Nitrogen Use Efficiency of Potato

A Thesis

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Chapter 1

Nitrogen Use Efficiency and its Mechanisms in Potato and Other Crops

Importance, current understanding and possible mechanisms

A Review

Abstract:

Potatoes are the third greatest production food crop by ton in the world. High fertilizer rates normally applied to potato contribute to environmental pollution and water eutrophication. One solution is the creation of nitrogen use efficient (NUE) varieties of potato which maintain industry-standard yield with less applied nitrogen (N). However, little progress has been made toward NUE variety development due to conflicting definitions of what constitutes NUE and a lack of efficient screening methods. Identifying effective screening methods could streamline selection by finding causal phenotypes, secondary traits, or genes which could be integrated into existing breeding systems. Work in N uptake as well as discrete genes which increase NUE in other crops could point a way forward for future research in potato. This review attempts to standardize the NUE equation and its component variables; explore interesting and proven methods of NUE research and discuss existing research potato phenotypic traits and mechanisms which may confer NUE.

Keywords: NUE, nitrogen use efficiency, potato, root, nutrient efficiency, uptake, utilization.

1 – Introduction

1. 1 – Current Potato Production.

Potato is one of the most productive crops in the world and the third most important food crop for direct human consumption (FAO 2018; cipotato.org). Potato tuber is a storage organ comprised of modified stem tissue (Fernie and Willmitzer 2001) which is rich in carbohydrate, dietary fiber, vitamin C, and potassium (King and Slavin, 2013). Cultivated potato in the U.S. is a single species — *Solanum tuberosum*. *S. tuberosum* includes many cultivars that are most often autotetraploids with 48 chromosomes (2n=4x=48). Wild and landrace potato types, such as *S. chacoense, S. tarijense, S. phureja*, *S. commersonii, S. kurtzianum,* and *S. microdontum* are usually diploid (2n=2x=24) (Errehbi et al. 1998; Hirsch et al. 2013) and rarely used in large scale production systems in the U.S.

Contemporary industrialized potato production methods are uniform, row systems which are reliant on high levels of various inputs. Potato is typically grown in sandy soils, which require high inputs of water and fertilizer — especially nitrogen (N) (Errebhi et al. 1998; Sharifi et al. 2007) — due to poor water retention and low nutrient holding ability. *S. tuberosum* varieties also have very small rooting systems (Goffart et al. 2008; Lesczynski and Tanner 1976) which are inadequate for acquisition of nutrients and require disproportionately large levels of irrigation (Wishart et al. 2013; Wishart et al. 2014) compared to other major crops, even in less sandy soils with high OM and better water holding capacity. All of these aspects of potato cultivation create a situation in which only 40 to 60% of the available N (Zebarth and Rosen 2007) and as little as ~10% of applied

phosphorus (White et al. 2005) is absorbed by the average potato crop. While older N recommendations for potato were modest, indicating a preplant application of 67-137 kg N ha^{-1} for maximum early tuber growth (Westerman and Kleinkopf 1985), current recommendations are as high as 280 kg ha^{-1} of N (Rosen and Eliason 2005). Compounding this, farmers often feel incentivized by inexpensive fertilizer prices to apply higher rates of N than recommended as a form of 'yield insurance' (Sheriff 2005). Conversations with professors at the University of Minnesota Twin Cities as well as Minnesota area farmers indicate N applications greater than 336 kg ha^{-1} are routinely applied to potato crops in the Midwest United States. Such high application rates can actually reduce yields in potato (De Jong et al. 2011; Errebhi et al. 1998; Kleinkopf et al. 1981) by prolonging the vine growth/tuber set phase and subsequently delaying tuber bulking.

Perhaps due to the low price of N fertilizer, this incredibly porous system was not seen as problematic until recently. Recently, however, concern about the health and economic costs of agricultural runoff in drinking water (Temkin et al. 2019), the highly publicized 'Gulf Dead Zone' (Rabalais et al. 2002), and local waterway eutrophication (i.e. algal blooms) have contributed to a change in public awareness and an increased environmental regulation on agricultural inputs (mda.state.mn.us). All of this has prompted a surge of research in reduced input agricultural systems and nitrogen use efficiency (NUE) (Good et al. 2004). This research effort is being heralded as a second Green Revolution (McAllister et al. 2012). Increasing NUE in potato through breeding has been identified as the most elegant solution to this problem for potato production systems (Zebarth and Rosen 2007).

In this review, we will outline the history of NUE scholarship, for form and meaning of the NUE equation, past NUE studies in potato, studies and mechanisms of N uptake, and a simple understanding of phenotypic plasticity as it relates to potato NUE studies.

1.2 – Defining N use efficiency: constituent parts and many names.

Broadly speaking, studies which screen for NUE are consistent in their definition. NUE has a long scholarship, particularly in cereals. NUE is normally defined as yield available-N^{-1} (Equation 1). Available N is most often the N applied or, in some cases, the actual N which is plant available as ascertained via lab tests of soil (Bock 1984; Moll et al. 1982; Xiaorong et al. 2016). There is some contention about the definition of NUE in potato however.

Tiwari et al. (2018), for their review of NUE in potato, compiled a table of 12 common efficiency terms used in potato NUE research. To understand why this is, and to realize a consistent and final definition for this chapter, we needed to trace back NUE work to its origin. Novoa and Loomis (1981) cites several papers which discuss 'physiological efficiency,' 'agronomic efficiency,' and 'recovery fraction' (Hamid 1972; van Keulen 1977; Pearman et al. 1977) which correspond to what are now known as use, utilization and uptake efficiencies. Moll et al. (1982) reasserted the theory that NUE could be expressed as the product of two constituent parts but renamed them to N uptake efficiency (NUpE) and N utilization efficiency (NUtE) . Moll et al. (1982) also pointed out how the equation could be changed and further expanded to fit other needs, such as partitioning NUtE into the product of N partitioned to grain and grain produced per unit translocated.

Equation 1

$$\frac{(NUE)}{\frac{Yield}{Applied\ N}} = \frac{(NUtE)}{\frac{Yield}{Plant\ N}} \; x \; \frac{(NUpE)}{\frac{Plant\ N}{Applied\ N}}$$

Bock (1984), rather than Moll et al. (1982), is often cited as the source of the NUE equation in papers, perhaps due to its publication in a popular textbook of the time, "N in Crop Production." In that book, Bock returned to the older terminology of referring to NUpE and NUtE as N recovery efficiency and physiological efficiency, respectively. However, for the purposes of this paper N uptake efficiency (NUpE) and N utilization efficiency (NUtE) will be the preferred names for these concepts, as has become standard in the field.

One of the most influential papers on NUE in potato was Errebhi et al. (1998), which used total plant dry weight as "yield," citing Bock (1984). Errebhi et al. (1998) was working with some wild-type and landrace germplasm which did not set tubers or have typical yield characteristics, so they defined yield in terms of the entire plant mass. This resulted in NUtE being defined as total dry weight/plant N-content. Errebhi et al. (1998) found this unique definition of NUtE to be unresponsive across all genotypes tested — a result which is inconsistent with prior and subsequent NUE work. A later study which used Errebhi et al. (1998)'s methodologies found the same result (Sharifi et al. 2007) and for this reason, we recommend defining yield in potato as either tuber wet weight or tuber dry weight. Otherwise, NUtE does not vary across genotypes and NUE becomes simply a product of NUpE. Note that if a per unit area of yield is used, the same unit area must be used for N applied. Recent work on NUE in potato has reached the same conclusion about the

definition of NUE yield in potato (Baye Berihun Getahun, PhD thesis, Wageningen University, 2017; Tiwari et al. 2018).

Regardless of how it is defined, there is a history of NUE study in potato which must be fully understood in order to judge the value of new avenues for study which are laid out in section two. The purpose of this chapter is not only to review literature on NUE in potato, but to review studies which found mechanisms of NUE in other crops that might be promising directions for research in potato NUE.

1.3 – NUE research in potato: Identifying individuals, but not mechanisms.

Prior screening methods for exploring NUE in potato focus on evaluating characteristics of a set of genotypes grown under multiple N treatment levels. These experiments usually yield mixed results (Errebhi et al. 1998; Kleinkopf et al. 1981; Sattelmacher et al. 1990a; Zebarth et al. 2004; Zebarth et al. 2008; Zvomuya et al. 2002). The experiments also share another trait in common: their small scale. In a breeding program with hundreds to thousands of unique genotypes, the screening-by-dose method would multiply screening resource requirements by the number of N treatment levels you chose and how many repetitions it required. A lower-cost screening method has been developed in hydroponics (Sharifi et al. 2007) but has not been widely adopted. There is insufficient evidence that traits seen in hydroponics carry over to field performance and more research is needed.

Studying the mechanisms of NUE is difficult and costly due to the complex nature of the many interacting genetic, environmental and cultural practices, which all contribute to N use in crops (Dawson et al. 2007). Past efforts screening for NUE in potato that use

multiple N rates create entire N response curves (Kleinkopf et al. 1981; Rosen et al. 2004). Recent efforts, however, have economized by employing two N rates (Zebarth et al. 2004; Zebarth et al. 2008). Even with two N rates, this method is still costly over a large set of genotypes. Identifying discrete genes and phenotypes which are correlated with or directly confer NUE in normal growing conditions could dramatically reduce this cost. This may eliminate the need for multiple N treatments altogether and still allow for the selection of genotypes predicted to express high NUE.

There is evidence for the efficacy of root phenotype correlation with yield. A field-based screening method for potato roots has shown nearly all root and stolon traits, such as root length, root weight, stolon length, etc. to be highly correlated with yield (Wishart et al. 2013). However, this method has not been implemented under multiple fertility rates for the study of NUE. Root phenotypes are relatively easy to screen for and are potential sources of increased uptake (Sattelmacher et al. 1990b). Physiological mechanisms of NUE, which are often more difficult to screen for, have not been studied in potato. Physiological constraints could be just as influential on nutrient use and uptake efficiency as any root or canopy phenotype and many factors come together to reduce plant N uptake well below physical capacity based on the plant and N availability (Glass 2003).

Section two of this chapter aims to detail the few existing mechanisms of increased NUE in potato that have been identified, as well as many mechanisms of N uptake from other species which could inspire avenues of potato NUE research. We also discuss the nature of trait plasticity and the potential for varieties which could adapt to stress when it is encountered but display normal growth when provided ideal conditions.

2 – Mechanisms

2.1 – Uptake of Nitrogen

Nitrogen uptake is a complicated function of plant physiology with multiple mechanisms controlling its rate. N distribution in the soil is complex, owing to many simultaneous competing chemical reactions and factors such as cation/anion charge, plant root distribution, soil water content, etc. (Davidson et al. 1978). Nitrogen is highly mobile with water and present in many forms throughout the soil, but it is especially abundant in the top layers of the soil where organic matter is highest (Chai et al. 2015; Nieder and Benbi 2008; Stein and Klots 2016). Plant N uptake increases in response to available light, transpiration, and N supply, with these mechanisms functioning both independently and in concert with each other (Huffaker and Rains 1978). The increases in uptake in response to N supply may also be regulated by increases in N reductase activity (Rao and Rains 1976) signaled by malate (Ben-Zioni et al. 1970) synthesized in shoots during the reduction of nitrate and translocated to roots where it increases preferential uptake of nitrate in the rhizosphere (Ben-Zioni et al. 1971). It seems likely that efficiency of the uptake system is related to regulation around these mechanisms.

Nitrogen fertility rate can have unexpected results in potato. Prior research has shown that N deficiency results in larger chloroplasts and abundant starch levels in potato, the opposite of what has been observed in grain crops like millet (Lutman 1934). Additionally, there is an inverse relation between N fertilization and sugar production in plants (Hewitt 1963), including potato leaf tissue (Wen 2019). Mechanistically, this suggests that, in potato, N

deficiency increases chloroplast size, which in turn, increases sugar production and levels in leaves resulting in greater sugar translocation to the tuber for storage as starch. This increased production of tuber starch could be measured as a form of NUtE.

NUtE is a somewhat opaque measure of how efficiently biomass is formed, N's effect on carbohydrate partitioning, nitrate reduction efficiency, and remobilization of protein N from senescent tissues (Novoa and Loomis 1981). Cells have a long-recognized ability to store N for later metabolic use during times of stress (Aslam et al. 1976; Ferrari et al. 1972). Both NUtE and NUpE ratios use total plant N measurement to calculate their influence on total NUE, but we have no way to differentiate stored N versus N which was incorporated into plant tissues. The ability to store N may be related to a method commonly seen in other plants for increasing NUtE – remobilization. Remobilization can be so efficient in oat, for example, that a given plant can acquire all the N necessary for its life cycle in vegetative phase alone (Leopold 1961). We know that leaf senescence in tubers during the later lifecycle of potato results in a reduction of leaf and vine N levels while tuber levels continue to grow (Kleinkopf et al. 1981; Rosen et al. 1993). It is likely a large percent of that N is from remobilization from stem tissue. Thus, potato vine termination methods and timing could have significant implications on NUtE in potato.

Wheat (*Triticum aestivum*), oat (*Avena sativa)* and corn (*Zea mays*) are frequent subjects of N studies, and their N uptake is well understood. Morgan and Jackson (1988/1989) found down-regulation of N uptake in wheat and oat. In both studies, N uptake was significantly increased when N levels were very low, with influx increasing by as much as a tenfold in the lowest N conditions and efflux decreasing significantly as well. Increasing

available N resulted in a slight increase in total influx followed by a steep down-regulation and subsequent decrease in N influx. Glutamine seems to play a central role in down-regulation of transcription for genes, which encode for influx high-affinity transport systems (HATS) of both ammonium and nitrate.

Rawat et al. (1999) working in *Arabidopsis thaliana*, found that mRNA levels for the *AtAMT1* gene rapidly decreased when ammonium nitrate (NH_4NO_3) supply was increased for plants previously in N stressed conditions. NH_{4^+} influx likewise declined along with *AtAMT1* mRNA. However, NH_4^+ influx was maintained at high levels when the ammonium to glutamine conversion was blocked with methionine sulfoximine. The conclusion was that products of ammonium metabolism, not ammonium itself, are responsible for down-regulation of N uptake in both ammonium and nitrate forms. *HvNRT2* in barley, which encodes for nitrate HATS was found to have similar behavior, down-regulating as a result of glutamine presence in the cytoplasm, which comes from metabolism of ammonium, (Vidmar et al. 2000) and subsequently decreasing influx of NO_3^-. This glutamine N influx feedback loop was confirmed for ammonia in an alga, *Chara australis* (Ryan and Walker 1994) as well. While these experiments utilize different species, including monocots and dicots, and find different causal genes, the relationship between glutamine presence in the cytoplasm and a down-regulation of the full N uptake capacity of the plant is clear and likely present in potato as well.

Fan et al. (2016) found a non-glutamine related mechanism in the identification of a spliced form of *OsNRT2.3*, a nitrate transporter gene found in rice (*Oryza sativa)*. *OsNRT2.3b* splice-form senses pH and acts as a switch to activate or deactivate nitrate transport

activity. Utilizing *OsNRT2.3b* overexpressing mutant lines, it was found that high expression of *OsNRT2.3b* enhances pH-buffering by regulating the uptake of ammonium vs. nitrate and subsequently increases N, iron and phosphorus uptake of the plant. The overall effect was an increase of NUE by 40%. Ammonium typically has an inhibitory effect on the uptake of nitrate (Huffaker and Rains 1978) and high cytoplasmic levels of ammonium may have an inhibitory effect on the rate of ammonium influx as well (Rawat et al. 1999). Miao et al. (1993) examined HATS for ammonium (NH_4^+) and nitrate (NO_3^-) in rice and concluded that exposure to high ammonium levels down-regulates transport gene expression. It was further hypothesized that increased expression of *OsNRT2.3b* in rice could help rice plants adapt to varied N supply forms such as those that occur in climates with frequent oscillations in soil moisture. Soil moisture levels affect the form of available N, changing between nitrate in wetter soil and ammonium in drier soil.

Being able to better cope with fluctuating soil moisture levels could also be adaptive for combating the conditions we expect will become more common from climate change. There are clearly exciting pathways for increasing N uptake which have been identified in other crops but not explored in potato.

2.2 – Plant phenotype plasticity: adaption to the environment of the moment.

Phenotypic plasticity is ability of some plants to produce different and distinct phenotypes depending on the environment they are in. A fairly universal example is the increasing of internode length in response to inadequate light. Internode elongation increases exploration of the environment so that an area with greater light intensity can be found — at which point the tissues of the plant which are exposed to that greater light intensity begin growing

with shorter internode lengths to create greater leaf density. Many of the phenotypes described in the previous section do not arise in response to the stressor they are adapted to combat — they are always present and often a liability if the stressor is not present. An example of that would be increased transpiration, which can increase N uptake but comes at the cost of increased susceptibility to drought. In potato, there is evidence of such immediate phenotypic adaptation in regard to nitrogen and root size.

Sattelmacher et al. (1990b) showed increased root mass and surface area of a high uptake variety of potato, but only in reaction to a low N environment. In normal N levels, this same variety had normal root mass compared to other potato. Considering the small rooting structures typical of cultivated potato, the mechanism for this phenotypic plasticity would seem a promising direction for study. Increased plant growth below ground will come at a metabolic cost, however, which could decrease above ground growth (Novoa and Loomis 1981). However, there are mechanisms for increasing plant tissue size and surface area which are effectively metabolically free, such as increased root aerenchyma size. Such a mechanism for metabolically free tissue mass increase could be considered a form of increased utilization efficiency.

Aerenchyma are gaseous spaces in the root cortex (Postma and Lynch 2011). By increasing the bulk of roots without increasing the metabolic cost of producing roots, root cortical aerenchyma allows for an effectively 'free' way to increase the total soil area explored and therefore, the nutrients available. Root cortex aerenchyma also decreases internal impedance to the transport of oxygen and N (Jackson and Armstrong 1999). The formation of these aerenchyma is triggered by stressors such as hypoxia (Jackson and Armstrong

1999); drought (Zhu et al. 2010a); and N (Drew et al. 1989), phosphorus (Fan et al. 2003) and/or sulfur (Bouranis et al. 2003, Bouranis et al. 2006) deficiency. In many ways this adaptation, or plasticity, is ideal for its ability to express only when needed at low/no metabolic cost and in reaction to a multitude of stressors, all of which it can aid in alleviating.

Some emerging research has found the ability to express plasticity of a trait to be costly to the overall fitness of the plant when the trait is not expressed (Weijschede et al. 2006). However, plasticity of root hair length in Z. *mays* actually conferred an equal to greater advantage over universally long-haired varieties in low phosphorus environments (Zhu et al. 2010b), indicating the cost of plasticity was low and the adaptive quality a net gain. The need for further research into these adaptive responses remains.

3 – Discussion

While the need for N efficient germplasm in potato is clear, the high cost of screening and the poor understanding of the mechanisms responsible have limited any effort to breed for NUE in potato. While increased root mass in reaction to low-N as a mechanism for NUE is an interesting idea, it has only been shown hydroponically in one variety (Sattelmacher et al. 1990). There is a clear need to identify discrete phenes that are responsible for NUE so that newer, more high-throughput screening methods (E.g. Nigon et al. 2014; Paez-Garcia 2015; Wasson et al. 2016) can be used to find NUE germplasm candidates.

While the complexities of working on tetraploid genetics are beyond the scope of this paper, discrete genes and genetic basis for mechanisms of NUE have been shown in other crop plants and need to be identified in potato. Regardless of what type or mechanism of efficiency a breeder may be aiming for, many of these traits have been shown to be either highly controlled by specific QTL or at least reasonably heritable through either standard breeding practices or genetic tools (Lynch and Brown 2012; Hong et al. 2004). While more complex root phenotypic traits are not as easily introgressed or selected for as discrete N efficiency genes outlined earlier in the paper, they still provide a way forward. Genetic or metabolistic testing which shows the potential for NUE at any N rate would be the most ideal and high-throughput way of screening for NUE in potato germplasm, but without NUE phenes and genes experimentally identified, the potential success of efforts such as GWAS, QTL or other marker-based technologies is very limited. It is our hope this work can serve as hypothesis generating material for future experiments in potato and point the way toward potentially rich areas of study for NUE trait mechanics and screening methods. We developed the goal of assessing potato roots in field conditions at two discrete nitrogen levels as a way of bridging the gap between work around root phenotypes in hydroponics and newer two-level NUE fertility screening. That work is detailed in chapter two.

Chapter 2

Effect of reduced nitrogen on potato yield, size distribution, and skin quality

Understanding the role of nitrogen use, uptake, and utilization efficiency and their associated root growth characteristics.

Abstract:

Potato (*Solanum tuberosum*) production typically occurs on sandy soils with only 40-60% of the applied nitrogen (N) acquired by the crop. Nitrogen fertilization rates in potato are upwards of 336 kg N ha^{-1}. Increased N use efficiency (NUE) and its component parts, N utilization efficiency (NUtE) and N uptake efficiency (NUpE), could drastically reduce fertilizer rates and losses to the environment. We grew 12 advanced breeding selections from the University of Minnesota red potato breeding population and two elite checks under two N rates, 101 kg N ha^{-1} and 202 kg N ha^{-1}. We compared NUE, NUpE and NUtE in low and high N using 45 day after planting (DAP) root phenotypes and harvest yield and skin quality metrics. We found that NUtE correlated with NUE and yield in low N and NUpE correlated with NUE and yield in high N. Low N favored smaller tubers <6.35 cm in diameter (USDA small), while high N favored tubers between 6.35cm to 8.26cm diameter (USDA medium). Nitrogen did not significantly affect skinning and redness but did significantly affect skin lightness, with low N resulting in slightly lighter skin color. Finally, we found that greater total root mass, stolon root, or basal root, correlated with greater yield and NUE, but did not correlate with measures of N uptake.

Keywords: NUE, NUpE, NUtE, Nitrogen, Efficiency, Use, Utilization, Uptake, Skinning, Potato, Yield.

1 – Introduction

Potato (*Solanum tuberosum*) is the third most important food crop (i.e. direct human consumption) in the world (FAO 2018). As the global population increases (United Nations 2015), assuming that potato retains its current level of importance in the human diet, potato production must rise to meet demand. US potato production systems require high nutrient inputs and, most often, sandy soils. Current nitrogen (N) fertilizer recommendations for 120-day potatoes grown in the Midwest United States range from 269 kg N ha^{-1} to more than 336 kg N ha^{-1} (Franzen et al. 2018; Laboski et al. 2006; Rosen 2018; Stark et al. 2004). Farmers will often apply excess N due to low fertilizer costs and perceived added value as 'yield insurance' (Sheriff 2005).

Due in part to potato's small rooting system (Goffart et al. 2008; Lesczynski and Tanner 1976; Wishart 2013; Wishart 2014), potato typically absorbs only 40-60% of available N with the remainder potentially lost to environment by leaching, denitrification and volatilization (Zebarth and Rosen 2007). The goal of increased production may therefore provoke environmental consequences as N is now seen as a limiting nutrient for eutrophication (Howarth and Marino 2006). Leached N from the Upper Midwest United States is a major contributor to the hypoxic dead zone in the Gulf of Mexico (Rabalais et al. 2002). The environmental consequences of potato production systems could be effectively mitigated by increasing crop N uptake efficiency (NUpE), a component part of N use efficiency (NUE).

NUE has been studied extensively in potato (Errebhi et al. 1998; Hewitt 1963; Kleinkopf et al. 1981; Klienkopf 1985; Mohammad et al. 1999; Sharifi et al. 2007; Sattelmacher et al. 1990b; Westerman and Sattelmacher et al. 1990a; Zebarth et al. 2004; Zebarth et al. 2008; Zvomuya et al. 2002) but authors differ in their definition of NUE and the units used in its calculation (Tiwari et al. 2018). NUE is typically defined across many crops in the general equation as:

Equation 2

$$NUE = \frac{Yield}{Available\ N}$$

However, the definition of 'yield' often varies by crop and study. In potato alone, 'yield' may be defined as tuber wet weight; tuber dry weight or total plant dry weight (Tiwari et al. 2018). Each of these definitions is limited: plant weight and dry weight don't reflect edible or marketable yield, while tuber wet weight, can fluctuate depending on environmental factors. Tuber-specific root structures may be responsible for water uptake by tubers (Kratzke and Palta 1985) and tuber water content can fluctuate in response to water stress (Levy et al. 2013). Our experiment utilizes tuber dry matter as numerator for NUE in order to reduce confounding factors, gain insight on phenotypic drivers of yield and be in line with the recent work in the field (Getahun 2017).

NUE is typically modeled as the product of its two components: N utilization efficiency (NUtE) and N uptake efficiency (NUpE) (Bock 1984; Moll 1982). The relationship among NUE, NUtE and NUpE we will use in our study is shown in Equation 3:

Equation 3

$$\frac{(NUE)}{\frac{Tuber\ Dry\ Matter}{Applied\ N}} = \frac{(NUtE)}{\frac{Tuber\ Dry\ Matter}{Plant\ N}} \; x \; \frac{(NUpE)}{\frac{Plant\ N}{Applied\ N}}$$

Applied N is a standard denominator across many NUE studies (Tiwari et al. 2018), sometimes specified as N fertilizer use efficiency but still most often abbreviated as NUE.

As unitless statistics, NUE and NUtE provide a comparison of dry matter units produced per single unit of N applied to the plot or found in the plant, respectively. NUpE can be understood as the fraction of N applied that was taken up by the plant. This can be calculated as the N present in all plant tissues in a plot divided by total N applied to that plot area.

Potatoes differ in their N uptake abilities (Zebarth and Rosen 2007). One hypothesis for a trait which may drive these differences is the size of the root system (Sattelmacher et al. 1990b). Wild or landrace potatoes generally have a much larger and more expansive root systems than cultivated potato. For this reason, N uptake studies are most often performed under an assumption that wild and landrace varieties may have better N scavenging ability at low nutrient levels (Errebhi et al. 1998, Wishart et al. 2013; Wishart et al. 2014). However, evidence shows that cultivars and experimental lines of tetraploid potato clones yield more than wild and landrace sub-species and alternative *Solanum spp*. (usually diploids) at low fertility levels (Sattelmacher et al. 1990a). This is likely because wild and landrace varieties often do not yield well in the U.S. even under the best growth conditions. For instance, Errebhi et al. (1998) found that tuber dry matter was 85% of total plant dry

matter in potato cultivars but just 8% in wild germplasm lines. However, when root mass was compared in two elite potato clones in hydroponics there was evidence to support the idea that larger root mass might increase N uptake ability (Sattelmacher et al. 1990b). Furthermore, excavation of whole mature potato plants from the field has demonstrated variance among root characteristics in elite clones and suggested that these characteristics affect yield (Wishart et al. 2013; Wishart et al. 2014).

20% of the potatoes grown in Minnesota are fresh market red-skinned potatoes (USDA NASS), often grown on sandy well drained soils in the center of the state. Therefore, this research endeavors to understand the potential diversity present in current University of Minnesota, commercial, fresh market, red-skinned potato breeding population for yield, NUE, NUtE, NUpE, and root phenotypes. Because of the importance of low skin abrasion and dark red skin color in this market class of potato, we also studied the effect of low-N fertility on skin removal by abrasion at harvest (skinning) and color.

2 – Methods

2.1 – Germplasm: We selected 10 red-skinned, fresh market potato clones from among the University of Minnesota Twin Cities potato breeding program's experimental lines. These experimental lines, which had been evaluated and advanced from earlier preliminary performance trials, were chosen for diversity in pedigree and represented crosses between parents originating from multiple university programs including University of Minnesota, Colorado State University and North Dakota State University (Table 1). Additionally, elite cultivars Red LaSoda and Dakota Rose were planted as checks for comparison. Tubers of

the experimental lines and check varieties used for planting the experimental plots had been field grown in Minnesota at least one generation (See Table 1) prior to planting in 2017 and were not certified as disease-free. We assumed disease accumulation within the seed tubers of both experimental lines and checks to be equal. Visual inspection did not reveal any overt disease presence in seed tubers.

2.2 – Field Design, Soil Testing and Planting: Our experiment was conducted at the University of Minnesota Sand Plains Research Farm in Becker, MN (45.390561, -93.890786), on flat, excessively drained, Hubbard-Mosford complex, loamy sand soil (Web Soil Survey, USDA-NRCS) with ~1.7% OM, ~5.0 pH, prior to planting in 2017. Average soil nutrient levels in field at planting in 2017 were as follows: 3.85 mg N kg^{-1} soil in the top 15cm of soil, 2.13 mg N kg^{-1} soil between 15cm and 61cm deapth, 84.8 mg P kg^{-1} soil P, 111.7 mg K kg^{-1} soil, and 282.3 mg Ca kg^{-1} soil.

Our experiment followed a split-block design with whole-blocks containing two N treatments and sub-blocks containing all experimental lines and checks, randomized by plot. Plots were two rows, 6.1m long with 91cm row spacing, 30cm plant spacing within rows. Plots abutted each other lengthwise and 2.4m wide alleys separated tiers of plots to reduce plot mixing during harvest. Tubers from a cultivar of distinct white skin color (Cascade) were used to mark the end of plots. Border plots of Red Norland from certified seed were planted along the upper and lower boundary of each block. Due to year-to-year differences in available field space, there were three blocks in year one and four in year two.

Diammonium phosphate and potash were applied prior to planting at the following rates: 224 kg ha^{-1} of 0-0-60 potash; 224 kg ha^{-1} of 0-0-22 K-Mag; 336 kg ha^{-1} 18-46-0 diammonium phosphate. Plots were amended with side-dressed urea at hilling (approximately 20-24 days after planting) to establish two rates of total applied N: 101 kg N ha^{-1} (low-N) and 202 kg N ha^{-1} (high-N). Pesticides and herbicides were applied according to maximum label recommendations and cycled to avoid resistance. These included: AgrimeckSC, BayThroid, Belay, Bravo, Carbaryl4L, Champ Formula 2, Corgen, Curzate, CurzateDF, Dimethoate, Endura, Linex, Luna, Permethrin, Previcur Flex, Priocar, Prowl H2O, Quadris, Radiant, Radiant, Rimon, Roper, and SencorDF. Weekly irrigation was scheduled by checkbook method, for a total of 18cm between June 5th and Aug 22nd in 2017 and a total of 21.7cm between May 21st and August 14th in 2018.

Six individual soil samples were taken for each sub-block and separated into two depths before bulking, 0 to 15cm and 15 to 61cm. Soil samples were taken in a zig-zag pattern across each treatment sub-block just prior to planting and before sidedressed N had been applied, then again just prior to harvest and before soil disturbance. Soil samples were tested for nitrate concentration at the University of Minnesota Research Analytical Lab (ral.cfans.umn.edu[1]) to study effect and efficacy of urea side-dressing for changing soil N concentrations.

2.3 – Statistical Analysis: The experiment was treated as a mixed restricted maximum likelihood (REML) model (Harville 1977) with treatment, variety, and their interaction as fixed, block (a.k.a. repetition) and year as random:

$$y_{ijkl} = \mu + T_i + V_j + TV_{ij} + Y_k + B_l + \varepsilon_{ijkl}$$

Where: T = N treatment effect, V = variety effect, Y = year effect, and B = block/rep effect. Data were analyzed in R-Studio v1.1.463 with R v3.5.2 ("Eggshell Igloo"). LME4 (Bates et al. 2015) and Tidyverse v1.2.1 (Wickham 2017) were the primary packages used to analyze, manipulate and visualize data. LME4 model: response ~ treatment * variety + (1|block) + (1|year). Best Linear Unbiased Estimates (BLUEs) were extracted from the model and used for all calculations and visualizations. Pearson correlation of BLUEs was calculated with cor() in base R and correlation p-values were calculated with cor.test(). Correlation p-values test against the null hypothesis that reported correlation is actually 0, therefore low correlations will have high p-values. Select correlations are featured in Table 3. Full Correlation tables can be found in appendix B. REML models do not produce p-values for random effects and cannot inform us about random effect significance. As such, we have included a scatterplot of yields by year with correlation, separated by treatment (Appendix figure B.7).

2.4 – 45 DAP Plant and Root "Shovelomics" Sampling: Plants were excavated at ~45 DAP, approximately during tuber set, prior to tuber bulking (Johnson, ed. 2008). A single individual plant per plot was selected randomly, excluding row-end plants. Plants washed by hand and dissected into root, shoot, and tuber components in the laboratory and then dried at 49°C for three to seven days. Additional plant phenotypic metrics were measured to assure plants had been sampled at a similar point in their lifecycle across plots and years (Table 2).

Sharifi et al. (2007) reported evidence that total root weight (TRW) correlates with root length (r=0.82 and r=0.96) and root surface area (r=0.87 and r=0.95) in low and high N,

respectively. For this reason, as well as the relative ease of taking TRW data, TRW was chosen as the primary root measurement for this experiment. The root attached to the plant when removed from the soil was called plant root or, once dried, total plant root dry weight (TRW). In 2017, the soil in a 30cm x 60cm x 40cm (L x W x D) area around the plant after excavation was removed and sorted to acquire all remaining potato roots (identified as "soil root" in the dataset). In year two the soil remaining in the plot after excavation was not examined for detached roots because statistical results from year one indicated that their effects were negligible. Instead roots attached to the plant were separated into "stolon root" and "basal root" categories by hand as defined in Wishart et al. 2013. Whole leaf (petiole and leaflets) samples were also taken at 40 DAP and 80 DAP to test for total nitrogen (ral.cfans.umn.edu[2]).

2.5 – Harvest and Postharvest: Vine growth was terminated by herbicide, Reglone and LI 700 adjuvant, at 90 DAP and plots were harvested mechanically two weeks after vine termination. Tubers were sorted by hand in year one and mechanically in year two into USDA small/medium/large sizes, which are diameters of <6.35cm, 6.35cm to 8.26cm, and >8.26cm, respectively (USDA 2011). Hollow heart/brown center was evaluated in a subsample of 12 USDA medium tubers from each plot.

Dry matter percent for tuber and vine samples at harvest were calculated from the weight of samples before and after drying at 49°C for three to seven days. Year one tuber dry matter data were lost. To account for this, tuber dry matter percent from year two was averaged per treatment by variety and those averages were used for year one tuber dry matter values and the calculation of BLUEs from the total dataset. Total dry matter

measurements were derived via multiplication of wet weights by dry matter decimal percentages and plant number per plot. Nitrogen % testing was performed via the combustion method at the University of Minnesota Research Analytical Lab (ral.cfans.umn.edu[2]). Mass of plant tissue N was derived by multiplication of vine N % and tuber N % by their dry weight counterpart.

2.6 – Imaging: 12 USDA medium potatoes were selected at random from each plot and arranged in a 3x4 grid in a Photosimile 200 with a Canon Rebel T6i camera using a 24mm lens, ISO 100, 1/30 sec shutter speed and aperture f/5.6. As the analysis of the image was not dependent on high color fidelity, pictures were saved in '.jpg' (lossy compression) to reduce file size and increase processing speed. An in-house custom R script utilizing EBImage (Pau et al. 2010) image analysis was used to analyze photographs and acquire skinning and skin color data based in the CIE L*a*b color space. Future and future.apply (Bengtsson, 2018) was also used to increase analysis speed by enabling multi-core processing.

3 – Results and Discussion:

3.1 – Experimental Design: Side-dressed urea was effective at creating treatment groups with different soil and plant tissue N concentrations. Pre-planting N distribution throughout the field was uniform, with sample depth the only significant effect. Nitrate was found at higher concentration in the shallow sample (0 to 15cm) than in the deep sample (15 to 61cm) (p=<0.001). Harvest soil samples, taken prior to any soil disturbance, showed significant N concentration differences which mirrored treatment sub-blocks (p=0.009),

but now with no significance of depth, indicating nitrate had already mobilized throughout the soil to a depth of 61cm (Figure 1). Year one petiole N concentrations responded significantly to treatment throughout the growing season at 40 DAP (p=0.008) and 80 DAP (p=<0.001).

3.2 – Yield: Yield was significantly affected by both Treatment (p= <0.001) and Variety (p= <0.001) (Table 3, Figure 2). There is no apparent correlation between low-N and high-N yield (r= 0.47, p=0.09). While yield was reduced for all varieties in low-N compared to high-N, the magnitude of that loss was highly variable. The interaction between N treatments and varieties was not significant (p=0.64) so we were unable to definitively identify varieties that were less affected by low N rates than others. However, some varieties maintained >95% of their yield in the low-N treatment group compared to high-N, while others lost nearly 40% of their yield (Figure 3).

MN1 was included in this trial because its female parent, MN96072-4, was identified as potentially N efficient (Rosen et al. 2007), where the measure of NUE was a maintenance of high yield levels in soils with lower-than-recommended N availability. This variety did maintain high yields in low-N in this experiment but did not stand out from other selected germplasm from the program, with low overall yield regardless of treatment.

As elite breeding clones, germplasm in this experiment had been selected for high yield in fields with the high recommended level of N for multiple generations. However, there was no correlation between high-N and low-N yield in this experiment, meaning that selection for high yield in high-N conditions does not result in proportionally high yield in low-N

conditions. It is conceivable from these results that low-N efficiency is neither selected for or against in the process of breeding elite cultivars, and current breeding populations may have a rich pool of low-N efficient varieties. It must also be conceded that current breeding populations may have an equally rich pool of low-N inefficient varieties, so cultivar testing should include a Low N trial to identify candidate varieties that could have poor yields in Low N conditions.

Low-N favored USDA small (<6.35cm diameter) tubers (Figure 4), though total yield at low-N did not correlate with USDA small tuber yield alone (r= 0.46, p=0.1) due to differences across varieties. USDA medium tubers (6.35cm to 8.26cm diameter) were favored in high-N conditions and seemed to be the primary driver of total yield in that treatment based on correlation (r=0.73, p=<0.01). Low-N USDA small tuber yield correlated with high-N USDA medium tuber yield (r=0.77, p=<0.01), and low-N USDA medium tuber yield correlated with high-N USDA large yield (r=0.71, p=<0.01) suggesting that increasing N resulted in increased yield through larger tubers and an extension of the tuber bulking phase. This suggests that the yield benefits from higher N rates are largely realized by increased bulking, not increased number of tubers. This is consistent with our observation that N rate is unrelated to 45 DAP tuber count. Prior research (Hewitt 1963) supports this observation of more and smaller tubers in low-N. Conversations with Minnesota farmers indicate that USDA smalls are the size preferred by consumers for fresh market reds. As shown in Figure 4, some varieties could produce more highly valued USDA smalls, by weight and result in a higher value yield in low-N.

3.3 – Efficiency: NUE and its constituent components, NUtE and NUpE, were all higher in low-N conditions for every variety (Figure 5) and significantly affected by both treatment and variety (Table 3). There was variability in amplitude of response to N stress, as is seen in many crops (Novoa and Loomis 1981) and potato in particular (Hewitt 1963). NUE was highly correlated with yield at both high-N (r=0.82, p=<0.01) and low-N (r=0.91, p=<0.01) conditions (Figure 6). NUE did not correlate with yield when treatment levels were taken overall (r=-0.18) because the relation of yield with N applied was non-linear. When N applied is reduced by half, yield was typically not reduced to the same degree and NUE was higher in low-N for all varieties. This resulted in two discrete linear groups when plotted (Figure 7).

There was a high correlation (r=0.94, p=<0.01) of NUE and NUtE in low-N, indicating efficient production of dry matter was a primary component of NUE in low-N. NUtE also had a clear and powerful linear relationship with yield in low-N (r= 0.80, p=0.02), but no correlation with yield in high-N (r= -0.28) (Figure 6). However, it is important to note that while prior research in corn (*Zea mays*) and wheat (*Triticum aestivum*) (Moll et al. 1982; Van Sanford and MacKown 1986) found this correlation as well, later research in wheat which used Moll's (1982) same methodology (Ortiz-Monasterio et al. 1997) found no correlation. So, it cannot be assumed that NUtE is always the low-N NUE driver in all conditions.

In relation to fertilizer applied, all varieties had <80% N uptake in the high-N group and >80% uptake in the low-N group (Figure 5 and 5) and all varieties showed increased uptake when N was limited. Despite decreased NUpE in high-N compared to low-N, total plant

N (i.e. absolute value of N uptake) was still higher in high-N (Figure 8). NUpE was the primary corollary of yield (r=0.82, p=<0.01) and NUE (r=0.86, p=<0.01) in high-N conditions, but not in low-N conditions.

To summarize: NUtE was the primary driver of NUE and yield in low-N, but it would seem that, given a high enough level of plant N, whatever mechanisms are responsible for NUtE are not as active within the plant and NUpE becomes the primary driver of NUE and yield in high-N.

Because NUtE (p=-0.22) and NUpE (p=-0.07) do not correlate with each other between low-N and high-N, there is potential that breeding varieties with favorable NUtE in low-N environments may not translate to high NUtE at other fertilizer rates. Similarly, for NUpE, it would seem varieties with high NUpE at one N level may not have that same superior uptake in different N conditions.

3.4 – Tuber Quality: All measures of skin quality had increased variation in low-N vs. high-N (Figure 10 and Figure 11). Lightness (L*) was significantly affected by both variety (p= <0.001) and treatment (p= <0.001), with treatment at low-N increasing tuber lightness (Figure 11). Though Redness (a*) decreased for some varieties in low-N, treatment was not significant overall (p= 0.52), only variety (p= <0.001). Skinning, likewise, was only significantly affected by variety (p= <0.001). In low-N, skinning was negatively correlated with NUE (r=-0.66, p=<0.01), NUtE (r=-0.72, p=<0.01), and 45 DAP total root weight (TRW) (r=-0.79, p=<0.01), indicating that plants with larger early root systems and greater production of tuber dry weight at harvest retained more skin. This

is likely a function of plant and tuber maturity, which is known to have an effect on skinning (De Jong et al. 2011, p. 135), with more mature tubers having less skin loss.

Given the higher variation of skin color and skinning metrics in low-N, it is possible that lower N conditions are appropriate for observing expression of greater variation in breeding populations for selection purposes. This is further supported by Figure 12, which shows that low-N skin quality traits correlated between year one and year two, while in high-N there was no year to year correlation. The increase in lightness for tubers at low-N is a concern for producers interested in growing with lower fertility but could explain seasonal variations often observed in potato tuber color in conventionally fertilized fields.

Brown center and hollow heart were so infrequent as to be statistically untestable, with fewer than ten instances of either across all 196 plots. Brown center and hollow heart are a continuum of one syndrome and considered functions of tuber size, tuber growth rate and N fertility (Johnson 2008, p. 239). However, the limited instances of either were not concentrated in either low-N or high-N.

3.5 – Root Traits: N treatment and potato variety significantly affected dry weight for both roots (TRW) and vines (VDW) at 45 days after planting (TRW: treatment p=<0.001, variety p=<0.001; VDW: treatment p=0.022, variety p=<0.01). TRW correlated with yield overall (Table 3). This supports the observations of Wishart et al. 2013 but contradicts Sharifi et al. 2005 and Sattelmacher 1990a. TRW was often lower in low-N (Figure 13), which was supported by prior research in the field (Geary et al. 2015; Sattelmacher et al. 1990b). TRW did not correlate with many metrics of increased N apart from 90 DAP

Tuber N (g) (Table 4). That correlation was consistent across both N levels and the experiment overall.

TRW weight corelated with NUtE only in low-N (r=0.91, p=<0.01), not in high-N (r=-0.40). Conversely, TRW correlated with NUpE only in high-N (r=0.80, p=<0.01), and not in low-N (r=0.34). Since TRW correlates with yield and NUE, this is expected but still significant. TRW correlated with 45 DAP tuber weight (r=0.68, p=*<0.01*) and 45 DAP Tuber Median Size (r=0.64, p=0.01) at low-N, just as NUtE does. However, TRW correlated with no early tuber traits in high-N but instead correlated with 90 DAP Vine Dry Weight (r=0.75, p=<0.01), as did NUpE, NUE and yield. Interestingly, 45 DAP tuber median size (TMS) correlated negatively with TRW (r=-0.68, p=0.01) and total yield (r=-0.72, p=<0.01) in high-N. Stolon root weight did positively correlate with several variables, but we believe that is due simply to stolon root weight representing the bulk of the TRW measurement and highly correlating with total root across all N levels and in the experiment (Table 3).

When evaluating plants at 45 DAP grown in both treatments, rather than seeing growth patterns associated with NUE, we were likely looking at plants in physiologically distinct lifecycle stages. We saw a pattern of total yield and its low-N drivers (NUE, NUtE, TRW) correlating with 45 DAP tuber wt. and tuber median size in low-N, but not 90 DAP vine traits. The opposite was true for high-N, where total yield and its high-N drivers (NUE, NUpE, TRW) correlated instead with 90 DAP vine weights and not with the 45 DAP tuber measurement. All of this leads us to conclude that varieties respond to N stress by altering their lifecycle, specifically the tuber initiation and/or tuber bulking stages and that much of

the different NUE by variety behavior we measured in this experiment is due to lifecycle stress responses. Additionally, there is ample work showing that increasing N can delay tuber initiation and bulking lifecycle stages (De Jong et al. 2011; Ivins and Brenner 1965; Johnson ed. 2008; Kleinkopf et al. 1981; Moorby and Milthorpe 1975) while also favoring greater vine growth (Sommerfeld and Knutson 1968). Lack of sufficient N in most plants can trigger senescence and translocation of N from top growth to sinks (Leopold 1968; Novoa and Loomis 1981).

In low-N, yield at harvest correlated with tuber fresh weight and median tuber size at 45 DAP, indicating that 45 DAP was already well into tuber set and perhaps even entering bulk phase. Moorby (1978) pointed out that, "Once the tubers are initiated the growth of all the other organs is retarded and the tubers become the dominant meristems and sinks for organic and inorganic nutrients." With reduced soil fertility, vine growth stalls and plants enter end of life phases of senescence, accounting for the lack of 90 DAP Vine correlation with yield and NUE in low-N. In the last growth phase, tubers cease to bulk, begin maturation and skin set. Skinning severity was negatively correlated with NUE, NUtE and TRW in low-N, indicating that larger 45 DAP plants with high tuber dry matter production were also more physiologically mature by harvest. In high-N, we believe that tuber set was delayed, and the tubers continued to bulk until vine termination.

The correlation of low-N USDA small tubers to high-N USDA mediums and low-N USDA mediums to high-N USDA larges support this theory of longer, more effective tuber bulking being a primary driver of yield in high-N. Ivins and Brenner 1965, wrote that, "Both the rate of tuber growth and the time of foliage senescence are related to the amount

of leaf growth made by the time of tuber initiation…" Within this life cycle difference framework, the correlation of 45 DAP roots and vines to yield and efficiencies can be explained as simply a function of increased overall plant size leading to a greater number of tubers.

4 – Conclusion

Within our subsample of 12 advanced red-skinned clones, we still found significant variations in yield, NUE, NUtE, NUpE and TRW. Based on this, it would be possible to identify and select for NUE amongst breeding program advanced germplasm. We did not observe any appreciable relationship between larger root systems and increased Uptake, as Sattelmacher (1990b) posited. It is more likely that the increased root fraction observed in that paper was related to the well-documented ability for increased N to increase vine fraction. In this way, Sattelmacher may have been observing stunted vines (Novoa and Loomis 1981), rather than prolific root growth, in response to N-stress. Regardless, when observed in field conditions, rather than hydroponics, there did not seem to be any specific root relationship to N level. Rather, larger roots were correlated with more yield at all N levels.

In high-N, NUtE levels were flat, and greater NUpE correlated with greater yield and NUE. Because no variety had an NUpE of >80% in high-N, we can assume N-stress was not a factor in plant lifecycle for high-N treatment plants. Based on this, it seems that potato do not become efficient utilizers when there is no shortage of N.

NUtE was a strong corollary with NUE and yield in low-N, and many varieties had NUpE values >90%. It appears that NUtE becomes a deciding factor in yield only once a deficit in available soil N is detected. Regardless, NUE correlated across treatment levels, indicating that there is no apparent tradeoff between breeding for high NUtE or high NUpE — which may best be accomplished by selecting for yield in N variable environments.

Low-N environments expose variation in skin quality phenotypes such as lightness, redness and skinning resistance that were more consistent across years. Selection for tuber skin quality of red-skinned, fresh market potato could benefit from observation in an N-reduced environment.

The fact remains that while greater total yields were found in the high-N system, it is a system which fails to absorb or use much of the N applied. The greatest uptake in high-N was 78% of applied N, but the worst was 58%. It should be noted that the actual source of that nitrogen could have been from residual soil organic matter. In low-N, however, the majority of those same varieties absorbed greater than 90% of applied N, and all varieties absorbed 80% or more.

N rate also had an effect on tuber size distribution, with low-N typically increasing the proportion of USDA smalls and high-N typically increasing the proportional yield of USDA mediums. This size distribution effect was somewhat independent of total yield or NUE however. This is exemplified in the size distribution of our two most efficient varieties in low-N, Red LaSoda and MN9 (Figure 4). This has implications for producers as well as breeders, as size distribution is one of the criteria upon which varieties are

selected. Inconsistencies in soil available N between environments could affect size distribution of some selections more than others, perhaps due to variability in tuber initiation time.

MN 9 (clone name MN13025PLWR-08R) was among the highest NUE varieties, with a .95 low-N to high-N yield ratio. In low-N, MN 9 absorbed an amount of N from the environment equal to 100% of the applied N. Lastly, MN 9 also produced significantly more USDA smalls in low-N, a desirable size for fresh market red potatoes. This clone was found to be in the University of Minnesota Twin Cites germplasm without any kind of selection in lower than recommended N level. From this research, we believe that there are potentially more clones in our, and other, breeding populations which could be valuable sources of NUE characteristics for crosses or direct release as-is. More work is needed that extends beyond a two-year span, incorporates more environments, and identifies more specific varieties. However, as regulatory hurdles around inputs increase and incentives for environmental stewardship become more common (mda.state.mn.us), we expect that reliably efficient varieties will be of increasing interest to growers.

Tables

Table 1: Germplasm used in this experiment. Clones were chosen in order to capture the most diverse pedigree possible from the advanced selections in the UMN fresh market, red-skinned potato breeding population.

Variety Code	Clone Name	Parents (F/M)			Generation prior to 2017
MN1	MN10020PLWR-08R	MN 96072-4	/	Colorado Rose	G7
MN2	Runestone Gold	MN Family #149	/	OP	Unknown
MN3	MN10008PLWR-06R	ND6002	/	Dakota Rose	G7
MN4	MN13032PLWR-08R	ND8555-5R	/	MN96013-1R	G4
MN5	MN13005WW-01R	CO99076-6R	/	COMN03021-1	G4
MN6	MN12004WW-01R	CO99076-6R	/	MN03505-3R	G5
MN7	MN13001PLWR-03R	ATMN03505-3	/	Dakota Rose	G4
MN8	MN10003PLWR-06R	CO98012-5R	/	Colorado Rose	G7
1MN9	MN13025PLWR-08R	MN96013-1	/	Dark Red Norland	G4
MN10	MN12006WW-01R	Dakota Rose	/	CO99076-6R	G5
MN11	MN13097PLWR-02R	ND4659	/	MN08122BW-1R	G4
MN12	MN12057PLWR-04R	ND8555-8R	/	Dakota Rose	G5
R. LaSoda	Red LaSoda	Triumph	/	Katahdin	G1
Dakota Rose	Dakota Rose	ND1196-2R	/	NorDonna	G1

Table 2: Phenotype names and descriptions. Variable key for Table 3 and supplemental correlation tables.

90 DAP/Harvest Phenotype	Description
Total Yield	(yield) Total fresh weight of harvested tubers per plot, reported as kilogram per hectare.
Tuber Dry %	Dry matter percent of typical tuber as found by dividing dry weight of 12 tuber subset by wet weight of same.
Tuber Dry Weight	Total dry weight of 12 tuber subset.
Skinning Severity	(Skinning) Data derived by digital photo analysis in R on a subset of 12 tubers from harvest. Percent of skinned area per tuber post-harvest. Derived by averaging skinned area over total area of 12 tubers from images.
Lightness (CIE L*)	(Lightness or L*) L* measurement from the CIELAB color space which represents a numeric measurement of lightness of color in as close an approximation to human vision as possible.
Redness (CIE a*)	(Redness or a*) a* measurement from the CIELAB color space which represents a numeric measurement of green-red values in as close an approximation to human vision as possible. Negative numbers are more green, positive more red.
Vine Wet Weight	Weight of the above ground material from 10 plants per plot just prior to vine termination.

Vine Dry %	Dry matter percent of typical above ground potato vine as found by dividing dry weight of 10 plant subset by wet weight of same.
Vine Dry Weight	Total dry weight of 10 plant's total above ground vine tissue.
Vine N %	% by weight of Nitrogen from a subsample of ground and bulked 10 plant dry vine subsample. As found by the combustion method.
Total Vine N	Total N present in above ground potato vine. Calculated by extrapolating total vine dry weight from 10 plant subsample and multiplying by Vine N %.
Tuber N %	% by weight of Nitrogen from a subsample of ground and bulked 12 tuber dry subsample. As found by the combustion method.
Total Tuber N	Total N present in tubers. Calculated by extrapolating total tuber dry weight from 12 tuber subsample and multiplying by Tuber N %.
Average Plant N %	Averaged of Tuber N % and Vine N %.
Total Plant N	(TPN) Sum of Total Tuber N and Total Vine N
40 DAP Petiole N %	% by weight of Nitrogen in a petiole and leaf tissue sample taken from the 4th node down from the terminal node at 40 days after planting. As found by the combustion method.
80 DAP Petiole N %	% by weight of Nitrogen in a petiole and leaf tissue sample taken from the 4th node down from the terminal node at 80 days after planting. As found by the combustion method.

45 DAP/Shovelomics Phenotype	Description
Total Plant Root Dry Weight	(TRW) Dry weight of all root tissue attached to an individual plant after excavation from the field.
Plant Root Fraction	Fraction of all root dry weight over total plant weight from shovelomics excavation of single plants per plot.
Soil Root Weight	Dry weight of all root present in a 1'x2'x16" block of soil around plant after plant removal. Extracted and sorted by hand. Only taken in year one due to time-intensive sampling.
Soil Root Fraction	Fraction of soil root dry weight over total plant weight from shovelomics excavation of single plants per plot in year 1.
Basal Root Weight	Dry weight of all root originating from the basal bulb – i.e. the first meristematic node of growth closest/attached to the mother tuber, as defined in Wishart, 2013, Fig. 1g Only taken in year two due to time-intensive sampling.
Basal Root Fraction	Fraction of basal root dry weight over total plant weight from shovelomics excavation of single plants per plot in year 2.
Stolon Root Weight	Dry weight of all root originating from stolon and stolon-stem junctions, as defined in Wishart, 2013, Fig. 1g. Only taken in year two due to time-intensive sampling.
Stolon Root Fraction	Fraction of soil root dry weight over total plant weight from shovelomics excavation of single plants per plot in year 2.

Stolon Weight	Dry weight of all stolon tissue attached to an individual plant after excavation from the field.
Stolon Length	Mean of subset 5 longest stolon found on an individual plant.
Tuber Dry Weight	Dry weight of all tuber tissue attached to an individual plant after excavation from the field.
Tuber Ct.	Total number of tubers >.5cm in diameter attached to an individual plant after excavation from the field.
Tuber Median Size	The median size of all tubers >.5cm in diameter attached to an individual plant after excavation from the field.
Vine Dry Weight	Dry weight of all vine (I.E. above ground) tissue attached to an individual plant after excavation from the field.
Stem Length	Length of longest stem of and individual plant

Table 3 (Opposite page): Significance and Selected Correlations. All variables, separated by 45 days after planting (DAP) and 90 DAP are listed alone with their mixed model *p*-value for Treatment, Variety and the interaction of the two. These correlations are reported for the both N level treatments combined, low-N treatment alone and high-N treatment alone.

Key:

Fraction = The named value divided by the total plant weight including that value

§ Year 1 Dry Matter based off mean of year two by variety and treatment, due to data loss;

† Year 1 only

‡ Year 2 only

		Mixed model (*p*)			Correlation (*r*) BLUEs														
					Combined Treatments					Low-N					High-N				
	Variable	Treat.	Var.	TxV	Yield	NUE	NUtE	NUpE	TRW	Yield	NUE	NUtE	NUpE	TRW	Yield	NUE	NUtE	NUpE	TRW
90 DAP / Harvest	Total Yield (kg)	<0.01	<0.01	NS	1	-0.18	-0.09	-0.33	**0.72**	1	**0.91**	**0.80**	0.56	**0.78**	1	**0.82**	-0.28	**0.83**	**0.83**
	USDA Small	0.003	<0.01	NS	-0.14	**0.78**	**0.73**	**0.66**	0.17	0.46	**0.63**	0.57	0.34	0.50	0.10	0.31	-0.23	0.36	0.30
	USDA Medium	<0.01	<0.01	0.04	**0.71**	**-0.63**	-0.54	**-0.65**	0.37	0.36	0.11	0.09	0.13	0.14	**0.73**	0.57	0.16	0.39	**0.69**
	USDA Large	0.046	0.006	NS	0.55	**-0.64**	-0.57	**-0.62**	0.12	0.28	0.04	0.03	0.05	0.05	0.07	-0.04	-0.39	0.19	-0.16
	Tuber Dry % §	NS	<0.01	<0.01	-0.42	**0.62**	**0.63**	0.49	0.05	-0.05	0.37	0.44	-0.05	0.45	-0.33	0.25	0.27	0.07	-0.20
	Tuber Dry Wt. (kg) §	<0.01	<0.01	NS	**0.89**	0.12	0.23	-0.13	**0.81**	**0.91**	1	**0.94**	0.49	**0.91**	**0.82**	1	-0.06	**0.86**	**0.73**
	Skinning Severity	NS	<0.01	NS	-0.29	-0.32	-0.46	-0.01	-0.50	-0.48	**-0.66**	**-0.72**	-0.05	**-0.79**	0.12	-0.02	-0.13	0.06	0.07
	Lightness (CIE L*)	<0.01	<0.01	NS	0.16	0.44	0.52	0.21	0.21	0.54	0.55	0.56	0.16	0.51	-0.24	**-0.59**	0.00	-0.48	-0.23
	Redness (CIE a*)	NS	<0.01	NS	0.27	-0.32	-0.37	-0.19	0.04	0.12	-0.06	-0.23	0.45	-0.16	-0.07	-0.26	0.02	-0.24	0.26
	Vine Wet Wt. (kg)	<0.01	0.004	NS	0.57	**-0.75**	**-0.77**	-0.56	0.32	-0.22	-0.48	**-0.65**	0.33	-0.39	**0.78**	**0.82**	-0.23	**0.79**	**0.80**
	Vine Dry %	<0.01	<0.01	NS	-0.06	0.28	0.41	0.01	-0.10	0.30	0.48	0.53	-0.02	0.37	**-0.60**	**-0.60**	0.21	**-0.62**	**-0.64**
	Vine Dry Wt. (g)	<0.01	0.003	NS	**0.60**	**-0.75**	**-0.76**	**-0.60**	0.32	-0.15	-0.42	**-0.61**	0.39	-0.37	**0.73**	**0.78**	-0.20	**0.74**	**0.75**
	Vine N %	<0.01	<0.01	NS	0.36	**-0.94**	**-0.87**	**-0.85**	-0.01	**-0.80**	**-0.82**	**-0.83**	-0.26	**-0.74**	-0.28	-0.36	-0.22	-0.17	-0.24
	Total Vine N (g)	<0.01	<0.01	NS	0.53	**-0.88**	**-0.84**	**-0.78**	0.17	-0.56	**-0.72**	**-0.86**	0.13	**-0.64**	0.54	0.53	-0.34	**0.61**	0.50
	Tuber N %	<0.01	<0.01	0.03	0.19	**-0.82**	**-0.90**	-0.54	-0.15	**-0.71**	**-0.81**	**-0.91**	-0.03	**-0.85**	0.31	0.12	**-0.78**	0.50	0.13
	Total Tuber N (g)	<0.01	<0.01	0.03	**0.86**	-0.41	-0.38	-0.42	**0.61**	**0.85**	**0.91**	**0.74**	**0.71**	**0.73**	**0.81**	**0.84**	-0.46	**0.94**	**0.65**
	Avg. Plant N %	<0.01	<0.01	NS	0.33	**-0.95**	**-0.92**	**-0.81**	-0.05	**-0.85**	**-0.90**	**-0.95**	-0.19	**-0.86**	0.00	-0.18	**-0.65**	0.20	-0.08
	Total Plant N (g)	<0.01	0.008	NS	**0.74**	**-0.71**	**-0.68**	**-0.65**	0.44	0.56	0.49	0.16	1	0.34	**0.83**	**0.86**	-0.56	1	**0.80**
	40 DAP Petiole N % †	<0.01	<0.01	NS	0.16	0.30	0.16	0.30	0.18	0.45	0.36	0.09	**0.63**	-0.09	0.27	0.32	0.11	0.10	0.56
	80 DAP Petiole N % †	<0.01	NS	NS	0.38	**-0.75**	**-0.61**	**-0.59**	-0.06	**-0.65**	-0.52	-0.49	-0.17	-0.24	0.03	0.07	-0.30	0.24	0.14
	NUE (Use)	<0.01	<0.01	NS	-0.18	1	**0.94**	**0.87**	0.19	**0.91**	1	**0.94**	0.49	**0.91**	**0.82**	1	-0.06	**0.86**	**0.73**
	NUtE (Utilization)	<0.01	<0.01	NS	-0.09	**0.94**	1	**0.65**	0.23	**0.80**	**0.94**	1	0.16	**0.91**	-0.28	-0.06	1	-0.56	-0.40
	NUpE (Uptake)	<0.01	0.002	NS	-0.33	**0.87**	**0.65**	1	0.05	0.56	0.49	0.16	1	0.34	**0.83**	**0.86**	-0.56	1	**0.80**
45 DAP / Shovelomics	Total Plant Root Wt. (g)	0.013	<0.01	NS	**0.72**	0.19	0.23	0.05	1	**0.78**	**0.91**	**0.91**	0.34	1	**0.83**	**0.73**	-0.40	**0.80**	1
	Plant Root Frac.	NS	<0.01	NS	0.36	-0.05	0.11	-0.26	0.50	0.30	0.43	**0.59**	-0.20	**0.66**	0.16	-0.04	-0.28	0.11	0.21
	Basal Root Wt. (g) ‡	NS	<0.01	NS	0.36	0.34	0.34	0.22	0.56	0.52	0.57	**0.59**	0.26	**0.62**	0.22	0.12	-0.33	0.34	0.56
	Basal Root Frac. ‡	NS	<0.01	NS	-0.11	0.11	0.20	-0.08	-0.02	0.08	0.13	0.25	-0.18	0.21	-0.49	-0.54	-0.11	-0.44	-0.26
	Stolon Root Wt. (g) ‡	NS	<0.01	NS	**0.68**	0.11	0.07	0.12	**0.92**	**0.74**	**0.76**	**0.72**	0.49	**0.88**	**0.63**	**0.60**	-0.24	**0.75**	**0.95**
	Stolon Root Frac. ‡	NS	<0.01	NS	0.23	-0.22	-0.12	-0.35	0.42	0.15	0.18	0.29	-0.15	0.41	-0.15	-0.06	-0.01	-0.04	0.34
	Soil Root Wt. (g) †	NS	NS	NS	0.01	0.51	0.55	0.28	0.11	0.53	0.50	0.42	0.24	0.30	0.27	0.37	0.51	-0.18	-0.09
	Soil Root Frac. †	0.024	0.035	NS	-0.16	0.40	0.41	0.26	-0.09	0.21	0.06	0.06	-0.02	-0.24	0.22	0.20	0.46	-0.24	0.01
	Stolon Wt. (g)	NS	<0.01	NS	0.34	0.31	0.34	0.18	0.37	0.54	0.55	0.49	0.34	0.39	0.26	0.34	0.12	0.21	0.46
	Stolon Length µ (cm)	NS	<0.01	NS	0.50	-0.09	0.00	-0.22	0.32	0.47	0.46	0.41	0.31	0.28	0.08	0.04	0.21	-0.07	0.27
	Tuber Dry Wt. (g)	NS	<0.01	NS	0.45	0.41	0.54	0.09	0.38	**0.81**	**0.91**	**0.85**	0.42	**0.79**	0.00	0.07	**0.60**	-0.26	-0.21
	Tuber Ct.	NS	<0.01	NS	0.21	0.41	0.31	0.43	0.31	0.47	0.46	0.28	0.57	0.28	0.44	0.55	-0.06	0.47	0.56
	Tuber Med. Size (cm)	NS	<0.01	NS	-0.09	0.38	0.52	0.09	0.05	0.37	0.57	**0.64**	0.04	**0.68**	**-0.72**	-0.53	0.30	**-0.60**	**-0.68**
	Vine Dry Wt. (g)	0.022	<0.01	NS	0.58	0.25	0.19	0.23	**0.77**	**0.72**	**0.78**	**0.62**	**0.63**	**0.69**	**0.73**	**0.73**	-0.26	**0.73**	**0.86**
	Stem Length (cm)	NS	<0.01	NS	**0.76**	-0.28	-0.17	-0.41	0.58	**0.60**	0.58	0.58	0.23	0.51	**0.65**	0.59	-0.13	0.54	**0.74**

Table 4: Select nitrogen uptake variable and total plant root dry weight correlations. A subset of correlations between nitrogen uptake related variables and plant root mass and fraction. Reported for the overall experiment as well as individual treatments.

	Correlation (*r*) BLUEs					
	45 DAP Total Plant Root Dry Weight (g)			**45 DAP Plant Root Fraction**		
Variable	**Overall**	**low-N**	**high-N**	**Overall**	**low-N**	**high-N**
45 DAP NUpE *	-0.01	0.23	0.24	-0.42	-0.27	-0.55
45 DAP Tuber N (g) *	0.51	0.59	0.37	0.42	0.45	0.29
45 DAP Vine N (g) *	0.28	0.22	0.23	-0.26	-0.29	-0.56
45 DAP Tuber N % *	-0.14	-0.37	-0.28	0.12	-0.06	0.02
45 DAP Vine N % *	0.15	-0.42	0.27	0.08	-0.49	0.15
90 DAP NUpE	0.05	0.34	**0.80**	-0.26	-0.20	0.11
90 DAP Tuber N (g)	**0.61**	**0.73**	**0.65**	0.27	0.17	0.08
90 DAP Vine N (g)	0.17	**-0.64**	0.50	0.20	-0.46	0.41
90 DAP Tuber N %	-0.15	**-0.85**	0.13	-0.04	**-0.61**	0.25
90 DAP Vine N %	-0.01	**-0.74**	-0.24	0.11	-0.48	0.14

* Year 1 data

Figures

Figure 1: Soil Nitrate Levels. Tested in year one only. Pre-planting N levels were uniform across the field with no effect of treatment and a significant effect of depth (p=<0.001). Harvest N levels, taken prior to any disturbance of the soil, were uniform across soil depth (p=0.47), but now with a significant effect of Treatment (p=0.009).

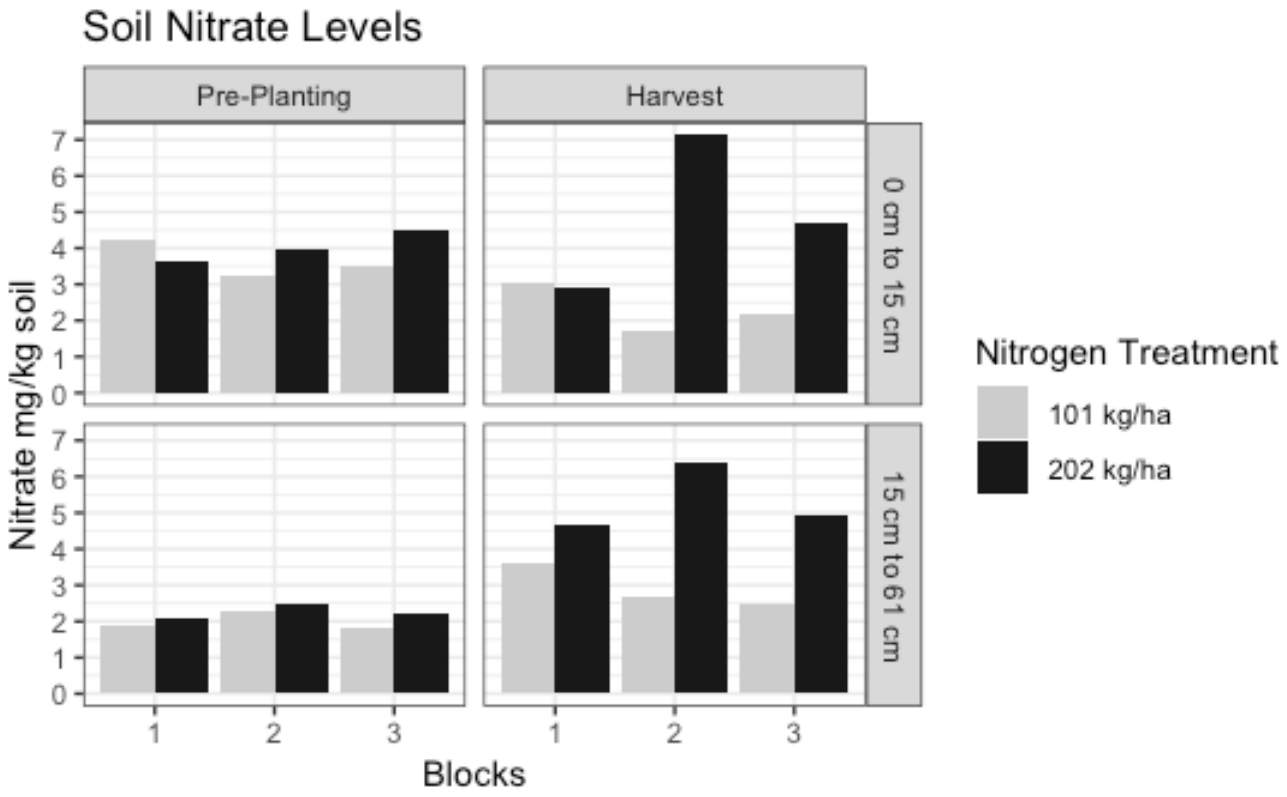


Figure 2: Yield Per Acre by Variety and Nitrogen Rate. The Mixed model derived (BLUE) yields for each variety in the experiment, separated by Nitrogen Rate.

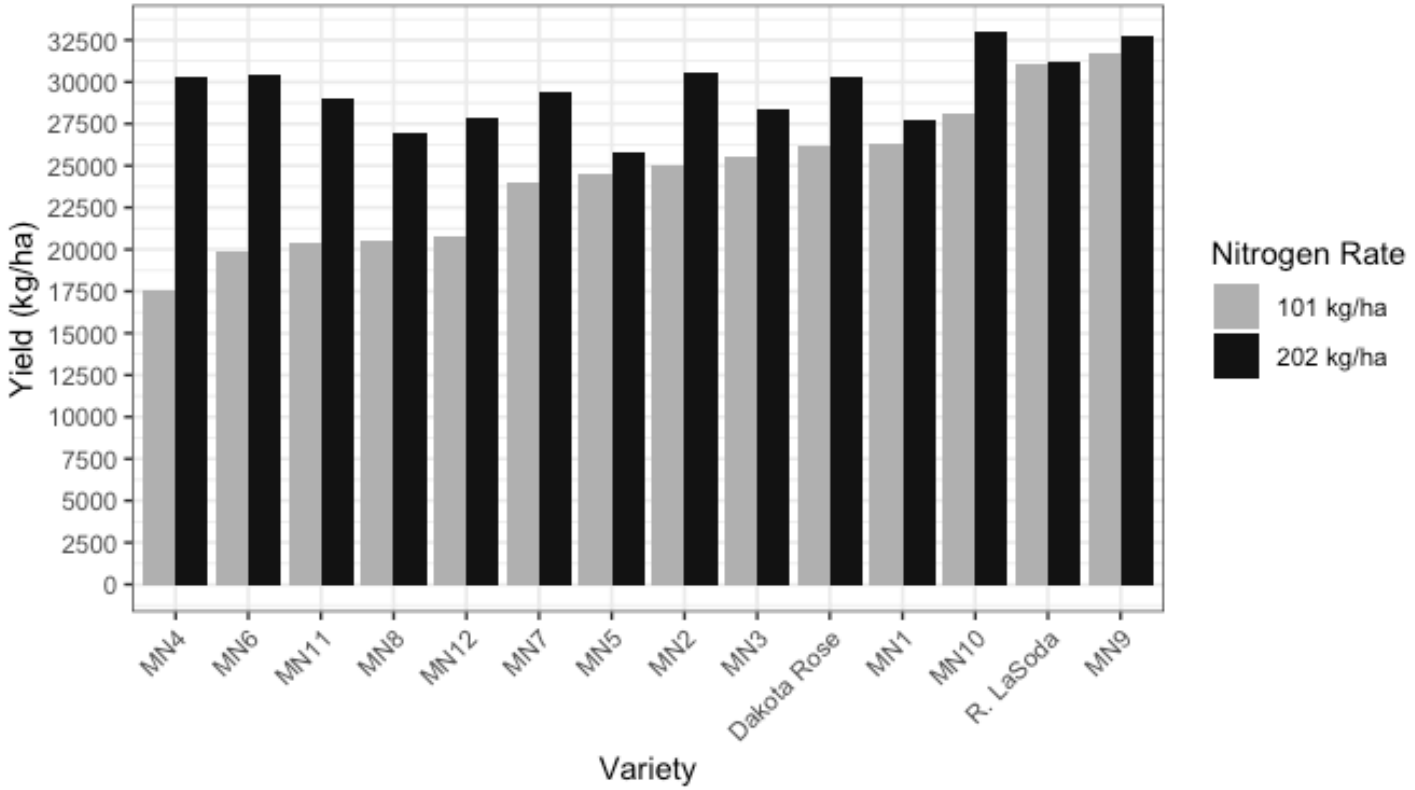


Figure 3: Percent of low-N (101 kg N ha^{-1}) yield to high-N (202 kg N ha^{-1}) yield. Uses the equation ((low-N yield / high-N yield) * 100). Some varieties lost >40% of their high-N yield when grown in low-N, while others lost < 5%.

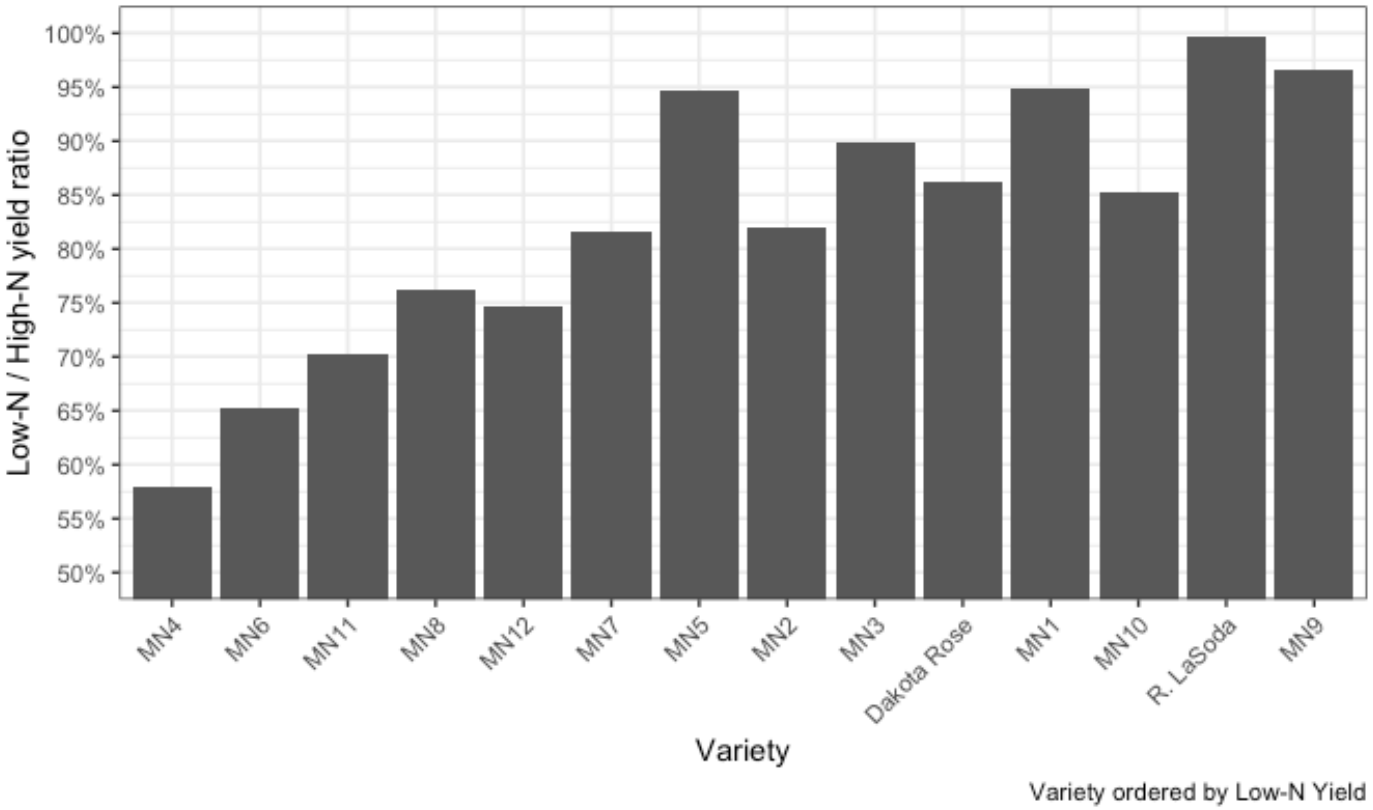


Figure 4: Market Class Yield Difference**.** Yield of each USDA size category is shaded to indicate its contribution to total yield per variety. Graph is separated by Nitrogen level. Low-N favored USDA smalls and high-N favored USDA Mediums in potatoes grown 90 days.

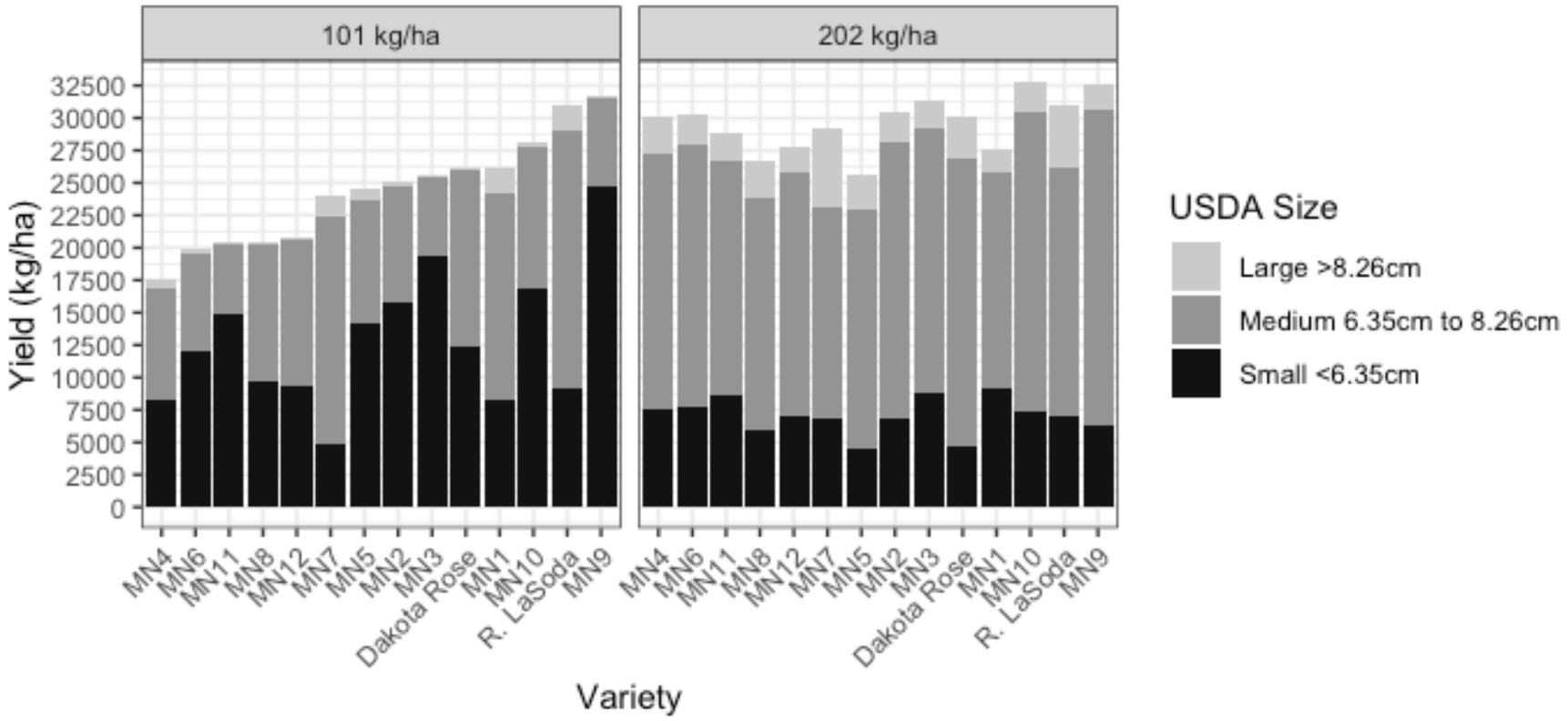


Figure 5: NUE, NUtE and NUpE% by Variety and Treatment. Efficiency values are inherently unitless but normally based on mass. Nitrogen Use Efficiency (NUE) is the units of tuber dry matter produced per single unit in nitrogen applied to the field. Nitrogen Utilization Efficiency (NUtE) is the units of tuber dry matter produced per single unit of nitrogen present in all plant tissues. Nitrogen Uptake Efficiency (NUpE) is the amount of nitrogen present in all plant tissues per single unit of nitrogen applied to the field – a measure of 1, or 100%, would mean all applied nitrogen was taken up by the plant.

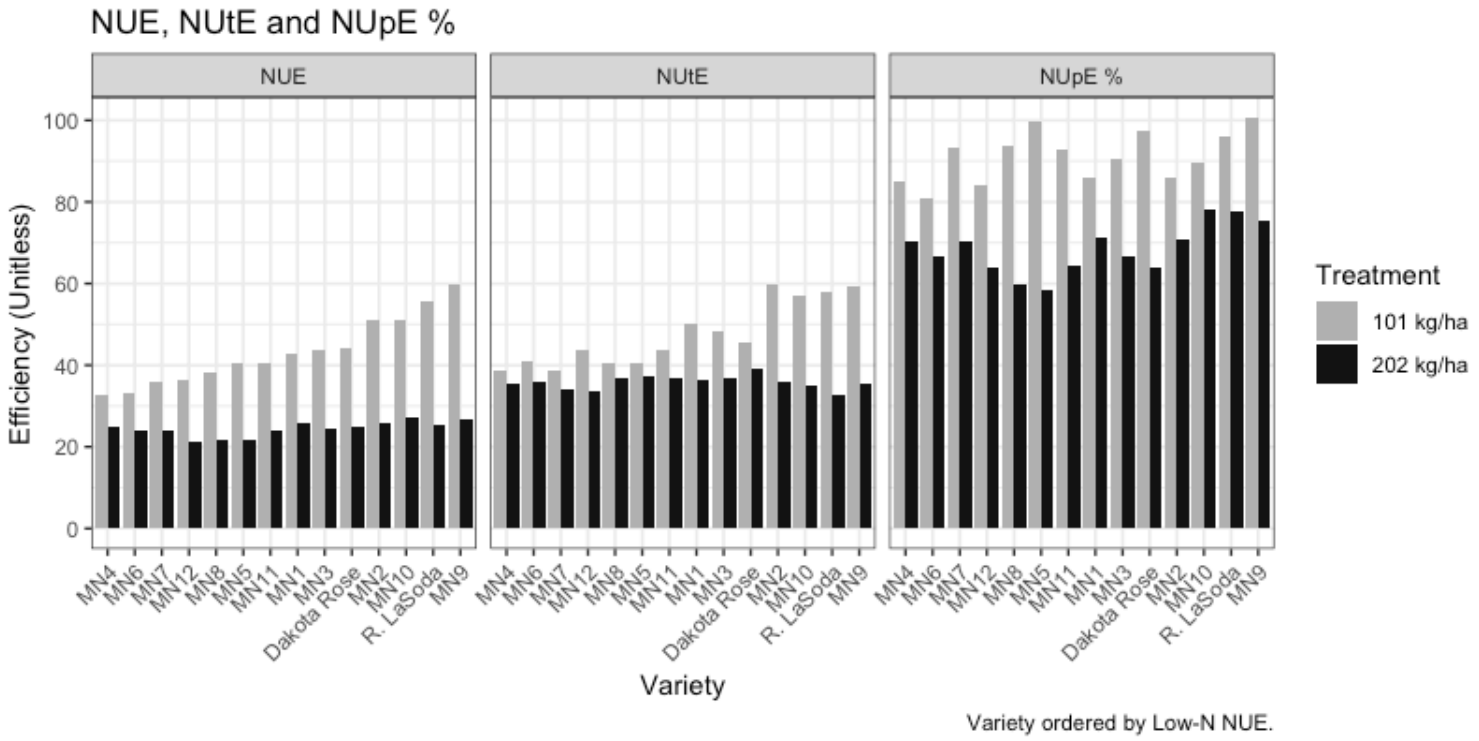


Figure 6: Correlation of Yield to NUE, NUtE and NUpE. In 101 kg N ha^{-1}, Nitrogen Use Efficiency (NUE) correlated with Nitrogen Utilization Efficiency (NUtE), r=0.94, but not Nitrogen Uptake Efficiency (NUpE). In 202 kg N ha^{-1}, NUE correlated with NUpE r=0.86, but not NUtE. This figure clearly shows the lack of NUtE differences across varieties in 202 kg N ha^{-1}, among other patterns.

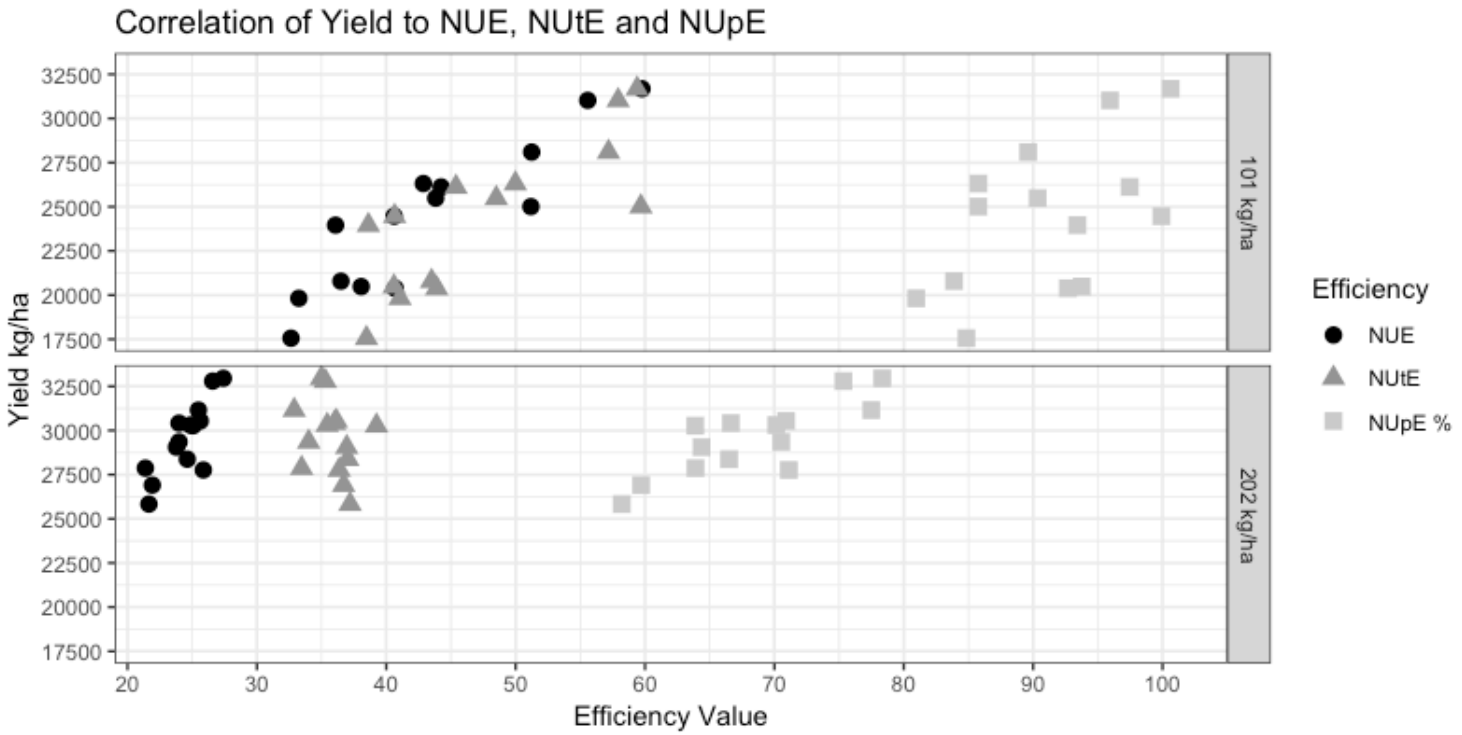


Figure 7: Nitrogen Use Efficiency correlation with Yield. While there was significant within-treatment correlations of NUE and Yield, there was no correlation overall due to the structure of the two linear groups, shown here.

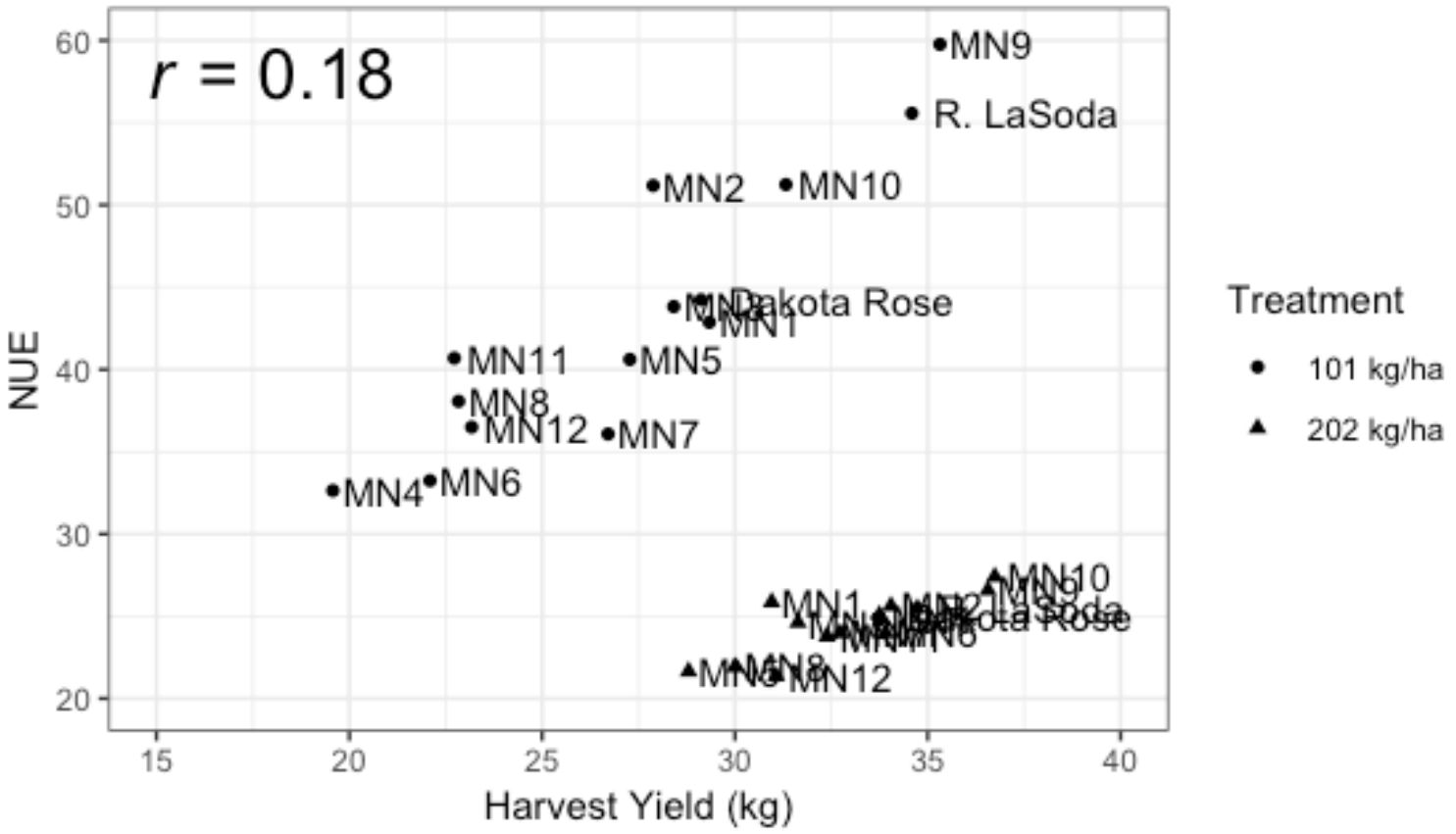


Figure 8: Total Plant Nitrogen Per Plot. High-N Nitrogen Uptake Efficiency was <80% for all varieties. Despite this inefficiency, the total grams of N absorbed per plot was still greater in high-N than low-N.

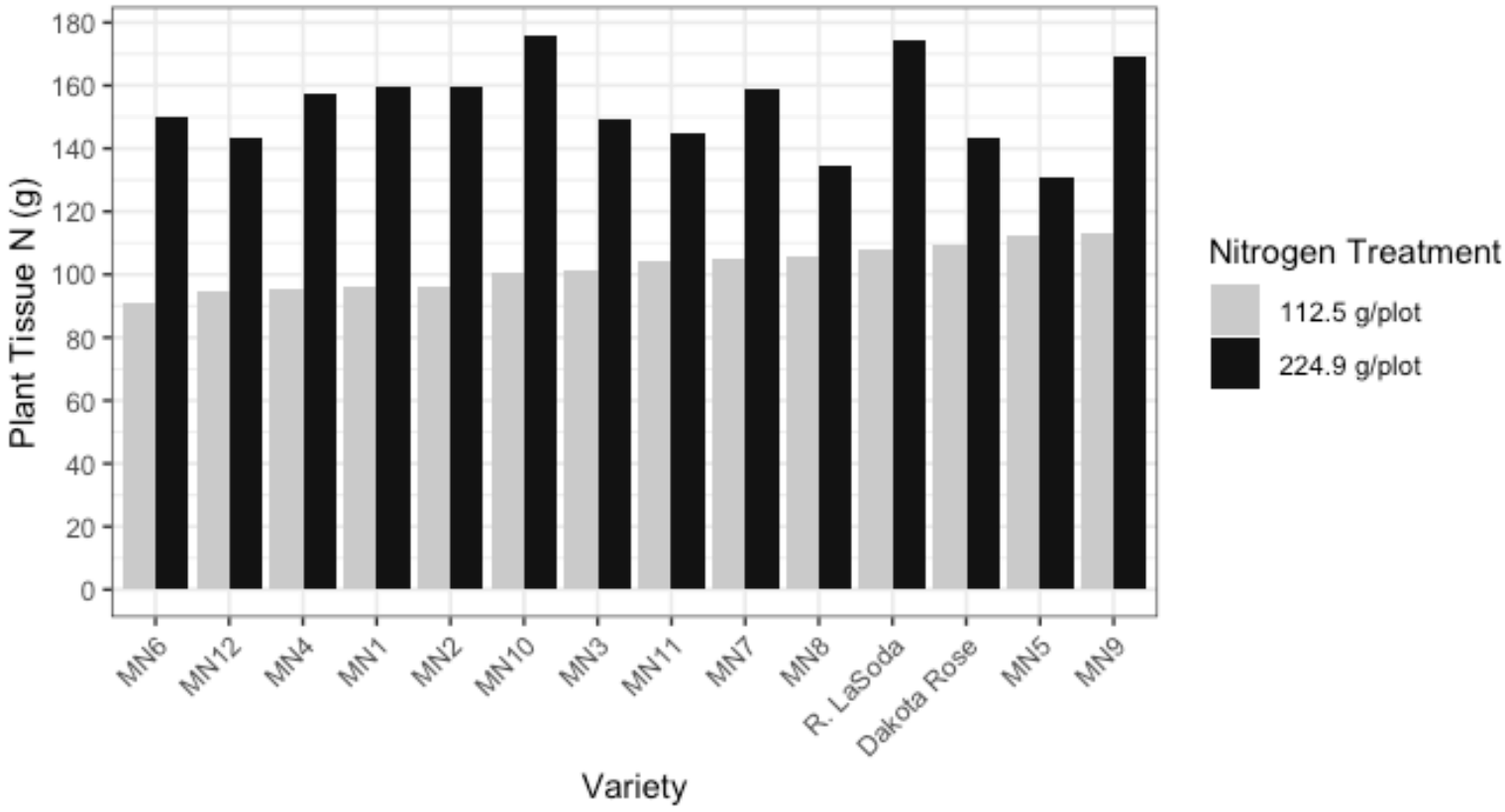


Figure 9: Select low-N to high-N Correlations. Efficiency was maintained via different mechanisms, as shown by the correlation of low-N Nitrogen Utilization Efficiency (NUtE) with high-N Nitrogen Uptake Efficiency (NUpE).

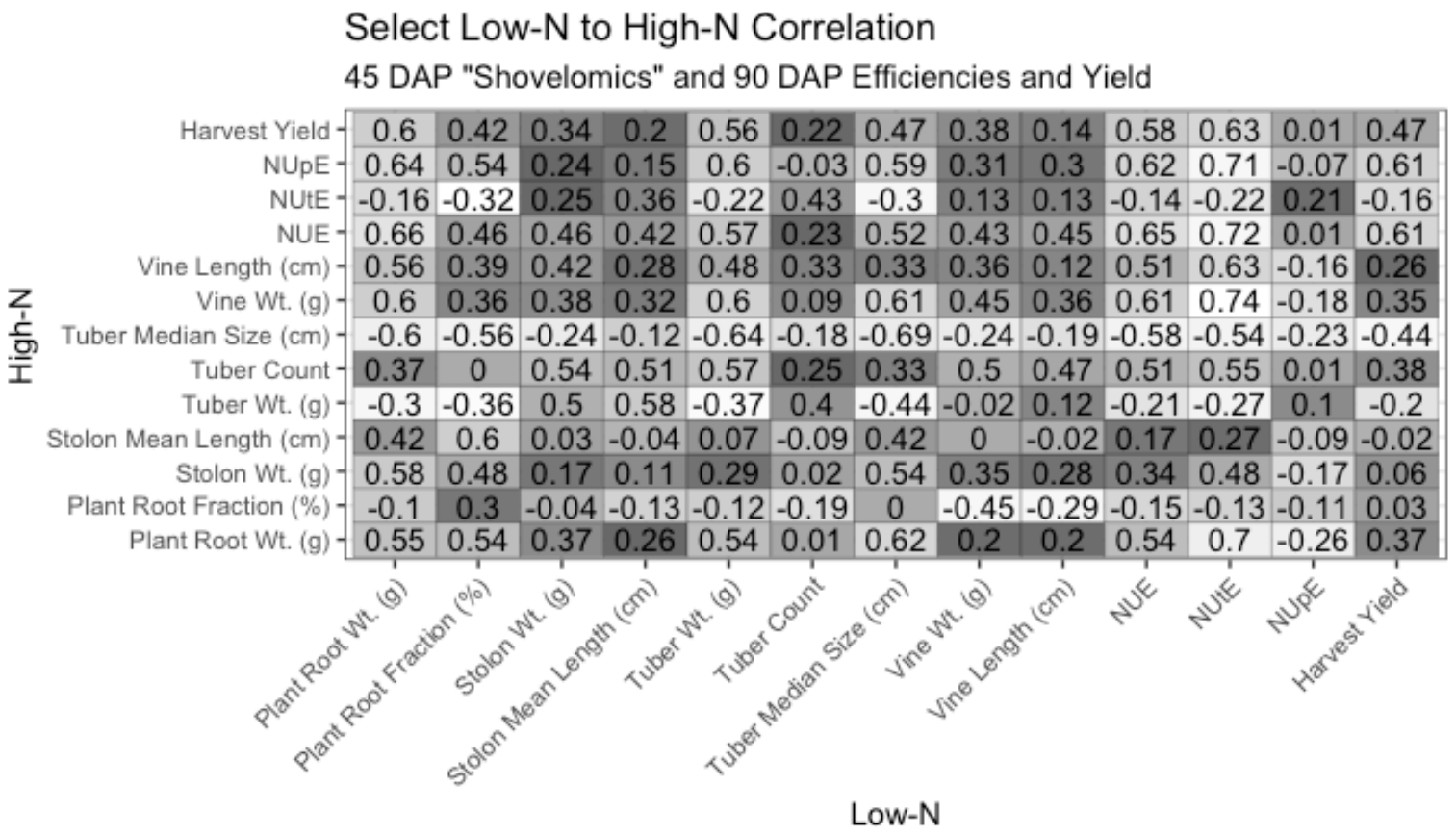


Figure 10: Coefficients of Variation for Skin Quality Traits. Variation was much greater – over 2x – for Skinning, Lightness, and Redness in the population when grown in low-N conditions.

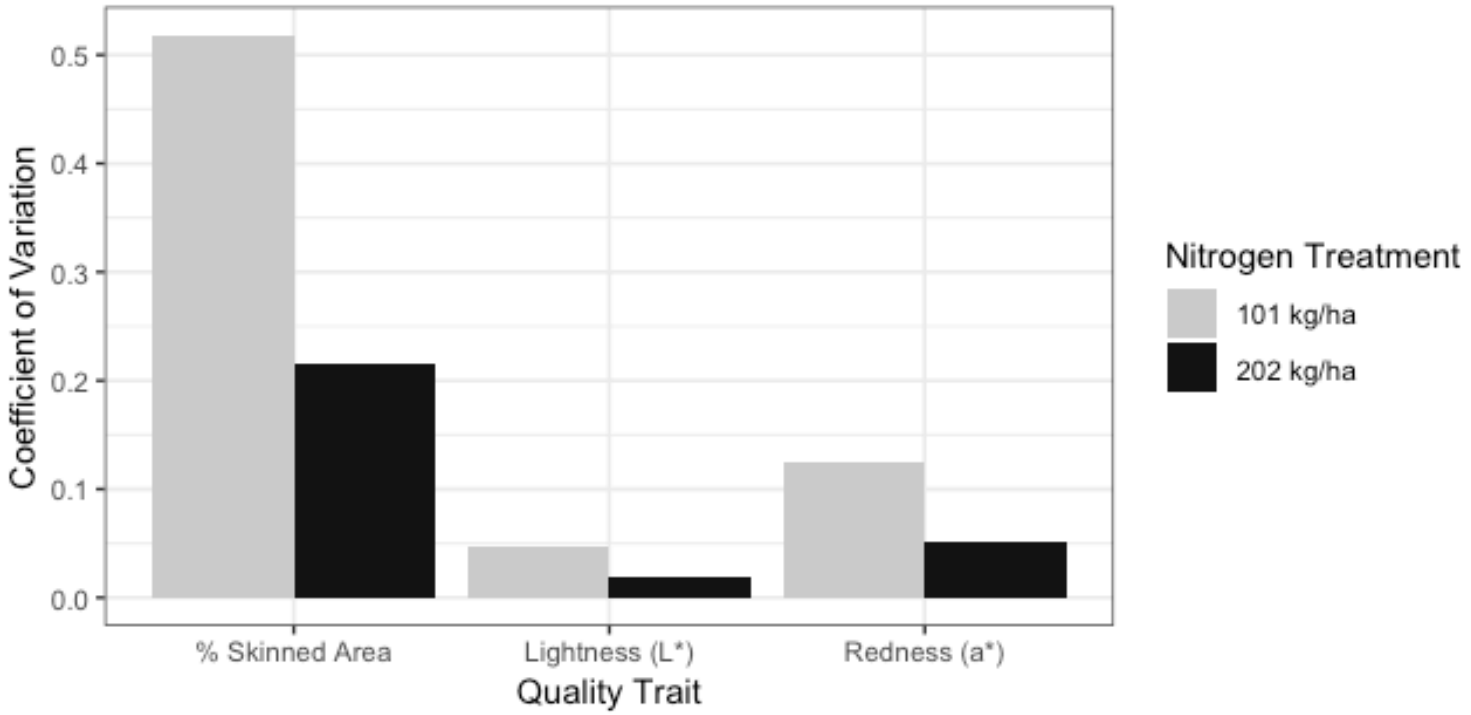


Figure 11: Skinning and Skin color by Nitrogen level. Distribution of values is broader and more spread out for all skin quality metrics in low-N (101 kg N ha^{-1}).

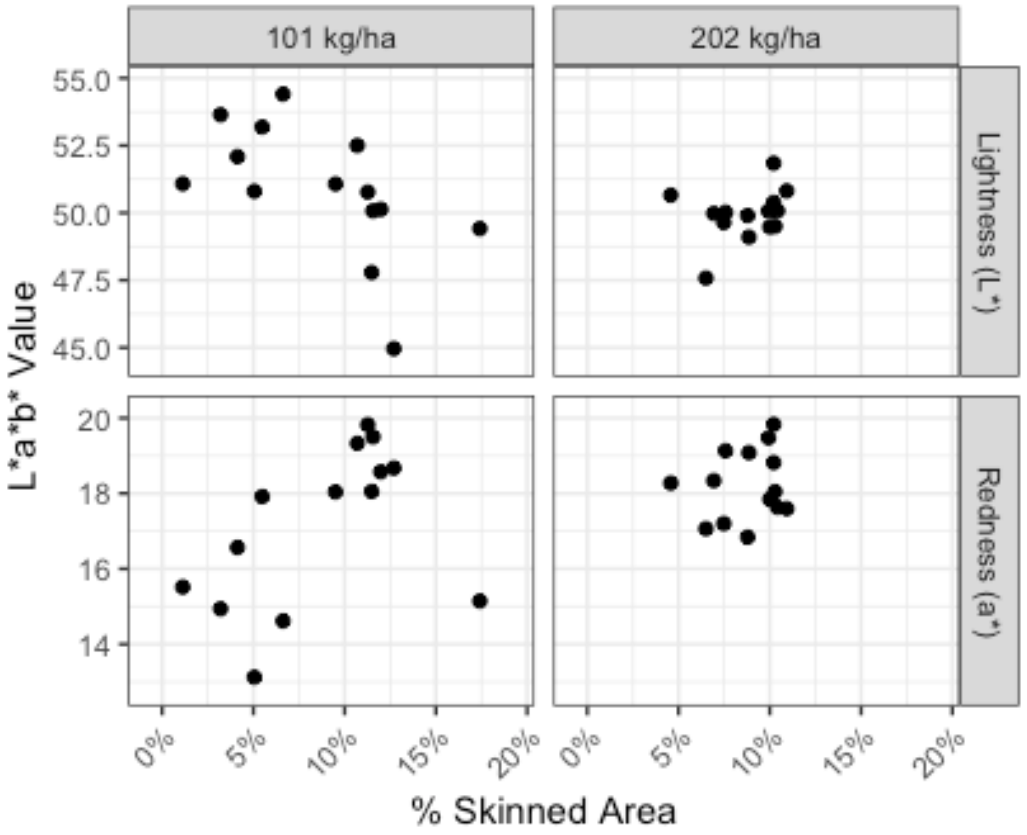


Figure 12: Year over Year correlation of Skin Quality Traits. Correlation between years was high in low-N only.

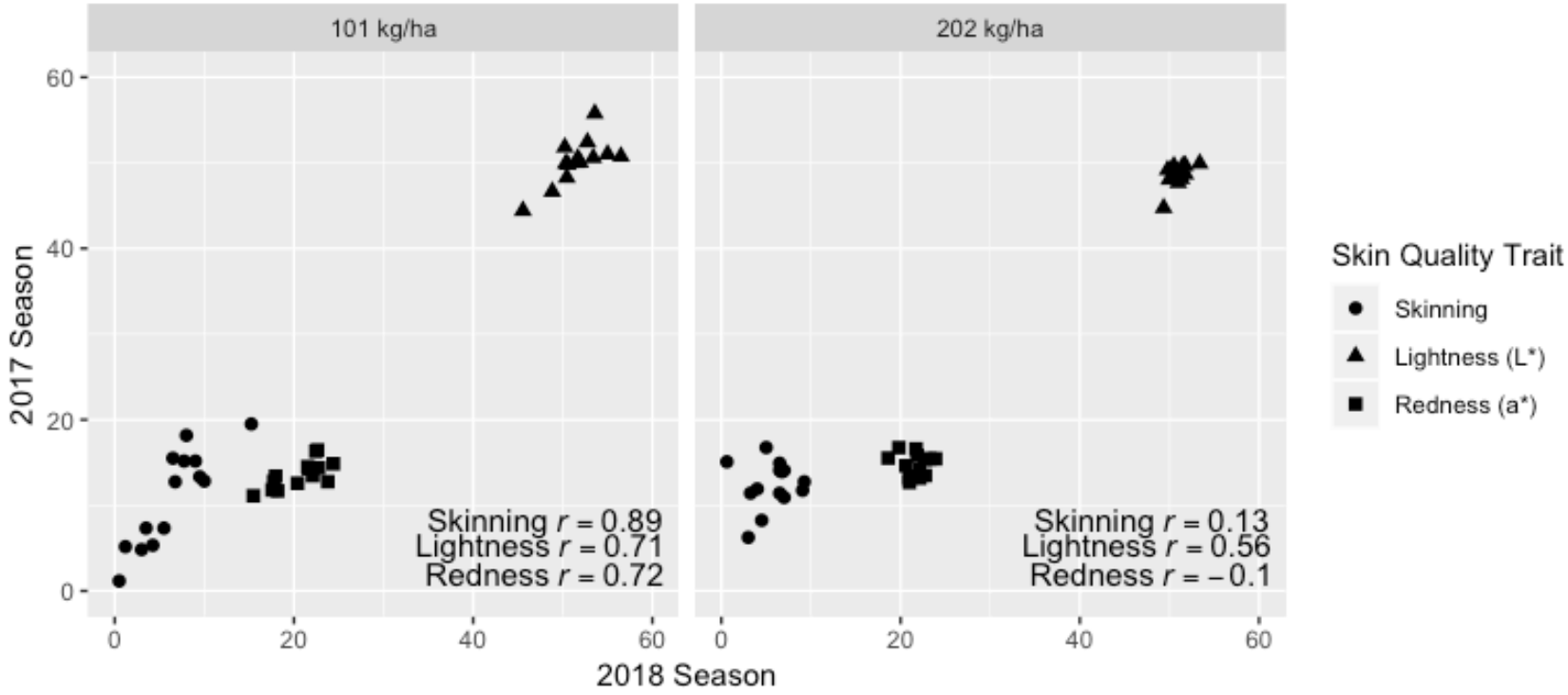
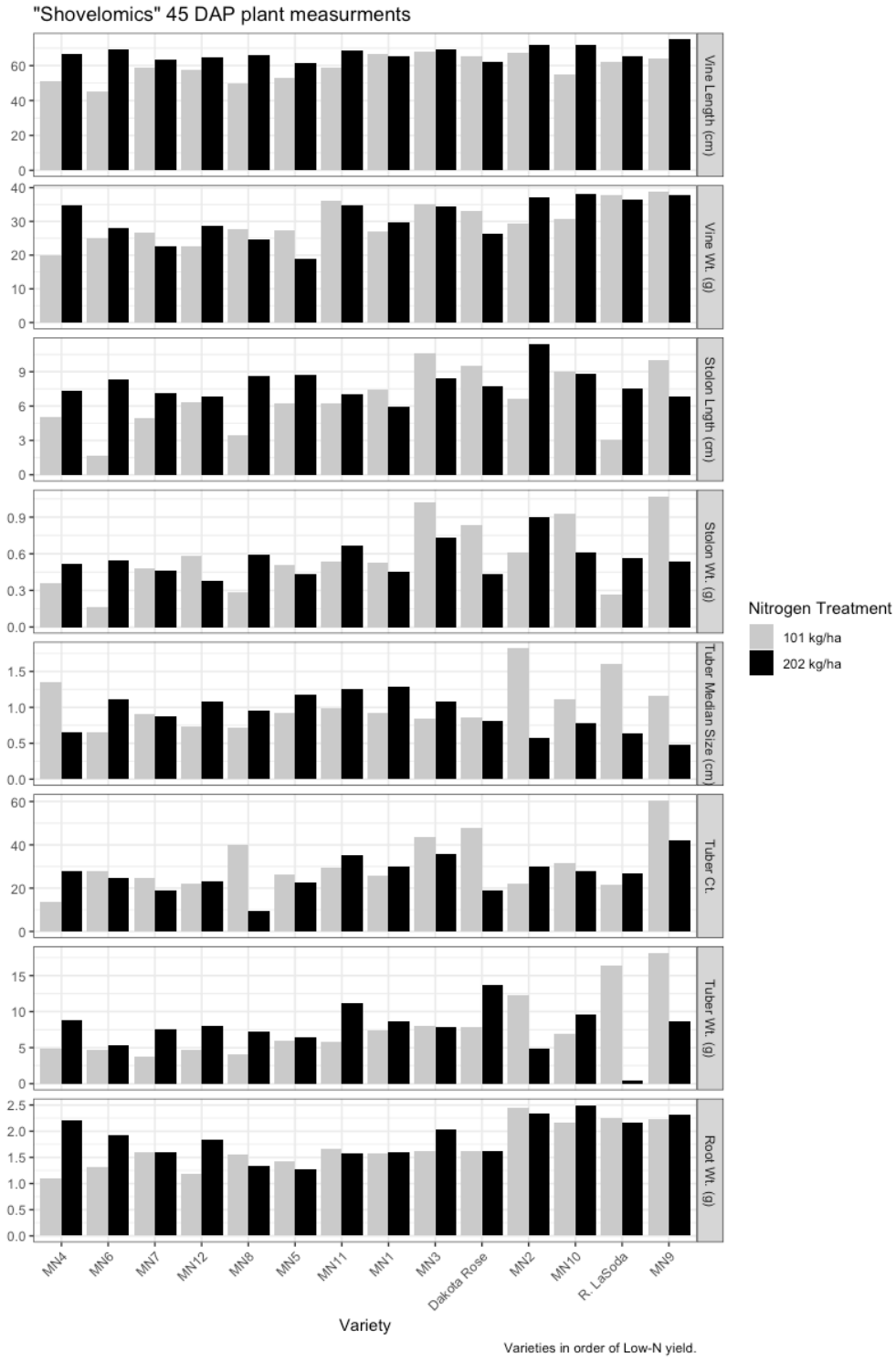


Figure 13: 45 DAP "Shovelomics" Plant Phenotypes. BLUEs values for all data collected from plants during Shovelomics phenotyping.



Bibliography

Anderson G. (1980) Assessing organic phosphorus in soils. In: Khasawneh FE, Sample EC, Kamprath EJ, eds. *The Role of Phosphorus in Agriculture.* Madison, WI, USA: ASA, CSSA, SSSA, 411-431.

Aslam, Muhammad, Ann Oaks & Ray C. Huffaker. 1976. Effect of Light and Glucose on the Induction of Nitrate Reductase and on the Distribution of Nitrate in Etiolated Barley Leaves. Plant Physiol., 58: 588-591

Bates, Terence R. and Lynch, Jonathan P. 2000a. Plant Growth and Phosphorus Accumulation of Wild Type and two Root Hair Mutants of Arabidopsis Thaliana (Brassicaceae). *American J. of Botany,* 87(7): 958-963.

Bates, Terence R. and Lynch, Jonathan P. 2000b. The Efficiency of Arabidopsis thaliana (Brassicaceae) Root Hairs in Phosphorus Acquisition. *American J. of Botany,* 87(7): 964-970.

Bennett, Elena M., Stephen R. Carpenter, Nina F. Caraco. 2001. Human Impact on Erodible Phosphorus and Eutrophication: A Global Perspective. *BioScience*, 51: 227-234

Ben-Zioni, Aliza, Y. Vaadia & S. Herman Lips. 1970. Correlations between Nitrate Reduction, Protein Synthesis and Malate Accumulation. Physiologia Plantarum, 23: 1039-1047

Ben-Zioni, Aliza, Y. Vaadia & S. Herman Lips. 1971. Nitrate Uptake by Roots as Regulated by Nitrate Reduction Products of the Shoot. Physiologia Plantarum, 24: 288-290

Bhat K. K. S. and Nye P. H. 1974. Diffusion of phosphate to plant roots in soil III. Depletion around onion roots without root hairs. *Plant and Soil,* 41, 383–394. doi:10.1007/BF00017265

Bock, B.R. 1984. Efficient use of nitrogen in cropping systems. In: Nitrogen in crop production, ed. R. D. Hauck, pp. 273-294. ASA, CSSA, and SSSA, Madison, WI.

Bonser, Amy M., Jonathan Lynch, Sieglinde Snapp. 1996. Effect of Phosphorus Deficiency on Growth Angle of Basal Roots in Phaseolus vulgaris. *The New Phytologist*, 132(2):281-288

Bouranis, D. L., S. N. Chorianopoulou, V. F. Siyiannis, V. E. Protonotarios, M. J. Hawkesford. 2003. Aerenchyma formation in roots of maize during sulphate starvation. Planta 217: 382

Bouranis, D. L., S. N. Chorianopoulou, C. Kollias, P. Maniou, V. F. Siyiannis, V. E. Protonotarios, M. J. Hawkesford. 2006. Dynamics of aerenchyma distribution in the cortex of sulfate-deprived adventitious roots of maize. *Annals of Botany,* 97: 695-704

Chai Juay Hua, 于贵瑞 Yu Guirui, 何念鹏 He Nianpeng, Wen Ke Ding, 李 杰 Li Jie, Fang Jiangping. 2015. Vertical Distribution of Soil Carbon, Nitrogen, and Phosphorus in Typical Chinese Terrestrial Ecosystems. *Chinese Geographical Science,* 25(5):549-560 doi: 10.1007/s11769-015-0756-z

Chu, W.K. and Chang, S. C. 1966. Surface activity of inorganic soil phosphorus. *Soil Science,* 101: 459-464.

cipotato.org/crops/potato/potato-facts-and-figures, accessed Jun 25th 2019

Davidson, James M., Donald A. Graetz, P. Suresh C. Rao, H. Magdi Selim. 1978. Simulation of Nitrogen Movement, Transformation, and Uptake in Plant Root Zone. *U.S. EPA Ecological Research Series.* EPA-600/3-78-029. URL: https://babel.hathitrust.org/cgi/pt?id=umn.319510028380407;view=1up;seq=1 On: 11/18/17

Dawson, Julie C., David R. Higgins, Stephen S. Jones. 2007. Characterizing nitrogen use efficiency in natural and agricultural ecosystems to improve the performance of cereal crops in low-input and organic agricultural systems. *Field Crops Research* 107: 89-101

De Jong, Hielke, Joseph B. Sieczka, Walter De Jong. 2011. The Complete Book of Potatoes, Timber Press, Portland Oregon and London. ISBN: 978-0-88192-999-7

Ryan, P. R., E. Delhaize, D. L. Jones. 2001. Function and Mechanism of Organic Anion Exudation from Plant Roots. *Annu. Rev. Plant Physiol. Plant Mol. Biol. 52:527-60*

Douglas Bates, Martin Maechler, Ben Bolker, Steve Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1), 1-48. doi:10.18637/jss.v067.i01

Drew M. C., C. Jiu He, Page W. Morgan. 1989. Decreased ethylene biosynthesis, and induction of aerenchyma, by nitrogen- or phosphate-starvation in adventitious roots of Zea mays L. *Plant Physiol.* 91: 266-271

Enwezor, W. O. and Moore, A. W. 1966. Phosphorus Status of Some Nigerian Soils. *Soil Science,* 102(5):322-328

Errebhi, Mohamed, Carl J. Rosen, Florian I. Lauer, Max W. Martin, John B. Bamberg, and David E. Birong. 1998. "Screening of Exotic Potato Germplasm for Nitrogen Uptake and Biomass Production." *American Journal of Potato Research* 75: 93-100.

Fan, Mingshou, Jinming Zhu, Christina Richards, Kathleen M. Brown and Jonathan P. Lynch. 2003. Physiological roles for aerenchyma in phosphorus-stressed roots. *Functional Plant Biol.* 30: 493-506

FAO. 2018. World Food and Agriculture – Statistical Pocketbook 2018. Rome. 254 Pp. License: Cc By-Nc-Sa 3.0 Igo.

Fernie, Alisdair R. and Willmitzer, Lothar. 2001. Molecular and Biochemical Triggers of Potato Tuber Development. *Plant Physiology*, 127: 1459-1465

Ferrari, Thomas E., Olin C. Yoder, Philip Filner. 1973. Anaerobic Nitrite Production by Plant Cells and Tissues: Evidence for Two Nitrate Pools. Plant Physiol., 51: 423-431

Franzen, David, Andrew Robinson, Carl Rosen. 2018. Fertilizing Potato in North Dakota. NDSU Extension Service. https://www.ag.ndsu.edu/publications/crops/fertilizing-potato-in-north-dakota#section-1, accessed Jan 2019

Geary, B., J. Clark, B. G. Hopkins, V. D. Jolley. 2015. Deficient, Adequate and Excess Nitrogen Levels Established in Hydroponics for Biotic and Abiotic Stress-Interaction Studies in Potato. Journal of Plant Nutrition, 38:1, 41-50, DOI: 10.1080/01904167.2014.912323

Gennari, Pietro, Food and Agriculture Organization of the United Nations. 2015. FAO Statistical Pocketbook 2015. pg. 31, http://www.fao.org/3/a-i4691e.pdf

Getahun, Baye Berihun. 2017. Genetic Diversity of Potato for Nitrogen Use Efficiency under Low Input Conditions in Ethiopia. Ph.D Thesis, Graduate School of Experimental Plant Sciences, Wageningen University, Wageningen, the Netherlands. Advisors: Richard G..F. Visser, C.G. van der Linden, Paul C. Struik, Anton J. Haverkort, Frits K. van Evert & Peter G. Vos.

Glass, Anthony D. M., 2003. "Nitrogen Use Efficiency of Crop Plants: Physiological Constraints upon Nitrogen Absorption." *Critical Reviews in plant Sciences,* 22(5):453-470

Goffart, J. P., M. Olivier, M. Frankinet. 2008. Potato Crop Nitrogen Status Assessment to Improve N Fertilization Management and Efficiency: Past–Present–Future. Potato Research 51: 355-383

Good, Allen, A. K. Shrawat, D. G. Muench. 2004. Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? *TRENDS in Plant Science,* 9:597-605

Hadley Wickham. .2017. tidyverse: Easily Install and Load the 'Tidyverse'. R package version 1.2.1. https://CRAN.R-project.org/package=tidyverse

Hamid, A. 1972. Efficiency of N uptake by wheat as affected by time and rate of application using N^{15} labeled ammonium sulfate and sodium nitrate. *Plant and Soil* 37: 340-389

Hammond, John P. and White, Philip J. 2011. Sugar Signaling in Root Responses to Low Phosphorus Availability. *Plant Physiology,* 156: 1033-1040

Harville, David A. 1977. Maximum Likelihood Approaches to Variance Component Estimation and to Related Problems. *Journal of the American Statistical Association,* 72: 320-338

Hewitt, E. J. 1963. The Essential Nutrient Elements, Requirements and Interaction in Plants. In: Steward, F. C. (ed.) Plant Physiology, a Treatise. Vol. 3, Chapter 2. Academic Press, New York.

Hirsch, Candice N., C. D. Hirsch, K. Felcher, J. Coombs, D. Zarka, A. Van Deynze, W. De Jong, R. E. Veilleux, S. Jansky, P. Bethke, D. S. Douches, C. R. Buell. 2013. Retrospective View of North American Potato (Solanum tuberosum L.) Breeding in the 20th and 21st Centuries. *G3* 3: 1003-1013.

Hirschi, Kendal D. 2004. The Calcium Conundrum. Both Versatile Nutrient and Specific Signal. *Plant Physiology.,* 136: 2438-2442

Ho, Melissa D., Juan Carlos Rosas, Kathleen M. Brown, Jonathan P. Lynch. 2005. Root architectural tradeoffs for water and phosphorus acquisition. *Functional Plant Biology,* 32: 737-748.

Hong Liao, Gerardo Rubio, Xiaolong Yan, Aiqin Cao, Kathleen M. Brown, Jonathan P. Lynch. 2001. Effect of phosphorus availability on basal root shallowness in common Bean. *Plant and Soil*, 232: 69-79.

Hong Liao, Xiaolong Yan, Gerardo Rubio, Steve R. Beebe, Mathew W. Blair, Jonathan P. Lynch. 2004. Genetic mapping of basal root gravitropism and phosphorus acquisition efficiency in common bean. *Functional Plant Biology.* 31: 959-970

Howarth, Robert W. and Marino, Roxanne. 2006. Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: Evolving views over three decades. *Limnol. Oceanogr.,* 51(1.2): 364-376.

mda.state.mn.us/environment-sustainability/minnesota-agricultural-water-quality-certification-program, accessed April 2019

Huffaker, R. C. & D. W. Rains. 1978. Factors Influencing Nitrate Acquisition by Plants; Assimilation and Fate of Reduced Nitrogen. In: Nielson, D. R. & McDonald, J. G. (eds.), Nitrogen in the Environment. Vol. 2, pp 1-43. Academic Press, New York.

Ivins, J. D., and P. M. Bremner. 1965. Growth, development, and yield in potato. Outlook Agric. 4:211-217

Jackson M.B. and Armstrong W. 1999. Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. *Plant Biol,* 1: 274-287

Johnson, Dennis, A. (ed) Potato Health Management, Second Edition. APS Press, St. Paul, Minnesota, 2008.

Johnson, Jane F., C. P. Vance, D. L. Allan. 1996. Phosphorus Deficiency in Lupinus albus: Altered Lateral Root Development and Enhanced Expression of Phophoeno/Pyruvate Carboxylase. *Plant Physiology.* 112(1):31-41

van Keulen, H. 1977. Nitrogen requirmens of rice with special reference to Java. Contr. Red. Inst. Agric. Bogor 30:67

King, Janet C., Joanne L. Slavin. 2013. White Potatos, Human Health, and Dietary Guidance. *Advanced Nutrition*, 4:393S-401S. doi: 10.3945/an.112.003525

Kleinkopf, G. E., D. T. Westermann, R. B. Dwelle. 1981. Dry Matter Production and Nitrogen Utilization by Six Potato Cultivars. *Agronomy J.*, 73: 799-802

Kratzke, Marian G. and Palta, Jiwan P. 1985. Evidence for The Existence of Functional Roots in Potato Tubers and Stolons: Significance in Water Transport to the Tuber. *Amer. Potato J.,* 62: 227-236

Laboski, Carrie A. M., John B. Peters, Larry G. Bundy. 2006. Nutrient Applications Guidelines for Field, Vegetable, and Fruit Crops in Wisconsin. UW Extension. http://www.soils.wisc.edu/extension/pubs/A2809_06.pdf, accessed Jan 2019

Leopold, A. C. 1961. Senescence in plant development. Science, 134: 1727-1732

Lesczynski, D. B. and Tanner, C. B. 1976. Seasonal Variation of Root Distribution of Irrigated, Field-Grown Russet Burbank Potato. Am Potato J, 53:69-78

Levy, David, Warren K. Coleman, Richard E. Veileux. 2013. Addaption of Potato to Water Shortage: Irrigation Management and Enhancement of Tolerance to Drought and Salinity. Am. J. Potato. Res., 90:186-206

Lutman, B. F. 1934. Cell size and Structure in Plants as Affected by Inorganic Elements. Univ. Vermont Agr. Expt. Sta. Bull., 383

Lynch J. P. and Beebe S. E. 1995. Adaptation of beans (Phaseolus vulgaris L.) to low phosphorus availability. *HortScience* 30, 1165–1171.

Lynch, J. P. 1998. Root architecture and phosphorus acquisition efficiency in common bean. *In* Phosphorus in Plant Biology: Regulatory Roles in Molecular, Cellular, Organismic, and Ecosystem Processes. Eds. J P Lynch and J Deikman. Rockville, USA. *American Society of Plant Physiologists.* 19, 81–91.

Lynch, Jonathan P. and Brown, Kathleen M. 2001. Topsoil foraging – an architectural adaptation of plants to low phosphorus availability. *Plant and Soil*, 237: 225-237

Lynch, Jonathan P. and Brown, Kathleen M. 2012. New roots for agriculture: exploiting the root phenome. *Phil. Trans. R. Soc. B* 367, 1598-1604. doi:10.1098/rstb.2011.0243

McAllister, Chandra H., P. H. Beatty, A. G. Good. 2012. Engineering nitrogen use efficient crop plants: the current status. *Plant Biotechnology J.* 10: 1011-1025.

Miao Yuan Wang, M. Y. Siddiqi, T. J. Ruth, A. D. M. Glass. 1993. Ammonium Uptake by rice roots: II. Kinetics of 13NH4+ influx across the plasmalemma." *Plant Phsiol.* 103: 1259-1267.

Miguel, M. A., A. Widrig, R. F. Veiria, K. M. Brown, J. P. Lynch. 2013. Basal root whorl number: a modulator of phosphorus acquisition in common bean (Phaseolus vulgaris). *Annals of Botany,* 112: 973-982.

Miller, Carter R., Ivan Ochoa, Kai Loenne Nielsen, Douglas P. Beck, Jonathan P. Lynch. 2003. Genetic variation for adventitious rooting in response to low phosphorus availability: potential utility for phosphorus acquisition from stratified soils. *Functional Plant Biol.,* 30: 973-985

Mohammad Rusan, M.J., S. Zuaraiqi, W. Quasmeh, I. Papadopoulos. 1999. Yield response and nitrogen utilization efficiency by drip-irrigated potato. Nutrient Cycling in Agroecosystems, 54: 243-249

Moll, R.H., E. J. Camprath, W. A. Jackson. 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. Agron J, 74:562-564

Moorby, J. 1978. The physiology of growth and tuber yield. p. 153-194. *In* P. M. Harris (ed.) The Potato Crop: The Scientific Basis for Improvement. Chapman and Hall Ltd. London.

Moorby, J., and F. L. Milthorpe. 1975. Potato. *In* Crop physiology: Some case histories. L. T. Evans (ed.) Cambridge University Press, London.

Morgan, M.A. & W. A. Jackson. 1988. Inward and Outward Movement of ammonium in Root Systems: Transient Responses during Recovery from nitrogen Deprivation in Presence of Ammonium. *J. of Exp. Botany,* 39(199):179-191

Morgan, M.A. & W. A. Jackson. 1989. Reciprocal Ammonium Transport Into and Out of Plant Roots: Modifications by Plant Nitrogen Status and Elevated Root Ammonium Concentration. *J. of Exp. Botany*, 40(211):207-214

Nieder, R. and Benbi, D. K. 2008. Carbon and Nitrogen in the Terrestrial Environment. BOOK: *Springer Science + Business Media B.V.* ISBN 978-1-4020-8432-4

Nigon, Tyler J., David J. Mulla, Carl J. Rosen, Yafit Cohen, Victor Alchanatis, Ronit Rud. 2014. Evaluation of the nitrogen sufficiency index for use with high resolution, broadband aerial imagery in a commercial potato field. *Precision Agric.,* 15: 202-226

Novoa, R. & Loomis, R. S. 1981. Nitrogen and Plant Production. Plant and Soil, 58:177-204

Ortiz-Monasterio, J. I., K. D. Sayre, S. Rajaram and M. McMahon. 1997. Genetic Progress in Wheat Yield and Nitrogen Use Efficiency under Four Nitrogen Rates. Crop Sci. 37:898-904

Paez-Garcia, Ana, C. M. Motes, W. R. Scheible, R. Chen, E. B. Blancaflor, M. J. Monteros. 2015. Root Traits and Phenotyping Strategies for Plant Improvement. *Plants*, 4: 334-355

Pau, G, F. Fuchs, O. Sklyar, M. Boutros, W. Huber. 2010. "EBImage—an R package for image processing with applications to cellular phenotypes." *Bioinformatics*, 26(7), 979–981. doi: 10.1093/bioinformatics/btq046.

Pearman, I., S. M. Thomas, G. N. Thorne. 1977. Effects of nitrogen fertilizer on growth and yield of spring wheat. Ann. Bot. 41: 108

Postma, J. A. and Lynch, J. P. 2011. Root Cortical Aerenchyma Enhances the Growth of Maize on Soils with Suboptimal Availability of Nitrogen, Phosphorus, and Potassium. *Plant Physiol.* 156(3): 1190-1201

Rabalais, Nancy N., R. Eugene Turner, William J. Wiseman Jr. 2002. Gulf of Mexico Hypoxia, A.K.A. "The Dead Zone." *Annu. Rev. Ecol. Syst.* 33:235–63.

ral.cfans.umn.edu/nitrate-and-nitrite[1], accessed May 9th, 2019

ral.cfans.umn.edu/total-nitrogen-plant[2], accessed May 9th, 2019

Rao, K. Prasad & Rains, D. William. 1976. Nitrate Absorption by Barley II. Influence of Nitrate Reductase Activity. Plant Physiol. 57: 59-62

Rawat, Suman R., S. N. Silim, H. J. Kronzucker. M. Y. Siddiqi, A. D. Glass. 1999 *AtAMT1* gene expression and NH_4^+ uptake in roots of *Arabidopsis thaliana*: evidence for regulation by root glutamine levels. *Plant J.* 19(2):143-152

Rosen, C., Errebhi, John Moncrief, Satish Gupta, H. H. Cheng, and Dave Birong. 1993a. Nitrogen fertilization studies on irrigated potatoes: Nitrogen use, soil nitrate movement and petiole sap analysis for predicting nitrogen needs. *Field Research in Soils (Soil Series #136) Misc. Pub. 79-1993, Minn. Ag. Station, Univ. of Minn.,* pp. 14-38, URL: https://www.extension.umn.edu/agriculture/nutrient-management/blue-books/full-version/1993-full.pdf

Rosen, Carl and Birong, Dave. 1993b. Calcium Nitrate as a Source of Calcium and Nitrogen for Potato Production. *Field Research in Soils (Soil Series #136) Misc. Pub. 79-1993, Minn. Ag. Station, Univ. of Minn.,* pp. 11-13

Rosen, Carl J and Eliason, Roger. 2005. Potatos. In: Nutrient Management for Commercial Fruit and Vegetable Crops in Minnesota. UMN Extension Service, pp. 18

Rosen, Carl, Christian Thill, Peter Bierman, Matt McNearney and Monica Carrasco. 2007. Effects of Nitrogen Fertility on Yield and Quality of Intermediate, Advanced, and Elite Selections from the University of Minnesota Potato Breeding Program. Unpublished.

Rosen, Carl, Christian Thill, Peter Bierman, Matt McNearney, and Monica Carrasco. 2004. Effects of Nitrogen Fertility on Yield and Quality of Intermediate, Advanced, and Elite Selections from the University of Minnesota Potato Breeding Program. *Minnesota Area II potato Research and Promotion Council & Northern Plains Potato Growers Association – Research Reports/Association Minutes* 160-178.

Rosen, Carl. 2018. Potato fertilization on irrigated soils. https://extension.umn.edu/crop-specific-needs/potato-fertilization-irrigated-soils, accessed Jan 2019

Rubio, Gerardo, L. Hong, X. Yan, J. P. Lynch. 2003. Topsoil Foraging and its Role in Plant Competitiveness for Phosphorus in Common Bean. *Crop Sci.* 43:598-607

Ryan, P. R. & Walker, N. A. 1994. The Regulation of ammonia uptake in *Chara australis. J. of Exp. Botany.* 45(277): 1057-1067

Sattelmacher, B., R. Kuene, P. Malagamba, U. Moreno. 1990a. Evaluation of tuber bearing Solanum species belonging to different ploidy levels for its yielding potential at low soil fertility. *Plant and Soil,* 129: 227-233

Sattelmacher, B., F. Klotz, H. Marschner. 1990b. Influence of the nitrogen level on root growth and morphology of two potato varieties differing in nitrogen acquisition. *Plant and Soil,* 123(2): 131-137

Schindler, D. W. 1977. Evolution of Phosphorus Limitation in Lakes. *Science,* 195: 260-262

Sharifi, Mehdi, Bernie J. Zebarth, Mohammad A. Hajabbasi & Mahmoud Kalbasi. 2005. Dry Matter and Nitrogen Accumulation and Root Morphological Characteristics of Two Clonal Selections of 'Russet Norkotah' Potato as Affected by Nitrogen Fertilization. *J Plant Nutrition,* 28:2243-2253

Sharifi, Mehdi, Bernie J. Zebarth, Warren Coleman. 2007. "Screening for Nitrogen-Use Efficiency in Potato with a Recirculating Hydroponic System" Communications in Soil Science and Plant Analysis, 38:3-4, 359-370, DOI: 10.1080/00103620601172357

Sharpley, A. N. 1994. Managing agricultural phosphorus for protection of surface waters: Issues and options. *Journal of Environmental Quality*, 23(3): 437-451

Sharpley, A. N., T. Daniel, T. Sims. J. Lemunyon. R. Stevens. R. Parry. 2003) Agricultural Phosphorus and Eutrophication, 2nd Ed. *USDA-ARS,* ARS--149. URL: www.ars.usda.gov/np/index.html.

Sheriff, Glen. 2005. Efficient Waste? Why Farmers Over-Apply Nutrients and the Implications for Policy Design. *Review of Agricultural Economics* 27 (4): 542-557

Snapp, S., R. Koide, J. Lynch. 1995. Exploitation of localized phosphorus-patches by common bean roots. *Plant and Soil*, 177: 211-218.

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. URL: https://websoilsurvey.sc.egov.usda.gov/. Accessed: 10/20/18.

Stark, Jeff, Dale Westermann & Bryan Hopkins. 2004. Nutrient Management Guidelines for Russet Burbank Potatoes. University of Idaho Extension. http://www.extension.uidaho.edu/nutrient/crop_nutrient/potato.html, accessed Jan 2019

Stein, Lisa Y. and Martin G. Klots. 2016. The Nitrogen Cycle. *Current Biology*. 26:94-98

Temkin, Alexis, Sydney Evans, Tatiana Manidis, Chris Campbell, and Olga V. Naidenko. 2019. Exposure-based assessment and economic valuation of adverse birth outcomes and cancer risk due to nitrate in United States drinking water. *Environmental Research*, https://doi.org/10.1016/j.envres.2019.04.009

Tillman, David, Kenneth G. Cassman, Pamela A. Matson, Rosamond Naylor, Stephen Polasky. 2002. Agricultural sustainability and intensive production practices. *Nature* 418: 671-677 doi:10.1038/nature01014

Tinker, P. B. 1976. The role of phosphorus in agriculture' *from:* National Fertiliser Development Centre of the Tennessee Valley Authority, Muscle Shoals, Alabama, l-3 June, 1976." *Food Policy,* November 1976:421-422

Tiwari, Jagesh K., Darren Plett, Trevor Garnett, Swarup K. Chakrabarti and Rajesh K. Singh. 2018. Integrated Genomics, Physiology and breeding approaches for improving nitrogen use efficiency in potato: translating knowledge from other crops. *Functional Plant Biology*, 45:587-605. https://doi.org/10.1071/FP17303

United Nations, Department of Economic and Social Affairs, Population Division. 2015. World Population Prospects 2015 – Data Booklet (ST/ESA/ SER.A/377). https://population.un.org/wpp/Publications/Files/WPP2015_DataBooklet.pdf, accessed Jan 2019

USDA. 2011. United States Standards for Grades of Potatoes. USDA Agricultural Marketing Service Fruit and Vegetable Programs Fresh Products Branch. https://www.ams.usda.gov/sites/default/files/media/Potato_Standard%5B1%5D.pdf

USDA National Agricultural Statistics Service. Potatoes 2017 Summary (September 2018). ISSN 1949-1514 Available at: potatoassociation.org/wp-content/uploads/2018/11/2018-NASS-Potato-Summary.pdf, accessed June 3rd, 2019

Van Sanford, D. A. & MacKown, C. T. 1986. Variation in nitrogen use efficiency among soft red winter wheat genotypes. Theor Appl Genet, 72:158-163

Vidmar, Joseph J., Degen Zhuo, M. Yaeesh Siddiqi, Jan K. Schjoerring, Bruno Touraine, Anthony D.M. Glass. 2000. Regulations of High-Affinity Nitrate Transporter Genes and High-Affinity Nitrate Influx by Nitrogen Pools in Roots and Barley. *Plant Physiology*. 123(1):307-318

Vitousek, Peter M. 1997. Human Alteration Of The Global Nitrogen Cycle: Sources And Consequences. *Ecological Applications*, 7(3): 737-750

Wasson, Anton, L. Bischof, A. Zwart. M. Watt. 2016. A portable fluorescence spectroscopy imaging system for automated root phenotyping in soil cores in the field. *J. of Experimental Botany,* 67: 1033-1043

Weijschede, Jelmer, J. Martinkova, H. de Kroon, H. Huber. 2006. Shade avoidance in *Trifolium repens:* costs and benefits of plasticity in petiole length and leaf size. *New Phytologist,* 172: 655-666

Wen, Guoqi, Athyna N. Cambouris, Annick Bertrand, Noura Ziadi, Haixiao Li, Mohamed Khelifi. 2019. Nitrogen Fertilization effect on the leaf chemical concentrations in Russet Burbank potato. Field Crops Research, 232:40-48.

Westerman, D. T. and Kleinkopf, G. E. 1985. Nitrogen requirements of potatoes. *Agronomy J.*, 77: 616-621

White, P. J., Martin R. Broadley, John P. Hammond, Andrew J. Thomson. 2005. Optimising the potato root system for phosphorus and water acquisition in low-input growing systems. *Asp Appl Biol,* 73:111–118

Wishart, Jane, Timothy S. George, Lawrie K. Brown, Gavin Ramsay, John E. Bradshaw, Philip J. White, Peter J. Gregory. 2013. "Measuring variation in potato roots in both field and glasshouse: the search for useful yield predictors in a simple screen for root traits." *Plant Soil,* 368:231-249.

Wishart, Jane, Timothy S. George, Lawrie K. Brown, Philip J. White, Gavin Ramsay, Hamlyn Jones, Peter J. Gregory. 2014. "Field phenotyping of potato to assess root and shoot characteristics associated with drought tolerance" *Plant Soil.* 378:351–363

Xiaorong Fan (范晓荣), Zhong Tang (唐仲), Yawen Tan (谭雅文), Yong Zhang (张勇), Bingbing Luo (罗冰冰), Meng Yang (杨锰), Xingming Lian (练兴明), Qirong Shen (沈其荣), Anthony John Millerc, Guohua Xu (徐国华). 2016. Overexpression of a pH-sensitive nitrate transporter in rice increases crop yields. *PNAS,* 113(26): 7118–7123. doi: 10.1073/pnas.1525184113

Zebarth, B J and Rosen, C. J. 2007. Research Perspective on Nitrogen BMP Development for Potato. *American Journal of Potato Research* 84*:*3-18

Zebarth, B. J., G. Tai, R. Tarn, Hielke De Jong, P. H. Milburn. 2004. Nitrogen Use Efficiency Characteristics of Commercial Potato Cultivars. *Canadian J.of Plant Science,* 84(2): 589-598

Zebarth, B. J., T. Richard Tarn, Henry De Jong, Agnes M. Murphy. 2008. Nitrogen Use Efficiency Characteristics of Andigena and Diploid Potato Selections. *Am. J. Pot. Res.,* 85: 210-218

Zhu, Jinming, K. M. Brown, J. P. Lynch. 2010a. Root cortical aerenchyma improves the drought tolerance of maize (Zea mays L.). *Plant Cell Environ,* 33: 740–749

Zhu, Jinming, Chaochun Zhang, Jonathan P. Lynch. 2010b. The utility of phenotypic plasticity of root hair length for phosphorus acquisition. *Functional Plant Biology*, 37: 313-322.

Zvomuya, F., Carl. J. Rosen, J. Creighton Miller. 2002. Response of Russet Norkotah Clonal Selections to Nitrogen Fertilization.c *Amer. J. of Potato Res.,* 79: 231-23

Appendix A: Experiment details.

A.1 - Soil characteristics, soil composition tests and irrigation water nitrate test:

Field 7, UMN Sand Plains Research Farm, Becker, MN.
Soil: Hubbard-Mosford complex, Loamy Sand/Sandy Loam, Excessively drained, >80" to water table, 0-3% slope. (Soil Survey Staff, NRCS)
Pre-Plant Soil Test (means): OM 1.6%, pH of 5.0, 3.85 mg/kg (NO3-/Soil) @ 0-6", 2.13 mg/kg (NO3-/Soil) @ 6-24"
Post-Plant Soil Test (means): OM 1.4%, pH of 5.1, 3.7 mg/kg (NO3-/Soil) @ 0-6", 4.1 mg/kg (NO3-/Soil) @ 6-24"
Irrigation Water Test: 5.2 mg NO3-/L, pH 7.5

A.2 Experiment Work Schedule:

2017 DAP	2017 Date	2018 DAP	2018 Date	Action	Data
	5/3/17			Pre-Plant Soil test	Bray P, K, OM, pH, Ca, Mg, SO4S, NO3-
0	5/5/17	0	5/8/18	Planting	
17	5/22/17	14	5/22/18	Emergence	
20	5/25/17	24	6/1/18	Urea Application -> Hilling	
31	6/5/17	35	6/12/18	Stand Count	Stand Count
40	6/14/17	---	----------	Leaf Sample for Petiole N content	
46	6/20/17	*49*	*6/26/18*	*Shovelomics Rep 1*	
47	6/21/17	*50*	*6/27/18*	*Shovelomics Rep 2*	
53	6/27/17	*51*	*6/28/18*	*Shovelomics Rep 3*	
48	6/22/17	*52*	*6/29/18*	*Begin Taking Shovelomics Data*	
--	----------	*56*	*7/3/18*	*Shovelomics Rep 4*	
--	----------	*57*	*7/4/18*	*Finish Taking Shovelomics Data*	
88	8/1/17	*91*	*8/7/18*	*HI and Haulm Sample for N content*	
90	8/4/17	*93*	*8/9/18*	*Vine kill*	
110	8/23/17	*107*	*8/23/18*	*Harvest*	

A.2 Cultural Practices

Task	Date (2017)	Notes
Fertilize	13-Apr	224kg/he 0-0-60: broadcast
Fertilize	17-Apr	200#/ac 0-0-22: broadcast
Plow	19-Apr	Moldboard
Field Cultivate	24-Apr	
Fertilizer	24-Apr	300#/ac 18-46-0: broadcast
Field Cultivate	28-Apr	
Field Cultivate	3-May	
Field Cultivate	5-May	
Plant	5-May	
Pesticide	5-May	8 oz/ac Quadris + 12 oz/ac Belay: Tank-Mix, in-furrow at planting
Herbicide	17-May	0.5#/ac SencorDF + 1.0pt/ac Linex + 1.5pt/ac Prowl H2O: Tank-Mix, broadcast
Fertilizer	25-May	Side dressing to create 101 kg N ha^{-1} and 202 kg N ha^{-1} treatments, as detailed in methods.
Hilled	25-May	
Foliar Pesticide	16-Jun	3.2oz/ac Curzate + 8oz/ac Radiant: broadcast
Foliar Pesticide	23-Jun	5.05oz/ac Endura + 1 pt./ac Previcur Flex + 2 oz/ac BayThroid + 12 oz/ac Rimon
Foliar Pesticide	29-Jun	1.5#/ac Roper + 2pt/ac Champ Formula2 + 1pt./ac Dimethoate + 12oz/ac Rimon
Foliar Pesticide	7-Jul	10oz/ac Luna + 3oz/ac CurzateDF + 2 oz/ac AgrimeckSC
Foliar Pesticide	14-Jul	1.5pt/ac Bravo + 5 oz/ac Coragen + 8oz/ac Permethrin
Foliar Pesticide	22-Jul	8oz/ac Prioxar + 8oz/ac Radiant
Foliar Pesticide	28-Jul	1.5pt./ac Bravo + 2pt/ac Champ Formula2 + 2pt/ac Carbaryl4L + 1pt/ac Dimethoate
Vine Kill	4-Aug	2pt/ac Reglone + 2pt/100gal. LI-700 adjuvant: broadcast
Vine Kill	11-Aug	2pt/ac Reglone + 2pt/100gal. LI-700 adjuvant: broadcast
Chop Vines	18-Aug	
Harvest	23-Aug	Grimmie harvester

A.2 Cultural Practices *cont.*

Task	Date (2018)	Details
Fertilizer	27-Apr	200#/ac 0-0-22: broadcast
Fertilizer	30-Apr	200#/ac 0-0-60: broadcast
Field Cultivate	1-May	
Fertilizer	1-May	300#/ac 18-46-0 (DAP): broadcast
Field Cultivate	1-May	
Plant	10-May	
Pesticide	10-May	8 oz/ac Quadris + 12 oz/ac Belay: Tank-Mix, in-furrow at row closure
Till Ends	17-May	
Herbicide	19-May	0.5#/ac SencorDF + 1.0pt/ac Linex + 1.5pt/ac Prowl H2O: Tank-Mix, broadcast
Till alleys	21-May	
Fertilizer	1-Jun	
Hilled	1-Jun	
Move Pipe	14-Jun	
Foliar Pesticide	15-Jun	1.5pt/ac Bravo + 12oz/ac Rimon
Foliar Pesticide	22-Jun	1.5#/ac Roper + 2pt/ac Badge + 1pt./ac Dimethoate + 12oz/ac Rimon
Till alleys	27-Jun	
Till alleys	28-Jun	
Foliar Pesticide	29-Jun	10oz/ac Luna + 3oz/ac CurzateDF + 2 oz/ac AgrimeckSC
Foliar Pesticide	6-Jul	1.5pt/ac Bravo
Foliar Pesticide	13-Jul	8oz/ac Priaxor + 5 oz/ac Coragen + 8oz/ac Permethrin
Foliar Pesticide	21-Jul	1.5pt./ac Bravo + 2pt/ac Badge + 8oz/ac Radiant
Vine Kill	2-Aug	2pt/ac Reglone + 2pt/100gal. LI-700 adjuvant: broadcast
Vine Kill	8-Aug	2pt/ac Reglone + 2pt/100gal. LI-700 adjuvant: broadcast
Chop Vines	13-Aug	
Harvest	23-Aug	

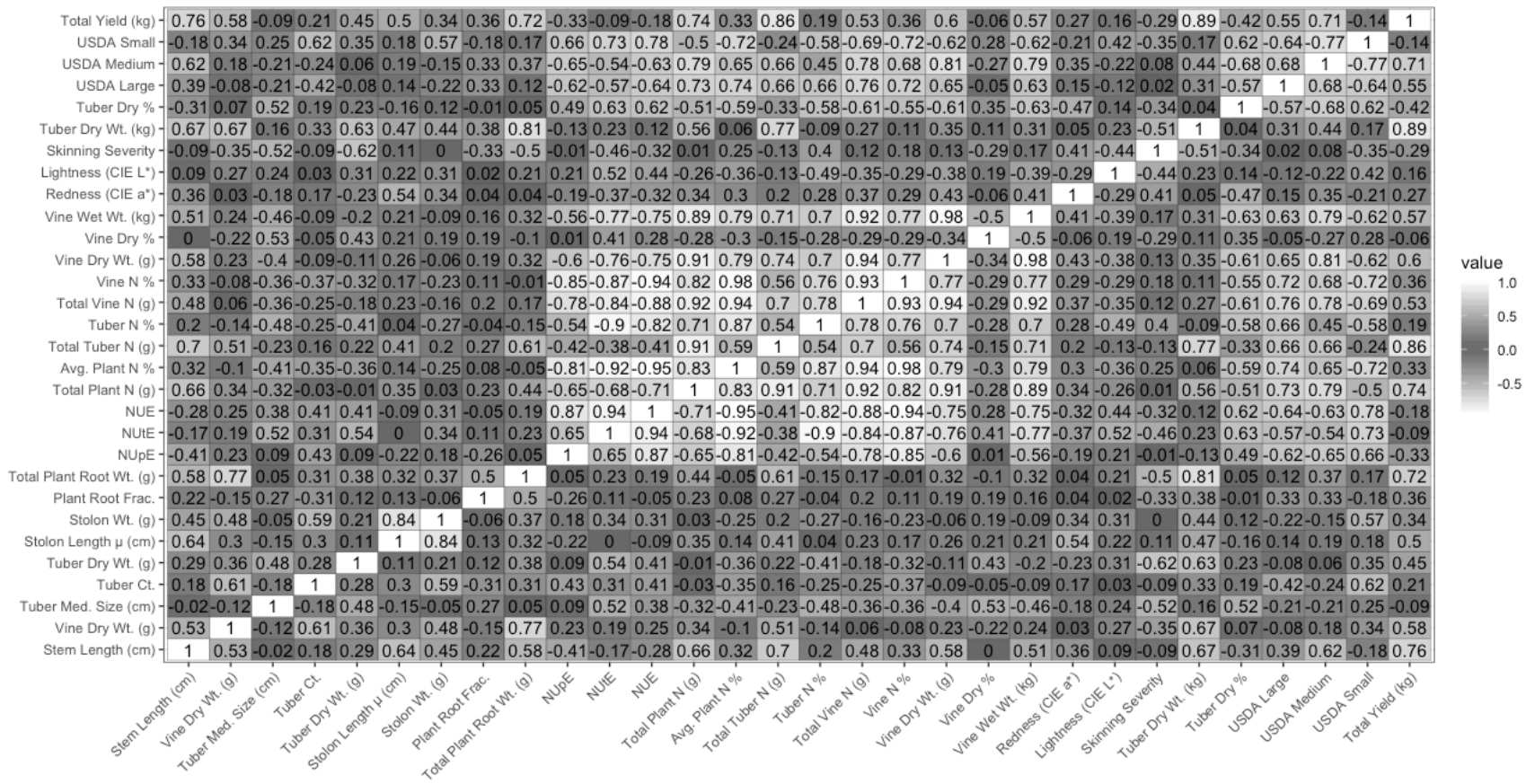
A.3 Irrigation

Date (2017)	Amount (cm)
5-Jun	0.762
8-Jun	0.762
10-Jun	0.762
19-Jun	1.524
26-Jun	1.524
3-Jul	0.762
6-Jul	1.524
8-Jul	0.762
10-Jul	1.27
12-Jul	0.762
15-Jul	0.762
17-Jul	1.27
20-Jul	0.762
24-Jul	1.27
27-Jul	0.762
29-Jul	0.762
31-Jul	1.27
22-Aug	0.762
Total:	18.034

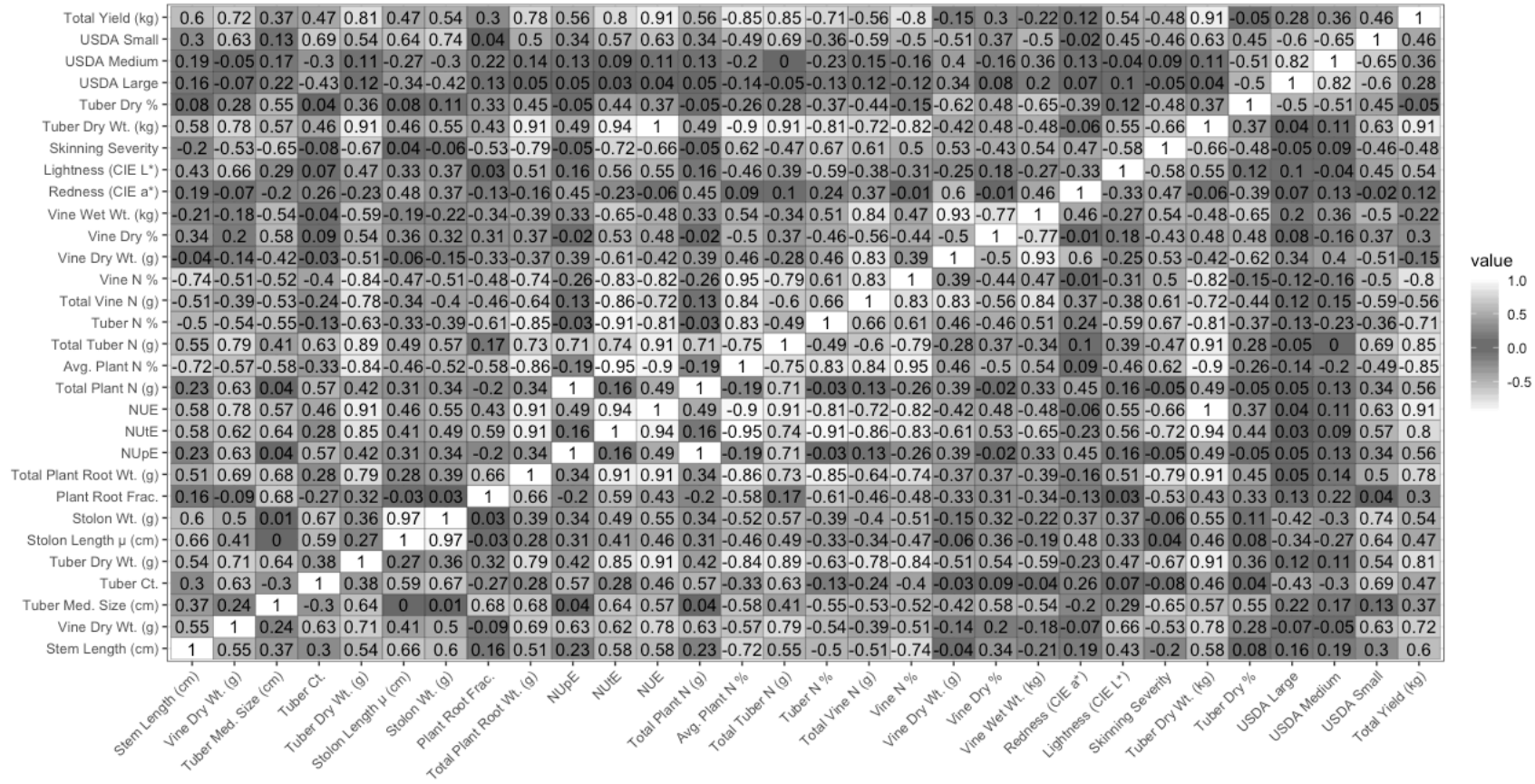
Date (2018)	Amount (cm)
23-May	1.27
29-May	1.27
5-Jun	0.762
8-Jun	1.27
14-Jun	1.524
21-Jun	1.524
28-Jun	0.762
5-Jul	0.889
9-Jul	1.778
12-Jul	0.762
16-Jul	1.778
19-Jul	0.635
23-Jul	1.27
26-Jul	1.524
30-Jul	1.524
2-Aug	1.016
9-Aug	1.524
14-Aug	0.635
Total:	21.72

Appendix B: Correlation Tables and Figure

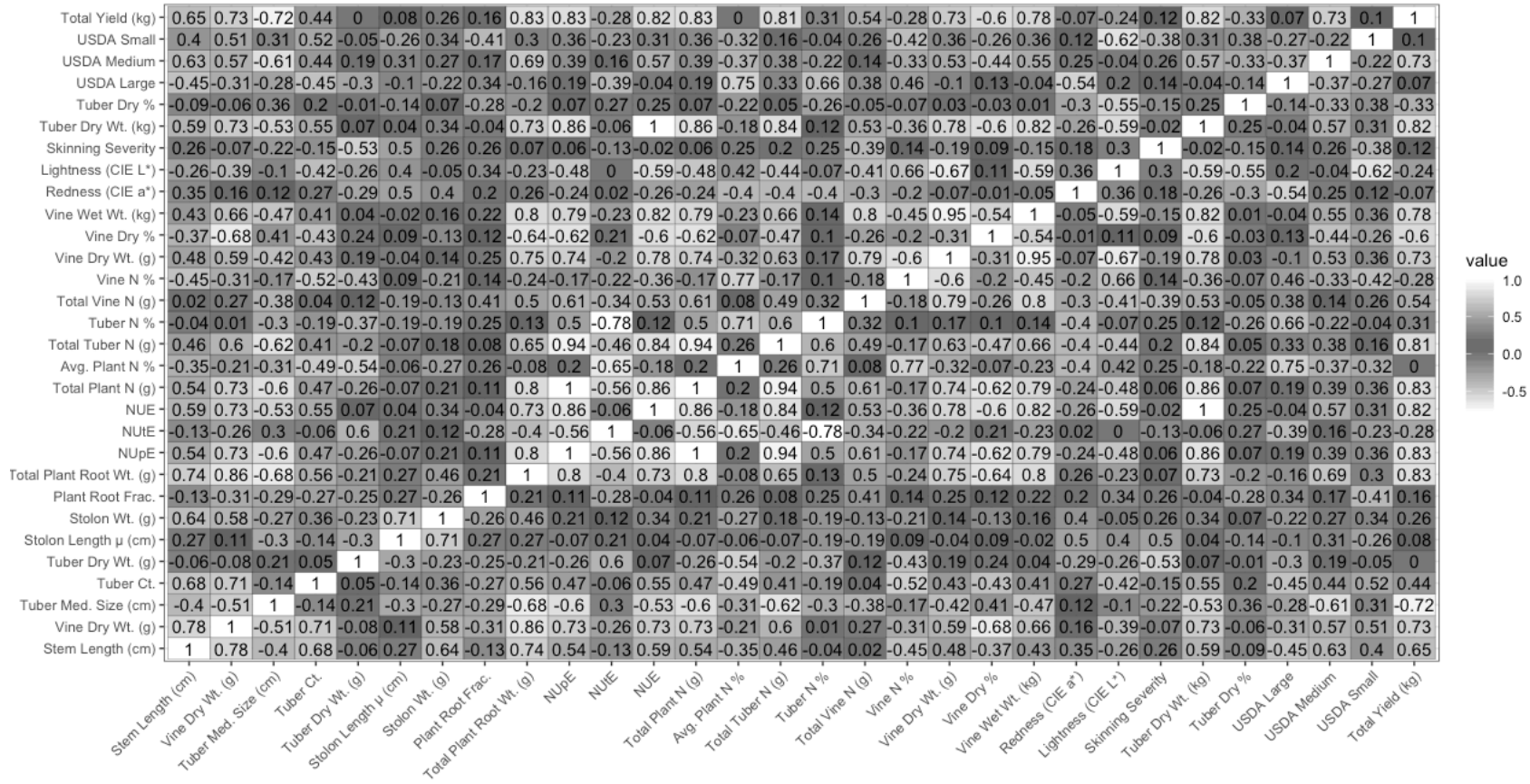
B.1 – Correlation of all variables, which are ordered and correspond to those from Table 3. This table encompasses both years and both treatments.



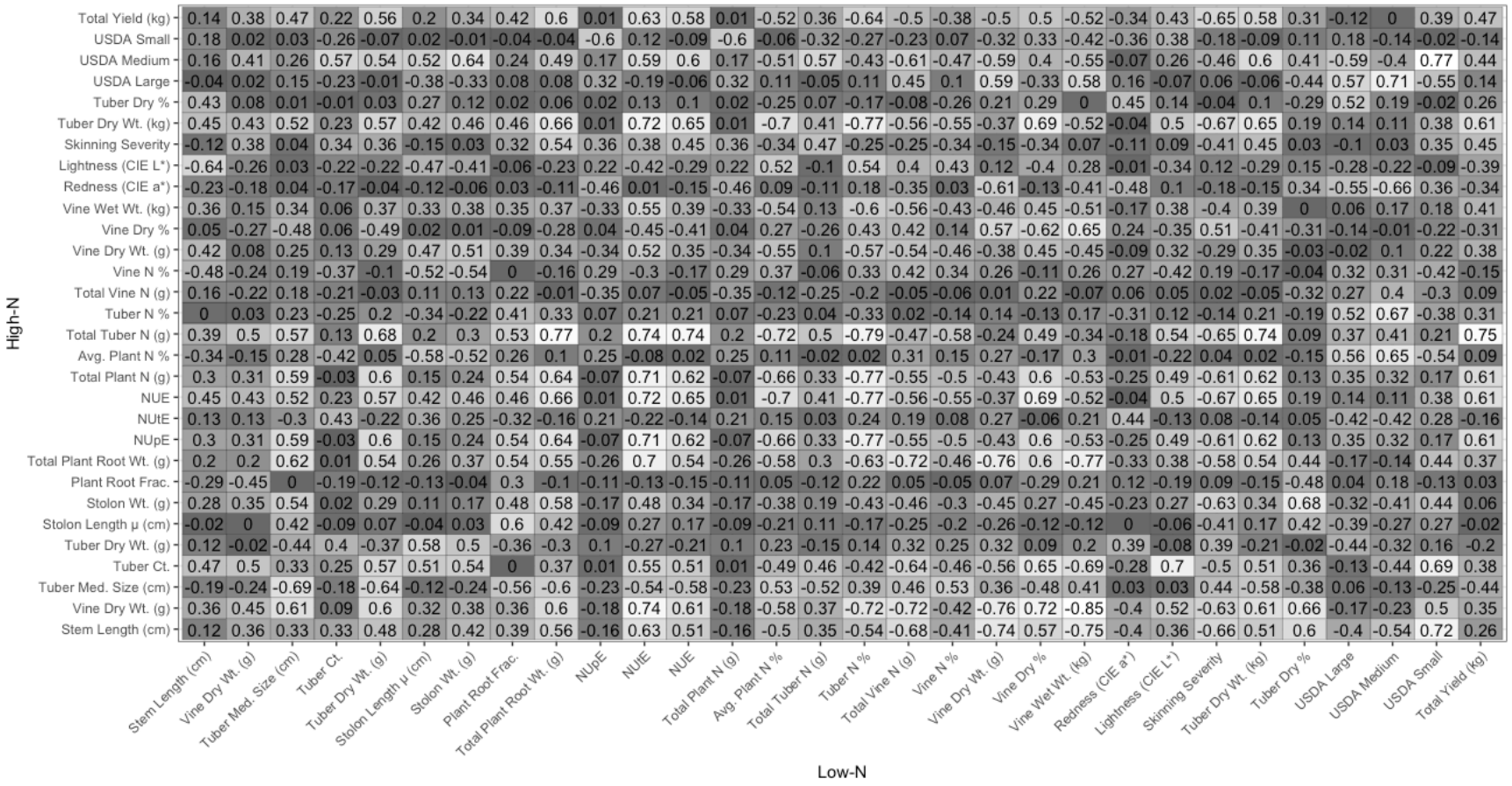
B.2 – Correlation of all low-N variables, which are ordered and correspond to those from Table 3. This table encompasses both years and only data taken from low-N plants.



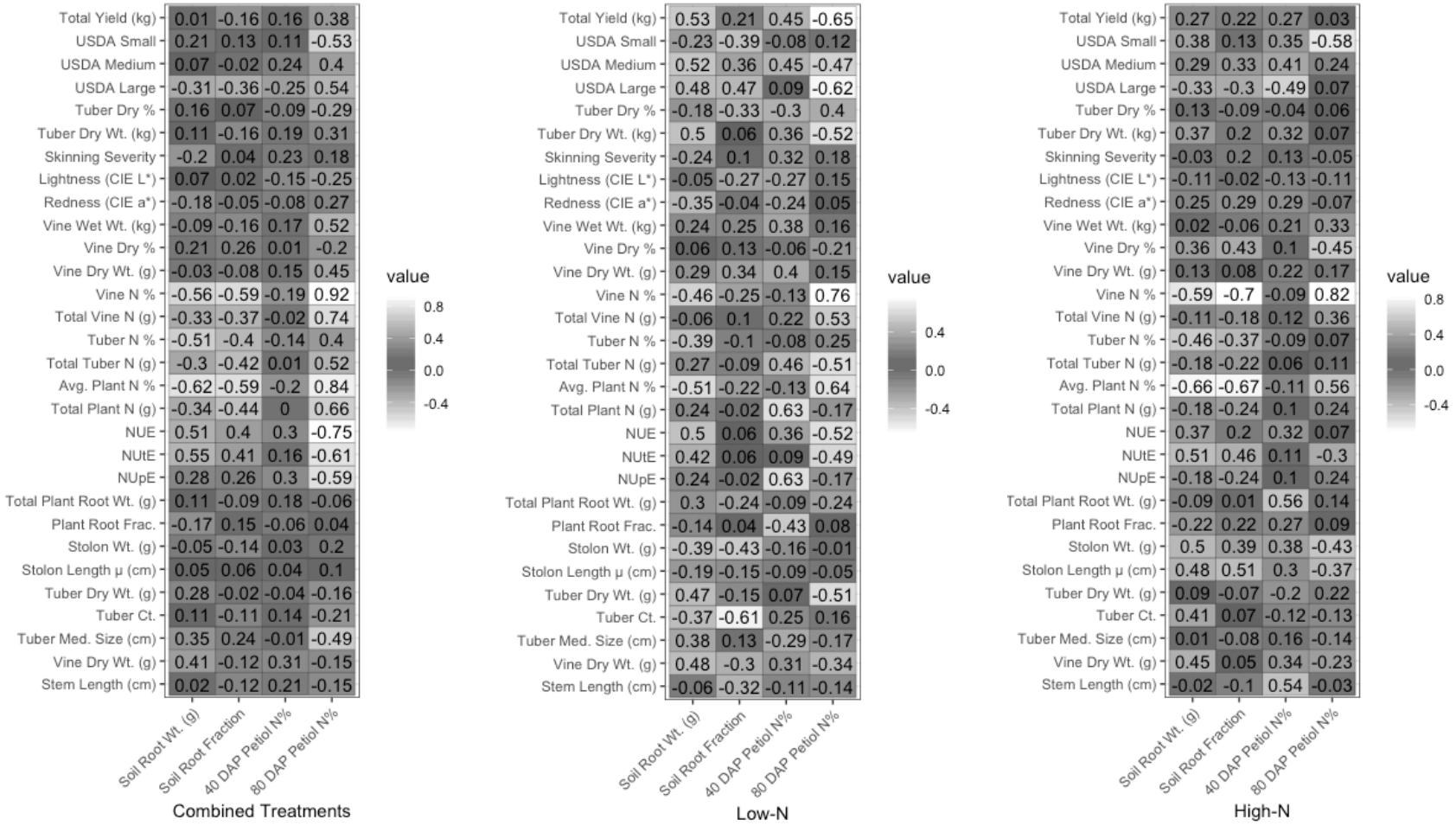
B.3 – Correlation of all high-N variables, which are ordered and correspond to those from Table 3. This table encompasses both years and only data taken from high-N plants.



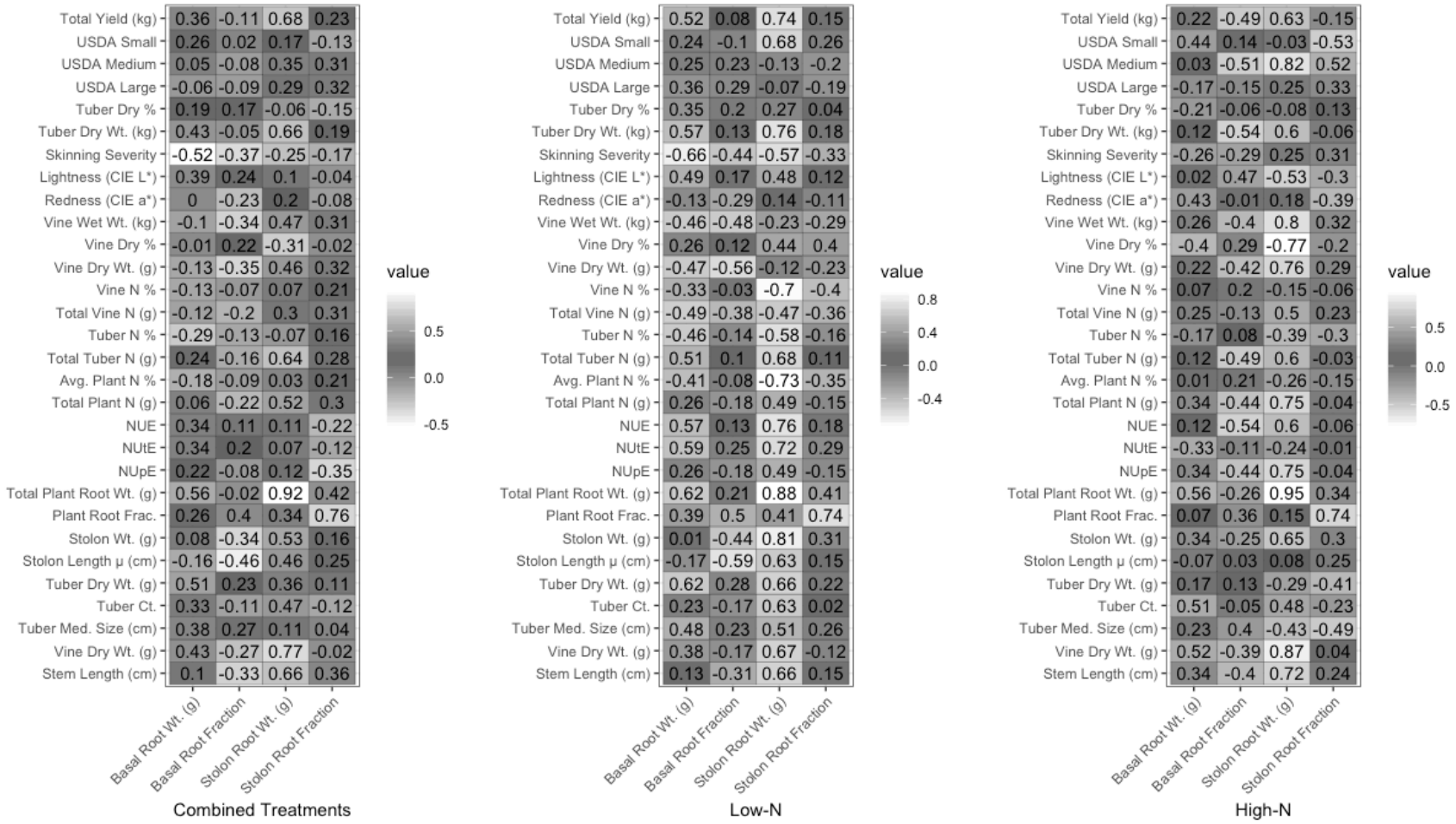
B.4 – Correlation of all low-N and high-N variables to each other, which are ordered and correspond to those from Table 3. Data taken from high-N plants is on the vertical axis and data from low-N plants is on the horizontal axis.



B.5 – Correlation of Y1-only data points: soil root wt., soil foot fraction, 40 DAP petiol N% and 80 DAP petiol N%.



B.6 – Correlation of Y2-only data points: basal root wt., basal root fraction, stolon root wt., stolon root fraction.



B.7 – Correlation scatterplots of yield between years, separated by treatment.