Market Class Differences in Response to Applied Nitrogen in Spring Barley

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Abstract

Nitrogen (N) is essential for tiller formation and development of kernels in spring barley (*Hordeum vulgare L.*). In order to address the issue of growing N fertilizer prices and better N management practice field trials were conducted in three locations at the University of Idaho R&E centers for two growing seasons (2021-2022). Three classes of spring barley i.e., feed, malt, and food were planted at 50, 101, 151, 202 kg N per hectare for feed and malt barley and 0, 34 67, 101, 134 kg N per hectare for food barley. It measured the effect of N fertilizer rates on Grain Yield (GY), Nitrogen Uptake (NUp) and Nitrogen Use Efficiency (NUE). Nitrogen rate and variety had a highly significant effect on grain yield. A higher N rate was also associated with a bigger N uptake. In two of the three locations, significant differences in N uptake for all the varieties were observed. N rate affected NUE in one of the three locations for both trial periods. Another study utilized ground sensors and aerial sensors to predict GY and NUp. Normalized vegetative differences index (NDVI) and Chlorophyll Content Index (CCI) were recorded at tillering and flowering stage using GreenSeeker (GS), unmanned aerial vehicle (UAV) mounted sensor, SPAD meter, and MC-100 meter respectively. We observe a highly significant effect of the N rate on GS NDVI readings. GS was also found to be better than SPAD meter, MC-100 and UAV at predicting Grain Yield (GY) and Nitrogen Uptake (NU).

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Dedication

I dedicate this thesis to my family for their constant love and support, my greatest strength, mom and dad, my siblings Kriti and Kripesh, and the newest addition to our family, my nephew, Nivu for always putting a smile on my face. Special appreciation goes out to my friends- Anamika, Asmita, Prativa, Srijana, Anisha, Sudip, Pradip, Pratap, and Simanta. Finally, to my love, Bishal.

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Chapter 1: Introduction

Overview of Cereal Crops

The term cereal is a derivative from Latin word ‘cerealis’ meaning ‘grain’ which is a type of fruit called a caryopsis, composed of the endosperm, germ, and bran. Cereal grains are also known as staple food crops as they are grown in greater quantities and provide more food energy than any other type of crops worldwide (Sarwar et al., 2013). Cereal grains have been the major component of the human diet for thousands of years and played a leading role in shaping human civilization.

The natural form of cereals, is the whole grain, are rich in vitamins and minerals, carbohydrates, proteins, fats, and oils. When the grain kernels are dehulled the remaining endosperm consists mostly of carbohydrates. More than 50 % of the world's daily caloric intake is derived directly from cereal grain consumption (Awika, 2011). Cereals are relatively inexpensive to produce, are easily stored and transported, and do not deteriorate readily if kept dry (McKevith, 2004). Rice (*Oryza sativa L.*), wheat (*Triticum aestivum L.*), and maize (*Zea mayz L.*) are the top three cereal crops produced globally, followed by barley (Hordeum vulgare L.) (FAOSTAT, 2020).

Evolution of Barley

The origin of barley domestication is dated to about 10,000 years ago in archaeological sites in the Fertile Crescent of the Near East which spread to North Africa, further east and north in Asia, and to Europe. The wild form of the domesticated barley was used as a human food source even before its domestication. Soon after domestication, selection of agronomically valuable traits such as spring growth habit, two to six row spikes, and hull-less caryopsis spread quickly to all cultivated barleys (Sato, 2020). Currently, barley is an important crop in cool and dry regions, in western North America, northern Europe, the Middle East, North Africa, and the Andean region of South America.

According to Harlan (1968), barley seeds along with wheat, and grapes were brought by Columbus on his second voyage to the Americas. The first barley growers in an American settlement attempted growing barley along the Atlantic seaboards characterized by humid summers. The eastern coastal areas of the United States were not favorable for the varieties Chevalier and Thrope, two rowed barleys commonly grown in England, and they were best suited to Eastern Provinces of Canada. Favorable growing conditions were found in the Eastern states where the Hanna and the six-rowed varieties of the Continent were best suited, making New York a pivotal production region.

Soon after, barley was introduced into all the colonies, where it was in demand as a grain for the brewing industry. From the Mississippi valley to Pennsylvania, considerable acreage of barley production was developing. Around 1873, the Wisconsin University experimental farm began distributing barley variety; its culture spread rapidly from farm to farm. The center of production consistently moved westward; by 1879 a large quantity of barley was grown in eastern Oregon, Washington, and California.

Barley Production and Importance

Barley (*Hordeum vulgare L.*.2n = 14, diploid, HH genomes) belongs to the grass family Poaceae (order Poales). Currently, barley is the fourth most important cereal crop grown in the world with the production of 149,465,000 metric tons (USDA, 2023). In the last decade, Europe has produced around 60% of the world barley with Asia and Americas producing 15% and 13%, respectively. United States ranks 9th with the production of 3,896,000 metric tons which is 3% of the total world barley production. Yield increment from 3.2 metric tons per hectare in 2021/22 to 3.9 metric ton per hectare in 2022/23 was observed. Similarly, production increased from 2,615 (2021/22) to 3,796 (2022/23) which represents an almost 45% increase (USDA, 2023). Barley production in the US is largely concentrated in the upper mid-west and in the higher elevation areas with shorter growing seasons (Rogers et al., 2017). North Dakota, Idaho, Montana, Colorado, Washington, Wyoming, and Arizona are among the major barley producing states. Idaho is the largest barley producing state contributing 33% of the total US barley production (USDA,2023).

Barley grain is used as feed for animals, malt, and human food. It is still a major food in several regions of the world which are characterized by harsh living conditions and are dependent on low production systems. However, the most significant use of barley is in the malting and animal feed industry (Grando & Macpherson, 2005). Since early times barley has been recognized as a high energy food. Its genus name, Hordeum, is derived from the word by which Roman gladiators were known, “hordearii,” or “barley men,” consuming barley gave them strength and stamina (Percival, 1921). Generally, wholegrain barley consists of approximately 70% starch, 10-20 % protein, 5-10% beta-glucan, 2-3 % free lipids, 11-34% total dietary fiber and 3-20 % soluble dietary. With major minerals like iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), calcium (Ca), phosphorus (P), and potassium (K), the total mineral content is approximately 2.5% in a covered/hulled barley. Beta-glucan constitutes approximately 75 % of the barley endosperm cell wall and has been shown to decrease the cholesterol level in blood sugar leading to reduced risk of coronary heart diseases (Sullivan, Arendt & Gallagher, 2013).

In the last 40 years, food barley consumption has decreased considerably. This is mainly due to the better product quality and palatability of food products prepared from wheat and rice compared to barley (Newman & Newman, 2006; Grando & Macpherson, 2005). For human consumption, barley grain is first processed to produce pearled barley and may be further processed to grits, flakes, and flour. Pearled barley, grits, and flour are used in preparing many dishes across the world such as bakery products, baby food, breakfast cereals, stews, rice substitute, soy sauce, soups, and porridge (Baik & Ullrich, 2008).

According to Ullrich (2010), barley has been associated with the malting industry for a long time and is the most economically significant option for barley farmers worldwide. Malting is a process of controlled germination followed by drying. After water, malt is the most abundant ingredient used in the brewing industry and its characteristics and quality have a significant influence on the beer quality. The malting process results to a large increase of hydrolytic enzymes, partial degradation of endosperm cell walls and protein, and structural changes within the grain tissues that render starch and protein substrates readily extractable.

Barley is a major cereal crop for animal feed, especially in Northern areas, which are unsuitable for corn cultivation. They usually compete with corn and sorghum as feed grains (Ullrich, 2010). For cows, pigs, and poultry, barley apart from being an energy source, constitutes also as a protein source. The quality of feed barley varies with the animal species and is complicated to define. The feed quality is influenced by both physical grain quality indicators (color, grain weight and size, hull content, thousand grain weight and grain hardiness) and chemical composition (protein, carbohydrates, amino acids, non-starch polysaccharides, minerals, and vitamins (Bleidere & Gaile, 2012).

Spring Barley in Idaho

Barley is a short season, early maturing crop that is tolerant to drought, alkali, and saline soils (Jacobs, 2016). Barley is cultivated in both spring and winter season and winter cultivars usually mature earlier than spring cultivars (Bongard et al., 2021). The former is typically sown in the fall to be exposed to low temperatures during the winter. This exposure to cold temperature (vernalization) is essential for the seedlings to produce heads and grain kernels. However, it is important to note that barley is sensitive to winter conditions and typically cannot survive temperatures below -8 °C. Unlike winter barley, spring barley does not require exposure to winter temperatures and can be sown in the spring. Conventional tillage winter-wheat summer fallow rotation is the predominant cropping system in dryland areas of the Pacific Northwest where annual precipitation is less than 400 mm. Spring barley have been used to diversify and intensify winter wheat-based production systems in the Pacific Northwest (Lyon & Young, 2015). Idaho is one of the regions in the Pacific Northwest whose altitude, high desert climate, and agronomic conditions make it an ideal location for barley production. Although more than 75% of total Idaho barley production is malt, it is known for growing feed varieties too (Idaho State Department of Agriculture). Sixty percent of the total Idaho barley production occurs in the eastern crop reporting districts and the highest yield per acre occurs in the southwest and southcentral districts. Around 75% of Idaho's barley acreage is irrigated (Robertson & Stark, 1993).

Fertilizer management

Fertilizer management is difficult due to the tremendous variations in soil, environment, and growing conditions. University of Idaho (UI) fertilizer guides for crops like barley are based on old data from the 1980's. Fertilizer management guides need to be updated to better address modern cultivars with varied genetics and greater yield potential, changes in production practices, and to improve nutrient-use efficiency. Since the eighties barley acreage along with the dairy industry has grown rapidly in some concentrated areas, especially in southern Idaho.

The optimal N rate in UI guides varies from grower practices and production in other regions. Previous research included a yield goal as well as validation of inorganic nitrogen (N) (NH_4-N and NO_3-N) for making barley-N recommendations. Currently, N recommendations from UI and many other states are based on inorganic-N as determined through sampling at 0-30- and 30-60-centimeters depths several weeks prior to planting. We also need comprehensive estimates of crop nutrient removal (grain + residue) in Idaho's high-input irrigated systems, where overfertilization, even with precision agriculture, is typical.

Nitrogen Fertilizer

Nitrogen is an essential nutrient which is mainly needed for photosynthesis in plants. It is also an important component of nucleic acid and DNA. Even though nitrogen is abundant on Earth, much of it occurs in forms that are unavailable to plants. The core and mantle contain 91% of the Earth's N. Of the total N at the Earth's surface, a quarter is in rocks and three quarters is in the air. Only 0.03% of the N at the surface is easily available to plants. Nitrogen is biologically important across a range of environments, be it a field research plot, harsh environments like alpine or desert, or seawater

(Scharf, 2020). Nitrogen naturally occurs in the atmosphere as dinitrogen gas (N_2) which makes up to 78% of the Earth's atmosphere. Its plant available forms are ammonium (NH_4^+) and nitrate (NO_3^-).

Nitrogen is an integral component of amino acids, which are the building blocks for protein. Proteins in turn are present in the plant as enzymes that are responsible for metabolic reactions in plants (Oklahoma State University, Elements). Application of fertilizer N results in increased growth and higher biomass yield. It directly increases the amino acid composition of protein and thus nutritional quality of the produce (Maheswari et al., 2017). Optimal N rates leaf area and photosynthetic assimilation. Typically, the higher the leaf area and total leaf biomass, the higher the crop yield (Leghari et al., 2016).

Nitrogen cycle illustrates how manure, fertilizer, and plant residue moves through soil, crops, water, and air. The N cycle can be dived into two halves; N fixation, mineralization, and nitrification where N becomes available to plants and denitrification, volatilization, immobilization, and leaching where N is lost temporarily or permanently from the plant-soil system. Nitrogen fixation is the conversion of atmospheric N (N_2) to a plant available form. This occurs through two processes: biological and non-biological N fixation. The fixation by cyanobacteria and rhizobia infected roots of legumes is the biological N fixation (Johnson et al., 2005). However, the non-biological fixation happens through an industrial process developed by Haber and Bosch (1917). The Haber-Bosch process combines atmospheric N with hydrogen (H) to produce ammonia (NH_4-N) at high temperature and pressure. The process of degradation of organic matter with higher N content in soil into ammonium and nitrate (NO_3-N) available to plants is called mineralization. Following mineralization, nitrification occurs. Nitrification is the process by which soil microorganisms convert ammonium to nitrate to obtain energy. Nitrate is the most plant available form of N but is also highly susceptible to leaching losses.

Unprecedented levels of natural gas prices and the hike in price of ammonia since the beginning of 2021 are among the primary causes of increase in N fertilizer prices. According to Jasinski et al. (1999), on an average 85 % of the ammonia produced in the United States is used as fertilizer. Of the 11.5 million metric tons per year (Mt/yr) of N produced in the United States is used in fertilizers. To breakdown the total N fertilizer use- ammonia represents 32 percent, urea (46-0-0) and urea ammonium nitrate (UAN, 28/32-0-0) solutions together make up 37 percent, ammonium nitrate (NH_4NO_3; 33/34-0-0) 5 percent, and ammonium sulfate $(NH_4)_2SO_4$; 21-0-0-24) - 2 percent. The remaining N is supplied by multiple-nutrient fertilizers that contain varying quantities of N, phosphorus (P), and K. Barley uses more N than any other nutrient. Typically, 25-40 lb/acre of nitrogen at planting is recommended for application (Alley et al., 2009). Spring barley has the capacity to store up to around 1 mg of N per grain at a concentration of 2.4% (Agriculture and Horticulture Development Board). Nitrogen also accounts for the greatest nutrient cost (Robertson & Stark, 1993).

Nitrogen Use Efficiency

Nitrogen Use Efficiency (NUE) can be defined as a function of unit of yield achieved per unit of N input. NUE is a complex genetic trait which is a product of two second level traits, N uptake efficiency (NUpE) and N utilization efficiency (NUtE). NUpE is the ratio of N taken up by the crop compared to what is available from the soil and applied fertilizer. NUtE is the amount of grain produced per unit of N taken up (Moll et al., 1982; Hawkesford & Riche, 2020). Of the total N applied to agricultural fields only a fraction is absorbed and utilized by the plants. Omara et al. (2019) indicated that cereal NUE in 2015 was 35, 41, 30, and 20% for the world, the United States, China, and India, respectively. Although, the world cereal NUE has increased from 33% in 1999, the increment is not significant and is still low. It is necessary to identify the N loss pathways so that we can minimize these losses and increase efficiency of N use. Leaching and runoff, denitrification, volatilization, immobilization are the major N loss pathways from the plant-soil system. Idaho ranks fifth in United States in irrigated water use (USDA, 2017) and therefore up to 40% of N loss is due to leaching (mainly) and runoffs (partially). Furrow irrigation is still a popular irrigation practice in Idaho cropping systems, especially in southern Idaho. This may cause prolonged flooding in the field causing denitrification. About 5-35% of N loss occurs due to nitrification. Urea-based products are mostly used to fertilize crops throughout the United States (Walsh & Belmont, 2015). These fertilizers hydrolyze and convert it to ammonium and then into ammonia which may be lost in the atmosphere. To summarize, Idaho's favorable climatic and geographic conditions make it suitable for spring barley cultivation. However, the lack of updates on the fertilizer guidelines, especially on the NUE poses a challenge on efficient barley production. Our study addresses this issue and contributes to a revision of the University of Idaho's fertilizer guidelines and fertilizer management literature for barley.

Objectives

With continuing concern of N losses through various pathways in the plant-soil system, it is necessary to identify the optimum N rates for barley production, especially in the semi-arid irrigated conditions that prevail in southern Idaho. This thesis will discuss the spring barley yield and grain quality response to various N rates for modern barley cultivars used for feed, malt, and food purposes.

Additionally, we will evaluate the use of remote sensing tools which comprise handheld sensors and unmanned aerial vehicle (UAV)-based sensors for better N management.

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Chapter 2: Spring Barley Yield, Protein, Nitrogen Uptake, and Nitrogen Use Efficiency Response to Nitrogen Rates

Abstract

Nitrogen (N) is the most important nutrient for tiller formation and development of kernels in barley. However, the rising prices and growing environmental concern necessitate a judicious use of N fertilizer that focuses on Nitrogen Use efficiency (NUE). Field trials were conducted in three locations at the University of Idaho Research and Extension Centers for two growing seasons (2021-2023). Nitrogen was applied at 0, 50, 101, 151, 202 kg N ha^{-1} to feed (Altorado), and malt (Voyager) barley and 0, 30, 67, 101, 134 kg N ha^{-1} for food (Goldenhart) barley. Each treatment was replicated four times in a randomized complete block design (RCBD). Nitrogen rate and variety had a highly significant effect on grain yield. A higher N rate was also associated with a bigger N uptake. In two of the three locations, significant differences in N uptake for all the varieties were observed. N rate affected NUE in one of the three locations for both trial periods. The results indicate that the effect of N rate on grain yield, NUp and NUE parameters is location, time and variety specific.

Introduction

Barley has been an important rotational crop in the US Pacific Northwest (PNW) region. Winter wheat-summer fallow is widely practiced in southern Idaho due to available irrigation. Incorporation of spring barley in the prevailing winter wheat-summer fallow cropping system has the advantage of higher annual income, reduction of wind erosion, suppression of weeds, plant pathogens, and insect pests (Young & Thorne, 2004).

According to USDA (2023), about 87% of the total barley production in the United States in contributed by spring barley. Fertilizer application for spring barley depends upon previous fertilizer applications, soil type, level of soil organic matter, soil depth, length of growing season, pest control, and other management practices. The amount of fertilizer required to optimize yield also depends on the end use, for example malting, feed grain, or food grain (Mahler & Guy, 1992). According to Peterson, Widner & Nelson (2006), Idaho produces two-row and six-row malting types, feed, and new food barleys. Most malting varieties do not yield as well as feed varieties. Ideally, malting barley should have a low to moderate protein content; high percentage of plump kernels; high test weight; and minimal skinned and broken kernels (Robertson & Stark, 1993). Idaho feed barley typically is high yielding with excellent test weight and protein content. It is mainly utilized by the dairies and cattle feeding operations. Even though there are only a few food barley varieties currently grown in

Idaho, the number is expected to increase (Peterson, Widner & Nelson, 2006). This is because a barley fractionation plant is being constructed in the south-western region of Idaho for extracting beta-glucan for food barley.

Barley uses more nitrogen (N) than any other nutrient. With the increasing N fertilizer prices throughout the United States, growers are considering reductions in fertilization rates to achieve maximum profits. The variation in soil type, climate, variety, and crop management practices influences the N rates applied. High N increased aboveground dry matter at anthesis, by improving cumulative solar radiation intercepted by the crop which results in an increased dry matter production at maturity (Arisnabarreta & Miralles, 2006). Anbessa & Juskiw (2012) reported that the applied N affects the number of fertile tillers per plant and the number of kernels per spike depending on the type of barley spike characteristic. The two-rowed barley produces more fertile tiller per plant and six-rowed barley produces a superior number of kernels per ear.

Nitrogen use efficiency (NUE) is a key metric that reflects the effectiveness of N management in balancing crop production and resource conservation. Several studies have shown that applying N at the right rate and time can improve NUE by increasing N uptake, reducing losses, and optimizing plant growth and development. Seventeen spring barley genotypes were evaluated under field conditions in Canada where Anbessa et al. (2009) reported that both genotypic variability and environment can account for differences in NUE. Similarly, Grusak et al. (2015) reviewed the current state of knowledge on NUE in barley and other small grains in the USA, and identified several factors that can affect NUE, including genotype, soil type, weather conditions, and management practices. They suggested that a systems approach that considers interactions between these factors is needed to optimize NUE in small grain production. For example, a study by Hawkesford et al. (2013) in the UK showed that reducing N rates by 30% while applying it at the right time and form improved NUE in barley without negatively affecting yield. Another study by Efretuei et al. (2016) in Ireland found that soil N supply and rainfall significantly influenced NUE in barley, with higher NUE under low-N and high rainfall conditions.

Although several studies have been conducted on grain yield, N uptake and use efficiency for various barley cultivars, little work has been done on modern barley cultivars grown in the semi-arid western United States under irrigated conditions for a wide range of N rates. The objective of this chapter is to study the response of various N rates on yield, nitrogen uptake, and nitrogen use efficiency for modern spring barley cultivars used for feed, malt, and food purposes. This information will be pivotal for Idaho growers for making N fertilizer applications that will be agronomically and economically sound.

Materials and Methods

Experimental Locations

For both growing seasons 2021 and 2022, the plots were planted at three locations: Parma Research and Extension Center (REC), Kimberly REC, and Aberdeen REC. For Parma REC, field trails were located at 43.8013638 N, -11694220268 W at an elevation of 702m. This plot was seeded into Greenleaf-Owyhee silt loam with corn as the previous crop (2020) and beans (2021). At Kimberly REC, field trials were located at 42.5490451 N, -114.3443822 W at an elevation of 1192 m. This plot was seeded into DeA Declo loam soil. At Aberdeen REC, field trials were conducted at 42.9526155 N, -112.8279282 W at an elevation of 1342 m. This plot was seeded into Bahem silt loam soil.

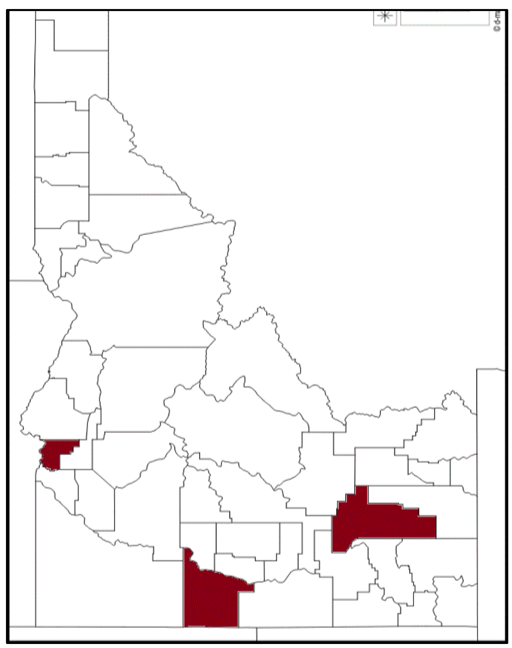

Figure 2.1 Study area map; red color represents three locations under study.

Experimental Design

The experimental design for this trial was a randomized complete block design with four replications. There were three barley classes/varieties (malt barley- ABI Voyager, feed barley- Altorado, and food barley- Goldenhart) and five nitrogen fertilizer rates as treatments. For all three locations for both growing seasons, uniform N rates were used. Nitrogen fertilizer was applied as urea (46-0-0) at planting at 0, 50, 101, 151, 202 kg N per hectare for feed and malt barley and 0, 34, 67, 101, 134 kg N per hectare for food barley, respectively. We categorized five N rates as very low for 0 kg N per hectare, low for 50/30 kg N per hectare, moderate for 101/67 kg N per hectare, high for 151/101 kg N per hectare, and very high for 202/134 kg N per hectare. We combined the data across treatments as barley class at different N rates. The rates were based on the current University of Idaho recommendations for yield goals.

ABI Voyager is a two-rowed barley variety which was released in 2011 by Busch Agricultural Resources. It had an average yield of 7,330 kg ha^{-1}, protein of 112 g kg^{-1}, and an average plant height of 97 cm in irrigated performance trials in Idaho, USA (Marshall et al. 2017). Goldenhart is a two-rowed spring hull-less food barley developed by the USDA-ARS, Aberdeen, ID in cooperation with the University of Idaho Agricultural Experiment Station and was released in 2017 (Hu et al. 2019). Altorado is an early maturing two-rowed feed barley. It has excellent standability, silage quality, and tonnage.

Soil Sampling

During the growing season, there were four soil samples collected. The first soil sampling was done before planting. For this, we took five sub samples (four corners and one middle point in the field) and combined them into a composite sample. The samples were immediately taken to the Brookside Laboratories, Inc. (2021) and Western Laboratories, Inc. (2022) for Parma. For Aberdeen and Kimberly, the samples were taken to Brookside Laboratories, Inc. The pre-plant soil test results for all three locations for both the growing seasons are reported in table 2.1. samples were analyzed for total exchange capacity, pH, organic matter, and micro and macro nutrient status.

Among the remaining three samples, the first soil sampling was taken around the Feekes 5 growth stage of spring barley, the second soil sampling was taken around the Feekes 10 growth stage, and finally the third sampling was done immediately after harvesting the barley field. The soil samplings were done for each plot into 0-30 cm and 30-60 cm depth using handheld soil sampling auger. Once the samples were pulled, they were dried in the oven at 37.8 °C for 2-4 days (depending on the moisture). These samples were analyzed for ammonium and nitrate concentrations in the Brookside Laboratories, Inc.

Table 2.1 Soil type, and soil characterization of top 60 cm soil before planting for 3 site-years; PH, OM = average, Soil residual N and K = potassium (top 60 cm), P= phosphorus (top 30 cm).

Site-year	pH	OM	Soil residual total N	P	K
		%	mg Kg^{-1}		
Parma, ID Soil type: Greenleaf-Owyhee silt loams, 0-1% slopes					
2021	8.0	1.9	22.7	14	420
2022	9.1	2.5	26	10	655
Aberdeen, ID Soil type: DeA Declo loam, 0-2% slopes					
2021	8.3	0.96	37.6	15	372
2022	8.2	1.16	22.4	18	400
Kimberly, ID Soil type: #10 Bahem silt loam, 1-4% slope					
2021	8.1	1.4	56.2	12	200
2022	8.2	1.6	26.2	18	414

Measurements

Barley was harvested at maturity with the Wintersteiger Classic (Wintersteiger, Inc., Salt Lake City, UT) small plot combine. The grains were analyzed for moisture, test weight, and total nitrogen content utilizing Inframatic 9500 NIR (Near Infrared) Grain Analyzer (PerkinElmer, Waltham, MA).

Grain nitrogen uptake (NUp) was calculated by multiplying yield and total N concentration of the grain expressed in kg ha^{-1}. Nitrogen use efficiency (NUE) was determined using the difference method as described by Varvel & Peterson (1990). This is done by deducting total N uptake from the unfertilized treatment (check plot) from total N uptake from fertilized treatment and then divided by the rate of N fertilizer applied.

Table 2.2 Planting and harvesting date, average temperature, and precipitation, from planting to harvesting for two years, three locations.

Site-year	Planting date	Harvest date	Average temperature, planting to harvest	Precipitation, planting to harvest
			C˚	mm
Parma, ID				
2021	April 12	July 22	19.51	56.64
2022	March 29	August 22	17.71	106.42
Aberdeen, ID				
2021	April 20	August 31	18.56	52.83
2022	April 8	August 16	14.89	73.91
Kimberly, ID				
2021	April 26	August 19	19.66	32.51
2022	April 4	August 9	15.59	80.26

Statistical analysis

Statistical analysis was done in RStudio (R version 4.2.2). Linear Mixed-Effects (lme) model was used to test the significance level at $P < 0.001$, $P < 0.01$, and $P < 0.05$. Analysis of variance (ANOVA) was conducted on the lme model where economically important traits like grain yield (GY), N uptake (NUp), and N uptake efficiency (NUE) were studied at different variety, year, location, and N rate as a fixed effect with replication as a random effect. Our hypothesis was that variety, year, location, and N rates affected the GY, NUp, and NUE. To better understand the effects of variety and N rates 'cld' function from Multcomp package was used to compare means when significance was observed in the ANOVA. For visualizing these effects, ggplot was used.

Results

Analysis of variance (ANOVA) on the effect of Variety, N rates, Environment, and Year

The effect of barley varieties, N rate, location, year, and their interactions on GY, GP, NUp, and NUE is described in table 2.3 and 2.4. When we combined the two years (table 2.3); variety, N rate and location had a strong effect on the GY, GP, NUp, and NUE. The two-way interaction between variety and N rate had no effect on GY, GP, and NUp, but had significance in NUE ($p<0.05$). Similarly, the two-way interaction between variety and location had a significant effect on all the parameters. In terms of Nitrogen and location, they had a significant effect on GY and NUp. For all four variables, GY, GP, NUp, and NUE, the three-way interactions are non-significant at 0.05 significance level.

When we combined the locations and looked at the effect of variety, N rate, and year (table 2.4); year had a stronger effect on GP only. The effect of the year was weaker for NUp and NUE to non-significant for GY. Two-way interaction between variety and year again strongly affected GP only. For all other traits there was non-significant effect on all four variables (GY, GP, NUp, and NUE).

Grain Yield and Grain Protein

The average grain yield (GY) ranged from 4,566 to 6,678 kg ha^{-1} (table 2.5). The highest GY was observed with very high nitrogen (N) rates of 202/34 kg N ha^{-1}. For both moderate and high N rates, the yield was statistically the same. However, these rates were significantly different from the very high N rates, showing a difference of 703 kg. When we analyzed GY based on locations, there was no significant difference in GY for all N rates in Aberdeen. In Kimberly, moderate, high, and very high N rates did not differ significantly from each other, but their GY was significantly higher than that of the very low and low N treatments. In Parma, GY associated with low and moderate N rates were statistically the same. GY was significantly higher than very high N rates and statistically similar to high N rates. Regarding varieties, ABI voyager and Altorado showed significantly higher yields in two out of three locations—Aberdeen and Kimberly. On the other hand, Goldenhart, a food barley variety, exhibited the lowest yield in both these locations. However, for Parma, all three varieties performed similarly in terms of GY.

For grain protein (GP), very low N rates showed the lowest GP of 11.2% and very high N rates showed the highest GP of 12.2%. Low, moderate, and high N rates had comparable GP of 11.7%, 11.8%, and 12%, respectively. Table 2.7 does not display notable variations in grain protein content due to different locations. The GP values appear consistent among Aberdeen, Kimberly, and Parma, irrespective of the nitrogen rates. Table 2.6 suggests that varieties do influence GP content across different locations and nitrogen rates. In Aberdeen, malt and feed barley; ABI Voyager and Altorado showed lower GP compared to Goldenhart which is a food barley. Conversely, in Kimberly and Parma, Goldenhart displayed higher GP than other two varieties.

NUp and NUE

Nitrogen uptake ranged from 96 kg ha^{-1} to 143 kg ha^{-1} (table 2.5). The lowest of 96 kg ha^{-1} was recorded for very low N rate and the highest 143 kg ha^{-1} was recorded for very high N rate. Moderate and high N rate NUp were statistically similar with a difference of only 2 kg ha^{-1}. Among the three varieties, Altorado demonstrated the highest NUp of 127 kg ha^{-1}, and Goldenhart with the lowest NUp of 110 kg ha^{-1}. This suggests that Altorado has the greatest ability to absorb nitrogen from the soil, making it more efficient in utilizing available nitrogen resources compared to the other two varieties. Parma exhibited the highest NUp followed by Aberdeen and Kimberly (table 2.7). Across all locations, very high N rate consistently resulted in the highest NUp and very low N rate consistently exhibited the lowest Nup. Goldenhart consistently showed the highest nitrogen uptake values for all three locations, indicating its efficient nitrogen absorption capability (table 2.9).

In terms of nitrogen use efficiency (NUE), as the nitrogen application rate increases from low to high, the NUE also increases. This suggests that the crop is utilizing the added nitrogen more efficiently. However, increasing N beyond a certain point does not significantly improve NUE in our case that was moderate N treatment. The very high N rate showed decreasing NUE of 24% (table 2.5). For Parma and Aberdeen, very high N rates again showed the lowest NUE % which was negative in the former (-19%) (table 2.8). When we combined the N rates, Aberdeen and Kimberly showed similar NUE values which were higher than Parma (fig 2.7). Parma had the lowest NUE than the other two locations. Among three varieties, ABI Voyager, a malt barley was able to utilize nitrogen to produce grains most efficiently (fig 2.6). When we further separated the NUE% of barley varieties based on locations, Goldenhart showed the highest NUE% in two out of three locations (fig 2.9). This value was however the lowest for Goldenhart in Parma.

Table 2.3 Analysis of variance of grain Yield (GY), grain protein (GP), nitrogen uptake (NUp), and nitrogen use efficiency (NUE) as affected by location, variety, N rates, and their interactions combined by years.

	GY	GP	NU	NUE
Variety (V)	***	***	*	**
N rate (N)	***	*	***	.
Environment (E)	***	***	***	*
V × N	NS	NS	NS	*
V × E	***	**	***	*
N × E	***	NS	**	NS
V × N × E	NS	NS	NS	NS

. Significant at 0.1 level of probability, * Significant at the 0.05 level of probability, ** Significant at the 0.01 level of probability, *** Significant at the 0.001 level of probability, NS, non-significant.

Table 2.4 Analysis of variance of grain Yield (GY), grain protein (GP), nitrogen uptake (NUp), and nitrogen use efficiency (NUE) as affected by year, variety, N rates, and their interactions combined by locations.

	GY	GP	NU	NUE
Variety (V)	***	***	*	**
N rate (N)	***	*	***	NS
Year (Y)	NS	***	*	.
V × N	NS	NS	NS	NS
V × Y	NS	***	NS	NS
N × Y	NS	NS	NS	NS
V × N × Y	NS	NS	NS	NS

. Significant at 0.1 level of probability, * Significant at the 0.05 level of probability, ** Significant at the 0.01 level of probability, ** Significant at the 0.001 level of probability, NS, non-significant.

Table 2.5 Mean grain yield (GY), grain protein (GP), nitrogen uptake (NUp) and nitrogen use efficiency (NUE) values for five N rates-year, location, and varieties combined. Values followed by different letters are significantly different.

N rate	GY	GP	NUp	NUE
	Kg ha $^{-1}$	%	Kg ha $^{-1}$	%
very low	4,566^{a}	11.2^{a}	96^{a}	.
low	5,467^{b}	11.7^{ab}	110^{ab}	20^{a}
moderate	5,975^{bc}	11.8^{ab}	122^{b}	30^{c}
high	5,948^{bc}	12.0^{ab}	124^{bc}	29^{c}
very high	6,678^{c}	12.2^{b}	143^{c}	24^{b}

Table 2.6 Mean grain yield (GY), grain protein (GP), nitrogen uptake (NUp) and nitrogen use efficiency (NUE) values for three barley varieties- year, location, and N rates combined. Values followed by different letters are significantly different.

Variety	GY	GP	NUp	NUE
	Kg ha^{-1}	%	Kg ha^{-1}	%
ABI Voyager	6,030[b]	11.2[a]	120[ab]	38[bc]
Altorado	6,442[b]	11.0[a]	127[b]	15[a]
Goldenhart	4,709[a]	13.0[b]	110[a]	23[b]

Table 2.7 Mean grain yield (GY), nitrogen uptake (NUp) and nitrogen uptake efficiency (NUE) values for three locations-year, N rates, and varieties combined. Values followed by different letters are significantly different.

Location	GY	GP	NUp	NUE
	Kg ha^{-1}	%	Kg ha^{-1}	%
Aberdeen	6,295[b]	11.7[b]	130[b]	32[b]
Kimberly	4,496[a]	10.4[a]	78[a]	35[b]
Parma	6,389[b]	13.3[c]	149[c]	9[a]

Table 2.8 Mean grain yield (GY), nitrogen uptake (NUp) and nitrogen uptake efficiency (NUE) values for five N rates and three locations-year and varieties combined. Values followed by different letters are significantly different.

N rate	Location	GY Kg ha^{-1}	GP %	NUp Kg ha^{-1}	NUE %
very low		5,386[a]	11.0[a]	105[a]	.
low		6,243[a]	11.2[a]	127[ab]	54[c]
moderate		6,486[a]	11.6[a]	133[ab]	33[b]
high		6,597[a]	12.2[a]	141[b]	28[b]
very high	Aberdeen	6,761[a]	12.3[a]	144[b]	16[a]
very low		2,509[a]	9.6[a]	45[a]	.
low		3,616[a]	9.8[a]	58[ab]	29[a]
moderate		5,044[b]	10.4[a]	79[bc]	37[b]
high		5,471[b]	11.0[a]	98[c]	38[b]
very high	Kimberly	5,843[b]	11.1[a]	111[c]	36[b]
high		5,775[a]	12.7[a]	139[ab]	.
very low		5,804[a]	13.3[a]	145[ab]	8[cd]
moderate		6,396[ab]	13.3[a]	153[ab]	17[d]
low		6,541[ab]	13.3[a]	133[a]	-7b
very high	Parma	7,431[b]	13.8[a]	173[b]	-19[ab]

Table 2.9 Mean grain yield (GY), nitrogen uptake (NUp) and nitrogen uptake efficiency (NUE) values for three varieties and five N rates of four locations- year and N rates combined. Values followed by different letters are significantly different.

Variety	Location	GY	GP	NUp	NUE
		Kg ha^{-1}	%	Kg ha^{-1}	%
ABI Voyager		7,086[b]	10.6[a]	135[ab]	26[a]
Altorado		7,311[b]	10.8[a]	143[b]	27[a]
Goldenhart	Aberdeen	4,487[a]	13.6[b]	112[a]	44[b]
ABI Voyager		4,996[b]	9.7[a]	82[b]	36[a]
Altorado		5,581[b]	9.7[a]	95[b]	42[b]
Goldenhart	Kimberly	2,912[a]	11.8[b]	58[a]	27[a]
ABI Voyager		6,007[a]	13.4[ab]	143[a]	53[c]
Altorado		6,434[a]	12.6[a]	143[a]	-25[a]
Goldenhart	Parma	6,727[a]	13.8[b]	160[a]	-1[b]

Discussion

Analysis of variance (ANOVA) on the effect of Variety, N rates, Environment, and Year

The response of grain yield to nitrogen (N) fertilization varies with site, climate, year, soil type, cultivar, N rate, source, timing, and application methods (Mandic et al. 2015, Rogers et al. 2017, Fikayo et al. 2019 and Pampana & Mariotti 2021). In our study, variety, N rate, and location had a strong effect on the GY, GP, NUp, and NUE (table 2.3). Oral et al. (2018) also demonstrated significant effect of nitrogen application rates and cultivars in barley grain yields. Climatic data from both growing seasons indicate that the year of 2022 was comparatively cooler and had higher average precipitation than 2021 from planting to harvesting in all three locations (table 2.3). Despite this difference in climatic conditions, we did not observe the effect of year in barley grain yields. Peltonen-Sainion et al. (2011) pointed out that increased temperature early to mid-growing season results in negative effect in the yield, however the result is positive if the temperature is higher later in the season when barley is closer to maturity. We believe this tradeoff between the negative and positive effect of climate on yield is negligible which is why we do not observe the effect of year on grain yield even though the average temperature in 2021 was higher. For both years, barley varieties were grown in irrigated condition in all three locations because of which despite the difference in precipitation, year may not have had significant effect in the grain yield.

Grain protein and nitrogen uptake were affected by year (table 2.4). Nitrogen uptake is the product of grain yield and N concentration in grain, the latter being factor of grain protein. Although GY was not affected by year GP was and this could be the reason for NUp being significantly affected by year as well.

Grain Yield and Grain Protein

Our results on GY and GP matches with Marshall et al. (2022) and Rogers et al. (2017). Marshall et al. (2022) conducted a study on spring barley irrigated nurseries in 12 site-years in southern Idaho and reported that Goldenhart had lower yield than ABI-voyager and Altorado. They also reported higher protein content for Goldenhart than the other two varieties. Ali et al. (2022) also observed an increase in barley grain yield and grain nitrogen with increasing N rates from 60 to 120 kg ha $^{-1}$. They did not report any changes in yield and grain nitrogen at 180 kg ha $^{-1}$. Increasing N rate after a point contributed to vegetative growth or straw yield rather than grain yield in barley varieties. We observed similar results where moderate, high N rate resulted in statistically similar yield response, higher N rates could have resulted in increased vegetative growth or straw yield rather than grain yield. In Aberdeen, all N rates showed comparable yields. This could be due to the higher average residual N in the soil (table 2.1). Even when we did not apply any nitrogen to our field, the soil N was so high that it contributed to similar yields as other N treatments.

A study by Walsh (2019) has validated that while grain yields may not consistently rise with application of nitrogen, the use of N fertilizer can still be advantageous in enhancing grain quality, including test weight and protein content. Our study supports these findings. When we look at the effect of N rates on GP (table 2.5) very high N rates showed the highest protein content. When we separated the proteins level by location, GP values increased with increasing N even though they were not statistically different from one another.

Nitrogen Uptake and Nitrogen Use Efficiency

NUE is the ability of crops to capture N from what is being applied and the efficiency with which that N is utilized to make grains. The primary objective of improving NUE is to apply less nitrogen while maintaining realistic yield goals (Dawson et al. 2008). Higher NUE indicates that a higher amount of biomass and grain yield per unit of N uptake was produced. In our study, the highest NUE was observed for moderate and high N rates which decreased significantly as the N rate was increased to very high N rates. This result was supported by Marino et al. (2004) and Mahajan et al. (2010) who saw that NUE decreased with higher N fertilization rates. Gaur et al. (1992) conducted a study in wheat where the N recovery efficiency decreased with higher N application levels from 120 to 360 kg ha $^{-1}$. They also reported that this variation might be due to other factors such as climate, cultivation, and N application rates. In the current study, different nitrogen rates displayed varying NUE across three locations, this suggests an environmental influence.

Several studies have demonstrated that NUp and NUE are higher in the new barley varieties compared with that of older varieties (Anbessa et al., 2009; Sylvester-Bradley and Kindres, 2009; Beatty et al. 2010). All three varieties used in our study were new and high yielding for Idaho and surrounding areas. Nitrogen uptake for feed barley was highest which was statistically similar to malt barley and significantly higher than food barley (table 2.6). Our findings align with Rogers et al. (2017) who observed higher nitrogen uptake among malt and feed barley as compared to the food variety.

The decrease in NUE with higher N rate applied can be attributed to reduction in various components, including N uptake efficiency, N utilization efficiency, and N retention efficiency (Dawson et al. 2008). In some of the cases in our study, even though there is a reduction in NUp we see higher NUE, and this could be due to the capacity of the crop to utilize and retain the applied nitrogen (table 2.8 and 2.9). The utilization of high N rates during early stages of plant growth can result in nitrogen losses due to the plant's low initial demand for N. After the N application, losses occur through competing pathways like leaching and volatilization. Therefore, aligning N application with crop's N requirements might not automatically enhance NUE. The crucial factor is to match the N availability with plant's actual N needs and uptake while minimizing losses. One solution to this problem could be topdressing nitrogen later in the season. Walsh and Walsh (2020) reported highest NUE with N applied at tillering and jointing stage of crop growth. Similarly, another study by Walsh & Christiaens (2015) found that nitrogen uptake increased while topdressing N fertilizer in 5 out of 8 site-years.

Conclusion

This study was designed to measure the temporal and spatial effect of application rate and variety on Grain Yield (GY), Nitrogen Uptake (NUp) and Nitrogen Use Efficiency (NUE). Variety, and N rate showed a highly significant effect on GY and NUp. The results also display a highly significant effect of location on GY, and NUp. However, the temporal variability does not have a highly significant impact on any of the variables. Consistent results are also seen in terms of interaction effects of location with N rate and with varieties.

The results illustrate that Goldenhart which is a food barley, has the least yield and Altorado, feed barley, has the highest yield among the three varieties. In terms of location, Kimberly has relatively lower production than Parma and Aberdeen. Even though N application and grain yield show a proportional relation in extremes, they have variable relations in moderate N rate application. They also display variability in terms of location, variety and year of cultivation.

Our results suggest that a higher N rate is directly proportional to N uptake. In Parma we find no significant difference in N uptake among the three barley varieties. As for Kimerly and Aberdeen, Goldenhart showed the least amount of N uptake while Atorado displayed the highest. In terms of Nitrogen Use Efficiency, in one of the locations, for both the years we observe a lower value for higher N rate. At the same time, we also see variable response for NUE to N rate in different locations and varieties.

In conclusion, the effect of N rate on the yield, NUp and NUE of barley is found to be location, time and variety specific. Traditional N-response trials are limited in their capacity to make a useful recommendation regarding suitable N rates. To measure and prescribe appropriate N rate and its impact, precision agriculture tools need to be utilized and explored in future research.

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Chapter 3: Use of Remote Sensing Tools for Nitrogen Management in Spring Barley

Abstract

Yield goals and quality are among the most important attributes for grain crops which are strongly affected by growing condition and applied fertilizer. Remote sensing is an efficient means for obtaining real time data for fertilizer management in crops. With a goal of exploring the potential of ground and aerial based sensors for nitrogen (N) management, field trials were conducted at multiple sites in Idaho for two growing seasons (2021 and 2022). Three classes of spring barley (Hordeum vulgare L.) i.e., feed, malt, and food were planted at 50, 101, 151, 202 kg N per hectare for feed and malt barley and 0, 34 67, 101, 134 kg N per hectare for food barley. Normalized vegetative differences index (NDVI) and Chlorophyll Content Index (CCI) were recorded at tillering and flowering stage using GreenSeeker (GS), unmanned aerial vehicle (UAV) mounted sensor, SPAD meter, and MC-100 meter respectively. We observe a highly significant effect of the N rate on GS NDVI readings. GS was also found to be better than SPAD meter, MC-100 and UAV at predicting Grain Yield (GY) and Nitrogen Uptake (NUp). Among the three varieties of spring barley Altorado displayed stronger correlation between grain yield and GS NDVI (r^2=0.55), UAV NDVI (r^2=0.42) and SPAD reading (r^2= 0.72). This data is valuable for early prediction of yield and quality of barley grains which will help growers to make a smart decision on N application and thus contribute to profitability.

Introduction

Potential yield and soil test results prior to planting are the two most important attributes based on which most of the growers in the PNW area calculate the required N fertilization rates for their field (Mahler & Guy, 1992). These rates could be inconsistent from field to field and year to year depending on numerous factors which are difficult to predict before fertilizer application. Large spatial and temporal variability in the field restricts efficient utilization of N fertilizer based on the recommendations. Recent technical advances have made spatially variable applications of nitrogen accessible to many crop producers. Methods based on in-season soil tests and laboratory analyses of tissue samples show good correlation with grain yield, however these methods are time-consuming and cumbersome especially in large field areas.

Precision agriculture has gained popularity in recent years for increasing farm efficiency and reducing input costs. Precision agriculture tools assist in plant stress detection, biomass, nutrient estimation, field mapping, weed management, and chemical spraying (Hassler & Basyal-Gurel, 2019). Remote sensing is one of the most important precision agriculture tools used in the field of water and nutrient management. Lintz & Simonett (1976) defined remote sensing as the acquisition of data of an object without touch or contact. Handheld sensors, unmanned aerial vehicles (UAVs), satellite imagery, and ground robots are among the most popular tools of remote sensing.

Most studies that examined the prediction of yield in cereal crops are focused on using satellite imagery. Satellite imagery has a low temporal and spatial resolution in comparison to unmanned aerial vehicles. The high temporal resolution provided by UAV sensors makes it possible to collect data at any time during a crop's growth period. Similarly, the high resolution multispectral and hyperspectral imaging provides quality data for estimating minute changes in plant-soil system which is difficult through satellite imageries (Herzig, et al., 2021). In this study, we used handheld and UAV mounted sensors to record normalized difference vegetative index (NDVI) and chlorophyll content. We compared readings from these sensors and compute their correlation with important yield attributes for different barley varieties. This study presents the possibility of N management based on the in-season prediction of grain yield and nitrogen uptake. The reflectance in red and near infrared range are compared to calculate the NDVI which determines how dense and green the crop is.

$$\mathrm{NDVI} = \frac{\text{Near Infrared} - \text{Red}}{\text{Near Infrared} + \text{Red}}$$

Bowen et al. (2005) conducted five field trials at multiple sites in Idaho where they used optical sensing instruments to predict in-season variable rate in barley. They concluded that GreenSeeker accurately predicted nitrogen requirement. As per the prediction, one of the field trails needed higher N than other two while the remaining two would not show any economical response despite added N. Colaco and Bramley (2018) reported N savings of 5-45% without significant impact on grain yield using sensor-based N application. They also discussed its positive impact on the environment. A recent study conducted in Europe by Fabbri et al. (2020) concluded that GreenSeeker can effectively be used for managing N fertilizer in barley. Similar yield to conventional practice was seen with less N fertilizer applied. This provides a strong support for our study. Similarly, another study conducted in California in malting barley reported that NDVI measured at tillering stage provides an estimate of crop response to in-season N application (Nelsen & Lundy, 2020).

Chlorophyll is an N sensitive compound and is strongly related to leaf N content. The Apogee Instrument MC-100 chlorophyll concentration meter exploits the difference of chlorophyll at different wavelengths to determine relative chlorophyll content (Parry et al., 2014). Chlorophyll content is measured as the ratio of transmittance at 931 nm, which is a near infrared wavelength outside the chlorophyll absorption range to transmittance at 653 nm which is a photosynthetically active wavelength within chlorophyll absorption range and is termed as chlorophyll content index (CCI). A CCI measurement near one indicates little to no chlorophyll in leaf sample whereas a CCI measurement greater than one indicates more chlorophyll. SPAD meter is a handheld spectrophotometer that measures the greenness or chlorophyll content of leaves i.e., CCI. It is much faster than tissue sampling for N in leaves (Singh, et al., 2020). There is a linear relationship between CCI value and leaf N concentration. Thus, it has been widely used to monitor leaf N status of several crops. Rafiqul et al. (2014) found positive correlation between CCI values and grain yield in wheat at different growth stages in Bangladesh. To help better understand the N management in different barley classes, further study using ground and aerial based sensors is needed.

The objectives of our study were to 1) study ground and aerial based sensor for N management 2) compare NDVI and chlorophyll content from handheld sensor, and 3) compare NDVI from handheld sensor and aerial sensor. Our study also examined the relationship between spectral indices with different crop growth parameters for food, feed, and malt barley. Knowing the performance of different cultivars in the early growing season saves cost and time for the growers. It also is valuable for making decisions on additional crop inputs i.e., fertilizer application. This thesis chapter will assess the feasibility of spring barley grain yield and N uptake with in-season crop reflectance measures.

Materials and Methods

Measurements

Vegetative parameters

Spring barley growth stages are described in table 3.1. For all three locations, barley vegetative parameters were assessed at Feekes 5 (f5) and Feekes 10 (f10) by measuring: i) plant height, ii) above ground biomass weight, and iii) above ground biomass N content. The barley plant height was determined by measuring the height of 10 randomly selected plants per plot. The biomass samples were collected by hand-cutting all barley plants near the soil surface within the 0.2 m^2 area in the middle of each plot. Plant samples were dried in the oven for 72 hours at 80°C and transferred to the lab for total N content analysis. Samples' N content analysis was performed using the AOAC method 990.3 at Brookside Laboratories, Inc (New Bremen, OH, USA).

Table 3.1 Spring Barley growth stages and development (Spring Barley Quick Facts 2015, Southern Idaho).

Stage	Feekes Scale	Description
Tillering	1	First leaf through coleoptile
	2	Beginning of tillering
	3	Tillers formed
	4	Beginning of erect growth
	5	Sheaths strongly erect
Stem extension and booting	6	First node detectable
	7	Second node detectable
	8	Flag leaf just visible
	9	Collar of flag leaf visible
	10	Boot swollen/first awn visible
Heading	10.1	First spikelet visible
	10.2	Heading ¼ complete
	10.3	Heading ½ complete
	10.4	Heading ¾ complete

Table 3.1 continued

	10.5	Heading complete
Flowering (prior to head emergence)	10.51	Beginning of flowering
	10.52	Flowering ½ complete
	10.52	Flowering complete
	10.54	Kernels watery ripe
Ripening	11.1	Medium milk
	11.2	Soft dough
	11.3	Kernel hard
	11.4	Harvest ripe

Ground-based sensor measurements

For measuring chlorophyll content in barley leaves we used SPAD-502 meter (Konica Minolta Sensing, Tokyo, Japan) and MC-100 chlorophyll concentration meter (Apogee Instruments, Inc., Logan, UT, USA). Measurement output for the SPAD-502 meter is SPAD units and for the MC-100 meter is CCI values. The top-most fully unfolded leaves from ten plants randomly selected within each plot were analyzed using the chlorophyll meter. Biomass volume and greenness was estimated as NDVI using GreenSeeker handheld optical sensor (Trimble Agriculture Division, Westminster, CO) by sensing the 2 middle rows of barley plants within each row at 70 cm above the canopy. Sensor measurements were taken at f5 and f10 growth stage.

Aerial sensor measurements

A quadcopter unmanned aerial vehicle (UAV) 3DR Solo was selected to carry camera payloads to acquire ultra-high-resolution imagery (fig x). A MicaSense red edge ™ 3 Multispectral Camera with an integrated Global Positioning System (GPS), with an accuracy of 2-3 meters, (MicaSense, Inc, Seattle, WA) mounted on the UAV was used to obtain the imagery. The camera was mounted on a Gimbal and as the camera's weight was similar to GoPro camera's weight, there was no need to add balance weight. The camera acquires 1.3-megapixel images in five spectral bands (rededge, Near Infrared, red, Green, and Blue) with 12-bit Digital Negative (DNG) or 16-bit Tag Image File Format (TIFF) radiometric resolution.

The UAV images were captured within 2 hours of solar noon with flight duration ranging from 15 to 20 minutes in sunny and cloud free conditions. Mission plane software was used to design the flight path and choose the flight and sensor parameters to ensure there is adequate overlap between acquired images for mosaicking. Two flight missions performed successfully at each location to coincide with Feekes 5 and Feekes 10 spring barley growth stages resulted in 6 flight missions per season. These growth stages were chosen because N fertilizer applied at these stages has potential to maximize grain yield and quality.

Figure 3.1 The Unmanned Aerial System 3DR Solo

Multispectral image acquisition and processing

The acquired multispectral images were processed using Micasense Atlas software (MicaSense, Inc, Seattle, WA) for mosaicking, georeferencing, and radiometric calibration. Micasense Atlas works in partnership with Pix4D Mapper image analysis software (Pix4D SA, Lausanne, Switzerland) to create aligned and georeferenced images from the multispectral data captured by the MicaSense red edge camera. Pix4D Mapper finds matching points between overlapping images and stitches them together to create a single ortho-rectified image of the entire study area. The accuracy of the output reconstructed images is typically 1-2 times the ground spatial resolution. After mosaicking, the images were subjected to radiometric calibration using the Red Edge Camera Radiometric Calibration Model in Atlas software. This calibration model uses a Calibration Reflectance Panel (CRP) to convert raw pixel values of an image into absolute spectral radiance values. The CRP, placed near the study area during each flight mission, was captured in images taken just before and after each flight. The output of the radiometric calibration model is a 5-layer, 16-bit ortho-rectified GeoTIFF image.

Statistical analysis

Statistical analysis was done in RStudio (R version 4.2.2). Linear Mixed-Effects (lme) model was used to test the significance level at $P < 0.001$, $P < 0.01$, and $P < 0.05$. Analysis of variance (ANOVA) was conducted on the lme model where readings from remote sensing tools (GS NDVI, UAV NDVI, SPAD, and MC-100 measurements taken at f5 and f10) were studied at different variety, N rate, and location as a fixed effect with replication as a random effect. Heat map was created using "metan" package and pearson correlation coefficient was studied for important traits. Furthermore, coefficient of determination was calculated and plotted using "ggplot" function for each variety.

Results

Pearson Correlation Coefficient and P-values

To be able to predict yield and other yield related traits, in-season crop sensor readings were studied. We compiled the Pearson correlation coefficient and p-values (fig 3.2) of important yield related traits and sensor readings. We saw a strong correlation between GS NDVI f5 and GY (0.86) and NUp (0.81). Similarly, the SPAD readings f5 showed a strong correlation with GY (0.59) and NU (0.71). The chlorophyll content estimation using MC-100, on the other hand, showed a negative correlation with both GY and NUp. Although UAV NDVI showed a good correlation with SPAD at f5 and f10, its correlation with GY and NUp was negative, and the p values were non-significant.

To better understand the relationship between crop sensor readings and yield attributes, we calculated the coefficient of determination (r^2) which measures the proportion of variation in dependent variable that is explained by independent variables. We then separated the measurements by barley varieties.

GS NDVI

Mean GS NDVI value in f5 growth stage increased with increase in N rates applied (fig 3.3). for f10, highest GS NDVI value was observed for moderate N rate while high and very high N rate had comparable values which were lower than Moderate N. Mean GS NDVI f10 ranged from 0.70-0.80. At f5 these values ranged from 0.800-0.6 and some even lower than 0.60 for very low N rates. Both Altorado (r^2= 0.55) and Goldenhart (r^2= 0.52) grain yield showed stronger correlation with GS NDVI f5. The correlation was weaker in f10 (fig 3.7). For N uptake, we did not see any difference in the r^2 values for Goldenhart barley at f5 and f10. Altorado barley's NDVI readings, however explained 62 % of the variation in N uptake (fig 3.8) at f5.

UAV NDVI

The UAV NDVI readings were plotted as box plots across five different N rates (fig 3.4). The UAV NDVI values increased with increasing N rates ranging from 0.75-0.85 at f5. For grain yield, Goldenhart showed stronger correlation with UAV NDVI f5 (r^2= 0.23) than other two varieties. All three varieties showed stronger correlation between N uptake and UAV NDVI at f5 (fig 3.14) even though these values were inferior to NDVI readings from handheld sensor. Altorado barley showed higher correlation (r^2= 0.29) of the three varieties in f5 followed by Goldenhart (r^2= 0.27) and ABI Voyager (r^2= 0.20).

Chlorophyll Meter

Altorado barley showed a strong linear positive correlation (r^2= 0.51) between SPAD readings and grain yield (fig 3.9) at f5. The correlation was weaker for the other two varieties. Altorado barley's SPAD readings also showed a strong correlation with N uptake (r^2= 0.72) at f5.

Although barley GY showed a positive linear correlation with MC-100 readings, the correlation was very weak for all three varieties (fig 3.11). This happened for f5 and f10. We saw similar trends for N uptake where the correlation was weak (r^2<0.01) (fig 3.12).

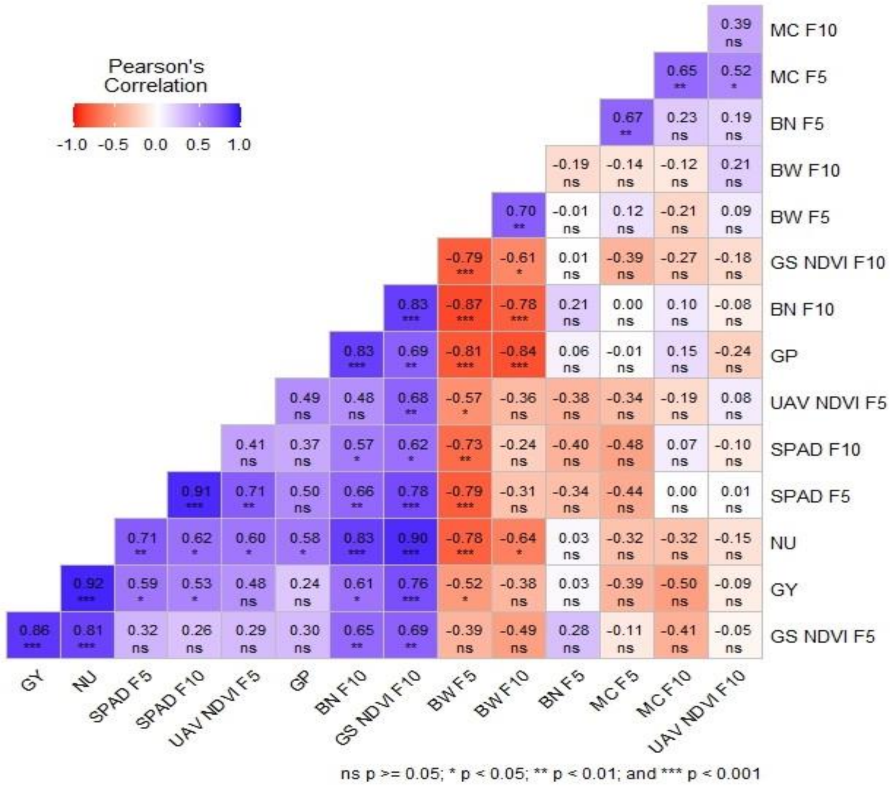

Figure 3.2 Pearson correlation coefficient of NDVI derived from UAV and GS with agronomic traits, grain yield, and nitrogen uptake. Color intensities indicate the degree of positive and negative correlation. Acronyms: BW, Biomass weight; UAV NDVI, Unmanned aerial vehicle NDVI; SPAD, Chlorophyll content by SPAD meter; GS NDVI, GreenSeeker NDVI; BN, Biomass N; GP, Grain protein; GY, Grain yield; MC, chlorophyll content by MC-100.

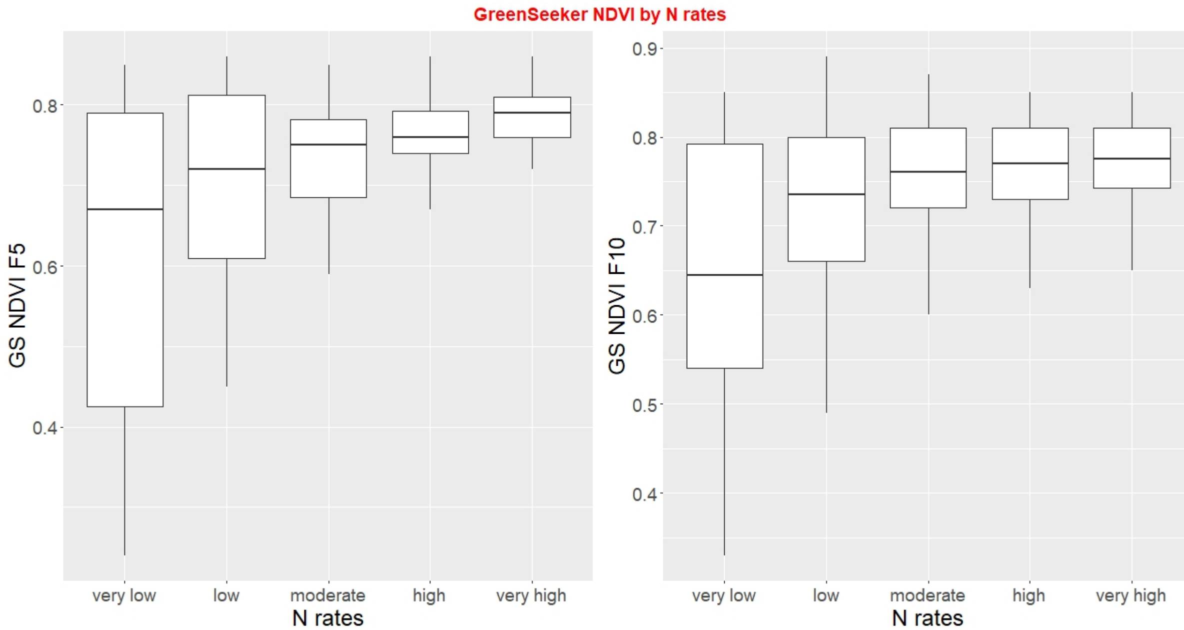

Figure 3.3 GreenSeeker NDVI readings for different N rates at Feekes 5 (left) and Feekes 10(right)- three locations, two years combined.

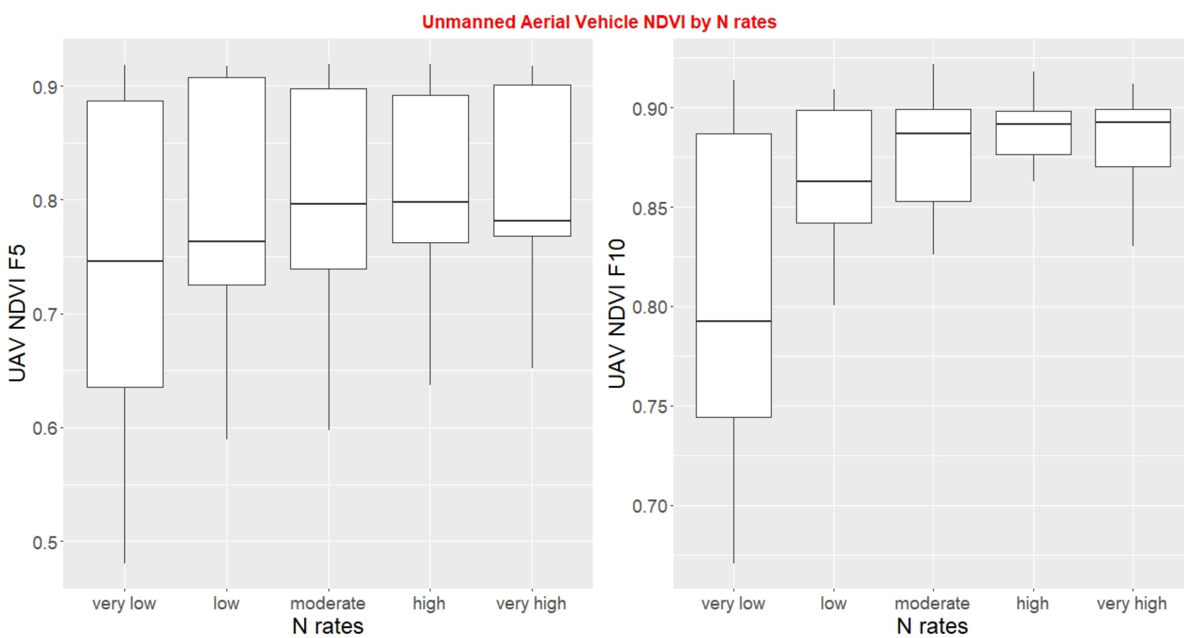

Figure 3.4 Unmanned aerial vehicle NDVI readings for different N rates at Feekes 5 (left) and Feekes 10(right)- three locations, 2022.

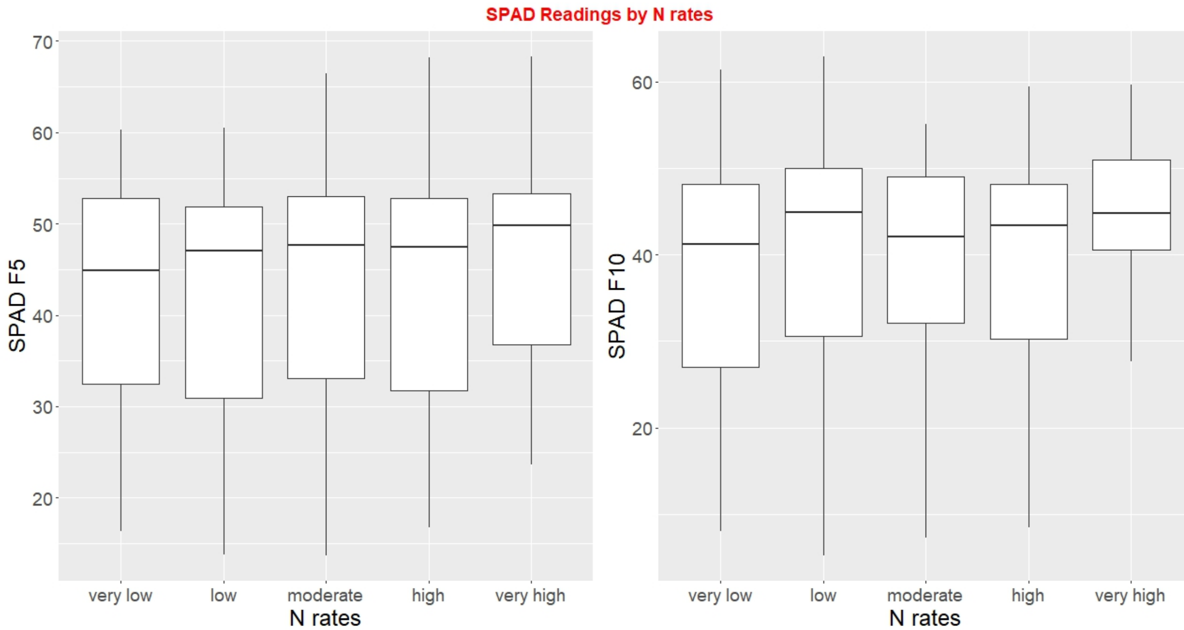

Figure 3.5 Chlorophyll content by SPAD meter readings for different N rates at Feekes 5 (left) and Feekes 10(right)- three locations, two years combined.

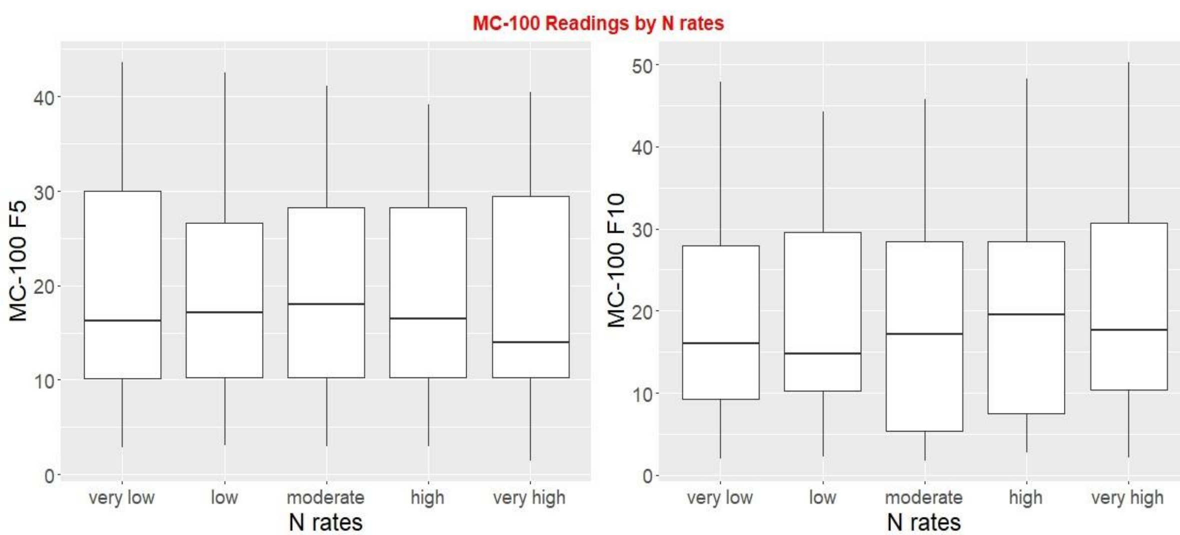

Figure 3.6 Chlorophyll content by MC-100 readings for different N rates at Feekes 5 (left) and Feekes 10(right)- three locations, two years combined.

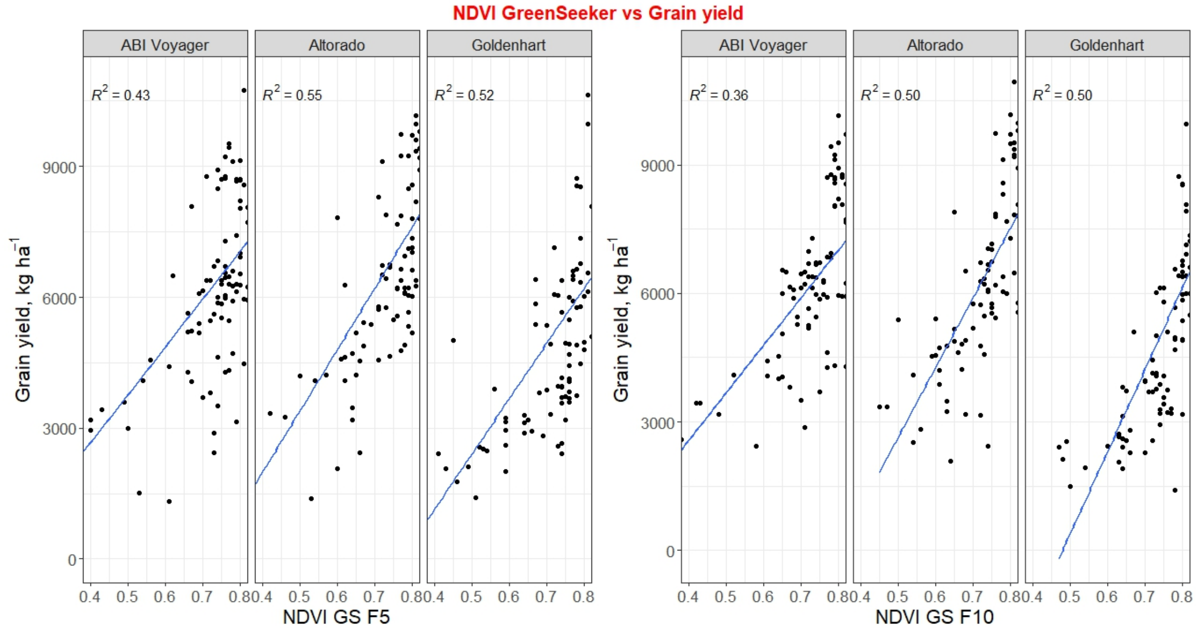

Figure 3.7 Correlation between grain yield and GreenSeeker NDVI at Feekes 5 (left) and Feekes 10 (right) separated by barley varieties- three locations and two years, combined.

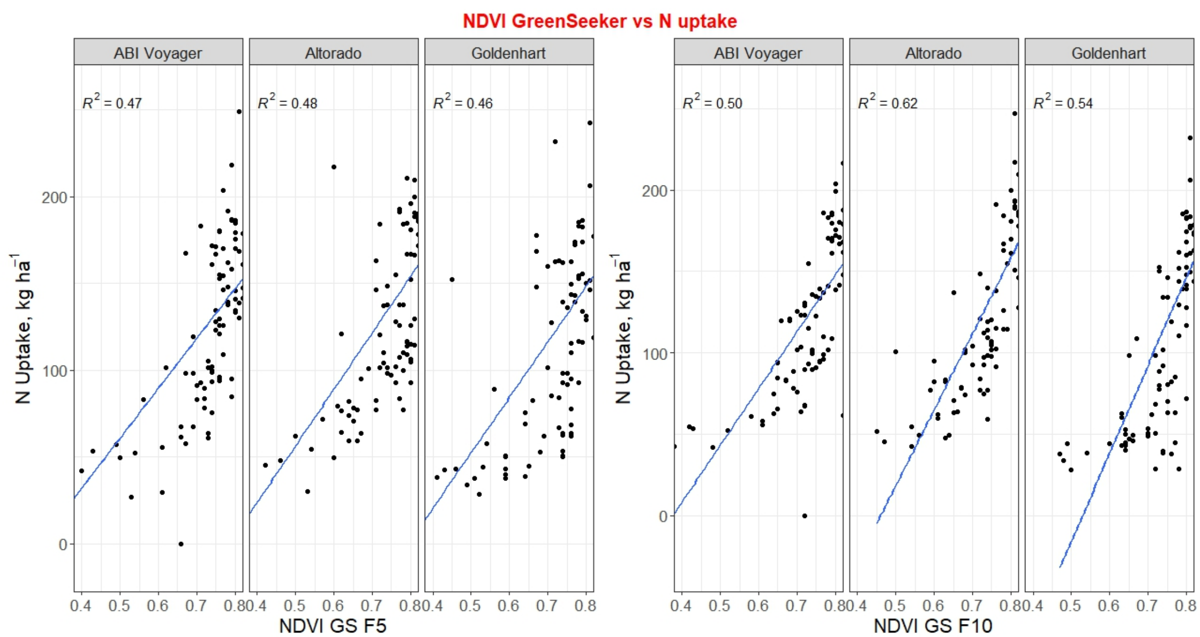

Figure 3.8 Correlation between N uptake and GreenSeeker NDVI at Feekes 5 (left) and Feekes 10 (right) separated by barley varieties- three locations and two years, combined.

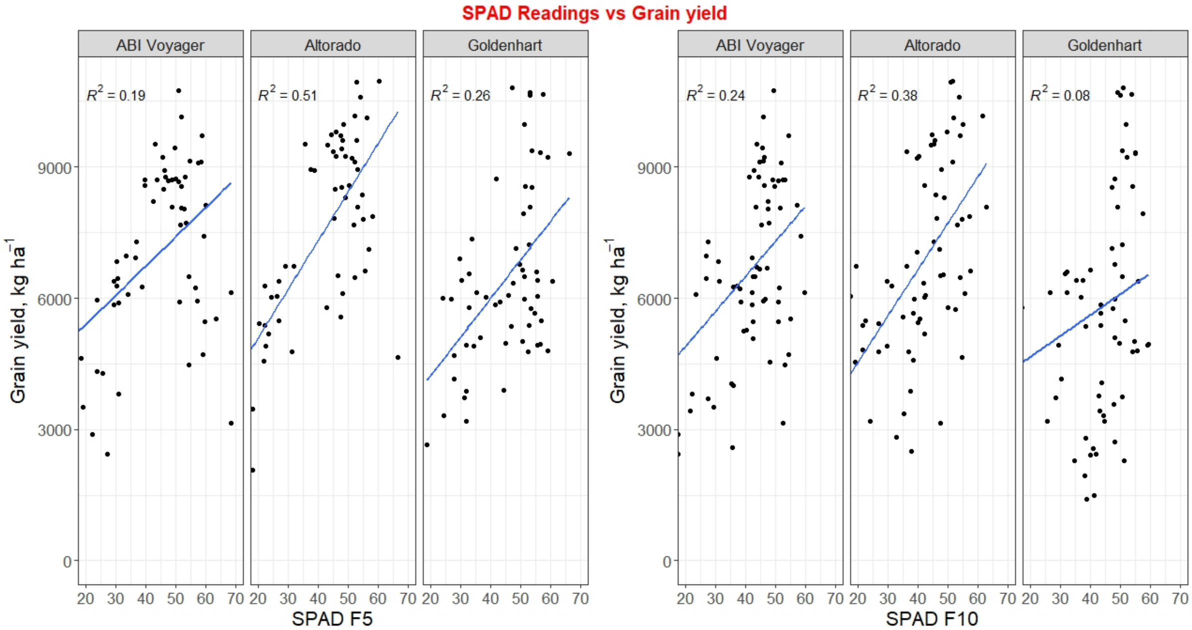

Figure 3.9 Correlation between grain yield and SPAD readings at Feekes 5 (left) and Feekes 10 (right) separated by barley varieties- three locations and two years, combined.

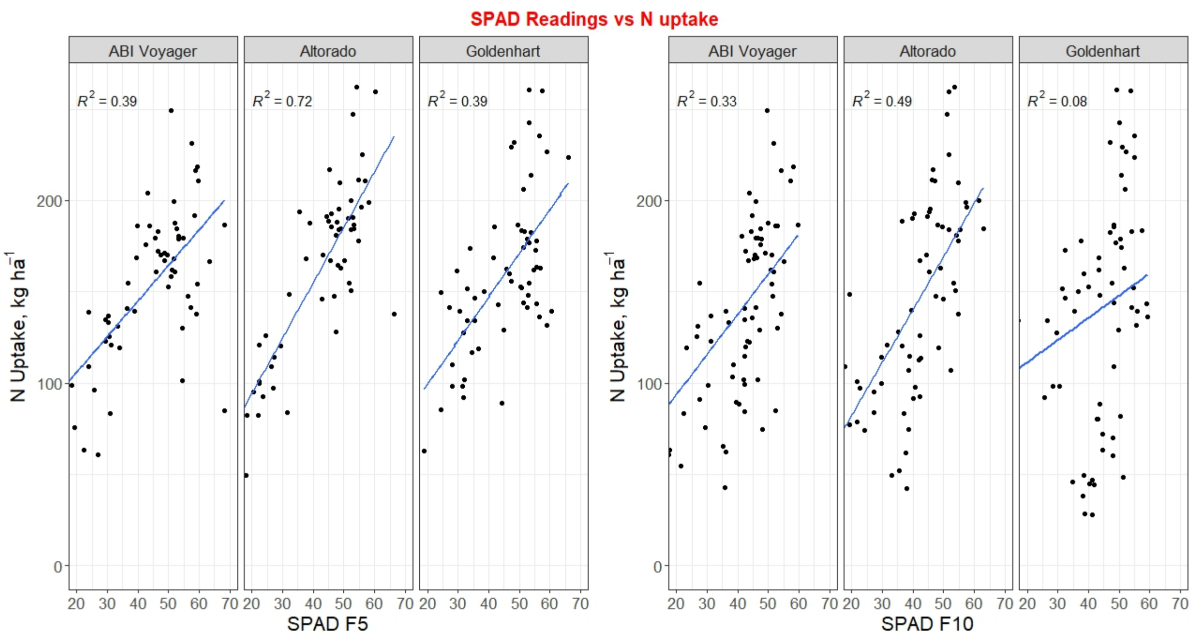

Figure 3.10 Correlation between N uptake and SPAD readings at Feekes 5 (left) and Feekes 10 (right) separated by barley varieties- three locations and two years, combined.

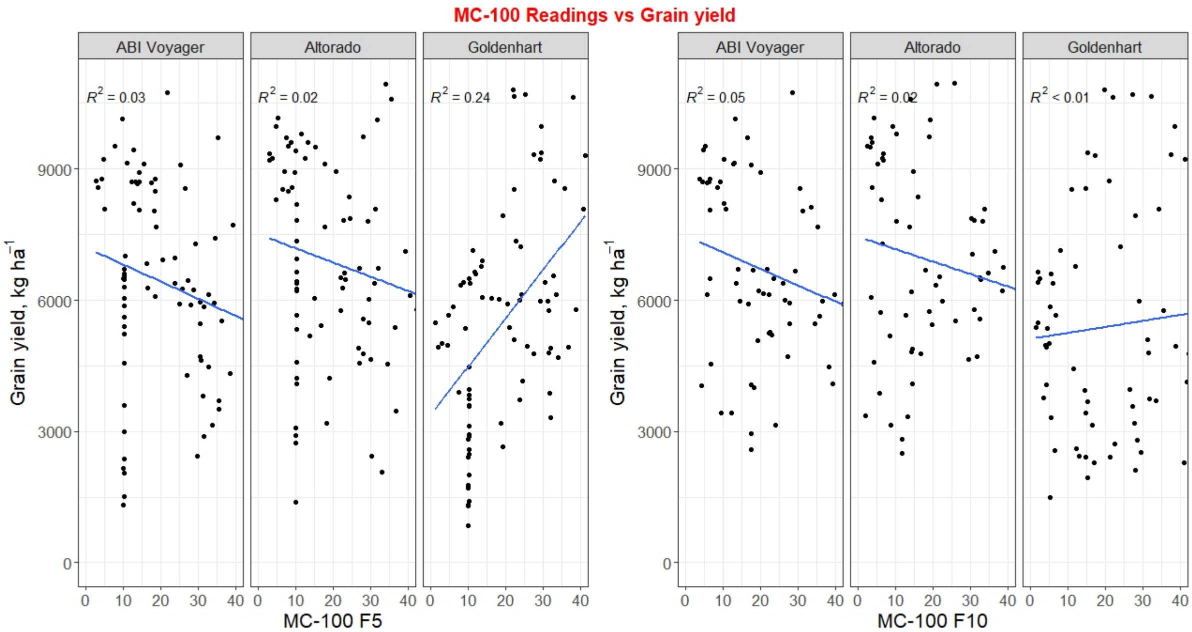

Figure 3.11 Correlation between grain yield and MC-100 readings at Feekes 5 (left) and Feekes 10 (right) separated by barley varieties- three locations and two years, combined.

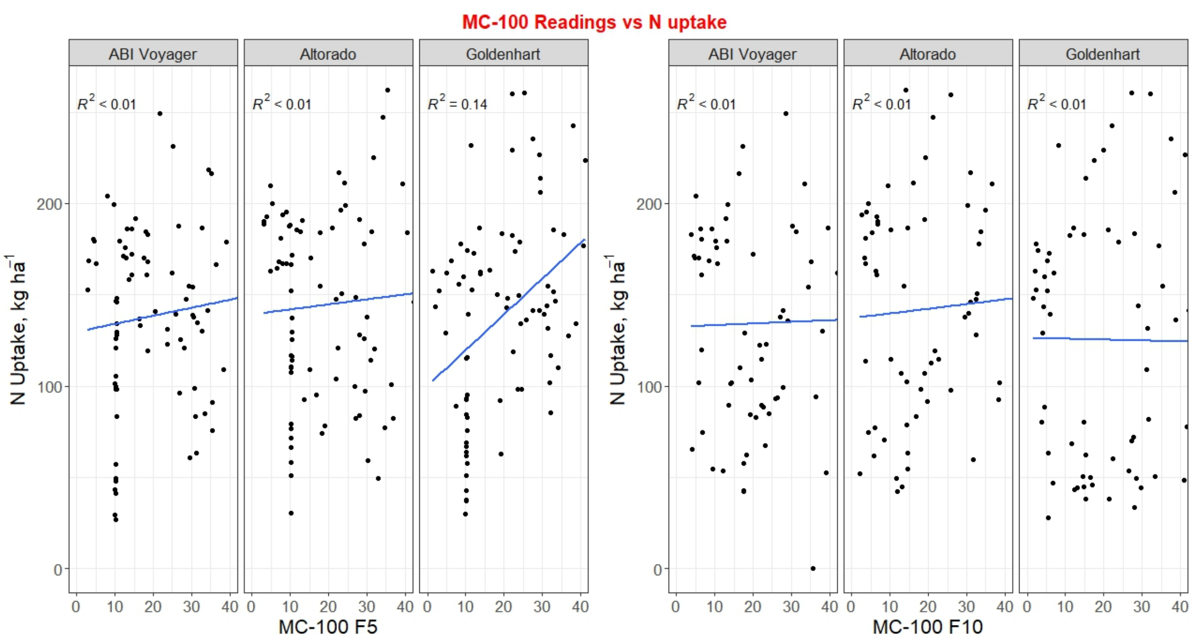

Figure 3.12 Correlation between N uptake and MC-100 readings at Feekes 5 (left) and Feekes 10 (right) separated by barley varieties- three locations and two years, combined.

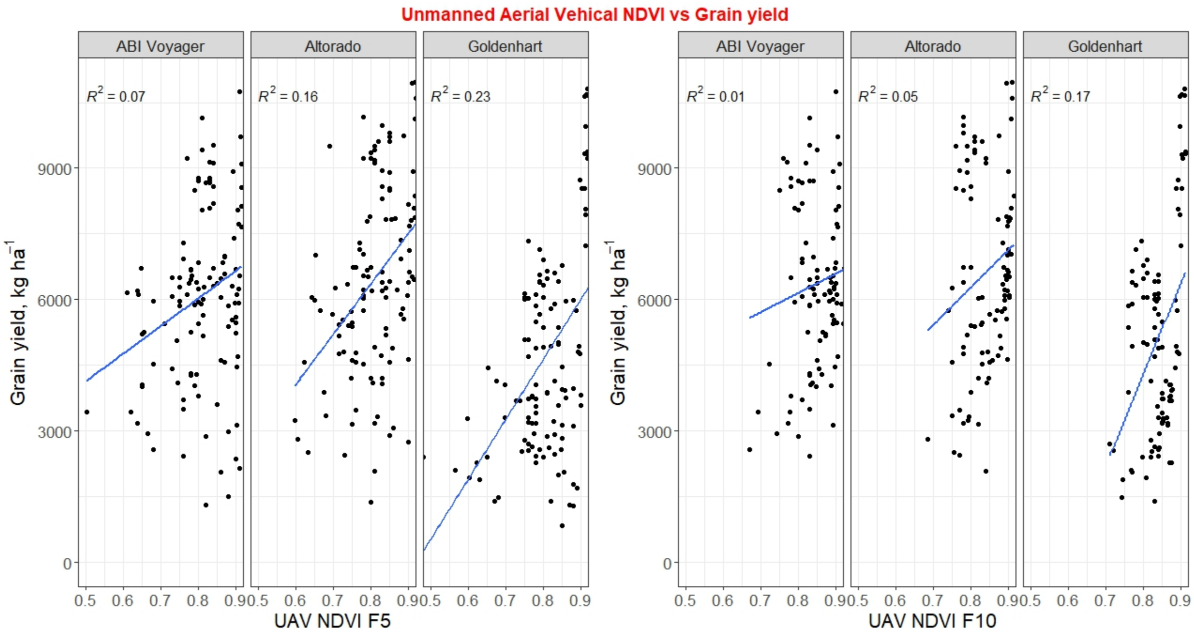

Figure 3.13 Correlation between grain yield and Unmanned aerial vehicle NDVI at Feekes 5 (left) and Feekes 10 (right) separated by barley varieties- three locations and two years, combined.

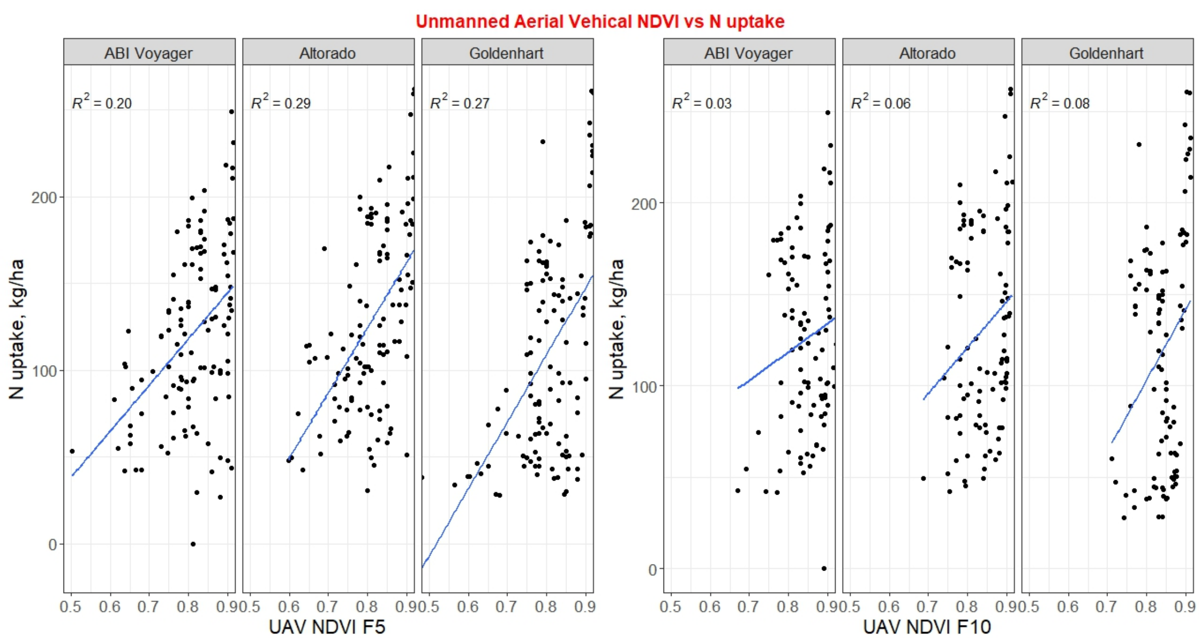

Figure 3.14 Correlation between N uptake and Unmanned aerial vehicle NDVI at Feekes 5 (left) and Feekes 10 (right) separated by barley varieties- three locations and two years, combined.

Discussion

GS NDVI

Holland et al. (2012) described the inverse square law for light. According to this law, the intensity of reflectance measured is inversely proportional to the square of the distance from the source. In the case of canopy reflectance, if the distance between the sensor and the crop (target) increases, the reflectance measured at the sensor would decrease. As barley matures and grows taller, the distance between sensor and crop decreases. This could be the reason for higher NDVI values at f10. The other reason for the lower NDVI reading at early growth stage is explained by Epee Misse & Gupta (2018). They described how during barley's early growth stage from tillering to stem elongation, which is usually Feekes 5 to 7, incomplete canopy is observed in the field. The incomplete canopy cover exposes bare soil which has NDVI value closer to zero and hence reduces the average NDVI reading. These findings were also supported by Fabbri et al. (2020). Another reason for the higher NDVI values at f10 could be the change leaf morphology as the crop reaches maturity. According to Campbell & Wynne (2011), reflectance of visible lights is dependent on the chlorophyll contained in the palisade layer of the leaf and the reflectance of NIR light depends upon the structure of mesophyll tissues. These internal properties of leaves are dependent on the crop growth stage. A study conducted in Australia on barley reported strong correlation between in-season NDVI readings and grain yield and nitrogen uptake (Misse & Gupta, 2018). Their correlation values match ours (fig 3.2).

UAV NDVI

While flying UAV, weather conditions play an important role. Clear sky is necessary to prevent shadows from the clouds. Similarly, wind velocity should be minimum to none for smooth flying of drone. The potential of UAV for gathering data in small plot research has not been widely adopted. Our study trials had 60 plots with an area of 30 m² each. The reason for the relatively weak correlation between UAV NDVI and grain yield could be due to small plot size. Many studies have explained the efficacy of UAV NDVI in larger plot size. Our findings coincide with Duan et al. (2017) who found that UAV NDVI were less accurate in predicting yield compared to spectral measurements obtained with handheld sensors. The reason being the background data from soil and objects like senesced leaves causing error during data extraction from aerial images. This should be especially important at later growing stages with mature barley (f10). Similar limitations were reported by Torres-Sanchez et al. (2013). Several research papers have studied the effect of flight altitude on the accuracy of yield prediction while using UAV-based imagery. Hassan et al. (2018) reduced the flight altitude to get higher image resolution and sufficient image overlap for superior quality orthomosaic generation. Decreasing the flight altitude of UAV flown in our study could have resulted in better efficacy in predicting yield and nitrogen uptake, especially later in the season.

Chlorophyll meter

One of the advantages of using chlorophyll meter for N monitoring is that the soil does not interfere with the signals that the sensor receives. Unlike NDVI values where readings are recorded by averaging the entire field, chlorophyll meter readings are taken by averaging a few sample plants. Even for those sample plants we record the data only from a single leaf. This difference between NDVI and chlorophyll meter readings was explained by Epee Misse & Gupta (2018) where they highlight the superiority of NDVI readings. Similar superiority was seen in our study (fig 3.2). Another reason for the superiority of NDVI readings to SPAD reading could be that the further accounts for both leaf greenness and biomass of crop while the latter accounts for leaf greenness alone (Ali et al. 2014). Many studies have shown reliable indication of N stress and grain yield prediction through its relationship with SPAD readings (Shukla et al., 2004 and Islam et al., 2014). Even though our NDVI readings were superior to SPAD readings, SPAD readings showed a strong correlation with yield and nitrogen uptake (fig 3.2).

According to Follett et al. (1992) factors such as moisture availability, soil profile N and cultivar differences may affect leaf greenness and hence the chlorophyll meter readings. Monostori et al. (2016) also reported that cultivar specific SPAD value can provide a more accurate estimate of the final yield in wheat. Similar could be the case in barley as well. In our study, we saw a better correlation of grain yield and nitrogen uptake with SPAD readings for Altorado which is a feed barley (fig 3.9 and 3.10).

Lower values could have been triggered because of adverse climatic conditions on chlorophyll content of the barley leaves. Extreme weather during our study period, high temperature and low precipitation, were recorded which could explain the SPAD meter readings (fig 3.5). This is further supported by Elsayed, Rischbeck & Schmidhalter (2017) who reported the effects of drought on the photosynthesis parameters impacting the chlorophyll content index measured by SPAD meter. Using MC-100 meter to access crop N status in vegetables crops like sweet pepper (de Souza et al, 2019), cucumbers are more popular than in small grain crops. Yost et al. (2021) used an average of 20 readings per plot from MC-100 meter to predict the N response in wheat field at 12 sites in Utah and reported its limited utility. In our study, we averaged 5 readings from each plot which could have resulted in poorer estimation of the entire plot and hence resulted in a weak correlation between MC-100 reading and both grain yield and nitrogen uptake (fig 3.11 and 3.12).

Conclusion

This study presents the possibility of N management using ground and aerial based sensors by comparing spectral indices obtained from the sensors and correlating it with yield and quality parameters. Overall, the N rate had a highly significant effect on the GS NDVI readings at both F5 and F10 stages. This suggests that higher N rate application leads to greater chlorophyll content. We found a higher correlation of NDVI obtained from GreenSeeker (GS) with grain yield. Correlation was stronger at flowering stage for grain yield (r= 0.86) and NUp (r = 0.81). The SPAD reading at flowering stage also displayed a higher correlation with Grain Yield (r=0.59) and NUp (r=0.79). However, a negative correlation was observed between GY and NUp. We did not find a significant p-value for the UAV reading and yield attributes. Based on these findings, the GreenSeeker was found to be superior to SPAD, MC-100 and UAV sensor at predicting GY and NUp in spring barley.

When we further breakdown the findings in terms of varieties, we observe a stronger correlation between grain yield and GS NDVI (r^2=0.55), UAV NDVI (r^2=0.16) and SPAD reading (r^2= 0.72) for Altorado. MC-100 reading for chlorophyll indicated a positive, linear but very weak correlation for all the three varieties. Higher correlations in all the varieties were observed at F5 than at F10. We recommend further studies in other barley varieties at different sites to improve the precision of yield and quality prediction. This study delivers valuable information to the barley growers on the utilization of spectral indices for estimating yield and protein. For future studies, we can use the prediction estimation from this study for variable rate application of nitrogen in barley and report its implications. Furthermore, we can use these findings and calculate the economic benefits associated with the use of remote sensing tools.

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Appendices

Appendix A

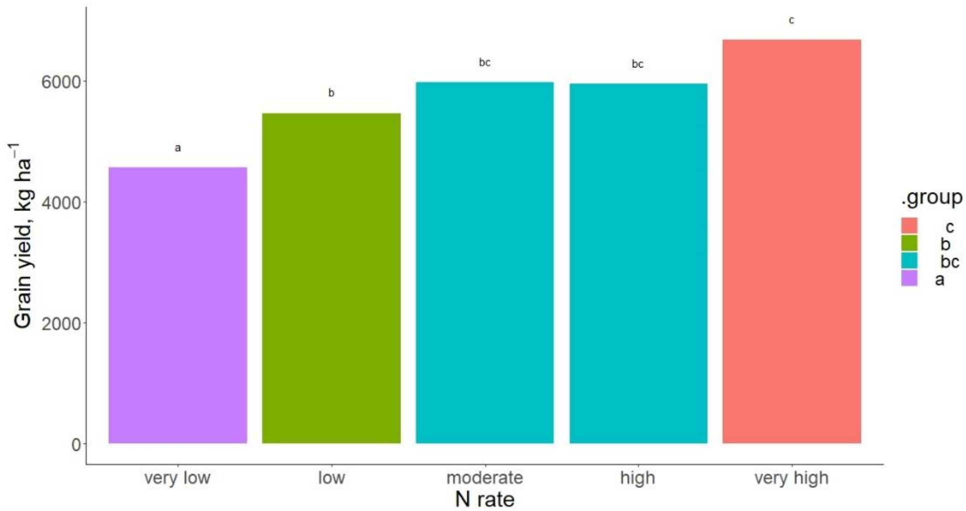

Figure A.1 Effect of N rates on grain yield (year, location, and variety combined). Five N rates are categorized as very low- 0 kg N per hectare, low- 50/30 kg N per hectare, moderate-101/67 kg N per hectare, high- 151/101 kg N per hectare, and very high- 202/134 kg N per hectare.

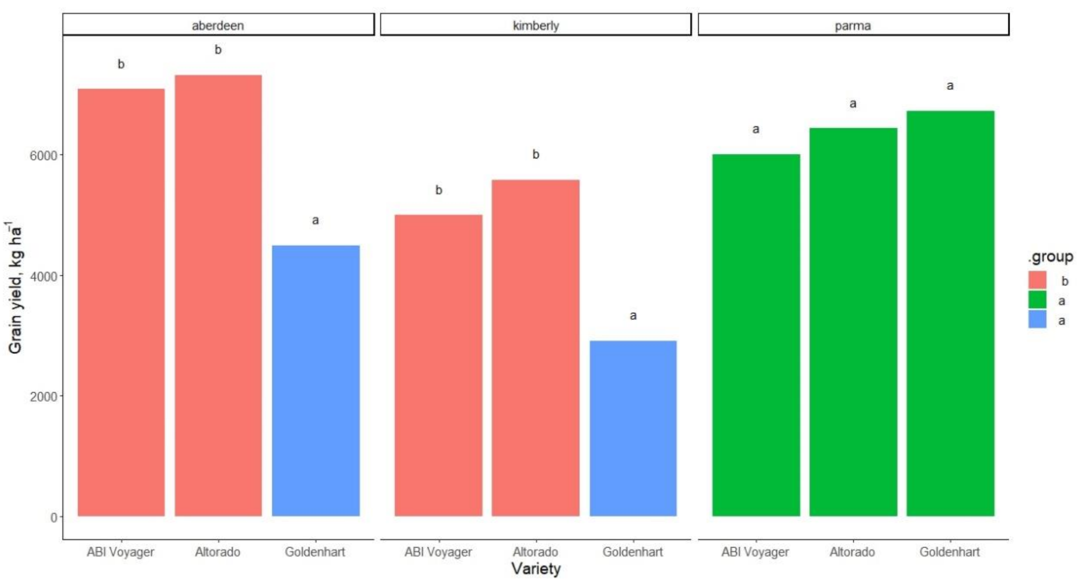

Figure A.2 Effect of variety on grain yield for three locations (year and N rate combined). ABI Voyager-malt barley, Altorado- feed barley, and Goldenhart- food barley.

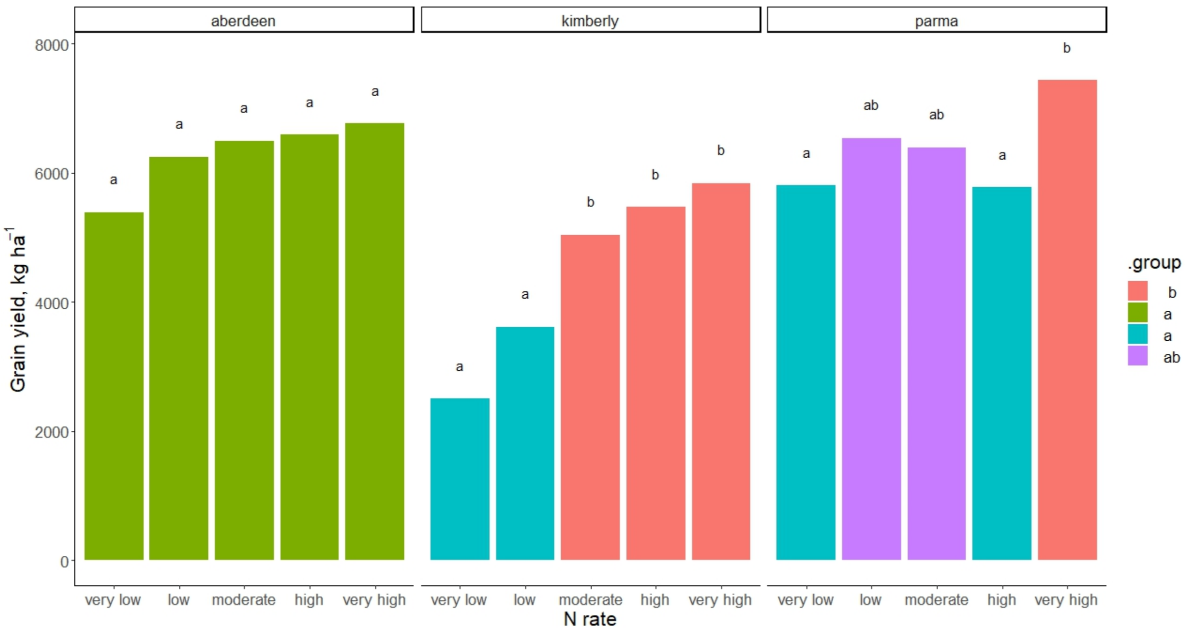

Figure A.3 Effect of N rate on grain yield for three locations (year and variety combined). Five N rates are categorized as very low- 0 kg N per hectare, low- 50/30 kg N per hectare, moderate-101/67 kg N per hectare, high- 151/101 kg N per hectare, and very high- 202/134 kg N per hectare.

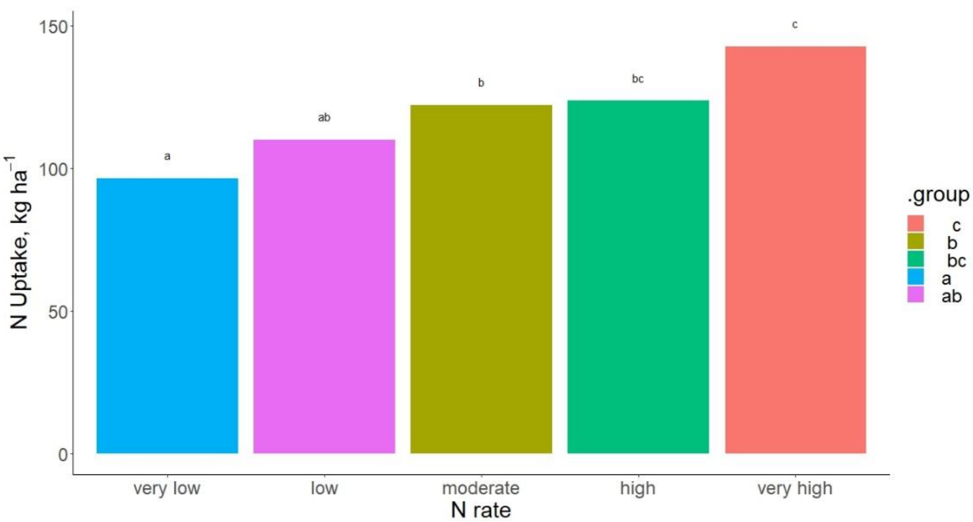

Figure A.4 Effect of N rates on N uptake (year, location, and variety combined). Five N rates are categorized as very low- 0 kg N per hectare, low- 50/30 kg N per hectare, moderate-101/67 kg N per hectare, high- 151/101 kg N per hectare, and very high- 202/134 kg N per hectare.

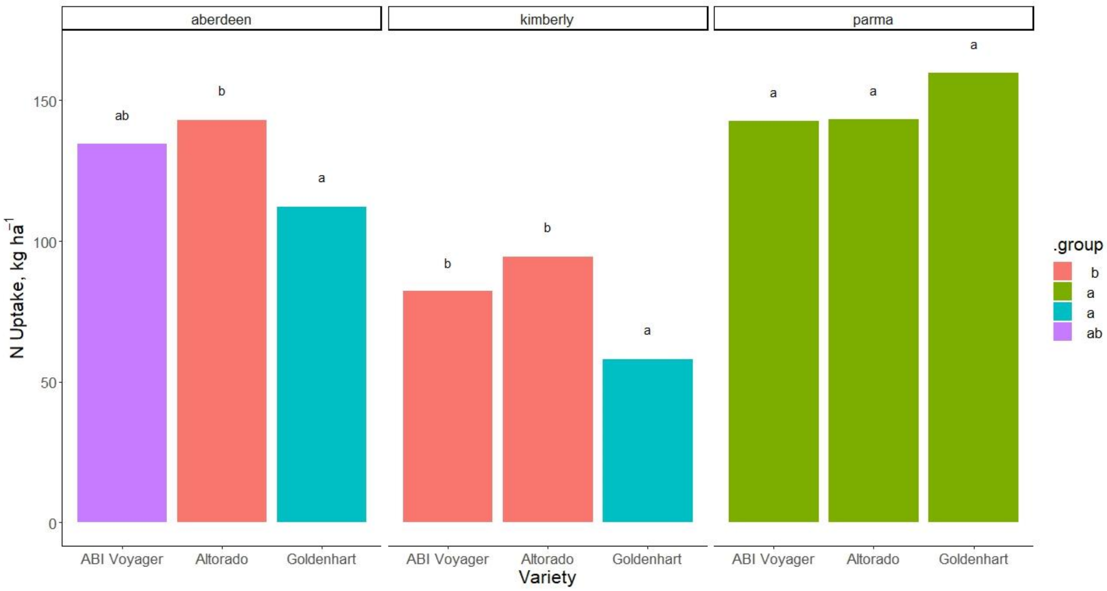

Figure A.5 Effect of variety on grain yield for three locations (year and N rate combined). ABI Voyager-malt barley, Altorado- feed barley, and Goldenhart- food barley.

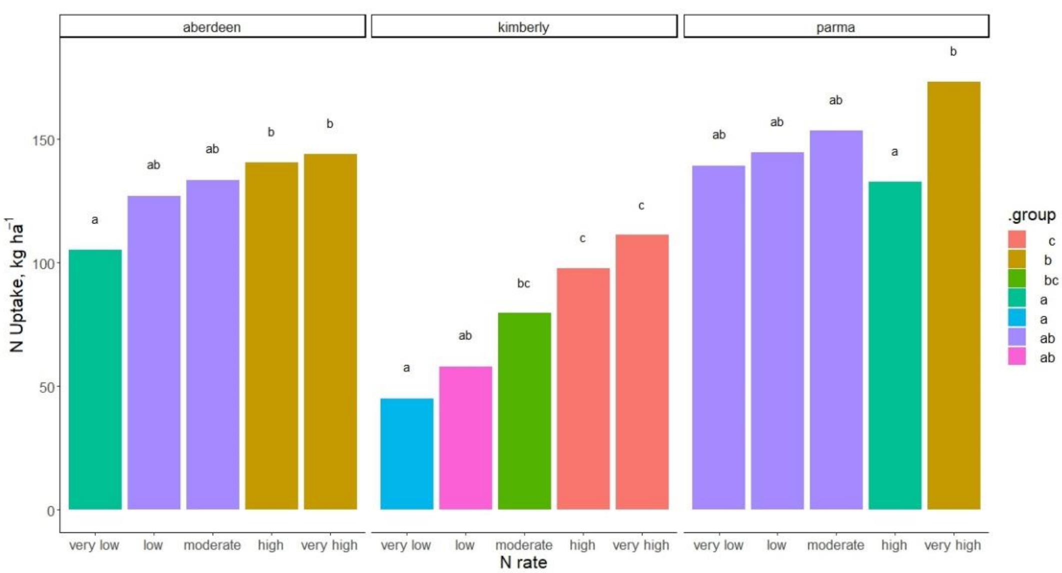

Figure A.6 Effect of N rate on grain yield for three locations (year and variety combined). Five N rates are categorized as very low- 0 kg N per hectare, low- 50/30 kg N per hectare, moderate-101/67 kg N per hectare, high- 151/101 kg N per hectare, and very high- 202/134 kg N per hectare.

Appendix B

Table B.1 Analysis of variance of GreenSeeker (GS) NDVI, unmanned aerial vehicle (UAV) NDVI, chlorophyll content by SPAD meter, chlorophyll content by MC-100 meter as affected by location, variety, N rates, and their interactions combined by years.

	Feekes 5				Feekes 10			
	GS NDVI	UAV NDVI	SPAD	MC-100	GS NDVI	UAV NDVI	SPAD	MC-100
Variety (V)	***	*	NS	NS	NS	***	NS	NS
N rate (N)	***	NS	NS	NS	***	*	NS	NS
Location (L)	***	***	***	***	***	***	***	***
V × N	*	NS	NS	NS	0.045	NS	NS	NS
V × L	***	NS	NS	NS	NS	***	NS	NS
N × L	***	NS	NS	NS	***	**	NS	NS
V × N × L	NS	NS	NS	NS	NS	NS	NS	NS

* Significant at the 0.05 level of probability, ** Significant at the 0.01 level of probability, *** Significant at the 0.001 level of probability, NS, non-significant.

Table B.2 Analysis of variance of GreenSeeker (GS) NDVI, unmanned aerial vehicle (UAV) NDVI, chlorophyll content by SPAD meter, chlorophyll content by MC-100 meter as affected by location, variety, N rates, and their interactions combined by years.

	Feekes 5				Feekes 10			
	GS NDVI	UAV NDVI	SPAD	MC-100	GS NDVI	UAV NDVI	SPAD	MC-100
Variety (V)	*	NS	*	NS	NS	*	NS	NS
N rate (N)	***	NS	NS	NS	***	NS	NS	NS
Year (Y)	NS	NS	***	***	**	***	***	*
V × N	NS	NS	NS	NS	NS	NS	NS	NS
V × Y	NS	NS	NS	NS	NS	NS	NS	NS
N × Y	NS	NS	NS	NS	NS	NS	NS	NS
V × N × Y	NS	NS	NS	NS	NS	NS	NS	NS

* Significant at the 0.05 level of probability, ** Significant at the 0.01 level of probability, *** Significant at the 0.001 level of probability, NS, non-significant