). A large variation in oil content was observed which was due to both genetic and environmental effects. The higher oil content of all lines was achieved in SK 2009. The mean value of oil content was highest in SK, which was 40.18 % in 2008 and 42.15 % in 2009. Correspondingly, the lowest protein content was found in SK for both 2008 and 2009. The negative correlation between oil and protein content has been reported in many previous studies (Grami *et al.* 1977; Lohaus and Moellers 2000). At the SK site, relatively low temperature in the seed filling stage tends to enhance the oil content while simultaneously decreasing the protein content. The difference of the oil content in NS and PEI was not significant. The wider range of values of oil content among genotypes (8.1 % in PEI 2009) than that observed among sites (5.8 % SK vs NS 2008) suggested that genotype had a larger impact on oil content. 070768EM had the highest oil content (37.76 % - 47.0 %) but the lowest protein content (25.85 % - 32.88 %) across all trials. 070714EM had the highest protein (30.53 % - 35.07 %) while the lowest oil content (31.84 % - 39.15 %) in all trials.

The seed oil of these 10 *B. carinata* accessions is primarily made up of palmitic (2.76 % to 3.56 %), oleic (6.68 % to 11.51 %), linoleic (15.44 % to 20.01 %), linolenic (10.90 % to 17.07 %), eicosenoic (6.11 % to 9.23 %) and erucic acid (33.78 % to 42.77 %). This finding is consistent with previous reports of *B. carinata* fatty acid composition (Velasco *et al.* 1998; Warwick *et al.* 2006). The influence of years and locations on fatty acid composition was relatively low when compared to the effect of genotypes. A similar pattern of low environmental effect on fatty acid composition was

also found in Crambe oil by Vollmann and Ruckenbauer (1993). Erucic acid is being considered as a major fat in *B. carinata* seed oil. Getinet *et al.* (1996) suggested that the change of the erucic acid content influences the contents of other fatty acids. The relationship among the essential fatty acid components in *B. carinata* oil (Table 6.17) supported this idea. The erucic acid was highly correlated with all the other essential fatty components except the palmitic acid. High erucic acid line 070714EM was found in this study, which contained approximately 39.17 % to 42.77 % erucic acid across the five year-sites.

6.6 Conclusion

In conclusion, the results have generally established that large genetic variations were found among the 10 lines for all traits measured. Parents of potential sources for genes of high yield, high seed weight, high oil content, high protein content and high erucic acid content were identified in this study. This information is the first step toward realizing the potential of this crop and provides the basic knowledge for further improvement of *B. carinata* germplasm through breeding.

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Chapter 7 Effect of Nitrogen Level and Seeding Rate on Growth and Seed Yield of *Brassica carinata*

7.1 Introduction

Brassica carinata A. Braun, also known as Ethiopian mustard, originated from Ethiopia, adapted well to the Mediterranean climate. The wide range of application of this plant includes annual crop for food, edible oil production and non-food use (biodiesel and solid bio-fuel) (Warwick *et al.* 2006). Recently, countries with semi-arid climate such as Spain (Velasco *et al.* 1999), India (Raj *et al.* 1998), Italy (Mazzoncini *et al.* 1993) and western Canada (Rakow and Getinet 1998), showed an increasing interest in this crop since it is highly drought and heat tolerant. Other positive agronomic traits of *B. carinata* include high resistance to blackleg (Gugel *et al.* 1990) and alternaria leaf spot, big seed size and good shattering resistance (Rakow and Getinet 1998), suggest that this crop could be developed as an oilseed crop in Canada. Current interest in *B. carinata* has raised the questions about the basic production practices such as seeding rate and nitrogen management for achieving maximum production and seed quality of this crop.

There is little information about the effect of seeding rate on the yield of Ethiopian mustard but the influence of seeding date has been studied by many researchers (Kaur and Sidhu 2004; Punia *et al.* 2001). Kaur and Sidhu (2004) examined the effect of three sowing dates (15 October, 15 November and 15 December) and three row spacings (30, 45 and 60 cm) on the oil quality of *B. carinata* in Ludhiana, India. They found that delayed sowing date and larger row spacing resulted in decreased oil content. Similar research was conducted by Punia *et al.* (2001) to evaluate the effect of crop geometry on seed yield of *B. carinata* during the winter season of 1997-98, 1998-99 and 1999-2000 in Hisar, India. Their research suggested that the optimum seed yield was achieved in 30 cm x 10 cm spacing with a population of 222,222 plants/ha (or 22 plants/m^2). Further studies on this area need to be carried out.

Numerous attempts have been made to evaluate the optimum nitrogen level for optimum production of *B. carinata*. Kaur and Sidhu (2004) reported that plant height, dry matter accumulation and seed yield of *B. carinata* were positively correlated with nitrogen application. Punia *et al.* (2001) observed that the plant height, pods per plant,

and seed yield significantly responded to N rate up to 100 kg/ha. Pramanik *et al.* (1996) noted that the growth, seed and oil yields of *B. carinata* significantly improved with increasing N rates up to 100 kg N/ha. Sharma *et al.* (2007) reported that the number of primary branches per plant, number of seeds per pods and TKW increased significantly with applied nitrogen up to 60 kg N/ha and also suggested that the maximum benefit and cost ratio 3.03 was achieved at 90 kg N/ha. Almost all investigations indicated that increasing N rate could increase seed yield substantially; however, nitrogen requirement can vary over a wide range depending on growing conditions. It is critical to consider the nitrogen requirements of *B. carinata* under the prevailing conditions.

Therefore, an attempt was made to assess the optimum nitrogen and seeding rate for the production of *B. carinata* under three varying environments in NS, PEI and SK. This was achieved by evaluating the effect of nitrogen and seeding rate on plant stand, flowering date, plant height, maturity date, seed yield, TKW, seed oil content, seed protein content and oil quality.

7.2 Materials and Methods

7.2.1 Experiment 1 (Seeding Rate Effect)

Three fields in Truro, NS, NSAC; AAFC Harrington, PEI and AAFC, Saskatoon, Saskatchewan, were selected for the 2008 and 2009 field trials. The effect of four seeding rates (50, 100, 200 and 400 seeds/m^2) were evaluated for *B. carinata* (070768EM). Fertilizer application schedule in three sites is listed in Table 7.1. The selected soil characteristics for each field are shown in Table 7.2 and 7.3. The pre-emergence herbicide Treflan 2.3 L/ha was applied in NS and PEI in both years of the study. The previous crop grown at Truro, NS and Harrington, PEI for the 2008 trial sites were spring cereals. The previous crops grown on the 2009 sites were flax in NS and red clover in PEI. In SK, the previous crops were oats and superb wheat in 2008 and 2009, respectively. Plots were seeded using a Hege plot drill (H and N Equipment Inc., Colwich, Kansas, USA) with double disc openers and press wheels in NS and PEI. In SK, plants were seeded with a 1.5 meter R-Tech hoe drill (AAFC, Saskatchewan, Canada) (packaged seed through a cone and splitter). In 2008, *B. carinata* was seeded on May 13, May 26 and May 15, in NS, PEI and SK, respectively. The seeded plot area was 7.5 m^2 (8 rows @ 15 cm x 6 m in

length) which were trimmed to 5 m in length after emergence. In 2009, the plants were seeded on May 5, May 21 and May 15 in NS, PEI and SK. The seeded plot size was 6.25 m^2 in NS and 7.5 m^2 in PEI. The row spacing was 15 cm and the distance between plots and blocks were 25 cm and 2.5 m for the NS and PEI sites for both 2008 and 2009. Plots at SK consisted of four 6 m rows spaced 30 cm apart. Plots were harvested with a Hege 125C plot combine (Hege USA, Colwich, Kansas, USA) with a harvest area of 5 m x 1.25 m for the NS and PEI sites. In SK, plants were also straight combined with a Hege combine (Hege, Germany).

Table 7.1 Fertilizer application schedule for *B. carinata* seeding rate study in three sites

Site	Date	Form	Method
NS 2008	May 12	200 kg/ha 0N-20P-20K + 240 kg/ha 21N-0P-0K-21S	Broadcast and incorporated
	June 28	140 kg/ha 34N-0P-0K	Topdressing
PEI 2008	May 25	200 kg/ha 0N-20P-20K + 240 kg/ha 21N-0P-0K-21S	Broadcast and incorporated
	July 7	140 kg/ha 34N-0P-0K	Topdressing
SK 2008	May 15	236 kg/ha 28.4N-14.2P-0K-11.8S	Incorporated
NS 2009	May 4	370 kg/ha 14N-14P-14K-10.19S	Broadcast and incorporated
	June 26	190 kg/ha 27N-0P-0K	Topdressing
PEI 2009	May 20	240 kg/ha 21N-0P-0K + 200 kg/ha 0N-20P-20K	Broadcast and incorporated
	July 15	150 kg/ha 34-0-0	Topdressing
SK 2009	May 15	114 19.5N-19.6P-0K-19.6S	Incorporated

Table 7.2 Soil characteristics of *B. carinata* seeding rate study in 2008

Location	pH	Organic Matter (%)	CEC (meq/100g)	P_2O_5 (kg/ha)	K_2O (kg/ha)	Ca (kg/ha)	Mg (kg/ha)	Sulfur (kg/ha)
NS	6.2	2.6	13.5	1033	266	2936	643	35
PEI	5.9	3.9	12.1	454	334	1866	228	48
SK	8.1	3.7	-	137	1229	-	-	19

Table 7.3 Soil characteristics of *B. carinata* seeding rate study in 2009

Location	pH	Organic Matter (%)	CEC (meq/100g)	P_2O_5 (kg/ha)	K_2O (kg/ha)	Ca (kg/ha)	Mg (kg/ha)	Nitrate-N(ppm)	% N	Sulfur (kg/ha)
NS	5.9	3.0	12.7	1562	348	2640	356	12.3	0.17	30
PEI	6.0	3.6	12.0	855	398	2612	148	33.1	0.24	30
SK	7.4	-	-	205	1301	-	-	24.8	-	30

7.2.2 Experiment 2 (Nitrogen Effect)

Three fields in Truro, NS, NSAC; AAFC Harrington, PEI and AAFC, Scott, Saskatchewan, were selected for the 2008 and 2009 field trials. Data in Scott is only available in 2008. Fertilizer application schedule in three sites is listed in Table 7.4. The selected soil characteristics for each field are shown in Table 7.5 and 7.6. The effect of N fertilizer rate was determined for one cultivar (070760EM) in all three sites. In 2008, seven N rates (0, 25, 50, 75, 100, 125 and 150 kg/ha) were evaluated. The previous crop in PEI and SK was barley and winter wheat, respectively. In NS, the site where the trial was planted was previously fallowed land. Plots were seeded using a Hege plot drill (H and N Equipment Inc., Colwich, Kansas, USA) with double disc openers and press wheels in NS and PEI. In SK, plots were seeded with a 1.5 meter R-Tech hoe drill (AAFC, Saskatchewan, Canada) (packaged seed through a cone and splitter). *B. carinata* was seeded at rate of 130 seeds/m^2 on May 13, May 26 and May 15, in NS, PEI and SK, respectively. The plot size was 15 m^2 (16 rows @ 15 cm x 5 m in length) in NS and PEI. In SK, the plot size was 7.5 m^2 (16 rows @10 cm x 5 m). Plots were straight combined with a Hege 125C (Hege USA, Colwich, Kansas, USA) at the NS and PEI sites and a Wintersteiger plot combine (Wintersteiger, Austria) at the SK site. Harvest area was 6.25 m^2 for all trials in NS and PEI and in SK the whole plot was harvested. In 2009, one more N rate (200 kg N/ha) was added into this study. The data was only available in two sites, NS and PEI. The crop previously grown in NS and PEI were flax and red clover, respectively. *B. carinata* was seeded on May 5 and May 21 in NS and PEI respectively. The seeding rate for this trial was 130 seeds/m^2 and plot size was 12.5 m^2 in NS and 15 m^2 in PEI. Plots were straight combined with a Hege 125C (Hege USA, Colwich, Kansas, USA) at the NS and PEI sites. The harvest area was 5 m^2 in NS and 6.25 m^2 in PEI. In both years, nitrogen application was split with half the N amount applied one week after seed germination and the other half applied at the start of flowering in NS and PEI sites. In Scott, SK the nitrogen fertilizer was mid-row banded at time of seeding in Scott. All N was banded at seeding since farmers do not split applications of fertilizer in western Canada. The nitrogen source was ammonium nitrate (34-0-0) for the NS and PEI sites. In SK the nitrogen source was urea (46-0-0).

Table 7.4 Fertilizer Application Schedule for *B. carinata* N study in three sites

Site	Date	Form	Method
NS 2008	May 12	200 kg/ha 0N-20P-20K	Broadcast and incorporated
PEI 2008	May 25	200 kg/ha 0N-20P-20K	Broadcast and incorporated
SK 2008	May 15	225 kg/ha of 23P-25K-17S	Incorporated
NS 2009	May 4	220 kg/ha 0N-20P-20K	Broadcast and incorporated
PEI 2009	May 20	200 kg/ha 0N-20P-20K	Broadcast and incorporated

Table 7.5 Soil characteristics of *B. carinata* nitrogen study in 2008

Location	pH	Organic Matter (%)	CEC (meq/100g)	P_2O_5 (kg/ha)	K_2O (kg/ha)	Ca (kg/ha)	Mg (kg/ha)	Sulfur (kg/ha)
NS	6.2	2.6	13.5	1033	266	2936	643	35
PEI	5.9	3.9	12.1	454	334	1866	228	48
SK	7.2	-	-	137	1530	-	-	20

Table 7.6 Soil characteristics of *B. carinata* nitrogen study in 2009

Location	pH	Organic Matter (%)	CEC (meq/100g)	P_2O_5 (kg/ha)	K_2O (kg/ha)	Ca (kg/ha)	Mg (kg/ha)	Nitrate-N(ppm)	% Nitrogen	Sulfur (kg/ha)
NS	5.9	3.0	12.7	1562	348	2640	356	12.3	0.17	30
PEI	6.0	3.6	12.0	855	398	2612	148	33.1	0.24	30

7.3 Data Collection

7.3.1 Measurements

Plant emergence was measured approximately three weeks after planting. Two counts were completed in each plot by placing two 0.25 m^2 quadrants in the plot, avoiding the outside rows. Plant height was measured on three plants per plot from the soil surface to highest point on the erect plant at time of maturity. Days to beginning of flowering was estimated visually as the date when approximately 10 % of plants had one flower open. Maturity date was also estimated visually as the date when approximately 95 % of the pods were brown. Lodging was visually evaluated using a scale of 1-5 with 1 being erect and 5 being flat on the ground. After drying to approximately 8 % moisture content seeds were cleaned using a Clipper (Clipper Seed Cleaning Co., Bluffton, IN) seed cleaner. Cleaned seed was weighed (g) and g/plot values were converted to kg/ha based on plot areas for each location. Climatic data (including weekly precipitation, temperature and sunshine hours) at three sites (NS, PEI and SK) and two years (2008 and 2009) were obtained from Environment Canada on-line data (http://www.climate.weatheroffice.ec.gc.ca/).

7.3.2 Biomass and Harvest Index

All plants from two quadrats, 0.25 m^2, were taken from each end of the plot to get a 0.5 m^2 sample. This sample was then dried and weighed to determine total biomass which was reported as kg/ha. The harvest index was calculated as the ratio of seed yield to the above-ground biomass and was expressed as a percentage.

7.3.3 Oil and Protein Content

Total seed oil and protein content was determined on 5.0 g of whole seed using a near-infrared reflectance (NIR) spectroscopy (FOSS NIR Systems model 6500 spectrometer, spinning cup autosampler, WIN ISI II calibration software, Technicon Canada Inc., Mississauga, ON). Samples were analyzed in bulk by combining blocks 1 and 2, as well as 3 and 4. Oil and protein contents are reported on a dry matter basis.

7.3.4 Fatty Acid Analysis

The lipid extraction and methylation process were generated based on the protocol of Budge *et al.* (2006) with minor modification. For detailed information, see chapter 4.

7.3.5 Statistical Analysis

Experiment 1 was laid out in a randomized completely block design (RCBD) with four replications. Seeding rates with four levels (50, 100, 200 and 400 seed/m^2) were tested. The experiment 2 was designed as a RCBD with four replications. The main factor in this study was N rate. The response variables from both experiments, including stand count, plant height, days to flowering, days to maturity, thousand kernel weight (TKW), seed yield, oil and protein content, fatty acid composition were collected and subjected to the PROC Mixed procedure in SAS version 9.1 (SAS Institute Inc., Cary, NC,USA, 2002-2003). Tukey test was used to compare the differences among treatments at 5 % significant level. PROC Mixed procedure in SAS version 9.1 was also used in analyzing the site and year effect. Minitab statistical software version 14 (Minitab Inc., USA, 1972-2004) was used for all regression analysis.

7.4 Results

7.4.1 Seeding Rate Study

7.4.1.1 Seed Yield

A summary of the seeding rate effects on seed yield in 2008 and 2009 at three sites is given in Table 7.7. Mean yield over six site-year was 1805 kg/ha and ranged from 909 kg/ha in NS 2008 to 2512 kg/ha in SK 2009. The results of analysis of variance (Table 7.8) showed that the response of seed yield depended on year and location (the interaction effect between seeding rate, site and year <0.0044). Therefore, the seeding rate recommendation may vary depending on geographical locations. The seed yield in NS was significantly influenced by the seeding rate effect during both years. In 2008, the seed yield ranged from 797 to 1103 kg/ha and were highly related to seeding rate (r^2 = 87.3 %) and the optimum seeding rate was 200 seeds/m^2 (Figure 7.1). In 2009, the seed yield increased dramatically as the seeding rate increased up to 100 seeds/m^2. Higher seeding rate requirement in 2008 may probably due to the lower plant stand and strong weed competition. The optimum plant density in these two years was around 80 plants/ m^2. In PEI and SK, variable plant densities as obtained under seeding rate of 50, 100 and 200 seeds/m^2 produced no significant differences in seed yield during 2008-2009, but significant reduction was recorded with seeding rate of 400 seeds/m^2 in PEI 2009 and SK

2008. This indicated that *B. carinata* has very strong yield compensation mechanism to adjust plant population. The regression analysis (Figure 7.1-7.3) in three sites showed that 200 seeds/m^2 produced highest seed yield in four out of six trials. This suggested that the most promising seeding rate would be around 200 seeds/m^2 across all sites.

Table 7.7 Summary of seeding rate effect on seed yield of *B. carinata* in three sites: NS, PEI and SK. 2008-09

Seeding	NS		PEI		SK	
Rate seeds/m^2	2008	2009	2008	2009	2008	2009
50	797b	1854c	944a	2459a	1908ab	2351a
100	837b	2427a	1056a	2549a	2051a	2406a
200	1103a	2122b	1080a	2193ab	2074a	2572a
400	897b	2276ab	984a	2004b	1790b	2718a
P-value	0.0004	0.033	0.873	0.0141	0.018	0.592
Mean	909	2134	1016	2301	1956	2512

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 7.8 Analysis of variance results for *B. carinata* seeding rate study across three sites: NS, PEI and SK. 2008-09

Effect	Num DF	Den DF	F Value	Pr > F
Site	2	69	97.81	<.0001
Year	1	69	568.90	<.0001
Site*Year	2	69	30.40	<.0001
Seeding Rate(SR)	3	69	5.24	0.0026
SR*Site	6	69	5.00	0.0003
SR*Year	3	69	6.29	0.0008
SR*Site*Year	6	69	3.50	0.0044

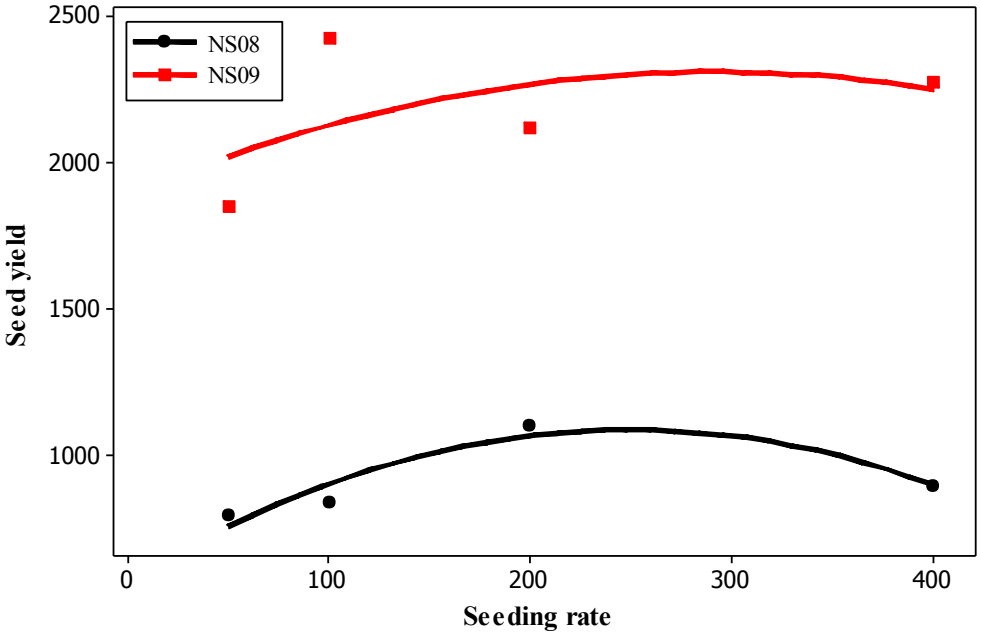

Figure 7.1 Relationship between *B. carinata* seed yield (kg/ha) and seeding rate (seeds/m^2) in NS. 2008-09

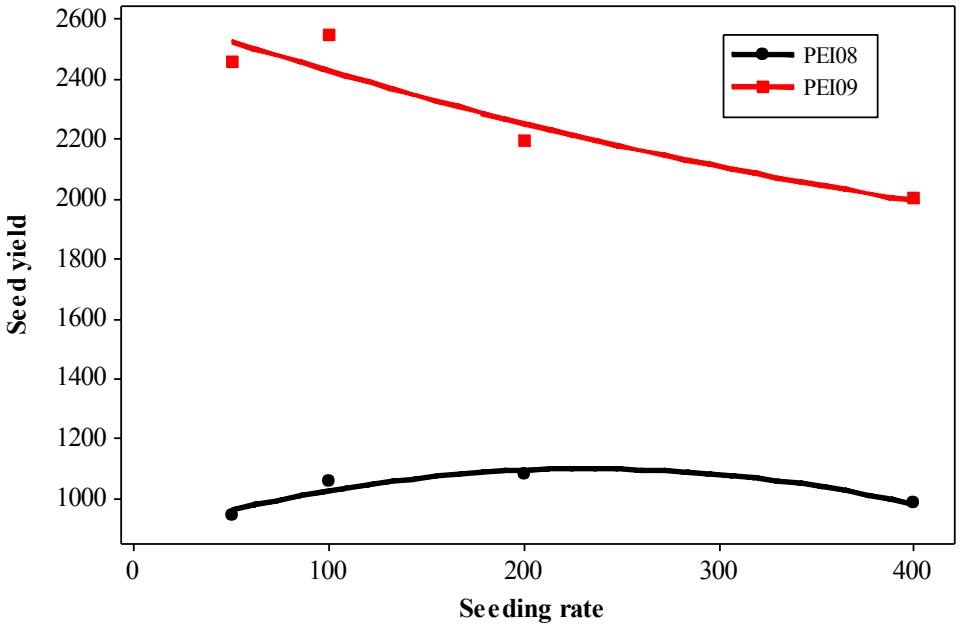

Figure 7.2 Relationship between *B. carinata* seed yield (kg/ha) and seeding rate (seeds/m^2) in PEI. 2008-09

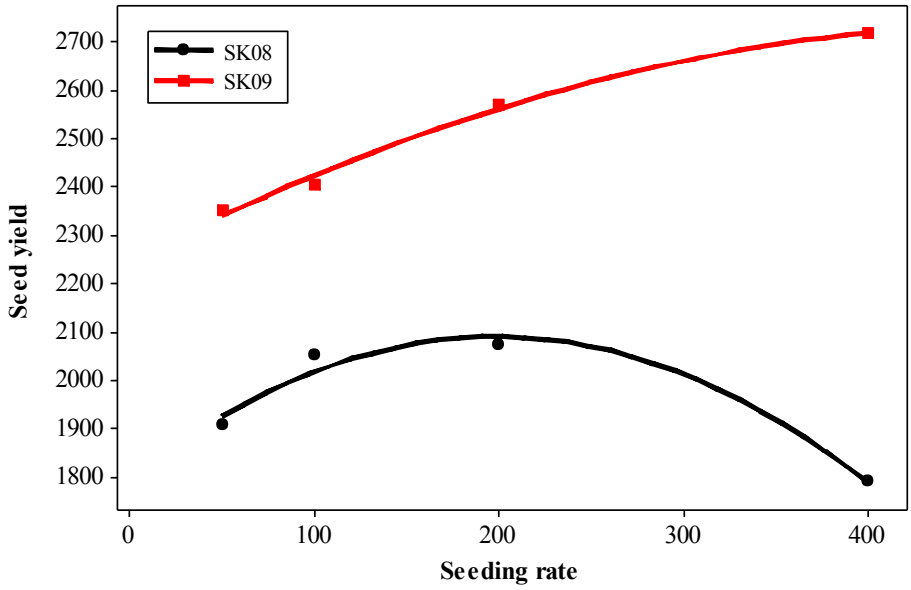

Figure 7.3 Relationship between *B. carinata* seed yield (kg/ha) and seeding rate (seeds/m^2) in SK. 2008-09

The regression equation between seed yield and seeding rate (SR):

y = 572.1 + 4.148SR - 0.008310 SR**2 (r^2 = 87.3 %) ----------NS08;

y = 1892 + 2.876SR - 0.00492 SR**2 (r^2 = 22.5 %) ---------- NS09;

y = 874.7 + 1.933SR - 0.004163 SR**2 (r^2 = 88.3 %) ---------- PEI08;

y = 2635 - 2.218 SR + 0.001547 SR**2 (r^2 = 87.7 %) ---------- PEI09;

y = 1797 + 2.953 SR - 0.007442 SR**2 (r^2 = 96.8 %) ---------- SK08;

y = 2246 + 1.982 SR - 0.001998 SR**2 (r^2 = 99.3 %) ---------- SK09

7.4.1.2 Plant Emergence

Seeding rate had a significant effect on plant stand. As the seeding rate increased, plant stand increased significantly at both NS and PEI sites in both years. However, plant stands in SK site were not correlated to seeding rate owing to the poor and uneven emergence due to the extremely dry conditions at seeding in 2009. When averaged over locations and densities, 63.8 % of the planted seeds emerged (Table 7.9). This varied from a low of 47 % at NS in 2008 to a high of 76 % at PEI in 2009. The percent emergence declined from 81 % to 49 % as planting density increased (Table 7.10). The significantly lower percent emergence at NS in 2008 may be due to strong weed competition.

Table 7.9 Overall percent emergences of *B. carinata* seed at sites in NS, PEI and SK. 2008-09

Site	Year	Emergence (%)
NS	2008	47
	2009	74
PEI	2008	62
	2009	76
SK	2009	60
Mean		64

Table 7.10 Percent emergence of *B. carinata* seed at different planting densities mean of 5 site-years. 2008-09

Seeding rate (seeds/m^2)	Plant stand (plants/m^2)	Emergence (%)
50	40.4	81
100	69.2	69
200	114	57
400	197.4	49

7.4.1.3 Plant height, Branches/plant, Pods/plant and Days to flower

The effect of seeding rate on plant height, number of branches/plant, pods/plant and days to flower are given in Tables 7.11-7.15. Plant height was not affected by the seeding rate in both NS and SK but was negatively correlated with seeding rate in PEI in 2008. In 2009, no significant difference of plant height was found at the PEI and SK sites but the negative relationship between plant height and seeding rate was observed in NS. The yield components, number of branches/plant and number of pods/plant, were negatively correlated with seeding rate. As seeding rate increased, the number of branches/plant decreased from 43 to 6 in NS 2008, 22 to 5 in PEI 2008, 13 to 10 in SK 2008, 20 to 3 in NS 2009 and 114 to 13 in SK 2009. The response of days to flowering was different between years. In 2008, it was not affected by seeding rate; however, it tended to decrease with the increasing seeding rate in all three sites in 2009 and was significantly shorter at the highest seeding rate compared to the lowest.

Table 7.11 Seeding rate effect on plant stand, branches/plant, and pods/plant in 2008 in NS, PEI and SK

Seeding Rate	Site								
	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK
seeds/m^2	Plant stand (plants/m^2)			Branches/plant			Pods/plant		
50	25d	32c	N/A	43a	22a	13a	162a	155a	N/A
100	47c	77b	N/A	14b	11ab	13a	76b	71ab	N/A
200	98b	114a	N/A	11b	7bc	12ab	60b	46b	N/A
400	173a	210a	N/A	6c	5c	10b	34c	26c	N/A
P-value	0.000	0.005	N/A	0.000	0.001	0.02	0.000	0.000	N/A

Means within a column followed by different letters are significantly different ($p < 0.05$) (N/A – Data is not available)

Table 7.12 Seeding rate effect on days to flower, days to maturity and plant height in 2008 in NS, PEI and SK

Seeding Rate	Site								
	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK
seeds/m^2	Days to flower			Days to maturity			Plant height (cm)		
50	54a	N/A	54a	N/A	N/A	107a	130a	131ab	107a
100	54a	N/A	53a	N/A	N/A	107a	128a	136a	109a
200	53a	N/A	52a	N/A	N/A	105ab	132a	123b	108a
400	53a	N/A	52a	N/A	N/A	103b	124a	105c	105a
P-value	0.347	N/A	0.084	N/A	N/A	0.025	0.632	0.000	0.911

Means within a column followed by different letters are significantly different ($p < 0.05$) (N/A – Data is not available)

Table 7.13 Seeding rate effect on TKW and seed yield in 2008 in NS, PEI and SK

Seeding Rate	Site					
	NS	PEI	SK	NS	PEI	SK
seeds/m^2	TKW (g)			Seed Yield (kg/ha)		
50	4.2a	4.13a	N/A	797b	944a	1908ab
100	3.9a	4.05a	N/A	837b	1056a	2051a
200	3.78a	3.9a	N/A	1103a	1080a	2074a
400	3.63a	3.68a	N/A	897b	984a	1790b
P-value	0.08	0.312	N/A	0.0004	0.873	0.018

Means within a column followed by different letters are significantly different ($p < 0.05$)
(N/A – Data is not available)

Table 7.14 Seeding rate effect on plant stand, branches/plant, and pods/plant in 2009 in NS, PEI and SK

Seeding Rate	Site								
	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK
seeds/m^2	Plant stand (plants/m^2)			Branches/plant			Pods/plant		
50	39d	41d	34a	20a	N/A	114a	178a	N/A	N/A
100	76c	80c	66a	13b	N/A	57ab	98b	N/A	N/A
200	144b	142b	66a	7c	N/A	34b	46c	N/A	N/A
400	281a	289a	72a	3c	N/A	13b	21d	N/A	N/A
P-value	<.000	<.000	0.399	<.000	N/A	0.024	<.0001	N/A	N/A

Means within a column followed by different letters are significantly different ($p < 0.05$)
(N/A – Data is not available)

Table 7.15 Seeding rate effect on seed yield, days to flower and plant height in 2009 in NS, PEI and SK

Seeding	Site								
Rate	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK
seeds/m^2	Seed yield (kg/ha)			Days to flower			Plant height (cm)		
50	1854c	2459a	2351a	54a	53a	52a	151a	153a	79a
100	2427a	2549a	2406a	53a	51ab	52a	156a	153a	79a
200	2122b	2193ab	2572a	54a	50b	51ab	148ab	152a	80a
400	2276ab	2004b	2718a	52b	50b	50b	136b	149a	67a
P-value	0.033	0.0141	0.592	0.041	0.0216	0.035	0.0285	0.07	0.329

Means within a column followed by different letters are significantly different ($p < 0.05$)

7.4.1.4 Oil and Protein Content

The oil and protein contents of *B. carinata* at different seeding rates are presented in Tables 7.16-7.17. When the data of the six site-years was averaged, the variation of the oil content was between 37.90 % (PEI 2008) and 45.43 % (SK 2008) and protein content ranged from 24.97 % (SK 2008) to 33.02 % (PEI 2008). The effect of seeding rates on *B. carinata* oil and protein content was not significant in all trials. The mean oil content in SK was approximately 4 % higher than NS and 7 % higher than PEI in 2008. In 2009, the oil content in NS and PEI was similar, which was 5 % lower than the SK site.

Table 7.16 Seeding rate effect on oil and protein content (%) of *B. carinata* seeds in 2008

Seeding	NS		PEI		SK	
Rate	Oil	Protein	Oil	Protein	Oil	Protein
seeds/m^2						
50	41.30a	29.35a	37.38a	33.95a	45.20a	25.02a
100	41.44a	28.77a	38.32a	32.62a	46.34a	24.48a
200	41.8a	28.0a	38.52a	32.34a	44.67a	25.14a
400	41.15a	28.06a	37.38a	33.15a	45.52a	25.25a
Mean	41.42	28.55	37.90	33.02	45.43	24.97
P-value	0.905	0.647	0.41	0.475	0.32	0.575

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 7.17 Seeding rate effect on oil and protein content (%) of *B. carinata* seeds in 2009

Seeding Rate seeds/m^2	NS		PEI		SK	
	Oil	Protein	Oil	Protein	Oil	Protein
50	39.26a	30.57a	40.39a	29.40a	43.11a	28.00a
100	38.99a	30.68a	40.38a	29.64a	45.36a	26.24a
200	40.32a	29.29a	40.20a	30.01a	44.66a	26.73a
400	38.54a	30.71a	38.53a	30.50a	45.47a	26.33a
Mean	39.28	30.31	39.88	29.89	44.65	26.83
P-value	0.825	0.728	0.420	0.672	0.123	0.417

Means within a column followed by different letters are significantly different ($p < 0.05$)

7.4.2 Nitrogen Effect Study

7.4.2.1 Seed Yield

Table 7.18 is a summary of the nitrogen effect on seed yield of *B. carinata* in all trials. The production of seed was significantly affected by the different rates of nitrogen supplies in all trials except the trial in PEI 2009. On average of the seed yield in five site-years, the lowest and highest yields lay among 500 kg/ha (0 nitrogen treatment in PEI 08) to 2204 kg/ha (150 kg N/ha in SK 2008). The total mean yield of all trials was approximately 1404 kg/ha. The significant interaction effect between nitrogen, year and site on seed yield found in this study suggested that the optimum N supply should vary according to the environments (Table 7.19). In NS, seed yield responded to applied N in both 2008 and 2009 (Figure 7.4). Seed yield in those plots receiving 125 kg N/ha were the highest in both years (1073 kg/ha in 2008 and 2035 kg/ha in 2009), which was 73 % (2008) and 64 % (2009) higher than the plots with no nitrogen applied. In 2008 at the PEI site, seed yield was positively correlated to N supply and the highest yield of 1192 kg/ha was achieved with 125 kg N/ha; however, no significant differences were found in 2009. The nitrogen effect was confounded with high amount of soil-available nitrogen from previous crop. In SK, the data from 2008 suggested that seed yield was positively correlated with nitrogen rates and began to level off at 75 kg N/ha. The nearly linear correlation between seed yield and nitrogen rate in four out of five sites (Figure 7.4) indicated that *B. carinata* is highly responsive to applied nitrogen.

Table 7.18 Effects of variation in level of nitrogen application on seed yield of *B. carinata*

N rate	Seed yield (kg/ha)					
(kg/ha)	NS(08)	PEI(08)	SK(08)	NS(09)	PEI(09)	Mean
0	619c	500d	1696c	1240d	1701a	1151
25	636c	620cd	1789bc	1456cd	1762a	1253
50	814bc	692c	1874b	1654bc	1597a	1340
75	975b	912b	1934ab	1868ab	1444a	1415
100	1006b	996b	2070ab	1892ab	1316a	1456
125	1073ab	1192a	2173a	2035a	1585a	1612
150	1291a	1364a	2204a	1832ab	1314a	1601
P-value	0.000	0.000	0.045	0.0025	0.05	
Mean	916	897	1964	1711	1531	1404

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 7.19 Analysis of variance results for seed yield of *B. carinata* in nitrogen response study in five site-years

Effect	Num DF	Den DF	F Value	Pr > F
Site	2	102	233.31	<.0001
Year	1	102	315.34	<.0001
Site*Year	2	102	3.96	0.0493
Nitrogen (N)	6	102	9.31	<.0001
N*Site	12	102	2.12	0.0216
N*Year	6	102	5.70	<.0001
N*Site*Year	12	102	5.04	0.0001

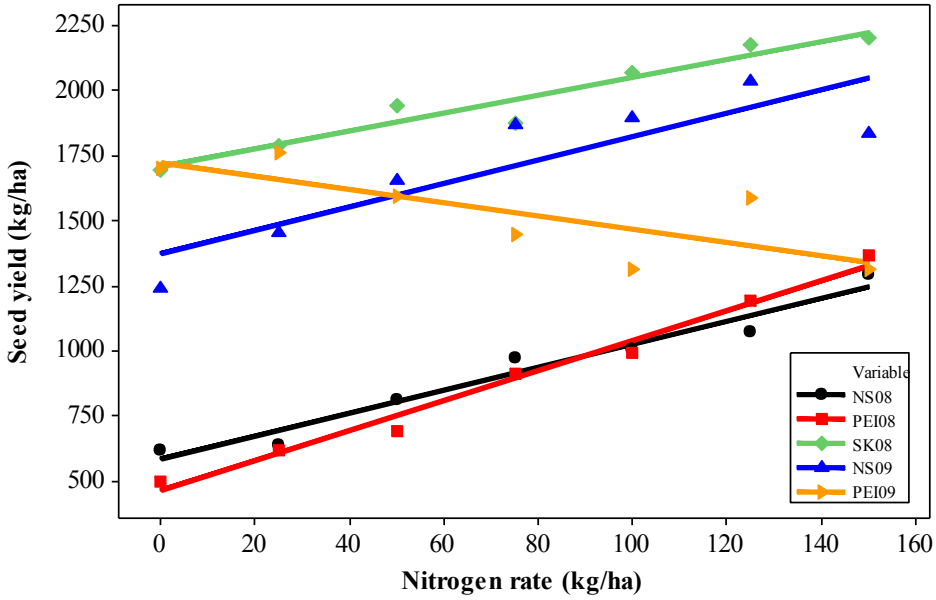

Figure 7.4 Regression analysis between seed yield and nitrogen rate in five site-years

Regression equation between seed yield and N rate (N):

$y = 586.1 + 4.399\ N$ ($r^2 = 96\ \%$) ---------- NS08;

$y = 463.7 + 5.77N$ ($r^2 = 93.6\ \%$) ---------- PEI08;

$y = 1371 + 4.531\ N$ ($r^2 = 76.9\ \%$) ---------- - NS09;

$y = 1724 - 2.566\ N$ ($r^2 = 60.5\ \%$) ---------- PEI09;

$y = 1705 + 3.5\ N$ ($r^2 = 93.6\ \%$) ---------- SK08

7.4.2.2 Plant height, Biomass and Harvest index

The nitrogen effect on plant height, biomass and harvest index is shown in Tables 7.21- 7.24. Applied N did not affect plant height at any of the three sites. Average plant height was 106 cm in NS 2008, 111 cm in PEI 2008, 98 cm in SK 2008, 139 cm in NS 2009 and 160 cm in PEI 2009. The effect of N rate on biomass accumulation differed among year-sites. In NS, biomass was significantly increased with the increase in nitrogen application from 0 to 75 kg N/ha in 2008 and from 0 to 100 kg N/ha in 2009. The maximum biomass (13900 kg/ha in 2008 and 10467 kg/ha in 2009) was achieved with 75 kg N/ha and 100 kg N/ha treatments, respectively. In PEI, biomass increased as N rate increased from 0 to 75 kg N/ha and highest value, 12133 kg/ha, at the 150 kg N/ha treatment in 2008. However, no difference was found in 2009. In SK, the N rate had no

effect on biomass accumulation in 2008. The data in 2009 is not available. The response of harvest index to N rate varied among years. In 2008, as N rate increased, the harvest index increased at both the NS and PEI sites. No significant difference of harvest index was observed in SK 2008 and PEI 2009. The data from the trial in NS 2009 showed that harvest index decreased from 0.28 to 0.18 as N rate increased from 0 to 125 kg N/ha.

7.4.2.3 Yield Components

The yield component of number of branches/plant and pods/plant was significantly affected by N rate (Table 7.20 and 7.23). As N rate increased, the number of branches/plant increased from 5 to 11 in NS 2008, 6 to 9 in PEI 2008 and 6 to 15 in NS 2009. The data of pods/plant was only collected for the 2008 NS and 2008 PEI site. The number of pods/plant increased from 31 to 55 in NS and 37 to 56 in PEI as the N rate increased from 0 to 125 kg N/ha.

Table 7.20 Nitrogen effect on plant stand, branches/plant and pods/plant for *Brassica carinata* in 2008 at NS, PEI and SK

N rate	Site								
(kg/ha)	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK
	Plant stand (plants/m^2)			Branches/plant			Pods/plant		
0	63a	116a	N/A	5c	6c	N/A	31c	37c	N/A
25	87a	92a	N/A	5c	7b	N/A	28c	39c	N/A
50	80a	116a	N/A	7bc	6b	N/A	37bc	41b	N/A
75	86a	95a	N/A	6c	8ab	N/A	33c	47ab	N/A
100	92.5a	94a	N/A	8ab	8ab	N/A	41abc	46ab	N/A
125	80.5a	114a	N/A	11a	9a	N/A	55a	56a	N/A
150	85a	87a	N/A	10ab	6b	N/A	52ab	41b	N/A
P-value	0.433	0.533	N/A	0.004	0.937	N/A	0.016	0.979	N/A

Means within a column followed by different letters are significantly different ($p < 0.05$)
(N/A – Data is not available)

Table 7.21 Nitrogen effect on days to flower, TKW and plant height for *B. carinata* in 2008 at NS, PEI and SK

N rate	Site								
(kg/ha)	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK
	Days to flower			TKW (g)			Plant height (cm)		
0	57a	N/A	55a	4.58a	3.98bc	4.66a	104a	106a	95a
25	58a	N/A	55a	4.6a	3.90c	4.54ab	101a	108a	99a
50	58a	N/A	55a	4.63a	4.33a	4.66a	108a	109a	100a
75	58a	N/A	55a	4.6a	4.28ab	4.48b	108a	110a	99a
100	58a	N/A	54a	4.58a	4.4a	4.49ab	109a	111a	99a
125	60a	N/A	54a	4.73a	4.3a	4.45b	107a	113a	94a
150	59a	N/A	55a	4.55a	4.2abc	4.38b	105a	119a	98a
P-value	0.399	N/A	0.451	0.868	0.028	0.026	0.242	0.098	0.468

Means within a column followed by different letters are significantly different ($p < 0.05$)
(N/A – Data is not available)

Table 7.22 N rate effects on seed yield, biomass and harvest index for *B. carinata* in 2008 at NS, PEI and SK

N rate	Site								
(kg/ha)	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK
	Seed yield (kg/ha)			Biomass (kg/ha)			Harvest index		
0	618.5c	500.0d	1696c	10500c	6400c	10715a	0.059bc	0.075c	0.163a
25	636.4c	620.0cd	1789bc	11500bc	7867bc	12865a	0.055c	0.083bc	0.142a
50	814.4bc	692.0c	1874b	11900b	8000bc	15285a	0.068b	0.083bc	0.133a
75	974.5b	912.0b	1934ab	12200ab	10400ab	14030a	0.078b	0.092bc	0.135a
100	1005.7b	996.0b	2070ab	12300ab	11467a	15275a	0.082ab	0.090bc	0.139a
125	1072.5ab	1192.0a	2173a	13000a	11867a	14200a	0.082ab	0.105ab	0.154a
150	1290.5a	1364.0a	2204a	13900a	12133a	16695a	0.093a	0.118a	0.142a
P-value	0.000	0.000	0.045	0.028	0.005	0.246	0.034	0.022	0.769

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 7.23 Nitrogen effect on plant stand, branches/plant and plant height for *B. carinata* in 2009 at NS, PEI and SK

N rate	Site								
(kg/ha)	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK
	Plant stand(plants/m^2)			Branches/plant			Plant height (cm)		
0	118a	127a	N/A	6c	N/A	N/A	133a	166a	N/A
25	108a	136a	N/A	6c	N/A	N/A	134a	154a	N/A
50	120a	123a	N/A	6c	N/A	N/A	137a	162a	N/A
75	107a	106a	N/A	9bc	N/A	N/A	141a	158a	N/A
100	110a	137a	N/A	8bc	N/A	N/A	140a	164a	N/A
125	111a	138a	N/A	10b	N/A	N/A	145a	162a	N/A
150	111a	123a	N/A	10b	N/A	N/A	146a	160a	N/A
200	109a	124a	N/A	15a	N/A	N/A	136a	153a	N/A
P-value	0.799	0.211	N/A	0.0001	N/A	N/A	0.0757	0.355	N/A

Means within a column followed by different letters are significantly different ($p < 0.05$)
(N/A – Data is not available)

Table 7.24 Nitrogen effect on seed yield, biomass and harvest index for *B. carinata* in 2009 at NS, PEI and SK

N rate	Site								
(kg/ha)	NS	PEI	SK	NS	PEI	SK	NS	PEI	SK
	Seed yield (kg/ha)			Biomass (kg/ha)			Harvest index		
0	1240d	1701a	N/A	4467d	9333	N/A	0.28a	0.19	N/A
25	1456cd	1762a	N/A	6200c	7733	N/A	0.24ab	0.24	N/A
50	1654bc	1597a	N/A	7133c	9000	N/A	0.22bc	0.18	N/A
75	1868b	1444a	N/A	8800b	8067	N/A	0.22bc	0.18	N/A
100	1892ab	1316a	N/A	9300ab	9267	N/A	0.18bc	0.16	N/A
125	2035a	1585a	N/A	10467a	9267	N/A	0.18c	0.17	N/A
150	1832b	1314a	N/A	8387b	7267	N/A	0.21bc	0.17	N/A
200	1880ab	1345a	N/A	8600b	7400	N/A	0.22bc	0.20	N/A
P-value	0.0038	0.05	N/A	<.0001	0.491	N/A	0.0332	0.147	N/A

Means within a column followed by different letters are significantly different ($p < 0.05$)
(N/A – Data is not available)

7.4.2.4 Oil and Protein Content

The oil and protein contents of *B. carinata* in different N rate trials are presented in Tables 7.25- 7.26. When the data of the three sites was averaged, the variation of the oil content lay between 34.81 % (NS 08) to 40.94 % (SK08) and protein content ranged from 26.61 % (SK 08) to 31.21 % (NS 08). Increases in N supply resulted in decreased oil content with increasing protein content in all trials. The correlation between N rate and oil (protein) content are highly significant except for trial in PEI 2009 (Figure 7.5 and 7.6). In 2008, the oil content decreased 4 % in NS, 3 % in PEI and 1% in SK, while the protein content increased 3 % in NS, 4 % in PEI and 5 % in SK. In 2009, the oil content declined approximately 4 % while the protein content rose 3 % in both NS and PEI. The strongly negatively correlation between oil and protein content (r^2 = 90.7 %) was found in all trials. The effect of N rate on fatty acid composition is listed in Table 7.27. Palmitic acid, stearic acid, oleic acid, linolenic acid and erucic acid were not affected by applied nitrogen in all trials. Linoleic acid and eicosenoic acid were affected by applied N but the influence was not consistent. Linoleic acid was positively correlated with increased N rates in four out of five trials; the only negative relationship was found in PEI 2009. Eicosenoic acid increased in NS 2008 and PEI 2009, but declined in PEI 2008 with the increased N rate. No difference was found in other trials.

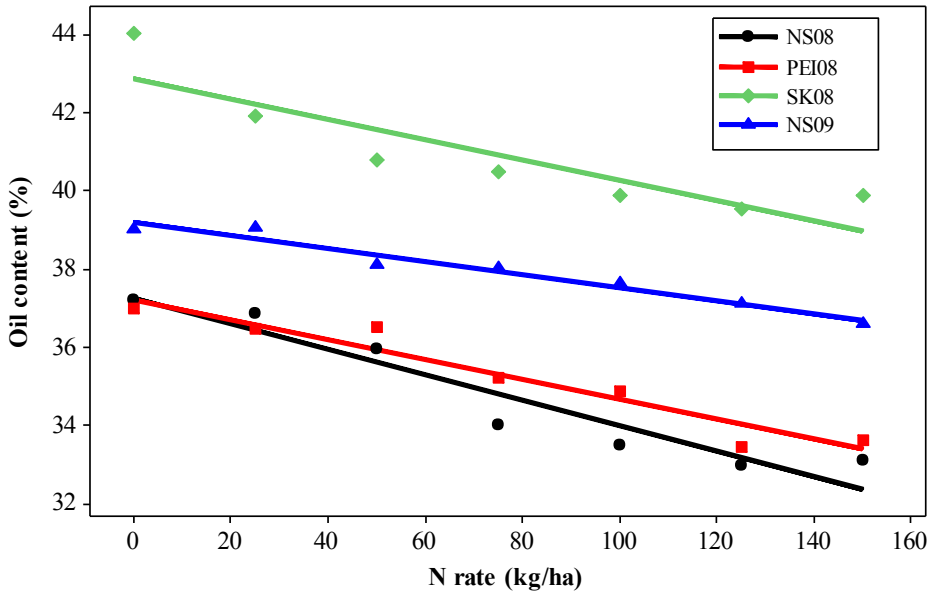

Figure 7.5 Correlation between N rate and oil content

The regression equation between N rate and oil content:

y = 37.24 - 0.03237 N (r^2 = 91 %) ---------- NS08;

y = 37.20 - 0.02533N (r^2 = 93 %) ---------- PEI08;

y = 42.88 - 0.02589 N (r^2 = 80 %) ---------- SK08;

y = 39.19 - 0.01660 N (r^2 = 96 %) ---------- NS09.

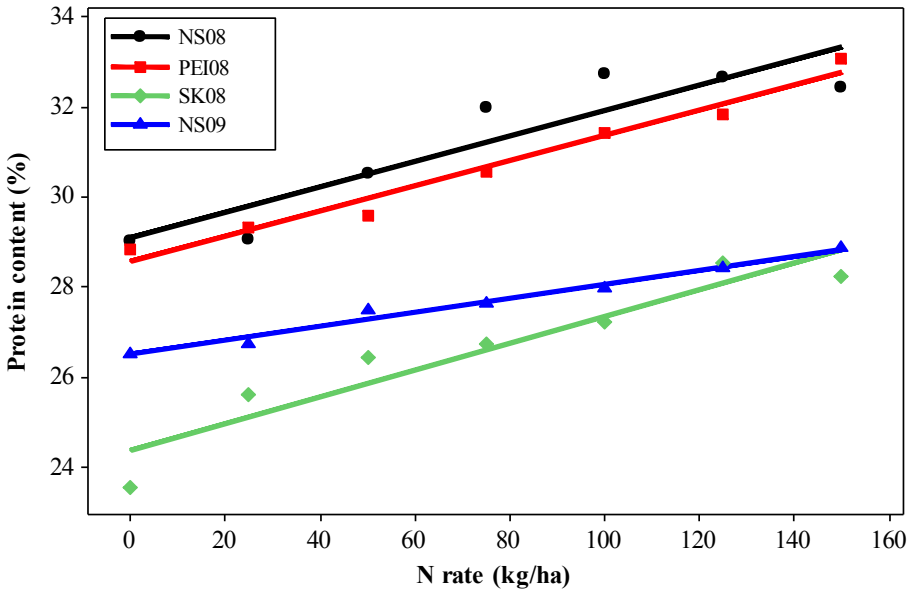

Figure 7.6 Correlation between N rate and protein content

The regression equation between N rate and protein content:

y = 29.10 + 0.02811 N (r^2 = 85 %) ----------NS08;

y = 28.56 + 0.02804 N (r^2 = 98 %) ---------- PEI08;

y = 24.39 + 0.02964 N (r^2 = 90 %) ---------- SK08;

y = 26.50 + 0.01560 N (r^2 = 98 %) ---------- NS09.

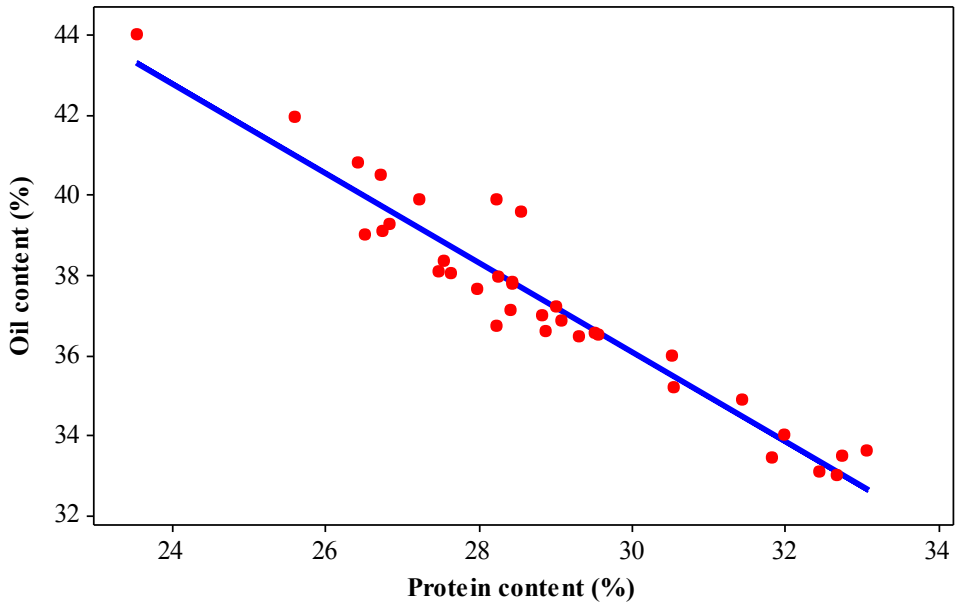

Figure 7.7 Correlation between the mean value of oil and protein content in 5 site-year trials (r^2 = 93%)

Table 7.25 Nitrogen effect on the oil (%) and protein content (%) in 2008

N rate	NS		PEI		SK	
(kg/ha)	Oil	Protein	Oil	Protein	Oil	Protein
0	37.23a	29.02b	36.98a	28.83d	44.03a	23.54e
25	36.88a	29.08b	36.46ab	29.32cd	41.93b	25.61d
50	35.97ab	30.53ab	36.53ab	29.57cd	40.79bc	26.42cd
75	34.01bc	32.00a	35.21bc	30.56bc	40.48bc	26.72c
100	33.49bc	32.74a	34.87cd	31.44b	39.89c	27.22bc
125	33.00c	32.67a	33.45d	31.84ab	39.56c	28.55a
150	33.09c	32.45a	33.63cd	33.07a	39.87c	28.23ab
Mean	34.81	31.21	35.30	30.66	40.94	26.61
P-value	0.023	0.0266	0.0052	0.0032	0.0033	0.0001

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 7.26 Nitrogen effect on the oil and protein content (%) in 2009

N rate	NS		PEI	
(kg/ha)	Oil	Protein	Oil	Protein
0	39.02a	26.52d	38.36a	27.56a
25	39.08a	26.74d	39.26a	26.83a
50	38.11ab	27.49cd	37.95a	28.26a
75	38.03ab	27.64cd	37.81a	28.45a
100	37.63ab	27.99bc	36.57a	29.51a
125	37.14b	28.41bc	37.78a	28.45a
150	36.60bc	28.88ab	36.75a	28.23a
200	35.38c	29.63a	35.95a	28.77a
Mean	37.62	27.91	37.55	28.26
P-value	0.0138	0.0033	0.194	0.496

Means within a column followed by different letters are significantly different ($p < 0.05$)

Table 7.27 Effect of N rate on fatty acid composition

N rate	16:0	18:0	18:1	18:2	18:3	20:1	22:1
N0(NS08)	3.18a	0.94a	8.83a	15.57b	13.00a	7.84a	40.44a
N7(NS08)	3.19a	0.86a	8.35a	16.93a	13.01a	6.97b	39.61a
P-value	0.935	0.078	0.117	0.038	0.834	0.084	0.589
N0(NS09)	3.22a	0.85a	9.32a	16.19a	12.61a	7.88a	40.50a
N7(NS09)	3.34a	0.86a	8.75a	17.33b	12.58a	7.53a	39.73a
P-value	0.644	0.701	0.263	0.036	0.372	0.053	0.566
N0(PEI08)	3.14a	0.93a	8.52a	14.88b	12.99a	8.00a	41.68a
N7(PEI08)	3.13a	0.83a	8.45a	15.85a	12.74a	7.59b	41.27a
P-value	0.059	0.053	0.136	0.035	0.068	0.026	0.243
N0(PEI09)	3.39a	0.91a	9.27a	17.91b	12.32a	8.00b	38.33a
N7(PEI09)	3.60a	0.98a	9.79a	18.88a	12.01a	8.34a	37.92a
P-value	0.171	0.05	0.088	0.032	0.234	0.04	0.103
N0(SK08)	3.30a	0.87a	8.42a	15.93b	13.27a	8.04a	39.26a
N7 (SK08)	3.35a	0.82a	7.68a	16.68a	14.42a	7.19a	38.50a
P-value	0.083	0.019	0.516	0.027	0.473	0.383	0.379

N0 = 0 kg N/ha; N7 = 150 kg N/ha

7.5 Discussion

7.5.1 Seeding Rate

Many growth processes occurring throughout the development of the plant affected the seed yield and quality (Morgan 1984). This includes the number and the architecture of branches, the number and growth rates of inflorescences and the number of pods/plant. Significant correlation between branches/plant and pods/plant with seeding rate found in this study suggested that increasing number of branches/plant and pods/plant was an important yield compensation factor of *B. carinata* for reduced plant stand. This result corresponded well to the findings reported by Agegnehu and Honermeier (1997) and Leach *et al.* (1999), which revealed that plants had the ability to modulate the vegetative growth by increasing the numbers of lateral branches for compensating a considerable degree of decreased plant density.

No significant difference of plant height due to seeding rates was observed in this study. This result was inconsistent with many earlier findings such as Hamid *et al.* (2002) work with soybean and Urbaniak *et al.* (2008b) studies on *camelina*, both of which found a positive relationship between the plant height and seeding rate. Even though the plant height was not significantly affected by the seeding rate treatments, the environment had a dramatic influence on plant height in this study. The significantly higher plant height in NS and PEI in 2008, which was about 20 cm higher than SK, could be caused by heavier vegetative growth due to more available moisture and which may have contributed to lower seed yields. The significantly lower plant height (76 cm) in SK 2009 could be explained by several reasons. One of the important factors is that the extremely dry condition during early growth stage depressed the apical dominance and then resulted in the shorter plants with more branches.

Significant seeding rate effect on seed yield was only observed in NS. However, regression analysis between seeding rate and seed yield revealed that the most promising planting rate for *B. carinata* would be around 200 seeds/m^2 across all sites. Seeding at a rate of 200 seeds/m^2 would result in 128 plants/m^2 if emergence is 64 % (average emergence in this study Table 7.9) and 94 plants/m^2 if emergence is 47 % (lowest emergence in this study). This seeding rate is satisfactory if emergence is greater than 40 % to achieve minimum of 80 plants/m^2.

7.5.2 Nitrogen Effects

The significant correlation between seed yield and N rate found in this study is in agreement with that reported by Kaur and Sidhu (2004) and Thakral *et al.* (1997), which indicate that *B. carinata* is a crop, responds well to applied nitrogen. Seed yield was significantly affected by applied N in all trials, but the response varied among sites. At both the NS (2008-09) and PEI (2008) sites, seed yields of *B. carinata* responded positively to N rates of up to 125 kg/ha at both the NS and PEI site. At the SK site, seed yield was maximized with an application of 75 kg N/ha. The lack of yield response in the 2009 PEI site was probably due to a sufficient soil-available N from the previous crop. Plotting mean seed yield against N rate gave nearly a linear relationship in all trials:

NS08 = 586.1 + 4.4 N ----------r^2 = 96 %;
PEI08 = 463.7 + 5.8 N ----------r^2 = 98.5 %;
SK08 = 1705 + 3.5 N ----------r^2 = 93.6 %;
NS09 = 1371 + 4.5 N ----------r^2 = 76.9 %;
PEI09 = 1724 - 2.6 N ----------r^2 = 60.5 %;

The regression equations indicate that each increase of 1kg nitrogen could achieve nearly 4.4, 5.8, and 3.5 kg more seed yield per hectare at the NS (2008-09), PEI (2008) and SK (2008) site respectively. However, the seed yield in PEI (2009) declined by 2.6 kg/ha per increase of 1 kg N /ha. *B. carinata* produced much higher yields in SK compared to NS and PEI sites. According to the regression function, the seed yield of *B. carinata* can achieve 1705 kg/ha without adding any nitrogen fertilizer in the field in SK, indicating a significant background level of available N. This amount was even higher than the seed yield achieved with 150 kg N/ha in NS and PEI in 2008. Less soil N leaching in SK could provide more soil available N for plant growth and contribute to the higher seed yield. Results of this study revealed that nitrogen fertilizers increased seed yield of *B. carinata* of 109 % in NS (2008), 64 % in NS (2009), 173 % in PEI (2008) and 30 % in SK (2008).

Furthermore, the amount of N supply greatly influenced the aboveground biomass accumulation. Higher biomass per plant under higher dose of N supply could be due to increased N supply, which escalated leaf area development, improved leaf area duration after flowering, and enhanced crop photosynthetic rate, contributing to the higher amount of carbohydrates source for biomass accumulation. Similar results were found by

Pramanik *et al.* (1996) and Thakral *et al.* (1997). This indicated that *B. carinata* is a high N requirement for optimum performance.

The harvest index increased significantly as the applied N increased up to the highest rate of fertilizer application in NS and PEI. However, in SK the harvest index of about 13.3-16.3 % was not significantly different among N levels. Similar harvest index ranges from 14-15 % for Indian mustard were reported by Kumar and Dhingra (1997). A slightly higher harvest index from 20 % to 22 % was reported by Kasa and Kondra (1986) in different *Brassica* spp. Increasing the N rate greatly increased the aboveground biomass more than seed yield in NS and PEI sites, which is reflected by the lower harvest index. The lower harvest index in NS and PEI might be due to the excessive vegetative growth since the plants were almost 10 cm taller in those two sites as compared with SK site. The comparatively lower biomass achieved in 2009 in NS and PEI might be attributed to low temperature during early growth phases. Gan *et al.* (2007) reported that the higher soil residual nitrogen had a greater influence on the seed yield than on straw yield. The results in SK support this result.

7.5.3 Oil and Protein Content

The effect of seeding rates on *B. carinata* oil and protein content was not significant at all sites. This result is consistent with previous studies on hemp seed (Vera *et al.* 2006) and rapeseed (Kondra 1975). A similar trend in oil and protein content with nitrogen application rate was noted in all trials. The plants without the N supply produced a higher oil content and lower protein content than any other treatments. The negative correlation between oil and protein content might be due to the competition for carbon skeletons between fatty acid and amino acid biosynthetic pathway. More amino acids than fatty acids was produced in the reductive amination and transamination processes and resulted in the increase of protein content and the decrease of oil content with the rise of nitrogen dose (Punia *et al.* 2001). This result was consistent with many previous findings on oilseed crop (Asare and Scarisbrick 1995; Hocking *et al.* 1997).

7.6 Conclusion

Overall, both factors (seeding rate and N supply) included in this study had an important role in *B. carinata* production. From the present finding, it can be concluded that *B. carinata* can be successfully grown at 200 seeds/m^2 with the aim of 80 plants/m^2 in all three geographic locations. A wide range in N fertilizer response among sites was due to large differences in available soil N. To achieve high yield, *B. carinata* required 125 kg N /ha in NS and PEI, while the maximum yield was attained at low rate of 75 kg N/ha in SK.

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Chapter 8 Conclusion

8.1 *Camelina sativa*

8.1.1 Genotype Evaluation

Large variations in maturity, seed yield, seed oil and protein and several fatty acids were observed among the tested *Camelina sativa* genotypes, which indicated the favorable agronomic potential of this specie. The genotype "CN30479" appears to be well adapted to most growing areas and has high yield potential. It would therefore be a good choice when choosing genotypes for a crossing program. Other genotypes with potential genes of interest to improve earliness (CN101985), high oil content (CN30476), high protein content (SRS933), high linolenic acid content (CN101989), were also identified in this study. Some of the genotypes which did not perform as consistently included CN101981 and CN30475. CN30475 ranked highest for seed yield in PEI 2008 but ranked lowest for yield in PEI 2009. Seed yield and oil contents of all camelina accessions tested were highest at the SK site. In general, average oil and protein contents for camelina ranged from 38.32 - 40.86 % and 27.0 - 29.56 %, respectively; high oil content was always associated with low protein content in all trials. The most abundant fatty acids in these 11 camelina genotypes were oleic (11.34 - 17.26 %), linolenic (30.51 - 36.20 %), linoleic (15.09 - 21.05 %) and eicosenic acid (11.79 - 15.01 %). A wider range of values of fatty acid was found among genotypes than that observed among sites; however, overall variation in individual fatty acids was relatively narrow. Downy mildew (*Peronospora parasitica* (Pers. (ex Fr.) Fr.) was observed at both the NS and PEI site; however, this disease did not appear to cause significant yield loss.

8.1.2 Nitrogen Response

Significant response of *C. sativa* to applied N was observed in this study. Plant height, number of pods/plant, seed yield, oil and protein content, and fatty acid composition were all influenced by N rate. The negative correlation between oil and protein content was found in all trials. Increasing N supply increased protein accumulation while decreasing oil accumulation. Fatty acid profile was also significantly influenced by the N supply. But the pattern was not consistent for five trials. More research should be carried out in this area. Generally, seed yield increased dramatically with increasing nitrogen at all sites; however, the optimum N supply varied among

locations due to the different soil types and environmental conditions. To achieve maximum seed yield, the optimum N supply was 125 kg/ha at the both NS and PEI site, but 100 kg N/ha in SK. However, taking into account seed oil contents, the 125 kg/ha applied N rate provided the highest oil yield in all four sites (Figure 8.1). Therefore, in view of the results, an applied N rate of 125 kg/ha would be the most appropriate option in all three sites.

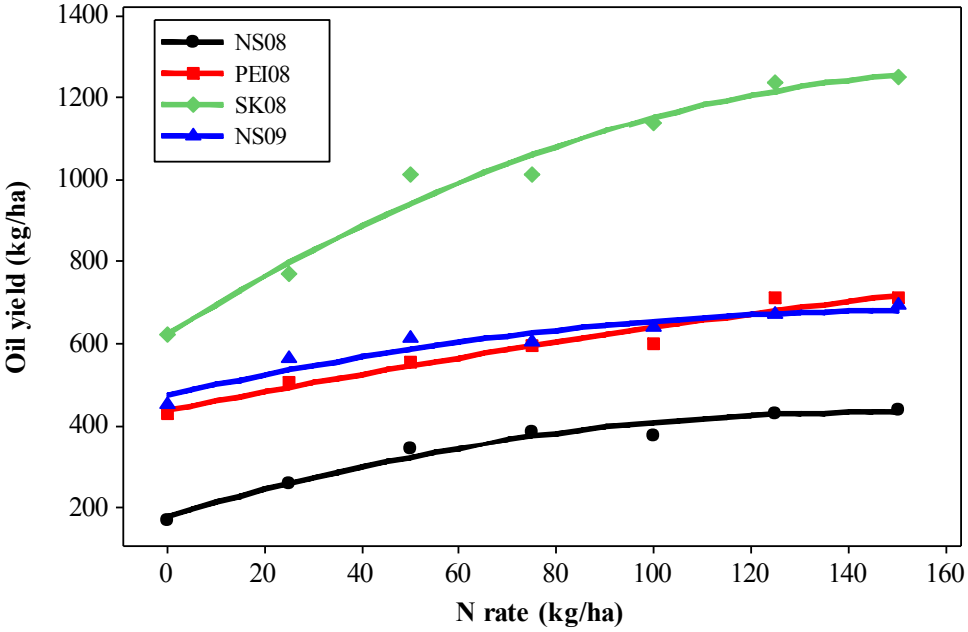

Figure 8.1 Relationship between *C. sativa* oil yield and N rate

Regression equation between oil yield and N supply:

y = 179.3 + 3.489 N - 0.01204 N**2 (r^2 = 96 %) ---------- NS08;

y = 437.9 + 2.301 N - 0.002876 N**2 (r^2 = 95 %) ---------- PEI08;

y = 623.3 + 7.459 N - 0.02166 N**2 (r^2 = 97 %) ---------- SK08;

y = 474.2 + 2.660 N - 0.008495 N**2 (r^2 = 92 %) ---------- NS09

8.1.3 Seeding Rate Effect

Studies on the effect of seeding rates showed that plant stands increased substantially with increasing seeding rates; however, the actual percentage of seeds emerging tended to decrease with increased seeding rate. The increased branches/plant and pods/plant number at the lower seeding rates suggested that *C. sativa* has the ability to compensate

for the reduced plant numbers by producing more branches and pods. The regression analysis indicated that the seed yield of *C. sativa* was highly correlated to seeding rate. The response of *C. sativa* seed yield was highly dependent on environmental conditions, therefore, the optimum seeding rates to achieve maximum seed yield varied across locations. The optimum plant density for achieving maximum seed yield in this study was 170, 280 and 150 plant/m^2 in NS, PEI and SK, respectively. Seeding rate did not affect oil and protein content in any trial.

8.2 *Brassica carinata*

8.2.1 Genotype Evaluation

Seeding rate, nitrogen and genotype all had a significant effect on growth and yield of *B. carinata*. Differences in plant stand, days to flower, days to maturity, TKW, seed oil and protein content, seed yield and fatty acid composition were found among the 10 genotypes at all sites. The mean seed yield of all 10 accessions was approximately 2500 kg/ha which is comparable to AC Vulcan, a *B. juncea* cultivar. Lines 070727EM, 050488EM and 050334EM had the highest seed yields of approximately 3000 kg/ha in PEI 2009 and lines 070756EM, 070732EM, 070768EM, 070760EM yielded more than 3000 kg/ha in SK 2009. Therefore, the *B. carinata* genotypes all had satisfactory yield performance. Many other positive attributes were also observed in these 10 genotypes. The oil content of line 070768EM (41.67 %) was significantly higher than all the other tested lines, thus, it could be an important source of gene to improve the oil content. Protein content up to 34.68 % and erucic acid content up 42.77 % to was found in line 070714EM. However, as compared with AC Vulcan, the earliest-maturing line 070714EM was 4 days later, while the latest-maturing line 070768EM was approximately 15 days later maturing than AC Vulcan. This later maturation would be the major limiting factor for cultivating in western Canada. More accessions should be evaluated in order to identify the potential genes of improving earliness. In conclusion, useful genetic variation in agronomic and seed quality characteristics was found among these 10 *B. carinata* genotypes and this offers the possibility of further improvement of this oilseed crop through breeding.

8.2.2 Nitrogen Response

Applied N had a significant influence on the number of branches/plant, aboveground biomass, harvest index, oil and protein content, and fatty acid composition depending on sites. The linear response of seed yield to N rate was seen up to the 150 kg/ha in four out of five sites. However, yields were similar among the 125 and 150 kg N/ha in NS (08 and 09) and PEI (08). In SK, the seed yield was not statistically different among 75, 100, 125 and 150 kg N/ha. The different response observed at the 09 PEI site was due to the high amount of soil-available N. Therefore, to achieve potential seed yield, *B. carinata* required 125 kg N /ha in NS and PEI, while the maximum yield was attained at a lower rate of 75 kg N/ha in SK. Negative correlation between N supply and seed oil yield in all trials suggested that the total oil yield should be taken into consideration. Figure 8.2 indicated that oil yield linearly responded to N supply up to 150 kg N/ha. Thus, the final decision for the amount of N supply is depends on the current oil prices and nitrogen costs.

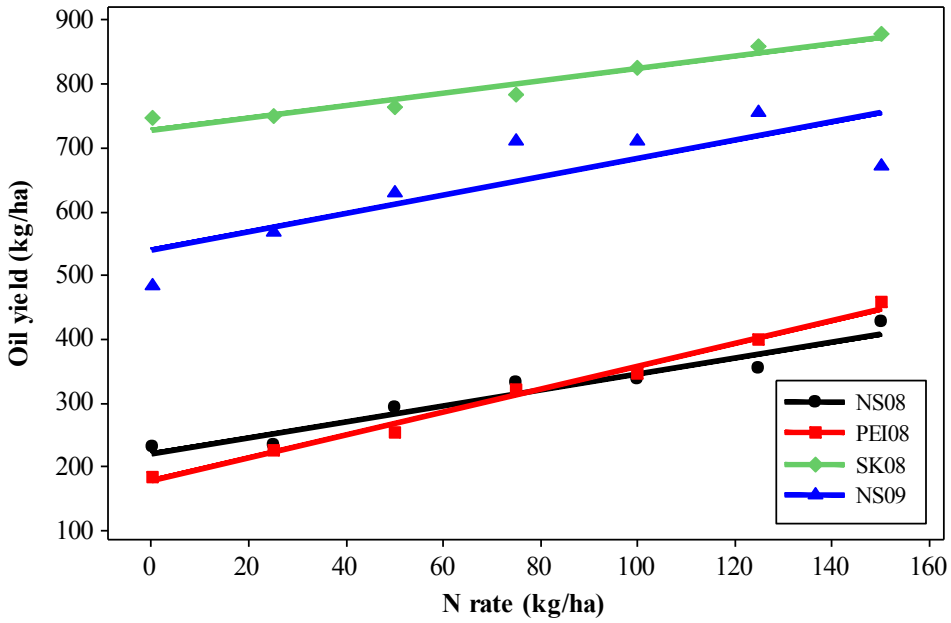

Figure 8.2 Relationship between *B. carinata* oil yield and N rate

Regression equation between oil yield and N supply (N):

$y = 221.8 + 1.248\ N$ ($r^2 = 94\ \%$) ---------- NS08;

$y = 177.7 + 1.802\ N$ ($r^2 = 99\ \%$) ---------- PEI08;

$y = 728.7 + 0.9661\ N$ ($r^2 = 95\ \%$) ---------- SK08;

$y = 538.6 + 1.450\ N$ ($r^2 = 70\ \%$) ---------- NS09

8.2.3 Seeding Rate Effect

Seeding rate had no influence on days to flower or days to maturity or plant height of *B. carinata*. No significant differences of oil and protein content were found among varied seeding rates either. The significant seeding rate effect was only observed in NS. Yield levels were similar across a wide range of plant densities in both PEI and SK. The regression analysis between seed yield and seeding rate observed in this study suggested that a seeding rate of 200 seeds/m^2 is the optimum rate for growing *B. carinata* in all sites. This seeding rate is satisfactory if emergence is greater than 40 % to achieve minimum of 80 plants/m^2.

8.3 Environmental Effect

In all trials, the site and year significantly affected all the measured variables, in particular seed yield performance. The variation of seed yield between three sites for all studies may be due to the genetic, environmental and agronomic factors as well as the interaction between them. Due to the different soil and climate in SK, the requirements for N and seeding rate were significantly lower to achieve maximum seed yield. These unequal requirements for production make it harder to compare the seed yield response to certain applied nitrogen and seeding rate among sites. Furthermore, the seed yield appeared to be highly dependent on the specific field site. In NS, the seed yield of all trials for *C. sativa* and *B. carinata* was significantly higher in 2009 than 2008. Severe weed competition and lower plant stands in 2008 appeared to be the critical factors which resulted in the lower seed yield. Generally, *C. sativa* yields up to 2000 kg/ha and *B. carinata* yields up to 2500 kg/ha within each experimental site indicated the agronomic potential of these two oilseed crops across Canada.

8.4 Growth Chamber Study

The growth chamber study showed that the effects of N rate and soil water regimes significantly influenced all the measured variables, which included plant height, root and shoot dry matter, root: shoot ratio, xylem pressure potential, yield components, photosynthetic parameters, net photosynthesis, transpiration rate, instantaneous water use efficiency and seed yield for both *C. sativa* and *B. carinata*. Net Photosynthesis (P_N) for both *C. sativa* and *B. carinata* increased hyperbolically with increasing photosynthetically active radiation (PAR). The nitrogen requirement for achieving maximum seed yield varied at different soil water availability regimes. Under low water availability, *C. sativa* and *B. carinata* had a nitrogen requirement of approximately 100 kg N/ha for achieving optimum yield. Whereas, in sufficient water conditions, an applied N rate of 150 kg/ha would likely be the most appropriate option for both crops.

8.5 Overall Assessment

Results of this study provide convincing evidence of the agronomic suitability of *C. sativa* and *B. carinata* in all three sites (NS, PEI and SK). In the future, it would be interesting to evaluate the seeding date and nitrogen application time in these regions. Genotype evaluation, particularly in terms of downy mildew (*Peronospora parasitica*) resistance for *camelina* and earliness for *B. carinata* is also recommended. A large sale study would also be valuable in herbicide screening since there is no herbicide registered for *C. sativa*. Overall, more work is needed to fully exploit the potential of these two oilseed crops; however, growing oilseed crops for profit will ultimately depend on market demand.

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Appendix A. Precipitation and Temperature Data for Truro, NS, (NSAC); Harrington, PEI, (AAFC) and Saskatoon, SK, (AAFC). 2008-09

Table A1. Weather Summaries for NS, PEI and SK in 2008

	Site								
	NS			PEI			SK		
	Precipitation (mm)	GDD	Sunshine Hours	Precipitation (mm)	GDD	Sunshine Hours	Precipitation (mm)	GDD	Sunshine Hours
May	103.2	112.9	498.45	127.8	117.8	503.96	2.5	177.6	532.65
June	118.9	303	505.76	61	306	512.54	68	299.9	548.78
July	83.8	469.8	509.52	34.4	489.8	515.71	93	397.4	547.9
August	223.5	390.2	468.02	240.2	406.1	471.79	19.5	398	490.85
September	157.1	268.1	404.65	156.8	273.6	405.93	13	201.7	412.29
Total	686.5	1544	2386.4	620.2	1593.3	2409.93	196	1474.6	2532.47

Table A2. Weather summaries for NS, PEI and SK in 2009

	Site								
	NS			PEI			SK		
	Precipitation (mm)	GDD	Sunshine Hours	Precipitation (mm)	GDD	Sunshine Hours	Precipitation (mm)	GDD	Sunshine Hours
May	69.8	152.6	498.13	107.8	158	503.63	10	117.4	532.21
June	85.2	314.7	505.71	111.6	304.2	512.5	81.5	298.1	548.68
July	62	398.7	509.78	126.2	404.9	515.97	58.5	336.7	548.25
August	146.4	425.7	468.37	185.8	441	486.57	90.5	340.9	491.33
September	39.4	213.1	405.04	65	255	406.35	31.5	333	412.8
Total	402.8	1504.8	2387.03	596.4	1563.1	2425.02	272	1426.1	2533.27

Appendix B. Severity Scale for Downy Mildew [*Peronospora parasitica* (Pers. (ex Fr.) Fr.] Disease

The 0- 9 visual rating scales for downy mildew infection:

Rate 0: No symptoms, no sporulation

Rate 1: Sporulation was only observed on pods. Less than 5 pods per branch were infected. No stem damage.

Rate 2: Sporulation was observed only on pods. More than 5 pods per branch were infected. No stem damage.

Rate 3: More than 5 pods per branch were infected. Slightly sporulation was observed on stem for 1-2 cm long and no distortion on stem.

Rate 4: 2-5 cm dense white mold coverage was observed on one or more branches and there was no or slight distortion on stem.

Rate 5: 2-5 cm dense white mold coverage was observed on one or more branches. The stem showed a twisted growth and some pods were dry out.

Rate 6: More than 5 cm dense white mold coverage was observed on one or more branches and some pods were dry out.

Rate 7: One of the branches was totally damaged and there was no pod on this branch.

Rate 8: More than one branch was fully covered by dense white mold and there was no pod on these damaged branches.

Rate 9: The whole plant was covered by dense white mold and there was no pod on whole plant.