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Influence of heterozygosity on nitrogen use efficiency in hybrid and purebred lines of *Brassica napus* (L.)

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Abstract

Consumption of nitrogenous fertilizers has increased dramatically over the past 50 years and is expected to rise ever further in the future as the global demand for agriculture outputs/products increases. Improving the nitrogen use efficiency (NUE) of crop plants is considered to be an important step for improving the sustainability of agricultural systems. 30 *Brassica napus* cultivars, 20 hybrid and 10 purebred lines, were grown in a greenhouse under two N levels, 0 and 200 kg N/ha N fertilization, then phenotyped. The influence of root traits with respect to seed mass and NUE were investigated and discussed, along with seed quality parameters among the cultivar groups. Additionally, Wilcoxon rank-sum tests on 36,456 polymorphic markers obtained from the Infinium *B. napus* 60K SNP chip were tested against phenotypic traits such as NUE and seed mass, to identify heterozygotic markers potentially associated with these traits under the contrasting N conditions. Heterozygotic markers that positively influence seed mass and NUE were identified, especially under conditions of no N fertilization. In addition, markers with which the absence influences seed mass and NUE were also identified. The methods used in this study represent a novel way to investigate heterosis in elite lines of *B. napus* and could be a useful tool for helping to improve and investigate phenotypic traits such as NUE. Future studies based on the results obtained herein are discussed.

Acknowledgements

I would like to thank Professor Rod Snowdon for allowing me to do my master's thesis in his department. Andreas Stahl for his help, supervision and guidance throughout the project, which he was kind enough to let me join. I would also like to thank Stavros Tzigos for assistance and guidance in the lab work, Birgit Samans for supplying information on SNP positions along the *Brassica napus* genome, and the many interns at the Department of Agronomy and Plant Breeding I, Justus-Liebig Universität, Gießen, for their help in data collection. Lastly, I would like to thank my family for supporting me in my educational goals.

Table of Contents

List of Figures

List of Tables

List of Equations

List of Abbreviations

BBCH = **B**iologische Bundesanstalt, **B**undessortenamt und **Ch**emische Industry, a number system describing the different stages of plant growth

FAO = Food and Agricultural Organization of the United Nations

N = Nitrogen

N1 = The treatment of no N fertilization

N2 = The treatment of 2 applications of 100 kg N/ha fertilization

NF = Nitrogenous Fertilizer

NFC = Nitrogenous Fertilizer Consumption

NIRS = Near Infrared Spectrophotometry

NUE = Nitrogen Use Efficiency

NupE = Nitrogen Uptake Efficiency

NutE = Nitrogen Utilization Efficiency

QTL = Quantitative Trait Loci

1 Introduction

1.1 *Brassica napus*

Brassica napus L., known by its common name as rape, is a member of the Brassicaceae family, and a very important oilseed crop. As of 2010, *B. napus* had an estimated annual value of C$15.4 billion in Canada (Rempel *et al.*, 2014), the largest *B. napus* producing country, in terms of both total production (Figure 1a) and area harvested (Figure 1b). *B. napus* is a amphidiploid species (AACC, 2n = 38), containing the full diploid genomes of *B. rapa* (AA, 2n = 20) and *B. oleracea* (CC, 2n = 18), that arose from interspecific hybridization followed by genome duplication enabling a stable genome. Similar events have occurred between another *Brassica* species, *B. nigra*, (BB, 2n = 16) producing *B. carinata* (BBCC, 2n = 34) and *B. juncea* (AABB, 2n = 36), described in a theory termed the Triangle of U (U, 1935). Evolutionarily speaking, *B. napus* is a very new plant species, thought to have appeared only after its parental species, *B. rapa* and *B. oleracea*, were cultivated in close geographical proximity (Friedt & Snowdon, 2010).

Production of *B. napus* was limited until the 1980's (Figure 1a), after the development of the so called "0" and "00" varieties beginning in the mid 1960's. Natural *B. napus* varieties had high levels of erucic acid (C22:1, cis 13-docosenoic acid), a fatty acid with bitter taste and health implications, and glucosinolates, which rendered the seed unusable for livestock feed after oil extraction (Friedt & Snowdon, 2010). Breeders in Manitoba, Canada reduced the erucic acid content from 28-42% to less than 1% creating the first "0" cultivars (Stefansson & Hougen, 1964). After the identification of the Polish "Bronowski" cultivar, with low levels of glucosinolates (Josefsson & Appelqvist,

1968), the Manitoba breeders were able to develop a “00” cultivar, “Tower”, with yields equivalent to the standard cultivar at the time, “Target” (Stefansson & Kondra, 1975). These “00” cultivars, with low levels of erucic acid and glucosinolates, became referred to as canola (can = Canada, ola = oil), a term which was later trademarked by the Western Canadian Oilseed Crushers Association in 1978 to distinguish these superior seeds from other rapeseed (canolacouncil.org). These breeding successes have since made *B. napus* the third largest vegetable oil source in the world (fas.usda.gov).

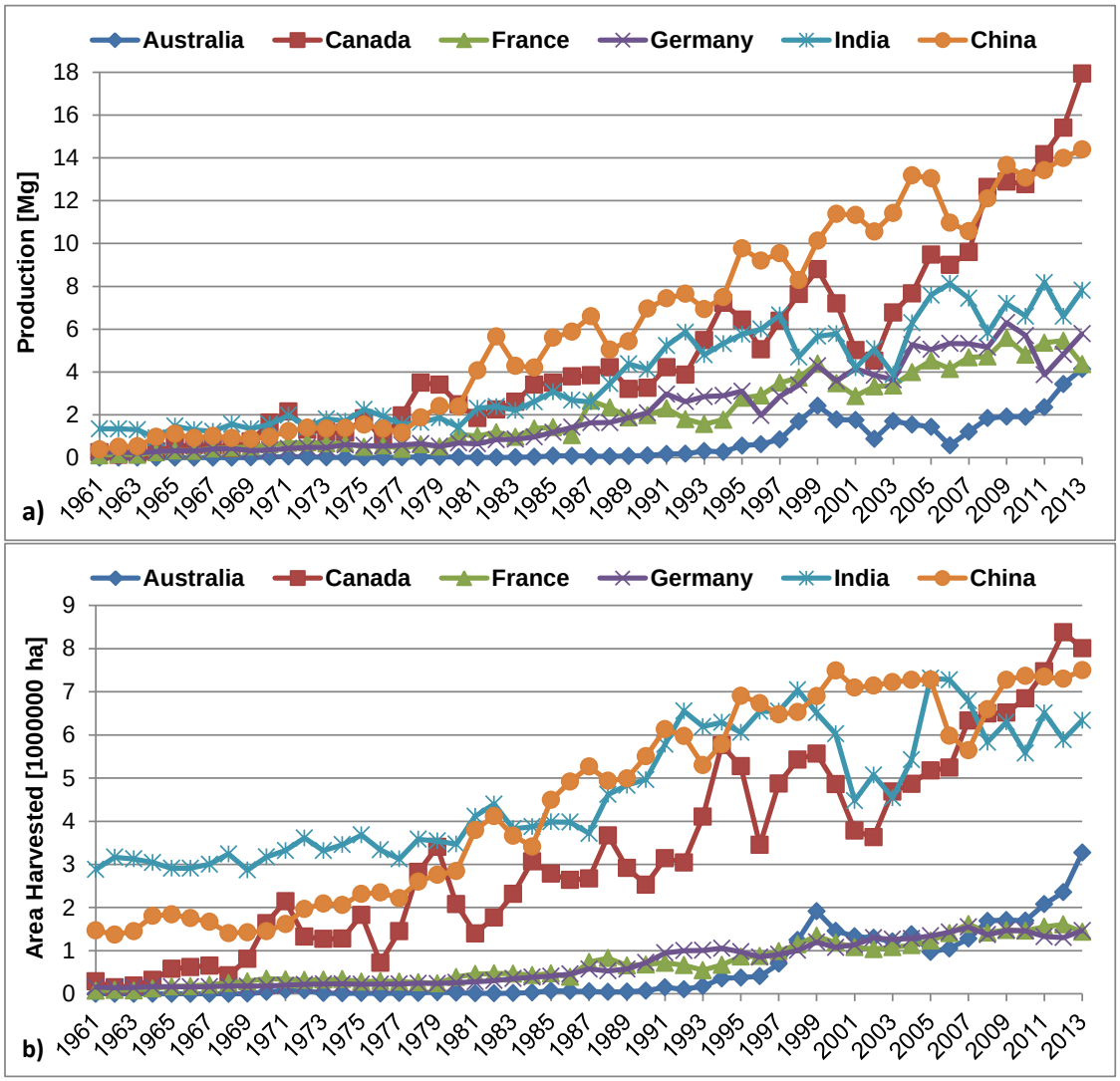


Figure 1: *Brassica napus* (a) production and (b) area harvested in Australia, Canada, France, Germany, India and China from 1961 to 2013. Data source is http://faostat.fao.org/faostat.

1.2 Fertilizer Use

Nitrogen (N) is an important part of the structure of both amino and nucleic acids, making it a macronutrient for all life on earth. The importance of nutrients for plant growth was first realized by the German botanist Carl Sprengel (1787-1859), in his Law of the Minimum, which states that the growth of an organism is limited by the scarcest nutrient, the concept behind fertilizer use. His ideas were later popularized by Justus von Liebig (1803-1873), “the father of the fertilizer industry”, who recognized nitrogen as one of the limiting nutrients. In 1913, the Haber-Bosch process was developed, allowing for the economic production of ammonia (NH_4) from atmospheric nitrogen (N_2) and, thus, industrial scale synthetic nitrogenous fertilizer (NF) production. Often the limiting element in plant growth, the application of synthetic NF has been used to greatly increase crop yields (Hatfield & Prueger, 2004), illustrated by Figure 2. This increase in yield due to the application of synthetic NF has been labeled as the “Detonator of the population explosion” (Smil, 1999), facilitating the 4.5 fold increase in the world population from 1.6 billion in 1900 to todays 7.2 billion (census.gov).

Since the 1960’s, worldwide consumption of NF has steadily increased (Figure 3). While the benefits of fertilizer use are obvious, their overuse has potential health and environmental problems, such as air and water pollution (Muhammad *et al.*, 2013). In addition, economic costs must also be considered. In cereal production, up to 67% of the NF is lost, representing an economic loss of $15.9 billion USD annually (Raun & Johnson, 1999). Combined with the high energy requirement for producing NF through the Haber-Bosch process, improved efficiency in nitrogen use has been suggested as a critical step required for the development of a sustainable agricultural system, with

which breeding programs should focus (Weisler *et al.*, 2001; Fess *et al.*, 2011). Breeding for crops with higher nitrogen use efficiency (NUE) could be used to both increase yields and help reduce the global demand for NF by allowing for sustained yields with less fertilizer input.

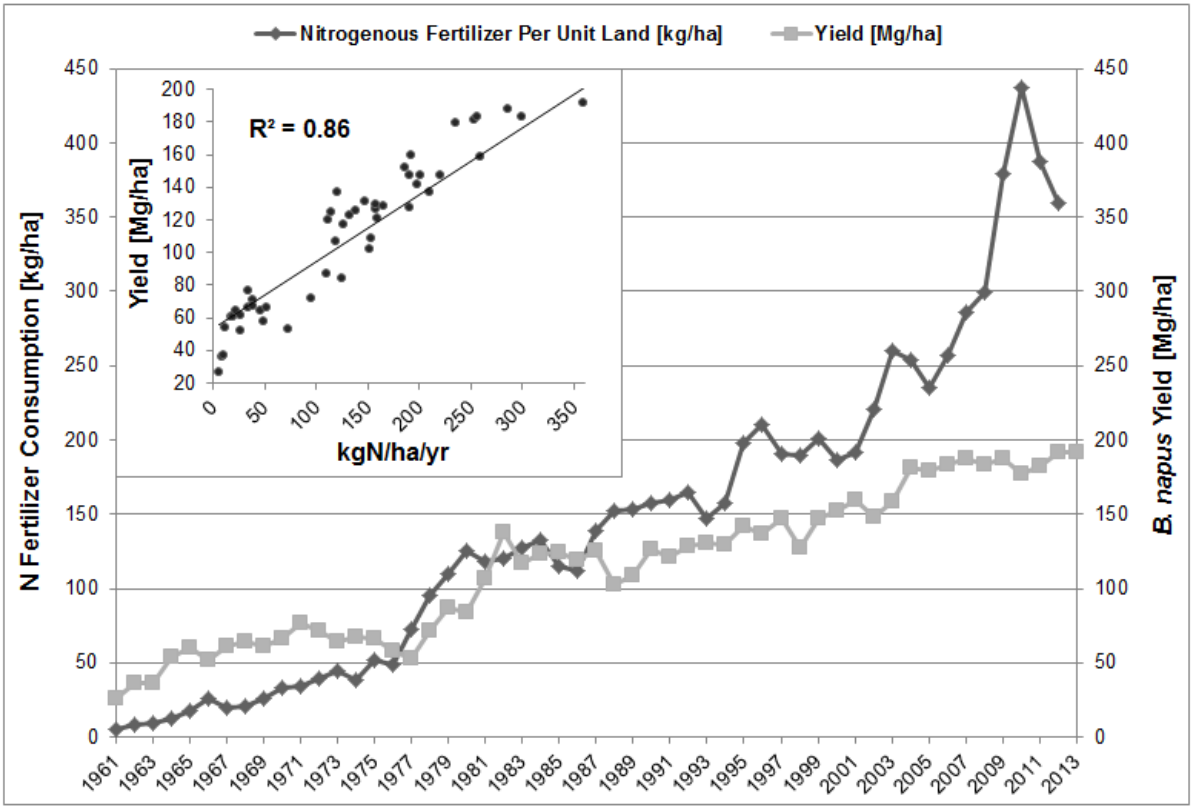


Figure 2: Correlation between nitrogenous fertilizer consumption and *Brassica napus* yield in China. Data source is http://faostat.fao.org/faostat.

1.3 Nitrogen Use Efficiency (NUE)

For crop plants, there are two ways researchers have calculated NUE. In the first method, which will be used in this study, the amount of N in the product produced is divided by the available N (Equation 1), giving a number that represents the proportion of available N the plant was able to allocate to its product. In the second method, the amount of product produced is divided by the available N (Equation 2), ignoring the products N content. Often, NUE is calculated using the latter, since data for the amount

of N in product often does not exist and since product produced per available N is more applicable to the farmer.

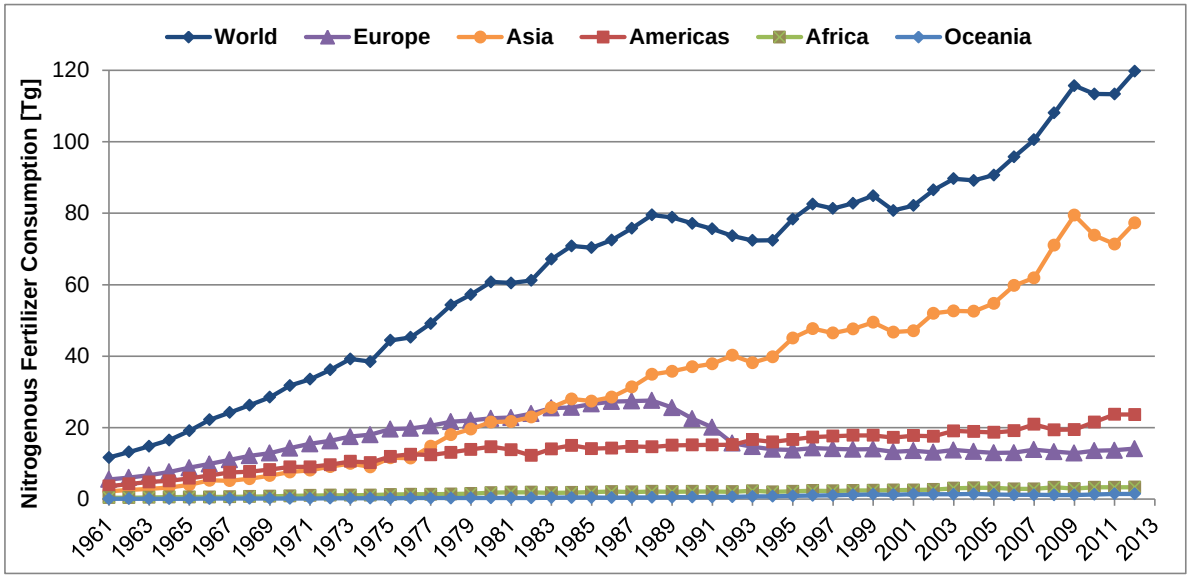


Figure 3: Worldwide and regional nitrogenous fertilizer consumption from 1961 to 2012. Data source is http://faostat.fao.org/faostat.

Equation 1: Nitrogen use efficiency (NUE) calculation, method 1.

$$N\ in\ Seeds / Available\ N = NUE$$

Equation 2: Nitrogen use efficiency (NUE) calculation, method 2.

$$Yield / Available\ N = NUE$$

Using data from the Food and Agricultural Organization of the United Nations (FAO) and defining NUE using Equation 2, as a region's total grain production divided by their total nitrogenous fertilizer consumption (NFC), Hatfield & Prueger (2004) showed a strong decrease of NUE in South America, China, Australia and Africa since 1961, with the USA decreasing slightly and Western Europe remaining the same. Lassaletta *et al.* (2014) calculated 50-year trends of NUE in 124 countries using Equation 1, by utilizing annual yield data for 178 primary crops and their corresponding

average N content, divided by total N input, which included synthetic NF, biological N fixation, manure application and atmospheric deposition. Their data showed diverse trends among the different countries. Canada and Australia, which originally had high NUE, decreased sporadically with increased N availability (Figure 4). China, which originally had a high NUE, has seen major decreases in NUE (Figure 4), coinciding with their tremendous increase in NFC per unit land (Figure 5). India, also decreased NUE with increasing available N levels but managed to halt further NUE decreases with total N inputs beyond ~70 kgN/ha/yr (Figure 4). Germany and France, which originally had low NUE, have seen a major increase in NUE (Figure 4), attributable to a decrease in NFC per unit land during 1988-1993 (Figure 5), and the ability to maintain general increases in crop yield, as can be seen with *B. napus* (Figure 6). Unfortunately data for NFC for specific crops does not exists and therefore NUE in *B. napus* cannot be estimated without making assumptions of equal fertilization among all crop land, which is known to be incorrect (Heffer, 2013). Even in countries such as China, it is not known how much of the increases in NFC is actually occurring in *B. napus* production or other major, NF intensive crops such as *Oryza sativa* (rice) and *Triticum* spp. (wheat).

NUE is an integration of both nitrogen uptake efficiency (NupE), how well the plant can acquire the available nitrogen in the soil, and nitrogen utilization efficiency (NutE), the fraction of acquired nitrogen that is used for seed production (Moll *et al.*, 1982). These are important to distinguish, because NUE can be limited by either factor. In *B. napus*, NutE is thought to be the limiting factor in its NUE. Relative to plant species from Poaceae and Fabaceae, the Brassicaceae have a high NupE (Laine *et al.*, 1993). Despite this, *B. napus* has a low NUE due to its low NutE, caused by N lost to the soil in

aborted leaves. This has been illustrated in investigations of N remobilization using ^{15}N labelled nitrogen (Schjoerring *et al.*, 1995; Rossato, 2001; Malagoli *et al.*, 2005b) and via N content determination of plant material (Hocking *et al.*, 1997; Leleu *et al.*, 2000). Recent studies under contrasting N fertilization (high and low) have shown differences based on the amount of available N, an approach which is critical for understanding NUE (Kant *et al.*, 2011), and which was used in this study. Under low N fertilization, NupE has a stronger effect on NUE in *B. napus*, while at high N fertilization, NutE is more important (Kessel *et al.*, 2012; Nyikako *et al.*, 2014). However, it should be noted that this also depends on genotype and environmental variation. Based on models from their ^{15}N labelled nitrogen experiments, Malagoli *et al.* (2005a) suggest that yield or N content could be increased by up to 15% through optimization of NutE in *B. napus*.

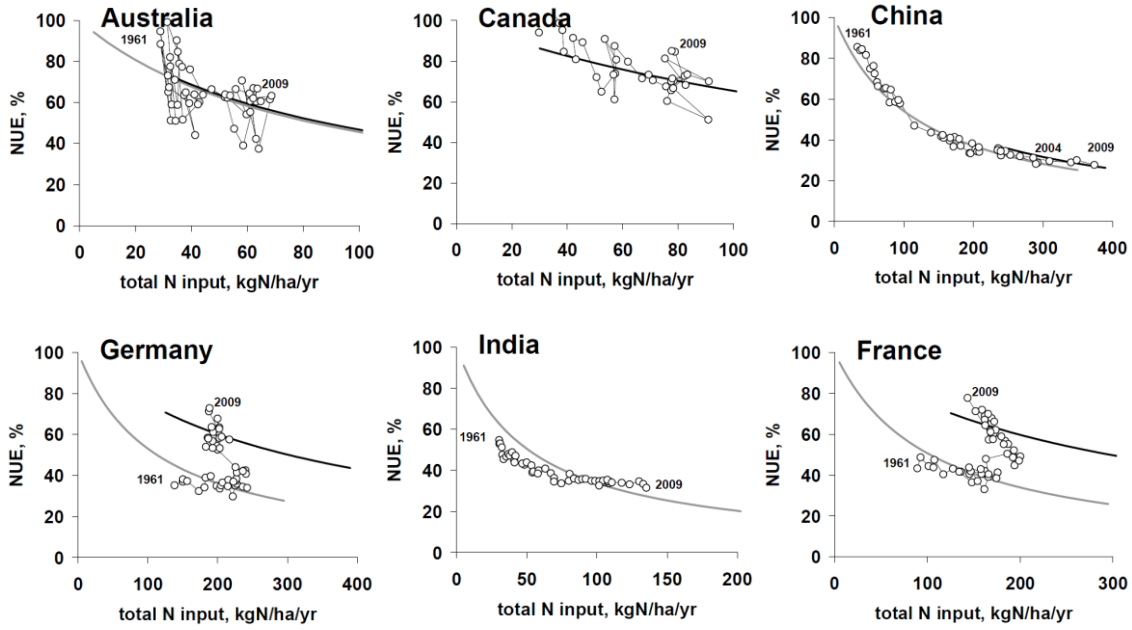


Figure 4: Nitrogen use efficiency (NUE) in Australia, Canada, China, Germany, India and France from 1961 to 2009. Taken with permission from Lassaletta *et al*. (2014) Supplemental Material 2 Results.

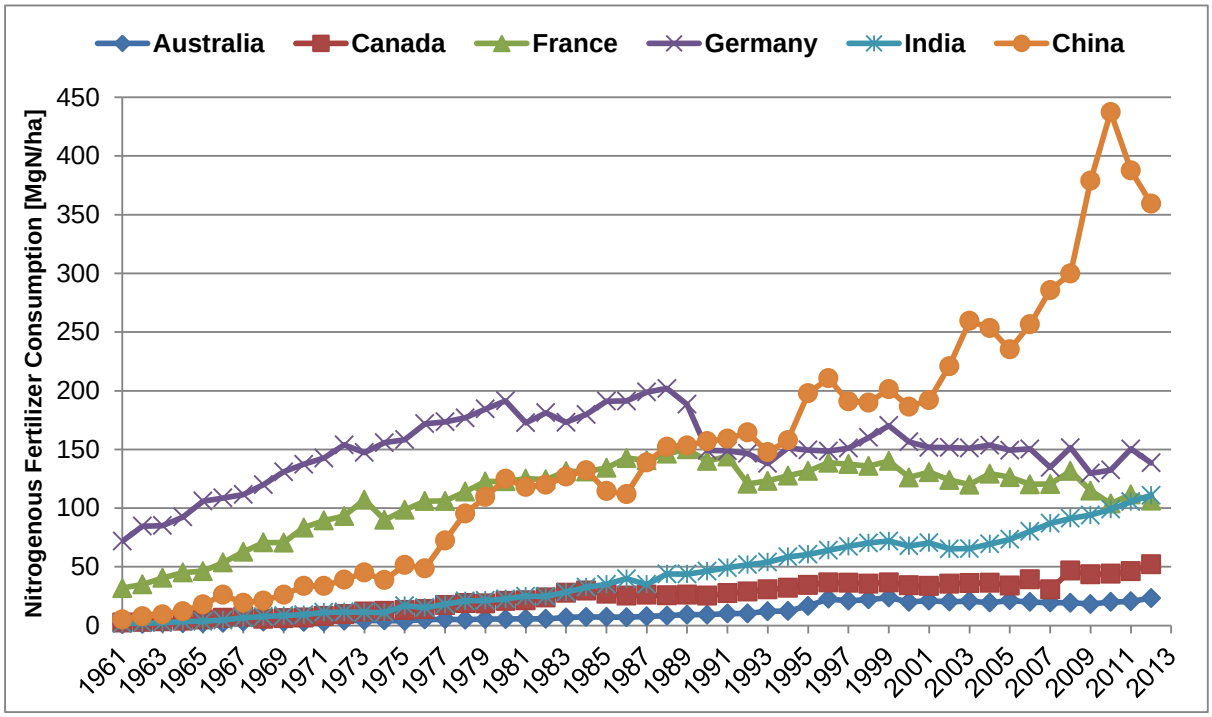


Figure 5: Nitrogenous fertilizer consumption per hectare for Australia, Canada, France, Germany, India and China from 1961-2012. Data source is http://faostat.fao.org/faostat.

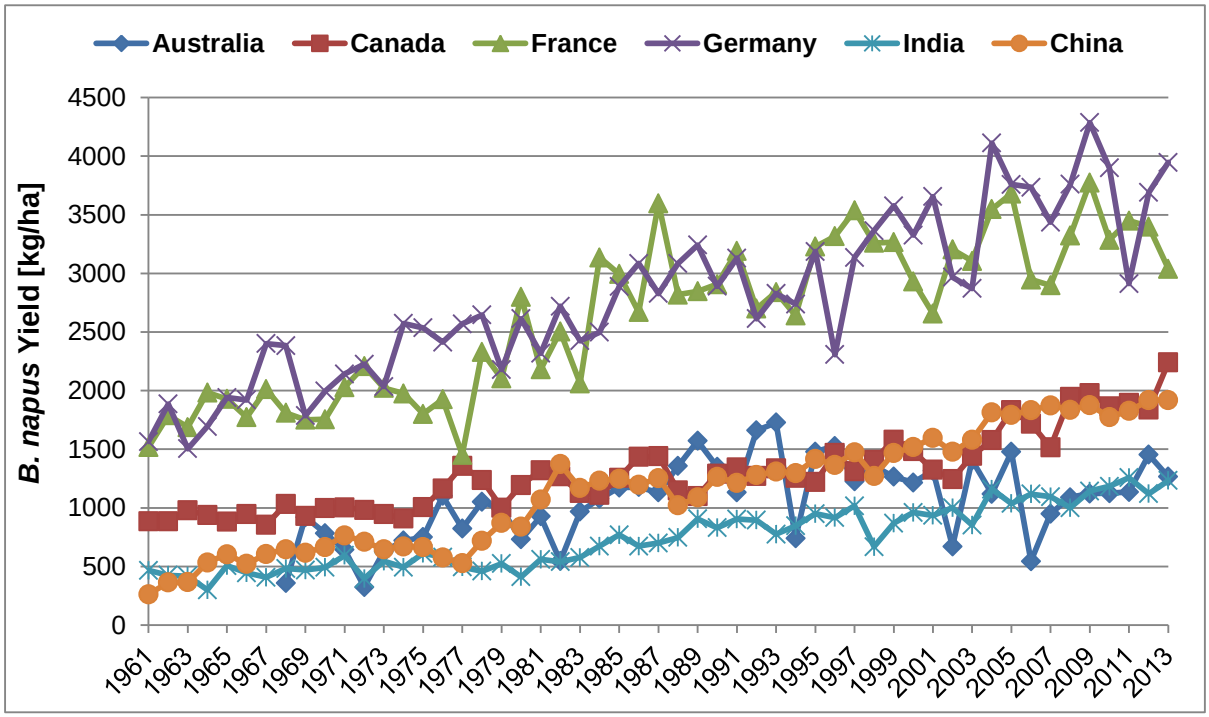


Figure 6: *B. napus* yield in Australia, Canada, France, Germany, India and China from 1961-2002. Data source is http://faostat.fao.org/faostat.

1.4 Heterosis & Hybrids

Hybrid vigor, the phenomenon of improved phenotypic performance in the offspring of crossed purebred/inbred lines, or heterosis, has been observed in various plant species for over a hundred years. Darwin described heterosis in *Brassica oleracea* in 1876, however, the term “heterosis”, was coined later in 1914 (Shull). Commercial hybrid seed, was first sold in 1924 (Crow, 1998), using a four-way hybrid breeding system developed by Jones (1922). Figure 7 illustrates the benefits of hybrid breeding programs to *Zea mays* (maize/corn) production in the USA. Hybrid production and utilization of heterosis have since become an important breeding aspect for many crops, including *B. napus*.

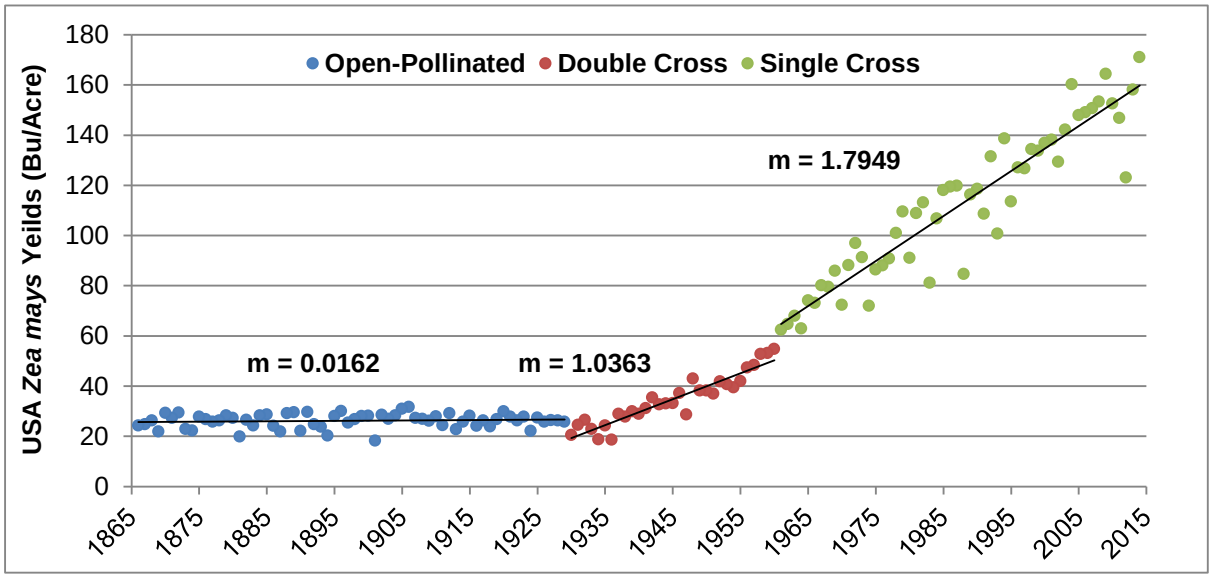


Figure 7: *Zea mays* (maize/corn) yields (bushels per acre) in the USA from 1866 to 2014. m = slope. Data source http://www.nass.usda.gov/.

It wasn’t until 1908 that hypotheses about heterosis were first proposed, Dominance (Davenport, 1908) and Codominance (East, 1908; Shull, 1908). In the Dominance hypothesis, heterosis occurs when slightly deleterious recessive alleles, present in the inbreed parents, are masked/complemented by the presence of a less-

deleterious dominant allele, *i.e.*, the opposite of inbreeding depression. According to the Codominance hypothesis, heterosis arises from the phenotypic superiority of heterozygotic loci. Since 1908, many investigations into heterosis have been conducted, but have yet to provide a complete understanding of the molecular mechanisms underlying the phenomenon. Instead, heterosis appears to result from a diversity of complex mechanisms (Schnable & Springer, 2013). Emerging models describe heterosis as "the cumulative positive effect of the differential expression of a variety of genes, on one or several yield-affecting metabolic pathways or overall energy-use efficiency" (Baranwal *et al.*, 2012).

In order to implement a hybrid breeding program, pollination must be controlled. For bisexual plants, such as *B. napus*, male sterility is the most efficient way to achieve this. There are many ways to produce male sterility, including mechanical castration, chemical gametocides, or biological pollination control. Biological pollination control can also be accomplished through several mechanisms: self-incompatibility, cytoplasmic-encoded male sterility, nuclear-encoded male sterility and environment-sensitive genetic male sterility. Kempe & Gils (2011) provide a historical review of all of these options, with a focus on new genetically engineered approaches.

In *B. napus*, hybrid cultivation has only relatively recently become popular. Despite numerous early documentations of male sterility from different sources (Thompson, 1972; Shiga & Baba, 1973; Bannerot *et al.*, 1974), problems such as instability, negative effects of the male sterility genes, and lack of suitable restorer or maintainer lines inhibited their use in *B. napus* (Friedt & Snowdon, 2010). However, in the 1990's a number of methods, which have since seen commercial success, were developed,

including the Ogura (OGU) system (Kao *et al.*, 1992) and the Male Sterility Lembke (MSL) system (Frauen & Paulmann, 1999). In 1995 the first hybrid winter-type *B. napus* varieties were registered (Frauen & Paulmann, 1999), and by the 2003/2004 season, a hybrid cultivar, “Talent”, became the most widely cultivated *B. napus* winter-type variety in Germany (Friedt & Snowdon, 2010). An increased yield stability and adaptation to low input cropping systems (Budewig & Lèon, 2003; Friedt *et al.*, 2003) motivated farmers to make the switch from the open-pollinated purebred lines. Since their introduction in Germany, *B. napus* hybrids have consistently had a higher yield than purebred lines (Abbadi & Leckband, 2011). A recent focus of *B. napus* breeders is to utilize the heterosis of hybrid cultivars to help achieve a higher NUE and adaptability to lower levels of N fertilization (Friedt *et al.*, 2003; Gehringer *et al.*, 2007).

1.5 Purpose

With the recent release of the genomes of *B. napus* (Chalhoub *et al.*, 2014), and its two parental species *B. rapa* (Wang *et al.*, 2011) and *B. oleracea* (Liu *et al.*, 2014), new avenues for research on heterosis in *B. napu*s have opened. In addition, a high density *B. napus* Infinium SNP array with over 50,000 SNPs was developed in 2011, by an international Brassica SNP consortium in cooperation with Illumina Inc. (San Diego, CA, USA) and released in 2012 (Snowdon & Iniguez Luy, 2012; Edwards *et al.*, 2013). This new information is a valuable resource for both *B. napus* researchers and breeders, and was exploited in this study.

The purpose of this study was to measure NUE and related phenotypic characteristics, such as root mass and seed yield, in thirty varieties of both new and old, hybrid and purebred *B. napus* cultivars (Table 1), under both high (N2; 2x 100 kg N/ha)

and no nitrogen fertilization (N1). In addition, marker analysis with the 60 k Illumina SNP chip was conducted on all varieties in order to investigate possible relationships of heterozygosity to phenotypic traits such as NUE and seed yield. The goal was to identify markers and regions of the *B. napus* genome with which heterozygosity correlates with improved phenotypic traits, something which could be advantageous to hybrid breeders. This study tested the hypothesis that heterozygosity within specific regions of the *B. napus* genome contributes to improved phenotypic traits, such as NUE or seed yield, and that these regions can be identified using a large data set of SNP markers of known genomic location, information which could be utilized as a selection tool in hybrid breeding programs.

2 Materials & Methods

2.1 Plant Growth

Thirty *B. napus* varieties, 20 hybrid and 10 purebred lines, both old and new (Table 1), were grown in a greenhouse (Figure 8b). For each experimental replicate of genotype and fertilizer treatment, nine plants were grown in containers of 0.16 m^2 surface area (Figure 8a), filled with 147.5 kg of soil with a dry matter content of 88.2% (130.1 kg dry mass; Table 2), in the layout described in Figure 9, with each being repeated once (n=2). Two fertilizer treatments were given, no N fertilization (N1) and 200 kg N/ha (N2), administered through two applications of 100 kg/ha N fertilization by applying 1.6 g N to each container (Equation 3) at growth stages of BBCH 18 and 53-55 (Julius Kühn Institut, 2001). The amount of N available from the soil was calculated by adding up the amount of available N per kg of soil from nitrate (NO_3) and ammonium (NH_4) and assuming the organic N is unavailable (Table 2). Some of the organic N may become

mobilized, and available for the plants, but since the amount is not known, it is excluded from use in Equation 4. Plants which received no N fertilization (N1) had 677 mg/plant of available N (Equation 4), while the plants which received 200 kg N/ha (N2) had 1033 mg/plant of available N (Equation 5). A timeline for actions taken during the growth of the *B. napus* plants, including fertilization dates, can be found in Table 3. Information on fertilizers used, including the presown fertilizer applied to all soils, is described in Table 4.

Table 1: Information on genotypes used in the study. MSL = Male Sterility Lembke, OGU = Ogura, SC = Safe-Cross, GMS = Genic Male Sterility, NPZ = Norddeutsche Pflanzenzucht , DSV = Deutsche Saatveredelung AG, MTO = Monsanto Deutschland GmbH, SW = SW Seed, today Syngenta Hadmersleben GmbH, LG = Limagrain GmbH, SYN = Syngenta Seeds GmbH, BCS = Bayer Crop Sciences AG.

	Hybrid Type	Breeder	Year of Release
A. new Hybrids			
NPZ 1203 Z (HZH)	MSL	NPZ	-
Troy (HZH)	MSL	DSV	-
Marathon	MSL	DSV	2013
Mercedes	MSL	NPZ	2013
Avatar	MSL	NPZ	2011
DK Exstorm	OGU	MTO	2011
Inspiration	OGU	DSV	2011
Genie	MSL	DSV	2011
Mascara	SC	SW	2011
Artoga	OGU	LG	2010
Sherpa	MSL	NPZ	2010
Compass	MSL	DSV	2009
NK Linus	GMS	SYN	2009
Visby	MSL	NPZ	2007
B. older Hybrids			
Exocet	OGU	DSV	2005
Taurus	MSL	NPZ	2004
Baldur	MSL	NPZ	2002
Elektra	MSL	BCS	2002
Ryder	OGU	SW	2000
Artus	MSL	NPZ	1997

	Hybrid Type	Breeder	Year of Release
C. new Lines			
Patron	-	BCS	2012
Trinity	-	SW	2012
Adriana	-	LG	2007
Lorenz	-	NPZ	2005
Oase	-	DSV	2004
D. older Lines			
Pacific	-	LG	2003
Californium	-	MTO	2002
Aviso	-	SW	2000
Express	-	NPZ	1993
Lirajet	-	DSV	1989

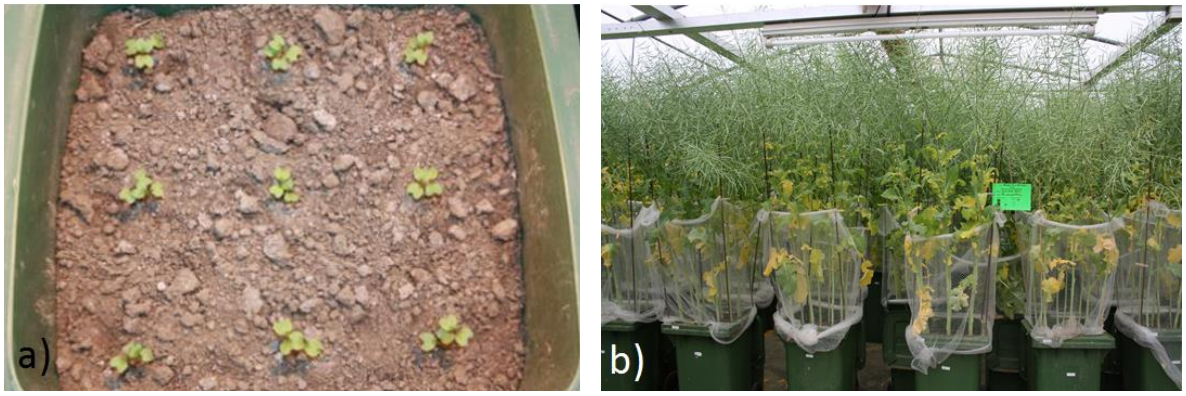


Figure 8: Photographs of (a) the plant density and (b) the containers. Photographs taken by Andreas Stahl.

Replication I							
N2	N1		N2	N1		N2	N1
1 Major	24 Major		25 Groß Lüsewitzer	48 Groß Lüsewitzer		49 Madrigal	72 Madrigal
2 Pacific	23 Pacific		26 Californium	47 Californium		50 Trinity	71 Trinity
3 Marathon	22 Marathon		27 Lirajet	46 Lirajet		51 Baldur	70 Baldur
4 Troy	21 Troy		28 Patron	45 Patron		52 Mercedes	69 Mercedes
5 Elektra	20 Elektra		29 Mascara	44 Mascara		53 Lorenz	68 Lorenz
6 NPZ 1203 Z	19 NPZ 1203 Z		30 Compass	43 Compass		54 Ryder	67 Ryder
7 DK Exstorm	18 DK Exstorm		31 Adriana	42 Adriana		55 Aviso	66 Aviso
8 NK Linus	17 NK Linus		32 Exocet	41 Exocet		56 Sherpa	65 Sherpa
9 Avatar	16 Avatar		33 Artus	40 Artus		57 Express	64 Express
10 Oase	15 Oase		34 Genie	39 Genie		58 Taurus	63 Taurus
11 Artoga	14 Artoga		35 Visby	38 Visby		59 Inspiration	62 Inspiration
12 Lirajet	13 Lirajet		36 Oase	37 Oase		60 Pacific	61 Pacific
Replication II							
N2	N1		N2	N1		N2	N1
73 Major	96 Major		97 Expert	120 Expert		121 Expert	144 Expert
74 Sherpa	95 Sherpa		98 Express	119 Express		122 Taurus	143 Taurus
75 Aviso	94 Aviso		99 Baldur	118 Baldur		123 Elektra	142 Elektra
76 NK Linus	93 NK Linus		100 Mascara	117 Mascara		124 Trinity	141 Trinity
77 Ryder	92 Ryder		101 Marathon	116 Marathon		125 Visby	140 Visby
78 Lirajet	91 Lirajet		102 Artus	115 Artus		126 Troy	139 Troy
79 Adriana	90 Adriana		103 Oase	114 Oase		127 DK Exstorm	138 DK Exstorm
80 Inspiration	89 Inspiration		104 Lorenz	113 Lorenz		128 Mercedes	137 Mercedes
81 Genie	88 Genie		105 Pacific	112 Pacific		129 Avatar	136 Avatar
82 Compass	87 Compass		106 Artoga	111 Artoga		130 Exocet	135 Exocet
83 Patron	86 Patron		107 Californium	110 Californium		131 NPZ 1203 Z	134 NPZ 1203 Z
84 Lirajet	85 Lirajet		108 Oase	109 Oase		132 Pacific	133 Pacific

Figure 9: Layout of containers grown in the greenhouse. Shaded containers were used as borders and not for phenotypic data analysis. N1 = no fertilizer treatment. N2 = 100 kg/ha applied at BBCH 18 and BBCH 53-55 (Figure 3).

Table 2: Values of specific soil characteristics.

Wet Soil Mass [kg]	Dry Soil Mass [kg]	Dry Matter Content [%]	P [mg/kg]	K_2O [mg/kg]	Total N [mg/kg]	NO3 [mg/kg]	NH_4 [mg/kg]	Organic N [mg/kg]
147.5	130.1	88.2	51.2	9.52	54.675	44.65	2.15	7.875

Equation 3: Calculation of N fertilization when 1.6 g N is added to container.

$$(1.6\ g\ N) \cdot \left(\frac{1\ kg}{1000\ g}\right) \cdot \left(\frac{1}{0.16\ m^2}\right) \cdot \left(\frac{10000\ m^2}{1\ ha}\right) = 100\ \frac{kg\ N}{ha}$$

Equation 4: Calculation of available N in soil (N1).

$$N1 = \frac{\left(44.65\ \frac{mg\ N}{kg\ soil} + 2.15\ \frac{mg\ N}{kg\ soil}\right) \cdot (130.1\ kg\ soil)}{9\ plants} = 677\ \frac{mg\ N}{per\ plant}$$

Equation 5: Calculation of available N with fertilization of 200 kg N/ha (N2).

$$N2 = 677\ \frac{mg\ N}{per\ plant} + \frac{2 \cdot (1600\ mg\ N)}{9\ plants} = 1033\ \frac{mg\ N}{per\ plant}$$

Table 3: Timeline for actions taken during the greenhouse growth of *Brassica napus* plants. BBCH = (**B**iologische Bundesanstalt, **B**undessortenamt und **Ch**emische Industry) *Brassica napus* life cycle stage according to (Julius Kühn Institut, 2001).

Date	Action
2013-10-30	Presowing fertilization of container
2013-11-04	Sowing Light were switched on from 8 a.m. to 9 p.m
2013-12-09	Supplementation of missing plants
2014-01-13	Light were switched on from 8. a.m. to 5 p.m.
2014-01-14	Thin out to final plant density of 9 plants per container
2014-03-06	1. N-Fertilization at BBCH 18 N1: no Fertilization N2: 1,6 g N via NH_4NO_3 in 1 L Water (=100 kg N/ha)
2014-03-10	Final container position
2014-03-31	From now watering to 75% Water capacity according to weight
2014-04-02	2. N-Fertilization at BBCH 53-55 N1: no Fertilization N2: 1,6 g N via NH_4NO_3 in 1 L Water (=100 kg N/ha)
2014-04-04	Application of Biscaya (Insecticide) 300 mL/ha in 600L/ha water
2014-05-27	Application of Proline (Fungicide) 0,7L/ha (because of powdery mildew)
2014-06-30	Start with container harvest
2014-07-15	Container harvest completed

Table 4: Fertilizers used in the growth of *Brassica napus* plants: (a) presown fertilizer and (b) nitrogenous fertilizer.

Fertilizer	Nutrient	Nurient Content [%]	kg / ha	Nutrient [g] / Container	Fertilizer [g] / Container
Triple-Superphosphat 50% P_2O_5	P	22.0	**100.00**	1.60	7.27
	S	14.0	0.00	1.02	
Patentkali (30% K_2O, 10% MgO, 17% S)	K	25.0	**400.00**	6.40	25.6
	Mg	6.0	0.00	1.54	
	S	17.0	0.00	4.35	

a)

Fertilizer	Fertilizer [g] / 100 mL	Nutrient Content [%]	Nutrient [g] / 100 mL	Nutrient [g] / ha	Nutrient [g] / Container
Ammonium molybdate (1kg/ha) NH_4^+	0.029	54.435	0.0160	1000	0.0160
$MnSO_4*H_2O$	0.492	32.5	0.1600	10000	0.1600
$ZnSO_4 * 7H_2O$	1.407	22.74	0.3200	20000	0.3200
$CuSO_4*5H_2O$	0.629	25.45	0.1600	10000	0.1600
H_3BO_3	0.183	17.48	0.0320	2000	0.0320

b)

N Fertilization (g)	N Fertilization (kg/ha)	Availible N Per Container (g)
1.6	100	6.1074

2.2 Phenotypic Data Collection

Plant phenotypes were measured on a per container basis but converted to a per plant basis. For plant roots all plants were used, however, for the shoots 2 of the nine plants were removed for use in another project. The scoring of root hairs was done subjectively, through visual estimation on a scale of 1-4, with 1 representing a low amount of fine roots and 4 a high amount of fine roots. Seed oil and protein masses were determined through near infrared spectrophotometry (NIRS) measurements (Tkachuk, 1981; Tillmann & Paul, 1998; Tillmann *et al.*, 2000) using a Unity SpectraStar 2500 (Brookfield, USA). For this study, Equation 1 was used to calculate NUE.

2.3 Genotypic Data Collection

Plants for genotypic analysis were grown for three weeks in a greenhouse, after which leaves were harvested for DNA extraction. For each genotype, 4 replicates (n=5) were grown for separate DNA extraction. DNA extraction was performed using a BioSprint 96 and BioSprint 15 with their Plant DNA Kit (www.qiagen.com). Leaf material was frozen in liquid nitrogen and lysed with a TissueLyser II (Qiagen) set at 30 rotations per second for 30 seconds. 500 µL RLT buffer was added to the frozen plant material and then vortexed. All samples were centrifuged for 5 minutes at 13000 rpm. 250 µL of the supernatant was then used for DNA extraction with the BioSprint 15 or 96, which used 200 µL isopropanol with 20 µL MagAttract Suspension G, 500 µL RPW buffer (with RNase) and 500 µL 97% ethanol as reagents for DNA extraction, with the DNA being dissolved in 60 µL of H_20 and stored at -20°C.

DNA concentration was determined using the Qubit 2.0 and their dsDNA Assay Kit using standards of known concentration and dsDNA binding fluorescent stains

(www.lifetechnologies.com). DNA quality was checked using gel electrophoresis (1% agrose gel with 0.5x TBE buffer) of 10 randomly chosen samples (Figure 10). Genotype replicates were then pooled together and sent to TraitGenetics GmbH, Gatersleben, Germany (www.traitgenetics.com) for SNP determination using the 60 K *B. napus* SNP Chip (Snowdon & Iniguez Luy, 2012; Edwards *et al.*, 2013).

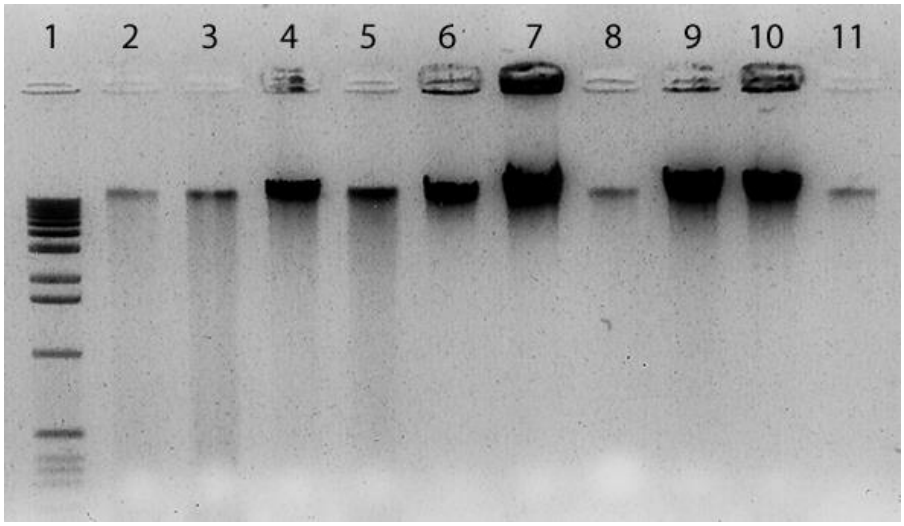


Figure 10: Gel electrophoresis of 10 randomly chosen DNA samples of varying DNA concentrations to check for quality.

2.4 Data Analysis

Data analysis was done with Excel, and the open-source statistical program R (www.r-project.org/). Marker data from the hybrid varieties were checked by analysing the markers of both parents. This allowed for determination of incorrect markers, if they did not match possible outcomes from the parents, and markers which may have been present as one copy, if one of the parents was "failed". There are a number of reasons to account for a "failed" marker: the absence of that SNP in the sample, errors in the equipment's ability to detect the SNP, such as insufficient light signal for detection, or the detection of more than two SNPs at the location. In the last situation since the DNA of five individuals was pooled, this could be due to multiple factors, such as

contamination from foreign pollen during seed production, or in the case of the hybrids, a situation in which marker combinations from the parents could produce two possible heterozygotes (*e.g.*, one parent is “AA” and the other is “CT”, resulting in offspring either “AC” or “AT”).

For our purposes, the SNP markers were converted to being either homozygous (“2”) or heterozygous (“3”), with hybrids having the additional options of markers determined to be incorrect (“4”), and those thought to be present in only one copy (“1”). The “failed” markers were converted to “0”. Genomic locations for 28,698 of the 52,157 markers (55%) were determined using a BLAST analysis. Markers that were monomorphic in our data set were filtered out, resulting in a loss of 15,701 of the 52,157 markers (30.1%), leaving 36,456 polymorphic markers. Wilcoxon rank-sum tests were conducted on all markers to test for their association with phenotypic traits such as NUE and seed yield using a statistical significance of $p < 0.01$ or $p < 0.05$.

3 Results

3.1 Phenotypic Data

3.1.1 Plant Masses & Seed Yield

The collection of winter-type *B. napus* hybrid and purebred cultivars exhibited phenotypic variation as expected. Genotypes showed variation in total plant mass, seed mass, root mass and root score (Figure 11; Figure 12). Under N2, there was an increase in plant masses and less variation compared to N1 (Figure 11; Figure 12). Patron is of particular interest due to its large root mass under both N treatments (Figure 11; Figure 12).

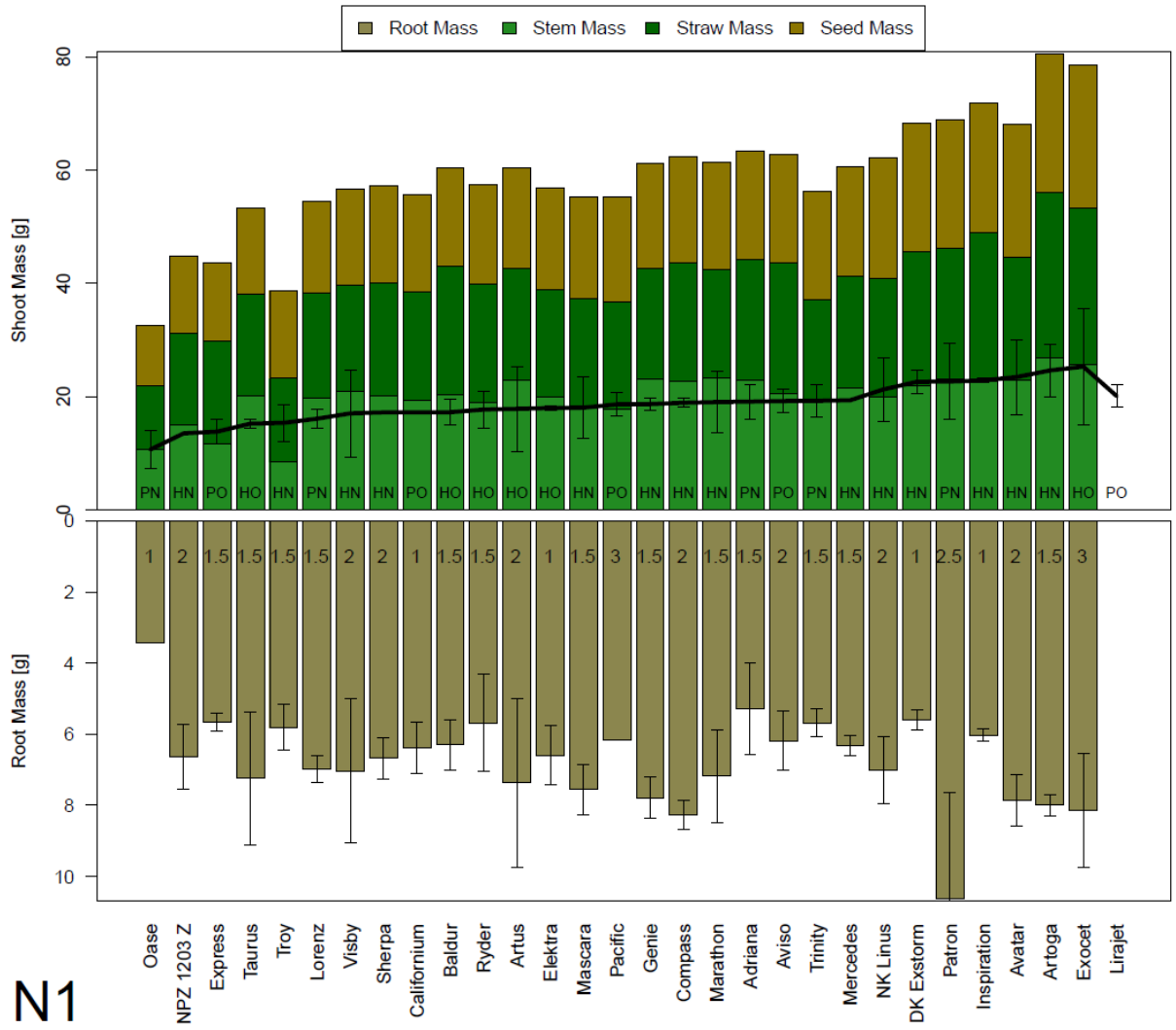


Figure 11: Plant masses of *B. napus* cultivars under no N fertilization. The top column and horizontal black line represent seed mass. The letters in the stem mass bars indicate the cultivar type and age; H = hybrid, P = purebred, N = new, O = old. The numbers in the root mass bars indicate the cultivars fine root score.

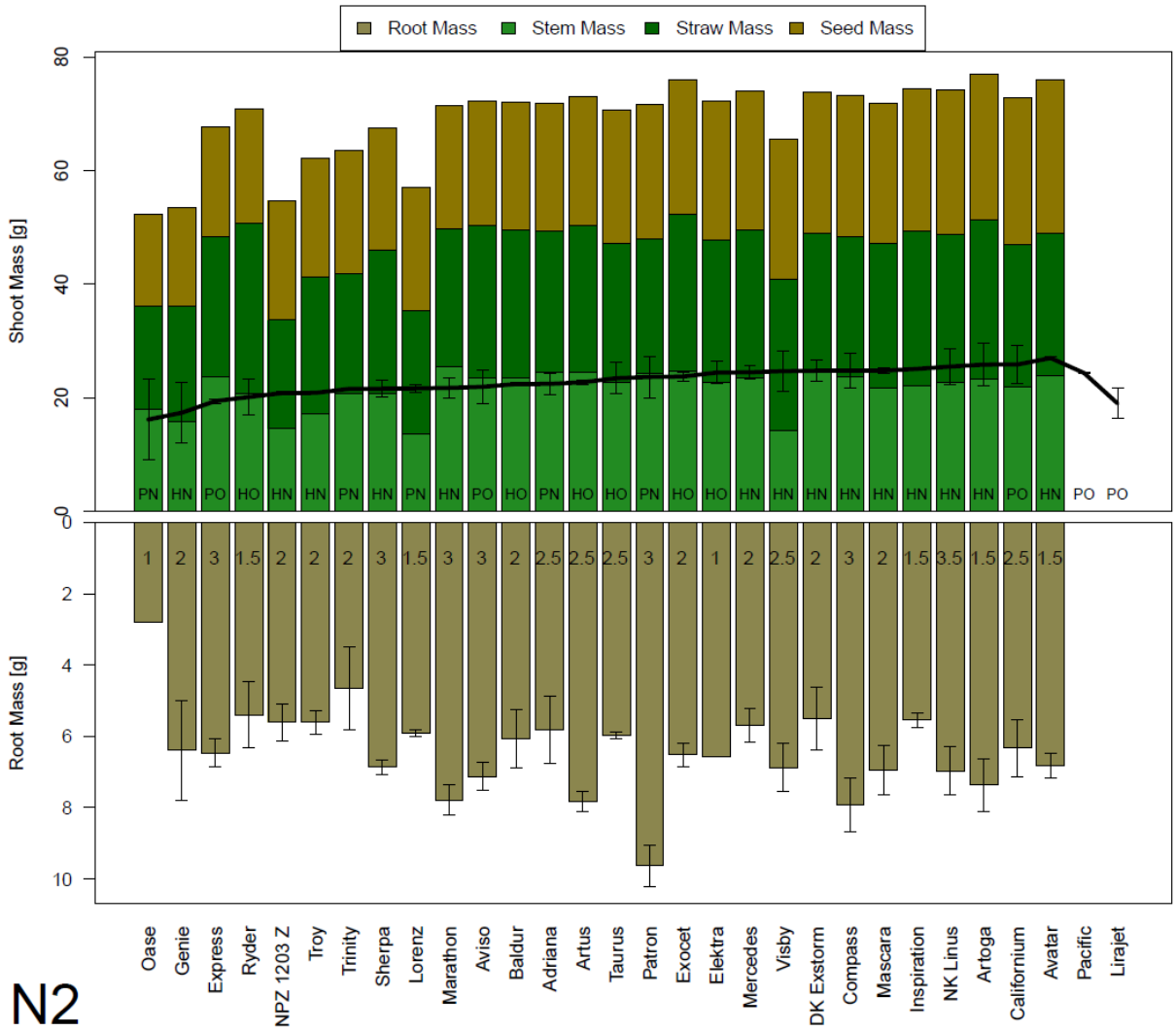


Figure 12: Plant masses of *B. napus* cultivars under 200 kg/ha N fertilization. The top column and horizontal black line represent seed mass. The letters in the stem mass bars indicate the cultivar type and age; H = hybrid, P = purebred, N = new, O = old. The numbers in the root mass bars indicate the cultivars fine root score.

Table 5 displays the phenotypic ranking of cultivars for seed mass and NUE under both N treatments. Among the top performers for seed mass and NUE under both N treatments there was high variation of breeders (Table 5). Under N1, Norddeutsche Pflanzenzucht cultivars represented many of the lowest performing cultivars for both seed mass and NUE, while under N2, Deutsche Saatveredelung cultivars represented many of the lowest performing cultivars for seed mass and NUE (Table 5). Under N1, the hybrids Exocet, Artoga, Avatar, Inspiration and the purebred Patron were the top performers for seed yield, respectively (Table 6a). Avatar, Californium, Artoga, NK Linus and Inspiration were the highest seed yield performers under N2, with Californium as the only one not a new hybrid (Table 6c). Comparing the top five seed mass performers to the bottom, root scores, plant masses and NUE were higher amongst the top five under both N treatments (Table 6a,c). Despite their nearly identical seed protein, seed, straw, stem and root masses, under N1, Argota and Exocet had different root structures. Artoga had a higher root length and a lower fine root score, compared to Exocet (Table 6a). Root score among the top five cultivars for seed mass ranged from 1-3 under N1 and 1.5-3.5 under N2 (Table 6). In the bottom five, root score ranged from 1-2 under N1 and 1-3 under N2 (Table 6).

Table 5: Ranking of *B. napus* cultivars for seed mass and nitrogen use efficiency (NUE) under no N fertilization (N1) and 200 kg/ha N fertilization (N2), along with cultivar type and breeder. NUE calculated using Equation 1. NPZ = Norddeutsche Pflanzenzucht , DSV = Deutsche Saatveredelung AG, MTO = Monsanto Deutschland GmbH, SW = SW Seed, today Syngenta Hadmersleben GmbH, LG = Limagrain GmbH, SYN = Syngenta Seeds GmbH, BCS = Bayer Crop Sciences AG.

	N1 Seed Mass		N2 Seed Mass		N1 NUE		N2 NUE	
1	HO Exocet	DSV	HN Avatar	NPZ	PN Patron	BCS	PO Californium	MTO
2	HN Artoga	LG	PO Californium	MTO	HO Exocet	DSV	HN NK Linus	SYN
3	HN Avatar	NPZ	HN Artoga	LG	HN Artoga	LG	PN Adriana	LG
4	HN Inspiration	DSV	HN NK Linus	SYN	HN NK Linus	SYN	HN Inspiration	DSV
5	PN Patron	BCS	HN Inspiration	DSV	PO Lirajet	DSV	PN Trinity	SW
6	HN DK Exstorm	MTO	HN Mascara	SW	HN Avatar	NPZ	HN Mascara	SW
7	HN NK Linus	SYN	HN Compass	DSV	PO Aviso	SW	HN Compass	DSV
8	PO Lirajet	DSV	HN DK Exstorm	MTO	HN Inspiration	DSV	HN Avatar	NPZ
9	HN Mercedes	NPZ	HN Visby	NPZ	HN DK Exstorm	MTO	HN Mercedes	NPZ
10	PN Trinity	SW	HN Mercedes	NPZ	PN Adriana	LG	HN NPZ 1203 Z	NPZ
11	PO Aviso	SW	HO Elektra	BCS	PN Trinity	SW	HO Artus	NPZ
12	PN Adriana	LG	PO Pacific	LG	HN Mascara	SW	PO Pacific	LG
13	HN Marathon	DSV	HO Exocet	DSV	HO Ryder	SW	PO Aviso	SW
14	HN Compass	DSV	PN Patron	BCS	PO Pacific	LG	HO Elektra	BCS
15	HN Genie	DSV	HO Taurus	NPZ	HO Elektra	BCS	HN Troy	DSV
16	PO Pacific	LG	HO Artus	NPZ	HN Marathon	DSV	PN Patron	BCS
17	HN Mascara	SW	PN Adriana	LG	HO Artus	NPZ	HO Ryder	SW
18	HO Elektra	BCS	HO Baldur	NPZ	HN Mercedes	NPZ	HN Artoga	LG
19	HO Artus	NPZ	PO Aviso	SW	HN Compass	DSV	HN Visby	NPZ
20	HO Ryder	SW	HN Marathon	DSV	HN Troy	DSV	HO Baldur	NPZ
21	HO Baldur	NPZ	PN Lorenz	NPZ	HN Sherpa	NPZ	HO Exocet	DSV
22	PO Californium	MTO	HN Sherpa	NPZ	HO Baldur	NPZ	HO Taurus	NPZ
23	HN Sherpa	NPZ	PN Trinity	SW	HN NPZ 1203 Z	NPZ	HN DK Exstorm	MTO
24	HN Visby	NPZ	HN Troy	DSV	PO Californium	MTO	HN Sherpa	NPZ
25	PN Lorenz	NPZ	HN NPZ 1203 Z	NPZ	HN Genie	DSV	PN Lorenz	NPZ
26	HN Troy	DSV	HO Ryder	SW	PN Lorenz	NPZ	PO Lirajet	DSV
27	HO Taurus	NPZ	PO Express	NPZ	HN Visby	NPZ	PO Express	NPZ
28	PO Express	NPZ	PO Lirajet	DSV	HO Taurus	NPZ	HN Marathon	DSV
29	HN NPZ 1203 Z	NPZ	HN Genie	DSV	PO Express	NPZ	HN Genie	DSV
30	PN Oase	DSV	PN Oase	DSV	PN Oase	DSV	PN Oase	DSV

Table 6: Top five and bottom five *B. napus* cultivar performers for (a) seed mass under no N fertilization (N1), (b) Nitrogen use efficiency (NUE) under N1, (c) seed mass under 200 kg N/ha fertilization (N2) and (d) NUE under N2. NUE calculated using Equation 1.

a) N1 Seed Mass

	Genotype		Root Score [1-4]	Root Length [cm]	Root Mass [g]	Stem + Straw Mass [g]	Seed Mass [g]	Seed Oil Mass [g]	Seed Protein Mass [g]	NUE
1	**Exocet**	**HO**	3	55	8.1	53.3	25.3	12.5	3.8	0.901
2	**Artoga**	**HN**	1.5	62.5	8.0	56.0	24.6	12.1	3.7	0.873
3	**Avatar**	**HN**	2	57.5	7.9	44.6	23.4	11.9	3.5	0.833
4	**Inspiration**	**HN**	1	62.5	6.0	48.9	22.9	11.5	3.5	0.818
5	**Patron**	**PN**	2.5	55	10.6	46.2	22.6	11.1	4.0	0.940
26	**Troy**	**HN**	1.5	62.5	5.8	23.3	15.4	7.3	2.8	0.665
27	**Taurus**	**HO**	1.5	55	7.2	38.0	15.2	7.5	2.5	0.580
28	**Express**	**PO**	1.5	67.5	5.7	29.9	13.8	6.8	2.4	0.569
29	**NPZ 1203 Z**	**HN**	2	55	6.6	31.3	13.5	6.2	2.6	0.623
30	**Oase**	**PN**	1	60	3.4	23.9	10.6	4.8	2.0	0.467

b) N1 NUE

	Genotype		Root Score [1-4]	Root Length [cm]	Root Mass [g]	Stem + Straw Mass [g]	Seed Mass [g]	Seed Oil Mass [g]	Seed Protein Mass [g]	NUE
1	**Patron**	**PN**	2.5	55	10.6	46.2	22.6	11.1	4.0	0.940
2	**Exocet**	**HO**	3	55	8.1	53.3	25.3	12.5	3.8	0.901
3	**Artoga**	**HN**	1.5	62.5	8.0	56.0	24.6	12.1	3.7	0.873
4	**NK Linus**	**HN**	2	55	7.0	40.9	21.3	10.2	3.6	0.840
5	**Lirajet**	**PO**	NA	NA	NA	NA	20.1	8.7	3.5	0.836
26	**Lorenz**	**PN**	1.5	65	7.0	38.3	16.1	7.6	2.6	0.604
27	**Visby**	**HN**	2	60	7.0	39.7	17.0	8.3	2.5	0.595
28	**Taurus**	**HO**	1.5	55	7.2	38.0	15.2	7.5	2.5	0.580
29	**Express**	**PO**	1.5	67.5	5.7	29.9	13.8	6.8	2.4	0.569
30	**Oase**	**PN**	1	60	3.4	23.9	10.6	4.8	2.0	0.467

c) N2 Seed Mass

	Genotype		Root Score [1-4]	Root Length [cm]	Root Mass [g]	Stem + Straw Mass [g]	Seed Mass [g]	Seed Oil Mass [g]	Seed Protein Mass [g]	NUE
1	**Avatar**	**HN**	1.5	50	6.8	48.9	27.0	13.1	4.4	0.675
2	**Californium**	**PO**	2.5	50	6.3	47.0	25.8	11.5	4.9	0.752
3	**Artoga**	**HN**	1.5	60	7.4	51.2	25.8	12.4	4.1	0.629
4	**NK Linus**	**HN**	3.5	62.5	7.0	48.7	25.5	12.0	4.6	0.710
5	**Inspiration**	**HN**	1.5	60	5.5	49.3	25.1	11.3	4.5	0.691
26	**Ryder**	**HO**	1.5	50	5.4	50.8	20.1	8.5	4.1	0.629
27	**Express**	**PO**	3	60	6.5	48.0	19.4	9.3	3.6	0.556
28	**Lirajet**	**PO**	NA	NA	NA	NA	19.0	7.8	3.6	0.563
29	**Genie**	**HN**	2	45	6.4	36.1	17.4	8.0	3.2	0.490
30	**Oase**	**PN**	1	55	2.8	41.7	16.2	7.0	3.1	0.488

d) N2 NUE

	Genotype		Root Score [1-4]	Root Length [cm]	Root Mass [g]	Stem + Straw Mass [g]	Seed Mass [g]	Seed Oil Mass [g]	Seed Protein Mass [g]	NUE
1	**Californium**	**PO**	2.5	50	6.3	47.0	25.8	11.5	4.9	0.752
2	**NK Linus**	**HN**	3.5	62.5	7.0	48.7	25.5	12.0	4.6	0.710
3	**Adriana**	**PN**	2.5	42.5	5.8	49.4	22.4	9.8	4.5	0.695
4	**Inspiration**	**HN**	1.5	60	5.5	49.3	25.1	11.3	4.5	0.691
5	**Trinity**	**PN**	2	50	4.7	41.9	21.6	9.0	4.4	0.686
26	**Lirajet**	**PO**	NA	NA	NA	NA	19.0	7.8	3.6	0.563
27	**Express**	**PO**	3	60	6.5	48.0	19.4	9.3	3.6	0.556
28	**Marathon**	**HN**	3	47.5	7.8	49.7	21.7	10.3	3.6	0.553
29	**Genie**	**HN**	2	45	6.4	36.1	17.4	8.0	3.2	0.490
30	**Oase**	**PN**	1	55	2.8	41.7	16.2	7.0	3.1	0.488

3.1.2 Seed Traits

Phenotypic differences in seed traits existed between both N treatments and cultivar groups. There was a general increase in traits such as seed mass (Figure 13; Figure 17), seed oil mass (Figure 14), seed protein mass and seed protein content (Figure 15) under N2. For seed mass and seed oil mass, there were no significant differences among cultivar groups. However, it was clear that the new hybrids performed the best overall (Figure 13; Figure 14; Figure 17), under both N levels. Under N1, the new purebreds had a higher seed oil content than the old purebreds. However, under N2 the new purebreds had lower seed oil content than the old purebreds (Figure 14). Excluding outliers, there appears to be major decreases in seed protein content in the new hybrids compared to the other cultivar groups, under both N treatments, but not in total seed protein mass (Figure 15). Seed protein content ranged from 10.7 – 19.2 %, with much variation within and among cultivars groups and between N fertilizer treatments (Figure 15). Harvest index, calculated as seed mass divided by the total plant mass, shows a clear increase from old to new in purebreds and hybrids under both N fertilization treatments (Figure 16).

The new hybrids Artoga and Avatar were in the top three for seed yield under both N treamtents (Figure 17). In addition, DK Exstorm, Inspiration and NK linus also performed well under both N treatments (Figure 17). In comparison, the purebred Californium did very well for seed mass under N2, but poorly under N1 (Figure 17). It is clear, by their position above the diagonal line, that most cultivars, with the exception of Exocet, Lirajet and Genie, perform better under N fertilization (Figure 17).

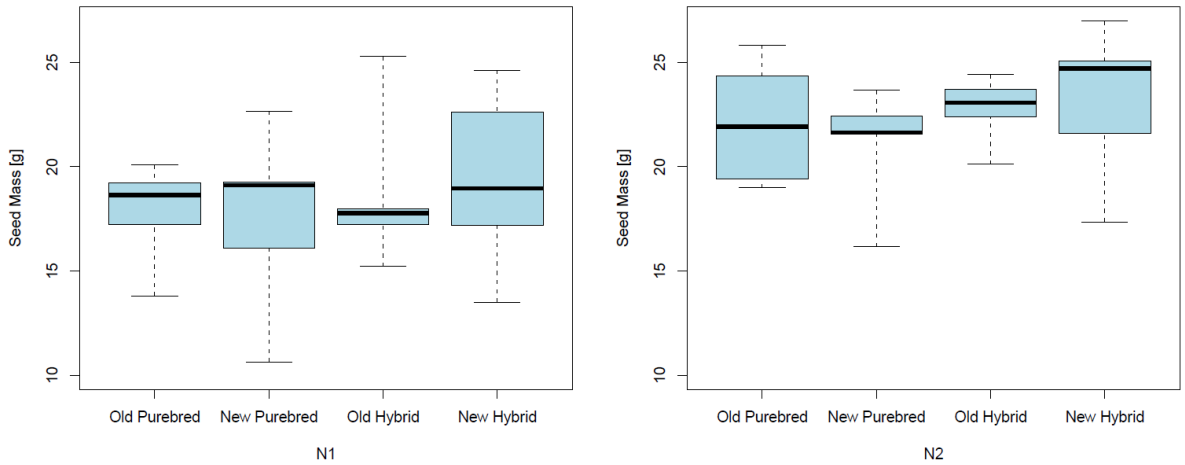
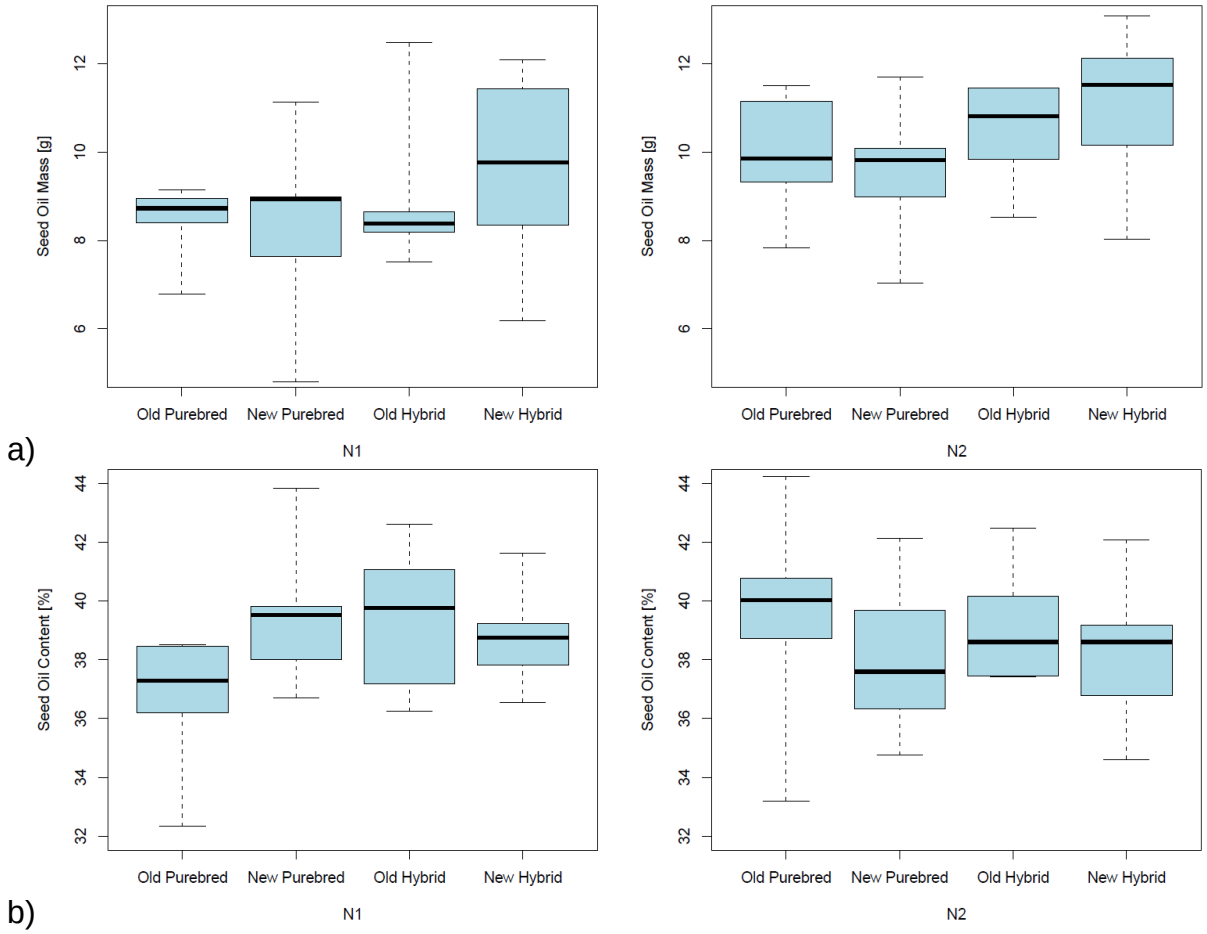


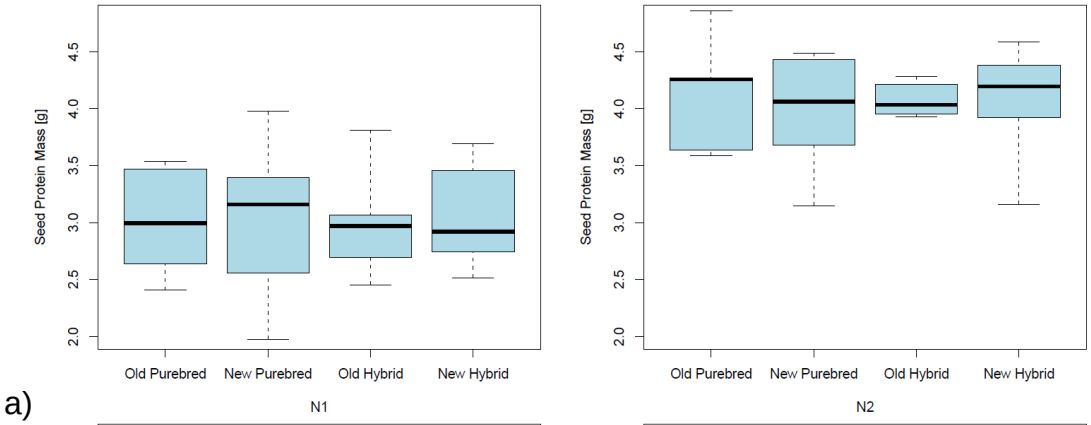
Figure 13: Seed mass of *B. napus* cultivar groups under no N fertilization (N1) and 200 kg/ha N fertilization (N2).



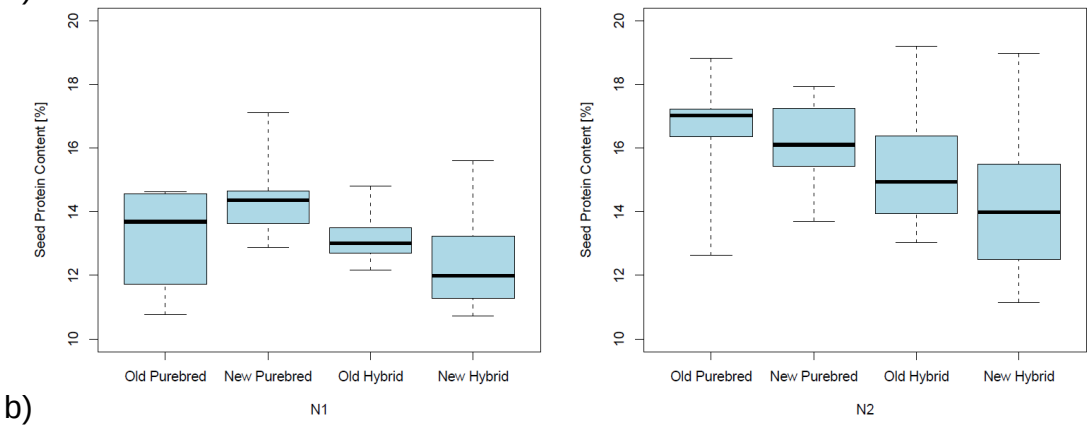
a)

b)

Figure 14: (a) Seed oil mass and (b) seed oil content of *B. napus* cultivar groups under no N fertilization (N1) and 200 kg/ha N fertilization (N2).



a)



b)

Figure 15: (a) Seed protein mass and (b) seed protein content of *B. napus* cultivar groups under no N fertilization (N1) and 200 kg/ha N fertilization (N2).

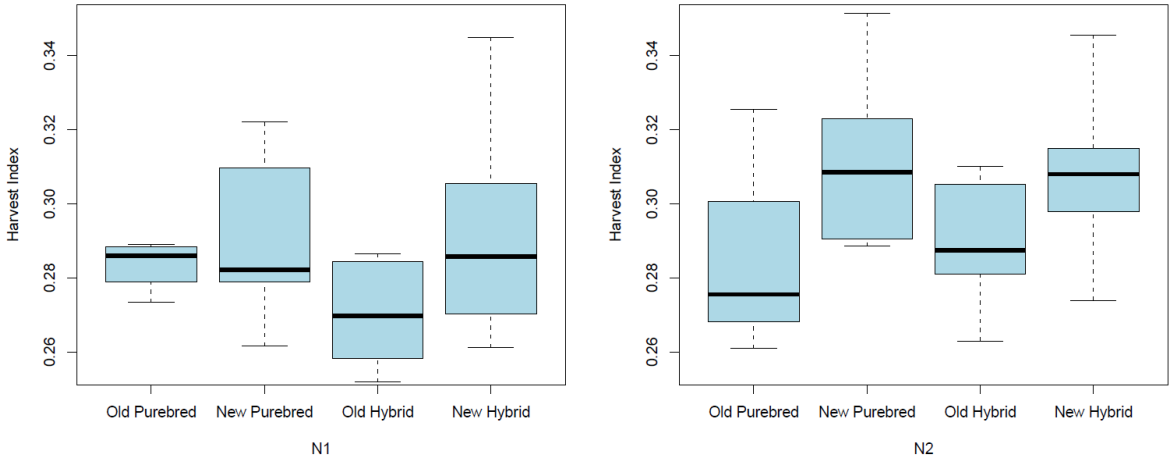
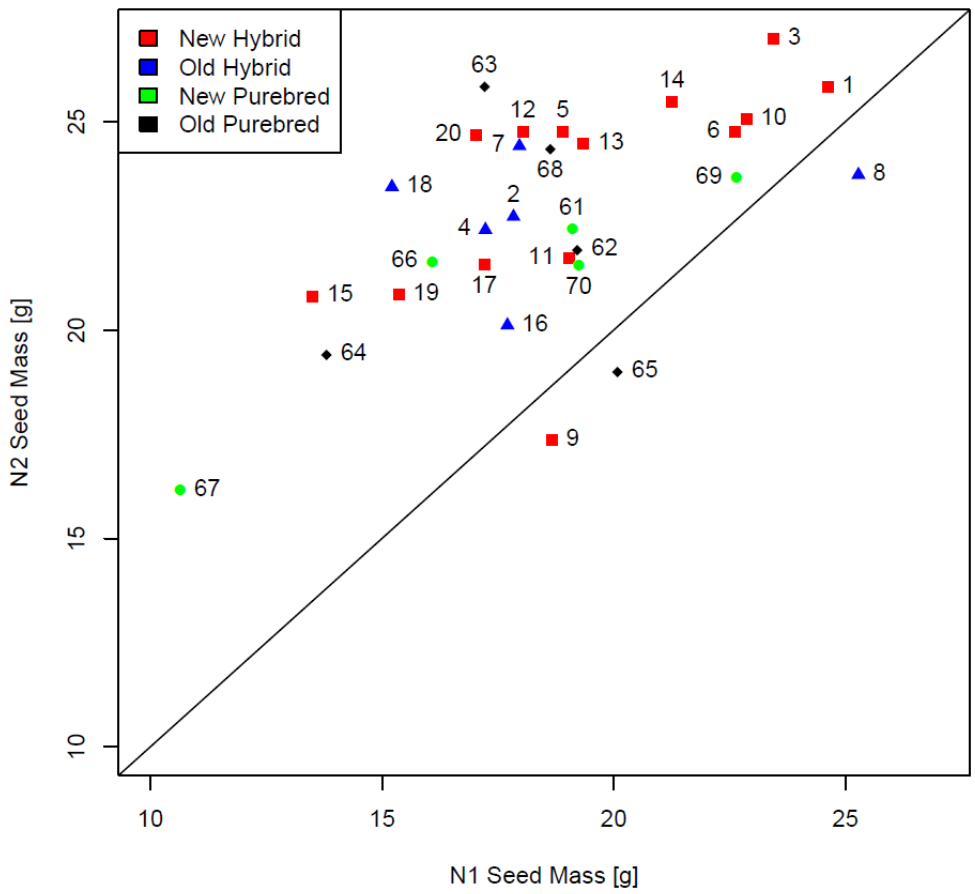


Figure 16: Harvest index of *B. napus* cultivar groups for harvest index under no N fertilization (N1) and 200 kg/ha N fertilization (N2).



Cultivar Number	Cultivar
1	Artoga
2	Artus
3	Avatar
4	Baldur
5	Compass
6	DK Exstorm
7	Elektra
8	Exocet
9	Genie
10	Inspiration
11	Marathon
12	Mascara
13	Mercedes
14	NK Linus
15	NPZ 1203 Z
16	Ryder
17	Sherpa
18	Taurus
19	Troy
20	Visby
61	Adriana
62	Aviso
63	Californium
64	Express
65	Lirajet
66	Lorenz
67	Oase
68	Pacific
69	Patron
70	Trinity

Figure 17: Seed mass under no N fertilization (N1) and 200 kg/ha N fertilization (N2).

3.1.3 Root Traits

Although root mass did not differ much between the two N levels, root length was slightly lower and fine root score was higher under N2 compared to N1 (Figure 18). This was especially seen in root score between the old purebred cultivars under the different N treatments. However, these changes were not universal among all cultivars. Artus, NK Linus, Visby and Patron had higher root length under N2 than N1, and Avatar and Exocet had lower fine root scores under N2 than N1 (Table 7), illustrating variability in root structure responses under the different N treatments among *B. napus* cultivars. There was a large difference in root mass variation between the old and new purebreds (Figure 18). Excluding the new purebred Patron, which had a root mass much greater

than all other cultivars, the hybrids had high root mass under both N treatments, and especially under N1 (Figure 19).

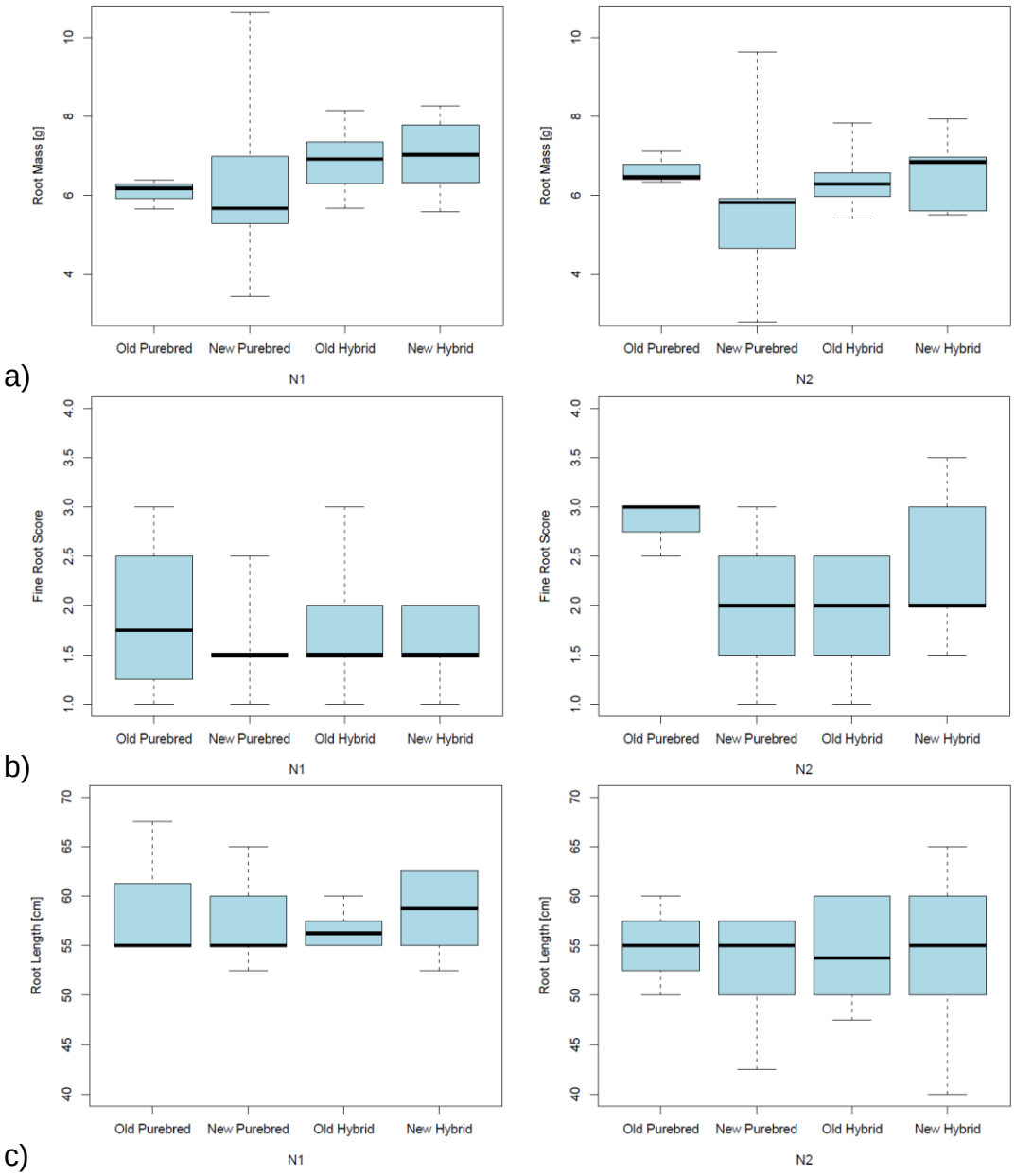


Figure 18: (a) Root mass, (b) fine root score and (c) root length of *B. napus* cultivar groups under no N fertilization (N1) and 200 kg/ha N fertilization (N2).

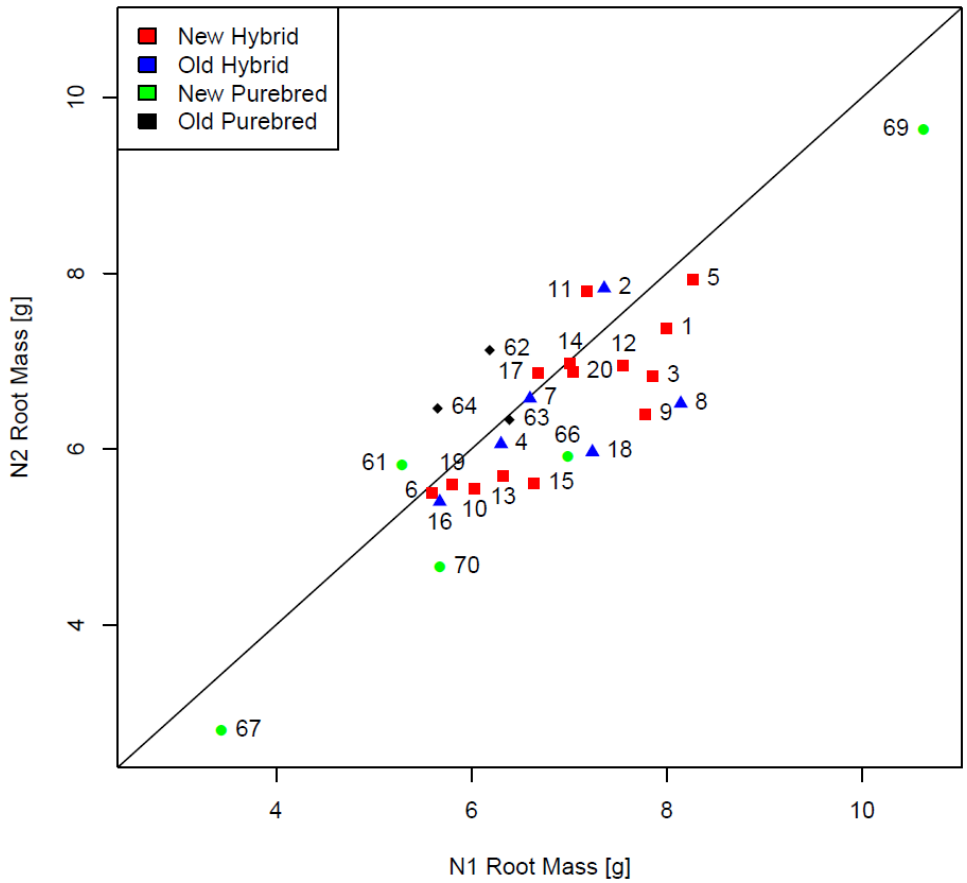
Table 7: Differences in root traits from no N fertilization to 200 kg N/ha N fertilization in (a) hybrid and (b) purebred cultivars.

a)

Genotype	Δ Root Length	Δ Root Mass	Δ Fine Root
Artoga	-2.5	-0.63	0
Artus	2.5	0.47	0.5
Avatar	-7.5	-1.03	-0.5
Baldur	0	-0.25	0.5
Compass	-2.5	-0.34	1
DK Exstorm	-2.5	-0.09	1
Elektra	-5	-0.03	0
Exocet	-7.5	-1.63	-1
Genie	-15	-1.39	0.5
Inspiration	-2.5	-0.48	0.5
Marathon	-5	0.61	1.5
Mascara	-2.5	-0.61	0.5
Mercedes	-7.5	-0.63	0.5
NK Linus	7.5	-0.04	1.5
NPZ 1203 Z	0	-1.03	0
Ryder	-5	-0.28	0
Sherpa	0	0.19	1
Taurus	0	-1.27	1
Troy	-22.5	-0.20	0.5
Visby	5	-0.16	0.5

b)

Genotype	Δ Root Length	Δ Root Mass	Δ Fine Root
Adriana	-10	0.53	1
Aviso	0	0.94	1
Californium	-5	-0.06	1.5
Express	-7.5	0.81	1.5
Lirajet	NA	NA	NA
Lorenz	-7.5	-1.07	0
Oase	-5	-0.65	0
Pacific	NA	NA	NA
Patron	2.5	-0.99	0.5
Trinity	-5	-1.02	0.5



Cultivar Number	Cultivar
1	Artoga
2	Artus
3	Avatar
4	Baldur
5	Compass
6	DK Exstorm
7	Elektra
8	Exocet
9	Genie
10	Inspiration
11	Marathon
12	Mascara
13	Mercedes
14	NK Linus
15	NPZ 1203 Z
16	Ryder
17	Sherpa
18	Taurus
19	Troy
20	Visby
61	Adriana
62	Aviso
63	Californium
64	Express
65	Lirajet
66	Lorenz
67	Oase
68	Pacific
69	Patron
70	Trinity

Figure 19: Root mass under no N fertilization (N1) and 200 kg/ha N fertilization (N2).

3.1.4 Nitrogen Use Efficiency

NUE, calculated using Equation 1, was lower under N2 than N1 (Figure 20), with the exception of a few cultivars (Figure 21), although there was less variation in cultivar groups under N2 (Figure 20). The highest NUE for both N1 and N2 was among the purebred cultivars. However, they were each adapted for only one of the N treatments and did poorly in the other (Figure 21). NUE was high under both N treatments for the new hybrids Inspiration, NK Linus and Avatar, as well as the new purebred Adriana (Figure 21). No significant increases or decreases were observed in NUE among the cultivar groups (Figure 20).

Correlation plots show a number of differences among cultivar groups and N treatments. Under N1, root mass and the fine root score correlated positively with traits such as seed mass and NUE (Figure 22). This effect is seen in all cultivar groups except the new hybrids, which show little correlation in these traits (Figure 23). Among the purebreds, there was an increase in correlation between root score and seed mass from old to new, however, among the hybrids this correlation decreased from old to new (Figure 23). Under N2, much of the correlations of root characteristics to seed mass and NUE were absent (Figure 22). New purebreds had a negative correlation of root length with NUE under N fertilization. However, correlation plots should be interpreted with caution as the sample size is quite low (especially in the purebred lines), and should be used only as a guide for possible further investigations.

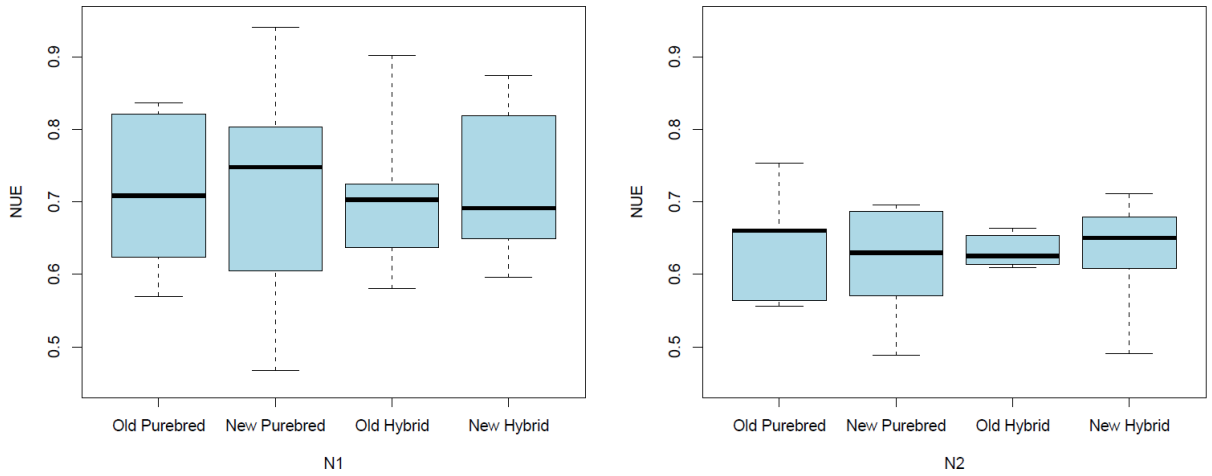
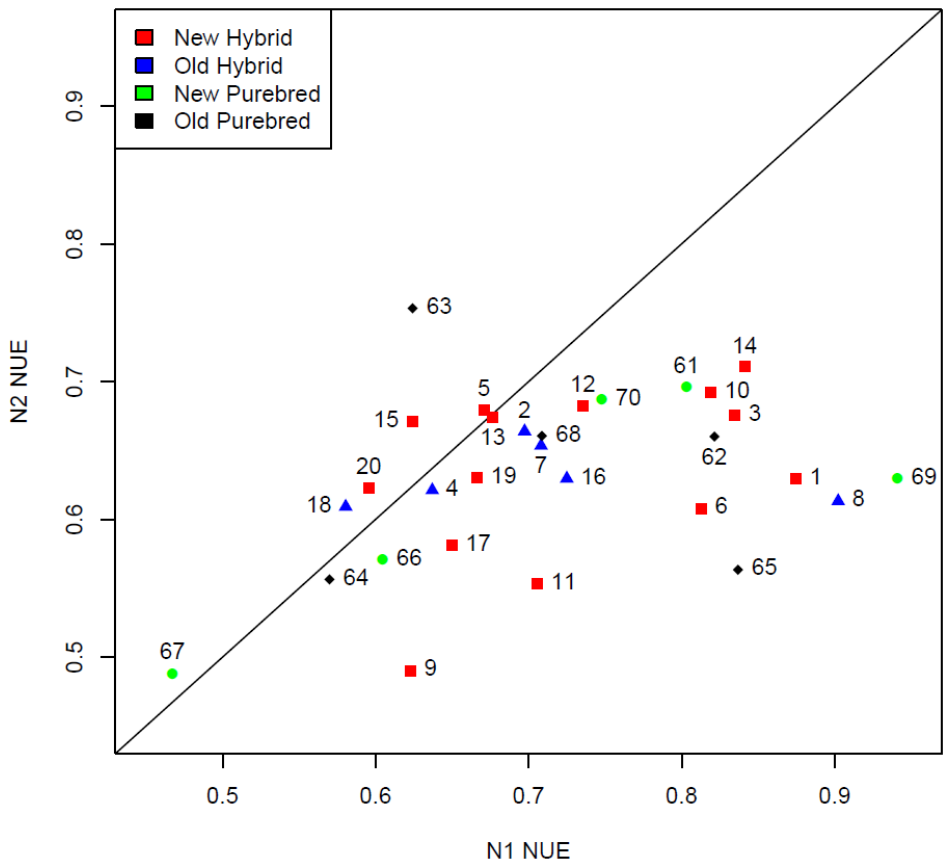


Figure 20: Nitrogen use efficiency (NUE) of *B. napus* cultivar groups under no N fertilization (N1) and 200 kg/ha N fertilization (N2). NUE calculated using Equation 1.



Cultivar Number	Cultivar
1	Artoga
2	Artus
3	Avatar
4	Baldur
5	Compass
6	DK Exstorm
7	Elektra
8	Exocet
9	Genie
10	Inspiration
11	Marathon
12	Mascara
13	Mercedes
14	NK Linus
15	NPZ 1203 Z
16	Ryder
17	Sherpa
18	Taurus
19	Troy
20	Visby
61	Adriana
62	Aviso
63	Californium
64	Express
65	Lirajet
66	Lorenz
67	Oase
68	Pacific
69	Patron
70	Trinity

Figure 21: Nitrogen Use Efficiency (NUE) under no N fertilization (N1) and 200 kg/ha N fertilization (N2). NUE calculated using Equation 1.

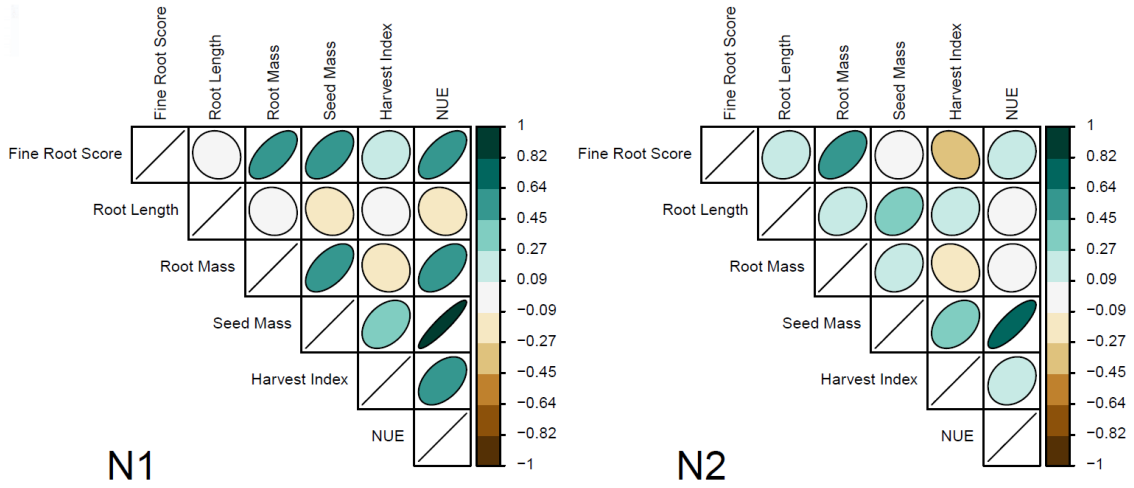


Figure 22: Correlation plots for specific phenotypic traits among B. napus cultivars under no N fertilization (N1) and 200 kg N/ha (N2). NUE calculated using Equation 1.

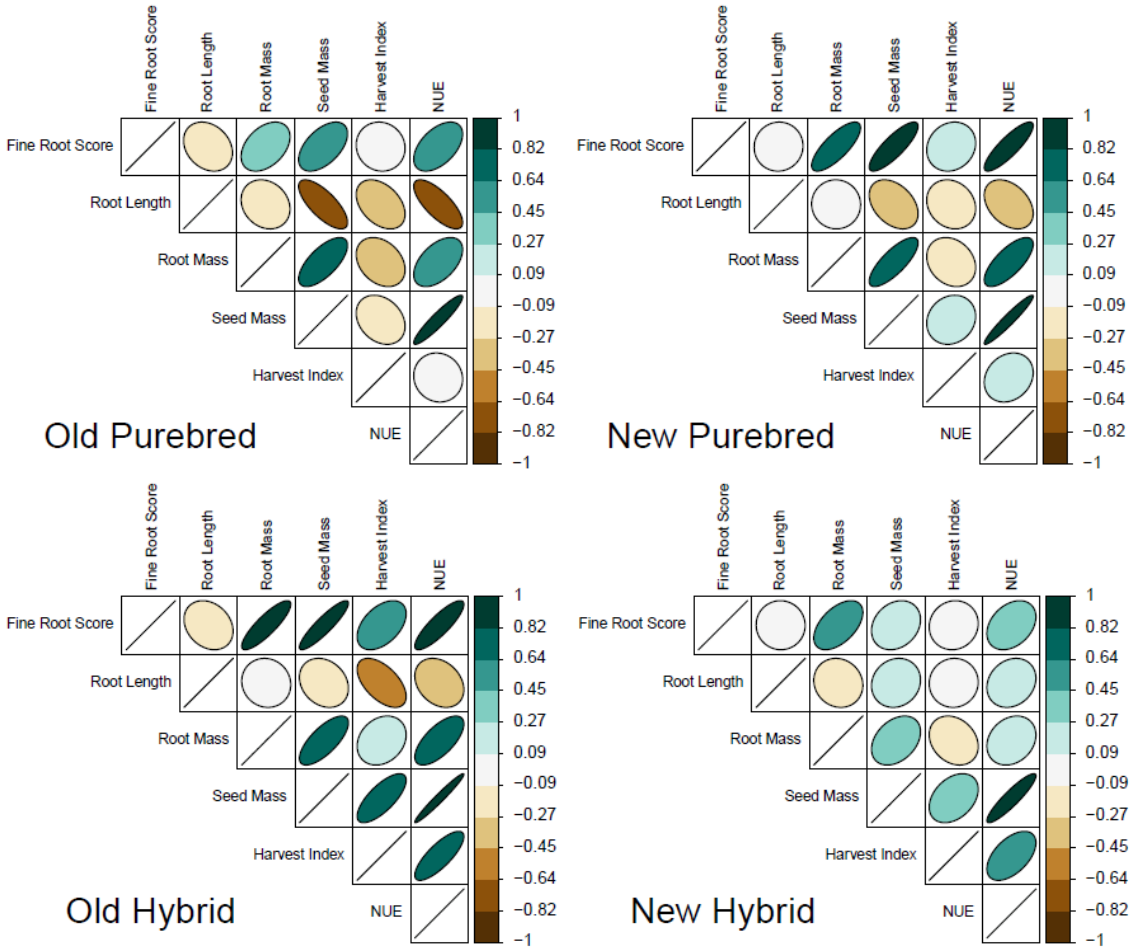


Figure 23: Correlation plots for specific phenotypic traits among *B. napus* cultivar groups under no N fertilization (N1). NUE calculated using Equation 1.

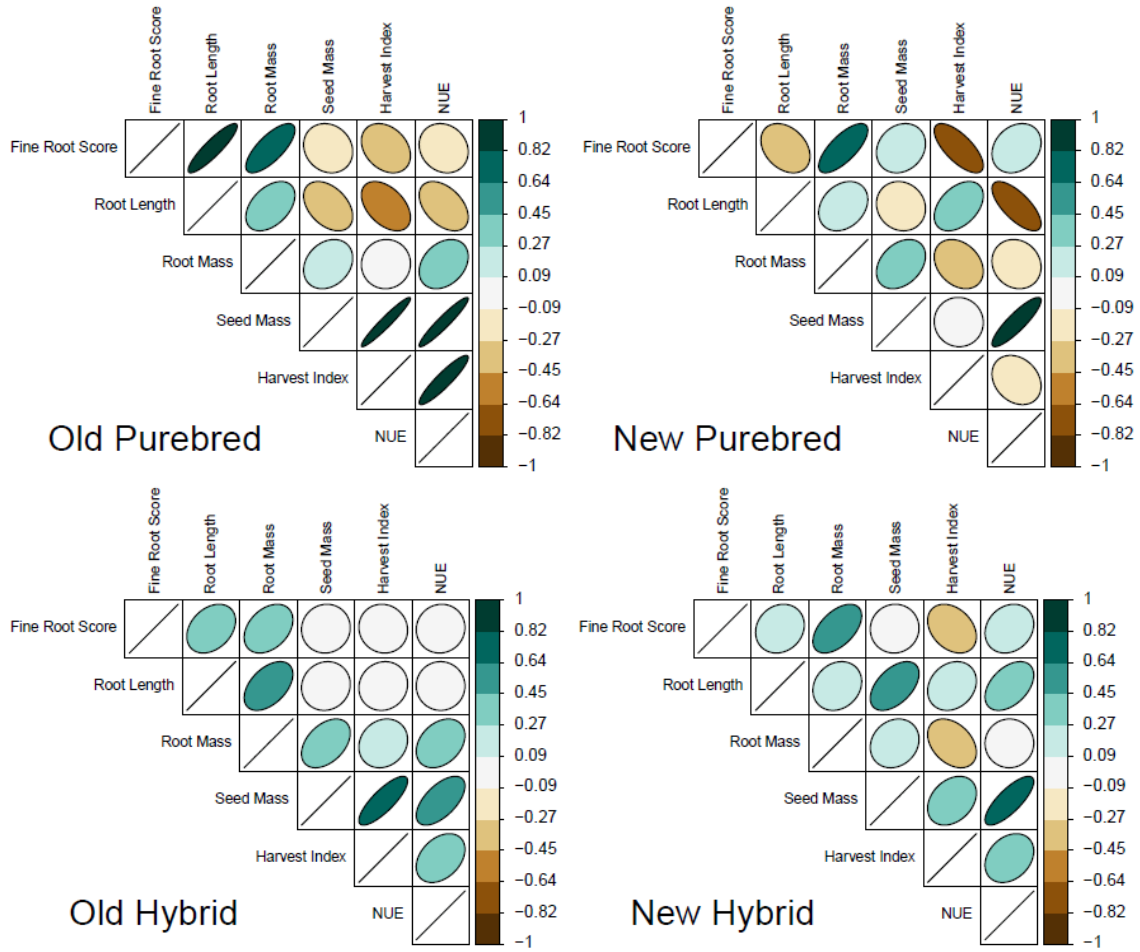


Figure 24: Correlation plots for specific phenotypic traits among *B. napus* cultivar groups under 200 kg/ha N fertilization (N2). NUE calculated using Equation 1.

3.2 Genotypic Data

Purebred lines had very low levels of heterozygosity, ranging from 0.2 – 8.6 %, with the exception of 2 purebred lines (Figure 25), at 29.3 and 24.2 % (Table 8). Heterozygosity in the hybrids ranged from 23.5 – 54.6 % (Table 8), distributed throughout the A and C genome (Figure 25). Of the 52157 SNP markers that were assayed, 5480 – 9030 markers were not detected, and for the hybrid corrections utilizing their parents markers, 193 – 5107 markers were determined to be incorrect, resulting in a failure range from 10.7 – 22.3 % (Table 8).

Table 8: Marker data information for each of the cultivars used in this study along with the parental lines of all hybrid cultivars. L = purebred, H = hybrid, X = parent 1, Z = parent 2.

	L01	L02	L03	L04	L05	L06	L07	L08	L09	L10
Missed	5677	5693	6172	5630	8077	5770	6682	5825	5783	5810
Homozygote	46377	45435	42071	46438	31149	45899	41856	46210	46215	42820
Heterozygote	103	1029	3914	89	12931	488	3619	122	159	3527
Total	52157	52157	52157	52157	52157	52157	52157	52157	52157	52157
Heterozygosity [%]	0.2	2.2	8.5	0.2	29.3	1.1	8.0	0.3	0.3	7.6
Failed [%]	10.9	10.9	11.8	10.8	15.5	11.1	12.8	11.2	11.1	11.1

	H01	H02	H03	H04	H05	H06	H07	H08	H09	H10
Missed	6416	5947	7838	6431	5670	5408	6224	5560	6272	6878
One-copy	1083	1195	794	958	944	1287	973	1199	951	1090
Homozygote	34049	33221	30227	30083	31849	35581	30967	35411	32385	31511
Heterozygote	10075	9626	9638	13158	12625	9688	12389	9694	11836	9906
Incorrect	534	2168	3660	1527	1069	193	1604	293	713	2772
Total	52157	52157	52157	52157	52157	52157	52157	52157	52157	52157
Heterozygosity [%]	28.7	28.0	31.1	42.4	38.5	26.3	38.8	26.5	35.5	30.4
Failed [%]	13.3	15.6	22.0	15.3	12.9	10.7	15.0	11.2	13.4	18.5

	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20
Missed	7669	6162	5522	6537	6236	5763	6246	6912	6685	5952
One-copy	875	1059	817	1674	783	3328	759	785	761	1766
Homozygote	29202	34912	34839	29400	33621	31066	31099	26646	30702	31382
Heterozygote	11410	8458	10684	9439	10939	9747	11285	14976	11543	10608
Incorrect	3001	1566	295	5107	578	2253	2768	2838	2466	2449
Total	52157	52157	52157	52157	52157	52157	52157	52157	52157	52157
Heterozygosity [%]	37.9	23.5	30.0	30.4	31.8	28.3	35.4	54.6	36.7	32.0
Failed [%]	20.5	14.8	11.2	22.3	13.1	15.4	17.3	18.7	17.5	16.1

	X01	X02	X03	X04	X05	X06	X07	X08	X09	X10
Missed	5674	5825	5848	5819	5925	5860	5819	5772	5925	6544
Homozygote	46307	44790	44484	42511	44220	46199	42511	46098	44220	41701
Heterozygote	176	1542	1825	3827	2012	98	3827	287	2012	3912
Total	52157	52157	52157	52157	52157	52157	52157	52157	52157	52157
Heterozygosity [%]	0.4	3.3	3.9	8.3	4.4	0.2	8.3	0.6	4.4	8.6
Failed [%]	10.9	11.2	11.2	11.2	11.4	11.2	11.2	11.1	11.4	12.5

	X11	X12	X13	X14	X15	X16	X17	X18	X19	X20
Missed	5943	6417	5848	6417	5839	9440	5822	5819	5839	5822
Homozygote	45032	42947	44484	42947	45247	39129	44014	42511	45247	44014
Heterozygote	1182	2793	1825	2793	1071	3588	2321	3827	1071	2321
Total	52157	52157	52157	52157	52157	52157	52157	52157	52157	52157
Heterozygosity [%]	2.6	6.1	3.9	6.1	2.3	8.4	5.0	8.3	2.3	5.0
Failed [%]	11.4	12.3	11.2	12.3	11.2	18.1	11.2	11.2	11.2	11.2

	Z01	Z02	Z03	Z04	Z05	Z06	Z07	Z08	Z09	Z10
Missed	6142	6996	5703	6421	6243	6015	5965	6094	5691	6094
Homozygote	45569	41483	45777	43948	44103	46062	45929	45595	46369	45595
Heterozygote	446	3678	677	1788	1811	80	263	468	97	468
Total	52157	52157	52157	52157	52157	52157	52157	52157	52157	52157
Heterozygosity [%]	1.0	8.1	1.5	3.9	3.9	0.2	0.6	1.0	0.2	1.0
Failed [%]	11.8	13.4	10.9	12.3	12.0	11.5	11.4	11.7	10.9	11.7

	Z11	Z12	Z13	Z14	Z15	Z16	Z17	Z18	Z19	Z20
Missed	5985	5836	5553	9030	5679	6241	5861	6013	5802	7915
Homozygote	46039	45329	46550	41665	46012	45738	46086	43473	45203	33530
Heterozygote	133	992	54	1462	466	178	210	2671	1152	10712
Total	52157	52157	52157	52157	52157	52157	52157	52157	52157	52157
Heterozygosity [%]	0.3	2.1	0.1	3.4	1.0	0.4	0.5	5.8	2.5	24.2
Failed [%]	11.5	11.2	10.6	17.3	10.9	12.0	11.2	11.5	11.1	15.2


Chr. 1
Chr. 2
Chr. 3
Chr. 4
Chr. 5
Chr. 6
Chr. 7
Chr. 8
Chr. 9
Chr. 10
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L02
L03
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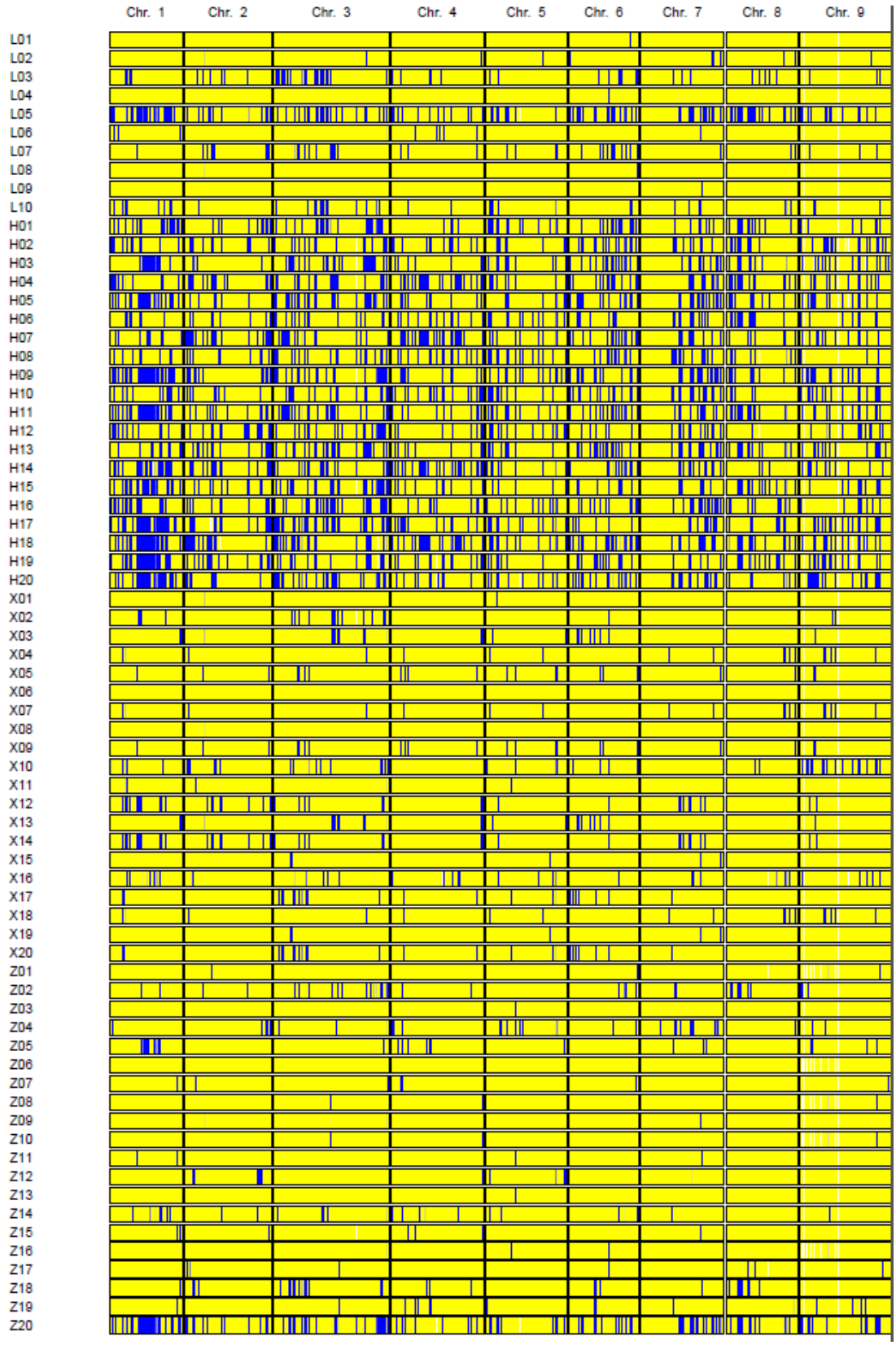
a)


Chr. 1
Chr. 2
Chr. 3
Chr. 4
Chr. 5
Chr. 6
Chr. 7
Chr. 8
Chr. 9
L01
L02
L03
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Z20

b)

Figure 25: (previous pages) Map of the *B. napus* (a) A genome and (b) C genome displaying heterozygosity (blue bars) of the 10 purebred lines, 20 hybrids and their parents.

R code was successfully used to identify markers which are associated with specific phenotypic traits using Wilcoxon rank-sum tests (Table 13). This was performed with seed mass under N1 and N2 (Table 10; Table 11) and NUE under N1 and N2 (Table 10; Table 12). By using Wilcoxon rank-sum tests it is possible to identify differences between the markers one wants to target, *e.g.*, between homozygous and heterozygous markers (*e.g.*, Table 11a), or between markers which may be present in one copy *vs*. two (*e.g.*, Table 11b), or between missing and present markers (*e.g.*, Table 11c). Incorporating this into R code enabled the ability to perform these tests on all 36,456 markers which showed variation, in a high-throughput fashion. In addition to identifying significant markers, the marker type of each genotype at that particular marker is displayed from lowest to highest in the tables, according to their rank for that phenotype (see Tables 9-12). Along with this, a score for each marker type present was calculated by determining the average ranking, amongst the 30 cultivars. This can help give an idea of the positive or negative correlations that the particular marker type might incur. In the last column, the original SNP variation in the 30 genotypes is listed. In total, this enables the examination of each marker with more detail to determine if there are positive or negative correlations with specific marker types, and to help determine potential false positives.

For NUE under N1, heterozygosity had positive impact on NUE for all but two of the markers identified as significant at $p < 0.01$, (Table 10a). Whereas under N2, heterozygosity had a negative impact on NUE for all but two of the significant markers

(Table 12a). In addition, Wilcoxon pairwise rank sum tests identified 5 markers in which their absence had a negative impact on NUE under N2 (Table 12b). For seed mass under N1, heterozygosity had a positive impact on seed mass for most of the significant markers (Table 9a). In addition 2 markers were identified whose absence had a positive impact on seed mass under N2 (Table 9c). For seed mass under N2, heterozygosity had both positive and negative impacts on seed mass for the markers identified as significant (Table 11a). Additionally there were seven markers with which their absence had negative or positive effects on seed mass under N2 (Table 11c).

At $p < 0.01$, there were very few markers that were significant between each test, except for NUE and seed mass under N1, which saw 28 of the 30 NUE markers also associated with seed mass (Table 13; Table 14). At $p < 0.05$, there was a much greater percentage of markers similar between each comparison. However, only 32 of the 294 NUE markers under N1 were shared with NUE under N2 (Table 13; Table 14). In total, 29 of the markers were shared among all tests (seed mass and NUE under both N treatments) when run at $p < 0.05$ (Table 15), and of those mapped to the *B. napus* genome, all were located in a small region on chromosome C 05.

Table 9 (following pages): Markers associated with seed mass under no N fertilization using Wilcoxon rank-sum tests with significance set to $p < 0.01$. For each marker, the genotype's marker type is displayed from lowest to highest (Table 5), along with a calculated score for each marker present, and the original SNP variation in the 30 genotypes. (a) markers in which homozygosity and heterozygosity differed, (b) markers in which single copy differed from two copies, and (c) markers in which presence and absence differed. "0" = missing, "1" = single copy, "2" = homozygotic, "3" = heterozygotic, "4" = incorrect.

a)

	Marker	Chr	Pos	Marker Order (Lowest -> Highest)	0	1	2	3	4	Variation
1	Bn-A01-p1067371	1	652869	2 2 2 4 2 2 2 2 2 2 2 2 2 0 2 2 2 2 2 2 2 2 2 3 2 3 0 3 3	21.0		13.4	27.8	4.0	G K failed
2	Bn-A01-p1770291	1	1310265	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3			14.5	29.5		T Y
3	Bn-A01-p1770482	1	1310457	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3			14.5	29.5		C M
4	Bn-A01-p2403471	1	1894127	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 2 3 3			14.2	27.3		A R
5	Bn-A01-p2421445	1	1912045	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 2 3 3			14.2	27.3		G R
6	Bn-A02-p6147730	2	3316152	2 2 2 2 2 2 1 2 2 2 2 2 2 2 2 2 3 2 2 2 2 3 2 3 2 3 2 3 3		7.0	13.3	25.2		G K T
7	Bn-A02-p6147823	2	3316245	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 2 3 2 3 2 3 2 3 3			13.1	25.2		A G R
8	Bn-A03-p27377729	3	25635715	2 2 2 2 0 2 1 4 0 2 4 2 1 3 2 2 2 1 2 2 2 3 0 3 3 2 3 3 3 3	12.3	12.7	12.3	24.9	9.5	C T Y failed
9	Bn-A04-p313091	4	257015	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3			14.0	28.7		T Y
10	Bn-A04-p313410	4	257334	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3			14.0	28.7		T Y
11	Bn-A04-p313935	4	257859	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3			14.0	28.7		C Y
12	Bn-A04-p314440	4	258364	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3			14.0	28.7		G R
13	Bn-A04-p314536	4	258460	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3			14.0	28.7		G R
14	Bn-A04-p314872	4	258796	2 2 2 2 2 2 2 2 2 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3		11.0	14.2	28.7		T Y
15	Bn-A04-p324144	4	268118	2 2 2 2 2 2 2 2 2 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3		11.0	14.2	28.7		C Y
16	Bn-A04-p325019	4	268690	2 2 2 2 2 2 2 2 2 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3		11.0	14.2	28.7		K T
17	Bn-A04-p326007	4	269680	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3			14.0	28.7		K T
18	Bn-A04-p664994	4	540419	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3			13.7	27.5		A M
19	Bn-A04-p665932	4	542345	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3			13.7	27.5		G R
20	Bn-A04-p669182	4	545975	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3			13.7	27.5		G R
21	Bn-A04-p726356	4	602860	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 0 2 2 3 2 3 3	24.0		13.7	28.7		A R failed
22	Bn-A04-p757668	4	628867	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 0 2 2 3 2 3 3	24.0		13.7	28.7		G R failed
23	Bn-A04-p783339	4	654736	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3			13.7	27.5		G K
24	Bn-A04-p784708	4	656105	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3			13.7	27.5		T Y
25	Bn-A04-p785628	4	657029	2 2 2 2 2 2 2 2 2 2 1 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3		11.0	13.8	27.5		A R
26	Bn-A04-p792399	4	663829	2 2 2 2 2 2 2 2 2 2 1 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3		11.0	13.8	27.5		G S
27	Bn-A04-p809984	4	686044	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3			14.0	28.7		A R
28	Bn-A04-p810039	4	686099	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 1 2 2 3 2 3 3		24.0	13.7	28.7		T Y
29	Bn-A04-p810166	4	686226	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 1 2 2 3 2 3 3		24.0	13.7	28.7		A R
30	Bn-A04-p810935	4	686983	2 2 2 2 2 2 2 2 2 2 1 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3		11.0	13.8	27.5		C Y
31	Bn-A04-p813173	4	689173	2 2 2 2 2 2 2 2 2 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3		11.0	14.2	28.7		A R
32	Bn-A04-p814033	4	690032	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3			14.0	28.7		C M
33	Bn-A04-p928350	4	798300	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3			14.0	28.7		T Y
34	Bn-A04-p1718248	4	1443179	2 2 2 2 2 2 2 2 2 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3		11.0	14.2	28.7		A M
35	Bn-A04-p2282199	4	1718920	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3			13.7	27.5		T Y
36	Bn-A04-p2152528	4	1829861	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3			13.7	27.5		T Y
37	Bn-A04-p2152171	4	1830215	2 2 2 2 2 2 2 2 2 2 1 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3		11.0	13.8	27.5		A M
38	Bn-A04-p2151567	4	1830804	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3			13.7	27.5		G R
39	Bn-A04-p2146459	4	1836075	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3			13.7	27.5		C Y
40	Bn-A04-p16104616	4	16299897	2 2 2 2 2 2 2 2 2 3 2 2 3 2 2 3 2 3 2 2 2 3 3 2 3 2 3 3 3			11.8	21.9		C T Y
41	Bn-A04-p16166839	4	16368214	2 2 2 2 2 2 2 2 2 3 3 1 3 2 2 2 2 3 2 2 3 3 3 2 3 2 3 3 3		12.0	11.5	21.4		A C M
42	Bn-A06-p24557193	6	23504975	3 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3 2 2 2 3 3 2 3 2 3 3 3 3			12.3	22.0		A C M
43	Bn-A07-p15816246	7	17723487	2 2 2 1 2 2 2 2 2 1 2 2 2 2 2 2 2 2 2 2 2 4 3 2 4 3 3 3		7.5	13.0	28.0	25.5	G R
44	Bn-A07-p17047263	7	18952770	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 2 2 3			14.2	27.3		A M
45	Bn-A08-p8783951	8	7646302	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 2 3 3			13.6	27.8		T Y
46	Bn-A08-p10031059	8	8030469	2 2 2 2 2 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 2 3 3		7.0	13.9	27.8		G R
47	Bn-A08-p16562035	8	14030897	2 2 2 3 2 2 2 2 2 2 2 2 3 3 2 2 2 3 2 2 2 3 2 3 3 2 3 3 3			12.2	21.3		A G R
48	Bn-A10-p7444203	9	5779828	3 3 2 4 3 2 2 2 2 4 2 2 4 2 2 2 2 2 2 2 2 2 4 2 2 2 2 2 2			17.7	2.7	12.8	G K T
49	Bn-A10-p10613361	10	11975797	2 3 2 3 2 2 3 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2			17.1	5.3		C M
50	Bn-A10-p11311425	10	12610488	2 3 2 3 2 2 3 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2			17.1	5.3		T Y
51	Bn-A10-p13028260	10	13064168	2 3 2 3 2 2 3 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2			17.1	5.3		A R
52	Bn-A10-p13190230	10	13228578	2 3 2 3 2 2 3 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2			17.1	5.3		A R
53	Bn-A10-p13335021	10	13379963	2 3 2 3 3 2 3 3 3 2 2 2 2 2 2 3 3 2 2 2 2 2 2 2 2 2 2 2 2			18.0	8.5		C T Y
54	Bn-A10-p14327285	10	14264190	2 2 2 2 2 2 2 2 3 3 2 2 2 2 2 2 2 2 2 2 2 3 3 2 2 2 3 3 3			13.0	22.3		A G R
55	Bn-A10-p14365581	10	14309162	2 2 2 2 2 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 2 3 3 3			13.3	24.2		C T Y
56	Bn-A10-p14384277	10	14329379	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3			14.5	29.5		C Y
57	Bn-scaff_20210_1-p161976	11	8221432	2 3 2 3 3 2 3 3 3 3 3 2 3 3 2 3 3 3 2 2 3 2 2 2 2 2 2 2 2			19.4	11.1		C T Y
58	Bn-scaff_17517_1-p330218	11	30559418	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3 1 2 2 3 3 3 2		24.0	13.0	25.8		A G R
59	Bn-scaff_17517_1-p330557	11	30559758	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3 2 2 2 3 3 3 2			13.4	25.8		C T Y
60	Bn-scaff_17517_1-p330624	11	30559825	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3 1 2 2 3 3 3 2		24.0	13.0	25.8		C T Y
61	Bn-scaff_17517_1-p332460	11	30561660	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3 2 2 2 3 3 3 2			13.4	25.8		G K T
62	Bn-scaff_17036_1-p179776	11	32259345	2 2 2 2 2 2 2 3 2 2 3 2 2 4 2 3 3 3 2 2 2 3 3 4 3 2 3 3 3 3			10.8	21.2	19.0	C T Y
63	Bn-scaff_15794_2-p80390	11	32593360	2 2 2 2 4 2 3 2 2 2 2 2 2 2 2 3 3 3 2 2 3 3 3 3 2 2 4 3 3 3			11.6	21.4	16.0	C T Y
64	Bn-scaff_15877_1-p617921	13	4284222	2 3 2 3 3 2 3 3 2 2 2 2 3 2 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2			17.7	8.1		C T Y
65	Bn-scaff_16534_1-p1458867	14	4097414	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3 2 3 2 2 3 2 3			13.5	25.6		G K T
66	Bn-scaff_16534_1-p1482507	14	4120900	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3 2 3 2 2 3 2 3			13.5	25.6		C T Y
67	Bn-scaff_16534_1-p1500199	14	4138594	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3 2 3 2 2 3 2 3			13.5	25.6		A G R
68	Bn-scaff_15908_1-p557100	14	5715418	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 2 3 2 3			13.8	26.3		A G R
69	Bn-scaff_18903_1-p556332	14	47723216	2 2 2 2 2 2 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 2 3 3 2 3			13.4	23.8		T Y

a) continued…

	Marker	Chr	Pos	Marker Order (Lowest -> Highest)	0	1	2	3	4	Variation
70	Bn-scaff_20901_1-p660528	15	3377431	2 2 2 2 2 2 2 2 2 3 2 2 2 2 2 2 2 2 2 2 3 2 3 2 3 2 3 3			13.4	24.0		C Y
71	Bn-scaff_20901_1-p653492	15	3384467	2 2 2 2 2 2 2 2 2 3 2 2 2 2 2 2 2 2 2 2 3 3 2 3 2 3 3 3			12.3	24.3		C T Y
72	Bn-scaff_18181_1-p960226	15	6841151	2 4 2 0 2 2 2 2 2 2 2 2 2 2 2 2 0 2 2 3 3 2 2 2 2 3 3 3 3	11.0		13.5	26.2	2.0	A T W failed
73	Bn-scaff_25878_1-p8784	16	21049946	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 2 3 2 2 3			13.8	26.3		G R
74	Bn-scaff_15746_1-p84688	16	21099359	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 2 3 2 2 3			13.8	26.3		K T
75	Bn-scaff_18439_1-p208544	16	24118166	3 3 2 3 3 2 3 3 3 3 2 3 2 2 2 2 2 4 2 2 2 2 3 2 2 2 2 4 2 2 2			18.8	8.1	22.5	T Y
76	Bn-scaff_15892_1-p1657890	16	27476177	2 3 2 3 3 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2			17.1	5.0		C Y
77	Bn-scaff_21711_1-p18343	17	31801361	2 2 2 3 2 2 2 2 2 3 2 2 2 2 3 3 2 2 2 2 3 3 3 2 2 3 3 3 3			12.3	21.0		A G R
78	Bn-A08-p20245485	18	19536129	2 3 2 3 3 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2			17.2	4.5		A R
79	Bn-A01-p1423163	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3			14.5	29.5		A M
80	Bn-A01-p1578682	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3			14.5	29.5		C M
81	Bn-A01-p1746226	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3			14.5	29.5		G K
82	Bn-A01-p1773289	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3			14.5	29.5		A R
83	Bn-A01-p2177864	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3			14.5	29.5		K T
84	Bn-A01-p2448162	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 2 3 3			14.2	27.3		T W
85	Bn-A01-p2504370	NA	NA	0 0 0 2 2 2 0 2 2 2 2 2 2 2 2 3 2 0 2 0 2 2 3 2 3 0 3 2 3 3	11.0		14.0	25.0		G K T failed
86	Bn-A03-p14927037	NA	NA	2 0 2 0 0 2 2 2 2 4 2 2 2 2 2 3 3 2 2 2 2 3 2 3 3 2 3 0 3 3	9.8		13.3	23.8	10.0	A G R failed
87	Bn-A04-p1001237	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3			13.7	27.5		A R
88	Bn-A04-p1007534	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3			13.7	27.5		T Y
89	Bn-A04-p1009976	NA	NA	2 2 2 2 2 2 2 2 2 2 1 2 2 2 2 2 2 2 2 2 2 2 4 2 2 3 2 3 3		11.0	13.8	28.7	24.0	C Y
90	Bn-A04-p1048288	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3			13.7	27.5		A M
91	Bn-A04-p262404	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3			14.0	28.7		C M
92	Bn-A04-p288906	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3			14.0	28.7		T Y
93	Bn-A04-p312324	NA	NA	2 2 2 2 2 2 2 2 2 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3		11.0	14.2	28.7		C Y
94	Bn-A04-p320972	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3			14.0	28.7		A R
95	Bn-A04-p322820	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3			14.0	28.7		K T
96	Bn-A04-p534430	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3			13.7	27.5		K T
97	Bn-A04-p534845	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3			13.7	27.5		A R
98	Bn-A04-p541336	NA	NA	2 2 2 2 2 2 2 2 2 2 4 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3			13.8	27.5	11.0	A R
99	Bn-A04-p761383	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3			13.7	27.5		K T
100	Bn-A04-p808834	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3			14.0	28.7		G K
101	Bn-A04-p926916	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 0 2 2 3 2 3 3	24.0		13.7	28.7		T Y failed
102	Bn-A04-p932865	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3			14.0	28.7		A R
103	Bn-A04-p980952	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3			13.7	27.5		G K
104	Bn-A04-p982068	NA	NA	2 2 2 2 2 2 2 2 2 2 1 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3		11.0	13.8	27.5		A R
105	Bn-A06-p24284014	NA	NA	2 2 2 2 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 2 3 3		7.0	13.9	27.8		T Y
106	Bn-A06-p24319329	NA	NA	2 2 2 2 2 0 2 2 1 2 0 2 2 2 1 0 0 2 2 2 3 3 2 3 3 3 2 0 3	16.6	13.0	11.9	25.5		G K T failed
107	Bn-A06-p25343454	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 0 2 2 2 2 2 3 2 3 3 3	18.0		13.4	27.8		A G R failed
108	Bn-A06-p25345586	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 4 2 2 2 2 2 3 2 3 2 3 3			13.4	27.8	18.0	A C M
109	Bn-A06-p5944090	NA	NA	2 0 2 4 2 2 2 2 2 2 2 2 2 2 2 2 4 2 2 3 3 2 2 2 2 3 3 3 3	2.0		13.5	26.2	11.0	A T W failed
110	Bn-A07-p16406072	NA	NA	2 3 2 3 3 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2			17.1	5.0		T Y
111	Bn-A08-p20244218	NA	NA	2 3 2 3 3 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2			17.2	4.5		T Y
112	Bn-A08-p4876592	NA	NA	2 3 2 3 2 2 3 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2			17.1	5.3		T Y
113	Bn-A08-p8589613	NA	NA	2 2 2 2 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3 3		7.0	13.9	27.8		T Y
114	Bn-A08-p8703472	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3 3			13.6	27.8		T Y
115	Bn-A08-p8716833	NA	NA	3 2 2 3 3 2 2 3 2 3 3 3 2 3 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2			18.2	9.1		G K T
116	Bn-A10-p10996646	NA	NA	2 3 2 3 2 2 3 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2			17.1	5.3		G R
117	Bn-scaff_15746_1-p403163	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 2 3 2 3			13.8	26.3		T Y
118	Bn-scaff_15892_1-p114893	NA	NA	3 3 2 3 3 2 2 2 3 2 2 2 2 2 2 2 2 4 2 2 2 2 2 2 2 2 2 2 2			17.8	4.2	18.0	A R
119	Bn-scaff_15892_1-p114985	NA	NA	3 3 2 3 3 2 2 2 3 2 2 2 2 2 2 2 2 4 2 2 2 2 2 2 2 2 2 2 2			17.8	4.2	18.0	K T
120	Bn-scaff_15892_1-p115171	NA	NA	2 3 2 3 3 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2			17.1	5.0		G R
121	Bn-scaff_15892_1-p122157	NA	NA	2 3 2 3 3 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2			17.1	5.0		K T
122	Bn-scaff_15892_1-p122759	NA	NA	2 3 2 3 3 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2			17.1	5.0		C Y
123	Bn-scaff_16064_1-p1442106	NA	NA	2 3 2 3 3 2 3 3 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2			17.9	5.8		G S
124	Bn-scaff_16209_1-p129602	NA	NA	2 2 2 2 2 2 2 4 2 3 1 4 1 4 2 0 3 2 2 2 3 3 1 3 0 3 3 1 3	21.0	19.3	9.9	22.9	11.3	A G R failed
125	Bn-scaff_16244_1-p25214	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 0 2 2 2 3 3 2	23.0		13.4	26.5		G K T failed
126	Bn-scaff_16244_1-p28539	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3 2 2 2 3 3 2			13.4	25.8		A G R
127	Bn-scaff_16244_1-p8089	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3 2 2 2 3 3 2			13.4	25.8		C T Y
128	Bn-scaff_16534_1-p1474844	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3 2 3 2 2 3			13.5	25.6		C T Y
129	Bn-scaff_17517_1-p353394	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 0 2 2 2 3 3 2	23.0		13.4	26.5		A G R failed
130	Bn-scaff_17517_1-p354243	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3 2 2 2 3 3 2			13.4	25.8		A C M
131	Bn-scaff_17517_1-p354525	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 3 2 2 2 3 3 2			13.4	25.8		C T Y
132	Bn-scaff_18439_1-p203550	NA	NA	3 3 2 3 3 2 3 3 3 2 3 2 2 2 2 2 4 2 2 2 2 3 2 2 2 4 2 2 2			18.8	8.1	22.5	K T
133	Bn-scaff_18656_1-p53047	NA	NA	2 2 2 2 2 2 2 2 2 2 1 2 2 2 0 2 2 1 2 2 2 2 3 2 2 3 2 3 3	15.0	14.5	13.5	27.5		C Y failed
134	Bn-scaff_18656_1-p54366	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 0 2 2 3 2 3 3	24.0		13.7	28.7		A R failed
135	Bn-scaff_18656_1-p54679	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 3 3			13.7	27.5		T W
136	Bn-scaff_20901_1-p653379	NA	NA	2 2 2 1 2 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 3 2 3 2 3 3 3 3		4.0	13.2	24.4		C T Y
137	Bn-scaff_20901_1-p857332	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 3 2 2 3 2			13.9	26.0		A G R
138	Bn-scaff_22350_1-p24061	NA	NA	2 2 2 2 2 2 2 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 3 2 3 2 3 3			13.7	24.6		G K
139	Bn-scaff_26946_1-p37150	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 0 2 2 3 2 3 3	24.0		13.7	28.7		T Y failed
140	Bn-Scaffold000203-p50466	NA	NA	2 2 2 2 4 2 2 2 2 2 2 2 2 2 3 4 2 2 2 3 3 3 2 3 2 0 0 3 3	27.5		11.7	23.7	11.0	C T Y failed

b)

	Marker	Chr	Pos	MarkerOrder (Lowest -> Highest)	0	1	2	3	4	Variation
1	Bn-A04-p560622	4	441599	2 2 2 2 2 2 2 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 1 2 1 1		28.7	14.2	11.0		C Y
2	Bn-scaff_17487_1-p416211	19	6588547	2 2 2 4 3 3 3 3 2 3 4 2 2 3 2 2 2 2 2 2 2 3 2 3 1 2 1 3 1 1		27.8	14.3	13.8	7.5	A G R
3	Bn-scaff_15701_1-p214816	19	10183356	2 2 2 2 3 2 3 3 2 2 4 2 3 2 2 2 2 2 2 2 2 3 2 2 1 2 1 3 1 1		27.8	13.7	13.8	11.0	A G R
4	Bn-scaff_28725_1-p150634	19	10403607	2 0 2 2 3 2 3 3 2 2 4 2 3 2 2 2 2 2 2 2 2 3 2 2 1 2 1 3 1 1	2.0	27.8	14.3	13.8	11.0	C Y failed
5	Bn-scaff_17869_1-p416439	19	12959541	2 0 2 2 3 2 3 3 2 2 4 3 3 2 2 2 2 2 2 2 2 3 2 2 1 2 1 3 1 1	2.0	27.8	14.5	13.6	11.0	A C M failed
6	Bn-scaff_17888_1-p183402	19	15811513	3 3 2 3 3 2 2 3 2 3 3 3 3 3 2 2 3 3 2 3 2 3 3 3 2 2 1 2 1 1		28.7	15.9	12.8		G K T
7	Bn-scaff_17888_1-p173470	19	15823395	3 3 2 3 3 2 2 3 2 3 4 3 0 3 2 2 0 3 2 3 2 3 3 3 1 2 1 2 1 1	15.0	27.8	15.0	12.5	11.0	A G R failed
8	Bn-scaff_15701_1-p121620	NA	NA	2 2 2 2 3 2 3 3 2 2 0 2 3 2 2 2 2 2 2 2 2 3 2 2 1 2 1 3 1 1	11.0	27.8	13.7	13.8		G K T failed

c)

	Marker	Chr	Pos	MarkerOrder (Lowest -> Highest)	0	1	2	3	4	Variation
1	Bn-scaff_18310_1-p635376	NA	NA	2 3 2 3 3 2 2 2 2 3 2 3 2 2 2 2 2 3 2 2 2 3 3 2 3 2 0 0 3 0	28.3		13.5	15.0		C T Y failed
2	Bn-scaff_18936_1-p358822	NA	NA	2 2 2 2 3 2 4 3 2 3 2 3 2 2 2 2 2 3 2 2 2 2 2 3 2 2 3 2 0 3 0 0	28.7		13.6	15.9	7.0	A G R failed

Table 10: Markers associated with nitrogen use efficiency (NUE) under no N fertilization using Wilcoxon rank-sum tests with significance set to $p < 0.01$. For each marker, the genotype's marker type is displayed from lowest to highest (Table 5), along with a calculated score for each marker present, and the original SNP variation in the 30 genotypes. (a) markers in which homozygosity and heterozygosity differed, and (b) markers in which single copy differed from two copies. "0" = missing, "1" = single copy, "2" = homozygotic, "3" = heterozygotic, "4" = incorrect. NUE calculated using Equation 1.

a)

	Marker	Chr	Pos	Marker Order (Lowest -> Highest)	0	1	2	3	4	Variation
1	Bn-A01-p2403471	1	1894127	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3 2			14.1	27.7		A R
2	Bn-A01-p2421445	1	1912045	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3 2			14.1	27.7		G R
3	Bn-A04-p556991	4	437301	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 2 3 2 2 2 3 3 2			13.6	25.0		C M
4	Bn-A04-p664994	4	540419	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 3 3 2			13.8	26.8		A M
5	Bn-A04-p665932	4	542345	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 3 3 2			13.8	26.8		G R
6	Bn-A04-p669182	4	545975	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 3 3 2			13.8	26.8		G R
7	Bn-A04-p783339	4	654736	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 3 3 2			13.8	26.8		G K
8	Bn-A04-p784708	4	656105	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 3 3 2			13.8	26.8		T Y
9	Bn-A04-p785628	4	657029	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 1 2 2 2 3 2 2 2 3 3 2		18.0	13.6	26.8		A R
10	Bn-A04-p792399	4	663829	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 1 2 2 2 2 3 2 2 2 3 3 2		18.0	13.6	26.8		G S
11	Bn-A04-p810935	4	686983	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 1 2 2 2 2 3 2 2 2 3 3 2		18.0	13.6	26.8		C Y
12	Bn-A04-p2282199	4	1718920	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 3 3 2			13.8	26.8		T Y
13	Bn-A04-p2152528	4	1829861	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 3 3 2			13.8	26.8		T Y
14	Bn-A04-p2152171	4	1830215	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 1 2 2 2 2 3 2 2 2 3 3 2		18.0	13.6	26.8		A M
15	Bn-A04-p2151567	4	1830804	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 3 3 2			13.8	26.8		G R
16	Bn-A04-p2146459	4	1836075	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 3 3 2			13.8	26.8		C Y
17	Bn-A10-p13335021	10	13379963	2 2 3 3 2 3 3 3 2 3 3 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2			18.4	7.6		C T Y
18	Bn-A01-p2448162	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 3 3 2			14.1	27.7		T W
19	Bn-A04-p1001237	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 3 3 2			13.8	26.8		A R
20	Bn-A04-p1007534	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 3 3 2			13.8	26.8		T Y
21	Bn-A04-p1048288	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 3 3 2			13.8	26.8		A M
22	Bn-A04-p534430	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 3 3 2			13.8	26.8		K T
23	Bn-A04-p534845	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 3 3 2			13.8	26.8		A R
24	Bn-A04-p541336	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 4 2 2 2 2 3 2 2 2 3 3 2			13.6	26.8	18.0	A R
25	Bn-A04-p557097	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 2 3 2 2 2 3 3 2			13.6	25.0		K T
26	Bn-A04-p761383	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 3 3 2			13.8	26.8		K T
27	Bn-A04-p980952	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 3 3 2			13.8	26.8		G K
28	Bn-A04-p982068	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 1 2 2 2 2 3 2 2 2 3 3 2		18.0	13.6	26.8		A R
29	Bn-scaff_16064_1-p1442106	NA	NA	2 2 3 3 2 2 3 3 2 3 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2			17.6	7.2		G S
30	Bn-scaff_18656_1-p54679	NA	NA	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 3 3 2			13.8	26.8		T W

b)

	Marker	Chr	Pos	MarkerOrder (Lowest -> Highest)	0	1	2	3	4	Variation
1	Bn-scaff_17487_1-p1909011	19	8186901	2 2 3 3 2 3 2 2 3 3 3 3 3 3 3 2 2 1 3 3 2 1 1 2 2 2 2 1 1 2		24.0	16.1	11.3		A G R
2	Bn-scaff_17487_1-p1912492	19	8191474	2 2 3 3 2 3 2 2 3 3 3 2 3 3 3 2 2 1 3 3 2 1 1 2 2 2 2 1 1 2		24.0	15.8	11.3		G K T
3	Bn-scaff_17487_1-p1927920	19	8201860	2 2 3 3 2 3 2 2 3 3 3 3 3 3 3 2 2 1 3 3 2 1 1 2 2 2 2 1 1 2		24.0	16.1	11.3		A C M
4	Bn-scaff_17487_1-p1938942	19	8218945	2 2 3 3 2 3 2 2 3 3 3 2 3 3 3 2 2 1 3 3 2 1 1 2 2 2 2 1 1 2		24.0	15.8	11.3		G K T

Table 11: Markers associated with seed mass under N fertilization of 200 kg N/ha using Wilcoxon rank-sum tests with significance set to $p < 0.01$. For each marker, the genotype's marker type is displayed from lowest to highest (Table 5), along with a calculated score for each marker present, and the original SNP variation in the 30 genotypes. (a) markers in which homozygosity and heterozygosity differed, (b) markers in which single copy differed from two copies, and (c) markers in which presence and absence differed. "0" = missing, "1" = single copy, "2" = homozygotic, "3" = heterozygotic, "4" = incorrect.

a)

	Marker	Chr	Pos	Marker Order (Lowest -> Highest)	0	1	2	3	4	Variation
1	Bn-A01-p10720611	1	9633959	3033222222202020222222020222222	14.5		17.6	2.7		C Y failed
2	Bn-A01-p23472380	1	19346196	2322223222322213222333233333333		15.0	10.8	20.6		A G R
3	Bn-A01-p24707425	1	20475601	2222420222222222222222222240333	17.0		13.6	29.0	15.5	C Y failed
4	Bn-scaff_22466_1-p746149	3	10371383	2202222222222243233331333333320	16.5	22.0	9.9	22.5	15.0	C G S failed
5	Bn-A03-p27377729	3	25635715	2202420242122222232131323333303	13.0	17.7	11.5	24.8	7.0	C T Y failed
6	Bn-A04-p6340854	4	7570612	2232222222232223332222223333233333			12.3	21.0		G R
7	Bn-A04-p6422450	4	7624947	2202222222232224322222233323333	3.0		12.3	23.7	15.0	G R failed
8	Bn-A06-p2676798	6	2619418	2222222223232242232323333233323			11.6	21.9	15.0	A C M
9	Bn-A06-p11239689	6	10450560	2222220222223220222331333333223	11.5	22.0	11.7	23.2		A G R failed
10	Bn-A07-p15428458	7	17342826	2222222222222222232222222223333323			13.5	25.4		T Y
11	Bn-Scaffold000827-p421	8	13961418	2222222222232222323233333330323	27.0		11.0	21.8		A C M failed
12	Bn-A08-p16562035	8	14030897	2222222222232222323233232333323			11.6	22.3		A G R
13	Bn-A10-p5183890	10	4769396	3332222222222222222222222242220	30.0		16.1	2.0	26.0	A G R failed
14	Bn-A10-p5679914	10	5324731	3332222222222222222222242220	30.0		16.1	2.0	26.0	A G R failed
15	Bn-A01-p18704834	10	5877343	3332222222222222222220222242220	26.0		15.9	2.0	26.0	C T Y failed
16	Bn-A01-p18890238	10	6093820	3332222222222221022222422242220	23.0	15.0	15.9	2.0	24.0	A C M failed
17	Bn-A01-p19107003	10	6319979	3332222222222221222222222242220	30.0	15.0	16.2	2.0	26.0	A G R failed
18	Bn-A01-p19108849	10	6324178	3332222222222221222222222242224		15.0	16.2	2.0	28.0	A G R
19	Bn-A10-p13164379	10	13206159	2222222222222223223223023333323	22.0		11.5	23.8		G K T failed
20	Bn-A10-p13169213	10	13211013	2222222222222123222223421333323		18.5	11.7	24.6	22.0	G K T
21	Bn-A10-p14365581	10	14309162	2222222222222223223223222232333			13.0	25.3		C T Y
22	Bn-A10-p14429854	10	14376659	2222222222222222223223222232233			13.6	24.8		C T Y
23	Bn-A01-p23538267	11	34626593	2322223222242222022233323333333	16.0		11.1	21.8	11.0	A G R failed
24	Bn-scaff_16352_1-p361447	13	15479874	0322222222202223323233333443334	6.0		9.8	20.4	27.0	C T Y failed
25	Bn-scaff_17799_1-p1308909	16	35299460	2222222222222222222222222222323			14.5	29.0		T Y
26	Bn-scaff_18202_1-p176275	17	23395310	2222222222222022323222223323333	13.0		12.1	24.6		A G R failed
27	Bn-A01-p18635275	NA	NA	3332222222222222422222422242220	30.0		15.9	2.0	21.3	G K T failed
28	Bn-A01-p18636804	NA	NA	3332222222222222222222222242220	30.0		16.1	2.0	26.0	A G R failed
29	Bn-A04-p6277634	NA	NA	2202222222232223022222233323330	16.3		12.3	22.8		K T failed
30	Bn-A04-p6428686	NA	NA	2222222222232223322222233323334			11.9	22.0	30.0	T Y
31	Bn-A05-p6480907	NA	NA	2222224222222222222233422323323			13.0	25.2	14.5	A G R
32	Bn-A05-p6480981	NA	NA	2222224222222224222333223232323			12.8	24.7	11.5	G K T
33	Bn-A05-p6534544	NA	NA	2222224222222220222333223232323	16.0		12.8	24.7	7.0	G K T failed
34	Bn-A05-p673050	NA	NA	2222020220220232222233323333223	8.8		12.5	24.1		G K T failed
35	Bn-A06-p10594170	NA	NA	2222220222223220232332333333223	11.5		11.9	22.7		G K T failed
36	Bn-A10-p14431375	NA	NA	2222222222222222222232222232233			13.8	26.5		A G R
37	Bn-A10-p4968727	NA	NA	3332222222222221222222222242220	30.0	15.0	16.2	2.0	26.0	C T Y failed
38	Bn-A10-p4972939	NA	NA	3332222222222222222222222242220	30.0		16.1	2.0	26.0	C T Y failed
39	Bn-scaff_18202_1-p182631	NA	NA	2222220222223223232222233233333	7.0		12.4	23.3		A T W failed
40	Bn-scaff_18275_1-p1372103	NA	NA	2222222222222222222222323223223			13.9	25.8		A C M

b)

	Marker	Chr	Pos	MarkerOrder (Lowest -> Highest)	0	1	2	3	4	Variation
1	Bn-A08-p16514789	NA	NA	2322233020023021212112131141014	14.4	23	10.7	10.4	27	G K T failed

c)

	Marker	Chr	Pos	MarkerOrder (Lowest -> Highest)	0	1	2	3	4	Variation
1	Bn-A01-p5522870	1	5082549	2222332222222324322233222000200	27.4		12.9	13.5	15.0	A G R failed
2	Bn-A09-p29968302	9	27779148	2232224232422222202240022400004	24.5		12.4	6.0	19.0	A G R failed
3	Bn-scaff_16197_1-p3241707	18	31032064	3232333323233223222203220220000	26.3		15.5	9.0		C T Y failed
4	Bn-A05-p673050	NA	NA	2222020220220232222233323333223	8.8		12.5	24.1		G K T failed
5	Bn-scaff_16197_1-p3111917	NA	NA	3232333323233223222203223220000	26.8		15.5	10.4		A G R failed
6	Bn-scaff_20646_1-p246974	NA	NA	3322122232222323220233030340304	24.0	5.0	10.6	15.7	28.0	C T Y failed
7	Bn-scaff_27076_1-p79989	NA	NA	0002322222222223221223222222222	2.0	18.0	17.4	13.7		C M failed

Table 12: Markers associated with nitrogen use efficiency (NUE) under N fertilization of 200 kg N/ha using Wilcoxon rank-sum tests with significance set to $p < 0.01$. For each marker, the genotype's marker type is displayed from lowest to highest (Table 5), along with a calculated score for each marker present, and the original SNP variation in the 30 genotypes. (a) markers in which homozygosity and heterozygosity differed, and (b) markers in which presence and absence differed. "0" = missing, "1" = single copy, "2" = homozygotic, "3" = heterozygotic, "4" = incorrect. NUE calculated using Equation 1.

a)

	Marker	Chr	Pos	MarkerOrder (Lowest -> Highest)	0	1	2	3	4	Variation
1	Bn-A01-p10720611	1	9633959	3 0 0 3 3 2 2 2 2 2 0 0 2 2 2 2 2 2 2 0 2 2 2 0 2 2 2 2 2 2	12.0		18.2	3.3		C Y failed
2	Bn-A03-p24971161	3	23385545	2 3 3 2 3 2 3 2 2 2 2 3 3 3 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2			18.0	8.6		A G R
3	Bn-A03-p24971360	3	23385744	2 3 3 2 3 2 3 2 2 2 2 3 3 3 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2			18.0	8.6		A G R
4	Bn-A03-p25009919	3	23409723	2 3 3 2 3 2 3 2 3 2 3 2 3 3 3 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2			18.4	8.7		C T Y
5	Bn-A03-p25009925	3	23409729	2 3 3 2 3 2 3 2 3 2 3 2 3 3 3 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2			18.4	8.7		A G R
6	Bn-A03-p25024647	3	23417138	2 3 3 2 3 2 3 2 3 2 3 2 3 3 3 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2			18.4	8.7		A G R
7	Bn-A03-p25025360	3	23417857	2 3 3 2 3 2 3 2 3 2 3 2 3 3 3 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2			18.4	8.7		C T Y
8	Bn-A03-p26815896	3	25244510	3 3 3 2 3 2 3 3 4 3 3 4 2 4 2 4 2 2 2 2 2 3 2 2 2 2 2 2 2 2			20.3	7.7	12.8	A G R
9	Bn-A03-p26822384	3	25252640	3 3 3 2 3 2 3 3 4 3 3 3 0 4 2 4 2 2 2 2 2 3 2 2 2 2 2 2 2 2	13.0		20.8	8.1	13.0	C T Y failed
10	Bn-A03-p26931199	3	25395191	3 3 3 2 3 2 3 3 4 3 3 3 2 4 2 4 2 2 2 2 2 3 2 2 2 2 2 2 2 2			20.3	8.1	13.0	A G R
11	Bn-A07-p9450863	7	10818136	2 3 2 2 3 2 3 3 3 3 3 3 2 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2			18.3	9.0		T Y
12	Bn-A07-p9468382	7	10825039	2 3 2 2 3 2 3 3 3 3 3 3 2 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2			18.3	9.0		K T
13	Bn-A07-p12345399	7	14471603	2 2 4 2 2 2 2 2 1 2 0 2 3 2 2 0 4 2 2 2 3 3 4 3 3 3 3 2 3 3	13.5	9.0	11.3	24.1	14.3	C T Y failed
14	Bn-A07-p13154645	7	15234994	3 1 3 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 0 2 2 2 2 2 2	24.0	2.0	17.2	3.0		G R failed
15	Bn-A10-p1125156	10	2416749	3 3 3 2 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 1 2 2 0 2 2 2 4 2 1 0	19.3	24.5	15.5	2.0	27.0	A G R failed
16	Bn-scaff_17721_1-p675626	12	43658302	3 3 3 2 3 2 3 3 3 2 3 2 2 3 2 2 3 2 2 2 2 3 2 2 2 2 4 2 2 2			18.8	9.0	27.0	A G R
17	Bn-A03-p24998976	NA	NA	2 3 3 2 3 2 3 2 3 2 3 3 3 2 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2			18.4	8.7		A C M
18	Bn-A03-p26121502	NA	NA	2 3 3 2 3 2 3 3 2 2 2 3 2 3 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2			18.0	8.5		A G R
19	Bn-A03-p26822285	NA	NA	3 3 3 2 3 2 3 3 4 3 3 3 2 4 2 4 2 2 2 1 2 3 2 2 2 2 2 2 2 2		20.0	20.3	8.1	13.0	A G R
20	Bn-A07-p10409623	NA	NA	3 3 3 2 3 2 3 3 3 3 3 2 2 3 2 2 3 2 2 2 3 3 3 2 2 2 2 2 2 2			19.5	10.9		A G R
21	Bn-A07-p13162255	NA	NA	3 1 3 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 1 2 2 2 2 2 2		13.0	17.2	3.0		G R
22	Bn-A10-p1023294	NA	NA	3 3 3 2 2 2 2 2 2 2 2 1 2 2 2 0 2 2 2 2 0 2 4 2 2 2 0 2 2 2	21.3	12.0	16.4	2.0	23.0	C T Y failed
23	Bn-scaff_15763_1-p1048413	NA	NA	3 1 3 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 1 2 2 2 2 2 2		13.0	17.2	3.0		K T
24	Bn-scaff_19899_1-p357670	NA	NA	2 2 2 2 2 2 2 2 2 2 3 2 2 2 2 3 3 2 2 2 3 3 2 2 3 2 3 2 3 3			12.7	22.0		C T Y

b)

	Marker	Chr	Pos	MarkerOrder (Lowest -> Highest)	0	1	2	3	4	Variation
1	Bn-scaff_20125_1-p306182	15	10902367	0 0 0 2 3 2 2 2 2 2 4 2 2 2 2 2 2 2 2 3 2 3 4 3 2 2 2 2 2 2	2.0		16.9	17.8	17.0	A M failed
2	Bn-scaff_15763_1-p622996	16	20178116	0 0 0 2 3 2 2 3 2 3 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2	2.0	11.0	18.1	12.5		A R failed
3	Bn-A04-p5971620	NA	NA	0 0 0 2 3 2 2 2 2 3 0 2 2 2 2 2 2 2 2 2 2 2 3 2 2 2 2 3 2 2	4.3		17.5	16.0		C T Y failed
4	Bn-A06-p17010360	NA	NA	0 2 0 2 0 2 2 2 0 2 2 2 3 3 3 2 3 2 2 2 2 3 2 2 2 3 3 2 2 2	4.5		15.5	20.4		C T Y failed
5	Bn-scaff_15844_1-p163992	NA	NA	2 0 2 2 0 2 2 2 0 2 0 3 2 2 2 3 3 2 2 3 3 2 3 2 3 2 3 2 3 2	6.8		14.6	21.1		A G R failed

Table 13: Number of markers associated with seed mass or nitrogen use efficiency (NUE), under either no N fertilization (N1) or N fertilization of 200 kg N/ha (N2), identifying differences between homozygous or heterozygous markers, one-copy or two-copy and presence or absence. Test were performed using a significance level of (a) $p = 0.01$ and (b) $p = 0.05$.

a)

$p < 0.01$		Homozygous *vs.* Heterozygous	One Copy *vs.* Two Copy	Presence *vs.* Absence
N1	Seed Mass	140	8	2
	NUE	30	4	0
N2	Seed Mass	40	1	7
	NUE	24	0	5

b)

$p < 0.05$		Homozygous *vs.* Heterozygous	One Copy *vs.* Two Copy	Presence *vs.* Absence
N1	Seed Mass	610	64	36
	NUE	294	23	27
N2	Seed Mass	635	7	82
	NUE	548	0	71

Table 14: The number of similar markers identified as significant for the test of homozygous *vs.* heterozygous, with seed mass and nitrogen use efficiency (NUE) under no N fertilization (N1) and N fertilization of 200 kg N/ha (N2). Test were performed using a significance level of (a) $p = 0.01$ and (b) $p = 0.05$.

a)

$p < 0.01$	N1 Seed Mass	N2 NUE
N1 NUE	28	0
N2 Seed Mass	3	1

b)

$p < 0.05$	N1 Seed Mass	N2 NUE
N1 NUE	183	32
N2 Seed Mass	103	326

Table 15: Markers identified as significant in all Wilcoxon rank-sum tests (seed mass and nitrogen use efficiency (NUE) under no and 200 kg N/ha fertilization) at $p < 0.05$. NUE calculated using Equation 1.

	Marker	Chr	Pos
1	Bn-scaff_16792_1-p35017	15	11084495
2	Bn-scaff_16792_1-p34725	15	11084787
3	Bn-scaff_16792_1-p34579	15	11084933
4	Bn-scaff_16792_1-p34528	15	11084984
5	Bn-scaff_16792_1-p26742	15	11092753
6	Bn-scaff_16792_1-p24255	15	11095200
7	Bn-scaff_16792_1-p21478	15	11097979
8	Bn-scaff_16792_1-p21096	15	11098361
9	Bn-scaff_21821_1-p9352	15	11134712
10	Bn-scaff_21821_1-p18474	15	11145768
11	Bn-scaff_21821_1-p18810	15	11146104
12	Bn-scaff_21338_1-p6152	15	11465022
13	Bn-scaff_21338_1-p8681	15	11467551
14	Bn-scaff_21338_1-p12356	15	11471226
15	Bn-scaff_21338_1-p18825	15	11476353
16	Bn-scaff_21338_1-p22459	15	11480018
17	Bn-scaff_21338_1-p156935	15	11576396
18	Bn-scaff_21338_1-p194830	15	11618495
19	Bn-scaff_16792_1-p22894	NA	NA
20	Bn-scaff_16792_1-p27067	NA	NA
21	Bn-scaff_16792_1-p54796	NA	NA
22	Bn-scaff_21338_1-p138987	NA	NA
23	Bn-scaff_21338_1-p151286	NA	NA
24	Bn-scaff_21338_1-p66728	NA	NA
25	Bn-scaff_21338_1-p67288	NA	NA
26	Bn-scaff_21338_1-p71093	NA	NA
27	Bn-scaff_21338_1-p71950	NA	NA
28	Bn-scaff_21338_1-p72824	NA	NA
29	Bn-scaff_21821_1-p32954	NA	NA

4 Discussion

4.1 Phenotypic Variation for NUE

Phenotypic data show that indeed some cultivars have a higher NUE and seed yield than others. As expected, due to the progress of breeding programs and hybrid vigor, the new hybrids performed the best for seed and oil yield. This was not the case for NUE, calculated using Equation 1. It was, in fact, purebred lines that scored the

highest NUE under both N treatments. However, these purebred lines scored high under only one of the N treatments, and had an average or poor score under the other. The hybrids were best suited for both N conditions, for root mass, NUE and especially seed and oil yield, the most important traits for farmers. This suggests that the hybrids may have gene combinations optimized for more variable environmental conditions, potentially due the presence of heterozygotic alleles, *i.e.*, heterosis through codominance. It is clear that in general, the hybrids outperform the purebreds in seed yield at both N levels, results which have been seen in other NUE studies (Kessel *et al.*, 2012), and a trend which has been seen in Germany since their adoption (Abbadi & Leckband, 2011).

4.2 Seed Quality

NIRS results indicate that breeding has caused changes in seed quality among *B. napus* cultivars. This is not surprising since seed yield and oil content are often the main goals of *B. napus* breeders, and come at the expense of lower seed protein content. Breeding progress could also be seen in the measurements of the harvest index, which saw increases among the new cultivars, both purebred and hybrid. Compared to the old purebreds, new purebreds had a higher seed oil content under N1, but lower under N2. Since seed oil and seed protein content are known to have a negative correlation (Grami *et al.*, 1977), it can be assumed that under N fertilization, there is less carbon for allocation to oil synthesis, due to an increased consumption of carbon for N metabolism. This can also be seen in the lower seed protein content among all cultivar groups upon N fertilization. Among the hybrids, increased seed mass and seed oil mass coincide with decreased seed protein content compared to purebreds, results which are in line

with Koeslin-Findeklee *et al.* (2014) studies on NUE in *B. napus* purebreds and hybrid cultivars. This suggests that breeders goals of increasing seed oil yield may reduce NUE by decreasing seed protein content, and could help explain why the highest NUE scorers under the two N levels were purebreds. In response, increasing both seed oil and protein content has become a goal *B. napus* breeders are exploring by identifying quantitative trait loci (QTL) which control oil content independently of protein content (Zhao *et al.*, 2006). These changes in seed protein content, and thus, N content, impact the calculation of NUE depending on how one measures it. Using Equation 2, NUE is calculated ignoring seed N content, using yield as the relevant parameter. Although Equation 2 is more applicable to farmers and often the only way to calculate NUE with available data, by not accounting for changes in seed N content, calculating NUE with Equation 1 and Equation 2 give different results. Van Sanford & Mackown (1986) distinguished these two methods as NUE for yield (NUEY) and NUE for protein (NUEP), noticing the difference in ranking among cultivars between the two.

4.3 Root Traits in the Context of NUE

One approach to improve NUE, which has attracted some scientists and breeders, is to focus on root characteristics, reviewed by Garnett *et al.* (2009). In this study, root traits were correlated with seed yield and NUE, but not always, which suggests that using root mass or fine root score as an indicator of seed yield or NUE may depend on the genetic background of the plant. As such, root traits might not always be a good selection tool for a breeder, since its applicability may be dependent on the plants NupE and NutE. Under N1, there was a much stronger correlation of seed mass and NUE with root traits compared to N2, suggesting that focusing on root traits may be on benefit

when breeding for *B. napus* crops suitable to low input systems. However, especially with new hybrids, there is little correlation under both N treatments, suggesting that root characteristics have become less important for seed yield and NUE over time, perhaps due to an increased NupE or NutE caused by cell physiological aspects, such as enzyme activity.

Results demonstrate that increases in available N through fertilization cause a shift in *B. napus* root structure, decreasing length and increasing fine root score. This is a little unexpected since high ratios of soil carbon to nitrogen have been shown to inhibit lateral root formation in *Arabidopsis* (Malamy & Ryan, 2001), a close relative of *B. napus*. However, the opposite reaction has been observed in some grass species (Robinson & Rorison, 1987), illustrating the variability of plant root structure responses to different environmental N levels. Interestingly, there were dramatic differences in root mass variation between the old and new purebreds, suggesting a possible diversification of breeding goals, such as resistance and optimization under different environments. Notably, there was less variation in both plant masses and NUE under N fertilization, likely due to the fact that many of these cultivars, especially the new ones, were bred under condition of high N fertilization. Additionally, with the exception of a few cultivars, it appears that even though breeders are not directly selecting for it, root mass seems to be increasing over time with some of the new purebreds and new hybrids.

Variation in root structure was also observed between cultivars, even those with similar seed yields, suggesting there may be diverse strategies to NupE and NutE with respect to root structure among *B. napus* cultivars. Future studies investigating the differences in NUE among *B. napus* cultivars, may benefit by combining phenotype

analysis of root traits such as root mass and fine root score with tools to investigate NupE and NetE, such as the use of ^{15}N labelling to model N flux as described by Salon *et al.* (2014), or the nitrate update modeling described by Le Deunff & Malagoli (2014) and Malagoli & Le Deunff (2014). The identification of diversity in NupE and NutE along with root traits in *B. napus* cultivars would be of great benefit to breeders for improving NUE and helping to expand our knowledge of it.

4.4 Heterozygosity and NUE

Genotypic analysis using the statistical program R allowed for the creation of reusable code for easy manipulation of large data sets with high-throughput. The use of computer language code for data analysis should allow for future, similar projects to be done in a much faster fashion, as the code can be rerun on new data sets with only small changes to the code, and could further be optimized for easier reusability if desired for continual reuse, which may be of benefit to a breeding/research program.

To my knowledge, this is the first study to investigate the effect of SNP heterozygosity on phenotypic traits in elite *B. napus* cultivars and may provide a valuable tool in the future for investigating heterosis. Markers in which heterozygosity may have an effect on a seed mass or NUE, both positively and negatively were identified. In addition, it was also possible to identify markers with which the presence or absence may have a phenotypic effect. Under N1, heterozygosity of significant markers mainly had a positive impact on NUE. However, under N2 they appeared to have a negative impact, indicating that heterozygosity is more beneficial under lower N levels. Similarly, there were many more markers in which heterozygosity positively impacted seed mass under N1 than N2. This further supports the idea of hybrid cultivars as being

an important tool for breeding for low input systems. Markers with which the absence positively or negatively impacts seed mass or NUE also represent genomic locations of particular interest.

At $p < 0.01$, 28 of the 30 markers associated with NUE were also associated with seed mass under N1 but only 1 of 24 under N2, illustrating how at low N levels, the genomic regions involved in NUE have a high impact on seed yield compared to high N levels. At $p < 0.05$, only 32 of the 294 NUE markers under N1 were shared with NUE under N2 (Table 13; Table 14), suggesting that under the different N treatments the genomic regions associated with NUE are very different, illustrating the complexity of the trait. In addition there were 18 markers associated with NUE and seed mass under both N levels and located in a small (534 kbp) region on chromosome C 05, representing a target region for further investigations into NUE.

A legitimate criticism of this study is the potential of false positives from the Wilcoxon rank sum tests, due to population structure. In an attempt to compensate for this, the marker types of each genotype according to their phenotypic rank is listed for all significant markers. While there was much diversity among the top performing cultivars for seed mass and NUE, under N1, a number of the bottom performing cultivars for seed mass and NUE came from Deutsche Saatveredelung. Under N2, the bottom three performing cultivars for both seed mass and NUE came from Norddeutsche Pflanzenzucht, indicating that many of the marker types which negatively impact seed mass or NUE under both N treatments could be false positives if they are primarily coming from a single breeding company. As such, the significance of these markers should be taken with a high degree of skepticism.

In conclusion, this strategy could potentially be a useful tool for hybrid plant breeding programs to identify markers useful for selection and help to breed *B. napus* varieties with a higher NUE or seed yield. It could also theoretically be utilized to predict optimal parent combinations for hybrid development, similar to the concept of genomic selection (Heffner *et al.*, 2009), but would require much larger and more diverse data sets, from plants grown in numerous environments, to enable better scoring of each maker type. Using genome wide SNP marker data to predict the general combining abilities of purebred lines, for creating superior hybrids, has been explored in other crops, such as *Zea mays* (maize/corn; Riedelsheimer *et al.*, 2012). Along with this, a more appropriate method for calculating marker scores could be to calculate the average phenotypic value of that marker type, instead of the average ranking amongst the cultivars, as was done in Tables 9-12.

Future studies should focus on replicating and confirming the results. Tests under field conditions would provide data that correlate more with the farmer conditions and which would be more applicable for breeders. Another focus could be on further investigations of the individual markers, which should be in close proximity to genes relating to the phenotype and may be useful for helping to elucidate genes which may play a role under different environmental conditions, such as for NUE at high and low N availability. *e.g.*, Orsel *et al.* (2014) identified 16 cytosolic glutamine synthase genes in the *B. napus* genome which are differentially regulated under different N levels and could help explain why there were so few similar markers identified as significant between N1 and N2 for NUE. Connections between the markers identified in this study and the genes identified by Orsel *et al.* (2014), Avice & Etienne (2014) or the QTLs

identified by Bouchet *et al.* (2014) and Basunanda *et al.* (2010) would help validate the results of this study and the usefulness of its methodology.

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