COMPARATIVE EFFECTS OF WATER DEFICIENCY AND APPLIED NITROGEN ON *CAMELINA SATIVA* L. CRANTZ AND *BRASSICA NAPUS* L.

by

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Abstract

This study compared key aspects of the physiology and agronomy of *Camelina sativa* and *Brassica napus* under controlled environments and at multiple field locations in 2013 and 2014. The controlled environment study showed that the response of both crops to N depended on the soil water status. Camelina maintained higher photosynthesis at N level over 125 kg ha^{-1} under water deficit. There was no N*water status interaction effect on biomass or seed yield of either crop. A higher shoot/root ratio in camelina indicates a better adaptability to imposed water deficit. In the field, response to N by both crops showed the optimum N rates for crop performances varied with soil-climatic conditions. Camelina and canola required 75 to 125 kg ha^{-1} and 125 to 175 kg ha^{-1} N, respectively, to optimize their performances. Future experiments should evaluate field level water use efficiency by both crops to confirm the controlled environment results.

List of Abbreviations and Symbols Used

CDI	Crop Development Institute
DAP	Days after planting
E	Transpiration
ET	Evapotranspiration
GLM	General linear model
g_s	Stomatal conductance
K	Potassium
MMT	Million metric tons
N	Nitrogen
NIR	Near inferred spectroscopy
NS	Nova Scotia
NUE	Nitrogen use efficiency
P	Phosphorous
PEI	Prince Edward Island
$P\text{n}$	Photosynthetic rate
S	Sulphur
SFA	Saturated fatty acids
TKW	Thousand Seed Weight
USDA-FAS	United States Department of Agriculture-Foreign Agricultural Service
$\text{WUE}i$	Instantaneous water use efficiency

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Chapter 1: Introduction

1.1 Background

Oilseed crops are characterized as those whose oil is the most important and valuable component within the seeds (Blumenthal et al., 2008). This feature turns them into the leading suppliers of superior quality and specialty vegetable oils (Sarwar et al., 2013) which can be utilized for diverse food and industrial purposes. The meal remaining after extraction of the oil is a valuable source of protein for livestock feed (Jensen et al., 1996). Common oilseed crops include cotton (*Gossypium herbaceum* L.), soybean (*Glycine max* L.), mustard (*Brassica nigra* L.), crambe (*Crambe cordifolia* Stev.), safflower (*Carthamus tinctorius* L.), sunflower (*Helianthus annuus* L.), flaxseed (*Linum usitatissimum* L.), peanut (*Arachis hypogaea* L.) and canola (*Brassica napus* L.) (Sarwar et al., 2013). Among them, soybeans, sunflower, and canola are the main oilseed crops grown globally (Wittkop et al., 2009).

Recently, there has been an increasing interest in exploring and developing alternative oilseed crops due to rising demands for vegetable oil for food and non-food uses. Over a period of 14 years, the consumption of vegetable oil worldwide has experienced a steady increase. According to the United States Department of Agriculture-Foreign Agricultural Service (USDA-FAS), to date, 172.99 million metric tons (MMT) of vegetable oil are domestically consumed in 2014, which almost doubles the amount consumed in 2001 (90.85 MMT). This consumption accounts for over 97% of total annual vegetable oil production. This growth is mostly driven by the increased demand for vegetable oils in regions with high populations such as China, India and the Far East where vegetable oils are an essential component of the diet (Koha et al., 2012).

Although, there are various crops grown as oil producing crops, the Canadian oilseed market is dominated by canola (*Brassica napus* L.), soybean and flaxseed [Agriculture and Agri-Food Canada (AAFC), 2012]. With the increasing demand for vegetable oils, these crops are likely to expand in acreage considerably. However, most of these major oilseed crops have limitations in regard to climatic adaptability, nutritional input requirements and application of oil products. Putnam, et al. (1993) stated that the major oilseed crops cannot perform well in marginal lands with low moisture, low fertility or saline soils. For instance, the production potential of soybean is dependent upon current climatic conditions including solar radiation, temperature and water availability (DeAvila et al., 2013); it does not adapt well to more northern regions in North America, Europe, and Asia (Putnam et al., 1993). Canola can grow better under northern climates but requires high nitrogen rates and is susceptible to insects and diseases (Putnam et al., 1993). Because of potential disease problems, canola cannot be successively grown in the same field and a one-in-four year rotation in any particular field is highly recommended (Livingston et al., 1995). Oils extracted from flaxseed are mainly used for industrial purposes and the remaining meal is used for animal feed only (Mridula et al., 2011).

In addition, the pressure on production has resulted in a lack of agricultural diversity that threatens agronomic and economic sustainability (Johnson et al., 2012). The requirement for greater diversification of crops, including oilseeds, as a result, has been significantly increased (Wittkop et al., 2009).

In order to overcome the challenges from the increasing demand on vegetable oils and agricultural diversification, it is necessary to develop an alternative oilseed crop with diverse food and non-food applications of the seed oil, reduced input requirements, high nutrient efficiency, disease tolerance and good adaptability on marginal soils and in northern climates. A good example of such an alternative crop is camelina (*Camelina sativa* L. Crantz) (Wittkop et al., 2009). The question involved in introducing a new crop is the environmental conditions under which profitable production with good quality may be undertaken (Ireland, 1940). However, there is little information available regarding the relative performance (e.g. grain yield and seed oil yield) of camelina in comparison with other oilseed crops, especially with canola, over a wide range of agricultural inputs (e.g. nitrogen (N) fertilizer and water) and target productivity levels.

1.1 Literature review

1.1.1 General description of camelina and canola

1.1.1.1 *Camelina sativa* L. Crantz

Camelina sativa L. Crantz (Camelina), also known as false flax, Dutch flax, German sesame, Siberian oilseed and "Gold of pleasure" (Putnam et al., 1993; Vollmann et al., 1996), is a member of the *Brassicaceae* family, but it does not cross pollinate with canola or mustard. It is grown as an herbaceous annual or winter annual crop with a short growing season (85-100 days) requirement (Putnam et al., 1993; Francis & Warwick, 2009).

The camelina plant has a taproot and its erect and branched hairy woody stem can typically reach 90cm in height (Putnam et al., 1993; Zubr, 1997). Leaves are arrow-shaped and sharp-pointed, 2-8 cm long and 2-10mm wide, with a few hairs and smooth edges (Francis & Warwick, 2009; Zubr, 1997). Predominantly self-pollinated flowers are small and pale yellow in colour with four petals (Francis & Warwick, 2009; Zubr, 1997). The seed pods are pear-shape and 7 - 9 mm long, containing 8-10 yellow or brownish yellow seeds (Francis & Warwick, 2009; Zubr, 1997). Seeds typically contain 27 to 32% protein and 38 to 43% oil (Gugel & Falk, 2006; Zubr, 1997) with exceptional levels of omega-3 a-linolenic acid (Shukla et al., 2002; Dubois et al., 2007) and relatively low erucic acid

(Putnam et al., 1993) and saturated fatty acids (SFA) (~10% of the oil) (Abramovic & Abram, 2005; Dubois et al., 2007).

According to archaeological excavations, camelina was first grown in the late Neolithic (Knörzer, 1978; Zinger, 1909) and was well established in the Bronze Age (1500-400 B.C.) in Europe (Bouby, 1998; Schultze-Motel, 1979; Zubr, 1997). In the Iron Age, camelina, together with flax and cereals, became a substantial crop (Gugel & Falk, 2006; Zubr, 1997) but the importance of camelina cultivated as a food crop with unique agronomic properties was neglected in the Middle Ages (Knörzer, 1978; Robinson, 1987). It is likely that camelina was first introduced into North American as a weed in flax (Francis & Warwick, 2009; Putnam et al., 1993). In Canada, camelina was first reported in 1863 in Manitoba (Francis & Warwick, 2009). Until the 1980s, camelina was considered as a potential crop (Plessers et al., 1962; Robinson, 1987), but it was only cultivated on a small scale in Europe and Russia (Zubr, 1997). Significant commercial cultivation began only in the late 1990s.

With a long cultivation history across the European continent, camelina shows more flexible adaptability and better performance under different climatic and soil conditions compared with other Brassica crops (such as rapeseed and mustard) (Gugel & Falk, 2006). It was reported that camelina grows best in cold semi-arid climate zones in steppes or prairies (Francis & Warwick, 2009). It successfully grows in most soil types (Gugel & Falk, 2006). Zubr (1997) described camelina as a crop with cold and drought tolerance, disease resistance and low-fertilizer requirements. These distinct characteristics make camelina a potential oilseed crop for temperate and water-limited cultivation areas.

In recent years, it has gained increasing attention as a renewed oilseed crop, being used both for food and industrial purposes, with favorable agronomic traits and specialty oil characteristics. Camelina has shown great promise as an alternative oilseed crop.

1.2.1.2 Canola

Canola also belongs to the *Brassicaceae* family and is grown as an annual crop around the world. Most current varieties of canola are *Brassica napus*, or *B. rapa* species (Ehrensing, 2008). Canola is primarily self-pollinating; however, *B. rapa* canola, which is a diploid species with a strong self-incompatibility, is predominantly cross-pollinated (Brown et al., 2008).

Canola plants have a long and slender taproot and branched stem, which can reach 120-180 cm high depending upon variety and environmental conditions (Colton & Sykes, 1992). The stem usually terminates in an elongated spike. Canola has waxy and smooth leaves that are in dark bluish-green in colour. Flowers are yellow in colour with four sepals and petals which are diagonally opposite of each other (OECD, 1997). Seeds are developed in a silique which is two-celled and elongated with a prominent mid-vein (Bailey, 1976)

Canola was first developed from rapeseed in Canada during the 1960s. Rapeseed was cultivated primarily as a source of erucic acid. This compound is not edible but valuable in high performance industrial lubricants (Brown et al., 2008). World War II called for vast quantities of lubricating oil, thus cultivated rapeseed acreage increased worldwide, especially in Canada. After the war, there was a dramatic decrease in industrial grade rapeseed oil demand, which drove farmers to look for other uses for the plant and its products (Brown et al., 2008). However, the high concentrations of erucic acid in the oil became the major disadvantage in using rapeseed for human consumption. At this point, Canadian plant breeders used conventional plant breeding techniques to remove undesirable compounds and produce edible oil containing low levels of erucic acid (< 2%) and glucosinolates (< 30 µmol/gram) (Ehrensing, 2008). In 1978, the Canola Council of

Canada (formerly Rapeseed Association of Canada) coined the word “Canola” (for Canadian oil low acid) as the registered trademark for edible rapeseed oil (Ehrensing, 2008). Now canola is widely accepted as the generic name given to rapeseed varieties that are grown for edible oil. Mature canola seeds are high in oil content (40 - 45%) and protein content (20- 25%) (Ghanbari-Malidarreh, 2010). It is the most important and valuable oilseed crop in Canada.

Canola can be successfully grown in cool areas with nutrient rich and well-drained soils and a moist environment (Brown et al., 2008). Both spring and winter varieties (*B. napus* and *B. rapa*) have been developed. Canola plants usually require an average of 100-125 days from seeding to maturity (Brown et al., 2008). Canola normally develops quickly and competes well with annual weeds, but is susceptible to disease, such as sclerotinia stem rot [*Sclerotinia sclerotiorum* (Lib.) deBary] and blackleg [*Leptosphaeria maculans* (Sowerby) P. Karst.] (Ehrensing, 2008).

1.2.2 Nitrogen

The significance of N as a macronutrient has long been recognized (Miller, 1939). Nitrogen serves as the constitute element of many important organic compounds such as amino acids, proteins and nucleic acids. It is also a component of compounds such as chlorophyll and alkaloids (Fageria1 & Baligar, 2005). To optimize the crop productivity of non-leguminous field crops, it is fundamental to supply N adequately (Miller et al., 2001; Jaynes et al., 2001). In oilseed production, N accounts for the largest energy input with regards to fertilizer supply (Gan et al., 2008). However, mismanagement of N not only can significantly affect agricultural production, but it can also cause environmental issues. For example, shortage in N supply can restrict crop growth, resulting in a decrease in crop yield; excessive supply of N can contaminate groundwater (Jaynes et al., 2001). Therefore, appropriate N fertilizer management is critical to balance the factors between crop N requirements and the need to minimize environmental contamination. To establish such improved N fertilizer management, a good understanding of the effects of N on crop physiology and growth is necessary.

1.2.2.1 Nitrogen and crop physiological responses

Nitrogen increases growth and yield by influencing a series of metabolic processes and physiological parameters, such as stomatal conductance, photosynthesis and chlorophyll content. For example, the stomatal conductance (g_s) in beans (*Phaseolus vulgaris* L.), coffee (*Coffea arabica* L.) and winter wheat (*Triticum aestivum* L.) increased with N availability under well-watered conditions (Shimshi, 1970; Tesha & Kumar, 1978; Shangguan, 1997). Sugiharto et al. (1990) found a positive correlation between plant photosynthetic capacity and N concentration in leaves and suggested that most of the N was used to synthesize the components of the photosynthetic apparatus. Yoshida (1972) reported that N functions to maintain photosynthetic activity during grain fill. A low photosynthetic rate under limited N availability conditions resulted in decreases in chlorophyll content and protein synthesis (Cechin et al., 2004). Rubisco, for example, which is representative of leaf protein and plays an important role in carbon assimilation, was significantly affected by N deficiency (Seemann et al., 1987). Karic et al. (2005) observed an insufficient use of photo-assimilates to synthesize organic N compounds and sugar accumulation under limited N levels.

Physiological responses and biochemical changes of a wide range of crops to N availability have been well documented. However, there is little information available in the literature regarding the physiological responses of canola and camelina to N effects.

1.2.2.2 Nitrogen and crop growth and production

1.2.2.2.1 *Camelina sativa* L. Crantz

Camelina has similar soil fertility requirements to other Brassica species, with the same yield potential. Generally, camelina's requirement for N fertilizer are moderate to low compared with other crops such as canola and sunflower (Putnam et al., 1993). Zubr (1997) pointed out that camelina could successfully grow at a rate of 100 kg N ha^{-1}. Camelina, grown in trials in the northern United States, showed good response to an N rate of 90 kg N ha^{-1} (Budin et al., 1995). Jackson (2008) claimed that farmers needed to apply 90 kg N ha^{-1} to optimize seed yield.

In the past decades, research has been conducted to evaluate camelina responses to different N rates, based on yield variations. In a two-year field study, it was found that N fertilizer increased camelina seed yield by 1.1 - 2.2 times, compared to that in unfertilized plots (Končius & Karčauskienē, 2010) in Lithuania. Bugnărug et al. (2000) in Romania reported that camelina yield was increased by 58% with 100 kg N ha^{-1} application, compared to control crops with no additional N applied. A number of studies have shown that seed yield has a linear response to N fertilizer. Maximum seed yield was obtained with 120 kg N ha^{-1} applied in Germany (Agegnehu & Honermeier, 1997). A more recent study conducted by Jiang et al. (2013) in the Maritime Provinces in Canada also found that seed yield was linearly correlated with N rates, up to 120 kg ha^{-1}; this result was consistent with previous work conducted by Urbaniak et al. (2008) in the same region.

The pattern of N influencing seed yield of camelina is the same as that for canola; it affects growth parameters, such as the number of branches and pods per plant and number of seeds per pod (Končius & Karčauskienē, 2010). Agegnehu & Honermeier (1997) found that the number of branches, number of pods as well as the number of seeds per pod developed on a single plant increased significantly as the N dose increased. Similar results were also reported by Končius & Karčauskienē (2010).

The N effects on protein and oil content of camelina seed have been examined by different research groups (Agegnehu & Honermeier, 1997; Gugel & Falk 2006; Urbaniak et al. 2008; Končius & Karčauskienē, 2010; Lošák et al. 2011; Jiang et al. 2013; Johnson and Gesch 2013; Kirkhus et al. 2013). Results showed a positive correlation between protein content and N input and an opposite effects of N input was found for oil content.

1.2.2.2.2 Canola

Canola requires higher amounts of N compared to most other grain crops at the vegetative growth and seed production stages (Hocking et al., 1997). About 25% more N is required by canola compared to wheat in order to produce the same grain yield (Hocking & Stapper, 2001). Numerous studies have shown that high rates of applied N significantly increase growth and yield of rapeseed (Bilsborrow et al., 1993; Cheema et al., 2001; Kumar et al., 2001; Karamzadeh et al., 2010). Nitrogen enhances seed yield via the positive effects on a number of yield components, such as number of branches and pods per plant, seeds per pod and 1000-seed weight (Karamzadeh et al., 2010; Ahmad et al., 2011). However, canola yield response to N rates varied according to different environmental conditions, including climate, soil profile characteristics, residual fertility (especially nitrate) and soil water content. For instance, under sub-humid environments in western Canada, canola crops responded positively to N fertilizer with a rate of 180 kg N ha^{-1} (Brandt et al., 2002). Under environments with a low-yield potential, the amount of N fertilizer required to achieve the maximum seed yield was 120 kg N ha^{-1} for canola species (Ahmad et al., 2011; Gan et al., 2007). A study conducted in a humid region in the north of Iran, in 2009, reported that the highest seed and oil yields were achieved with 161 kg N ha^{-1} (Karamzadeh et al., 2010).

The oil content in canola seed was also closely related to the amount of available N, including fertilizer N and soil residual N. The highest seed and oil yield occurred at about 200 kg N ha^{-1} (Ibrahim et al., 1989; Jackson, 2000).

Excessive N, on the other hand, could negatively affect seed quality (Cheema et al., 2001). An over application of N could increase N concentration in seeds and decrease oil content, thus reducing the commercial and industrial value (Chamorro et al., 2002).

1.2.2.3 Nitrogen use efficiency

Nitrogen Use Efficiency (NUE) can be defined as the ratio of grain weight to N supply (Moll et al., 1982). In oilseed crop production, from a scope of sustainability in agriculture, fertilizer application, especially N fertilizer, should be well managed to achieve higher NUE, reduce environmental risk, and improve the oilseed commercial value.

The NUE can be divided into two processes: N uptake efficiency, defined as the ability of plants to uptake N from the soil in the forms of NO^{3-} and NH^{4+}; and N utilization efficiency, which means the ability of the plant to use N to produce grain yield (Hirel et al. 2007). Canola has been reported to have a relatively low NUE due to poor N utilization in productive tissues and not because of ineffective N uptake (Svečnjak et al. 2006). Poor N utilization leads to a low N-harvest index which is mainly because of the grain sink limitations (Hocking et al. 1997). However, N uptake, transport and assimilation varies within crop species. A greenhouse study demonstrated different N metabolism and/or uptake patterns between camelina and calendula (*Calendula officinalis* L.) (Johnson et al. 2012). The N concentration in camelina root tissues remained constant with increasing rate of N application, whereas N content in the shoots increased. Johnson et al. (2012) assumed that additional N was stored in shoot tissue and was utilized for growth.

1.2.3 Water

Water plays a multi-functionary role in plants (Gardner, et al. 1985) and significantly affects or determines crop development, biomass accumulation and grain yield (Deng et al. 2004; Micheletto et al. 2007). Typically, the content of water in the mass of plant tissues is ranges from 70 to 90% depending on species, ages and the environment (Gardner, et al. 1985). Water availability or deficiency for crop plants can result in a successful harvest or failure in crop yield, respectively. Borsani et al. (2001) highlighted that globally, water deficiency continues to cause great economic losses in agriculture.

To maintain agricultural production sustainably, it is essential to understand soil water status and water behavior in plants.

1.2.3.1 Physiological importance and functions of water in plants

Water availability affects almost every physiological process in plants in direct or indirect patterns (Kramer et al., 1995). McIntyre (1987) suggested that water should be considered as a primary factor in regulating plant growth. Decreasing water content is usually accompanied by loss of turgor and wilting, cessation of cell enlargement, decrease in chlorophyll content (Sharma et al., 1993; Paclik et al., 1996; Jaleel et al. 2009), closure of stomata (Rao et al. 1987), reduction in photosynthetic and transpiration rates (Kramer et al., 1995). Water deficiency also negatively affects cell growth, causing separation of membrane proteins (Jaleel et al. 2009) and changes in the physical organization of the membrane (McKersie et al. 1996). The important functions of water in plants can be summarized as the following (Kramer et al., 1995):

(1) Constituent: water qualitatively constitutes 70-90% of the fresh weight of most herbaceous plant tissue and over 50% of the fresh weight of woody plants.

(2) Solvent: water in plants acts as the solvent in which gases, minerals, and other

solutes can dissolve in and are transported to plant cells and tissues.

(3) Reactant: water is the raw material for photosynthesis and some hydrolytic processes in germinating seeds.

(4) Structural support: water provides the "turgor pressure" to maintain turgor which is essential for cell enlargement and growth.

(5) Evaporative cooling: leaf temperature is controlled by transpiration.

1.2.3.2 Water requirements and water deficiency effects

1.2.3.2.1 *Camelina Sativa* L. Crantz

Camelina is described as a promising crop with better tolerance of water deficits than flax and is better suited to drier regions (Putnam et al., 1993). Vollmann, et al. (1996) conducted a study to evaluate 32 camelina genotypes in regard to their agronomic performance in 1993-1994 in Australia. They found a decrease in seed yield when water stress occurred during the flowering stage; while sufficient rainfall during the seed filling period increased oil content. More recent work done by Hergert et al. (2011) in the United States reported that irrigation of camelina at rates from 185 to 500 mm resulted in seed yield increases from 560 to 2800 kg ha^{-1}; with a range from 202 mm to 517mm of cumulative water use, camelina yielded 582 and 2867 kg ha^{-1} seeds, respectively. A linear correlation between camelina seed yield and seasonal crop evapotranspiration (ET) was detected by Hunsaker et al. (2012) in an arid environment in Arizona in the United States and the highest yield occurred at about 470–490 mm of seasonal ET, which was considerably lower than those reported for traditional oilseed crops (such as soybean and sunflower) grown in the same region (Aiken et al. 2011).

In further research on camelina production potential, understanding its water requirement and responses to water deficiency are increasingly important. However, there is insufficient information in the literature regarding the physiological and growth responses to water availability. More detail and specific studies on camelina response to water deficiency are necessary in order for camelina to be grown economically.

1.2.3.2.2 Canola

Canola is an important oilseed crop and has been cultivated in many arid and semiarid areas throughout the world; as with all field crops, its physiological processes, yield production and yield components are significantly affected by water availability.

Data obtained by Kauser et al. (2006) revealed decreases in canola leaf chlorophyll A, carotenoids and quantum yield of photosystem II due to a water deficiency. Din et al. (2011) evaluated physiological responses of 5 canola varieties to drought stress and found a significant decrease in chlorophyll A & B content and an increases in proline (osmosis-regulating substance) accumulation among 4 *B. napus* genotypes under water deficit conditions. Decreases in water supply also resulted in a reduction in leaf osmotic potential (Kauser et al., 2006), lower stomatal conductance (g_s) and photosynthetic rate (Kauser et al., 2006; Qaderia et al., 2012; Shabani et al., 2013). A decreased ratio of photosynthesis rate to transpiration rate was also detected when leaf vapor pressure deficit increased (Shabani et al., 2013). The prolonged water stress also reduced the stem height, leaf number, leaf area and dry matter of individual organs and a whole plant (Qaderia et al., 2012).

In order to perform well, canola requires a considerable amount of water during multiple growth stages. For example, at the flowering stage, the ET of *B. rapa* is up to 8 mm per day and the crop is particularly sensitive to drought during pod elongation, as well as at seed germination (Carlsson et al., 2007). Failure of appropriate water supply can significantly impact canola growth and grain yield. Din et al. (2011) reported a greater reduction in grain yield when stress was imposed at flowering stage. The effect of water deficiency was more significant during the reproductive stage than during vegetative growth of rapeseed (Ghobadi et al., 2006).

Appropriate irrigation schedules can significantly increase the yield in many regions. Gilliland and Hang (2001) in the United States reported a significant yield increase in two canola cultivars under 5 irrigation schedules in spring. The study carried out by Seyedmohammadi et al.(2013) indicated that the highest seed yield, biological yield, harvest index, oil yield, total silique number, plant height, lateral branch number, silique length, 1000-seeds weight were produced with 6 days irrigation interval. However, Pouzet (1995) suggested that because water supplies are becoming limited, irrigation of rapeseed is not an economically efficient practice.

1.3 Statement of goals

1.3.1 Scope

The overall goal of this project are to use field and greenhouse trials and laboratory facilities to explore the potential of camelina as a new oilseed crop with unique and attractive properties that are not present in other field crops. The project was to provide a foundation for the understanding the integrated effects of soil water availability and N application on the oilseed cropping system. The results from this study contributed to the development of optimum strategies for managing water and N fertilizer that contribute to the high grain yield of camelina and canola.

1.3.2 Objectives

To achieve the overall goal, experiments were conducted with the following objectives:

(1) to evaluate the comparative physiological responses (photosynthesis, transpiration, and stomatal conductance) of camelina and canola in response to different levels of soil water potential and N availability (Chapter 2);

(2) to examine the comparative effects of water deficiency and N availability on camelina and canola growth (biomass, grain yield, oil and protein content in the seeds) (Chapter 3);

(3) to determine the comparative effects of applied N on grain yield, yield components, oil and protein contents and yields in canola and camelina seeds (Chapter 4).

Chapter 2: Comparison of camelina (*Camelina sativa* L. Crantz.) and canola (*Brassica napus* L.) physiological responses to water deficiency and applied nitrogen under controlled environments

2.1 Introduction

Evaluating crop physiological status can indicate the productivity and adaptability to environmental stress (Chapin, 1991; Colombo & Parker, 1999). Water availability in soil is one of the most important abiotic limiting factors that affects crop performance (Deng et al., 2004; Micheletto et al., 2007). A reduction in soil water availability to a growing plant immediately affects its metabolic processes and physiological functions, such as stomatal closure, leading to a decrease in photosynthetic carbon assimilation and transpiration (Rao et al. 1987), and a decrease in chlorophyll content (Jaleel et al. 2009). Paclik et al. (1996) and Sharma et al. (1993) reported that chlorophyll A + B content in *Brassica napus* cultivars was reduced by 38% due to water stress, compared with adequately watered plants. Drought stress also negatively affected cell growth, causing separation of membrane proteins (Jaleel et al. 2009) and changes in the physical organization of the membrane (McKersie et al. 1996). For Brassica crops, water deficiency accelerates the process of flowering and fruit drop, thus decreasing seed yield (Gan, et al. 2004; Sinaki, et al. 2007).

Nitrogen (N) fertilizer plays a vital role in increasing non-legume crop growth and grain yield. To maximize the productivity of crops, N must be supplied adequately (Miller et al. 2001). In oilseed production, N accounts for the largest energy input in regard to fertilizer supply (Gan et al. 2008). Nitrogen increases growth and yield by influencing a series of metabolic processes and physiological parameters, such as stomatal conductance (g_s), photosynthesis (*P*n) and transpiration (*E*) and chlorophyll content. For example, the stomatal conductance in beans (*Phaseolus vulgaris* L.), coffee (*Coffea arabica* L.) and winter wheat (*Triticum aestivum* L.) increased with N availability under well-watered conditions (Shimshi, 1970; Tesha & Kumar, 1978; Shangguan, 1997). Sugiharto et al. (1990) found that plant photosynthetic capacity was positively correlated with N concentration in leaves. Yoshida (1972) reported that the functions of N were to maintain photosynthetic activity during grain fill stage. A low photosynthetic rate under limited N availability conditions indeed resulted in decreases in chlorophyll content and protein synthesis (Cechin et al. 2004).

Historically, the vast majority of studies on oilseed crops were concerned with plant physiological responses to single factorial effects of either water deficiency or N availability. Physiological responses of a wide range of crops to either water deficiency or N availability have been well documented. However, the influence of water and N on plant physiological processes often interact. The interaction of water deficiency and N rates on the physiology of crops, especially for oilseed crops, has received relatively little attention. Understanding interactions of drought stress and N availability on plant growth could assist farmers to better manage the input resources and improve crop production with greater environmental sustainability.

As a renewed high value oilseed crop in Canada, with favorable agronomic traits and specialty oil characteristics (Zubr, 1997; Gugel & Falk, 2006), there is little information available in the literature regarding the responses to the interactive effects of water and N supply on the physiological traits of camelina in comparison with canola. With such considerations, a greenhouse experiment was designed with these two crop species and 6 nitrogen rates under three water availability conditions.

The objectives of the present study were to clarify the physiological responses including chlorophyll content index (CCI), photosynthesis (*P*n), transpiration (*E*) and stomatal conductance (g_s) of the two oilseed crops under different combinations of soil water potential and applied N rates in controlled environments.

2.2 Methods and Materials

2.2.1 Experimental design

A greenhouse experiment was carried out as split-plot factorial with main plots arranged as a randomized complete block design with 6 replications. Three different levels of soil water availability (saturation, -60 cbar and -120 cbar in soil water potential) were the factors for the main plot and six N rates applied at 0, 25, 75, 125, 175 and 225 kg N ha^{-1} and two crop species (canola and camelina) were assigned to each of the subplots.

2.2.2 Crop selection

The canola hybrid "InVigor 5440" is highly consistent, with excellent yield potential and standability. In the 2005-2006 official WCC/RRC public co-op trials, it yielded 135% of the check varieties (46A65 and Q2) and contained higher oil than the checks. With medium plant height, it is an easily harvested high quality canola crop (Bayer Crop Science Canada, 2014). Camelina-CDI007 is a promising genotype of *Camelina sativa* L. Crantz line. With the highest yield potential, lowest glucosinolate content and the highest disease resistance, camelina-CDI007 shows good potential for cultivation in arid and cool environments.

2.2.3 Plant culture and growth conditions

Pro-Mix (PRO-MIX BX MYCORRHIZAE™, Premier Tech Horticulture) was used as the growth medium in 6’ standard pots (14.0cm height and 15.0cm diameter). The characteristics of the growth medium are described in Table 2.1. Ten canola and camelina seeds were planted per pot at about 0.5 cm depth. When plants reached the 4-true-leaf stage, canola were thinned to 2 equidistant plants in each pot and camelina were thinned to 3 plants per pot. This was based on the optimum plant stand to achieve maximum seed yield (Brown, et al. 2008; Urbaniak, et al. 2008; Zubr, 1997). Greenhouse conditions were as follows: 16-hour photoperiod, with a mean day/night temperature of 22/18 °C. The light was supplied by HID- high pressure sodium lamps. The light intensity was 600 µE/m2/sec photosynthetic active radiations (PAR). Relative humidity was maintained at 70 % ± 5 %. When seeds in the top pods turned brown or black for canola and brown for camelina, the crops were harvested. Harvesting was done by hand.

Table 2.1: Characteristics of growth medium

N	P	K	Ca	Mg	pH
35ppm	25ppm	125ppm	88ppm	33ppm	5.5
Conductivity (mmho s^{-1}) 0.9					
water-holding capacity: 60-75% by volume					

Table 2.2: Description of experimental management information in 2013 and 2014

Year		**2013**	**2014**
Seeding date		10 Nov.	08 Apr.
1st 50% of N application		15 DAP	14 DAP
2nd 50% of N application		30 DAP (camelina)	32 DAP (camelina)
		39 DAP (canola)	39 DAP (canola)
Water deficiency imposition		29 DAP	29 DAP
Harvesting	**Biomass**	70 DAP (camelina)	70 DAP (camelina)
		80 DAP (canola)	82 DAP (canola)
	Grain	90 DAP (camelina)	92 DAP (camelina)
		105 DAP (canola)	106 DAP (canola)

*DAP: days after planting;
*Due to different growing-day requirements, camelina and canola were harvested on different DAP.

2.2.4 Nitrogen application

Nitrogen was applied in the form of ammonium nitrate (NH_4NO_3) at rates of 0, 0.126, 0.379, 0.632 and 0.885 g N pot^{-1}, corresponding to 0, 25, 75, 125, 175 and 225 kg N ha^{-1}, respectively. Ammonium nitrate was dissolved in 100mL of distilled water. This solution was applied to each treatment. The checks received 100 mL of distilled water. Nitrogen was applied in two doses: 50% applied at the 4-true-leaf stage and the other half at the early flowering period. Since there were different growing stages for the two oilseed crops, the second 50% of N was applied to the two crops on different days. The experimental management is described in Table 2.2. Potassium (K), phosphor (P) and other nutrients were supplied by the growth medium.

2.2.5 Water deficiency imposition

Water deficiency was imposed 30 days after planting when plants were at the early stem elongation stage. The effects of three different soil water potentials (saturation, -60 cbar and -120 cbar) were evaluated in this study. A cyclical water deficiency method was used to simulate a realistic drought response. Soil water deficit was gradually imposed by withholding water. This allowed soil water potentials to drop to target levels. Once the target soil water potentials were achieved, water was added to soil until it was saturated. Control pots received water daily to maintain soil in saturation. Soil moisture potential was measured daily by using *Watermark* soil moisture sensors (Spectrum Technologies, IL, USA).

2.3 Measurements

2.3.1 Chlorophyll content index

Chlorophyll content index (CCI) in plant leaves from each treatment were measured 7 days after water deficiency imposition by using a SPAD 502 Chlorophyll Meter. Six measurements were taken in each plot (2 leaves per camelina plant*3 plants per pot and 3 leaves per canola plant* 2 plants per pot). The CCI for each treatment in every replication was determined, based on the average reading of 6 measurements.

2.3.2 Gas exchange measurements

Photosynthesis (*P*n), stomatal conductance (g_s), and transpiration (*E*) were measured by an LCi Portable Photosynthesis System (ADC BioScientific Ltd.). The sixth fully expanded leaf from a branch tip of two plants of each treatment was selected for measurements. All parameters were measured from 12:00 pm to 2:00 pm, under a variety of weather conditions, when the target water potentials were reached.

2.3.3 Instantaneous water use efficiency (WUE*i*)

WUEi =Photosynthesis rate (*P*n)/ Transpiration rates (*E*) (Eamus, 1991)

2.4 Statistical analysis

Minitab 17 Statistical Software (Minitab Inc., USA, 2013) was used in the analysis of data. The General linear model (GLM) was employed to examine the effects of different soil water potential and applied N rates on physiological processes of camelina and canola. The significant level was set to be 0.05.

2.5 Results

2.5.1 Chlorophyll content index

The result of analysis of variance (Table 2.3) showed that a three-way interaction of crop species, water deficiency and applied N significantly affected chlorophyll content index (CCI).

Canola showed a higher CCI than camelina under each treatment except for controls. Chlorophyll content index of camelina averaged at 48.08 in controls and it decreased to 44.67 as water deficiency developed (Table 2.4). A significant difference was found when soil water potential was dropped to -120 cbar. However, water deficiency did not markedly affect the CCI of canola (Figure 2.1). The chlorophyll content index of canola at each water deficit level averaged at 49.62, 48.02 and 47.57, respectively.

Under well-watered condition, the chlorophyll content index of camelina ranged from 42.3 to 51.2 with no-additional N and 125 kg N ha^{-1} applications, respectively. A significant difference on the CCI of camelina was not observed among the applied N rates ranging from 75 to 225 kg ha^{-1} (Table 2.4). The CCI of camelina under water deficit conditions (-60 cbar and -120 cbar) peaked at 49.6 and 46.5, respectively, with an N application of 225 kg ha^{-1} but a significant difference was not found among all N treatments at each water deficit level (Table 2.4).

A strong correlation between applied N and the CCI of canola was found, with the value of coefficient determination over 98 % in all water deficit treatments (Figure 2.2). The highest CCI of canola under each water deficit treatment was 55.8, 53.9 and 53.4 respectively, at applied N rate of 225 kg N ha^{-1}. However, these values were not significantly different from those at applied N rate of 125 kg N ha^{-1} (Table 2.4).

The effects of water deficiencies and applied N on the CCI indicated that chlorophyll content in canola leaves was more sensitive to N availability and compared with camelina, a higher N rate was required by canola to reach its plateau. Water deficiency had a greater effect on chlorophyll content in camelina leaves than that in canola leaves.

Table 2.3: ANOVA table of the CCI of camelina and canola as influenced by water deficiency and applied N

Effects	F-Value	P-Value
Species (S)	52.95	0.000
Nitrogen (N)	228.07	0.000
Water deficiency (W)	49.32	0.000
S*W	49.64	0.000
S*N	8.87	0.000
W*N	2.52	0.007
S*W*N	**1.91**	**0.045**

*1. Error term used in main plot factor was W;
F test was Replication (Reps.)*W.
*2. Error term used in sub plot factors were S*N;
F test was Reps.*S*N*W+ Reps*S*N+ Reps. * S+ Reps.*N.

Table 2.4: Effects of applied N and water deficiency on the CCI of camelina and canola

Species	Applied N (kg ha^{-1})	Chlorophyll content index		
		Control	-60 cbar	-120 cbar
Camelina	**0**	42.3 m-o	43.5 lm	42.4 m-o
	25	45.3 i-m	47.4 g-k	43.2 l-n
	75	48.3 f-i	47.6 f-j	45.9 h-m
	125	51.2 b-f	48.6 e-i	45.6 i-m
	175	50.8 c-g	48.1 f-i	44.4 j-m
	225	50.6 c-g	49.6 d-h	46.5 h-l
	Mean	48.08	47.47	44.67
Canola	**0**	39.8 n-p	38.9 op	38.1 p
	25	43.8 k-m	42.4 m-o	43.0 l-n
	75	50.8 c-g	49.0 d-i	47.9 f-j
	125	52.6 a-d	51.2 b-f	50.7 c-g
	175	54.9 ab	52.7 a-d	52.3 a-e
	225	55.8 a	53.9 a-c	53.4 a-c
	Mean	49.62	48.02	47.57

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

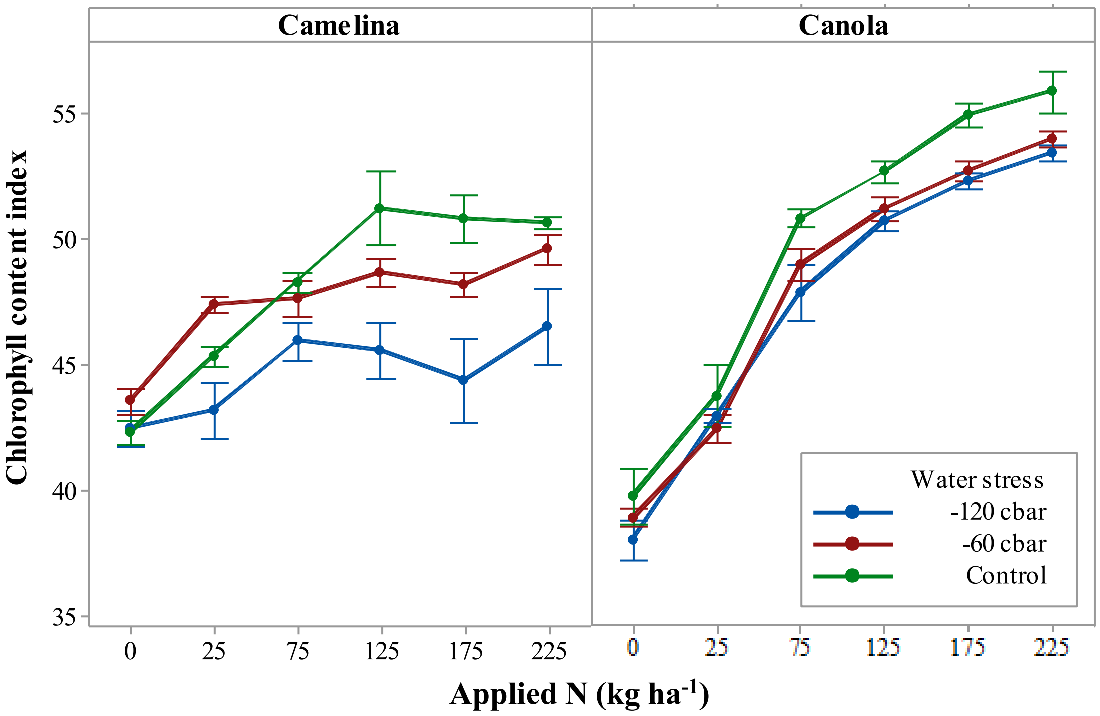
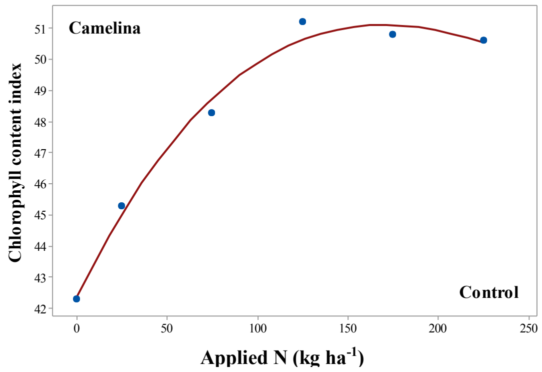
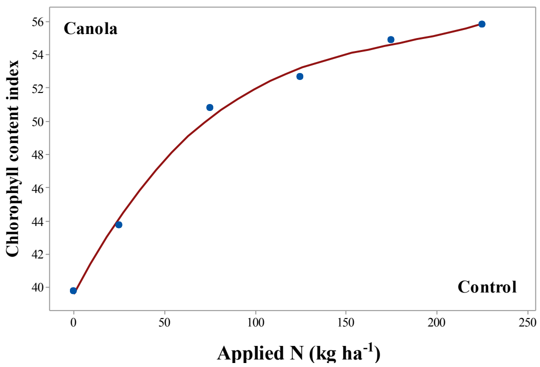
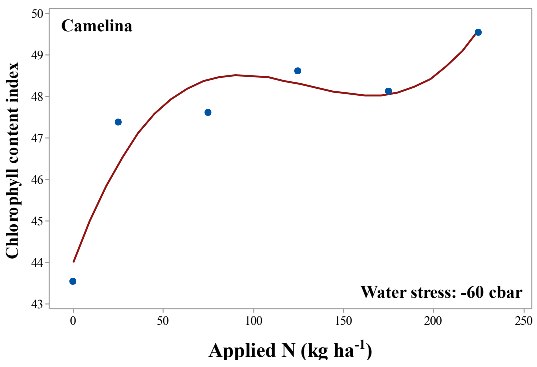
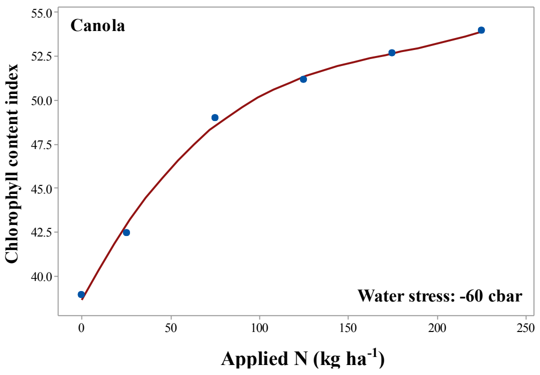


Figure 2.1: Effects of water deficiency and applied N on the CCI of canola and camelina (Bars are one standard error from the mean)









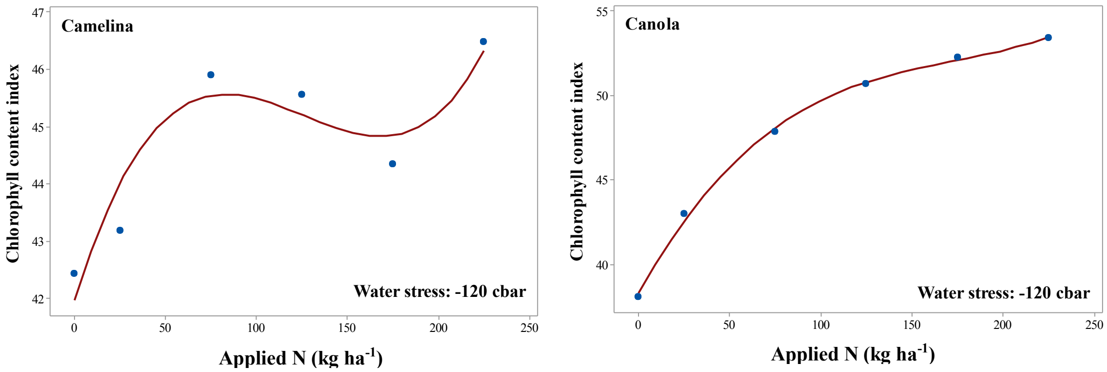


Figure 2.2 Regression analysis of N on chlorophyll content index of camelina and canola under different water deficit levels

Y (Camelina, Control) = 42.37 + 0.1182 N - 0.000482 N*2 + 0.000001 N**3 with R-Sq (adj) = 97.2%
Y (Camelina, -60 cbar) = 43.99 + 0.1198 N - 0.001007 N*2 + 0.000003 N**3 with R-Sq (adj) = 78.1%
Y (Camelina, -120 cbar) = 41.97 + 0.1034 N - 0.000928 N*2 + 0.000002 N**3 with R-Sq (adj) =72.3%
Y (Canola, Control) = 39.58 + 0.2084 N - 0.001036 N*2 + 0.000002 N**3 with R-Sq (adj) =98.9%
Y (Canola, -60 cbar) = 38.66 + 0.1939 N - 0.000965 N*2 + 0.000002 N**3 with R-Sq (adj) = 99.3%
Y (Canola, -120 cbar) = 38.27 + 0.1952 N - 0.001002 N*2 + 0.000002 N**3 with R-Sq (adj) = 99.6%

2.5.2 Photosynthesis

A three-way interaction of crop species, water deficiency and applied N significantly affected plant photosynthetic rate (*P*n) (Table 2.5).

Under well-watered conditions, the photosynthetic rate of canola was 10.37 μmol m^{-2} s^{-1} on average, which was higher than that of camelina (8.58 μmol m^{-2} s^{-1}) under the same situation (Table 2.6). However, the inverse was found under the significant influence of a water deficiency (Figure 2.3). The photosynthetic rates of camelina were 7.33 and 5.51μmol m^{-2} s^{-1} for each water deficit treatment (-60 cbar and -120 cbar), respectively. These values were higher than 6.41 and 4.70μmol m^{-2} s^{-1} for canola under moderate (-60 cbar) and severe (-120 cbar) water deficit conditions (Table 2.6).

The significance of applied N to the photosynthesis of camelina and canola was found but the effects differed among water deficit levels and crop species. The *P*n of camelina plants rose with an increase in applied N at all levels of water deficiency (Figure 2.3). The highest *P*n of camelina in controls, moderate (-60 cbar) and severe (-120 cbar) water deficiency was 9.65, 8.48 and 7.23µmol m^{-2} s^{-1}, respectively (Table 2.6). A significant difference of N effect on *P*n of camelina was not found when applied N was over 125kg ha^{-1} under well-watered condition and moderate (-60 cbar) water deficit condition (Table 2.6). Under severe (-120 cbar) water deficiency, a plateau was reached at applied N rate of 175 kg ha^{-1} (Figure 2.3).

In canola plants, N supply only contributed to the *P*n when adequate water was applied (Table 2.6 & Figure 2.3). In controls, the *P*n of canola peaked at 13.18 µmol m^{-2} s^{-1} with 225 kg N ha^{-1} application but it did not significantly differed from that at applied N rate of 175 kg ha^{-1} (Table 2.6). Under water deficit conditions, canola plants reduced their *P*n when N rates were elevated (Figure 2.3). A significant decrease in *P*n of canola was observed when N was over 175 kg ha^{-1} under moderate (-60 cabr) deficiency and 75 kg ha^{-1} under severe deficiency (Table 2.6).

The effects of water deficiency and applied N on the *P*n of camelina and canola indicated that the *P*n of canola was more sensitive to water deficiency and applied N than that of camelina. Camelina required less water and N than canola to maintain the *P*n at acceptable level.

Table 2.5: ANOVA table of photosynthesis of camelina and canola as influenced by water deficiency and applied N

Effects	F-Value	P-Value
Species (S)	0.13	0.714
Nitrogen (N)	2455.05	0.000
Water deficiency (W)	129.36	0.000
S*W	296.69	0.000
S*N	59.94	0.000
W*N	80.28	0.000
S*W*N	**84.74**	**0.000**

*1. Error term used in main plot factor was W;
F test was Replication (Reps.)*W.

*2. Error term used in sub plot factors were S*N;
F test was Reps.*S*N*W+ Reps*S*N+ Reps. * S+ Reps.*N.

Table 2.6: Effects of applied N and water deficiency on Pn of camelina and canola

Species	**Applied N (kg ha^{-1})**	***P*n (µmol m^{-2} s^{-1})**		
		Control	**-60 cbar**	**-120 cbar**
Camelina	**0**	6.74 h-l	5.87 m-o	3.99 r
	25	7.38 g-i	6.35 k-m	4.41 qr
	75	8.62 de	6.81 h-k	4.98 pq
	125	9.61 c	8.16 e-g	5.93 l-o
	175	9.65 c	8.48 de	6.49 j-m
	225	9.51 c	8.32 d-f	7.23 h-j
	Mean	8.58	7.33	5.51
Canola	**0**	7.52 f-h	6.62 i-m	6.26 k-m
	25	8.26 d-f	6.78 h-k	5.85 m-o
	75	9.04 cd	6.91 h-k	5.38 n-p
	125	11.58 b	6.57 i-m	4.13 r
	175	12.63 a	6.2 k-n	3.74 r
	225	13.18 a	5.37 op	2.85 s
	Mean	10.37	6.41	4.70

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

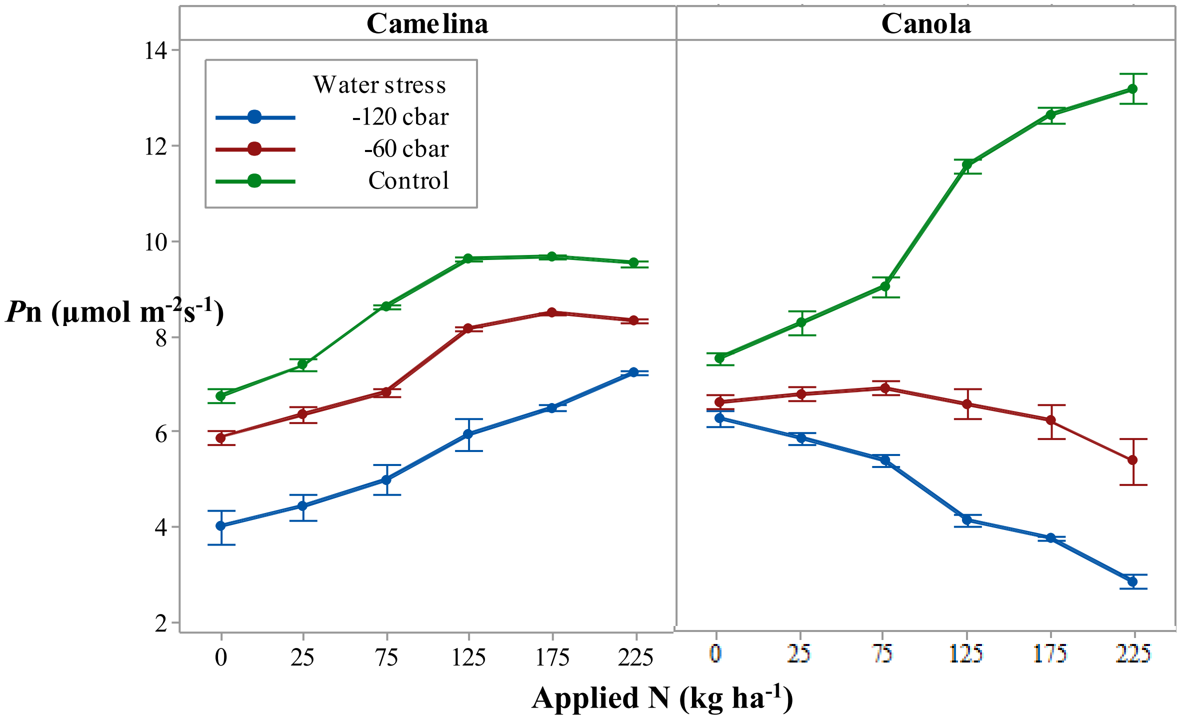


Figure 2.3: Photosynthetic rates of camelina and canola affected by water deficiency and applied N (Bars are one standard error from the mean)

2.5.3 Transpiration

A three-way interaction of crop species, water deficiency and applied N had significant effect on transpiration rate (*E*) of camelina and canola (Table 2.7).

Water deficiency significantly decreased transpiration rate of canola in all N treatment, however, a significant difference on camelina was only observed when applied N was over 125 kg ha^{-1} (Table 2.8 & Figure 2.4). Under water non-limiting conditions, the *E* of canola averaged at 3.75 mmol m^{-2} s^{-1}, which was higher than 2.98 mmol m^{-2} s^{-1} on average for camelina in the controls (Table 2.8). In contrast, the *E* of camelina under moderate (-60 cbar) and severe (-120 cbar) water deficiencies were 2.51 and 1.84 mmol m^{-2} s^{-1}, respectively, which were higher than those of canola (2.12 and 1.56 mmol m^{-2} s^{-1}) under water deficit conditions (Table 2.8).

With adequate water supplied, the *E* of camelina rose to 3.49 mmol m^{-2} s^{-1} with N increase to 175kg ha^{-1} (Table 2.8). Under moderate (-60 cbar) water deficiency, the *E* value of camelina slightly increased from 2.03 to 2.75 mmol m^{-2} s^{-1} with an increase in applied N from 0 to 175 kg ha^{-1}. Under severe (-120 cbar) water deficiency, the *E* value plateaued with applied N rate of 75 kg ha^{-1} (Table 2.8).

A positive effect of applied N on canola transpiration was found under well-watered conditions (Figure 2.4). Under this condition, the *E* value of canola rose to 4.69 mmol m^{-2} s^{-1} with 225 kg N ha^{-1} applied, but a significant difference was not found when N was applied at the rate of 175 kg ha^{-1} (Table 2.8). A negative correlation between water deficiency and *E* of canola was observed under water deficit conditions (Figure 2.4). A significant reduction in *E* of canola occurred at applied N rate of 175 and 75 kg ha^{-1} at moderate (-60 cbar) and severe (-120 cbar) water deficit levels, respectively (Table 2.8).

Table 2.7: ANOVA table of transpiration rates of camelina and canola

Effects	**F-Value**	**P-Value**
Species (S)	2.40	0.122
Water deficiency (W)	1822.40	0.000
Nitrogen (N)	29.23	0.000
S*W	262.15	0.000
S*N	30.62	0.000
W*N	79.24	0.000
S*W*N	**35.22**	**0.000**

*1. Error term used in main plot factor was W;
F test was Replication (Reps.)*W.
*2. Error term used in sub plot factors were S*N;
F test was Reps.*S*N*W+ Reps*S*N+ Reps. * S+ Reps.*N.

Table 2.8: Effects of applied N and water deficiency on E of camelina and canola

Species	Applied N (kg ha^{-1})	*E* (mmol m^{-2} s^{-1})		
		Control	**-60 cbar**	**-120 cbar**
Camelina	**0**	2.39 f-j	2.03 j-m	1.46 p-r
	25	2.61 e-h	2.49 e-i	1.66 n-q
	75	2.84 de	2.68 e-h	1.80 m-p
	125	3.09 d	2.75 d-f	1.99 k-o
	175	3.49 c	2.7 d-g	2.14 i-m
	225	3.46 c	2.34 h-l	2.03 j-n
	Mean	2.98	2.51	1.84
Canola	**0**	2.77 de	2.39 f-j	2.20 i-l
	25	3.07 d	2.35 h-k	2.22 i-l
	75	3.48 c	2.37 g-j	1.62 o-q
	125	4.07 b	2.11j-m	1.30 qr
	175	4.40 ab	1.98 l-o	1.16 rs
	225	4.69 a	1.55 pq	0.89 s
	Mean	3.75	2.12	1.56

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

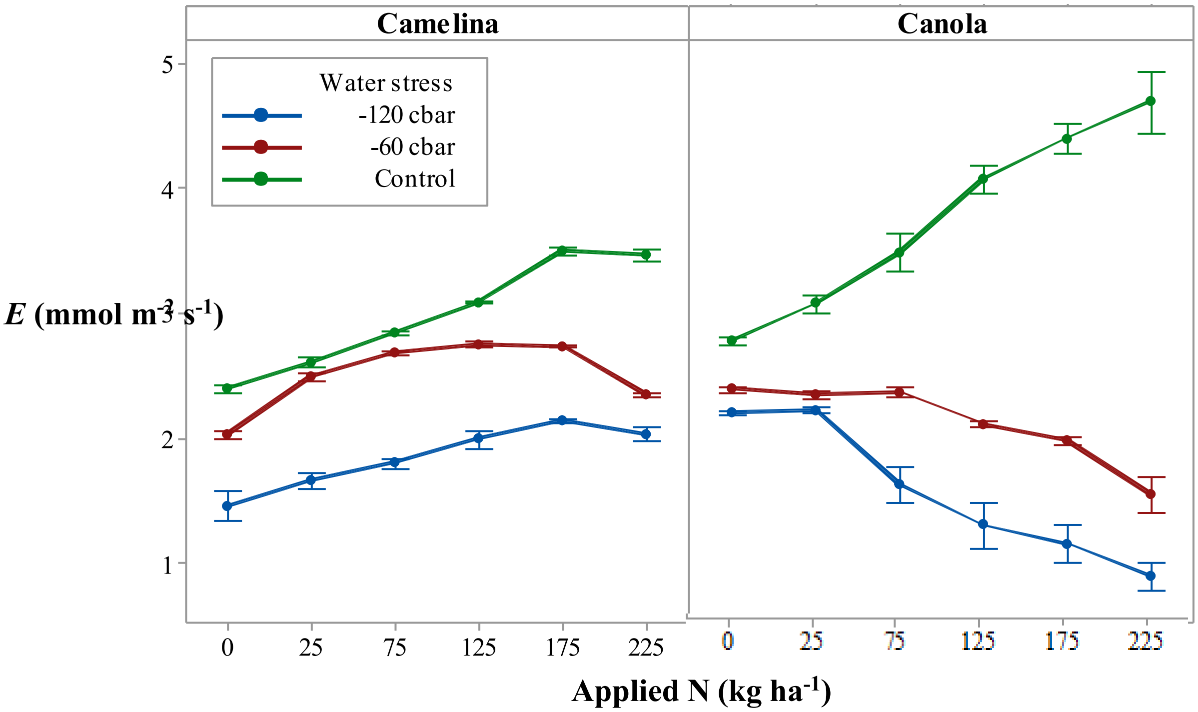


Figure 2.4: Transpiration rates of camelina and canola responses to water deficiency and applied N (Bars are one standard error from the mean)

2.5.4 Stomatal conductance

A three-way interaction of crop species, water deficiency and N availability significantly influenced stomatal conductance (g_s) (Table 2.9).

Water deficiency induced camelina stomatal closure, but different water levels did not make a significant difference in the closure of stomata (Table 2.10 & Figure 2.9). An increase in applied N rate enhanced stomatal conductance and a significant effect was observed under well-watered conditions at an applied N rate of 125 kg ha^{-1} (Table 2.10). Under limited soil moisture conditions, the g_s of camelina leveled out at applied N rate of 75 kg ha^{-1} (Table 2.10)

The applied N had a positive effect on g_s of canola when adequate water was supplied. The highest g_s value (0.27 mol m^{-2} s^{-1}) was recorded with 225 kg N ha^{-1} applied (Table 2.10) and a significant difference was detected until applied N rate decreased to 125 kg ha^{-1}. As water deficiency developed, applied N negatively affected stomatal conductance and at high N rates resulted in stomatal closure in canola plants (Figure 2.5). Approximately 50% and 60 % of stomatal closure were attributable to high N rates under moderate (-60 cbar) and severe (-120 cbar) water deficit conditions, respectively (Table 2.10). This indicated that soil water availability was the primary factor and the applied N would restrict gas exchange in canola leaves.

Table 2.9: ANOVA table of stomatal conductance of camelina and canola

Effects	F-Value	P-Value
Species (S)	137.22	0.000
Water deficiency (W)	389.19	0.000
Nitrogen (N)	19.80	0.000
S*W	114.67	0.000
S*N	36.66	0.000
W*N	21.80	0.000
S*W*N	**9.34**	**0.000**

*1. Error term used in main plot factor was W;
F test was Replication (Reps.)*W.
*2. Error term used in sub plot factors were S*N;
F test was Reps.*S*N*W+ Reps*S*N+ Reps. * S+ Reps.*N.

Table 2.10: Effects of applied N and water deficiency on g_s of camelina and canola

Species	Applied N (kg ha^{-1})	g_s (mol m^{-2} s^{-1})		
		Control	-60 cbar	-120 cbar
Camelina	**0**	0.04 l-p	0.04 n-p	0.03 op
	25	0.07 h-o	0.04 l-p	0.05 l-p
	75	0.11 f-j	0.08 g-m	0.07 i-o
	125	0.15 c-f	0.10 g-k	0.09 g-l
	175	0.16 c-e	0.11 e-i	0.10 g-j
	225	0.16 cd	0.10 g-k	0.11 f-j
	Mean	0.11	0.09	0.07
Canola	**0**	0.16 c-e	0.12 d-h	0.11 f-j
	25	0.17 c	0.16 d-i	0.11 f-j
	75	0.19 bc	0.12 d-g	0.08 g-n
	125	0.23 ab	0.08 g-m	0.05 k-p
	175	0.26 a	0.06 j-o	0.04 l-p
	225	0.27 a	0.04 m-p	0.02 p
	Mean	0.21	0.09	0.07

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

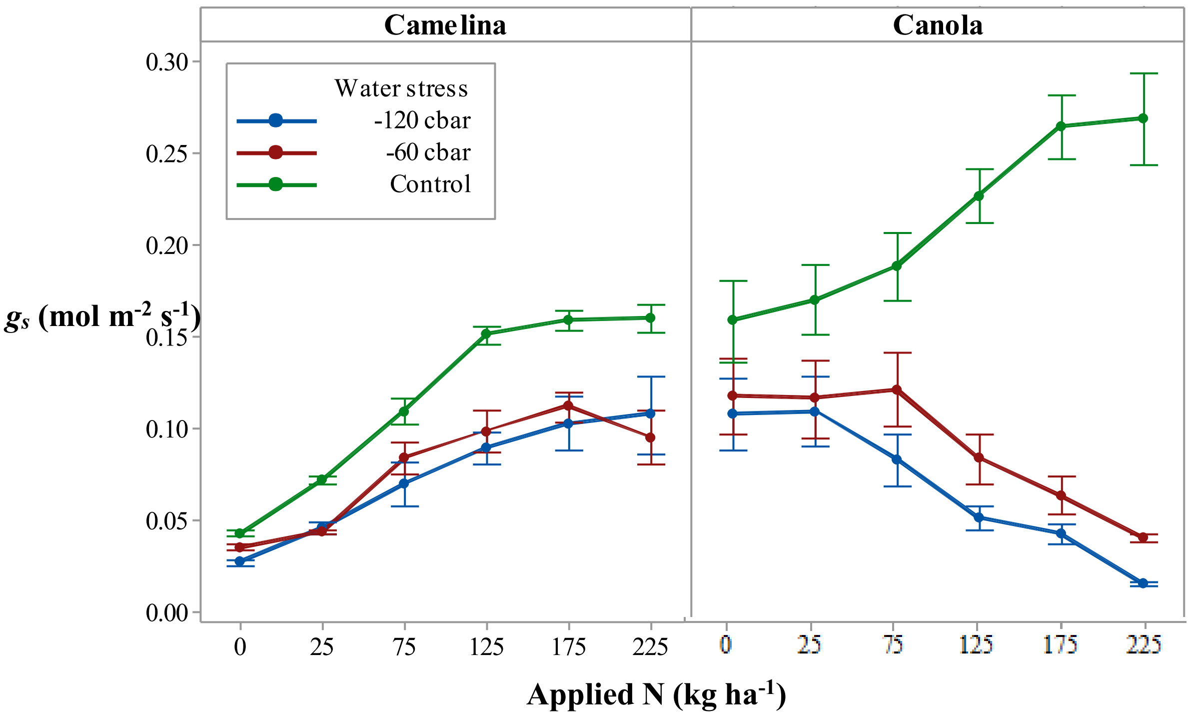


Figure 2.5: Stomata conductance of camelina and canola responses to water deficiency and N (Bars are one standard error from the mean)

2.5.5 Instantaneous water use efficiency

A three-way interaction of species, water deficiency and N significantly affected instantaneous water use efficiency (WUE*i*) (Table 2.11).

Canola and camelina plants under severe water deficit conditions showed the highest WUE*i*, while the lowest WUE*i* value was recorded in the control canola plants (Table 2.12 & Figure 2.9). Under well-watered conditions, the WUE*i* of camelina was greater than that of canola (Table 2.12). Water deficiency, however, increased WUE*i* of both species and canola showed higher WUE*i* than camelina under well-watered conditions (Table 2.12).

The WUE*i* of camelina was positively correlated with applied N up to 125 kg N ha^{-1} application under controlled conditions and N rates that were greater than 125 kg ha^{-1} negatively affected WUE*i* (Figure 2.6). A significant difference in WUE*i* was not observed in all N treatments in the control. In contrast, under water deficit conditions (-60 cbar and -120 cbar), a significant increase in the WUE*i* of camelina occurred at the applied N rate of 225 kg ha^{-1} (Figure 2.6).

Nitrogen application significantly increased WUE*i* of canola plants under severe deficit conditions (-120 cbar) (Table 2.12 & Figure 2.6). The highest WUE*i* of canola under severe deficiency (-120 cbar) was observed when the N rate was 175 kg ha^{-1}. A significant effect of applied N on WUE*i* of canola under moisture non-limiting conditions and -60 cbar soil water potential was not observed (Table 2.12 & Figure 2.6).

Table 2.11: ANOVA table of WUE*i* of camelina and canola

Factor	F-Value	P-Value
Species (S)	1.64	0.202
Nitrogen (N)	9.95	0.000
Water deficiency (W)	7.19	0.001
S*W	3.73	0.025
S*N	7.91	0.000
W*N	6.66	0.000
S*W*N	**9.09**	**0.000**

*1. Error term used in main plot factor was W;
F test was Replication (Reps.)*W.
*2. Error term used in sub plot factors were S*N;
F test was Reps.*S*N*W+ Reps*S*N+ Reps. * S+ Reps.*N.

Table 2.12: Effects of applied N and water deficiency on WUE*i* by camelina and canola

Species	Applied N (kg ha^{-1})	WUE*i**10^{-3}		
		Control	-60 cbar	-120 cbar
Camelina	**0**	2.83 d-h	2.89 c-h	2.71 h
	25	2.84 d-h	2.56 h	2.63 h
	75	3.03 c-h	2.54 h	2.75 gh
	125	3.11 b-h	2.97 c-h	2.97 c-h
	175	2.76 fgh	3.10 b-h	3.04 c-h
	225	2.76 fgh	3.55 a-e	3.60 a-d
	Mean	2.89	2.93	2.95
Canola	**0**	2.71 h	2.77 e-h	2.84 d-h
	25	2.69 h	2.89 c-h	2.64 h
	75	2.62 h	2.91 c-h	3.54 a-f
	125	2.86 d-h	3.10 b-h	3.88 ab
	175	2.88 c-h	3.12 b-h	4.03 a
	225	2.86 d-h	3.50 a-g	3.66 abc
	Mean	2.77	3.05	3.43

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

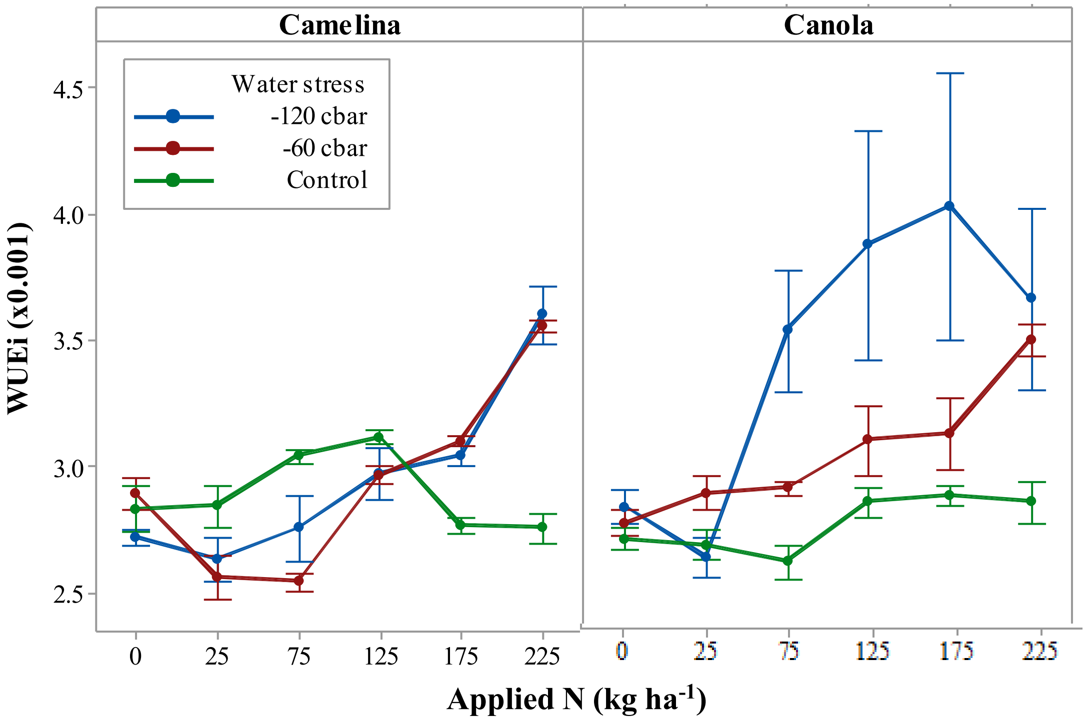


Figure 2.6: WUE*i* of camelina and canola under different water deficiency and N (Bars are one standard error from the mean)

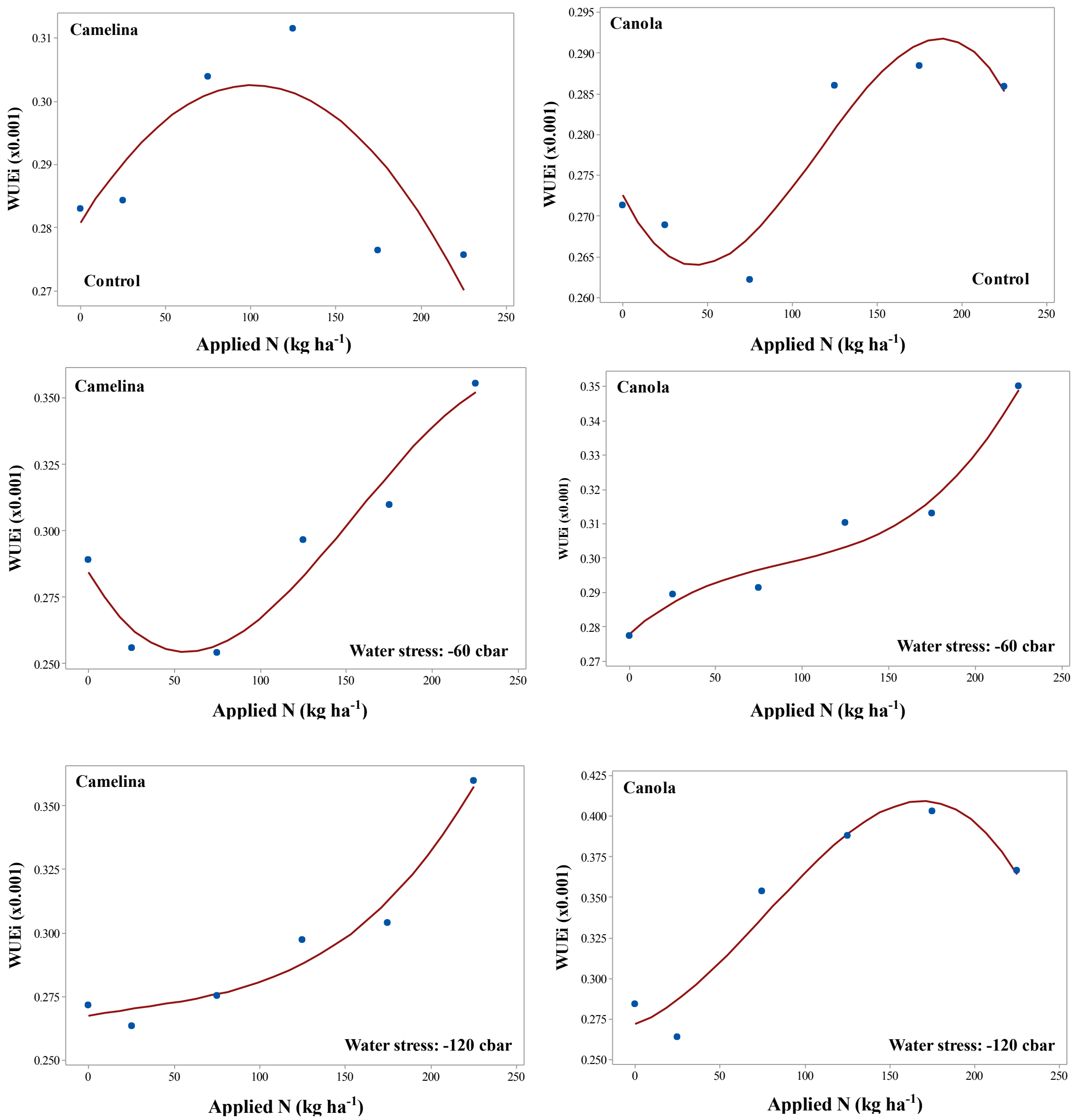


Figure 2.7: Regression analysis of N on water use effciency of camelina and canola under different water deficit levels

Y (Camelina, 0 cbar) = 0.2808 + 0.000430 N - 0.000002 N*2 with R-sq (adj) = 41.3%
Y (Camelina, -60 cbar) = 0.2843 - 0.001148 N + 0.000012 N*2 - 0.000001 N**3 with R-sq (adj) = 85.7%
Y (Camelina, -120 cbar) = 0.2673 + 0.000128 N - 0.000001 N*2 + 0.000001 N**3 with R-sq (adj) = 90.5%
Y (Canola, 0 cbar) = 0.2726 - 0.000439 N + 0.000006 N*2 - 0.000001 N**3 with R-sq (adj) = 67.5%
Y (Canola, -60 cbar) = 0.2779 + 0.000450 N - 0.000004 N*2 + 0.000001 N**3 with R-sq (adj) = 92.3%
Y (Canola, -120 cbar) = 0.2722 + 0.000367 N + 0.000010 N*2 - 0.000001 N**3 with R-sq (adj) = 84.8%

2.6 Discussion

2.6.1 Chlorophyll content

In the present study, water deficiency reduced chlorophyll content in camelina leaves. Previous studies (Paclik et al. 1996; Sharma et al. 1993) have shown a reduction of chlorophyll content in Brassica species as water deficiency developed. However, chlorophyll content in canola was not affected by water deficiency but positively correlated with N in this study. This indicates a closer association between canola leaf chlorophyll concentration and canola leaf N content, compared with camelina. This could be explained by the findings of Peterson et al. (1993), who found that the majority of leaf N is contained in chlorophyll molecules. Other studies also noted a decrease in chlorophyll content due to chlorophyll decomposition in plant leaves when N was deficit (Kowalczyk-Jusko & Koscik, 2002; Shaahan et al. 1999). The changes in chlorophyll content in crops' leaves were considered as possible reasons for the reduction of *P*n in this study. It was reported that low chlorophyll content directly limits photosynthetic potential (Richardson et al., 2002). Leaf chlorophyll content is a good indicator of photosynthetic activity (Naumann et al., 2008). However, although canola and camelina were under water deficit conditions, the CCI of each crop was greater than 40 in all water deficit treatments. According to Netto et al. (2005), this value indicated a great capacity to maintain the leaf green to conserve the photosynthetic pigments under water deficit conditions. Therefore, the change in chlorophyll content was not the main factor that caused the changes of *P*n.

2.6.2 Photosynthetic parameters (*P*n, *E* and g_s) and WUE*i*

The results in this study demonstrated that water deficiency decreased photosynthetic rate and transpiration rates in both camelina and canola. This was mainly attributed to stomatal closure. Stomata located on the leaves plays an important role in controlling O_2, water vapor and CO_2 flows into and out of the leaves (Kim et al. 2010), thus determining both the rates of net photosynthesis and transpiration (Condon et al. 2004). Ni and Pallardy, (1991) suggested that changes in the gas exchange characteristics could potentially be used as a screening indicator of drought tolerance in plants. Porporato et al. (2003) found that early closure of stomata was caused by "dry soil" and if the stomata close, gas exchange is immediately interrupted and then, stops. This process is an important barrier for water and gas loss (Mittler, 2006), leading to the reduction of photosynthesis and transpiration rates. The results in this study showed a significant reduction in photosynthetic rate and transpiration of canola and camelina and the relative decline of canola was greater than that of camelina. These observations indicated that photosynthetic parameters of canola were more sensitive to water deficiency than camelina. The plants overcome water deficiency by reducing the transpiration rate due to the partial closure of stomata to reduce water vapor. This is a mechanism to prevent water loss and maintain water balance (Aina et al., 2007). However, the data in this study suggested that this mechanism would also reduce canola photosynthesis and eventually seed yield would decrease if water deficiency was more sever. The photosynthetic parameters demonstrated that camelina still maintained higher photosynthetic and transpiration rates (Table 2.6 &Table 2.8) than canola under water deficit conditions, suggesting higher stomatal control efficiency. This was considered to be a strong adaptive mechanism to water deficit (Silva et al., 2013).

Water use efficiency corresponds with the performance of a crop growing under any environmental constraints. At the leaf level, instantaneous water use efficiency (WUE*i*) is defined as the ratio of photosynthetic rate (Pn) to transpiration rate. WUE*i* is closely related to crop transpiration, evapotranspiration (ET), and total water input into the system. The non-significant influence of water deficiency on camelina WUE*i* in this study indicated that camelina was more tolerant to water deficit conditions, and had greater adaptabilities to dry-land growth than canola. According to the data from a study in 2006 in Akron, Colorado, camelina showed the highest potential in dry-land production, whereas, canola had a strong linear curve responding to irrigation. These results suggested that canola was more suitable for limited and full irrigation than for dry-land production (Johnson et al., 2009).

2.7 Conclusion

Water deficiency and N availability significantly affected the physiological and metabolic processes of camelina and canola. Water deficiency decreased chlorophyll content in camelina plants but not in canola. Nitrogen application played an important role in increasing chlorophyll content in both crops. Water deficiency caused stomatal closure in both crops, leading to a decrease in photosynthesis and the transpiration processes. Under water deficit conditions, applied N negatively affected canola photosynthesis and transpiration but slightly increased those processes in camelina. In this study, the results indicated that camelina was more tolerant to water deficiency and potentially had greater adaptabilities to dry-land growth than canola.

Chapter 3: Comparative effects of water deficiency and applied nitrogen on camelina (*Camelina sativa* L. Crantz.) and canola (*Brassica napus* L.) growth and seed yield and quality under controlled environments

3.1 Introduction

Crop development, biomass accumulation and grain yield were significantly affected by water deficiency (Deng et al., 2004; Micheletto et al., 2007) and resulted in great economic losses in agricultural production throughout the world (Borsani et al., 2001). The severity of the influence of water deficiency on crops is dependent on the time of occurrence, frequency and duration of the deficiency (Robertson & Holland, 2004). The abilities of crops to tolerate drought are primarily determined by species, genotype and physiological and adaptive mechanisms (Bannayana et al., 2008; Gürsoy et al., 2012; Robertson & Holland, 2004). Many research studies have been carried out to verify the effects of water deficiency on growth and yield of oilseed crops. Farre & Faci (2006) found that water deficiency affected vegetative parts, total biomass and grain yield of corn (*Zea mays* L.) and sorghum [*Sorghum bicolor* (L.) Moench]. Bañuelos et al. (2002) reported that shoot dry matter of canola was significantly increased with water supply but root dry matter did not differ significantly among all treatments. However, an earlier study found rooting depth, root length and root density of canola and mustard was negatively correlated with soil water availability (Kirkegaard et al. 1997). Therefore, there remains a conflict effect of water deficiency on root biomass. Sinaki et al. (2007) assessed rapeseed responses to different levels of soil water deficiency and reported that the number of seed pods was the most significantly affected by water deficiency among yield components. For Brassica crops, water deficiency accelerates the process of flowering and fruit drop, thus decreasing seed yield (Gan, et al. 2004, Sinaki, et al. 2007).

Nitrogen is an important nutrient to plants and plays a vital role in increasing crop growth and grain yield. Oilseed crops require adequate N supply to maximize productivity (Miller et al, 2001). The highest canola seed and oil yield occurred with about 200 kg N ha^{-1} applied (Ibrahim et al., 1989; Jackson, 2000). Bugnărug et al. (2000) reported that camelina yield was increased by 58% with 100 kg N ha^{-1} application, compared to control crops with no additional N applied. Urbaniak et al., (2008) also found camelina seed yield was linearly correlated with N rates, up to 120 kg ha^{-1} in the Maritime Provinces in Canada. The application of N increased the seed yield, protein content, protein yield, and percentage of polyunsaturated fatty acids (PUFA); but decreased oil content and resulted in a reduction of monounsaturated fatty acids (MUFA) (Jiang et al. 2013). Nitrogen increases growth and yield by influencing a series of metabolic processes and consequently affecting various growth parameters such as stem length, branches per plant and seed pods per plant (Scott et al. 1973). A number of studies have suggested that an increase in N application could raise photosynthetic rate due to the positive effect of N on chlorophyll content (Field & Mooney, 1986; Evans, 1989; Huber et al. 1989; Connor et al. 1993) and thus increase plant growth. Nutrient availability in soil also influence the response of a plant to environmental stresses (Zareian et al. 2014). A study has reported that irrigation levels and N rates and their interaction had significant effects on water use efficiency of canola seed, oil, and protein yields (Hamzei, 2011). Nitrogen could improve water use efficiency and ease negative effects of drought stress on plant growth in arid systems by preventing cell membrane damage (Andrews et al., 1999; Saneoka et al., 2004).

However, the vast majority of studies have been carried out to evaluate the growth, seed yield and seed qualities of oilseed crops as influenced by a single factor such as water deficiency or N availability. Camelina is a renewed oilseed crop in Canada, with favorable agronomic traits and specialty oil characteristics (Zubr, 1997; Gugel & Falk, 2006), but there is little information available in the literature regarding the responses to the interactive effects of water and N on camelina growth traits in comparison with canola. With such considerations, a greenhouse experiment was designed with the two crop species and six N rates under three water status conditions. The objective of the present study was to clarify the growth and production including biomass, yield components, seed yield and seed quality of camelina and canola under different combined soil water potentials.

3.2 Methods and Materials

(See the details in the section of “Methods and Materials” in Chapter 2)

3.3 Measurements

(1) Root and shoot biomass

All of the aboveground portions of plants in each pot were collected. Roots were washed by hand after harvesting to evaluate the root dry matter and shoot: root ratio.

(2) Seed yield

The seeds were harvested by hand when plants were mature.

(3) Yield components

The data of yield components including plant height, number of branches per plant, number of seed pods per plant, thousand seed weight (TKW) and number of seeds per plant were collected.

(4) Oil and Protein Content in Seeds

Total oil and protein content in seeds were determined by using near-infrared reflectance (NIR) spectroscopy (Unity Scientific LLC, SpectraStar 2500X).

3.4 Statistical analysis

Minitab 17 Statistical Software (Minitab Inc., USA, 2013) was used in the analysis of data. The General linear model (GLM) was used to examine the effects of different soil water potential and N rates on camelina and canola biomass accumulation (root and shoot biomass), grain yield and yield components. The significant level was set to be 0.05.

3.5 Results

3.5.1 The root and shoot biomass

A three-way interaction of crop species, water deficiency and applied N significantly affected shoot and root biomass, respectively (Table 3.1).

Canola shoot biomass was significantly higher than that of camelina in each treatment. The effect of water deficiency did not significantly affected camelina shoot biomass. The average shoot biomass of the camelina under the three water status conditions was 8.2, 7.6 and 7.4 g per plant, respectively (Table 3.2). In contrast, water deficiency significantly decreased canola shoot biomass. The averaged shoot biomass of canola was in the range of 47.1 to 34.7 g per plant (Table 3.2). A significant difference in canola shoot biomass was observed between controls and severe (-120 cbar) water deficiency (Table 3.2). The different effects of water deficiency on camelina and canola shoot biomass indicated that camelina shoot growth was less sensitive to water deficiency than that of canola.

With additional N applied, camelina shoot biomass was significantly increased. The highest shoot biomass of camelina in the controls, moderate (-60 cbar) and severe (-120 cbar) water deficiency was 11, 9.8 and 8.9 g per plant with 175 kg N ha^{-1} application, respectively (Table 3.2). These values were not significantly different from the shoot biomass of camelina at applied N rate of 25 kg ha^{-1} (Table 3.2). It should be pointed out that a decreasing trend of camelina shoot biomass under water deficit conditions was observed when applied N rate was greater than 175 kg ha^{-1} (Table 3.2). The effect of applied N on canola shoot biomass was significant. Under well-watered conditions, the highest shoot biomass of canola (65.1 g/plant) was obtained at applied N rate of 125 kgha^{-1} (Table 3.2). The canola shoot biomass under water deficit conditions peaked at 52.6 and 49.3 g per plant under moderate (-60 cbar) and severe (-120 cbar) water deficiency, respectively, at applied N rate of 75 kg ha^{-1}. A notable decrease in canola shoot biomass was found at applied N rate of 225 kg ha^{-1} when water deficiency occurred (Table 3.2).

Table 3.1: ANOVA table of shoot and root biomass and the ratio of shoot/room of camelina and canola as influenced by water deficiency and applied N

Effects	P-value		
	Shoot biomass (g/plant)	**Root biomass (g/plant)**	**Ratio of shoot/root**
Species (S)	0.000	0.000	0.000
Water deficiency (W)	0.000	0.000	0.000
Nitrogen (N)	0.000	0.000	0.000
S*W	0.000	0.000	**0.010**
S*N	0.000	0.000	**0.000**
W*N	0.000	0.000	**0.003**
S*W*N	**0.000**	**0.000**	0.078

*1. Error term used in main plot factor was W;
F test was Replication (Reps.)*W.
*2. Error term used in sub plot factors were S*N;
F test was Reps.*S*N*W+ Reps*S*N+ Reps. * S+ Reps.*N.

Table 3.2: Effects of water deficiency and applied N on shoot biomass of camelina and canola

Species	Applied N (kg ha^{-1})	Shoot biomass (g/plant)		
		Control	-60 cbar	-120 cbar
Camelina	0	3.1 o	3.6 no	3.0 o
	25	6.9 m-o	8.8 lm	8.8 lm
	75	7.7 l-o	8.1 l-n	8.7 lm
	125	10.5 lm	8.2 l-n	8.5 lm
	175	11.0 lm	9.8 lm	8.9 lm
	225	10.2 lm	7.2 m-o	6.6 m-o
	Mean	8.2	7.6	7.4
Canola	0	12.3 l	11.1 lm	10.4 lm
	25	28.9 j	26.5 jk	24.1 k
	75	55.5 cd	52.6 de	49.3 ef
	125	65.1 a	46.9 fg	44.4 gh
	175	58.8 bc	45.6 fg	42.2 gh
	225	62.4 ab	40.7 hi	37.7 i
	Mean	47.1	37.2	34.7

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

Canola root biomass was significantly higher than that of camelina in each treatment. The effect of water deficiency did not significantly affect camelina root biomass. With the development of water deficiency, the root biomass of camelina averaged from 1.0 to 1.5 g per plant (Table 3.3). However, water deficiency significantly increased canola root biomass, which was elevated from 9.4 to 11.5 g per plant on average, as water deficiency developed (Table 3.3). This indicated that under water deficit conditions, the canola root system was likely to be larger while searching for more water.

A significant difference in camelina root biomass was not observed among all N treatments. The root biomass of camelina ranged from 0.5 to 1.7 g, 0.5 to 2.1g and 0.9 to 2.4g per plant under well-watered, moderate (-60 cbar) severe (-120 cbar) water deficit conditions, respectively (Table 3.3). In contrast, applied N significantly increased root biomass of canola. With adequate water supplied, the canola root biomass was in a range of 3.9 to 12.5 g per plant and the value plateaued at applied N rate of 175 kg ha^{-1} (Table

3.3). Under deficit conditions, the highest root biomass of canola was 16.8 and 18.7 g per plant under moderate (-60 cbar) and severe (-120 cbar) water deficit conditions, respectively, and both values were obtained at applied N rate of 175 kg ha^{-1} (Table 3.3). A negative effect of high N rate (225 kg ha^{-1}) on canola root biomass was observed under water deficit conditions.

Compared with canola, the non-significant change in camelina root biomass as influenced by water deficiency and applied N indicated that camelina better tolerates water deficiencies and requires lower N input.

Table 3.3: Effects of water deficiency and applied N on root biomass of camelina and canola

Species	**Applied N (kg ha^{-1})**	**Root biomass (g/plant)**		
		Control	**-60 cbar**	**-120 cbar**
Camelina	**0**	0.5 j	0.5 j	0.9 j
	25	0.7 j	0.9 j	1.0 j
	75	0.8 j	1.0 j	1.1 j
	125	0.9 j	1.3 i-j	1.4 i-j
	175	1.7 h-j	1.7 h-j	2.4 h-j
	225	1.6 h-j	2.1 h-j	2.4 h-j
	Mean	1.0	1.2	1.5
Canola	**0**	3.9 hi	4.2 gh	4.0 gh
	25	5.5 fg	6.6 fg	7.3 f
	75	10.6 e	12.6 c-e	14.9 bc
	125	11.4 de	14.9 bc	13.6 cd
	175	12.8 c-e	16.8 ab	18.7 a
	225	12.5 c-e	10.2 e	10.4 e
	Mean	9.4	10.9	11.5

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

3.5.2 The ratio of shoot/root biomass

Two-way interaction of crop species and water deficiency; crop species and applied N; and water deficiency and applied N had significant effects on the ratio of shoot/root biomass (Table 3.1).

The shoot/root ratio of camelina was greater than that of canola at all water deficit levels (Table 3.4). Water deficiency significantly decreased the shoot/root ratio of camelina and canola. With the development of water deficiency, the shoot/ root biomass ratio of both species ranged from 8.7 to 5.5 and 4.9 to 3.1 for camelina and canola, respectively (Table 3.4).

The shoot/root ratio of canola was in the range of 2.8 to 4.3 and it was not significantly affected by applied N (Figure 3.1). In contrast, camelina shoot/root ratio was significantly influenced by applied N. The highest ratio (9.9) was obtained when 25 kg N ha^{-1} was applied and a negative effect was observed when N application was greater than this rate (Figure 3.1).

As water deficiency developed, an increase in N application rates significantly decreased the shoot/root biomass ratio. With adequate water supplied, shoot/root ratios of the two crops increased with N rates up to 125 kg ha^{-1} (Table 3.5). Under water deficit conditions, applied N negatively affected the ratio when it was greater than 25 kg ha^{-1} (Table 3.5). The lowest shoot/root ratio was obtained when soil water potential was -120 cbar with 175 kg ha^{-1} N applied (Table 3.5).

Table 3.4: Effects of species and water deficiency on shoot/root biomass ratio of camelina and canola

Species	Water deficiency (cbar)	Ratio of shoot/root biomass
Camelina	Control	8.7 a
	-60	6.8 b
	-120	5.5 c
Canola	Control	4.9 c
	-60	3.5 d
	-120	3.1 d

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

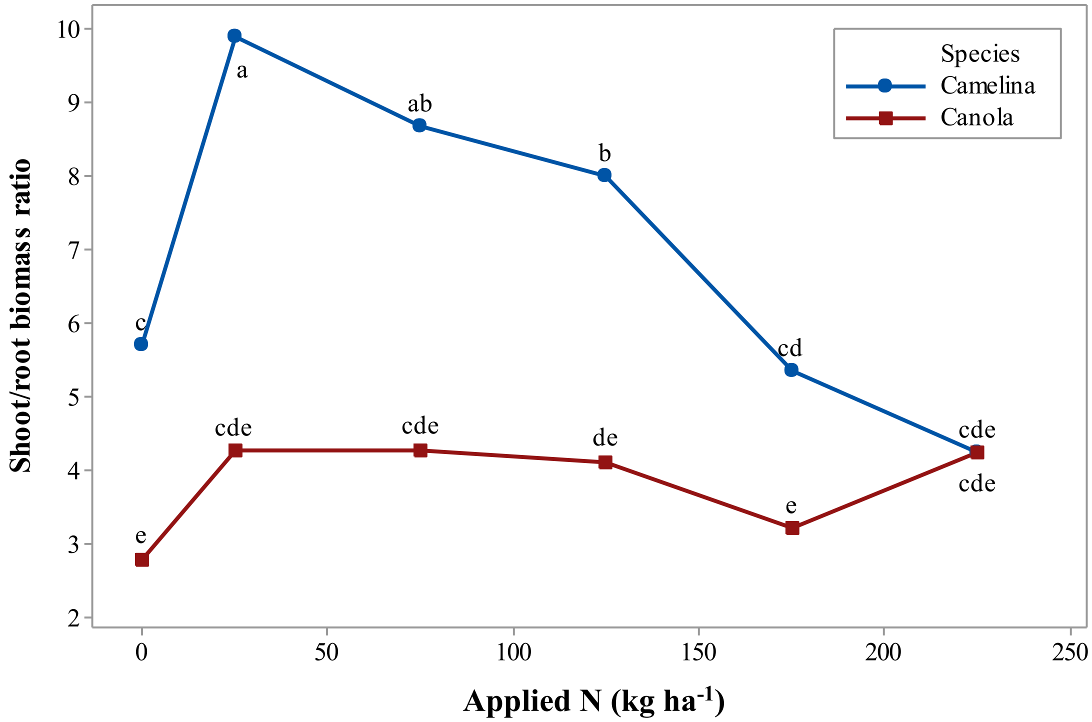


Figure 3.1: Effects of species and applied N on shoot/root biomass ratio of camelina and canola (Means with a common letter are not significantly different at the 5% level)

Table 3.5: Effects of interaction of water deficiency and nitrogen on shoot/root biomass ratio

Applied N (kg ha^{-1})	**Shoot/root biomass ratio**		
	Control	**-60 cbar**	**-120 cbar**
0	4.9 d-f	4.8 d-f	3.0 f
25	7.8 ab	7.2 a-c	6.1 b-d
75	7.8 ab	6.1 b-d	5.5 c-e
125	8.7 a	4. 7 d-f	4.8 d-f
175	5.6 c-e	4.2 d-f	3.0 f
225	5.7 cd	3.7 ef	3.2 f
Mean	6.8	5.1	4.3

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

3.5.2 Grain yield

The two-way interactions of crop species and water availability; and crops species and applied N significantly affected seed yield (Table 3.6).

Canola seed yield was negatively affected by soil water availability (Table 3.7). The highest canola seed yield (1555.82 kg ha^{-1}) was produced under moisture non-limiting conditions (Table 3.7). Canola seed yield significantly decreased from 1272 and 1158 kg ha^{-1} when soil water potential dropped to -60 cbar and -120 cbar, respectively (Table 3.7). On contrast, water deficiency did not significantly affect the seed yield of camelina (Table 3.7). The highest and the lowest camelina seed yields were 1112 and 937 kg ha^{-1}, obtained at moderate (-60 cbar) and severe (-120 cbar) water deficiency (Table 3.6). It should be pointed out that, under water deficit conditions, non-significant difference between camelina and canola seed yield was observed (Table 3.7).

The seed yield was significantly increased by N supply in both species (Table 3.8), with a greater seed yield responses (Figure3.2) in canola (R^2_{adj} = 54.6%) than in camelina (R^2_{adj} = 51.2 %). These indicated a closer relationship between N supplied and canola seed yield in comparison with camelina. Camelina and canola seed yields plateaued at applied N rate of 75 kg ha^{-1}. The maximum seed yield for each crop was 1497 and 1732 kg ha^{-1} for camelina and canola, respectively, and a significant difference was not observed between these values (Table 3.8). The highest amount of applied N (225 kg ha^{-1}) significantly decreased camelina seed yield but did not affect canola seed yield (Table 3.8).

Table 3.6: ANOVA table of grain yield of camelina and canola as influenced by water deficiency and applied N

Effects	**F-value**	**P-value**
Species (S)	50.29	0.000
Water deficiency (W)	16.42	0.000
Nitrogen (N)	69.17	0.000
S*W	**5.38**	**0.005**
S*N	**2.28**	**0.049**
W*N	1.140	0.336
S*W*N	0.740	0.682

*1. Error term used in main plot factor was W;
F test was Replication (Reps.)*W.
*2. Error term used in sub plot factors were S*N;
F test was Reps.*S*N*W+ Reps*S*N+ Reps. * S+ Reps.*N.

Table 3.7: The interactive effects of water deficiency and species on grain yield

Species	Water deficiency (cbar)	Seed yield (kg ha^{-1})
Canola	Control	1555.82 a
	-60	1272.27 b
	-120	1158.90 b
Camelina	Control	1095.63 bc
	-60	1112.68 bc
	-120	937.75 c

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

Table 3.8: The combined effect of crop species and applied N on seed yield

Applied N (kg ha^{-1})	Grain yield (kg ha^{-1})	
	Camelina	**Canola**
0	493.4 g	528.0 g
25	797.7 fg	1169.0 de
75	1215.3 c-e	1676.3 a
125	1497.4 a-c	1732.0 a
175	1307.6 b-d	1557.3 ab
225	980.8 ef	1311.4 b-d
Mean	1048.7	1329.0

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

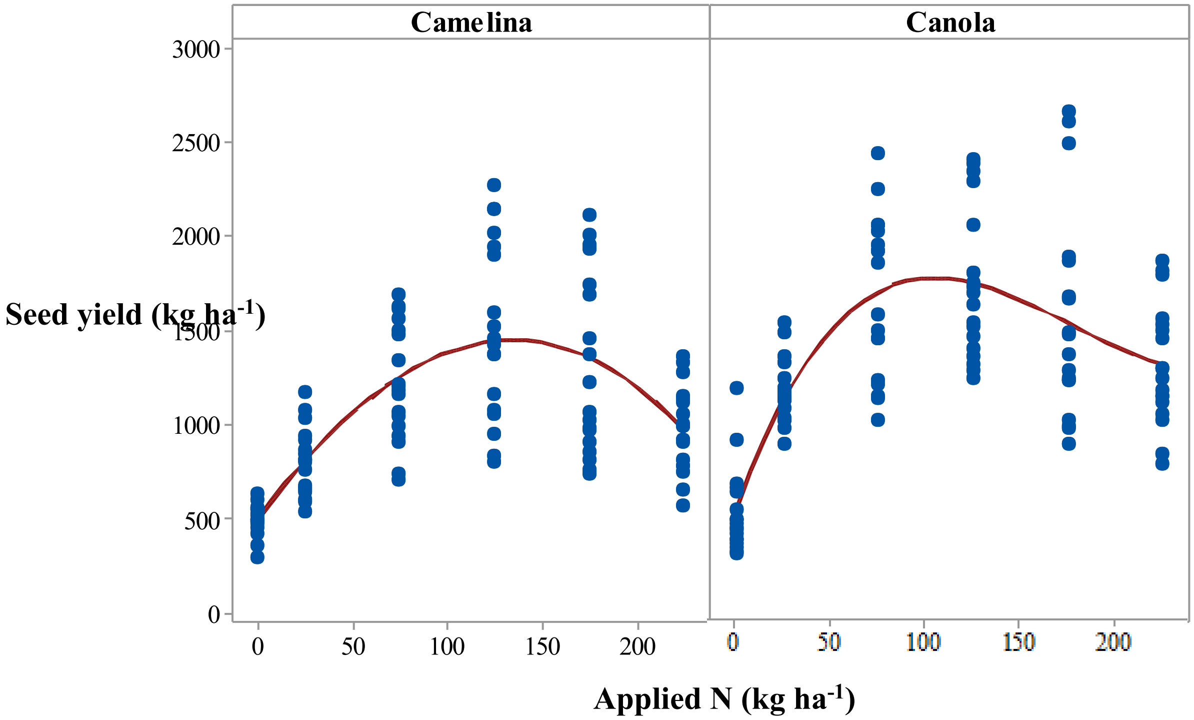


Figure 3.2: Regression analysis of applied N on seed yield of camelina and canola

Y (Camelina) = 479.4 + 14.43 N - 0.05433 N*2 with R-Sq (adj) = 51.2%
Y (Canola) = 553.1 + 27.17 N - 0.1850 N*2 + 0.000353 **3 with R-Sq (adj) = 54.6%

3.5.3 Yield components

A combined effect of crop species and applied N significantly affected plant height; crop species and applied N independently influenced the number of branches per plant and the number of seed pods per plant; the number of seeds per plant was significantly affected by water deficiency and the two-way interactions of crop species and applied N; and crop species and water deficiency significantly affected the TKW (Table 3.9).

Table 3.9: ANOVA table of yield component of camelina and canola as influenced by water deficiency and applied N

Effects	**P-value**				
	Plant height	**# branches /plant**	**# seed pods /plant**	**# seeds /plant**	**TKW**
Species (S)	0.000	**0.000**	**0.000**	0.000	0.000
Water deficiency (W)	0.314	0.539	0.379	**0.000**	0.003
Nitrogen (N)	0.000	**0.000**	**0.000**	0.000	0.085
S*W	0.531	0.193	0.210	0.409	**0.000**
S*N	**0.000**	0.121	0.102	**0.000**	0.103
W*N	0.343	0.978	0.938	0.331	0.566
S*W*N	0.872	0.979	0.983	0.594	0.994

*1. Error term used in main plot factor was W; F test was Replication (Reps.)*W.
*2. Error term used in sub plot factors were S*N; F test was Reps.*S*N*W+ Reps*S*N+ Reps. * S+ Reps.*N.

Plant height of canola increased with an increase in N supply. The highest canola plants was found at applied N rate of 125 kg ha^{-1} (Figure 3.3). A significant difference was not observed in the range of applied N rates from 25 to 175 kg ha^{-1} (Figure 3.3). Nitrogen supply did not significant affect camelina plant height. The plant height of camelina ranged from 72 to 78 cm on average.

Camelina produced more than 8 branches per plant, which was significantly greater than that of canola (2.75 branches per plant) (Table 3.10). This indicated a greater axillary branching ability in camelina plants. Applied N significantly enhanced branch number of the two species. The number of branches per plant ranged from 3.24 to 7.07 (Table 3.10). The number of branches per plant reached a plateau at an applied N rate of 75 kg ha^{-1} and a significant difference was not found if N rate was higher than this rate (Table 3.10).

Camelina produced 153 seed pods per plant, which was significantly higher than that of canola (58 seed pods per plant) (Table 3.11). Applied N had a positive effect on number of seed pods per plant. The highest and lowest numbers of seed pods per plant were 51 and 132, respectively. The plateau was reached at an applied N rate of 125 kg ha^{-1} (Table 3.11).

The effect of water deficiency on the number of seeds per plant was pronounced. The highest number of seeds was 1354 which were produced under moisture non-limiting condition, and this number dramatically decreased to 1056 when soil water potential was dropped to -120 cbar (Table 3.12). Camelina produced significantly higher amount of seeds than canola among all N treatments (Figure 3.4). Camelina seed number per plant was in the range of 749 to 2141, which were observed in the controls and applied N rate of 125 kg ha^{-1}, respectively (Figure 3.4). A high rate of applied N decreased camelina number of seeds per plant to 1358 at applied N rate of 225 kg ha^{-1} (Figure 3.4). The highest number of seeds per canola plant was obtained with N applied at 75kg ha^{-1} and exceeding amount of N was slightly decreased canola seed quantity but did not result in significant differences (Figure 3.4).

Water deficiency did not significantly affected the TKW of canola, but it had a pronounced effect on the TKW of camelina (Table 3.13). The highest 1000- seed weight of camelina was achieved under moderate (-60 cbar) water deficiency. Adequate water and severe water deficiency caused a decrease in camelina 1000-seed weight (Table 3.13).

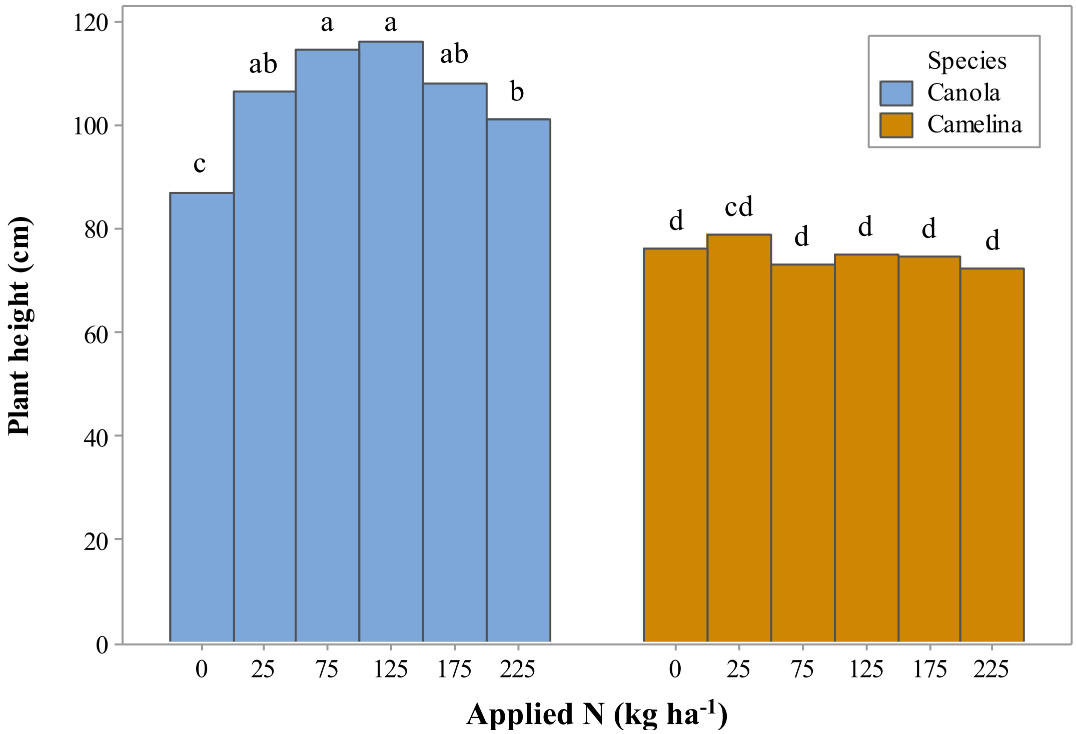


Figure 3.3: The effect of applied N on plant height of camelina and canola (Means with a common letter are not significantly different at the 5% level)

Table 3.10: Effects of species and applied N on number of branches per plant

Species	**Applied N (kg ha^{-1})**	**# Branches per plant**
Camelina		8.68 a
Canola		2.75 b
	0	3.24 c
	25	4.80 bc
	75	6.61 a
	125	7.07 a
	175	6.38 a
	225	6.19 ab

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

Table 3.11: Effects of species and applied N on number of seed pods per plant

Species	Applied N (kg ha^{-1})	#seed pods per plant
Camelina		153.44 a
Canola		58.03 b
	0	51.07 c
	25	93.58 b
	75	123.57 ab
	125	132.42 a
	175	123.57 ab
	225	110.20 ab

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

Table 3.12: Effects of water deficiency on number of seed per plant

Water deficiency (cbar)	# seed pods per plant
Control	1354.3 a
-60	1104.6 b
-120	1056.2 b

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

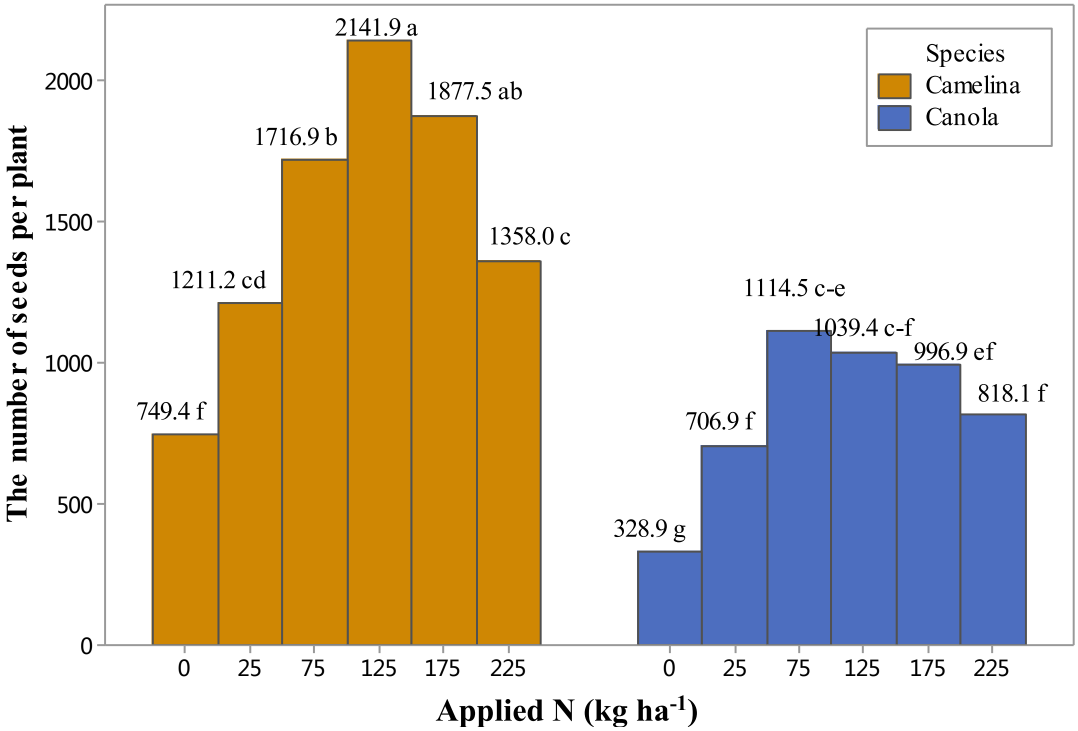


Figure 3.4: The effects of species and applied N on number of seeds per plant
(Means with a common letter are not significantly different at the 5% level)

Table 3.13: The effect of water deficiency on TKW of camelina and canola

Water deficiency (cbar)	TKW (g)	
	Camelina	Canola
Control	1.13 c	2.85 a
-60	1.41 b	2.82 a
-120	1.21 c	2.83 a

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

3.5.4 Oil content

The oil content in the seed was significantly affected by soil water availability and a two-way interaction of crop species and applied N (Table 3.14).

Water deficiency had a significant effect on seed oil content; however, data in Table 3.15 shows seed oil content was decreased by only 1% due to water deficiency. The highest seed oil content was 37.34 % under well-watered condition and the lowest oil content (36.44%) was observed under moderate (-60 cbar) water deficiency (Table 3.15).

Applied N was negatively correlated with canola seed oil content. With an increase in applied N, canola seed oil content was dramatically decreased from 41 % to 33 % (Figure 3.5). N application slightly decreased camelina seed oil content and a significant difference was found between controls and the highest N treatments. Among all N treatments (25 to 225 kg N ha^{-1}), a significant difference was not observed (Figure 3.5).

Table 3.14: ANOVA table of oil content of camelina and canola as influenced by water deficiency and applied N

Effects	F-value	P-value
Species (S)	8.44	0.004
Water deficiency (W)	**3.43**	**0.035**
Nitrogen (N)	33.49	0.000
S*W	0.72	0.487
S*N	**20.66**	**0.000**
W*N	0.56	0.848
S*W*N	0.67	0.748

*1. Error term used in main plot factor was W; F test was Replication (Reps.)*W.

*2. Error term used in sub plot factors were S*N; F test was Reps.*S*N*W+ Reps*S*N+ Reps. * S+ Reps.*N.

Table 3.15: The effects of water deficiency on seed oil content

Water deficiency (cbar)	Oil content (%)
Control	37.34 a
-60	36.44 b
-120	37.15 ab

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

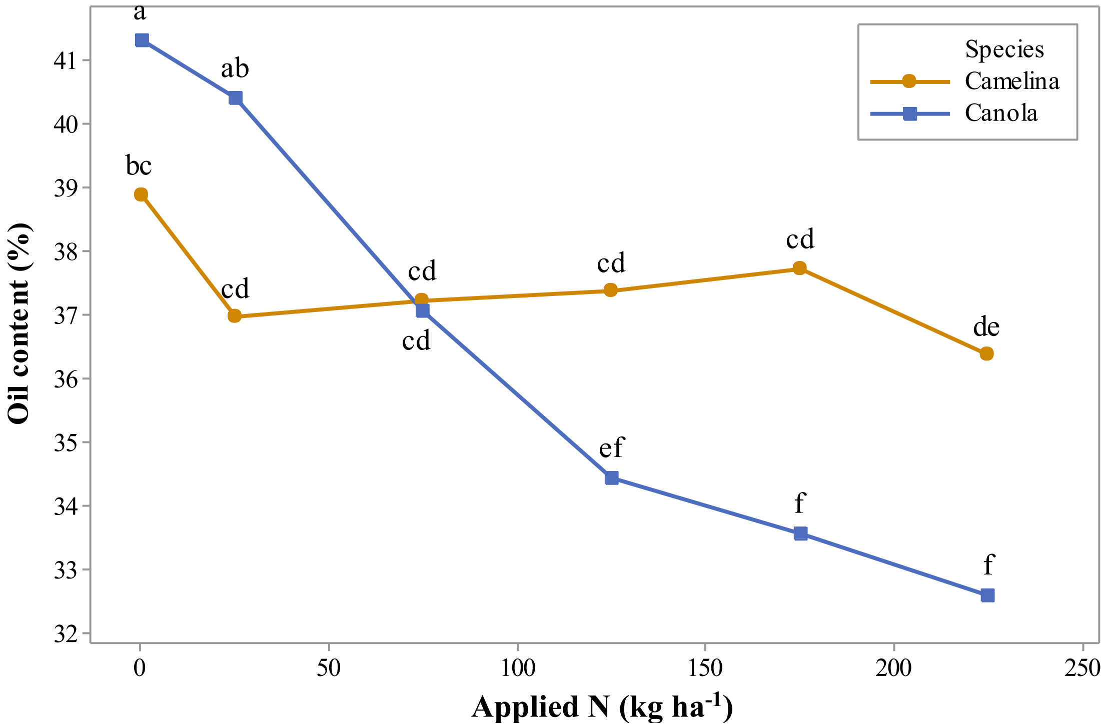


Figure 3.5: Effects of nitrogen on seed oil content of camelina and canola
(Means with a common letter are not significantly different at the 5% level)

3.5.5 Protein content

A two-way interaction of crop species and applied N significantly affected seed protein content (Table 3.16).

The protein content in camelina seeds was greater than that of canola seed among all N treatments (Figure 3.6). The protein content in the camelina seed was gradually increased with an increase in applied N up to 225 kg ha^{-1}, but the plateau was reached at 75 kg N ha^{-1} application rate (Figure 3.6). The protein content in the canola seeds was dramatically increased with an increase in applied N as high as 225kg ha^{-1}. The greater variability of canola protein content in response to applied N indicated that protein content in canola seeds was more responsive to applied N than camelina. The optimum applied N for canola to maintain high protein content was 125kg ha^{-1} (Figure 3.6).

Table 3.16: ANOVA table of protein content of camelina and canola as influenced by water deficiency and nitrogen

Effects	F-value	P-value
Species (S)	137.42	0.000
Water deficiency (W)	2.85	0.061
Nitrogen (N)	70.33	0.000
S*W	0.23	0.797
S*N	**13.76**	**0.000**
W*N	0.31	0.977
S*W*N	0.42	0.936

*1. Error term used in main plot factor was W;
F test was Replication (Reps.)*W.
*2. Error term used in sub plot factors were S*N;
F test was Reps.*S*N*W+ Reps*S*N+ Reps. * S+ Reps.*N.

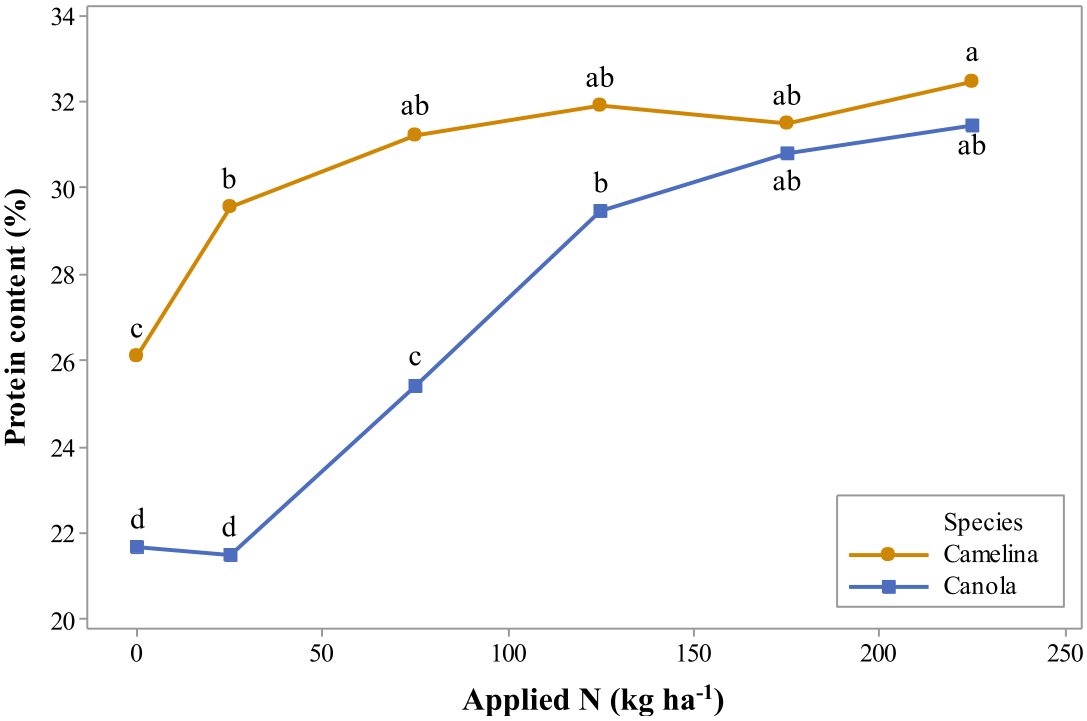


Figure 3.6: The effect of applied N on seed protein content of camelina and canola
(Means with a common letter are not significantly different at the 5% level)

3.6 Discussion

3.6.1 Shoot and root biomass

One of the important mechanisms for plants responding to their environments is allocation of resources between shoots and roots to optimize resource uses (Ågren & Franklin, 2003). In this study, water deficiency and applied N did not significantly affect camelina shoot and root biomass (Table 3.2 and Table 3.3). This suggested that changes in the soil N and water availabilities did not result in significant resource reallocation in the camelina plants. Such levels of water deficiency might not lead to a severe drought stress on camelina plants. This might infer a good adaptability of camelina. In contrast, canola plants showed a different responses mechanism to soil water and N availabilities. Applied N increased shoot and root biomass; while water stress decreased shoot biomass but increased root biomass. Similar results was reported by Bilibio et al. (2011), who found the green matter of *Brassica napa* L. was linearly decreased with water deficiency developed. Harris (1992) found proportional growth of roots when N was limited. This indicated that the biomass accumulation of canola was more responsive to their environments. Under such conditions, canola plants allocated more resource to roots, resulting in greater root growth rate than shoot. This was supported by the results from a number of studies, which showed a greater decrease in shoot biomass than in root biomass due to water deficiency (Sharp et al., 1988; Saab et al., 1990; Engels et al., 1994). The results in this study showed that due to water deficiency, shoot biomass was reduced by 9 % and 26 % and root biomass was elevated by 50 % and 22% for camelina and canola, respectively. These results indicated a great ability of camelina in resource allocation under water deficit conditions.

However, considering about the shoot/root biomass ratio, applied N effect on camelina was more significant than that on canola. This could be explained as the different response mechanisms between two crops. Brouwer (1962) emphasized that the balance between carbohydrates and N plays an important role in determining the magnitude of shoot and root growth. According to Pearsall (1923) and White (1937), the photo-assimilates was firstly utilized in the shoots; while the roots have the first call on N and soil moisture. The internal balance between labile N and carbon in root and shoot system determines how dry matter is being partitioned in the plant (Ericsson, 1995). According to the results from Chapter 2, water deficiency significantly decreased photosynthetic rate and this resulted in a decrease in carbohydrate supply. However, compared to camelina, larger canola canopy and greater shoot biomass retained a larger proportion of assimilates (internal carbon) which was contributed to shoot growth. This shoot growth could be comparable to root growth, thus change in shoot/root ratio was less significant.

3.6.2 Seed yield and yield components

Water deficiency significantly affected canola and camelina seed yield. The results indicated camelina required less water to achieve its highest yield than canola. French et al. (2009) reported a weak correlation between camelina yield and total ET and suggested that seasonal camelina water minimally required 333–423mm. This was significantly less than that typically needed by grain and vegetable crops (600–655 mm) (Hunsaker, et al., 2011).

Results from the present study indicated that applied N significantly influenced seed yield of both species. Nitrogen enhances seed yield via the influences on a number of yield components, such as number of branches and pods per plant, seeds per pod and 1000-seed weight (Karamzadeh, 2010). N responses of seed yield were closely related to the amount of available N, including fertilizer N and soil residual N. The positive correlation between seed yield and N rates in this study was in agreement with previous studies.

A number of researchers have shown that high rates of applied N significantly increase growth and yield of canola (Bilsborrow et al., 1993; Cheema et al., 2001; Karamzadeh, 2010; Kumar et al., 2001). With a range of N rates from 80 kg ha^{-1} to 120 kg ha^{-1}, canola seeds yield increased from 1686 kg ha^{-1} to 2310 kg ha^{-1} (Ahmad, et al. 2011). This positive correlation was consistent with other canola N response studies (Ghanbari-Malidarreh, 2010; Karamzadeh, et al. 2010, Pan, et al. 2012).

Camelina which is described as an oilseed crop with low-input requirement, has similar soil fertility requirements to other Brassica species, with the same yield potential. The pattern of N influencing seed yield of camelina is the same as that for canola. The result from this study showed positive correlation between camelina seed yield and N rates. This was consistent with a number of studies. Agegnehu et al., (1997) and Jiang, et al. (2013) found a maximum seed yield was obtained with 120 kg N ha^{-1} applied. Urbaniak et al., (2008a) also reported that seed yield was linearly correlated with N rates, up to 120 kg ha^{-1}. In a two-year field study, it was found that N fertilizer increased camelina seed yield by 1.1 - 2.2 times, compared to that in unfertilized plots (Končius et al., 2010).

3.6.3 Oil and protein content

Oil and protein in the seeds are considered as the most important components when evaluating seed quality. They can be significantly affected by N. In this study, a negative correlation between oil content in the seed of both species and N rates was clearly demonstrated. This was supported by Karamzadeh et al. (2010) who reported that the N rate increased from 92 kg ha^{-1} to 160 kg ha^{-1}, while canola seed oil content decreased from 47.42% to 39.84%. This negative effect on canola was consistent with many other previous studies (Ghanbari-Malidarreh. 2010; Kumar et al. 2007). A more recent study reported that in camelina seed, the oil content decreased from 42% to 35%, with an increase in N application from 0 to 120 kg ha^{-1} (Jiang et al. 2013).

Comparing the oil contents in the two oilseed species in this experiment, the average value in canola seed 5 to 7% greater than that of camelina. A similar result was also highlighted in the study conducted by Steppuhn et al (2009).

In regards to protein content, camelina contained higher protein in the seeds than that of canola under each N application rate. The seed protein content obtained in this study was comparable with previous results. Kuhlmann (1986) has reported protein content in camelina seed linear responded to N rates up to 120 kg ha^{-1} and protein content ranged from 23.5% to 30.1%. A similar result was also found by Urbaniak, et al (2008a).

3.7 Conclusion

Water and N fertilizer play key roles in oilseed production. Applied N affected seed yield, oil yield, seed qualities of camelina and canola. 175 kg N ha^{-1} could be applied to canola in order to achieve optimum seed and oil yield, and only 125 kg N ha^{-1} was required to maximize camelina yield. The characteristics of camelina and canola water requirement were significantly different. The result of this study suggested that canola required more water than camelina to achieved optimum biomass and seed yield. Camelina could successfully grow under -60 cbar soil water potential.

Chapter 4: Comparative effects of applied nitrogen on camelina (*Camelina sativa* L. Crantz.) and canola (*Brassica napus* L.) growth and seed quality as influenced by the combined effect of site and year

4.1 Introduction

The uninterrupted increase in global vegetable oil demand and consumption have placed great pressure on oilseed supplies. To satisfy this, three broad strategies are available: (1) expand farming land for oilseed crop cultivation; (2) improve productivity of current oilseed crops via agricultural management on the existing farmland or (3) introduce an alternative oilseed crop with desirable agronomic features into the existing cropping system. Among the three options, introducing a new oilseed crop is preferable because it avoids the large-scale disruption of existing ecosystems and reduces the impact on environment due to expanding production land and changing agricultural practice, particularly in fertilizer management.

To bring a new crop into a current cropping system, it is necessary to assess its agronomic traits associated with production management and compare with existing crops. In terms of management, the application of nitrogen (N) fertilizer is described as a key factor to maximize profitable production with acceptable quality (Otteson et al. 2007). The importance of N as an essential mineral elements for plant growth has long been recognized (Miller, 1939). In oilseed production, N is one of the most important nutrients and accounts for the largest energy input with regard to fertilizer supply (Nuttall & Malhi, 1991; Gan et al., 2008; Urbaniak et al., 2008a).

Numerous research projects have been conducted to evaluate N requirements in camelina to achieve maximum seed yield. However, variable results were observed as the soil-available N concentration, uptake, transport and utilization vary with different environmental variables, including precipitation, temperature, soil type and residual fertility especially N.

According to study carried out in Ireland by Crowley & Frohlich (1998), camelina seed yield was maximized with 75kg N ha^{-1} applied. Zubr (1997) in Denmark reported that camelina was successfully grown with 100 kg ha^{-1} N applied. A study performed in Romania clarify this point further that 100 kg ha^{-1} of N contributed to camelina seed yield increases of 58% (Bugnarug and Borcean 2000). However, camelina (summer type) grown in Germany required 120 kg ha^{-1} N to produce the highest yields (Agegnehu & Honermeier, 1997).

Researchers in the northern United States found that camelina grown in research trials responded well to an N rate of 90 kg ha^{-1} (Budin et al. 1995). McVay and Lamb (2008) similarly recommended that 90-100 kg ha^{-1} of N could be supplied to camelina in Montana. More recent studies carried out in four rain-fed sites in the Pacific Northwest (PNW) of the United States by Wysocki et al. (2013) reported that optimum applied N rates ranged from 0 to 90 kg ha^{-1} depending on annual precipitation and soil available N. In Chile, however, Solis et al. (2013) suggested that camelina might respond to high N rates under desirable environmental conditions that maximize seed yield potential. Camelina seed yield was predicted to increase with N fertilization up to 185 kg N ha^{-1} (Solis et al., 2013).

In Canada, Jiang et al. (2013) evaluated camelina performance with applied N in the Maritime Provinces and found that seed yield was linearly correlated with N rates up to 120 kg ha^{-1}; this result was consistent with a previous study conducted by Urbaniak et al. (2008a) in the same region. Camelina grown in western Canada, however, only required 100 kg N ha^{-1} to achieve maximum seed yield (Pan, 2009).

There is little literature reporting the mechanism by which N affects camelina seed yield. Agegnehu & Honermeier (1997) concluded that the number of seeds per pod, seed weight per plant, pod production per plant or per unit area are the major factors determining camelina seed production. N applied at various rates can consistently change the number of branches, the number of pods and the seed weight per plant as well as the number of seeds per pod (Agegnehu & Honermeier, 1997; Končius & Karčauskienē 2010; Solis et al., 2013).

The effects of N on camelina seed quality, including protein content and oil content in the seed, have been well documented (Agegnehu & Honermeier, 1997; Gugel & Falk 2006; Urbaniak et al. 2008a; Končius & Karčauskienē, 2010; Lošák et al. 2011; Jiang et al. 2013; Johnson and Gesch 2013; Kirkhus et al. 2013; Jiang et al., 2014). Results showed that N significantly increased protein content in camelina seed but negatively affected oil concentration. The optimum N rate for the highest protein content was 160 kg N ha^{-1} (Jiang et al., 2014) while oil content in the seed experienced a dramatic decease when N was applied above 120 kg ha^{-1} (Lošák et al., 2011).

Canola is an important source of vegetable oil or livestock feed, with high protein content in the meal after oil extraction; however, its susceptibility to various insects and diseases (Ehrensing, 2008) and significant fertilizer and moisture requirements (Hocking et al., 1997) limit its production. The N fertilizer recommendations for canola are high, ranging from 100 to 170 kg ha^{-1} (Svečnjak et al., 2006). As a major oilseed crop grown throughout the world, canola response to applied N is well demonstrated in the literature (Scarisbrick et al., 1980; Nuttall et al., 1992; Jackson, 2000; Ahmad et al., 2007; Gan et al., 2008; Ansar et al., 2013; Elewa et al., 2014).

However, there is a lack of information in the literature regarding the performance (e.g. seed and oil yields) of camelina in comparison with canola directly, over a wide range of agricultural inputs (e.g. N fertilizer). The objectives of this study was to compare the effects of applied nitrogen on the yield, yield components, and seed oil and protein contents of canola and camelina under various environments (sites*years).

4.2 Methods and Materials

4.2.1 Experimental sites

Sites at Canning (lat. 45.16^{o} N; long. 64.43^{o} W), NS (Lyndhurst Farms) and Truro (lat. 45.36^{o} N; long. 63.28^{o} W), NS (Dal-AC) were selected for this study in 2013 and Truro, NS (Dal-AC) and AAFC Harrington (lat. 46.33° N; long. 63.17° W), PEI were used in 2014. Soil characteristics (Table 4.1) and weather summaries (Table 4.2) from May to September in 2013 and 2014 are presented below. The soil was sampled down to a depth of 15 cm and five sub-samples from different locations within each plot were randomly sampled. The soil test fertility status was conducted by Modified Mehlich III extraction in the Department of Agriculture Laboratory Services at Truro. The inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Impact Analytical, US) was used in the soil fertility test.

Table 4.1: Characteristics of soil and previous crop grown in experimental sites in 2013 and 2014

Location	Previous crop	Organic matter (%)	pH	CEC (meq per100g)	P_2O_5	K_2O	Ca	Mg	S
					kg ha^{-1}				
Canning, NS (2013)	Soybean	2.6	5.9	11.4	1770	345	3174	221	31
Truro, NS NS (2013)	Barley	2.9	6.2	11.9	614	229	2854	442	29
Truro, NS NS (2014)	Barley	2.8	6.4	11.5	549	185	3019	559	18
Harrington, PEI (2014)	Barley	3.4	5.6	7.7	554	266	1472	108	29

Table 4.2: Precipitation, growing degree days (GDD), and monthly mean temperature at the tested locations in 2013 and 2014

Month	Precipitation (mm)				GDD (≥5 °C)				Mean temperature (°C)			
	Canning 2013	Truro 2013	Truro 2014	Harrington 2014	Canning 2013	Truro 2013	Truro 2014	Harrington 2014	Canning 2013	Truro 2013	Truro 2014	Harrington 2014
May	72.6	69.4	32.5	68.2	211.9	256.0	140.9	32.4	11.8	10.7	9.6	N/A
June	124.0	133.2	102.5	79.3	346.0	517.3	164.6	136.25	16.5	15.2	10.5	N/A
July	90.2	123.8	31.9	40.6	492.9	450.2	489.5	338.35	20.9	19.5	20.8	N/A
Aug.	39.0	47.2	46.6	120.6	424.1	378.2	406.3	250.5	18.7	17.2	18.1	N/A
Sept.	101.8	142.0	127.0	88.7	307.5	281.7	291.6	137.1	15.2	14.4	14.72	N/A
Total	427.6	515.6	340.5	397.4	1782.4	1883.4	1492.9	894.6	16.6	15.4	14.7	N/A

4.2.2 Experimental design

The experiment at all sites and years was laid out as 2*6 factor factorial in a randomized complete block design (RCBD) with four replicates. The experimental treatment factors consisted of two oilseed species (canola 'InVigor 5440' and camelina-CDI007) and 6 N application rates, designated as 0 (N_0), 25 (N_{25}), 75 (N_{75}), 125 (N_{125}), 175 (N_{175}) and 225 (N_{225}) kg N ha^{-1}. Different seasonal precipitation in experimental sites was considered as a factor when data was analyzed.

Camelina and canola were seeded with a Hege plot drill (H and N Equipment Inc., Colwich, Kansas, USA) at the Canning site in 2013, and a plot-seeder XXL (Wintersteiger AG Austria) at Truro site in 2013 and 2014 and Harrington site in 2014. Plots were sown 5 metres in length and 2.5 metres in width, consisted of 16 rows with a row spacing of 15 centimetres and a seeding depth of 0.5-1.0 centimetre. The seeding date in each site in both years is shown in Table 4.3.

In May each year, canola seeds were sown at rate of 3.75 kg ha^{-1} while the seeding rate for camelina was 5 kg ha^{-1}. In order to obtain good seed distribution in field, seeds were sown with a mixture of viable and dead seeds at a ratio of 1:1 and 3:2 for camelina and canola respectively. Dead seeds were produced by autoclaving and checked afterwards to ensure they were not viable.

Nitrogen fertilizers were broadcasted to each plot in the form of Dolomite-Ammonium Nitrate (27-0-0). Nitrogen applications for higher N treatments including N_{125}, N_{175} and N_{225} were split into two doses: 50% of N as a pre-plant application and the other half was top-dressed when each crop reached their early flowering stage (Late June to early

July) (Table 4.3). In addition, 20 kg ha^{-1} S and 30- 40 kg ha^{-1} P and K were applied pre-plant at all sites.

When 90% of pods were brown in colour and seeds of canola and camelina in the top pods turned brown or black and brown, respectively, the crops were harvested. The Hege 125C plot combine harvester (Hege USA, Colwich, Kansas, USA) was used to harvest the crops in all sites in both years. The harvest area was 6.25 m^2 (5m ×1.25m) at all sites and years with the exception of 12.5 m^2 (5m ×2.5m) at the Truro site in 2013. The schedule of crop management operations is summarized in Table 4.3. Harvested seeds were air dried for 72 hours at room temperature (20 °C). Contaminants in seeds were cleaned by using a mechanical seed cleaner (Clipper Seed Cleaning Co.).

Table 4.3: Description of crop management information in tested sites in 2013 and 2014

Canning, NS (2013)	May 07	May 07	June 21	Aug. 29
Truro, NS (2013)	May 21	May 21	July 4	Sept. 19
Truro, NS (2014)	May 23	May 23	July 02	Sept. 08
Harrington, PEI (2014)	May 30	May 30	July 15	Sept. 17

4.2.3 Data Collection

4.2.3.1 Plant density

Plant density was assessed by counting the number of plants in two subsamples with three rows in 0.5 metres (0.225 m^2) from each plot. Subsamples were chosen randomly but the outside rows were avoided. Plant stand was counted after harvesting.

4.2.3.2 Plant height

Plant height was measured on three randomly selected plants from each plot when plants were mature. The height from soil surface level to the highest point on the erect plants was measured.

4.2.3.3 Yield components

Six mature plants from each plot were randomly selected and sampled to quantify the number of branches per plant, number of seed pods per plant, number of seeds per plant, and thousand kernel weight (TKW).

4.2.3.4 Seed yield and seed quality

Seed yields were determined when seed moisture content reached approximately 8% with drying. Seed protein content and oil content were analyzed by near-infrared spectroscopy (NIRs) (Unity Scientific, Spectra Star 2500x) on 150g seed samples.

4.2.4 Statistical Analysis

Minitab 17 Statistical Software (Minitab Inc., USA, 2013) was used in the analysis of data. The General linear model (GLM) was used to test the effects of applied N on camelina and canola growth, grain yield and seed quality. The comparison of the differences among treatments was completed by Tukey. The significace level was set to be 0.05. A partial correlation coefficient was used to examine the correlations between seed yield and yield components after removing the effect of applied N.

4.3 Results

4.3.1 Plant density

Table 4.4 shows the significant interactive effect of location, crop species and N h on plant population in 2013; in 2014 the interactive effect of location and species on plant population was significant.

Camelina and canola grown at the Canning site showed higher plant density than that planted at the Truro site in 2013 (Table 4.5). At both sites, camelina had a higher plant stand than canola in 2013. There were 256 and 177 plants of camelina per square meter, on average, at Canning and Truro, respectively; while 175 and 134 plants of canola per square meter were counted at Canning and Truro, respectively in 2013 (Table 4.5).

A negative effect of applied N on plant density of the two crops was found at both sites in 2013, but the magnitude of the effect varied with crop species and growing environments (Table 4.5). At the Canning site, the highest density of camelina population (338 plants m^{-2}) was observed with 25 kg ha^{-1} N applied, while significant difference did not occur until N rate was applied at 225 kg ha^{-1} and 194 camelina plants/m^2 were grown under this N rate (Table 4.5). However, the camelina plant population at the Truro site responded to applied N from 0 to 225 kg ha^{-1} and a significant difference was not found in all N treatments (Table 4.5). The highest and lowest camelina plant populations were 205 and 163 plants m^{-2} with applied N rate of 75 kg ha^{-1} and 175 kg ha^{-1}, respectively at the Truro site in 2013 (Table 4.5). The applied N did not significantly affect canola plant population at Canning in 2013. The highest and lowest canola plant populations were 232 and 140 plants m^{-2}, observed in the checks and plots with N rate of 25 kg ha^{-1}, but a significant difference was not recorded in all N treatments (Table 4.5). High N rates (125, 175 and 225 kg ha^{-1}) and low N rate (25 kg ha^{-1}) did not significantly impact canola plant density at Truro in 2013 and the plant density plateaued at an applied N rate of 75 kg ha^{-1} with 140 plants m^{-2} (Table 4.5).

In 2014, the plant density of camelina population at Truro was 319 plants m^{-2} on average, which was significantly higher than that grown at Harrington, PEI (184 plants m^{-2} on average) (Figure 4.1). However, canola at Harrington, PEI had a higher plant population (167 plants m^{-2} on average) than that at Truro, which was 153 plants m^{-2} on average and significant difference was not observed between them (Figure 4.1).

Table 4.4: ANOVA table of applied N effects on plant density of camelina and canola in two locations in 2013 and 2014

Year	Location	Effect	F-Value	P-Value
2013	**Truro, NS Canning, NS**	Location (L)	30.47	0.000
		Species (Sp)	32.78	0.000
		Nitrogen (N)	2.57	0.034
		L*Sp	3.02	0.087
		L*N	0.47	0.798
		N*Sp	1.54	0.189
		L*N*Sp	**2.54**	**0.036**
2014	**Truro, NS Harrington, PEI**	L	27.16	0.000
		Sp	61.65	0.000
		N	1.44	0.222
		L*Sp	**41.03**	**0.000**
		L*N	0.26	0.934
		Sp*N	1.29	0.276
		L*Sp*N	0.61	0.695

Table 4.5: The interactive effect of location, species and N on plant density in 2013

Location	Applied N (kg ha^{-1})	Plant density (# plants m^{-2})	
		Camelina	Canola
Canning, NS	0	284 ab	232 a-d
	25	338 a	140 cd
	75	280 a-c	165 b-d
	125	239 a-d	143 b-d
	175	198 a-d	185 b-d
	225	194 b-d	185 b-d
	Mean	256	175
Truro, NS	0	186 b-d	155 b-d
	25	165 b-d	159 b-d
	75	205 a-d	140 c
	125	179 b-d	115 d
	175	163 b-d	126 d
	225	166 b-d	110 d
	Mean	177	134

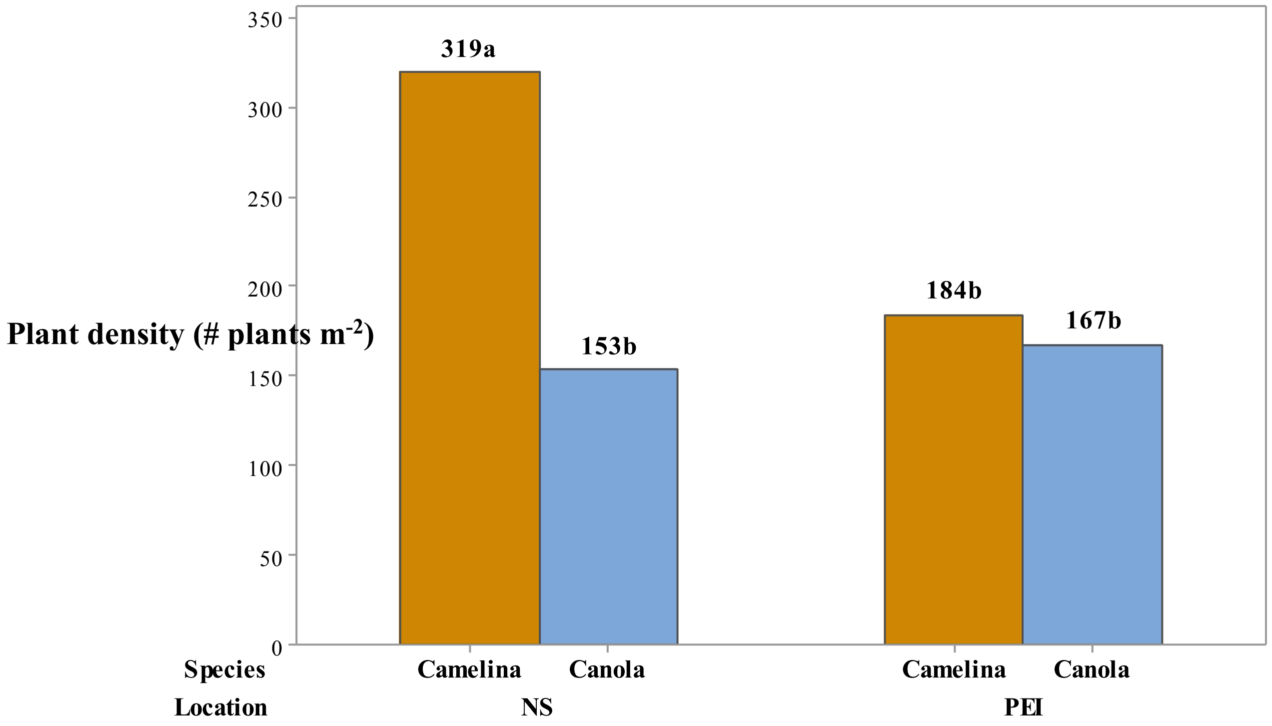


Figure 4.1: The combined effect of location and species on plant density in 2014
(Means followed by different letters are significantly different ($p < 0.05$)

4.3.2 Plant height

A significant effect of applied N on plant height of camelina and canola was observed in 2013 (Table 4.6). The interaction of location and crop species significantly affected plant height of camelina and canola in 2013 (Table 4.6). However, location, crop species and applied N had independent significant effects on plant height in 2014 (Table 4.6).

The plant height of canola grown at Truro in 2013 averaged 121 cm, higher than that at Canning (117 cm), but not significantly different between them. However, camelina at Canning in 2013 grew as high as 88 cm, on average, which was significantly higher than that at Truro (77 cm) (Figure 4.2). The overall plant height of canola was higher than camelina at both sites in 2013 (Figure 4.2). The same result was found in 2014 (Table 4.7).

The crops grown at Truro in 2014 were significantly higher than those at Harrington, PEI. The averaged plant height in each site was 118 cm and 104 cm at Truro, NS and Harrington, PEI, respectively (Table 4.7).

The applied N significantly increased plant height of both crops in 2013 and 2014 (Figure 4.3). The plant height in 2013 ranged from 87 cm in the checks to 109 cm in the entries with 225 kg ha^{-1} N applied (Figure 4.3: 2013). A significant difference was not observed when the N rates were greater than 75 kg ha^{-1} (Figure 4.3: 2013). In 2014, a significant difference was found in all N treatments, but the shortest plant (103 cm), growing in the controls significantly differed from the tallest plants in the plots with 125 kg ha^{-1} N applied (Figure 4.3:2014).

Table 4.6: ANOVA table of applied N effects on plant height of camelina and canola in two locations in 2013 and 2014

Year	Location	Effect	F-Value	P-Value
2013	**Truro, NS Canning, NS**	Location (L)	5.37	0.023
		Species (Sp)	707.48	0.000
		Nitrogen (N)	**24.77**	**0.000**
		L*Sp	**25.75**	**0.000**
		L*N	1.45	0.217
		Sp*N	2.08	0.078
		L*Sp*N	0.38	0.859
2014	**Truro, NS Harrington, PEI**	**L**	**47.31**	**0.000**
		Sp	**493.87**	**0.000**
		N	**3.09**	**0.014**
		L*Sp	0.57	0.452
		L*N	1.18	0.326
		Sp*N	1.71	0.144
		L*Sp*N	0.76	0.582

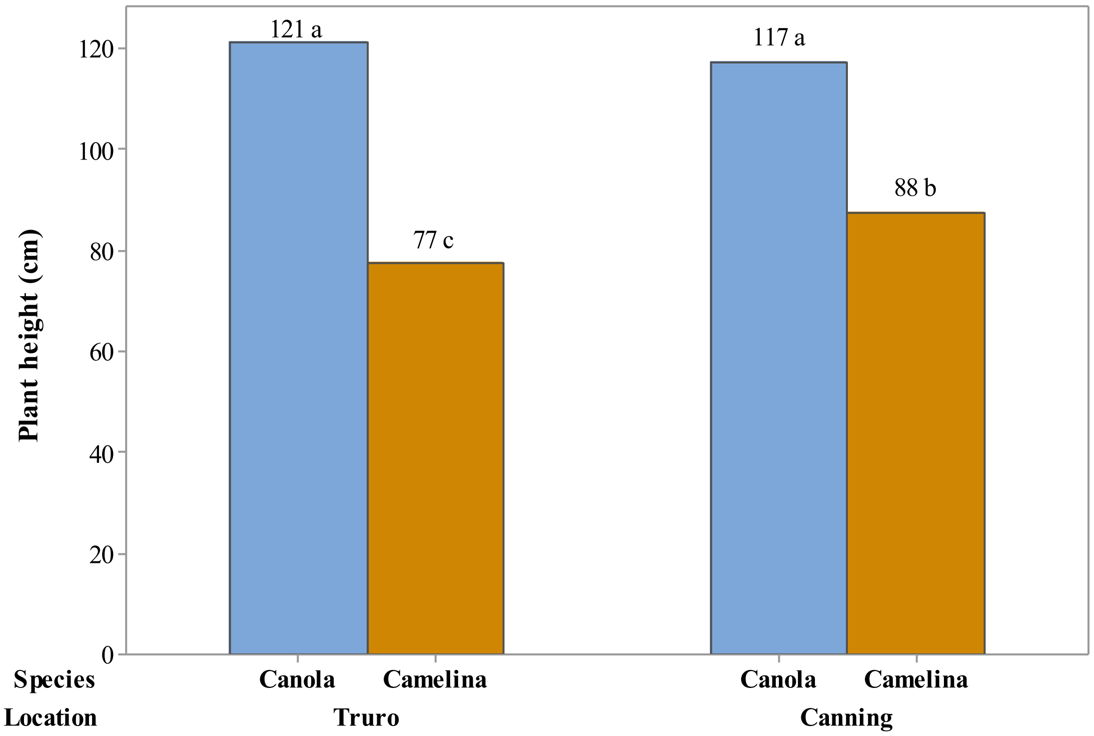


Figure 4.2: The interactive effect of location and species on plant height in 2013
(Means followed by different letters are significantly different with $p < 0.05$)

Table 4.7: The independent effect of location and crop species on plant height in 2014

Location	Species	Plant height (cm)
Truro, NS		118 a
Harrington, PEI		104 b
	Canola	133 a
	Camelina	89 b

(Means followed by different letters are significantly different with $p < 0.05$)

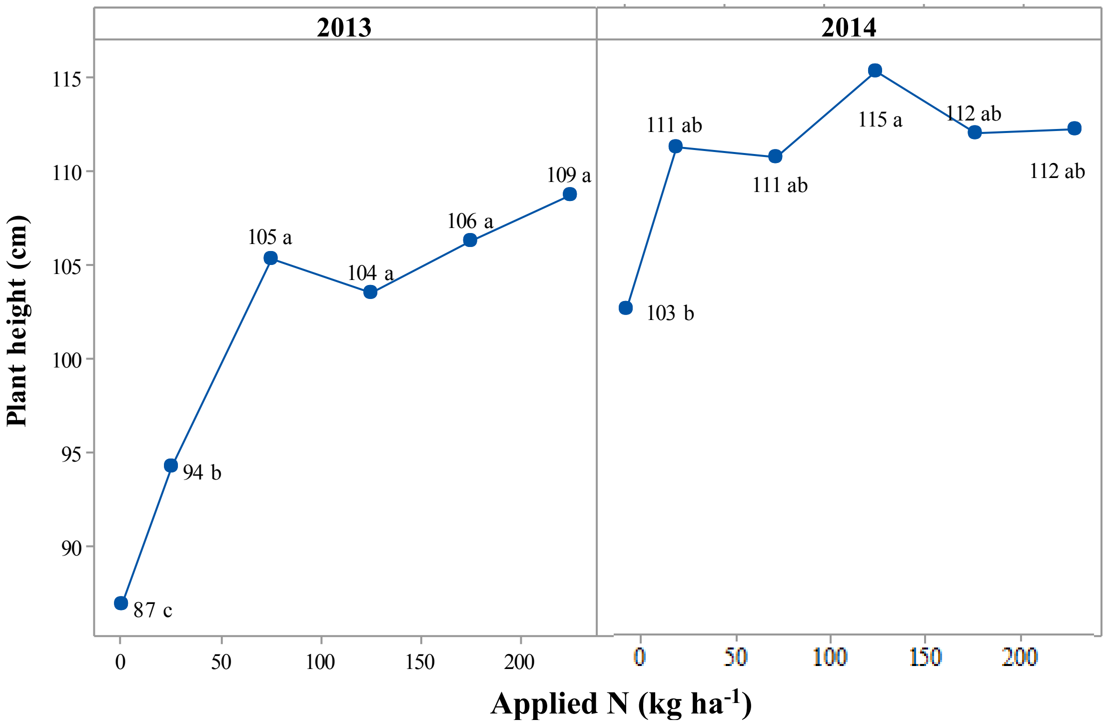


Figure 4.3: The effect of applied N on plant height in 2013 and 2014
(Means followed by different letters are significantly different with $p < 0.05$)

4.3.3 Seed yield

The analysis of variance (Table 4.8) showed that the interactive effect among experimental sites, applied N rate and crop species on seed yield was highly significant in 2013 and 2014. This indicated that the N effect on camelina and canola varied with specific environments.

Canola seed yield was greater than camelina in both Truro and Canning sites in 2013 (Table 4.9). The mean yields of camelina (1169 kg ha^{-1}) and canola (2522 kg ha^{-1}) at the Canning site were higher than those at Truro, NS (740 kg ha^{-1} and 1866 kg ha^{-1}for camelina and canola, respectively) in 2013 (Table 4.9). Camelina planted at Truro, NS in 2014 produced 1271 kg ha^{-1} seed on average which was less than canola (2486 kg ha^{-1}) in the same site; however, camelina yielded 1714 kg ha^{-1} which was more than canola (1209 kg ha^{-1}), on average, when grown at Harrington, PEI in 2014 (Table 4.10).

The positive effect of N was found at both sites in two years but the maximum N requirement was different. In 2013, the highest camelina and canola seed yields (1544 kg ha^{-1} and 3492 kg ha^{-1}, respectively) at the Canning site were obtained with 225 kg ha^{-1} N applied, but a significant difference was not found when N was applied at 75 kg ha^{-1} for camelina and 175 kg ha^{-1} for canola (Table 4.9). 47.2 % and 85.8 % variability in camelina and canola seed yields, respectively at the Canning site could be explained by the change in N levels (Figure4.4). The seed yield of camelina at Truro in 2013 positively responded to N rates ranging from 0 to 225 kg ha^{-1}. The maximum camelina seed yield was 1017 kg ha^{-1} produced at an applied N rate of 225 kg ha^{-1} and the minimum yield (453 kg ha^{-1}) was found in the check but, the difference was not significant. However, the highest seed yield of canola (2264 kg ha^{-1}) at Truro in 2013 was produced at N rates of 175 kg ha^{-1}; a significant difference was not seen at an N rate of 75 kg ha^{-1}. 52.0 % and 68.8 % variability in seed yields of camelina and canola, respectively, could be explained by the change of applied N levels (Figure 4.4).

In 2014, camelina seed yield at the Truro site responded to applied N rates as high as 225 kg ha^{-1} (Table 4.10) but a significant differences were not observed with all N rates. 77.4% variability in camelina seed yield at the Truro site could be explained by the change of applied N rates (Figure 4.5). The maximum and minimum seed yield of camelina (1572 kg ha^{-1} and 868 kg ha^{-1}, respectively) were produced at applied N rates of 225 kg ha^{-1} and 0 kg ha^{-1}. The seed yield of canola at Truro in 2014 was maximized at 3128 kg ha^{-1} with 225 kg ha^{-1} N applied, but the seed yield reached a plateau at N rates of 125 kg ha^{-1} (Table 4.10). 70.9% variability in canola seed yield at the Truro site could be explained by the change of applied N rates (Figure 4.5). It must be highlighted that camelina planted at Harrington, PEI produced the higher seed yield than canola in the same site. Both camelina and canola grown in PEI produced highest seed yield, 2344 kg ha^{-1} and 1804 kg ha^{-1}, respectively with additional N applied at 225 kg ha^{-1} for camelina and 175 kg ha^{-1} for canola. The seed yield of both crops reached a plateau at N rates of 75 kg ha^{-1} (Table 4.10). The regression model in Figure 4.5 showed that applied N had a strong relationship with R-Sq (adj) 82.3 % on the seed yield of camelina. However, the correlation between applied N and canola seed yield was weak with R-Sq (adj) 38.1% (Figure 4.5).

Table 4.8: ANOVA table of applied N effects on seed yield of camelina and canola in two locations in 2013 and 2014

Year	Location	Effect	F-Value	P-Value
2013	**Truro, NS** **Canning, NS**	Location (L)	147.54	0.000
		Species (Sp)	770.52	0.000
		Nitrogen (N)	53.9	0.000
		L*Sp	6.45	0.013
		L*N	7.69	0.000
		Sp*N	4.62	0.001
		L*Sp*N	**3.57**	**0.006**
2014	**Truro, NS** **Harrington, PEI**	L	58.67	0.000
		Sp	42.51	0.000
		N	38.2	0.000
		L*Sp	250.35	0.000
		L*N	1.19	0.324
		Sp*N	0.85	0.519
		L*Sp*N	**4.96**	**0.001**

Table 4.9: Nitrogen effect on seed yield of camelina and canola at Truro and Canning, NS in 2013

Location	Applied N (kg ha^{-1})	Yield (kg ha^{-1})	
		Camelina	Canola
Canning, NS	0	748 kl	1716 e-h
	25	668 kl	1716 e-h
	75	1200 h-k	2364 cd
	125	1476 f-j	2708 bc
	175	1380 g-j	3136 ab
	225	1544 f-i	3492 a
	Mean	1169	2522
Truro, NS	0	453 l	1440 f-j
	25	540 l	1520 f-j
	75	743 kl	1844 d-g
	125	753 kl	2138 c-e
	175	936 j-l	2264 c-e
	225	1017 i-l	1992 d-f
	Mean	740	1866

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

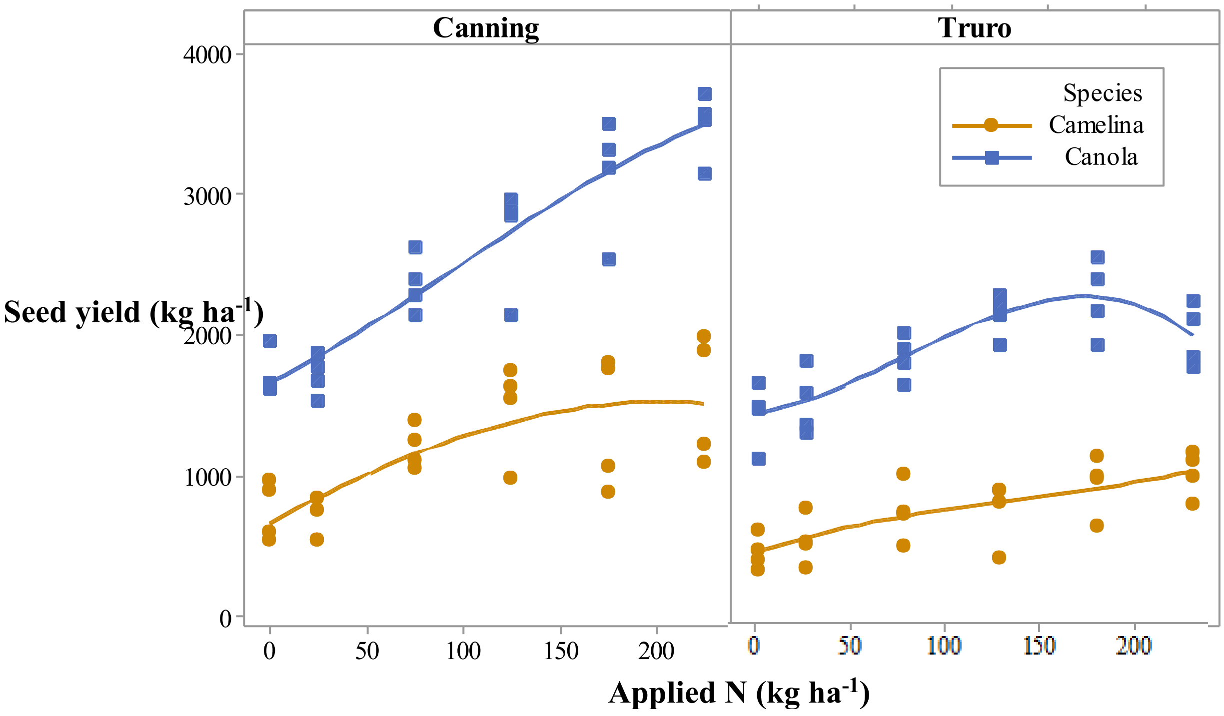


Figure 4.4: Regression analysis of the applied N effect on seed yield of camelina and canola at the Canning and Truro sites in NS in 2013

Y (Camelina at Canning) = 654.6 + 7.492 N - 0.01070N*2 - 0.000026 N**3 with R-Sq (adj) = 47.2%
Y (Camelina at Truro) = 450.7 + 4.619 N - 0.02038 N*2 + 0.000050 N**3 with R-Sq (adj) = 52.0%
Y (Canola at Canning) = 1655 + 6.781 N + 0.02525 N*2 - 0.000086 N**3 with R-Sq (adj) = 85.8%
Y (Canola at Truro) = 1436 + 2.358 N + 0.05889 N*2 - 0.000259 N**3 with R-Sq (adj) = 68.8%

Table 4.10: Nitrogen effect on seed yield of camelina and canola at Truro, NS and Harrington, PEI in 2014

Location	**Applied N (kg ha^{-1})**	**Yield (kg ha^{-1})**	
		Camelina	**Canola**
Truro, NS	0	868 hi	1808 c-f
	25	1048 g-i	1996 c-e
	75	1256 f-i	2340 bc
	125	1476 d-i	2828 ab
	175	1404 d-i	2816 ab
	225	1572 d-h	3128 a
	Mean	1271	2486
Harrington, PEI	0	956 hi	820 i
	25	1076 ghi	868 hi
	75	1736 c-g	1220 f-i
	125	2100 cd	1236 f-i
	175	2076 cd	1804 c-f
	225	2344 bc	1304 e-i
	Mean	1714	1209

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

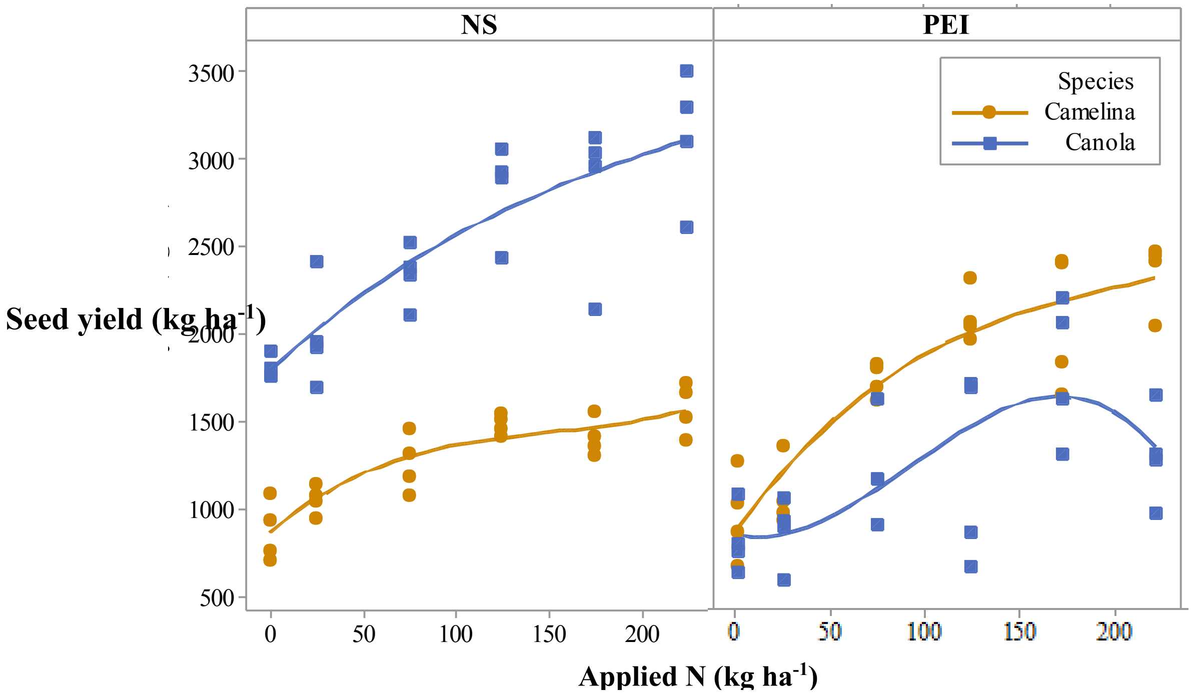


Figure 4.5: Regression analysis of the applied N effect on seed yield of camelina and canola in PEI and NS in 2014

Y (Camelina in NS) = 858.3 + 9.074 N - 0.05213 N*2 + 0.000114 N**3 with R-Sq (adj) = 77.4%

Y (Camelina in PEI) = 878.7 + 14.69 N - 0.05631 N*2 + 0.000086 N**3 with R-Sq (adj) = 82.3%

Y (Canola in NS) = 1785 + 9.898 N - 0.02497 N*2 + 0.000031 N**3 with R-Sq (adj) = 70.9%

Y (Canola in PEI) = 848.2 - 2.304 N + 0.1048 N*2 - 0.000376 N**3 with R-Sq (adj) = 38.1%

4.3.4 Yield components

Table 4.11 illustrates the effects of location, crop species and applied N on yield components including number of branches, seed pod and seeds per plant and thousand kernel weight (TKW). Number of branches per plant was significantly affected by N and crop species independently in 2013 and 2014 and the factor location in 2013 (Table 4.11). The interaction of location and applied N had significant effect on number of seed pods per plant in 2013 and 2014 (Table 4.11). The same parameter was also affected by the interactive effect of crop species and applied N in 2013 and interaction of location and crop species in 2014 (Table 4.11). A three-way interaction of location, crop species and applied N significantly affected the number of seeds per plant n 2013 and 2014 (Table 4.11). A three-way interaction of location, crop species and applied N had significant effect on TKW in 2014, which was only affected by location and two way interaction of crop species and applied N in 2013 (Table 4.11).

Table 4.11: ANOVA table of applied N effects on yield components (number of branches, seed pods and seeds and thousand kernel weight (TKW) of camelina and canola in two locations in 2013 and 2014

Year	**Location**	**Effect**	**P-Value**			
			Branches/ plant	**#Seed pods/plant**	**#Seeds/ plant**	**TKW**
2013	**Truro, NS Canning, NS**	Location (L)	**0.011**	0.013	0.046	**0.001**
		Nitrogen(N)	**0.000**	0.000	0.000	0.000
		Species (Sp)	**0.000**	0.000	0.031	0.000
		L*N	0.121	**0.012**	0.090	0.269
		L*Sp	0.084	0.162	0.004	0.176
		Sp*N	0.607	**0.003**	0.791	**0.021**
		L*Sp*N	0.935	0.255	**0.006**	0.060
2014	**Truro, NS Harrington, PEI**	L	0.349	0.001	0.070	0.000
		N	**0.031**	0.001	0.000	0.083
		Sp	**0.000**	0.000	0.000	0.000
		L*N	0.627	**0.032**	0.002	0.372
		L*Sp	0.727	**0.000**	0.000	0.000
		Sp*N	0.268	0.060	0.006	0.138
		L*Sp*N	0.523	0.183	**0.012**	**0.001**

In 2013, crops at Truro produced more branches than those grown at Canning in 2013 (Table 4.12). The numbers of branches of camelina plants in each year were 6.49 and 12.38 on average in 2013 and 2014, respectively, which were higher than those of canola (3.69 in 2013 and 3.63 in 2014) (Table 4.12). With applied N increased, the number of branches per plant wasincreased from 4.23 to 6.15, but a significant difference was not found when applied N was over 125 kg ha^{-1} in 2013. The least branch number, 5.77 on average, in 2014 was recorded at N rate of 25 kg ha^{-1} and a significant difference was not observed in all N treatments in 2014 (Table 4.12).

Table 4.12: The independent effect of location, applied N and crop species on number of branches per plant in 2013 and 2014

Location	**Species**	**Applied N (kg ha^{-1})**	**# of branches /plant**	
			2013	**2014**
Truro, NS			5.34 a	
Canning, NS			4.84 b	
	Camelina		6.49 a	12.38 a
	Canola		3.69 b	3.63 b
		0	4.23 c	7.16 ab
		25	4.33 c	5.77 b
		75	5.00 bc	8.88 a
		125	5.36 ab	8.47 ab
		175	5.48 ab	9.88 a
		225	6.15 a	7.90 ab

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

The applied N dramatically increased the number of seed pods per plant at both sites in 2013; however, a significant difference in the parameter between the two sites was not detected when an applied N rate was greater than 75 kg ha^{-1} (Figure 4.6). The highest numbers of seed pod per plant at Truro and Canning were 147 and 127, respectively, which were both obtained at N rate of 225 kg ha^{-1}, while the lowest seed pods per plant was found in the checks at the Truro site (69 pods/plant) and in the plots with N rate of 25 kg ha^{-1} at the Canning site (50 pods/plant) (Figure 4.6).

The number of seed pods of camelina and canola were positively correlated with applied N rates. Camelina produced more seed pods than canola in all N treatments in 2013 (Figure 4.7). The highest (178 and 97) and lowest (86 and 43) numbers of seed pods for camelina and canola were obtained at applied N rates of 225 kg ha^{-1} and 0 kg ha^{-1} and the plateau was reached at N rate of 175 kg ha^{-1} (Figure 4.7).

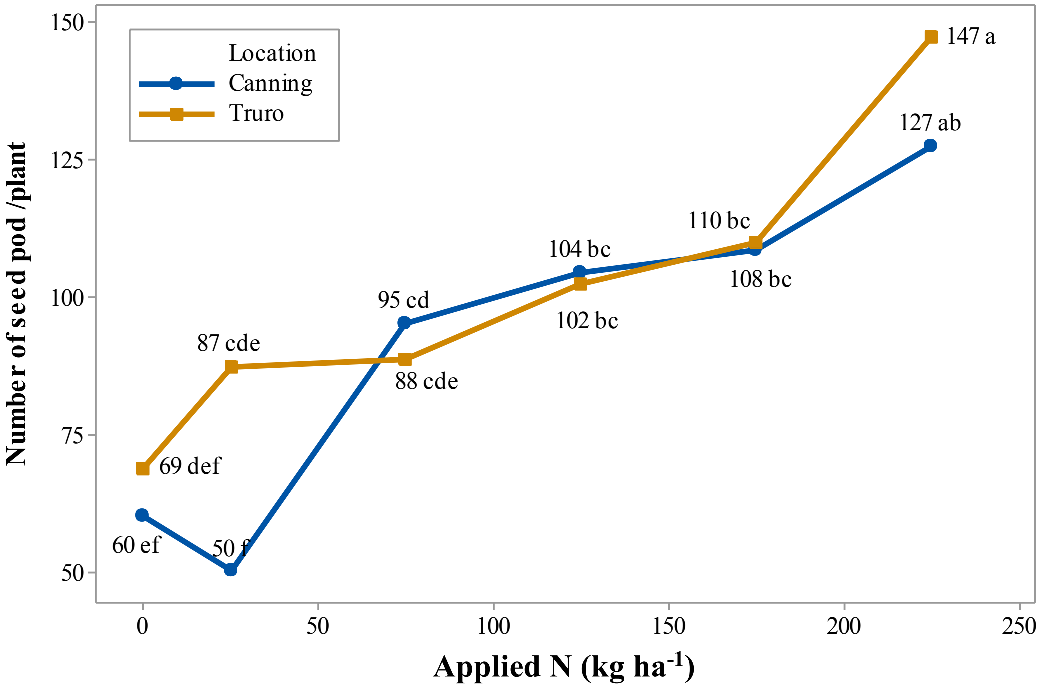


Figure 4.6: The interactive effect of location and applied N on # of seed pods /plant in 2013 (Means followed by different letters are significantly different with $p < 0.05$)

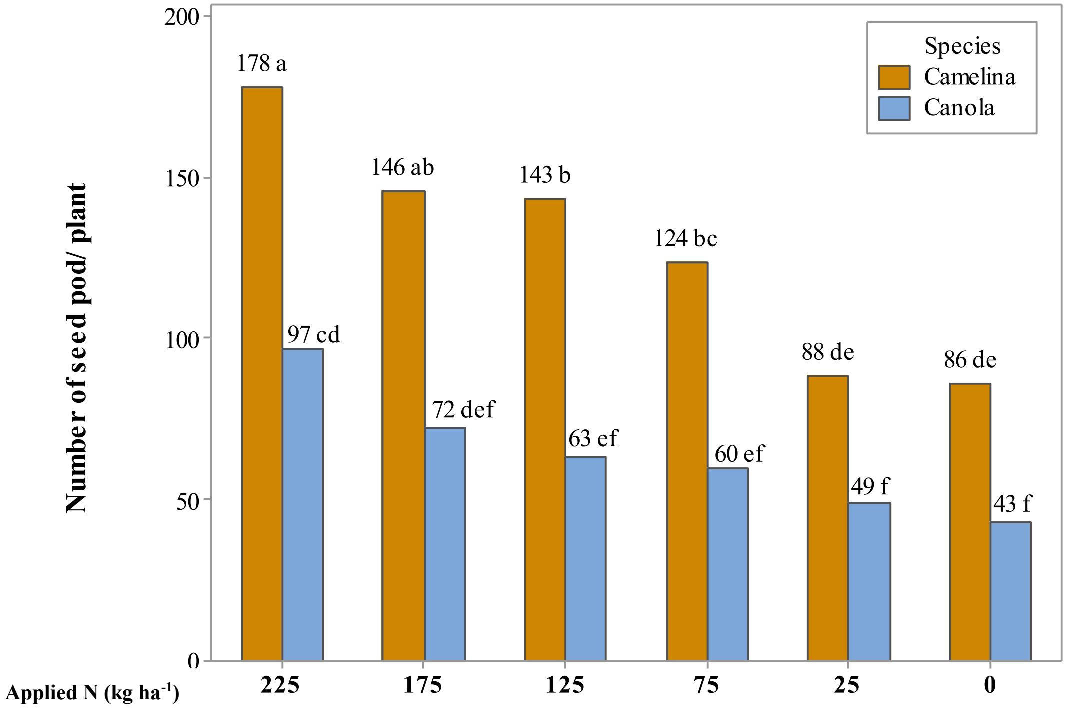


Figure 4.7: The interactive effect of crop species and applied N on number of seed pods /plant in 2013 (Means followed by different letters are significantly different with $p < 0.05$)

Camelina in PEI produced 315 pods/plant, on average, which was more than at Truro (215 pods/plant) in 2104 (Figure 4.8). A significant difference of the seed pod number of canola was not found at either site (Figure 4.8). The seed pod number per camelina plant was significantly higher than canola in PEI and NS sites in 2014 (Figure 4.8). Applied N at low rates had a positive effect on seed pod formation, while high N rates negatively affected seed pod number in PEI in 2014 (Figure 4.9). The number of seed pod was increased to 272 pods/plant with applied N increasing to 125 kg ha^{-1} then deceased to 184 pods/plant in PEI in 2014 (Figure 4.9). The lowest number of seed pods per plant was observed at an N rate of 25 kg ha^{-1} in PEI. Applied N did not significantly affect seed pod number per plant in NS in 2014 (Figure 4.9). The number of seed pods fluctuated between 114 and 179 pods/plant, but there was no significant difference in all N treatments (Figure 4.9).

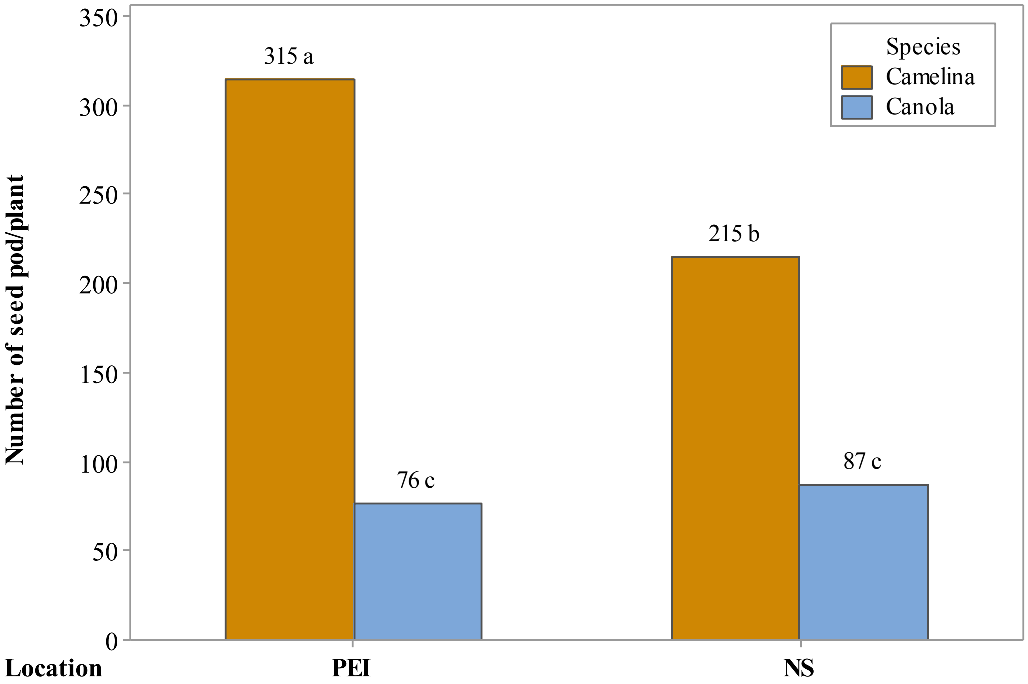


Figure 4.8: The interactive effect of crop species and location on number of seed pods/ plant in 2014 (Means followed by different letters are significantly different with $p < 0.05$)

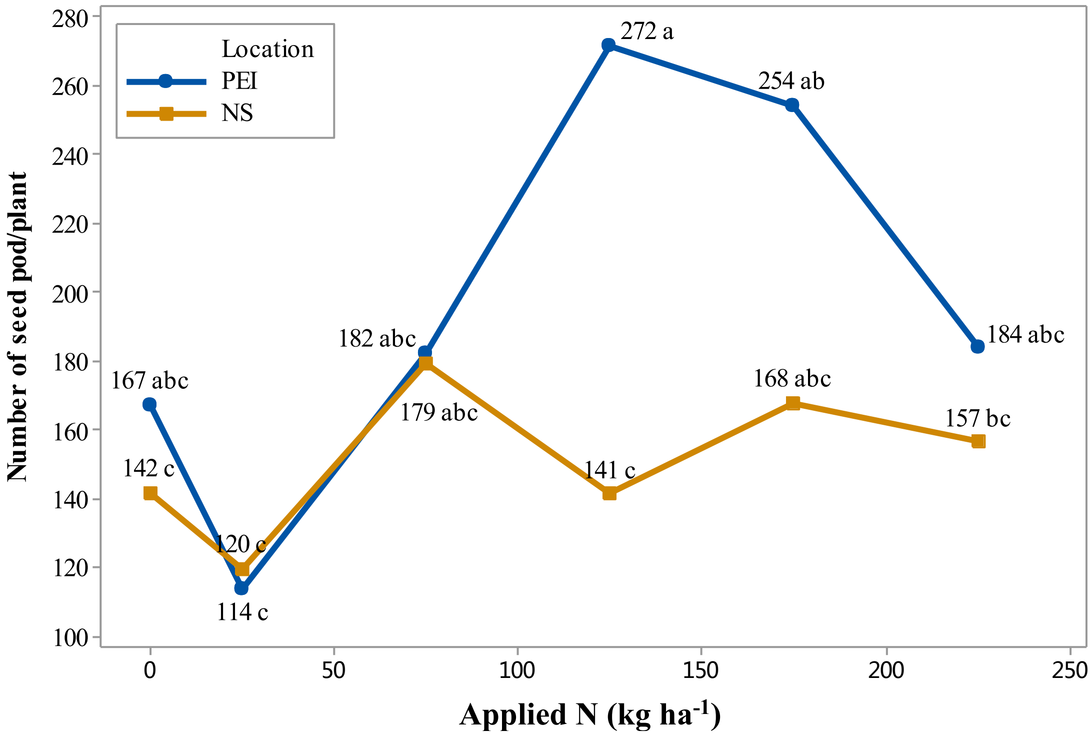


Figure 4.9: The interactive effect of applied N and location on number of seed pods /plant in 2014 (Means followed by different letters are significantly different with $p < 0.05$)

A three way-interaction of locations, crop species and applied N significantly affected the number of seeds per plant in 2013 and 2014. Camelina grown at Canning, NS produced less seeds (886 seeds/plant) than at Truro, NS (1084 seeds/plant) in 2013; while the number of canola seeds at Canning was 1094 seeds/plant, which was slightly higher than at Truro (1053 seeds/plant) (Table 4.13). In 2014, camelina at Harrington, PEI produced the most seeds (2473 on average) in each plant, compared with the numbers of camelina at Truro (1391) and canola at Harrington and Truro with 1098 and 1769 seeds/plant, respectively (Table 4.14).

The number of seeds per plant was positively correlated with applied N in two sites in 2013. The highest numbers of seeds per camelina plant at Canning and Truro were 1201 and 1720 seeds/plant which were occurred at N rate of 225 kg ha^{-1} (Table 4.13). However, the optimum N rated were 75 kg ha^{-1} and 175 kg ha^{-1} at Canning and Truro, respectively in 2013 (Table 4.13). The highest number of seeds per canola plant, 1756 and 1300 seeds/plant at Canning and Truro, respectively, were found at N rate of 225 kg ha^{-1} in 2013 (Table 4.13). A significant difference was not observed in all N treatments at Truro but occurred when applied N rate was lower than 175 kg ha^{-1} at Canning in 2013 (Table 4.13). The applied N generated different results in 2014. The applied N did not significantly affected the number of seed/plant of camelina at Truro and canola at Harrington and Truro in 2014 but a remarkable effect was found on camelina at Harrington (Table 4.14). The number of camelina seeds per plant peaked at 3747 with 125 kg ha^{-1} N applied, then the number was decreased with applied N rates increasing (Table 4.14).

Table 4.13 The interactive effect of location, crop species and applied N on number of seeds/plant in 2013

Location	**Applied N (kg ha^{-1})**	**Number of seeds/plant**	
		Camelina	**Canola**
Canning, NS	0	524 gh	715 e-h
	25	381 h	666 f-h
	75	934 c-g	1067c-f
	125	1091 c-f	1059 c-f
	175	1188 c-e	1307 a-c
	225	1201 a-f	1756 a
	Mean	886	1094
Truro, NS	0	655 e-h	746 d-h
	25	819 c-h	895 c-h
	75	944 c-g	1035 c-g
	125	1124 c-f	1214 b-e
	175	1245 a-d	1132 c-f
	225	1720 ab	1300 a-c
	Mean	1084	1053

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

Table 4.14 : The interactive effect of location, crop species and applied N on number of seeds per plant in 2014

Location	Applied N (kg ha^{-1})	Number of seeds/plant	
		Camelina	Canola
Harrington, PEI	0	2012 b-e	857 de
	25	1416 c-e	697 e
	75	2002 b-e	1071 de
	125	3747 a	1198 c-e
	175	3468 ab	1474 c-e
	225	2196 b-d	1297 c-e
	Mean	2473	1098
Truro, NS	0	1465 c-e	1015 de
	25	1148 c-e	1250 c-e
	75	1942 c-e	2116 b-e
	125	1442 c-e	1403 c-e
	175	1253 c-e	2271 a-d
	225	1097 c-e	2563 a-c
	Mean	1391	1769

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

Thousand kernel weight at Canning in 2013 was 2.25g, which was higher than 2.17g at Truro (Table 4.15). The TKW of canola on average was 3.22 g which was more than double to 1.19g of camelina TKW in 2013 (Table 4.15). The applied N did not affected camelina TKW but increased canola TKW. The highest TKW of canola was 3.4 g, recorded at N rate of 175 kg ha^{-1}, but significant difference was not observed at N rate of 125 kg ha^{-1} (Table 4.15). Non-effect of applied N on the TKW of canola at Truro and camelina at Harrington and Truro in 2014 (Table 4.16). The TKW of canola at Truro was enhanced by applied N and the plateau was reached at N rate of 125 kg ha^{-1} (Table 4.16).

Table 4.15: The effect of location and the combined effect of applied N and species on TKW (g) in 2013

Location	Applied N (kg ha^{-1})	TKW (g)	
Canning, NS		2.25 a	
Truro, NS		2.17 b	
		Camelina	**Canola**
	0	1.10 d	3.09 c
	25	1.14 d	3.18 bc
	75	1.20 d	3.08 c
	125	1.22 d	3.23 a-c
	175	1.23 d	3.40 a
	225	1.25 d	3.35 ab
	Mean	1.19	3.22

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

Table 4.16: The interactive effect of location, crop species and applied N on TKW in 2014

Location	Applied N (kg ha^{-1})	TKW (g)	
		Camelina	**Canola**
Harrington, PEI	0	1.14 e	2.54 d
	25	1.14 e	2.64 d
	75	1.21 e	2.76 cd
	125	1.31 e	2.74 cd
	175	1.27 e	2.64 d
	225	1.29 e	2.55 d
	Mean	1.23	2.64
Truro, NS	0	1.20 e	2.90 b-d
	25	1.19 e	2.88 b-d
	75	1.14 e	2.86 b-d
	125	1.11 e	3.26 ab
	175	1.09 e	3.16 a-c
	225	0.99 e	3.43 a
	Mean	1.12	3.08

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

4.3.5 Seed yield and yield components

The correlations of the variables after removing the effect of applied N are shown in Table 4.17 and Table 4.18. All correlations between yield components and seed yield of camelina were statistically significant at 0.01 level (Table 4.17). Similar results were also found on canola except for the one between number of seed pods/plant and seed yield which showed a weak correlation (Table 4.18). The correlation coefficients between plant stands and seed yield of camelina and canola were 0.068 and 0.159 which indicated that a weak or no linear relationship remains between plant stand and seed yield after the applied N effect within these variables have been removed. Camelina seed yield was highly correlated with seed pods/plant (0.661 Corr. Coeff.) followed by seeds/plant, branches/plant and the TKW (Table 4.17). However, the TKW showed the greatest correlation with canola seed yield (0.681 Corr. Coeff.) followed by branches/plant and seeds /plant (Table 4.18).

Figure 4.9 and Figure 4.10 present the relationship between seed yield and yield components and plant stand. The positive relationships between yield and branches/plant, yield and seeds/plant, and yield and the TKW were observed in camelina and canola (Figure 4.9 & Figure 4.10). The seed pods/plant of camelina had stronger positive effect on seed yield than that of canola (Figure 4.9 & Figure 4.10). The plant stand did not showed strong effect on seed yield of camelina but a small positive effect on canola seed yield (Figure 4.9 & Figure 4.10).

Table 4.17 Correlations between camelina seed yield and yield components after removing the effect of applied N

Variables	#Branches/ plant	#Seed pods/plant	#Seeds /plant	TKW	Plant stand
#Branches/plant	--				
Significance	--				
#Seed pods/plant	0.902^{**}	--			
Significance	0.000	--			
#Seeds/plant	0.823^{**}	0.962^{**}	--		
Significance	0.000	0.000	--		
TKW	0.116	0.264^{*}	0.297^{**}	--	
Significance	0.296	0.016	0.006	--	
Plant stand	-0.009	-0.103	-0.202	-0.204	--
Significance	0.935	0.352	0.067	0.064	--
Seed yield	**0.451****	**0.611****	**0.554****	**0.342****	0.068
Significance	**0.000**	**0.000**	**0.000**	**0.002**	0.540

**. Correlation is significant at 0.01 level

*. Correlation is significant at 0.05 level

Table 4.18: Correlations between canola seed yield and yield components after removing the effect of applied N

Variables	#Branches/ plant	#Seed pods/plant	#Seeds/ plant	TKW	Plant stand
#Branches/plant	--				
Significance	--				
#Seed pods/plant	0.670^{**}	--			
Significance	0.000	--			
#Seeds/plant	0.687^{**}	0.771^{**}	--		
Significance	0.000	0.000	--		
TKW	0.268^{*}	-0.252^{*}	-0.026	--	
Significance	0.014	0.022	0.814	--	
Plant stand	0.071	0.019	0.153	-0.035	--
Significance	0.521	0.866	0.167	0.754	--
Seed yield	**0.313****	0.138	**0.300****	**0.681****	0.159
Significance	**0.004**	0.213	**0.006**	**0.000**	0.152

**. Correlation is significant at 0.01 level

*. Correlation is significant at 0.05 level

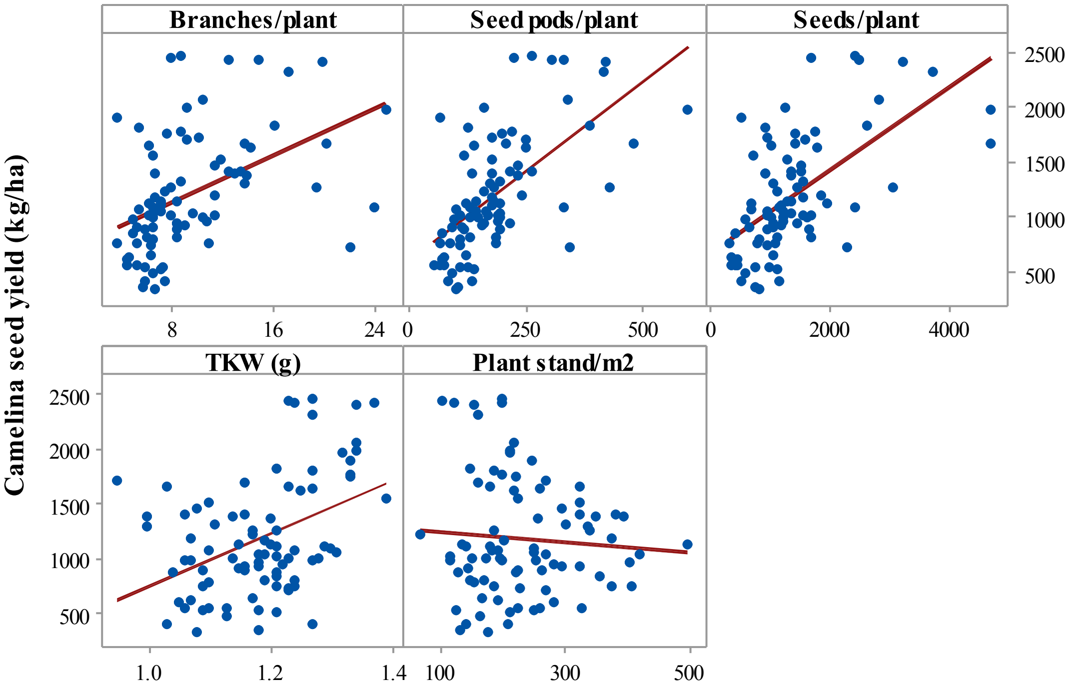


Figure 4. 9: Scatter plots between camelina seed yield and yield components and plant stand with significant relationships

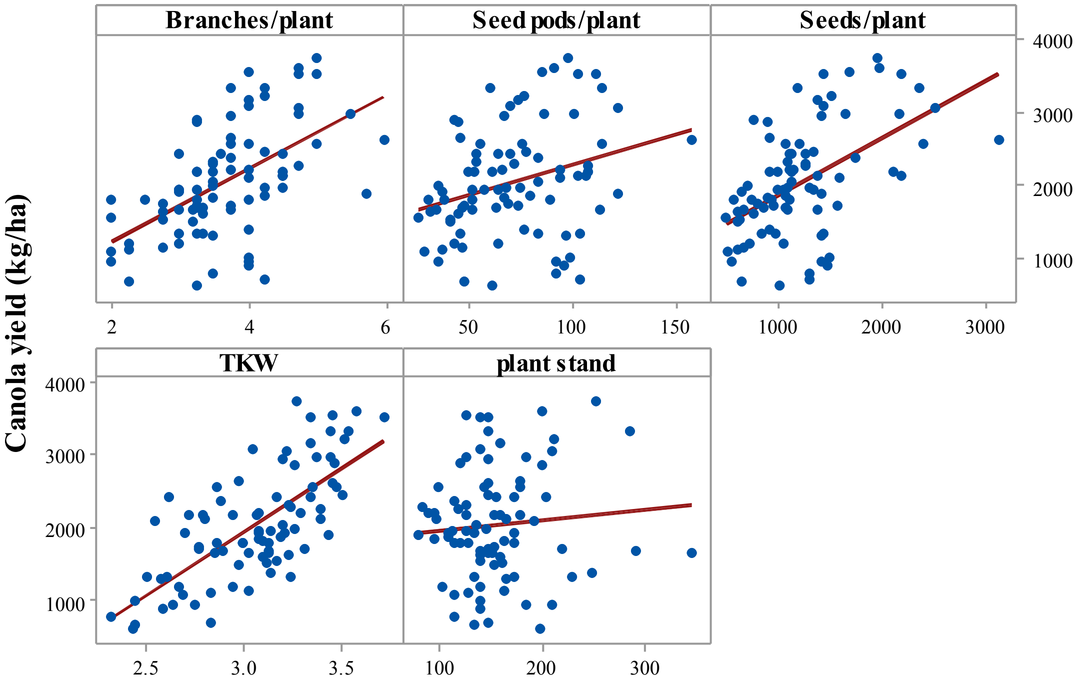


Figure 4. 10: Scatter plots between canola seed yield and yield components and plant stand with significant relationships

4.3.6 Oil content

Location, crop species and N each had a significant effect on seed oil content inboth years (Table 4.19). A statistically significant difference on oil content in the seeds between two sites was found in 2013 and 2014 (Table 4.20 & Table 4.21). However, the oil contents in the seeds at Canning and Harrington in 2014 were slightly higher at Truro in the same year (Table 4.20 & Table 4.21). Canola seeds contained 44.21 % of oil in 2013 and 42.90 % of oil in 2014, which were greater than that camelina contained in both years (34.69 % in 2013 and 36.17% in 2014) (Table 4.20 & Table 4.21). Negative effects of N on seed oil content was detected in both years. The highest seed oil content on average (40.86% in 2013 and 40.63% in 2014) were recorded when 25 kg ha^{-1} N applied, whereas, highest applied N rate (225 kg ha^{-1}) resulted in lowest oil content in the seeds (38.18 % in 2013 and 38.38% in 2014) (Table 4.20 & Table 4.21). The seed oil content of both crops decreased with an increase in applied N rate but then did not change when applied N rate was 125 kg ha^{-1} at Canning and Truro, NS in 2013 and Harrington, PEI in 2014. Camelina and canola oil content at Truro, NS in 2014 were leveled out at applied N rate of 125 kg ha^{-1} and 75 kg ha^{-1}, respectively (Table 4.22 & Table 4.23).

Table 4.19: ANOVA table of applied N effects on oil content in camelina and canola seeds in two locations in 2013 and 2014

Year	Location	Effect	F-Value	P-Value
2013	**Truro, NS** **Canning, NS**	**Location (L)**	**7.21**	**0.009**
		Species (Sp)	**2337.99**	**0.000**
		Nitrogen (N)	**23.24**	**0.000**
		L*N	0.79	0.557
		L*Sp	3.25	0.076
		Sp*N	0.99	0.432
		L*Sp*N	1.27	0.286
2014	**Truro, NS** **Harrington, PEI**	**L**	**50.81**	**0.000**
		Sp	**1656.81**	**0.000**
		N	**24.38**	**0.000**
		L*N	1.77	0.130
		L*Sp	0.02	0.896
		Sp*N	1.12	0.359
		L*Sp*N	0.74	0.597

Table 4.20: The effects of location, species and N on oil content in the seeds in 2013

Location	Species	Applied N (kg ha^{-1})	Oil content (%)
Canning, NS			39.71 a
Truro, NS			39.18 b
	Canola		44.21 a
	Camelina		34.69 b
		0	40.42 a
		25	40.86 a
		75	40.15 a
		125	38.75 b
		175	38.33 b
		225	38.18 b

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

Table 4.21: The effects of location, species and N on oil content in the seeds in 2014

Location	Species	Applied N (kg ha^{-1})	Oil content (%)
Harrington, PEI			40.13 a
Truro, NS			38.95 b
	Canola		42.90 a
	Camelina		36.17 b
		0	40.62 a
		25	40.63 a
		75	39.94 a
		125	39.06 b
		175	38.59 b
		225	38.38 b

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

Table 4.22: Nitrogen effect on oil content in the seeds of camelina and canola in two sites in 2013

N (kg ha^{-1})	Canning, NS		Truro, NS	
	Camelina	**Canola**	**Camelina**	**Canola**
0	35.76 a	45.92 ab	36.05 a	45.12 a
25	35.47 a	46.80 a	35.82 a	44.67 ab
75	35.41 a	45.67 a-c	34.86 ab	44.16 ab
125	34.34 ab	43.71 b-d	33.80 bc	43.16 bc
175	34.15 ab	43.28 cd	33.61 c	43.07 bc
225	33.54 b	42.52 d	33.49 c	42.42 c
Mean	34.78	44.65	34.60	43.76
P-value	0.008	0.000	0.000	0.000

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

Table 4.23: Nitrogen effect on oil content in the seeds of camelina and canola in two sites in 2014

N (kg ha^{-1})	Harrington, PEI		Truro, NS	
	Camelina	**Canola**	**Camelina**	**Canola**
0	38.61 a	44.52 a	36.57 a	43.18 a
25	38.27 ab	44.49 a	36.20 ab	43.17 a
75	37.14 b	44.21 a	35.94 a-c	42.45 ab
125	35.76 c	43.24 ab	35.31 b-d	41.94 ab
175	35.49 c	42.76 ab	34.97 cd	41.82 ab
225	35.35 c	41.66 b	34.43 d	41.40 b
Mean	36.77	43.48	35.57	42.32
P-value	0.000	0.012	0.000	0.005

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

4.3.7 Oil yield

The interaction of locations, crop species and applied N significantly affected crop oil yield in both years (Table 4.24). Camelina and canola planted at Canning, NS produced 405.73 kg ha^{-1} and 1117.18 kg ha^{-1} oil, respectively in 2013, which were higher than those (254.28 kg ha^{-1} for camelina and 813.79 kg ha^{-1} for canola) produced at Truro, NS (Table 4.25). At both Canning and Truro, NS, canola yielded more oil than camelina in 2013. A similar result was found at Truro, NS in 2014. Canola produced 1048.63 kg ha^{-1} oil, on average, more than double what was produced by camelina (450.72 kg ha^{-1}) in 2014 (Table 4.26). However, camelina grown at Harrington, PEI yielded 623.72 kg ha^{-1} oil, which was significantly greater than 522.08 kg ha^{-1} oil that produced by canola at the same site (Table 4.26). The low oil yield was mainly attributed to low seed yield.

The applied N had a positive effect on oil yield of camelina and canola, but the results varied with different experimental sites. In 2013, camelina oil yields at Canning and Truro were increased with applied N. The highest oil yield at Canning (521.93 kg ha^{-1}) was obtained at applied N rate of 225 kg ha^{-1}; however, a significant difference was not seen when applied N rate was 75 kg ha^{-1} (Table 4.25). The oil yield of camelina grown at Truro in 2013 positively responded to a wide N rates, ranging from 0 to 225 kg ha^{-1} and non-significant difference was detected (Table 4.25). With regard to canola in 2013, the highest oil yield, at Canning (1484.08 kg ha^{-1}) and at the Truro (960.12 kg ha^{-1}), were reached at applied N rates of 225 kg ha^{-1} and 175 kg ha^{-1}, respectively (Table 4.25). Canola oil yield reached a plateau at 125 kg ha^{-1} and 75 kg ha^{-1} at Canning and Truro, respectively.

In 2014, the highest oil yield of camelina was achieved at N rate of 225 kg ha^{-1} at both sites, 550.66 kg ha^{-1} and 831.34 kg ha^{-1} for Truro, NS and Harrington, PEI, respectively (Table 4.26). A significant difference of oil yield was not observed in all N treatments at Truro and was only detected when applied N rate was lower than 125 kg ha^{-1} (Table 4.26). Canola oil yield at Harrington, PEI in 2014 was significantly increased up to 770.48 kg ha^{-1} when additional N was applied at 175 kg ha^{-1}, while at Truro, NS, the highest canola oil yield (1293.32 kg ha^{-1}) was produced at applied N rate of 225 kg ha^{-1} and a significant difference was arisen when N rate decreased to 75 kg ha^{-1} (Table 4.26).

Table 4.24: ANOVA table of applied N effects on oil yield of camelina and canola in two locations in 2013 and 2014

Year	Location	Effect	F-Value	P-Value
2013	**Truro, NS** **Canning, NS**	Location (L)	140.19	0.000
		Species (Sp)	1095.88	0.000
		Nitrogen (N)	38.87	0.000
		L*N	15.62	0.000
		L*Sp	5.99	0.000
		Sp*N	5.54	0.000
		L*Sp*N	3.02	**0.016**
2014	**Truro, NS** **Harrington, PEI**	L	64.84	0.000
		Sp	127.74	0.000
		N	30.17	0.000
		L*N	1.31	0.269
		L*Sp	253.83	0.000
		Sp*N	1.12	0.359
		L*Sp*N	**4.12**	**0.002**

Table 4.25: Applied N effect on oil yield of camelina and canola in two different locations in 2013

Location	Applied N (kg ha^{-1})	Oil yield (kg ha^{-1})	
		Camelina	Canola
Canning, NS	0	268.26 k-n	789.03 e-g
	25	236.88 l-n	802.69 e-g
	75	424.6 i-m	1079.17 cd
	125	508.74 h-k	1186.18 bc
	175	473.95 h-l	1361.93 ab
	225	521.93 h-j	1484.08 a
	Mean	405.73	1117.18
Truro, NS	0	162.36 n	634.34 g-i
	25	195 mn	685.6 f-h
	75	258.25 k-n	822.42 e-g
	125	256.22 l-n	922.53 d-f
	175	312.69 j-n	960.12 c-e
	225	341.2 j-n	858.82 d-g
	Mean	254.28	813.97

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

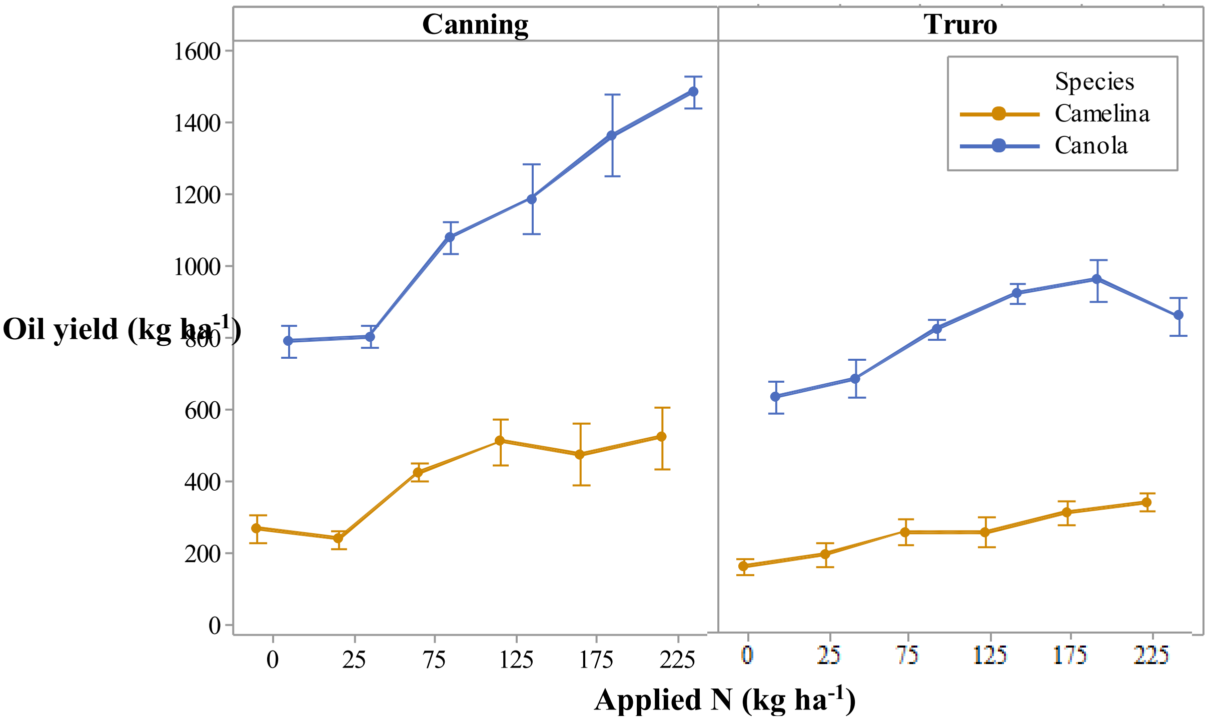


Figure 4.11: The effect of applied N on oil yield of camelina and canola at Canning and Truro, NS 2013 (Bars are one standard error from the mean)

Table 4. 26: The effect of applied nitrogen on oil yield of camelina and canola at Truro, NS and Harrington, PEI in 2014

Location	Applied N (kg ha^{-1})	Oil yield (kg ha^{-1})	
		Camelina	**Canola**
Truro, NS	0	314.47 j	781.28 c-f
	25	383.16 i-j	862.09 cd
	75	451.57 h-j	994.11 bc
	125	521.07 f-j	1185.14 ab
	175	483.41 g-j	1175.83 ab
	225	550.66 e-j	1293.32 a
	Mean	450.72	1048.63
Harrington, PEI	0	369.35 ij	363.29 ij
	25	411.64 ij	386.14 ij
	75	644.35 d-i	538.99 f-j
	125	750.61 c-g	532.00 f-j
	175	735.02 c-h	770.48 c-g
	225	831.34 c-e	541.56 f-j
	Mean	623.72	522.08

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

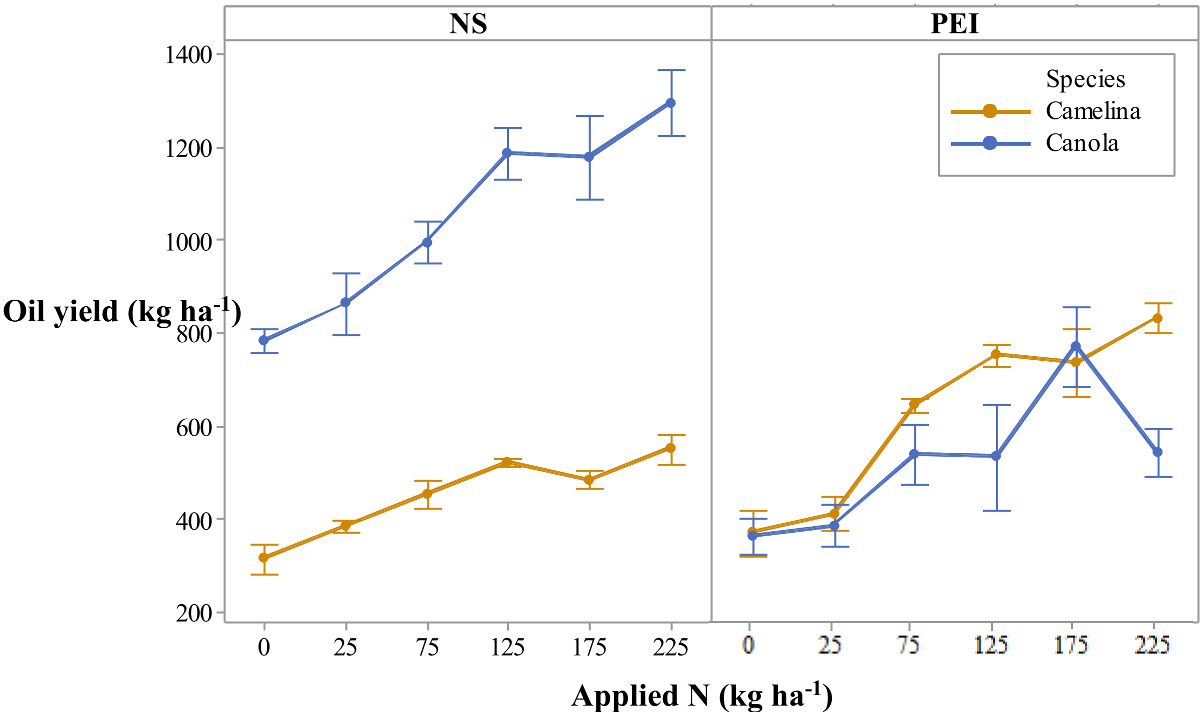


Figure 4. 12: The effect of applied N on oil yield of camelina and canola at Truro, NS and Harrington, PEI in 2014 (Bars are one standard error from the mean)

4.3.8 Protein content

The analysis variance showed that the interactive effect of location and N significantly affected protein content in the seed in both years. A two-way interaction of location and crop species significantly affected protein content in the seed in both years (Table 4.27). Crop species and N interactively affected seed protein content in 2014 (Table 4.27).

The protein content in camelina seeds was higher than that in canola seeds in both sites in 2013 and 2014 (Table 4.28). A Significant difference on protein content in camelina seed was not found at two sites in either year; however, the protein content in canola seed at Truro in 2013 was significantly different from that at Canning in 2013 (Table 4.28). A similar result was shown between two experimental sites in 2014 (Table 4.28).

The applied N significantly enhanced protein contained in the seed in each site in both years, however, the optimum N rate to achieve satisfactory protein content was dependent upon environmental conditions. The highest seed protein contents at each site was achieved at applied N rate of 225 kg ha^{-1} in 2013 and 2014 (Table 4.29). The lowest protein content was found when N rate was 25 kg ha^{-1} at two sites in 2013, while, in 2014, the lowest value was recorded in the check in two sites (Table 4.29). The protein content was leveled out at N rate of 125 kg ha^{-1}, except for Canning, NS in where significant difference on protein content was found when applied N rate was lower than 175 kg ha^{-1} (Table 4.29).

Camelina seeds contained more protein than canola seeds. The applied N significantly increased protein content in both crops. The lowest protein content was found at N rate of 25 kg ha^{-1} for camelina and 0 kg ha^{-1} for canola. The optimum applied N rate for each species to achieve acceptable protein content was 125 kg ha^{-1} and 175 kg ha^{-1} for camelina and canola, respectively (Figure 4.13).

Table 4.27: ANOVA table of applied N effect on protein content in camelina and canola seeds in two locations in 2013 and 2014

Year	Location	Effect	F-Value	P-Value
2013	**Truro, NS** **Canning, NS**	Location (L)	22.22	0.000
		Species (Sp)	1925.58	0.000
		Nitrogen (N)	77.19	0.000
		L*N	**3.86**	**0.004**
		L*Sp	**28.64**	**0.000**
		Sp*N	0.37	0.867
		L* Sp*N	0.18	0.970
2014	**Truro, NS** **Harrington, PEI**	L	43.35	0.000
		Sp	1867.87	0.000
		N	88.39	0.000
		L*N	**11.63**	**0.000**
		L*Sp	**8.22**	**0.005**
		Sp*N	**2.88**	**0.020**
		L*Sp*N	1.33	0.261

Table 4.28: The interactive effect of location and species on protein content in the seed in 2013 and 2014

Year	Location	Species	Protein content (%)
2013	**Truro, NS**	**Camelina**	27.40 a
		Canola	21.53 b
	Canning, NS	**Camelina**	27.49 a
		Canola	20.00 c
2014	**Truro, NS**	**Camelina**	26.45 a
		Canola	19.96 b
	Harrington, PEI	**Camelina**	25.85 a
		Canola	18.43 c

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

Table 4. 29: The combined effects of N and location on protein content in the seed in 2013 and 2014

Applied N (kg ha^{-1})	Protein content (%)			
	2013		2014	
	Truro, NS	Canning, NS	Harrington, PEI	Truro, NS
0	23.40 de	22.10 fg	19.22 e	21.93 cd
25	23.22 d-f	21.62 g	19.50 e	22.04 cd
75	23.33 d-f	22.42 e-g	21.13 d	22.54 bc
125	25.00 bc	24.36 cd	23.70 ab	23.70 ab
175	25.90 ab	25.63 ab	24.53 a	24.42 a
225	25.93 ab	26.36 a	24.8 a	24.61 a

Means within same year followed by different letters are significantly different ($p < 0.05$)

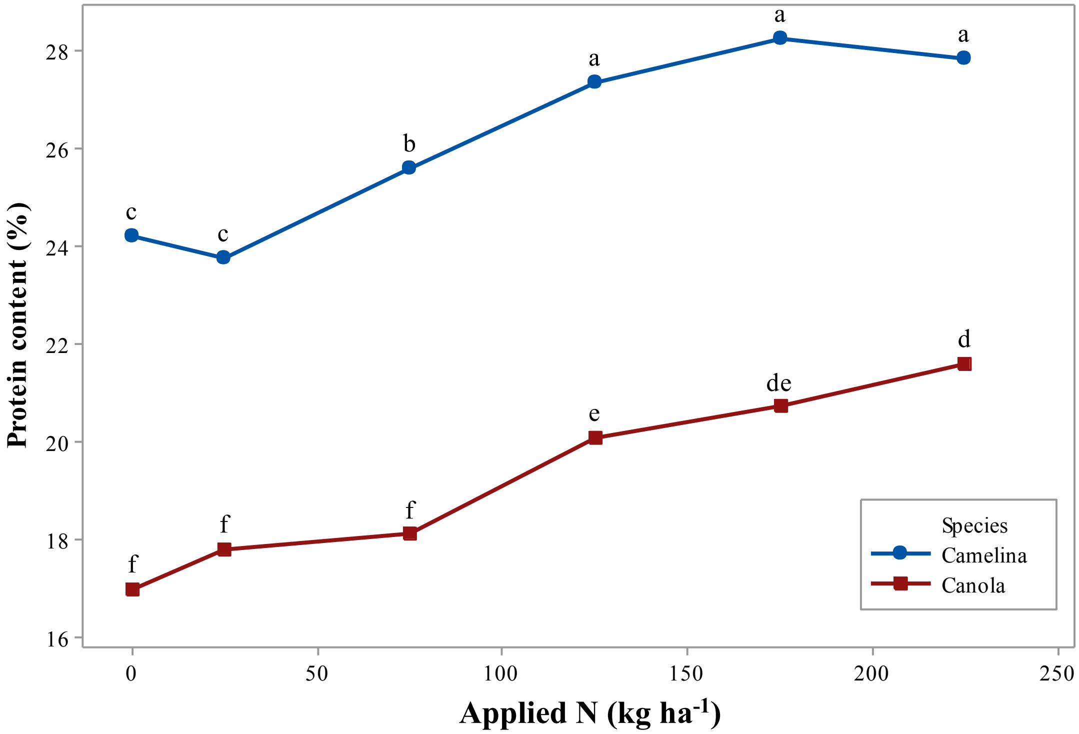


Figure 4.13: The interactive effect of N and crop species on protein content in the seeds in 2014 (Means within same year followed by different letters are significantly different ($p < 0.05$)

Table 4. 30: Nitrogen effect on protein content in camelina and canola seeds in two sites in 2013

N	Canning, NS		Truro, NS	
	Camelina	**Canola**	**Camelina**	**Canola**
0	25.83 d	18.36 c	26.36 b	20.45 b
25	25.46 d	17.79 c	26.19 b	20.25 b
75	26.23 cd	18.61 c	26.39 b	20.28 b
125	27.89 bc	20.83 b	27.96 a	22.05 a
175	29.61 ab	21.65 ab	28.83 a	22.98 a
225	29.95 a	22.76 a	28.67 a	23.18 a
Mean	27.49	20.00	27.40	21.53
P-value	0.000	0.000	0.000	0.000

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

Table 4. 31: Applied N effect on protein content in camelina and canola seeds in two sites in 2014

Applied N (kg ha^{-1})	Truro, NS		Harrington, PEI	
	Camelina	**Canola**	**Camelina**	**Canola**
0	25.56 c	18.29 d	22.81 c	15.64 b
25	25.05 c	19.03 cd	22.46 c	16.55 b
75	25.71 bc	19.38 b-d	25.44 b	16.82 b
125	27.06 ab	20.33 a-c	27.59 a	19.80 a
175	27.89 a	20.96 ab	28.61 a	20.45 a
225	27.45 a	21.78 a	28.22 a	21.38 a
Mean	26.45	19.96	25.85	18.44
P-value	0.000	0.000	0.000	0.000

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

4.3.9 Protein yield

Table 4.32 shows the significantly interactive effect among location, crop species and N on protein yield in 2013 and 2014 (Table 4.32). In both sites in 2013, canola produced more protein than camelina (Table 4.33). Camelina and canola at Canning, NS yielded 327.63 kg ha^{-1} and 516.00 kg ha^{-1} protein on average, which were higher than 205.67 kg ha^{-1} and 405.53 kg ha^{-1} for camelina and canola protein, respectively, at Truro, NS in 2013 (Table 4.33). Camelina protein yield at Harrington, PEI was higher than that at Truro, whereas, canola protein yield at Harrington, PEI was lower than that at Truro in 2014.

Camelina grown at Harrington in 2014 produced 456.19 kg ha^{-1} protein on average, which was significantly higher than canola protein (229.5 kg ha^{-1}) at the same site. At Truro, however, canola protein yield was 502.07 kg ha^{-1}, on average, which was greater than 338.25 kg ha^{-1} on camelina in 2014 (Table 4.34).

The applied N significantly increased protein yield of the two crops, however, the optimum N requirement was different due to species and locational difference. At Canning, NS, the highest and lowest protein yield were 462.46 kg ha^{-1} and 171.66 kg ha^{-1} for camelina and 796.01 kg ha^{-1} and 305.59 kg ha^{-1} for canola, which were observed at N rate of 225 kg ha^{-1} and 25 kg ha^{-1}, respectively in 2013. A significant difference of protein yield was found when applied N rate was lower than 125 kg ha^{-1} for camelina and 175 kg ha^{-1} for canola at Canning in 2013 (Table 4.33). At Truro, check plots produced the lowest protein, while the highest protein yield was found at N rate of 225 kg ha^{-1} for camelina to produce 462.46 kg ha^{-1} protein, and N rate of 175 kg ha^{-1} for canola to yielded 521.2 kg ha^{-1} protein in 2013 (Table 4.33). It must be pointed out that although statistical differences in protein yield at Truro in 2013 was not found between 225 kg ha^{-1} and 25 kg ha^{-1} for camelina and 225 kg ha^{-1} and 75 kg ha^{-1} for canola, the disparity of protein content between two applied N rates was approximately 150 kg ha^{-1} (Table 4.33).

The positive effect of applied N on protein yield was occurred at Truro, NS and Harrington, PEI in 2014. The lowest and highest camelina protein yield, 222.1 kg ha^{-1} and 430.9 kg ha^{-1} at Truro and 217.39 kg ha^{-1} and 662.15 kg ha^{-1} at Harrington, were produced in check plots and plots with 225 kg ha^{-1} N applied in 2014 (Table 4.34). The camelina protein yield was plateaued at applied N rate of 75 kg ha^{-1} at Truro and 125 kg ha^{-1} at Harrington in 2014 (Table 4.34). Canola planted at Truro in 2014 produced highest protein yield (681.83 kg ha^{-1}) with 225 kg ha^{-1} N applied and lowest protein yield (330.54 kg ha^{-1})

in the checks, and a significant difference was observed at applied N rate of 125 kg ha^{-1} (Table 4.34). Similarly, canola protein yield at Harrington responded to applied N to 225 kg ha^{-1}, but significant difference was recorded at applied N rate of 125 kg ha^{-1} at Truro and 25 kg ha^{-1} at Harrington in 2014 (Table 4.34).

Table 4. 32: ANOVA table of applied N effect on protein yield in camelina and canola seeds in two locations in 2013 and 2014

Year	Location	Effect	F-Value	P-Value
2013	**Truro, NS Canning, NS**	Location (L)	101.69	0.000
		Species (Sp)	283.74	0.000
		Nitrogen (N)	69.19	0.000
		L*N	9.12	0.000
		L*Sp	0.25	0.620
		Sp*N	3.37	0.009
		L* Sp*N	**2.85**	**0.021**
2014	**Truro, NS Harrington, PEI**	L	35.43	0.000
		Sp	5.86	0.018
		N	58.44	0.000
		L*N	1.6	0.173
		L*Sp	225.95	0.000
		Sp*N	1.47	0.210
		L*Sp*N	**7.05**	**0.000**

Table 4.33: Applied nitrogen effect on protein yield of camelina and canola in two different locations in 2013

Location	Applied N (kg ha^{-1})	Protein yield (kg ha^{-1})	
		Camelina	Canola
Canning, NS	0	195.26 h-j	315.17 e-h
	25	171.66 h-j	305.59 f-h
	75	315.22 e-h	440.29 c-f
	125	412.61 c-g	563.64 bc
	175	408.55 d-g	675.31 ab
	225	462.46 c-e	796.01 a
	Mean	327.63	516.00
Truro, NS	0	119.57 j	296.19 f-h
	25	141.80 ij	308.78 f-h
	75	197.73 h-j	374.43 d-g
	125	211.96 h-j	471.62 cd
	175	270.64 g-j	521.20 cd
	225	292.33 f-i	460.98 c-e
	Mean	205.67	405.53

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

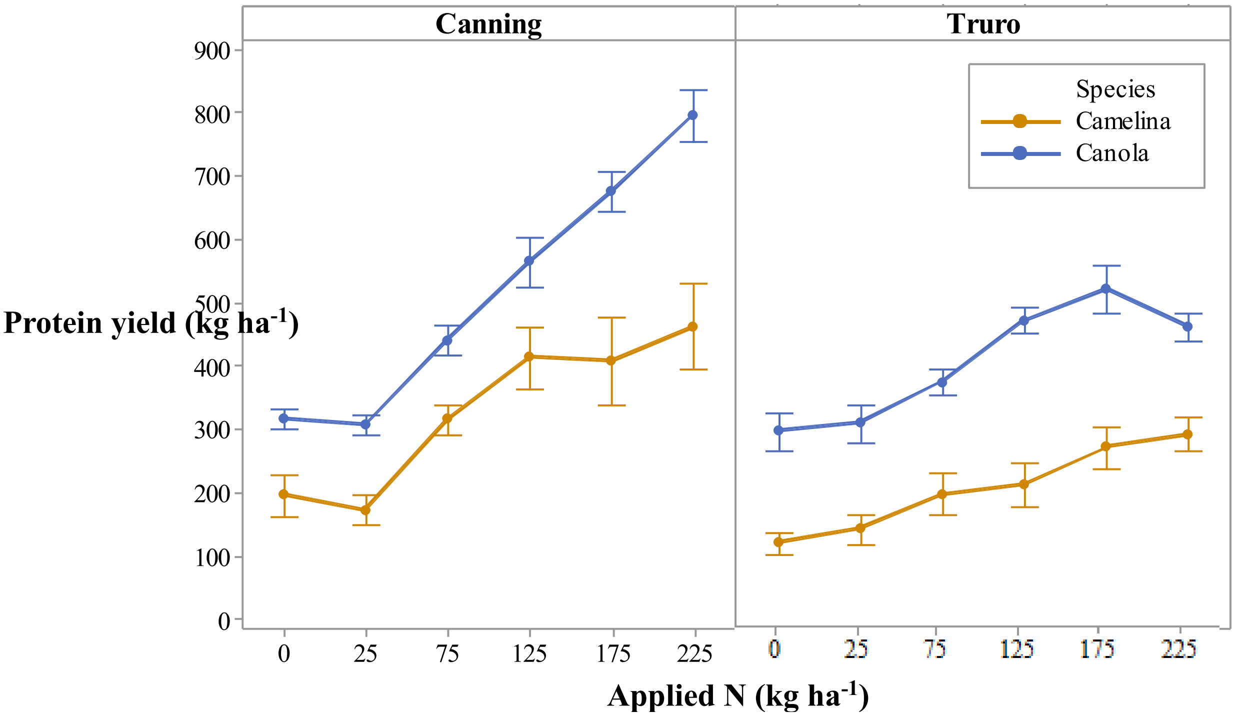


Figure 4.14: The interactive effect of location, crop species and N on protein yield in 2013 (Bars are one standard error from the mean)

Table 4.34: Applied nitrogen effect on protein yield of camelina and canola in two different locations in 2014

Location	Applied N (kg ha^{-1})	Protein yield (kg ha^{-1})	
		Camelina	Canola
Truro, NS	0	222.10 g-j	330.54 c-i
	25	262.44 e-j	379.69 c-h
	75	322.59 c-i	453.51 bc
	125	399.44 c-f	575.21 ab
	175	392.07 c-g	591.63 ab
	225	430.90 b-e	681.83 a
	Mean	338.25	502.07
Harrington, PEI	0	217.39 h-j	128.95 j
	25	242.25 f-j	143.70 j
	75	442.15 b-d	207.24 ij
	125	578.98 ab	249.58 f-j
	175	594.23 ab	369.64 c-i
	225	662.15 a	277.93 d-j
	Mean	456.19	229.50

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

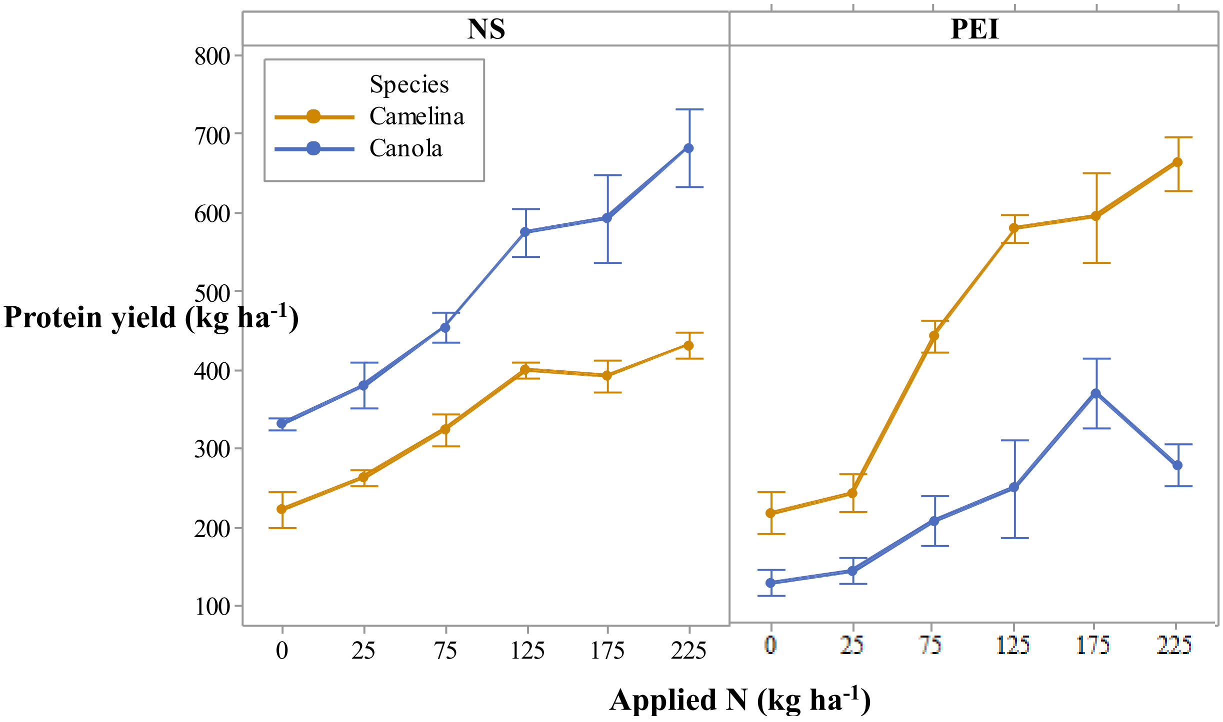


Figure 4.15: The interactive effect of location, species and N on protein yield in 2014 (Bars are one standard error from the mean)

4.4 Discussion

4.4.1 Plant height and plant density

The results in this study show the positive effect of applied N in increasing camelina and canola plant heights. Nitrogen plays an essential role in stimulating plant vegetative growth and stem elongation (Espindula et al., 2011). A similar positive correlation between plant height and N rates was reported in cereals including wheat (*Triticum aestivum* L.) (Zagonel & Fernandes, 2007), rice (*Oryza sativa* L.) (Buzetti et al., 2006), millet (*Panicum miliaceum* L.) (Soratto et al., 2007) and maize (*Zea mays* L.) (El-Murtada Hassan Amin, 2011). Gasim (2001) explained that this positive correlation probably was due to the fact that applied N significantly improved plant growth and increased the number and length of internodes; these were the contributors to progressive increase in plant height. Comparable results were also reported by Chandler (1969), Turkhede and Rajendra (1978) and Koul (1997). The results in this study also demonstrated that plant height was significantly affected by location and crop species. This could probably be explained as the different responses between camelina and canola to the various growing conditions, including soil properties, fertilizer residuals in the soil, precipitations and mean temperature in each site.

A study conducted by Urbaniak, et al. (2008b) suggested that the best seeding rate for camelina grown in the Maritime region to optimize seed yield was in the range of 400 to 600 seeds with a target plant density range of 198 to 234 plants per m^2. This result was comparable with the data obtained at the Canning site in 2013 (Table 4.5) and the Harrington site in 2014. According to the Canola Council of Canada (2013), the ideal plant density to achieve maximum yield potential was 70 to 140 plants per m^2, which was comparable with the final canola plant stands in all sites and years in this study.

The plant population in the experimental site in each year was lower than the seeding rate. This might be the result of natural ability of self-thinning and due to this, populations of camelina and canola decreased when plant size increased (Jiang, 2013). Although the effects of three-way interaction (location, species and N) on plant population of camelina and canola in 2013 were statistically significance, the plant stands of two crops were not markedly affected by N rates. Angadi et al. (2003) reported that environmental conditions significantly affected the expression of plasticity of canola. In the field, seed germinations and seedling survivals can be affected by many factors, including moisture, air, light, and temperature. The favorable germination temperature for canola seeds, however, was 10 °C, and temperatures declines below this point resulted in progressively poorer germination and emergence (Nykiforuk & Johnson-Flanagan, 1994). Camelina seed was reported to be able to germinate at temperatures as low as 1ºC and camelina seedlings were resistant to frost up to -11ºC during nights in Montana (Ehrensing & Guy, 2008). Multiple locations provided different conditions for camelina and canola seed germination and seedling growth, and higher camelina population was observed in each site in 2013 and 2014. These results suggest that camelina seed is capable of germinating and surviving in a variety of climatic and edaphic conditions.

4.4.2 Seed yield and yield components

Nitrogen fertilizers have a strong influence on productivity in most natural and agricultural ecosystems (Steer and Harrigan, 1986). In this study, seed yield of camelina and canola were significantly enhanced by applied N in both sites in each year. This positive effect of applied N on camelina and canola was supported by previous studies (Urbaniak et al., 2008a; Malhi et al. 2013; Jiang et al., 2014). It was suggested that additional N supply could improve leaf number (Svečnjak & Rengel, 2006), expand leaf area, prolong leaf duration after flowering, accelerate crop photosynthetic rate and improve carbon assimilate formation, resulting in increased seed yield (Wright et al., 1998).

Seed yield is a function of population density, number of pods per plant, number of seeds per pod and seed weight (Diepenbrock, 2000). This can be well demonstrated by the correlation coefficient between yield components and camelina and canola seed yields in this study. Agegnehu and Honermerier (1996) have achieved similar results on camelina. They found that camelina yielded 2300 kg ha^{-1} seeds at a population density of 182 plants m^{-2}, 6.4 branches per plant and 194 pods per plant. Steer & Harrigan (1986) found a reduction in yield and yield components such as the number of seeds per plant and number of branches per plant and seed weight when N was deficit. The correlation between seed yield and yield components in this study suggested that the number of seed pods per plant and TKW would likely be the most important yield components of camelina and canola, respectively. Allen and Morgan (1972) indicated that N increased yield via the effect on a number of growth parameters such as the number of branches/plant, the number of pods/plant, and seeds per plant. In this study, all the parameters of camelina and canola yield components were markedly increased with applied N rates increasing. The positive N effect on camelina was supported by Urbaniak et al. (2008a) and Jiang et al. (2014). Ahmadi and Bahrani (2009) observed a significant increase in the number of canola branches and seed pods and seed yield with increased N rates.

It is important to point out that camelina out-yielded canola significantly at Harrington, PEI in 2014. The data in this study showed that canola seed yield at the Harrington site was lower than that at the Truro site in 2014, while camelina seed yield at the Harrington site was greater than that at the Truro site in 2014. The notable decrease in canola seed yield might be attributed to two factors. The first one is most likely to be the TKW of canola. The data of canola TKW, obtained from the Harrington site, was lower than that in other sites and years. Due to high correlation between TKW and canola seed yield, the reduction in canola TKW could consequently decreased seed yield. Another reason for reduced canola yield was that canola plants might be under water deficit condition during the growing season which could progressively reduce its yield. By interpreting the weather data at Harrington, PEI in 2014, the rainfall in this site in June was much lower than that at the Truro site. This might cause water deficiency during this period. In June, both canola and camelina were in flowering stage and the reduced water availability could significantly impact on flower survivals, thus reduced the number of seed pods. Diepenbrock, (2000) suggested that the number of pods per plant is the most responsive of all the yield components; however, the number of seed pods was determined by the survived branches, buds, flowers and young pods rather than by the potential number of flowers and pods (McGregor, 1981). Masoud Sinaki et al. (2007) found that water stress occurred at flowering and pod developing stages caused the most yield loss on rapeseed. This was supported by Ahmadi and Bahrani (2009). The obtained data of the number of seeds per canola plant showed a significant reduction at the Harrington site in PEI (Table 4.14). This probably indicated a notable decrease in canola seed pods as the number of seeds per plant was highly correlated with the number of seed pods per canola plant (Table 4.18). As a consequence, canola seed yield was markedly reduced. In contrast, camelina might be tolerant to the same conditions and consequently, achieved high yield. Under water shortage situation, good performance was observed and seed yield was higher than canola in the same site in 2014. This results suggested that the water requirement for camelina to maintain its yield potential was lower than canola.

It should be highlighted that in this study much higher camelina yield in PEI was detected than that in NS. This suggested that the climatic-soil conditions in PEI might be suitable for camelina growth. According to previous research conducted in the Maritime Provinces in Canada, camelina seed yield in PEI was greater than that in NS (Table 4.35). The values of camelina seed yield shown in Table 4.35 were consistent with the result obtained in this study. This indicated the agronomic suitability of camelina in the PEI in Maritime Provinces of Canada.

Table 4. 35: Camelina seed yield in NS and PEI from 2005 to 2011 listed in the literature

Year	Location		Literature
	NS	PEI	
2005	1299 kg ha^{-1}	1647 kg ha^{-1}	Urbaniak et al. 2008a
2006	1096 kg ha^{-1}	1660 kg ha^{-1}	Urbaniak et al. 2008a
2008	886 kg ha^{-1}	1458 kg ha^{-1}	Pan, 2009
2009	1528 kg ha^{-1}	1906 kg ha^{-1}	Pan, 2009
2011	1435 kg ha^{-1}	1616 kg ha^{-1}	Jiang, 2013

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4.4.3 Oil and protein

Camelina seed oil concentration in this study ranged from 33% to 35% at Canning, NS; 33% to 36% at Truro, NS and 35% to 38 % at Harrington, PEI. These values are consistent with the results presented in previous studies conducted in Italy, Austria, the United States, south central Chile and Canada (Budin et al., 1995; Agegnehu & Honermeier, 1997; Putnam et al. 1993; Solis et al., 2013; Urbaniak et al., 2008a; Malhi et al. 2013; Jiang et al., 2014;). Zubr (2003) suggested that the difference in camelina seed quality can be attributed mainly to the effect of growing environments including climate and soil conditions. Roseberg & Shuck (2009) reported a range of camelina oil content from 31.2 to 35.0% and varied little due to soil water availability. This indicated that in this study various seasonal precipitation in each site might not result in differences in camelina oil content in each site. Soil fertility especially residual N can be attributed mainly to the variations in camelina oil content. The effect of N would be discussed more in the following. The oil content in canola seeds in this study ranged from 42% to 47% at Canning, NS; 41% to 45% at Truro, NS and 41% to 44% at Harrington, PEI which were comparable to the results reported that canola seed oil concentration ranged from 40% to 46% (Taylor et al., 1991) in previous research. Unlike camelina, changes in canola seed oil content is not only caused by applied N, but also by soil water moisture. Henry and MacDonald (1978) found a decrease in oil content and an increase in protein content in rapeseed due to water shortage.

The protein content in camelina seed shown in this study were in the range from 19% to 25% at Harrington PEI and 21% to 26% at Canning and Truro, NS. These values were lower than that reported in the literature. Urbaniak et al. (2008a) reported a range of protein content in camelina seed from 27% to 32%; Jiang (2013) found protein concentration ranging from 24% to 28% in the same region. The protein content in canola seeds was reported in the literature was approximately from 24% to 28 % (Aminpanah, 2013) which was higher than those obtained in this study. These variances might be due to differences in soil-climatic conditions in each experimental sites.

A significant N effect on oil and protein contents in camelina and canola was found in this study. The protein content in seed increased with increasing N rates, while oil content was decreased as N rates increasing. Previous studies on camelina (Urbaniak et al. 2008a; Jiang et al. 2013) and canola (Lemke et al. 2009) have also shown inverse effects of applied N on seed oil and protein contents. Rathke et al. (2005) described that this inverse relationship between protein and oil concentration was the result of carbon skeletons competition during carbohydrate metabolism. Gehringer et al. (2006) confirmed that the pathways for fatty acid and amino acid biosynthesis compete for carbon skeletons and energy. Malhi et al. (2013) suggested that due to lower carbohydrate levels in protein than in oil, N supply was increased in protein synthesis at the expense of fatty acid synthesis.

The oil and protein yield were significantly increased with applied N and the responses trends were nearly identical with yield responses to N (Figure 4.4, Figure 4.5, Figure 4.11 and Figure 4.12). In this study, the oil and protein yield were functions of grain yield and oil and protein contents in the seeds. Any variations in oil content and protein content or seed yield could result in significant difference in oil and protein yield. Having been discussed above, compared to the change in seed yield, the change in oil and protein contents were considered as a minor factor to cause significant differences in oil and protein yield. Narang and Gill (1992) made a good explanation that although applied N negatively affected grain oil percentage, it increases the grain yield thus enhanced the oil yield. Therefore, farmers should apply optimum N to achieve highest economic seed yield responses as this is likely to maximize total seed oil yield for both camelina and canola.

4.5 Conclusion

Applied N influenced variables such as plant height, seed yield, yield components, oil content and protein content. The stronger correlation between canola seed yield and applied N than that of camelina suggested that canola seed production more relied upon N fertilizer application than camelina. The optimum N application rates for camelina and canola to maximize the seed yield depended on climatic-soil conditions. For camelina, the optimum applied N rate was in the range of 75 to125 kg ha^{-1}; while canola required 125 to 175 kg ha^{-1} N. When determining a target N rate for the crop, climatic-soil conditions must be taken into consideration. The data obtained from Harrington, PEI indicated a high yield potential of camelina and under rain-fed condition with relative low seasonal precipitation, camelina can perform better than canola.

Chapter 5：Conclusion

5.1 Physiological responses to water deficiency and applied N

The greenhouse study showed that the effects of crops species, soil water regimes and applied N significantly influenced all the measured physiological variables, which included chlorophyll index, photosynthesis, transpiration rate and stomatal conductance. The chlorophyll content of camelina was more responsive to soil water availability. Photosynthesis, transpiration rates and stomatal conductance in the two crops were significantly decreased by the interactive effects of water deficiency and applied N. The relative decline in each parameter in canola was greater than that in camelina, suggesting that camelina had a higher stomatal control efficiency than canola. It was concluded that the influence of water deficiency on stomatal conductance of both species had greater weight than chlorophyll level in regulating photosynthesis during water deficiency; where water supply was sufficient, canola had a more active physiological mechanism than camelina; however, under water deficit conditions, camelina showed better adaptability by maintaining its physiological activities.

5.2 Instantaneous water use efficiency

The instantaneous water use efficiency (WUE*i*) in this study was defined as the ratio of the rate of carbon assimilation (photosynthesis) to the rate of transpiration (Chapter 2). Camelina showed a higher WUEi than canola under well-watered conditions; while the result were the inverse when water was deficit. Applied N did not significantly affect the WUE*i* of camelina but markedly increased the WUE*i* of canola under severe water deficiency.

5.3 Biomass and seed yield responses

Under controlled conditions, applied N did not influence camelina biomass but did positively affected that of canola, suggesting a more pronounced effect of N supply on canola biomass accumulation. The shoot biomass of the two species decreased by 9 % and 26 % but the root biomass increased by 50 % and 22% for camelina and canola, respectively, due to a water deficit. This indicated a greater ability of camelina in resource allocation than canola under water deficit conditions. This might be one important mechanism for camelina to tolerate a water deficit. With such a mechanism, the effect of water deficiency did not significantly affect camelina seed yield.

5.4 Applied N effect

The present study provided comparative effects of applied N on the performance of camelina and canola under controlled environments and in the field. The parameters include the number of branches per plant, the number of pods per plant, the number of seeds per plant, 1000-seed weight, seed yield, seed oil and protein contents, and oil and protein yields.

Applied N was positively correlated with yield components (the number of branches per plant, the number of pods per plant, the number of seeds per plant) of the two species under controlled environments and different soil-climatic conditions. Applied N had positive and negative effects on protein and oil contents, respectively in both crop species in controlled environments and in the field. Higher protein content was negatively correlated with oil content. Seed yield, oil and protein yield from both species were positively correlated with applied N; and a greater seed yield responses in canola than that in camelina was found in both greenhouse and field trials. These indicated a closer relationship between N supplied and canola seed yield in comparison with camelina. The optimum N application rates for camelina and canola were in the ranges of 75 to 125 kg ha^{-1} and 125 and 175 kg ha^{-1}, respectively, depending on soil-climatic conditions. This indicated the importance of soil-climatic conditions to each crop. The study indicated the prospects for extending the production of camelina as it could be better adapted to low soil moisture conditions and low N availability.

5.5 Further study

It would be important to conduct a trial to evaluate the combined effect of irrigation and N supply on camelina and canola, in terms of physiological responses, growth, seed yield and quality in the field. To introduce a relative new crop into the current cropping system, it is important to examine the resource use efficiency (e.g water and N use efficiencies) by this crop in comparison to current crops. In the future, evaluating and comparing camelina and canola water requirements and N uptake mechanisms would contribute to the study of water and N use efficiencies by both crops.

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