NATURAL VARIATION IN CAMELINA NITROGEN RESPONSES

by

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DEDICATION

I hereby dedicate my work to my family - my mother, father, and brother, whose unwavering love and support have been the foundation of my life. I would also like to express my gratitude to my advisor for his invaluable guidance and support throughout this journey. My friends Katie Sparks, Yi Zhou, and Maral Etesami have been a constant source of encouragement, and I dedicate this work to them as well. Their unwavering support and motivation have been a source of strength in my success, and for that, I am forever grateful.

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ABSTRACT

Camelina (*Camelina sativa* L.*Crantz*) is an oilseed crop with the potential to be planted for biofuel production. It is crucial to select camelina genotypes with higher nitrogen use efficiency (NUE) so that the superior cultivar has higher crop productivity. To select genotypes of camelina that exhibit higher biomass yield and nitrogen use efficiency, two field experiments were conducted in 2021 and 2022 in Sidney, MT with different nitrogen regimes, low (unfertilized) and high (fertilized). Distinct projects were carried out, one of them emphasizing canopy area and normalized difference vegetation index (NDVI), and the other focusing on biomass yield and NUE. The experiments highlighted the response of camelina to nitrogen application and the variation among genotypes. The study identified canopy image analysis effectively differentiated the canopy size and growth rate of camelina genotypes under two nitrogen regimes, demonstrating the influence of nitrogen on camelina growth. The NDVI measurement proved to be useful in evaluating plant health and greenness, offering a time-saving and efficient approach. Additionally, some of the genotypes were identified that exhibited high canopy area, NDVI, and nitrogen use efficiency in both 2021 and 2022, providing potential for enhancing crop productivity. This study reveals the potential to use canopy area, NDVI for biomass yield and nitrogen use efficiency screening in camelina.

CHAPTER ONE

GENERAL INTRODUCTION

Camelina (*Camelina sativa* L.*)* is an oilseed crop from the *Brassicaceae* family native to Eastern Europe and Western Asia (Vollmann & Eynck, 2015). Camelina was introduced to cultivation in North Africa (Tunisia), Australia (Tasmania, South Australia, Victoria, Western Australia), North America (USA, Canada), and South America (Argentina, Uruguay) (Sydor et al., 2022). Camelina was commonly grown in Europe until the Middle Ages, and for a long time camelina was known primarily in North America as a weed (false flax).Recently, camelina is recognized for its value as an oilseed crop, and its cultivation is increasing (Guy et al., 2014).Camelina is characterized as a short-season crop which requires 85 to 100 days to mature and has high adaptability to various climatic and soil conditions (Hunter & Roth, 2010). It is well adapted to production in the temperate climate zone (Hunter & Roth, 2010). Camelina is an annual plant with both spring and winter biotypes. The spring biotype is the most widespread globally and is grown as an early summer annual oilseed crop, but camelina can also be grown as a winter annual in milder climates(Hunter & Roth, 2010). Camelina attains heights of 0.3-0.9 m, has branched stems that become woody at maturity, and stems are generally smooth or only sparsely hairy near the base (Hitchcock & Cronquist, 2018). Leaves are arrow-shaped, sharp-pointed, 5 to 8.9 centimeters long with smooth edges. Camelina produces prolific small, pale yellow or greenish-yellow flowers with four petals. Seed are contained in pear shaped pods known as silicles resembling flax bolls and have a squared off tip (Klinkenberg, 2008). The

seeds have a rough surface and are small, with 1000- seed weight in the range of 0.8- 2.0 grams (Ehrensing & Guy).

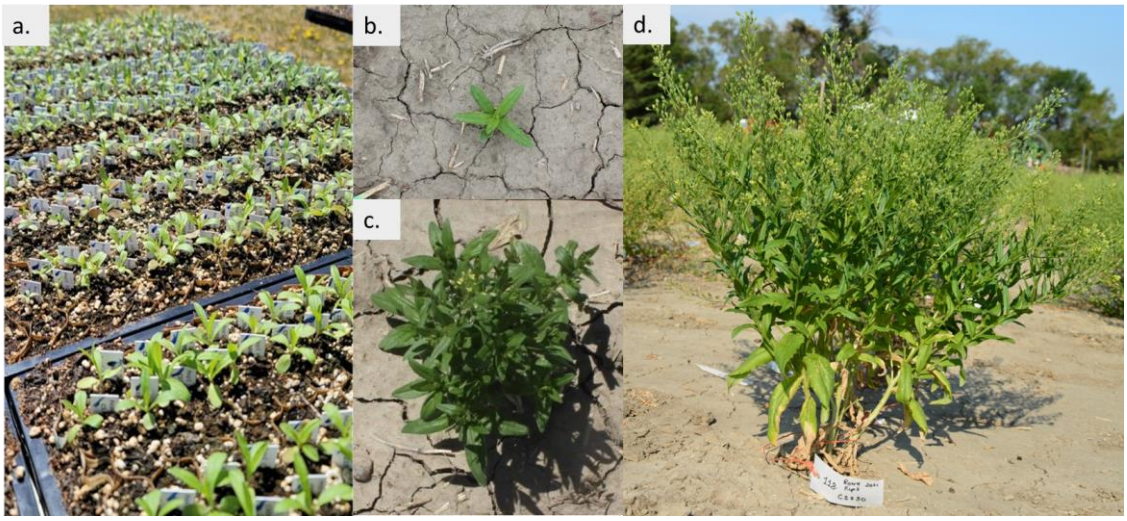

Figure 1. 1. (a) Camelina seedlings (5-leaf stage), (b) rossette stage of camelina after transplantation, (c) camelina at initiation of flowering, and (d) camelina during pod setting.

Because of the oil content research is inclined towards the development of camelina as a crop. The oil content of the seed, on a dry weight basis, is typically between 30 and 40 percent, which contains about 64 percent polyunsaturated, 30 percent monounsaturated, and 6 percent saturated fatty acids (McVay & Lamb, 2008a). Camelina oil can be used in both edible and industrial products. Historically, the seeds of camelina were crushed and boiled to release oil for food, medicinal use, and lamp oil (Ehrensing & Guy, 2008). More recently camelina has been grown as a source of vegetable oil high in omega-3 fatty acids. Camelina has been marketed in Europe as salad dressing and as cooking oil and has been approved for use in cattle, chicken, and pig feed in USA (Ehrensing & Guy, 2008). It is also used in cosmetics, skincare products, soaps,

and soft detergents (Ehrensing & Guy, 2008). The oil has been used successfully as an adjuvant in agricultural spraying applications, and as biodiesel (Ehrensing & Guy, 2008). The oil content in camelina seeds are rich in ω-3 (α-linolenic acid ;C18:3 ω-3) and ω-6 acids (linoleic acid ;C18:2 ω-6), phytosterols, and phenolic compounds, which makes it attractive for the production of food and biofuels (Berti et al., 2016). These high levels of long-chain hydrocarbons in camelina oil are used for an aviation biofuel and have been reported to reduce CO2 emissions compared with traditional petroleum jet fuels (Belayneh et al., 2015; Kwiatek et al., 2021; Shonnard et al., 2010; Walia et al., 2018; Yang et al., 2016). This raw material made it attractive for the production of food and it's potential to be planted for advanced biofuel production on marginal land in Northern Great Plains(NPG) and also as a rotation crop on fallow land (Shonnard et al., 2010).

Camelina Production and Distribution

The earliest discoveries of camelina as a plant was in Central Europe dated to 4000 BCE in Auvernier, Switzerland (Zohary & Hopf, 2000). Further discoveries in south-eastern Europe dated back to 1800–1200 BCE (Kroll, 1991), while camelina was also found in Scandinavia between 500 BCE and 1000 CE (Larsson, 2013). In southeast Europe and southwest Asia, it is believed to have originated as weed in flax and some grain crops (Haldane, 1990).

In recent decades camelina has been grown widely since the interest in low-input oilseed crop elevated, with the majority of commercial production occurring in North America, Russia and Europe (Gugel & Falk, 2006; Guy et al., 2014; Shonnard et al., 2010; Zubr, 1997). In 2020, Canada cultivated approximately 4,050 hectares of camelina, predominantly in Saskatchewan (Eynck et al., 2021). The United States produced about 9,700 hectares of camelina in 2007,

primarily in Montana (McVay & Lamb, 2008b), which is similar to Europe's production of 10,000 hectares. In 2019, Russia had the largest cultivation of camelina, with an estimated 75,600 hectares (Kon’kova et al., 2021). Global camelina yields vary due to weather conditions, cultivars, and other parameters (Arshad et al., 2022). Yield of camelina seed in Canada is about 3 tons per hectare (Zanetti et al., 2017), while in the United States, it is 2.3 tons per hectare (Gesch et al., 2018). In Russia and Europe, camelina yields are approximately 0.69 tons per hectare and 3.3 tons per hectare, respectively (Arshad et al., 2022; Gesch et al., 2018; Kirkhus et al., 2013; Zanetti et al., 2017). The figure below shows the cultivation of camelina in different parts of the world (Figure.1.2 (a)), also illustrates the yield of camelina seed (Figure.1.2. (b)).

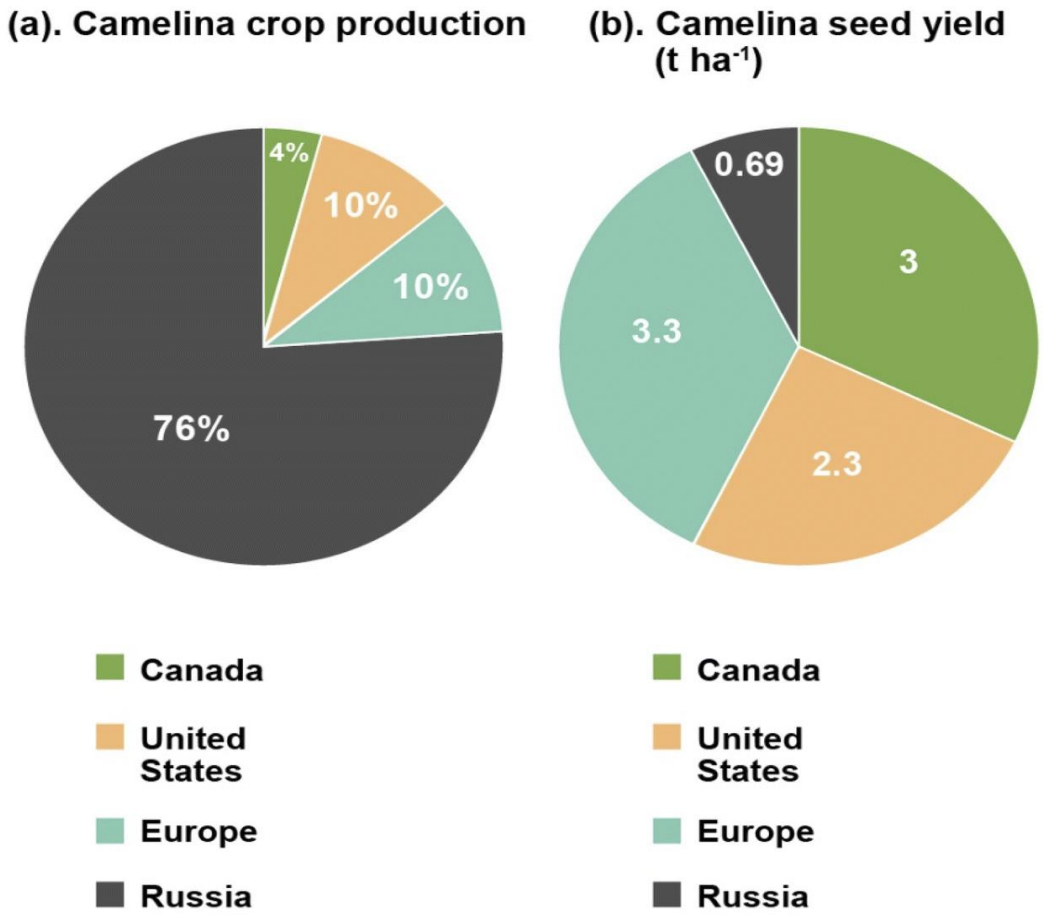

Figure 1. 2. Comparison of (a) camelina annual crop production (Eynck et al., 2021; Kon'kova et al., 2021; McVay & Lamb, 2008b), and (b) camelina seed yield (Adiele et al., 2021; Gesch et al., 2018; Kirkhus et al., 2013; Zanetti et al., 2017)

In North America , the acreage expanded to 8100 hectares in the Northern Great Plains in 2011 (Nass, 2012). In Montana, there was no commercial production of camelina before 2004, and the production increased quickly to more than 20,000 hectares in 2007 and to around 30,000 ha in 2009 (Pilgeram, 2007).Similarly the grain yields of camelina was reported in different countries ranging from 1.65 to 3.58 t ha^{-1} in Austria (Vollmann et al., 1996; Vollmann et al., 2007) and from 2.87 to 3.64 t ha^{-1} in Denmark (Zubr, 1997). While in the United States, grain

yield was reported from 0.70 to 1.76 t ha^{-1} in Montana (McVay & Lamb, 2008a), from 0.66 to 1.87 t ha^{-1} in Rosemount, Minnesota (Putnam et al., 1993), from 0.79 to 2.20 t ha^{-1} in North Dakota, and about 1.10 t ha^{-1} in Arizona (French et al., 2009).

Role of Nitrogen Fertilizer in Camelina

Several authors have reported positive yield response to nitrogen (N) fertilizer application. The estimation of application dose is influenced by location, soil type and genotype (Malhi et al., 2014; Solis et al., 2013; Zubr & Matthäus, 2002).A prerequisite to maintaining high crop productivity under lower N fertilization input is to determine whether it is possible to select for genotypes that are adapted to low or high N fertilization, or that can perform well under both N fertilization conditions (Hirel et al., 2007).

In oilseed production, nitrogen accounts for the largest energy input (Mohammed et al., 2017), reflecting the need to improve N use efficiency and minimize production costs (Chen et al., 2015; Gan et al., 2008). Compared with other biofuel crops such as rapeseed, camelina required less energy input in dryland farming systems (Keshavarz-Afshar et al., 2015). One study showed that N has the biggest share of production costs in camelina production when N was applied at a rate of 75 kg ha^{-1} (Chen et al., 2015).

Nitrogen is essential for a crop's metabolic activity and transformation of energy, and chlorophyll and protein synthesis. It also affects uptake of other essential nutrients and helps in the better partitioning of photosynthates to reproductive parts thereby increasing the seed: stover ratio(Singh & Meena, 2004). Camelina has been described as a crop with low capital expenditure, modest chemical inputs, and the ability to achieve moderate yields on less fertile soils (Solis et al., 2013). However, under N deficiency, camelina plants are thin and upright, and

the leaves are small and pale-yellow green. Ripening tends to be premature, and fewer pods and seed-bearing branches are developed (Agegenehu & Honermeier, 1997). Nitrogen application promoted the onset and development of yield components such as branches plant^{-1}, pods plant^{-1}, pods per unit area, seed weight plant^{-1}, and seeds pod^{-1}in camelina (Agegenehu & Honermeier, 1997; Stolarski et al., 2019). Camelina cultivar selection and applied N levels are important factors in obtaining optimum yield (Urbaniak et al., 2008). During the vegetative stage, the leaves represent a major nitrogen source and sink with the remobilization of nutrients from older to younger leaves or senescing leaves to reproductive tissues during bolting, flowering and seed fill (Jensen et al., 1996). As a result, N deficiency reduces plant growth by restricting leaf area development (Albert et al., 2012; Gammelvind et al., 1996), branching (Momoh et al., 2004) and dry matter accumulation. A positive linear relationship between seed yield and applied N rate up to 100 kg ha^{-1} was found by (Wysocki et al., 2013). However, significant variation in N requirement for camelina production have been documented under different environmental conditions in the world. Camelina has been shown to increase in seed yield with the application of 80 kg N ha^{-1} in Montana (McVay & Lamb, 2008a) .The optimum N input for camelina was found to be between 60 and 80 kg ha^{-1}. Crowley and Fröhlich (1998) found that camelina yields peaked by using 75 kg ha^{-1} N in Ireland. Camelina seed yield was maximized at 100 kg ha^{-1} N in Europe (Zubr 1997) and 90 kg ha^{-1} N in US (Budin et al. 1995). Most recent research showed that optimum yields of camelina required relatively high N application. For example, the maximum seed yield of camelina was attained with the application of 185–300 kg ha^{-1} N in Chile (Solis et al. 2013). The optimum N rate for the highest yield ranged from 120 to 160 kg ha^{-1} N in eastern Canada (Jiang et al. 2013) and the maximum seed yield was achieved at a rate

of 170 kg ha^{-1} N on the Canadian prairies (Malhi et al. 2014). In general, there are many production in different areas of the world (Hirel et al., 2007).However, further research is needed to optimize the N fertilization rate and to increase N use efficiency ; moderate amount of fertilization was reasonable for efficient uptake of nitrogen (N) in spring camelina, but higher level of N do not always result in higher yield (Johnson et al., 2019).

Brassicas are highly responsive to N application (Hocking et al., 1997); however, for optimizing seed yield they require relatively high rates of mineral N fertilizers (Malagoli et al., 2005). Managing N application for uptake and utilization efficiency requires an understanding of growth and resource allocation in response to N limitation. Brassicas have relatively high N uptake during vegetative growth until flowering followed by reduced N uptake during flowering and finally incomplete N translocation from the leaves and stems to the developing seeds (Wiesler et al., 2001). Although brassicas have a high capacity for N uptake, many species have a low nitrogen use efficiency and remobilization during the vegetative phase partly due to freeze-induced abscission of N-rich leaves during cold winter months; this is different for the spring and winter varieties, the spring varieties are susceptible to cold (Albert et al., 2012; Malagoli et al., 2005; Rossato et al., 2001). During the vegetative stage, the leaves represent a major nitrogen source and sink with the remobilization of nutrients from old to younger leaves or senescing leaves to reproductive tissues during bolting, flowering and seed fill (Jensen et al., 1996). In canola, nitrogen mobilized from leaves and stems contributes 70% of the total N required for seed filling with the remainder mobilized from other tissues ,22% from inflorescence and 8% from roots (Malagoli et al., 2005). Nitrogen uptake is usually greatest during the vegetative stage and declines at flowering and pod fill stages in canola (Rossato et al., 2001).Abiotic factors that

affect the uptake, assimilation and allocation capacity during the pre-bolting period will modulate the reproductive performance and seed yield of oilseed brassicas (Jackson, 2000; Malagoli et al., 2004; Malagoli et al., 2005). For example, removal of 50% of the leaves present at the end of the vegetative stage resulted in a 30% decrease in seed yield in canola (Noquet et al., 2004). Comparing camelina seed yields to those of other Brassicaceae oilseeds, including *Brassica napus* and *B. juncea* found in most cases that yields were comparable with fewer inputs (Robinson, 1987).

Selection of Genotypes of Camelina

Cultivars differ in their absorption and translocation of soil moisture, plant nutrients, photosynthates, and most importantly, interactions with environmental factors (Sintim et al., 2016). Both selection and breeding for better genotypes and transgenic techniques to develop varieties with changed gene expression have been used to increase the nitrogen use efficiency (NUE) in Brassica crops (Abberton et al., 2016; Chen et al., 2015; Fischer et al., 2013). It is important to establish its genetic potential and cultivation peculiarities for maximum yield considering the broad range of camelina uses (Bonjean A 1999). Brassica populations exhibit significant genetic variation of NUE (Ahmad et al., 2008). An evaluation of genotypic variation for plant traits contributing to N efficiency under low versus high N supply might be required to enable selection of genotypes showing optimal adaptation to the given growing conditions (Ceccarelli, 1994).

Various parameters in plants affect the yield of the crops, such as flowers, vegetation indices (VIs), plant height, and canopy area (Bai et al., 2016; Sun et al., 2018; Tattaris et al., 2016; Thorp et al., 2016). The canopy area and NDVI (Normalized Difference Vegetative

Indices) vary with the condition and health of plant, under stress conditions, plant physiology and structural properties undergo complex changes, which in turn alter the reflectance spectra of leaves (Wahabzada et al., 2016). By applying appropriate spectral analysis, the differences in the reflectance signal can be used to characterize the plant's physiological state and to assess plant genotype-specific responses to biotic and abiotic stresses (Wahabzada et al., 2015). The vegetation biomass directly or indirectly reflects crop vigor and photosynthetic capacity thus can be naturally used to indicate crop yield (Siegmann & Jarmer, 2015).

Relevance and Importance of the Research

My research aims to select camelina genotypes with high nitrogen use efficiency and yield. The growth and greenness; and biomass yield are examined to identify the desirable camelina lines. This research explores the image analysis to evaluate the growth and greenness of camelina population with wide genetic backgrounds. Comparing the plants grown under low and high doses of nitrogen fertilizers provides information on response of camelina genotypes to different nitrogen doses. The work should be beneficial in identifying camelina lines that are efficient in producing greater biomass yield and higher nitrogen use efficiency.

Summary

The research projects were carried out in years 2021 and 2022 in Sidney, Montana. The first project involved in remote sensing techniques, and the second project involved with measuring biomass yield and nitrogen use efficiency (NUE). The details of the studies are reported in this thesis as Chapter 2 and Chapter 3 respectively. The goals of the research were 1) to evaluate different genotypes of camelina for health and growth under low and high N regimes

using remote sensing, and 2) to select elite camelina genotypes based on biomass yield and nitrogen use efficiency.

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CHAPTER TWO

SELECTION OF CAMELINA GENOTYPES WITH REMOTE SENSING DEVICES FOR HIGH PRODUCTIVITY

Introduction

Phenotyping and quantifying nitrogen response can aid in the identification of target traits to screen for N use efficiency in N-limited conditions and serve as a precursor to breeding for those traits for improved oilseed productivity. Therefore, understanding the effects of N deprivation on growth, development and physiology of oilseed is essential for optimum N management and seed productivity (Seepaul et al., 2016).

Plant breeders usually rely on traditional approaches when collecting phenotypic data, such as using a measurement stick to collect plant height data and visual ratings to evaluate flowering intensity, to collect phenotypic data to evaluate breeding lines. But these traditional phenotyping approaches are often low-throughput, labor-intensive, time-consuming, and sometimes subjective and/or destructive. These phenotyping bottlenecks have slowed the development of new cultivars (Furbank and Tester, 2011). Development of high-throughput phenotyping or phenomics technologies, using sensing and computer vison to collect data and evaluate plant traits qualitatively and quantitatively (Dhondt et al., 2013; Furbank and Tester, 2011), could alleviate these bottlenecks. Remote sensing (RS) can collect object or area related information from a distance without having direct physical contact (Shanmugapriya et al., 2019). Image-based phenotyping are usually connected to some greenness-related biophysical parameters in crop such as chlorophyll content, vegetation biomass and leaf area index, all of

which directly or indirectly reflect crop vigor and photosynthesis capacity thus can be naturally used to indicate crop health and yield (Siegmann & Jarmer, 2015).

Electromagnetic radiation is a form of energy released and absorbed by charged particles, which has specific electrical and magnetic properties. The wavelength range corresponding to the electromagnetic radiation is termed the 'electromagnetic spectrum.' The human eye can only detect small portion of the spectrum. The interaction of electromagnetic spectrum with any material can be used in qualitative and quantitative analysis of various materials. The most commonly used quantitative index to assess the vegetation condition is the Normalized Difference Vegetation Index (NDVI) and was introduced by Rouse (Rouse et al., 1974). NDVI is defined by the reflectance of Red band (625 nm to 740 nm) and NIR band (750 nm to 1400 nm). The Red channel is the strong chlorophyll absorption region while NIR channel has high vegetation canopy reflectance in this area. The absorption and reflection vary with different depths through vegetation canopies. Hence, this index can be applied to classify the crop land cover and vigorousness (Zhang et al., 2017). The Green Seeker handheld is an instrument that directly provides the NDVI index, contributing to a fast and targeted diagnosis of nutritional and physiological state, the incidence of stress, and the potential yield of crops. Unlike aerial and satellite imagery, this system provides information obtained locally and quickly by terrestrial determinations.

Despite the great interest in the implementation of image-based methods in agriculture, research is still in its early stages for applying the technology in alternative oil seed crops such as camelina (ANGELOPOULOU et al., 2020). Prediction of yield in oilseed rape is quite challenging, since oilseed rape has distinct developmental stages (e.g., seedling, bolting,

flowering, pod formation) that are very spectrally different (Domínguez et al., 2015) thus increasing uncertainties of yield prediction by spectral indices. Many studies showed that the presence of yellow flowers on top of the canopy in oilseed rape caused a decline in the relationship between rape spectra and biophysical parameters (Behrens et al., 2006; Fang et al., 2016) and timing to acquire spectral data for yield prediction in oilseed rape is important to achieve high accuracy (Piekarczyk et al., 2011).

Digital image analysis is also widely used to determine the morphometric parameters of plants. This technique supports the identification and discrimination of various taxa (Cope et al., 2012). The shapes of leaves, petals and whole plants are of great significance to plant science, as they can help to distinguish between different species, to determine plant health, and even to model climate change (Cope et al., 2012). The plant phenomics technologies are used in many studies to evaluate plant traits in field conditions, such as early vigor (Kipp et al., 2014; Sankaran et al., 2015; Sankaran et al., 2018), canopy area and temperature (Bai et al., 2016; Patrignani & Ochsner, 2015), plant height (Madec et al., 2017; Wang et al., 2018), heading and flower intensity (Sadeghi-Tehran et al., 2017; Zhang et al., 2020), yield (Donohue et al., 2018; Lai et al., 2018), and phenological stages (Yang et al., 2017). Different image-based plant traits, such as flowers, vegetation indices (VIs), plant height, and canopy area (Bai et al., 2016; Sun et al., 2018; Tattaris et al., 2016; Thorp et al., 2016), have been used to monitor and predict crop yield. Among these plant traits, yield and agronomic traits are of great importance to agronomists, plant breeders and physiologists.

The objectives of this study were to investigate if 1) NDVI and 2) canopy area images can be used for phenotyping and identifying camelina genotypes for high productivity.

Materials and Methods

Trial Site

The experiments were carried out at the Eastern Agricultural Research Center (EARC), irrigated farm (47˚73′ N, 104˚15′ W; 594 m asl), near Sidney, Montana, in 2021 and 2022. The soil is Savage clay loam (fine, smectitic, frigid Vertic Argiustolls) with less than 3% organic matter (OM) and pH of 7.8.

Experimental Design

The 212 accessions from the germplasm collection of camelina from Montana State University (MSU) and seven spring camelina cultivars (Ligena, Soshone, Calena, Licalla, Pronghorn, Suneson, and Blainecreek) in 2021 and five cultivars (Ligena, Soshone, Licalla, Pronghorn, Suneson, and Suneson) in 2022 were planted as single-plant trail in Split-plot Randomized Complete Block design with six replications. The main plots are nitrogen doses, and the split plots are accessions.

Camelina seeds were sown into paper pots in the greenhouse on April 22nd, 2021 and June 7th , 2022. After sowing, all the seeds were thinly covered with potting mix soil and then soaked in water. The camelina emerged three days after planting. More than 95% emergence was observed on April 26th, 2021, and on June 10th, 2022.Right after the emergence, thinning was carried out to thin the plants down to one seedling per paper pot on April 28th, 2021, and June 15th ,2022. The seedlings were fertilized with 1 tablespoon of 20-20-20 (N-P-K) per gallon of water ten days after emergence. A week before transplanting, seedlings were hardened by taking them out of the greenhouse and into the open outside environment. Before the field preparation

for transplantation, soil samples were taken for the initial soil residual nutrients determination. Four composite soil samples, which is four soil cores per composite, were taken across the whole field at 0-6-, 6-12-, 12-24-, and 24–36-inch depths. The field was divided into low and high N blocks. Urea fertilizer was applied at a target rate of 100 kg N per acre or 223 lbs urea per acre on May 10th, 2021 and on June 24th ,2022 to the high N block, and no nitrogen fertilizer was applied to the low N block. For field preparation, soil was roto-tilled at least twice to make a uniform and loose seedbed. The TerraTek single row transplanter was used to carry out transplantation. The seedlings were transplanted into the field on May 12th, 2021, and on June 27th ,2022. Each transplanted seedling was inspected to ensure that the roots were covered with soil. Single camelina plants were planted at a foot apart and three feet between the rows. Sprinkler irrigation of half an inch was applied the day after transplantation.

Data Collection

The data collection included NDVI and canopy RGB images. NDVI value provides the information on the leaf area and leaf chlorophyll content which relates to grain yield (Labus et al., 2002). An NDVI plant health rating between 0 and 0.33 indicates unhealthy or stressed plant material, 0.33 to 0.66 moderately healthy, and 0.66 to 1 very healthy. These numbers are just rules of thumb and vary based on type of plant and other conditions.

For NDVI, a handheld device Green Seeker was used to collect readings once every week after a week of transplanting for three weeks until flowering, which gives us the NDVI readings between 0-1. The NDVI device was held 0-1 cm over the canopy area while collecting the data. The NDVI- value is computed as:

(NIR – Red) / (NIR + Red)

For canopy images, RGB images of the camelina single plants were taken using a RGB camera, (Canon DS126311 and Nikon D7100). A picture frame of 1 ft^2 quadrat was prepared. It was placed as a base frame while taking pictures of camelina plant at the center. The frame was used as a reference to extract the green area to measure change in canopy growth over time. In 2022, the camera was placed 1m distance from the ground. This non-destructive method allowed us to monitor the progression of growth in individual plant to evaluate the plant. The pictures were taken after transplanting for three weeks until flowering, when the canopy areas become dense and plants are overlapping each other, and the frame is not visible when taking pictures.

Data Analysis

The Image Analysis was done with the software Image J for the images of 2021 and Plant Computer Vision (PlantCV) was used for image analysis in 2022. ImageJ is a freely available (https://imagej.nih.gov/ij/) Digital Image Analysis (DIA) program that was developed originally for medical research (Schindelin et al., 2015). The canopy images were transferred from the camera to computer folders and were processed individually by using ImageJ (Version 1.54c). The images are cropped after taking the measurements from the frame by: “select Analyze > Set Scale”, which helps setting the scale in the centimeter’s metric. The color threshold is then adjusted following the ImageJ user Guide (Ferreira & Rasband, 2012). The selected image particles were reselected and analyzed by the following operations: select “Analyze > Analyze Particles. The results were transferred to a Microsoft Excel worksheet (.xlsx format) for future analysis.

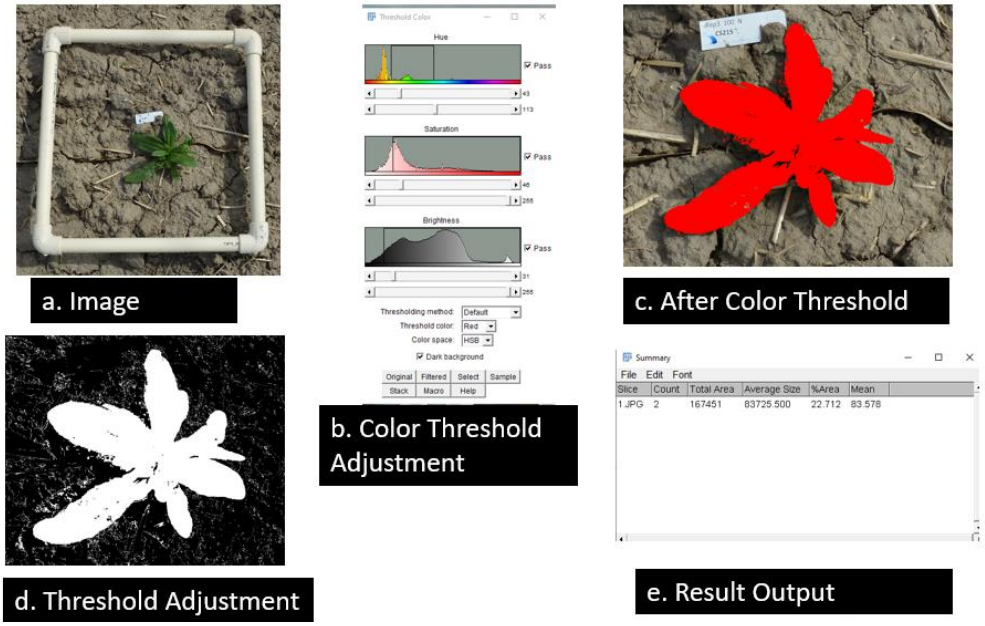

Figure 2. 1. Digital image analysis by ImageJ: (a) raw image capture; (b) ImageJ color threshold settings; (c)Image after cropping and applying color threshold; (d) green tissue in the image is selected; (e) green tissue is re-selected by "mask" function when analyzing the selected pixels.

Images from 2022 were processed using PlantCV an open-source, open-development suite of analysis tools capable of analyzing high-throughput image-based phenotyping data (Ferreira & Rasband, 2012). The pipeline is created in such a way that it works for all the images taken at a time-period in a batch. The results are received in .csv format in pixels. The reading for NDVI was taken through Greenseeker and the readings were saved in .txt file and later transferred to a Microsoft Excel worksheet (.xlsx format) for future analysis.

Analysis of variance (ANOVA) was performed on canopy area and NDVI for data of 2021 and 2022. The effects were considered statistically significant at $P < 0.05$. Tukey's HSD test was conducted for mean comparisons with 5% significance levels. All statistical analysis were performed using R version 4.2.3 (R core team, 2021). Data were fitted to a linear model to

test for normality. The canopy area data were not normally distributed and, therefore, square root transformed for the data. The 212 genotypes of camelina and spring varieties and nitrogen were explanatory variables and canopy area and NDVI were taken as response variables. Data were analyzed separately by year due to different configurations among them.

Results

Conditions in Sidney 2021 and 2022

The weather conditions of Sidney were slightly different in year 2021 and 2022 planting seasons, recording different temperatures, wind speed and rainfall (Table 2.1). The available nitrogen in the soil before the application of the nitrogen also varied in year 2021 and 2011 (Table 2.2).

Table 2. 1 Weather conditions in Sidney, MT in year 2021 and 2022.

Year 2021						
	Max Temp	Min Temp	Avg Bare Soil Temp	Avg Turf Soil Temp	Avg Wind Speed	Total Rainfall
Month	Degrees C	Degrees C	Degrees C	Degrees C	Kmph	mm
May	18.79	4.52	12.54	9.63	13.10	41.66
June	28.50	11.91	20.32	16.27	11.02	52.60
July	32.40	15.76	24.56	21.01	7.82	0.51
August	28.21	11.89	21.14	21.45	9.89	27.18
Year 2022						
June	24.28	10.75	17.22	17.42	12.50	67.97
July	29.21	14.03	23.73	21.55	8.30	45.57
August	30.57	13.73	23.76	19.59	8.78	3.63

Table 2. 2 Available soil nitrogen after fertilization in the field in year 2021 and 2022.

Available soil nitrogen after fertilization (lbs/Acre)		
Year	2021	2022
Low N	34.8	31
High N	134.8	131

Canopy Area

The canopy areas were assessed at three times at the early growing seasons each year. They were analyzed separately by year.

Year 2021:The analysis of variance (ANOVA) results showed that canopy area of camelina was significantly affected by genotype and nitrogen in all three time points of year 2021 (Table 2.3). The mean canopy area of the genotypes was higher when nitrogen was applied than no additional supply of nitrogen (Table 2.4). We did not detect an interaction between nitrogen and genotype.

Table 2. 3. ANOVA table showing the effects of genotype, nitrogen, and their interaction on canopy area taken at three time points on camelina in Sidney for year 2021.

Source of Variance	Df	2021Canopy Area(time1) 05/25/2021	2021Canopy Area(time2) 06/01/2021	2021Canopy Area(time3) 06/07/2021
		P>F	*P>F*	*P>F*
Genotype	215	0.0001	0.0116	0.0001
Nitrogen	1	<0. 0001	0. 0001	<0. 0001
Genotype x Nitrogen	215	0.7588	0.9387	0.9977

Table 2. 4. Mean comparisons table showing the effects of nitrogen on canopy area ($cm^{\wedge 2}$) taken at within each of three time periods on camelina in Sidney for year 2021.

Nitrogen	2021Canopy Area (time1)	2021Canopy Area (time2)	2021Canopy Area (time3)
High	4.329 a	6.490 a	9.968 a
Low	3.243 b	5.901 b	8.282 b

The mean canopy area varied by genotypes. Ligena, CS210 and CS023 had the highest mean canopy area at time 1, time 2 and time 3 respectively. The figure below represents the canopy area at time 1, which is a week after the transplantation (Figure 2.2). The results of differences in mean canopy area at high and low N regimes are shown in Table 2.5, where top 30 genotypes with higher canopy area were listed from all three time periods.

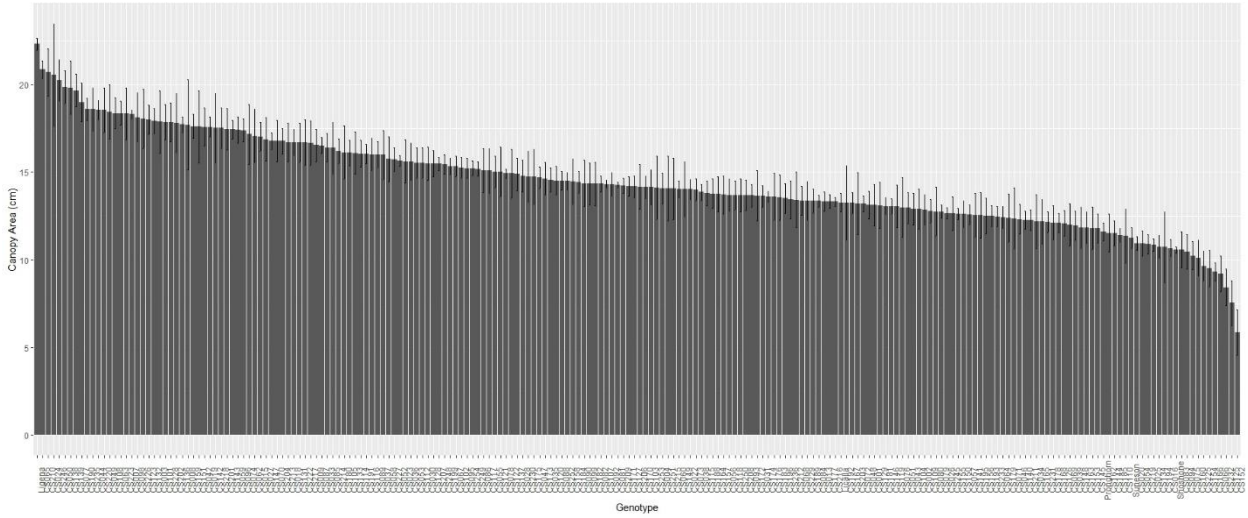

Figure 2. 2 . Effect of genotype on canopy area in square centimeters of camelina taken one week after transplantation (time 1) in year 2021.

Table 2. 5. Top 30 genotypes with the largest canopy areas at times 1,2, and 3 in Sidney, during 2021 growing season.

Time 1			Time 2			Time 3		
#	Genotypes	Canopy area	#	Genotypes	Canopy area	#	Genotypes	Canopy area
1	Ligena	22.318	1	CS210	58.963	1	CS023	128.420
2	CS065	20.868	2	CS018	57.744	2	CS217	118.550
3	CS210	20.708	3	CS024	56.931	3	CS050	115.632
4	CS024	20.545	4	CS222	55.581	4	CS133	115.289
5	CS246	20.243	5	CS173	55.526	5	CS009	115.118
6	CS050	19.846	6	CS037	55.441	6	CS204	114.328
7	CS138	19.829	7	CS030	54.357	7	CS098	114.249
8	CS139	19.665	8	CS114	54.311	8	CS249	113.810
9	CS077	18.980	9	CS007	53.835	9	CS065	112.809
10	CS190	18.594	10	CS077	53.499	10	CS114	112.521
11	CS235	18.576	11	CS008	53.269	11	CS220	112.305
12	CS044	18.553	12	CS226	52.609	12	CS147	112.141
13	CS220	18.548	13	CS132	52.585	13	CS007	111.969
14	CS049	18.451	14	CS128	52.276	14	CS101	110.650
15	CS108	18.374	15	CS217	51.752	15	CS146	110.411
16	CS063	18.370	16	CS009	51.429	16	CS201	110.077
17	CS223	18.343	17	CS201	51.377	17	CS033	109.877
18	CS007	18.308	18	CS043	51.127	18	CS192	109.664
19	CS098	18.133	19	CS246	50.927	19	CS142	109.583
20	CS226	18.037	20	CS143	50.733	20	CS246	109.142
21	CS123	18.003	21	CS213	50.577	21	CS248	108.790
22	CS132	17.928	22	CS096	49.855	22	CS143	108.490
23	CS003	17.877	23	CS035	49.838	23	CS128	108.232
24	CS101	17.863	24	CS233	49.736	24	CS228	107.617
25	CS228	17.857	25	CS230	49.717	25	CS116	107.524
26	CS202	17.811	26	CS058	49.607	26	CS188	107.399
27	CS136	17.724	27	CS151	49.603	27	CS093	107.104
28	CS008	17.703	28	CS220	49.574	28	CS027	107.034
29	CS159	17.622	29	CS050	49.384	29	CS071	106.874
30	CS151	17.593	30	CS019	49.299	30	CS223	106.516

Growth rates were calculated as the difference of canopy areas taken at different time points divided by the time lapse. The ANOVA results showed that growth rate of camelina was significantly affected by genotype and nitrogen between the 2nd and 3rd time points of year 2021 (Table 2.6).

Table 2. 6. ANOVA showing the effects of genotype, nitrogen and their interaction on canopy area growth rate between times 1 and 2 (Rate 1) and times 2 and 3 (Rate 2) in Sidney during 2021 growing season.

Source of Variance	Df	2021Canopy Area Growth Rate 1	2021Canopy Area Growth Rate 2
		P>F	*P>F*
Genotype	215	0.5191	0.0001
Nitrogen	1	0.1630	<0. 0001
Genotype x Nitrogen	215	0.9372	0.9910

The figure 2.3 below represents the mean canopy area growth rate at high and low N regimes taken between times 2 and 3, where the growth of canopy area is higher with high nitrogen and lower with low nitrogen. Figure 2.4 represents the variation of growth rate of 30 genotypes with larger canopy area growth rate between time 2nd and 3rd periods.

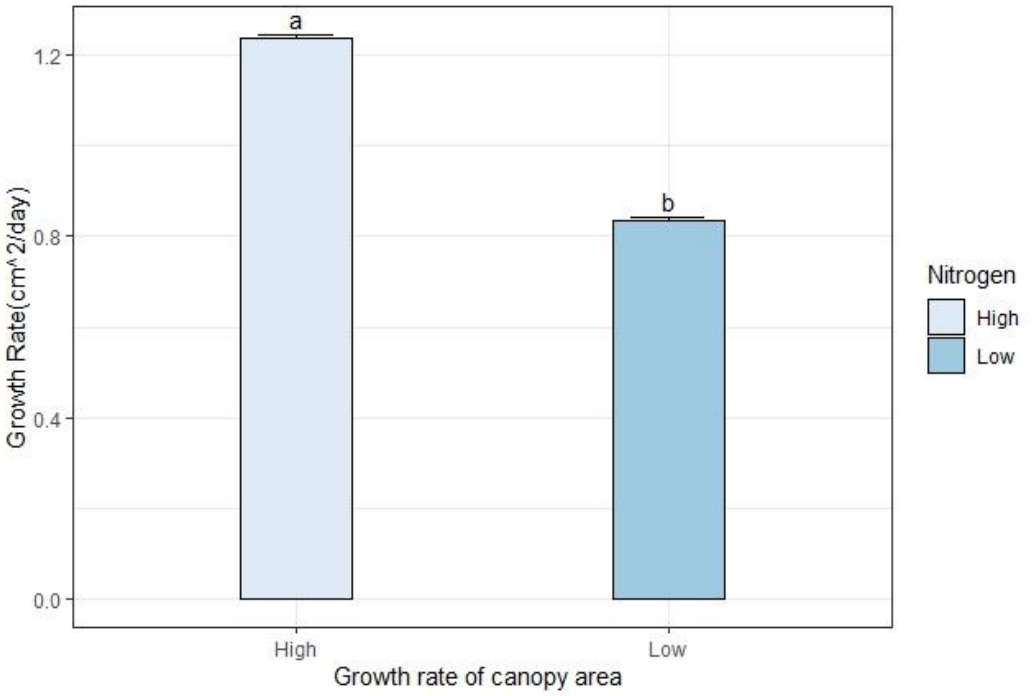

Figure 2. 3. Effect of nitrogen on canopy area growth rate (cm^2/day) of camelina taken between times 2 and 3 in year 2021.

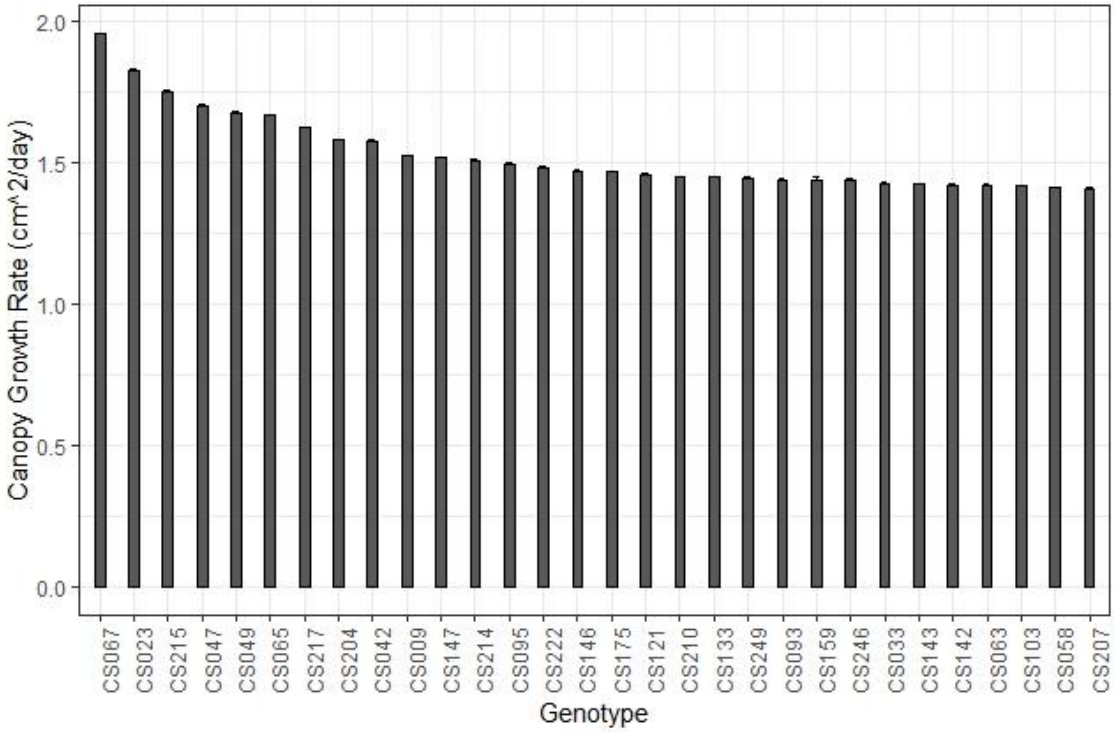

Figure 2. 4. Effect of genotype on canopy area growth rate (cm^2/day) of camelina taken between times 2 and 3 in year 2021.

Year 2022:The ANOVA results showed that canopy area of camelina was significantly affected by genotype and nitrogen in both time periods of year 2022 (Table 2.7). The mean canopy area of the genotypes was higher when nitrogen was applied than when there was no nitrogen application (Table 2.8). The mean canopy area varies with the genotypes, genotype CS144 (20.4755) and CS158 (69.173) had highest mean canopy area in time 1 and time 2 respectively (Table 2.9).

Table 2. 7. ANOVA showing the effects of genotype, nitrogen and their interaction on canopy area taken at two time periods on Camelina in Sidney for year 2022.

Source of Variance	Df	2022Canopy Area(time1) 06/02/2022	2022Canopy Area(time2) 07/15/2022
		P>F	*P>F*
Genotype	215	0.0002	0.0060
Nitrogen	1	<0.0001	0.0001
Genotype x Nitrogen	215	0.7782	0.8380

Table 2. 8. Mean comparisons showing the effects of nitrogen on canopy area (cm^2) within two time periods on camelina in Sidney for year 2022.

Nitrogen	2022Canopy Area(time1) 06/02/2022	2022Canopy Area(time2) 07/15/2022
High	14.09 a	55.26 a
Low	5.81 b	37.20 b

Table 2. 9 Top 30 genotypes with the largest canopy areas at times 1, and 2 in Sidney, 2022.

2022 Canopy Area taken at different times in cm^2					
Time 1			Time 2		
#	Genotypes	Mean	#	Genotypes	Mean
1	CS144	20.476	1	CS158	69.173
2	CS220	18.229	2	CS157	67.225
3	CS166	18.138	3	CS144	67.128
4	CS075	17.063	4	CS166	65.160
5	CS087	16.488	5	CS050	63.658
6	CS024	16.446	6	CS067	63.018
7	CS109	16.182	7	CS192	58.983
8	CS215	16.030	8	CS024	56.770
9	CS223	15.465	9	CS132	54.207
10	CS077	15.246	10	Shoshone	53.529
11	CS050	15.011	11	CS220	52.533
12	CS116	14.699	12	CS094	52.042
13	CS182	14.693	13	CS202	50.613
14	CS222	14.676	14	CS116	50.517
15	CS203	14.315	15	CS075	49.553
16	CS094	14.171	16	CS143	49.412
17	CS230	13.983	17	CS042	48.741
18	CS201	13.733	18	CS230	48.252
19	CS105	13.630	19	CS049	47.353
20	CS254	13.454	20	CS053	46.265
21	CS158	13.255	21	CS223	46.154
22	CS211	13.153	22	CS215	45.200
23	Suneson	13.142	23	CS218	45.157
24	CS157	12.990	24	CS055	44.331
25	CS092	12.964	25	CS226	44.224
26	CS131	12.831	26	CS108	43.758
27	CS034	12.734	27	CS102	43.633
28	CS066	12.700	28	CS137	42.589
29	CS053	12.555	29	CS188	42.303
30	CS086	12.512	30	CS105	42.094

Table 2. 10. ANOVA showing the effects of genotype, nitrogen, and their interaction on growth rate of canopy area (cm^2day^{-1}) taken at two time periods in Sidney, 2022.

Source of Variance	Df	2022 Canopy Area Growth Rate 1
		P>F
Genotype	215	0.0001
Nitrogen	1	0.0001
Genotype x Nitrogen	215	0.3069

Figure 2.5 represents the mean canopy area growth rate taken between times 1 and 2, where the growth of canopy area is higher with high nitrogen and lower with low nitrogen (Table 2.8).

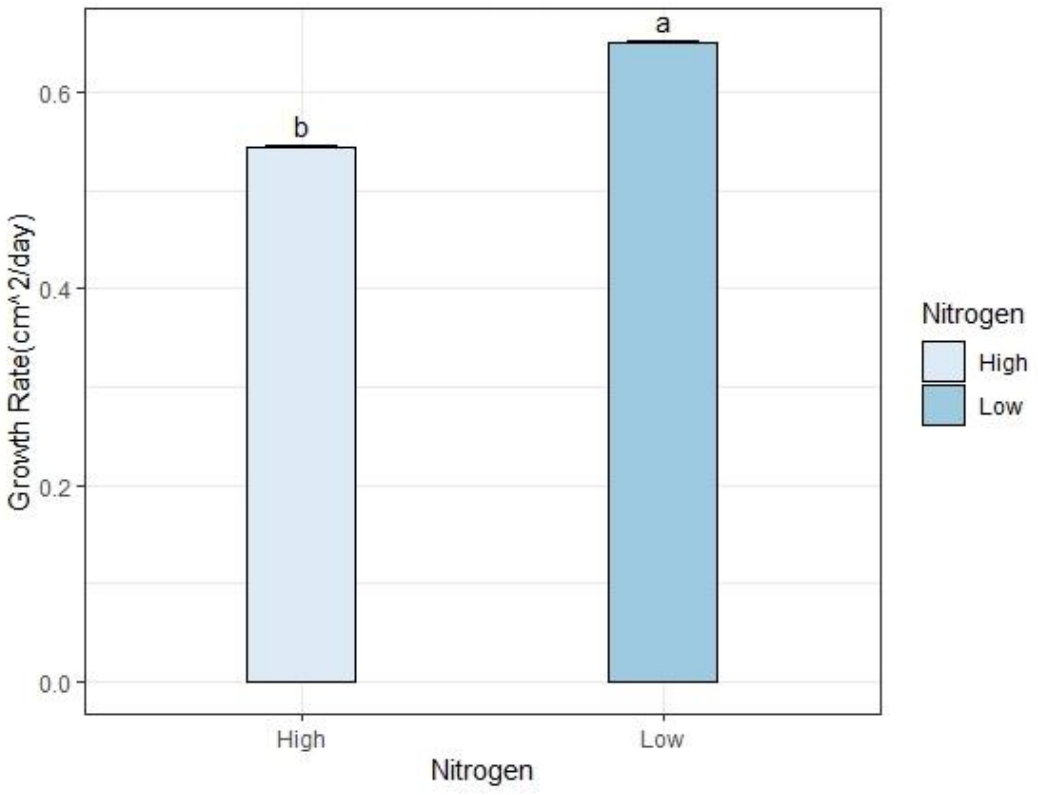

Figure 2. 5. Effect of nitrogen on canopy area growth rate (cm^2/day) of camelina taken in year 2022.

Normalized Vegetative Index (NDVI)

The canopy of the plants was assessed also by NDVI for each year 2021 and 2022. The data were analyzed separately by year. The NDVI readings were taken three times after transplantation, once every week, for three weeks.

Year 2021:The ANOVA results (Table 2.11) show that NDVI is highly significantly affected by the genotypes in all three time periods. NDVI is significant with nitrogen during first and second period. There is no significance with NDVI and interaction between genotype and nitrogen.

Table 2. 11 .ANOVA showing the effects of genotype and nitrogen on Normalized Difference Vegetation Index (NDVI) taken at three time points in Sidney for year 2021.

Source of Variance	Df	2021 NDVI (time1) 06/15/2021	2021 NDVI (time2) 06/24/2021	2021 NDVI (time3) 06/28/2021
		P>F	*P>F*	*P>F*
Genotype	215	0. 0001	0. 0001	0. 0001
Nitrogen	1	0.0135	0.0006	0.1040
Genotype x Nitrogen	215	0.4439	0.5263	0.3773

Table 2. 12. Top 30 genotypes with the highest NDVI values across three points (time 1, 2, and 3) in Sidney 2021.

2021 NDVI								
#	Genotypes	NDVI 1	#	Genotypes	NDVI 2	#	Genotypes	NDVI 3
1	CS133	0.918	1	CS254	0.975	1	CS133	0.942
2	CS202	0.915	2	CS023	0.947	2	CS245	0.930
3	CS019	0.910	3	CS220	0.940	3	CS229	0.903
4	CS008	0.906	4	CS019	0.933	4	CS023	0.897
5	CS030	0.905	5	CS215	0.932	5	CS101	0.897
6	CS223	0.904	6	CS201	0.926	6	CS030	0.896
7	CS042	0.904	7	CS036	0.925	7	CS201	0.879
8	CS044	0.901	8	CS121	0.924	8	CS071	0.872
9	CS235	0.900	9	CS033	0.919	9	CS210	0.870
10	CS087	0.900	10	CS101	0.918	10	CS202	0.866
11	CS003	0.899	11	CS142	0.915	11	CS248	0.857
12	CS016	0.895	12	CS024	0.908	12	CS003	0.855
13	CS204	0.894	13	CS015	0.905	13	CS215	0.855
14	CS004	0.889	14	CS075	0.904	14	CS220	0.853
15	CS018	0.887	15	CS202	0.902	15	CS122	0.850
16	CS142	0.884	16	CS210	0.901	16	CS235	0.847
17	CS137	0.882	17	CS166	0.891	17	CS193	0.844
18	CS217	0.879	18	CS077	0.888	18	CS230	0.843
19	CS220	0.878	19	CS037	0.886	19	CS144	0.841
20	CS190	0.876	20	CS003	0.882	20	CS254	0.839
21	CS131	0.871	21	CS230	0.876	21	CS024	0.838
22	CS116	0.870	22	CS071	0.862	22	CS025	0.830
23	CS056	0.869	23	CS217	0.860	23	CS033	0.826
24	CS051	0.869	24	CS030	0.859	24	CS162	0.824
25	CS216	0.868	25	CS188	0.854	25	CS222	0.817
26	CS143	0.868	26	CS122	0.853	26	CS166	0.817
27	CS236	0.867	27	CS050	0.852	27	CS116	0.808
28	CS170	0.867	28	CS236	0.844	28	CS027	0.805
29	CS037	0.866	29	CS096	0.842	29	CS236	0.804
30	CS039	0.866	30	CS126	0.839	30	CS019	0.803

Table 2. 13 Mean comparisons showing the effects of nitrogen on genotypes in NDVI taken at three time periods in Sidney for year 2021.

Nitrogen	2021 NDVI (time1) 06/15/2021	2021 NDVI (time2) 06/24/2021	2021 NDVI (time3) 06/28/2021
Low	0.793 a	0.733 a	0.668 a
High	0.772 b	0.698 b	0.652 a

The mean of NDVI readings had a trend of decreasing with the time after transplanting (NDVI1 vs. NDVI2 vs. NDVI3; Figure 2.6; Table 2.13). The mean NDVI of camelina planted at low nitrogen regimes was slightly higher than with nitrogen supply (Table 2.13).

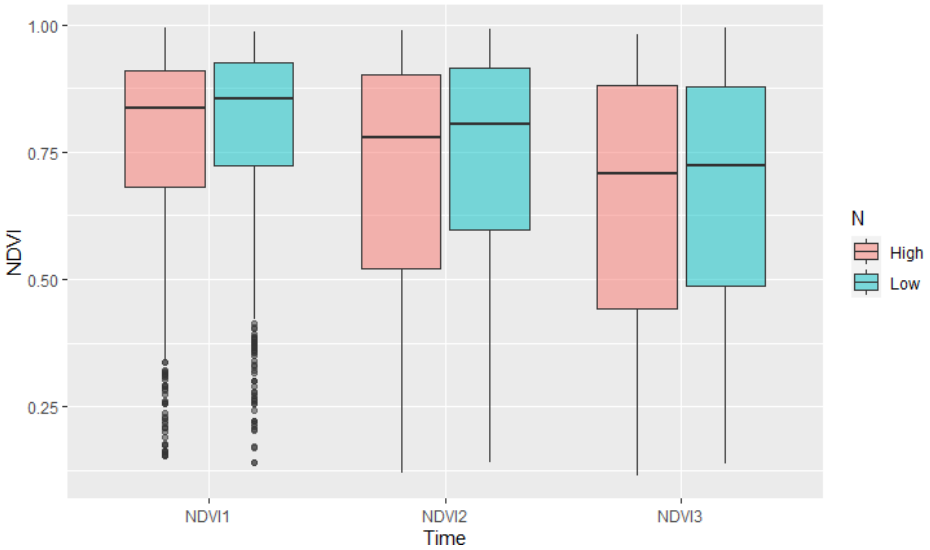

Figure 2. 6. Effect of nitrogen on NDVI of camelina at three time periods after transplantation in year 2021, first week as NDVI 1, second week as NDVI 2 and third week as NDVI 3.

Year 2022:The ANOVA results (Table 2.14) shows that NDVI is significantly affected by the genotypes in all second and third time periods. NDVI is significant with nitrogen during

first and third period. There is no significance with NDVI and interaction between genotype and nitrogen.

Table 2. 14 . ANOVA showing the effects of genotype and nitrogen on Normalized Difference Vegetation Index (NDVI) taken at three time points in Sidney for year 2022.

Source of Variance	Df	2022 NDVI (time1) 07/21/2022	2022 NDVI (time2) 07/28/2022	2022 NDVI (time3) 08/04/2022
		P>F	*P>F*	*P>F*
Genotype	215	0.7094	0.0057	0.0826
Nitrogen	1	0. 0001	0.6204	0. 0001
Genotype x Nitrogen	215	0.5932	0.8840	0.9060

Table 2. 15. Top 30 genotypes with the highest NDVI values across three points (time 1, 2, and 3) in Sidney 2022.

2022 NDVI								
#	Genotypes	NDVI 1	#	Genotypes	NDVI 2	#	Genotypes	NDVI 3
1	CS009	0.786	1	CS050	0.892	1	CS202	0.755
2	CS250	0.774	2	CS164	0.845	2	CS245	0.745
3	CS052	0.767	3	CS182	0.831	3	CS126	0.743
4	CS033	0.743	4	CS133	0.827	4	CS085	0.739
5	CS084	0.740	5	CS228	0.806	5	CS137	0.734
6	CS122	0.738	6	CS062	0.803	6	CS220	0.723
7	CS094	0.736	7	CS019	0.802	7	CS101	0.707
8	CS050	0.732	8	CS047	0.802	8	CS253	0.705
9	CS137	0.725	9	CS030	0.797	9	CS230	0.700
10	CS024	0.725	10	CS077	0.793	10	CS254	0.698
11	CS129	0.722	11	CS116	0.790	11	CS102	0.697
12	CS163	0.717	12	CS220	0.790	12	CS175	0.694
13	CS144	0.716	13	CS109	0.784	13	CS067	0.694
14	CS146	0.713	14	CS254	0.784	14	CS142	0.693
15	CS030	0.705	15	CS071	0.782	15	CS133	0.686
16	CS081	0.697	16	CS132	0.780	16	CS112	0.681
17	CS218	0.693	17	CS137	0.775	17	CS030	0.676
18	CS119	0.692	18	CS084	0.768	18	CS051	0.668
19	CS027	0.689	19	CS230	0.762	19	CS165	0.668
20	CS028	0.686	20	CS009	0.758	20	CS076	0.667
21	CS046	0.685	21	CS049	0.755	21	CS008	0.662
22	CS102	0.684	22	CS175	0.754	22	CS105	0.647
23	CS116	0.681	23	CS115	0.745	23	CS025	0.645
24	CS196	0.679	24	CS039	0.743	24	CS222	0.642
25	Ligena	0.676	25	CS087	0.739	25	CS055	0.640
26	CS139	0.676	26	CS085	0.736	26	CS044	0.638
27	CS191	0.670	27	CS245	0.734	27	CS218	0.637
28	CS051	0.667	28	CS159	0.734	28	CS071	0.637
29	CS214	0.664	29	CS188	0.727	29	CS223	0.637
30	CS121	0.663	30	CS064	0.726	30	CS210	0.637

Table 2. 16. Mean comparisons showing the effects of nitrogen on genotypes in NDVI taken at three time periods in Sidney for year 2022.

Nitrogen	2022 NDVI (time1) 07/21/2022	2022 NDVI (time2) 07/28/2022	2022 NDVI (time3) 08/04/2022
Low	0.631 a	0.606 a	0.557 a
High	0.506 b	0.600 a	0.496 b

The mean NDVI reading was higher for high N than low N at one week after transplantation (time1), but it was higher for low N (time2) than high N at second week after transplantation (Table 2.16; Figure 2.7).The mean NDVI of camelina planted at low nitrogen regimes was slightly higher than that of those provided with nitrogen, except at second week after transplantation where mean NDVI of low N was similar to that of higher N.

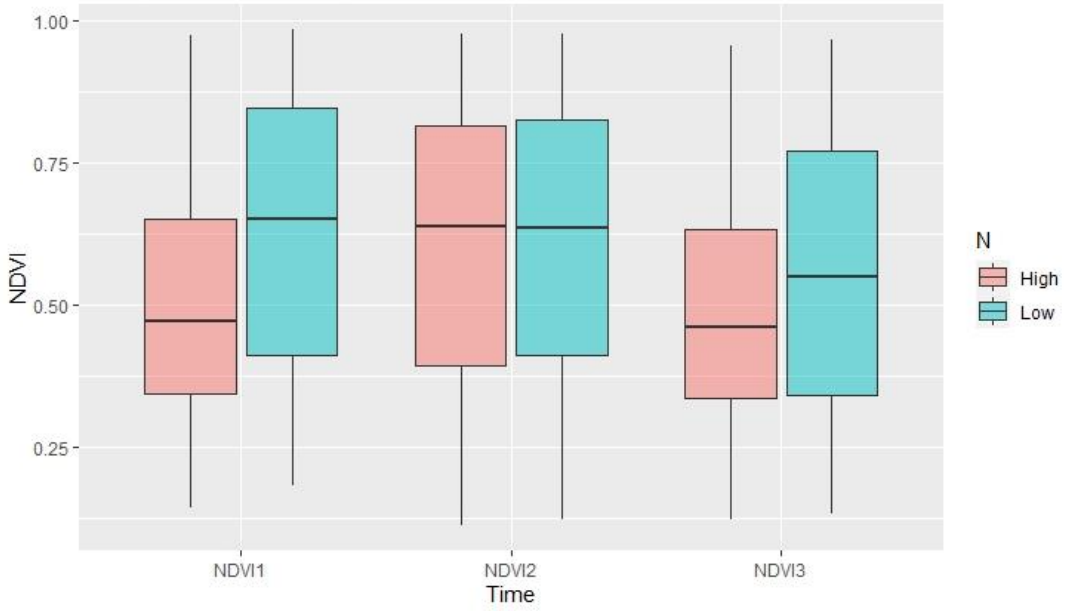

Figure 2. 7. Effect of nitrogen on NDVI of camelina at three time periods after transplantation in year 2022, first week as NDVI 1, second week as NDVI 2 and third week as NDVI 3.

Discussion

Identification of Lines that Have Robust Vegetative Growth

The canopy area significantly increased by nitrogen applied in the field in both years 2021 and 2022. The growth rate calculated using image analysis were also significant with the nitrogen applications in both years. Therefore, it is crucial to consider the effect of nitrogen on growth of camelina during early season as it is essential for optimum N management and seed productivity (Seepaul et al., 2016). Also, canopy areas and growth rates measured were significantly different among genotypes in 2021 and 2022, resembling Brassica populations that exhibit significant genetic variation (Ahmad et al., 2008). However, the interaction between genotype and nitrogen was not seen in camelina.

Similarly, the NDVI measurements, which help classify the crop cover and vigorousness (Zhang et al., 2017), suggest that nitrogen had significant effects on camelina during vegetative

state before flowering in 2021 and 2022. The NDVI, interpretation can aid in quickly and accurately diagnosing the nutritional and physiological state, stress levels, and potential yield of crops (Gutiérrez-Soto et al., 2011). As indicated by Labus et al. (2002), NDVI value provides the information on the leaf area and chlorophyll content which may relate to grain yield. The presence of yellow flowers in a vegetation canopy can lead to a decrease in NDVI values caused by red light (yellow = green + red) (Behrens et al., 2006; Piekarczyk et al., 2011; Shen et al., 2009). In this study, the NDVI seems to decrease later in the vegetative stage, which corresponds to the beginning of flowering stage. In research performed in canola, the NDVI data acquired between the six-leaf stage and the beginning of flowering were correlated to canola seed yield ($R2 = 0.35$; $p < 0.001$) (Holzapfel et al., 2007).

Here, several genotypes were selected based on high canopy area and NDVI because they are reflective of health ,vigor and greenness of the plant thus can be used to indicate crop yield (Siegmann & Jarmer, 2015). The few genotypes were selected resulting high canopy area and NDVI. For elucidation of plant N response across the entire vegetation, destructive measurements are not appropriate. Instead, non-destructive high-throughput techniques, for example by unmanned aerial vehicles, can be a feasible option, and these initial image-based findings provide a foundation for those future efforts. Those data, coupled with elucidation of genome diversity (Voss-Fels & Snowdon, 2016). The few genotypes were selected resulting high canopy area and NDVI. In this study, there were differences in the genotype ranking based on canopy area and NDVI at different measuring time points, which was likely caused by the differences in morphology and phenology of different biotypes of camelina. As for instance we

can see different stages of growth in camelina and can note the dates of flower initiation and pod formation (Figure 2.8).

Figure 2. 8. Images changing morphology and phenology of genotype CS070 in week 1 (06/02/2022) (a); week 2 (07/15/2022) (b); week 3 (07/22/2022) (c) ;and week 4 (07/29/2022) (d),in 2022.

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CHAPTER THREE

SELECTION OF CAMELINA LINES FOR HIGH NITROGEN USE EFFICIENCY

Introduction

Although camelina is a low-input new oilseed crop, it responds well to fertilization (Putnam et al., 1993). Various researchers have observed the effect of applied nitrogen (N) on yield, protein and oil content (Gugel & Falk, 2006; Jiang et al., 2013; Johnson & Gesch, 2013; Kirkhus et al., 2013; Lošák et al., 2011; Urbaniak et al., 2008). While focusing on maximizing seed and oil yield response, most studies lack information on balancing agronomic performance against the environmental risk associated with nitrogen fertilization, e.g., nitrate-N and ammonium-N remaining in the soil after harvest, which can be transported off-site (Randall et al., 1997). Furthermore, nitrogen is one of the most expensive nutrients to supply, therefore it is essential to measure and maximize nutrient use efficiency (Good et al., 2004). Nitrogen application should provide enough N to optimize yield while minimizing any unused N that could be lost to the environment (Robertson & Vitousek, 2009). The efficiency of a crop utilizing nitrogen fertilizer determines economic sustainability of cropping system and response of crops to applied N and use efficiency is important criteria for evaluating crop (Neupane et al., 2018). Spring camelina was relatively efficient at taking up N at moderate levels of fertilization, but higher levels do not necessarily translate to higher yields (Johnson et al., 2019). The selection of plants with more efficient nitrogen usage is, therefore, an important research goal in achieving greater agricultural sustainability (Gifford et al., 1984). This challenge must be approached through the selection of the high nitrogen use efficient crops and applying optimal rates of N fertilizers.

Nitrogen (N) is a macronutrient that significantly affects yield and growth in plants; it is involved in the metabolism and transformation of energy, chlorophyll, and protein synthesis. It also affects uptake of other essential nutrients and helps in the optimal partitioning of photosynthates to reproductive parts which increases the seed: stover ratio (Singh A, 2004). However, plant requirements for N vary with cultivar, growth stage of plant, N utilization efficiency, soil type, climate, and type of N application (Berry et al., 2010; Sidlauskas & Tarakanovas, 2004). The observed variability in the nutrient use efficiency among plants is partly due to the inherent natural genetic variability within the germplasm (Baligar et al., 2001). The variability influences nutrient use efficiency of plants is based on the nutrient uptake, incorporation, and utilization efficiency of the plant (Baligar VC 2015).

Definitions of nitrogen use efficiencies have been grouped or classified as agronomic efficiency, physiological efficiency, agro-physiological efficiency, apparent recovery efficiency, and utilization efficiency (Fageria & Baligar, 2001; Fageria & Baligar, 2003).

Agronomic efficiency: defined as the economic production obtained per unit of nitrogen applied and calculated by:

$$\text{Agronomic efficiency (AE; kg kg}^{-1}) = ((Gf - Gu)/Na)$$

where Gf is the grain yield of the fertilized plot (kg), Gu is the grain yield in the unfertilized plot (kg), and Na is the quantity of nitrogen applied (kg).

Physiological efficiency: defined as the biological yield obtained per unit of nitrogen uptake and calculated by:

$$\text{Physiological efficiency (PE; kg kg}^{-1}) = ((Yf - Yu)/(Nf - Nu))$$

where Yf is the total biological yield (grain plus straw) of the fertilized plot (kg), Yu is the total biological yield in the unfertilized plot (kg), Nf is the nitrogen accumulation in the fertilized plot in grain and straw (kg), and Nu is the nitrogen accumulation in the unfertilized plot in grain and straw (kg).

Agro-physiological efficiency: defined as the economic production (grain yield in case of annual crops) obtained per unit of nitrogen uptake and calculated by:

$$\text{Agro-physiological efficiency (APE; kg kg}^{-1}) = ((Gf - Gu)/(Nf - Nu))$$

where Gf is the grain yield in the fertilized plot (kg), Gu is the grain yield in the unfertilized plot (kg), Nf is the nitrogen accumulation by straw and grain in the fertilized plot (kg), and Nu is the nitrogen accumulation by straw and grains in the unfertilized plot (kg).

Apparent recovery efficiency is defined as the quantity of nitrogen uptake per unit of nitrogen applied and calculated by:

$$\text{Apparent recovery efficiency (ARE; \%)} = ((Nf - Nu)/Na) \times 100$$

where Nf is the nitrogen accumulation by the total biological yield (straw plus grain) in the fertilized plot (kg), Nu is the nitrogen accumulation by the total biological yield (straw plus grain) in the unfertilized plot (kg), and Na is the quantity of nitrogen applied (kg).

For field had no fertilizer applied, the NUE may be evaluated as:

$$\text{Nitrogen Use Efficiency (NUE; kg kg}^{-1}) = (Y/N)$$

where Y is the biomass yield of the unfertilized plot (kg), and N is the quantity of nitrogen available in field (kg) (Badr et al., 2016; Hammad et al., 2017; Jin et al., 2012; Lu et al., 2016; Mon et al., 2016).

The yield estimation under different N regimes is commonly used as the indicator of NUE. In this study, experiments were conducted with two nitrogen doses and with different genotypes to study NUE and select genotypes by their performance on these conditions.

Materials and Methods

Data Collection

In the same study described in Chapter 2, after pod setting the plants were bagged and tied well to minimize seed loss. The plants were uprooted or cut near to the ground surface after the plants reached physiological maturity. The roots were removed from the uprooted plants, and the plants were kept in a greenhouse and airdried. The above ground biomass was weighed after airdrying, and agronomic (NUE) were calculated.

Data Analysis

The data for biomass were taken and saved to a Microsoft Excel worksheet (.xlsx format), the data was also used to calculate Nitrogen Use Efficiency (NUE).

The statistical analysis was performed using R version 4.2.3 (R core team, 2021). The biomass yields of the 212 genotypes of camelina plus the check and spring varieties were accessed for their response to N and data were fitted to a linear model to test for normality. Since the biomass data were not normally distributed, the square root transformation was used for data transform. The analysis of variance (ANOVA) was performed on biomass after transformation to determine the effects of N and genotype. The NUE was calculated with agronomic efficiency (AE; kg kg^{-1}) = ((Gf – Gu)/Na)

where Gf is the biomass yield of the fertilized plot (kg), Gu is the biomass yield in the unfertilized plot (kg), and Na is the quantity of nitrogen applied (kg). Because the difference of biomass yield between high N and low N treatment were small and negative in some cases, and since the ANOVA showed that N effect was not significant, NUE was calculated using the biomass yields from the unfertilized plots, i.e.,

$$\text{NUE (kg kg}^{-1}\text{)} = \text{Plant Biomass/ N}$$

where, Plant Biomass is the biomass yield of the unfertilized plot (g), and N is the quantity of nitrogen in the field (kg) (Moll et al., 1982)

ANOVA was performed for NUE. Data were tested for normality and square root transformation was performed. The effects were considered statistically significant at $p < 0.05$. Tukey's HSD test was conducted for mean comparisons with 5% significance levels.

Results

Biomass

The biomass of MSU collection of 212 genotypes of camelina and check spring varieties with different nitrogen treatments were assessed for year, N, genotype, and their interactive effects for 2021 and 2022.

The ANOVA results showed that biomass of camelina was significantly affected by year, genotype and interaction between genotype and year (Table 3.1), but no significant nitrogen effects. Therefore, NUE was calculated using the biomass from unfertilized plots only and analyzed by individual years (Table 3.2).

Table 3. 1. ANOVA table showing the effects of year, genotype, nitrogen, and its interaction on biomass yield (grams) in Sidney on year 2021 and 2022.

Source of Variance	Df	Biomass in grams
		P>F
Year	1	0.0001
Genotype	215	0.0001
Nitrogen	1	0.8681
Year x Genotype	215	0.0222
Year x Nitrogen	1	0.4689
Genotype x Nitrogen	215	0.3453
Year x Genotype x Nitrogen	215	0.4743

Biomass and Canopy Imagery

In this experiment we could see that the correlation of biomass with canopy area varied with measuring time points with coefficients of correlation ranging between 0.24 and 0.50. The correlation coefficient with the collective canopy area of year 2021 and year 2022 in time point 1 and 2 (Ca1 and Ca2) was 0.50 and 0.24, respectively, and the correlation of canopy area at third time point (Ca3) in 2021 is 0.29. Canopy area at time point 1 (Ca1) was quite a decent predictor when including data from both years (Figure 3.1).

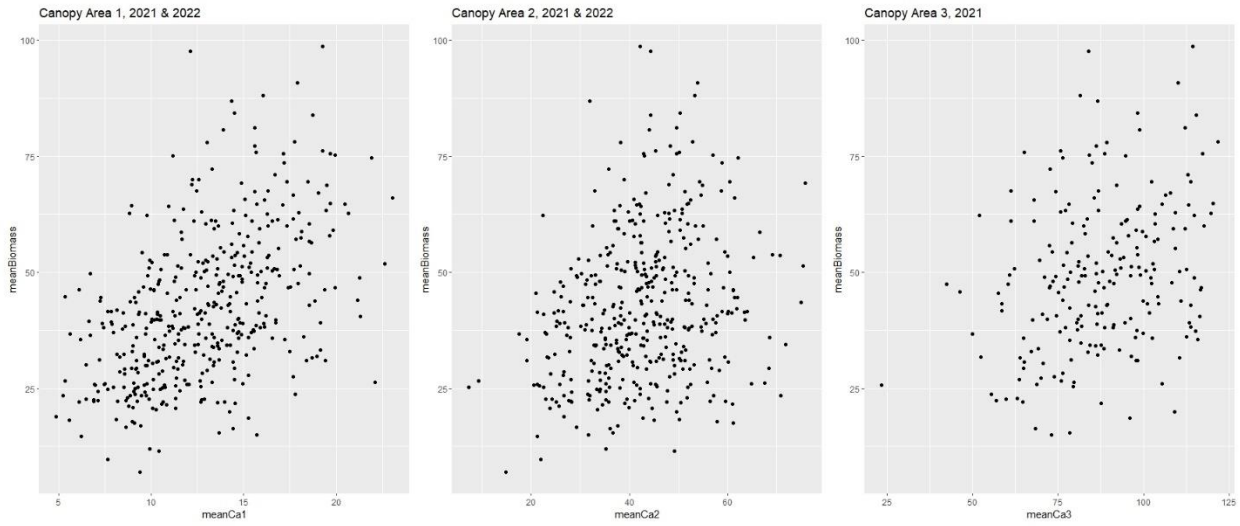

Figure 3. 1 Correlation of Canopy Area taken at different time points in year 2021 (time 1,2 and 3) and year 2022(time 1 and 2) with Biomass.

Nitrogen Use Efficiency (NUE)

ANOVA results showed that NUE of camelina was significantly affected by genotype in 2021 and 2022 (Table 3.2). The NUE varied greatly among genotypes (Figure 3.2; Figure 3.3). The highest NUE in g plant^{-1} kgN^{-1}was of genotype CS144 (0.36444) and CS248 (0.3454) in 2021 and 2022, respectively (Table 3.3).

Table 3. 2. ANOVA table showing the effects of genotype on NUE (g plant^{-1} kgN^{-1}) in Sidney for years 2021 and 2022.

Source of Variance	Df	2021 NUE	2022 NUE
		P>F	*P>F*
Genotype	215	<2.20E-16	0.03434

Table 3. 3 Top 30 genotypes with the highest NUE (g plant^{-1} kgN$^{-1)}$ in Sidney in 2021 and 2022.

2021 and 2022 Genotypes with NUE					
2021			2022		
#	Genotypes	Mean	#	Genotypes	Mean
1	CS144	0.3644	1	CS248	0.3454
2	CS229	0.3508	2	CS088	0.3120
3	CS230	0.3277	3	CS133	0.3082
4	CS142	0.3249	4	CS047	0.3035
5	CS215	0.3142	5	CS213	0.2942
6	CS143	0.3076	6	CS071	0.2781
7	CS101	0.3032	7	CS067	0.2758
8	CS254	0.3020	8	CS142	0.2666
9	CS150	0.3010	9	CS009	0.2662
10	CS217	0.2982	10	CS131	0.2650
11	CS202	0.2846	11	CS060	0.2622
12	CS235	0.2832	12	CS116	0.2561
13	CS075	0.2800	13	CS063	0.2552
14	CS185	0.2716	14	CS246	0.2538
15	CS193	0.2701	15	CS044	0.2445
16	CS030	0.2641	16	CS058	0.2391
17	CS133	0.2630	17	Shoshone	0.2340
18	CS122	0.2568	18	CS189	0.2309
19	CS210	0.2564	19	CS103	0.2295
20	CS226	0.2515	20	CS162	0.2284
21	CS220	0.2511	21	CS183	0.2275
22	CS071	0.2511	22	CS037	0.2263
23	CS162	0.2457	23	CS217	0.2244
24	CS049	0.2379	24	CS220	0.2236
25	CS218	0.2326	25	CS210	0.2229
26	CS201	0.2310	26	CS145	0.2184
27	CS192	0.2309	27	CS136	0.2151
28	CS060	0.2305	28	CS081	0.2150
29	CS042	0.2299	29	CS027	0.2122
30	CS044	0.2284	30	CS182	0.2073

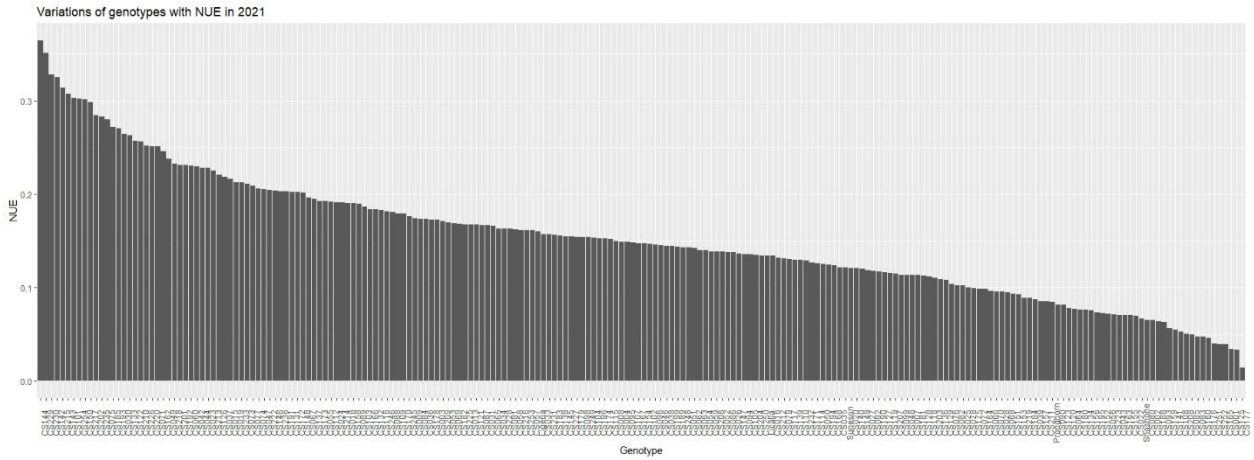

Figure 3. 2 Mean NUE of camelina genotypes on NUE in year 2021.

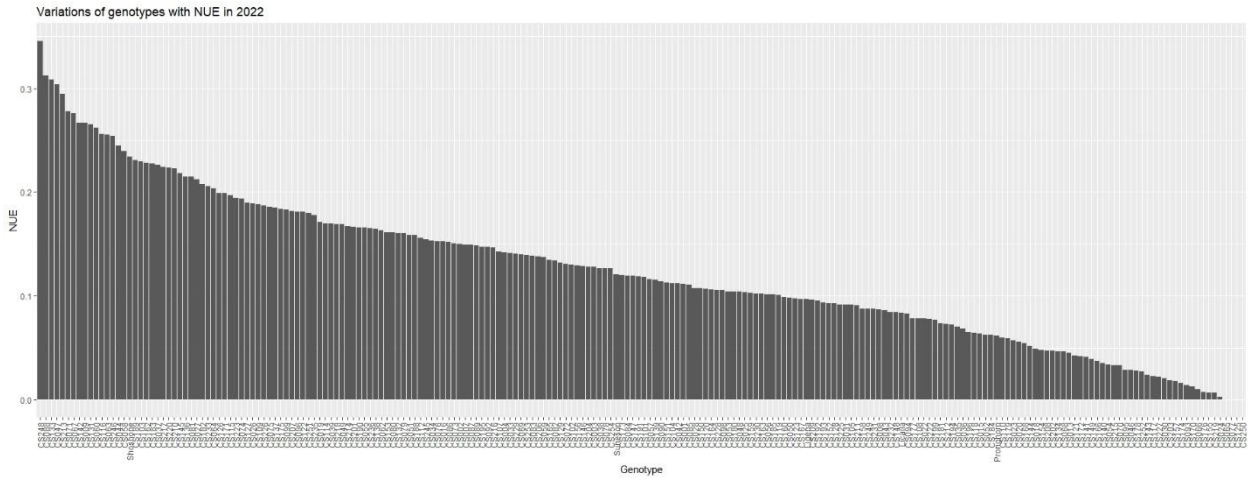

Figure 3. 3 Mean NUE of camelina genotypes on NUE in year 2022.

Discussion

Camelina as an important, under-utilized oilseed crop has great potential for biodiesel production and other industrial applications. The estimation of the crop biomass harvested from above the ground is vital in improving the agronomic management efficiency and predicting crop yield (Chen et al., 2010; Cilia et al., 2014; Gilles et al., 2008). In one of the previous studies in

oilseed rape, higher plant biomass until flowering and increase of number of seeds per plant were identified as the major contributors for higher seed yield, and thus enhanced NUE (Stahl et al., 2019).

Plant biomass accumulation is mainly determined by the fast-growing organs, which are termed as growth center, those falls on stem and branches in budding and early flowering periods, but shifts to reproductive organs during flowering and podding periods in oilseed crop (Chi-yun, 1975; Li et al., 2016; Schjoerring et al., 1995).The result of this study suggested that the genotype influence significantly on the above ground biomass yield of the camelina, indicating that genotype selection is important. The comparative biomass yield was higher in 2021 than that of 2022. There were weak to moderate correlations between biomass and canopy area with maximum coefficients of correlation ranging between 0.24 and 0.50.

The nitrogen use efficiency (NUE) varies among the genotypes. The NUE was calculated with the biomass of crops with low N, the plant utilizes the nitrogen available in the soil during their growth period when nitrogen input is not provided. Nitrogen applied at pre-sowing has the highest benefit to increase seed yield and NUE (Li et al., 2016). The result suggested there was highest growth in genotype CS144 with 0.3644g increase in biomass per lbs of N available in soil in 2021 meanwhile, highest growth was found in genotype CS248 with 0.3454 g increase in biomass per lbs of N available in soil in 2022.The NUE is lower in 2021 than 2022 because the nitrogen availability varied in the field in both years.

The determination of NUE in crop plants is an important approach to evaluate fertilizers and their role in improving crop yields (Baligar et al., 2001).In this study 30 genotypes with higher NUE were selected from 2021 and 2022 (Table 3.4.a.; 3.4.b.). Among those there are total

of 9 genotypes that appeared doing well in both year 2021 and 2022, they are CS14, CS217, CS133, CS210, CS220, CS071, CS162, CS060, and CS044. These genotypes are expected to yield higher that warrant for further yield evaluation.

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CHAPTER FOUR

SUMMARY

The research was conducted in Sidney on Camelina aimed to select genotypes that are efficient in producing higher biomass yield and high NUE. Canopy image analysis and NDVI measurements were evaluated as remote, fast, and non-destructive methods for screen camelina genotypes in the field.

Canopy image analysis was able to differentiate the canopy size and growth rate of camelina genotypes in high and low N regimes. The difference in growth rate with high N and low N showed us influence of nitrogen in the growth of crop. The correlation with biomass and canopy area varied with measuring time points with a stronger at early growth stage measurements. The rank order of canopy size and growth rate at different time points indicates difference among the genotypes and effect of nitrogen in growth. Other research has showed that the canopy images were efficient in identifying the growth stage, such as initiation of flowering stage, flowering stage, and pod setting stages without using any destructive measurement, with much more efficient way. For canopy area and growth analysis through images has been accurate and less biased. Though it took long period of time for image analysis in 2021, increasing the consistency in the height of the camera and using same camera for retrieving the images helped making a pipeline which could provide results in few minutes in 2022.

NDVI was a useful measure to identify the greenness of the plants. The NDVI above 0.6 when plants were healthier and was much lower NDVI indicate the plants being damaged or dead. There was a decline in the NDVI as the plants grew in this study likely due to plant bolding or flowering.

Accurate estimation of above-ground biomass production is crucial for enhancing agronomic management efficiency and predicting crop yield. Final biomass yield and NUE data aid in identifying the optimal amount of fertilization required, particularly in addressing nitrogen deficiencies in crops. Notably, certain genotypes demonstrated higher biomass yield and nitrogen use efficiency (NUE). These genotypes, including CS014, CS217, CS133, CS210, CS220, CS071, CS162, CS060, and CS044, possess characteristics that hold promise for enhancing crop productivity.

The results of remote sensing technology and traditional biomass measurements contribute valuable insights into the relationship between camelina biomass yield, canopy growth rate, NDVI and NUE. The remote sensing technologies provided nondestructive methods of screening camelina genotypes and traditional biomass measurement aided with those results for selection of genotypes with the potential to enhance crop yield in camelina.

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