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**Detailed phenotypic assessment of genetic variation for  
nitrogen uptake and utilisation efficiency traits in a  
diverse panel of *Brassica napus* L.**

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for a Doctorate Degree in Agricultural Sciences  
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Environmental Management

by

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>> The important thing is not to stop questioning <<

*Albert Einstein*

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## List of abbreviations

AE	Agronomic efficiency
AR	Apparent nitrogen recovery
C18:1	Oleic Acid
C18:3	Alpha Linolenic acid
C22:1	Erucic acid
CoV	Coefficient of Variation
DAS	Days after sowing
DaysAfter0101	Days after Jan 1 <sup>st</sup>
DM	Dry matter
DW	Dry weight
GSL	Glucosinolates
Gw	Grain weight
G <sub>WC</sub>	Grain weight of unfertilised control
G <sub>WF</sub>	Grain weight with fertiliser
HN	High nitrogen
LeavesMassF	Mass of leaves at flowering
LN	Low nitrogen
LSD	Least significant difference
MR	Main raceme
NconcLeavesF	Nitrogen concentration in leaves at flowering
NconcRoot	N concentration of roots ( <i>in vitro</i> experiment)
NconcShoot	N concentration of shoots ( <i>in vitro</i> experiment)
NconcSiliquesF	Nitrogen concentration in siliques at flowering
NconcSiliquesM	Nitrogen concentration in siliques at maturity
NconcStemF	Nitrogen concentration in stems at flowering
NconcStemM	Nitrogen concentration in stems at maturity
NcontBiomassF	N content in complete biomass at flowering
NcontLeavesF	N content of leaves at flowering
NcontRoot	N content of roots ( <i>in vitro</i> experiment)
NcontShoot	N content of shoots ( <i>in vitro</i> experiment)
NcontSiliquesF	N content of siliques at flowering
NcontSiliquesM	N content of siliques at maturity
NcontStemM	N content of stems at maturity
NcontStemsF	N content of stems at flowering
N <sub>Fertilised</sub>	Nitrogen fertilisation
NFL	Nitrogen fertilisation level
Nharv_Nsupply	Ratio of N in plant at maturity to N supplied
NHI	Nitrogen harvest index
No	Number

NoLeaves	Number of leaves at flowering
NoSB	Number of side branches
NRE	Nitrogen remobilisation efficiency
Ns	Nitrogen supplied
N <sub>soil</sub>	Soil content of nitrate and ammonium
Nt	Total nitrogen in the plant
NUE	Nitrogen use efficiency
NupE	Nitrogen uptake efficiency
NutE	Nitrogen utilisation efficiency
Oilconc	Oil concentration of seeds
OilYield	Oil yield
PE	Physiological efficiency
RE	Reinshof
RH	Rauischholzhausen
RO	Rotenkirchen
S	Sulphur
SB	Side branches
SD	Standard deviation
SeedNconc	N concentration of seeds
SeedNyield	N yield of seeds
SiliquesMassF	Mass of siliques at flowering
SiliquesMassM	Mass of siliques at maturity
StemMassF	Mass of stems at flowering
StemMassM	Mass of stems at maturity
SY	Seed yield
UI	Usage index

# 1. Introduction

## 1.1. Winter oilseed rape: Evolution, history and economic importance

Oilseed rape (*Brassica napus* L,  $2n=4x=38$ , AACC; also known as canola) is a member of the genus *Brassica* within the family *Brassicaceae*. The amphidiploid *Brassica napus* is the result from the recent spontaneous interspecific hybridisation between *Brassica rapa* ( $2n=2x=20$ , AA) and *Brassica oleracea* ( $2n=2x=18$ , CC) (U 1935). Hence, *B. napus* contains the full intact chromosome complement of *B. rapa* and *B. oleracea* (Parkin et al., 1995; Sharpe et al., 1995; Axelsson et al., 2000). Most probably the initial allopolyploidization first occurred in the Mediterranean region, after co-cultivation of its diploid progenitors in close geographical proximity (Friedt and Snowdon, 2010). Recently the published *B. napus* genome sequence revealed that – due to genome multiplication during the origin of angiosperms – *B. napus* has been subject to 72-fold genome multiplications and therefore carries genes with high redundancy (Challhoub et al., 2014). Moreover, by comparison of orthologous genes between *B. napus* and *B. rapa* or *B. oleracea* respectively the same study illustrated that *B. napus* is most probably not older than 7500 to 12500 years. Thus, from the evolution point of view oilseed rape is a very young plant species with a short domestication history.

Cultivation of oilseed rape was first documented in the Middle Ages, and subsequently spread across the world (Allender et al., 2010). In general, diversification of *Brassica* oilseeds conferred a broad potential to adapt to different agroclimatic regions (Allender et al., 2010; Bus et al., 2011; Snowdon et al., 2006). Rapeseed has a strong eco-geographical differentiation into spring versus winter forms. This differentiation is under genetic control of mechanisms that control the vernalisation requirement and onset of flowering. In Europe the in autumn-sown winter form is predominant, whereas in North America (in particular Canada) and northern parts of China, spring forms which do not require vernalisation and are not winter hardy are widely sown due to the climatic conditions. In Asia and Australia, intermediary types of oilseed rape are suitable (Bus et al., 2011; Snowdon et al., 2006) and are grown during the rain-rich, mild winter.

Oilseed rape production showed a strong increase in production within the last four decades, promoted by two major driving forces: On the one side, the improvement of the oil and meal quality by plant breeding, and on the other side political decisions to promote the substitution of fossil energy resources by renewable energy resources. The seed quality improvement was essential to allow the use of oilseed rape for human and animal nutrition. Oil from earlier rapeseed cultivars contained up to 50% erucic acid (C22:1) in the seed oil and had a high glucosinolate content. Erucic acid has a bitter taste and – even more important – in high doses can deposit other fatty acids in the heart and lead to cardiac dysfunction (Kramer et al., 1983; Kramer et al., 1988), which rendered the oil of *B. napus* unusable for human

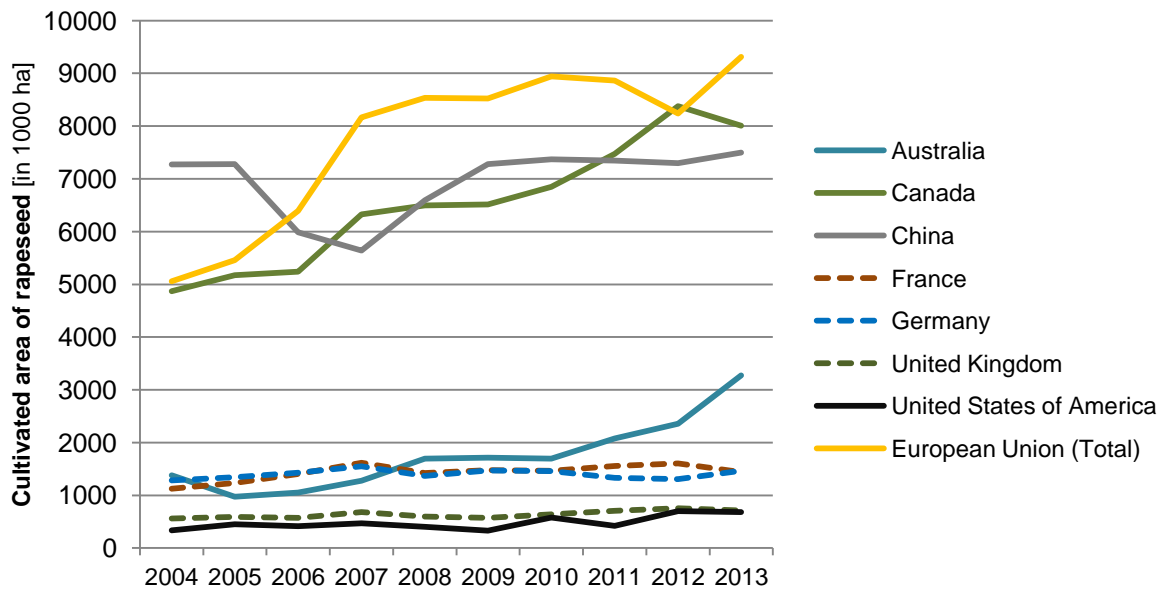


nutrition. However, this was overcome by the identification of a spontaneous mutant of the German spring type cultivar Liho with low quantities of erucic acid. Discovery and introgression of the responsible mutations into Canadian spring rapeseed provided the basis for so called 0-quality rapeseed cultivars, with an erucic acid content of less than 1%, that were released to the Canadian market (Downey and Harvey, 1963; Harvey and Downey, 1964; Stefansson and Hougen, 1964). Today in Germany the allowable maximum erucic acid limit for the release of new varieties is 2% of the total fatty acid content. Furthermore, a high glucosinolate content in the seeds made the original rapeseed meals unusable for animal nutrition. Glucosinolates are sulfur-rich secondary metabolites and lead to toxic byproducts when digested in monogastric animals, potentially causing liver and/or kidney damage or lymph dysfunction (Wittkop et al., 2009; Snowdon et al., 2010; Snowdon, et al., 2006). With the identification of the Polish spring-type oilseed rape variety Bronowski in 1969, this disturbing factor for a healthy use in nutrition was also overcome (Josefsson and Appelqvist, 1968). Five years later Tower, the first 00-variety, which combines both zero erucic acid and low glucosinolate content, was released to the Canadian market and initiated the beginning of strong increases in oilseed rape production, transforming it into a major oil crop in the world within just a few decades (Stefansson and Kondra, 1975). Moreover, a genetic improvement of the oil composition towards a higher content of (poly-) unsaturated fatty acids has matched the latest interest of human nutrition and health, making rapeseed oil one of the world's most widely used frying oils.

Besides the use as oil for human nutrition, rapeseed is also used for many other purposes. The prominent example is the politically motivated use of methyl esters based on rapeseed oil as diesel substitute, particularly in Europe. In this regard, the compulsory addition of biodiesel to fossil diesel has created by far the most relevant market for biodiesel. In 2014 in Germany 15.38 M metric tons of biodiesel were added in total to fossil diesel, whereas only 0.02 M metric tons biodiesel were used as pure biodiesel (UFOP, <http://www.ufop.de/biodiesel-und-co/biodiesel-preis/>). Independent of the category of oil use, the extracted and pressed residues, commonly known as meal or cake, contain a highly valuable protein which is widely used as a livestock feed, particularly for cattle feeding.

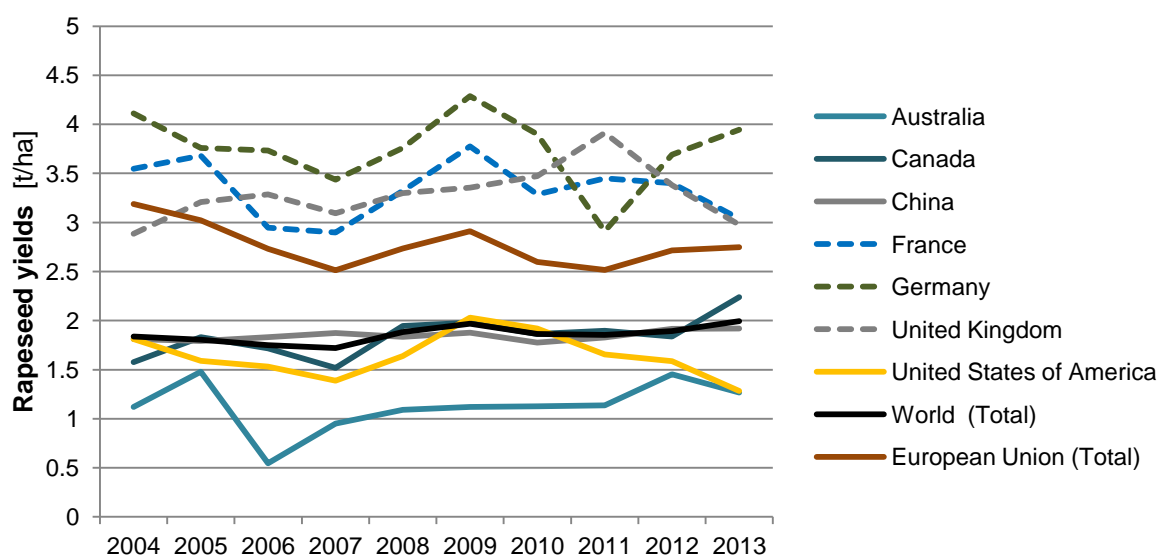
Behind soybean (ten year average production of 236.1 M metric tons, 2004-2013) and oil palm, rapeseed (57.6 M metric tons, 2004-2013) is the third most important oilseed crop in the world (FAOSTAT data, 2015: <http://faostat.fao.org/>). On a ten year average (2004-2013) rapeseed was produced on 30.94 M ha worldwide, mainly in Canada (6.53 M ha), China (6.96 M ha), EU (7.75 M ha), Australia (1.75 M ha) and the United States of America (0.48 M ha). Within the European Union (EU), France (1.43 M ha) and Germany (1.40 M ha) are the countries with the biggest oilseed rape production area (Figure 1).

Oilseed rape has a high value in crop rotations, with a strong positive influence on yields of subsequent cereals such as wheat (Christen et al., 1992) and barley (Christen and Sieling, 1993). In many regions of central and northern Europe, Canada and Australia, it is the only dicotyledonous crop regularly used in crop rotations, thus imparting an essential role in soil rejuvenation and management of monocotyledonous cereal diseases and pests (Ryan et al., 2006; Kirkegaard et al., 1997).



**Figure 1:** Production area of rapeseed between 2004 and 2013 in 1000 ha (own diagram based on FAOSTAT data, 2015: <http://faostat.fao.org/>)

Beyond the influence of the climatic conditions in the different production areas, soil, production systems and intensities lead to enormous differences in seed yields. While Australia (1.22 t/ha), United States (1.69 t/ha) and China (1.86 t/ha) are below the five year world average of 1.91 t/ha, Canada (1.96 t/ha) and the EU (2.70 t/ha) exceed this level significant. Countries within the EU, particularly Germany (3.74 t/ha), the United Kingdom (3.42 t/ha) and France (3.39 t/ha), represent the highest yielding regions for oilseed rape (Figure 2).



**Figure 2:** Average seed yields of rapeseed between 2004 and 2013 in t/ha (own diagram based on FAOSTAT data, 2015: <http://faostat.fao.org/>)

The abovementioned yield statistics from FAO also include spring types and other species of Brassica oilseed species (e.g. turnip rape, mustard, etc.), however, winter oilseed rape is by far the most important of these in Europe. Hence, the actual oilseed yields from *B. napus* can be assumed to be higher than indicated by FAO, probably exceeding 4 t/ha. In several regions yields of more than 5-6 t/ha are reported. These differences also require consideration of different fertilisation inputs and application strategies. The issue of nitrogen fertilisation, in relation to achievement of high yields, is dealt with in the following chapter.

## 1.2. Nitrogen use efficiency in the context of oilseed rape

### 1.2.1. How to define nitrogen use efficiency?

Although the definition of NUE is quite well established, its estimation is a more complex issue, since this process can be approached at different levels (reviewed by Good et al., 2004, Rathke et al., 2006; Han et al., 2015). Depending on the harvested parts of the crop, the scientific question and cultivation conditions several methods and definitions have been used for measuring NUE (summarized in Good et al., 2004 and Rathke et al., 2006). As explained in Table 1, the agronomic efficiency measures how efficiently the fertiliser is converted into grain yield, while the apparent nitrogen recovery indicates how efficiently nitrogen was acquired from the soil. In contrast, the physical efficiency describes how efficient the plant was in capturing plant nitrogen in grain yield (Craswell et al., 1984). Total NUE is split into two components that can be evaluated at the canopy or plant levels: i) the ability of the plant to capture the N from the soil (nitrogen uptake efficiency, NupE), and ii) the

ability to use the absorbed N to produce seeds (nitrogen utilization efficiency, NutE) (Moll et al., 1982). The latter includes the capacity of the plant to remobilize N into the seeds (nitrogen remobilization efficiency, NRE) (Masclaux-Daubresse et al., 2010). Other definitions, like the N usage index, account for the absolute increase in biomass (Siddiqi et al., 1981).

From a physiological point of view, NUE can be considered as the nitrogen/carbon balance in the shoots at harvest, by measuring the relationship between the biomass and the N content of the shoots (Good et al., 2004). Finally, for a plant-scale consideration, N flux measurements based on isotope labelling techniques ( $^{15}\text{N}$ ) allow precise assessment of N dynamics throughout the plant organs.

**Table 1:** Definitions of nitrogen use parameters

Term	Formula	Definition	Literature
Agronomic efficiency	$AE = (Gw_F - Gw_C) / N_F$	Gw <sub>F</sub> : Grain weight with fertiliser Gw <sub>C</sub> : Grain weight of unfertilised control N <sub>F</sub> : Applied nitrogen fertiliser	Craswell et al., 1984
Apparent nitrogen recovery	$AR = (N_{F\text{uptake}} - N_C) / (N_F \times 100)$	N <sub>F</sub> uptake: plant nitrogen with fertiliser N <sub>C</sub> : uptake: plant nitrogen of unfertilised control N <sub>F</sub> : Applied nitrogen fertiliser Gw <sub>F</sub> : Grain weight with fertiliser Gw <sub>C</sub> : Grain weight of unfertilised control	Craswell et al., 1984
Physiological efficiency	$PE = (Gw_F - Gw_C) / (N_F \text{ uptake} - N_C \text{ uptake})$	N <sub>F</sub> uptake: plant nitrogen with fertiliser N <sub>C</sub> : uptake: plant nitrogen of unfertilised control	Craswell et al., 1984
Nitrogen use efficiency	$NUE = Gw / N_s$	Gw: Grain weight N <sub>s</sub> : Nitrogen supplied	Moll et al., 1982
Nitrogen uptake efficiency	$NupE = N_t / N_s$	N <sub>s</sub> : Nitrogen supplied N <sub>t</sub> : Total nitrogen in the plant	Moll et al., 1982
Nitrogen utilization efficiency	$NutE = Gw / N_t$	Gw: Grain weight N <sub>t</sub> : Total nitrogen in the plant	Moll et al., 1982
Usage index	$UI = Sw \times (Sw / N)$	Sw: shoot weight N: Nitrogen in shoots	Siddiqi et al., 1981

### 1.2.2. Nitrogen use efficiency - a major global challenge in agriculture

As for all non-legume crops, nitrogen (N) is the plant nutrient that must be fertilised to oilseed rape in high quantities for sufficient productivity (Hocking and Stapper, 2001; Rathke et al., 2005; Jackson, 2000; Sieling and Christen, 1999; Sieling and Christen, 1997). Elevated N fertilisation has substantially increased yields and thus helped to secure agricultural commodity production over the last decades (Tilman et al., 2002). The rising world population is further increasing demand for food and non-food agricultural commodities,

necessitating a continued maintenance of high yields. On the other hand, some reports estimate that a recovery by rapeseed plants usually does not exceed 50% to 60% (Smil, 1999; Schjoerring et al., 1995; Malagoli et al., 2005a), which is only around half that for cereals (Sylvester-Bradley and Kindred, 2009). This is not necessarily the result of over-fertilisation by farmers, but rather describes the narrowed acquisition and utilisation efficiency of plant-environment interactions within agricultural production systems. Unused nitrogen can escape from the production system (Sieling et al., 1999) and cause environmental damage in other ecosystems (Galloway and Cowling, 2002), including contamination of ground and drinking water by leaching of nitrate ( $\text{NO}_3$ ), or its deposition by run off or erosion into rivers, lakes and oceans (Galloway et al., 2004; Billen et al., 2013; Sebilo et al., 2013). Furthermore, emissions of nitrogen oxides or volatile ammonia in form of ammoniac ( $\text{NH}_3$ ) act as potential greenhouse gases, hence their reduction is very desirable in a climate change context (Venterea et al., 2012). Besides those gasses emitted directly from agricultural fields, energy consumption during mineral N fertiliser production by the Haber-Bosch process causes additional carbon dioxide emissions. Taken together, these factors pose a considerable challenge in the quest to increase yields while simultaneously reducing environmental impacts. Nitrogen use efficiency (NUE) is therefore a focal subject for agricultural production in general (Hawkesford, 2012; Kant et al., 2011) and oilseed rape production in particular. This has led to regulatory farming policies to limit the N balance surplus and cap N inputs. For example, Denmark has implemented strict regulation of N inputs in agricultural production systems (Hutchings et al., 2014), forcing farmers to more efficiently utilise N resources to achieve high yields. Since oilseed rape is also the primary feedstock for European biodiesel production, legislation in the EU expects greenhouse gas generation resulting from oilseed rape production to be reduced up to 50% by 2017 (RED, 2009; DüV, 2007). Since N fertilisation is the major single factor influencing the ecological footprint of oilseed rape, achieving this aim will depend on a better understanding and improvement of nitrogen use (Rathke and Diepenbrock, 2006). Finally, the energy dependent production also results in potentially higher N fertiliser prices. Already today, N fertiliser is among the major single costs in oilseed rape production (Orsel et al., 2014), which additionally encourages NUE improvement from an economic point of view.

An enhanced NUE involves consideration of mineral soil N (Henke et al., 2009) together with more precise fertiliser application (Sieling and Kage, 2010), for example based on plant nitrogen demand by on-field, high-throughput phenotyping tools (Erdle et al., 2013; Mistele and Schmidhalter, 2010; Samborski et al., 2009) coupled with plant growth models (Adam et al., 2012; Martin et al., 2012; Henke et al., 2008; Müller et al., 2008; Müller, 2009). Agronomic improvement by breeding for more N-efficient varieties is another promising strategy towards sustainable agriculture (Rathke et al., 2006).

### **1.2.3. Nitrogen uptake of oilseed rape**

The acquisition of mineral ions by the plant depends on their availability in the soil solution and the ability of the roots to take up the minerals. While the former depends on exogenous factors like additional fertilisation by farmers, the latter might be modifiable by breeding.

The water soluble nitrate ( $\text{NO}_3^-$ ) form of N usually has the highest concentration among mineral N in the soil (Wolt, 1994) and is the main source of mineral N absorbed by plants. Therefore N nutrition depends essentially on the soil hydric reserves, the volume of soil penetrated by the roots and the capacity of N absorption per unit root length. In a narrower sense, N uptake can be understood as the active transport process to carry N over the plasmalemma membrane into the cell interior, a process achieved by nitrate and ammonium transporters (reviewed in Xu et al., 2012). In a broader sense N uptake includes morphological properties of the roots (fine roots, root length, surface, etc.) which are suggested to determine NupE of rapeseed more than N uptake per root surface (Kamh et al., 2005).

Soon after seedling emergence, the root system develops and mineral N is efficiently absorbed from the soil and stored into the vegetative biomass. Rapeseed plant biomass increases dramatically over this first period and shows a high NupE at early stages, with up to 100 kg N/ha being absorbed (Rossato et al., 2001). This makes it a valuable catch crop during autumn. N acquisition depends strongly on water availability, since in moderate climate conditions where oilseed rape is grown water soluble nitrate is the predominant form. In this regard the root system has a central function for water and nutrient uptake. Although this role has been recognised for a long time (Sharp and Davies, 1979), its degree of relevance is still not conclusively clarified. Although on the one hand it is suggested that root length density is not a critical factor for nutrient acquisition (Kage, 1997), other studies see roots more relevant (Ulas et al., 2012; Ulas et al., 2015) and even suggest that poor rooting explains to some extent the stagnation of yield for major crops (White et al., 2015). In fact the root system is strongly influenced by and interacts with soil-environmental factors and, thus, has to be considered as a trait complex with a low heritability. Moreover, a direct selection for particular root traits in breeding or even prebreeding programs is hindered by their soil-hidden nature that does not allow large scale phenotyping. Hence, a better understanding of the inheritance of root traits associated with more efficient nutrient acquisition is desired.

#### **1.2.3.1. Genetic mapping of root traits associated to nitrogen acquisition**

Several genetic studies have addressed the genetic control of root system vigor in *B. napus* in the context of adaptation to various environments (Rahman and McClean 2013), especially drought (Fletcher et al., 2015) and phosphorous stress (Yang et al., 2010; Shi et

al., 2013). Using linkage analysis on a RIL population and a DH population, Yang et al. (2010) and Shi et al. (2013) detected QTL clusters for root length, lateral root number, root surface area, root biomass and root volume that were specific to low-phosphorous treatments. In reciprocal crosses between spring-type and winter-type *B. napus*, Rahman and McClean (2013) hypothesized a trigenic dominant control of root vigour, based on the segregation ratio of F2 populations. Furthermore, by the use 225 DH lines derived from a cross between the annual variety IMC106RR (Cargill) and biennial variety Wichita, potential pleiotropic relationships with flowering time were found for root vigour, root biomass and root length, suggesting common genetic control (Rahman and McClean 2013; Fletcher et al., 2015). Such studies provide first insights into potentially interesting variation and its underlying genetic control, although these studies have their limitations. The study of Rahman and McClean (2013) phenotyped the roots in pots which might limit the root growth system, especially at advanced developmental stages. In contrast, the study of Fletcher et al. (2015) was conducted under field conditions and used the vertical root pulling force (Hayes and Johnson, 1939; Landi et al., 2002) as an indirect proxy for the root system size. Nevertheless, care must be taken not to overestimate the importance of major QTL in mapping populations from strongly differentiating parents, since such QTL are often influenced by strong phenological differences that can be caused by the specific genetic background of the parents, particularly in crosses between spring and winter forms.

#### **1.2.3.2. Genes associated to nitrogen acquisition and nitrogen sensing**

Besides the morphological root traits influencing – for example – the root surface, distribution in the soil volume and rooting depth, other traits as the activity of signalling and transport mechanisms are relevant for NupE. Although extensively studied in *Arabidopsis thaliana*, little information is available on genes responsible for variation in root-related traits and their regulation in response to N availability in rapeseed. In *A. thaliana*, root growth response to the availability of nitrate involves two distinct pathways (Zhang and Forde 1998; Zhang and Forde 2000). On the one hand, a direct effect of external nitrate on the MADS-box transcription factor *ARABIDOPSIS NITRATE-REGULATED 1* (Zhang and Forde, 1998) and, on the other hand, a systemic inhibitory effect by a basic leucine zipper and a LIM transcription factor, depending on the plant internal N status, were described by Tranbarger et al. (2003). More recently, the N-responsive *CLAVATA3/ESR (CLE)* peptides and the *CLAVATA1 (CLV1)* leucine-rich repeat receptor-like kinase signalling module were identified to play a crucial role in development of the lateral root system in N-poor environments (Araya et al., 2014). Additionally, the nitrate transporters *NRT1.1* (Remans et al., 2006a; Krouk et al., 2010) and *NRT2.1* (Little et al., 2005; Remans et al., 2006b) are known to be crucial in

nitrate sensing, independently of their uptake function (Malamy and Ryan 2001; Miller et al., 2007). Previous studies investigating root responses of *B. napus* to nitrogen availability were exclusively conducted in early developmental stages. As far as known, no studies till today have elucidated the detailed genetic control and variation of root growth and nitrogen responses at adult developmental stages in winter oilseed rape.

#### **1.2.4. Nitrogen utilisation of oilseed rape**

The process of N remobilisation begins relatively early during oilseed rape plant development, but nevertheless coincides with N acquisition due to the sequential progression of senescence after the onset of flowering. During the remobilisation phase, N from older leaves is continuously translocated to younger ones and promotes the initiation of foliar primordia as early as the end of autumn. During winter, a part of the leaf area produced during this first period can be destroyed by freezing, leading to important N losses of 2-3.5% of the fallen leaves' dry weight (Malagoli et al., 2005a).

After the beginning of vegetation in spring, N uptake is again predominant. At this time, leaves and stems are still the sink for uptaken N. The flowering stage onwards, however, leaves are no longer produced and the onset of senescence occurs quickly, with leaves showing different remobilisation ability depending on their position on the main stem (Malagoli et al., 2005a). In parallel, photosynthesis activity is partly ensured by the pod area index (PAI). In addition, the N pool is also maintained through the N re-absorption from leaves that have fallen during autumn, with an uptake efficiency that can reach 40% of the N content from the fallen leaves (Dejoux et al., 2000).

##### **1.2.4.1. Nitrogen utilisation in the context of senescence**

NutE is closely related to beginning and intensity of leaf senescence. However, it is still unclear if an early or late senescence is beneficial for an increased NutE. In the last case the 'stay-green' phenotype, describing plants with altered chlorophyll catabolism (Gregersen et al., 2013), often refers to delayed leaf senescence in crop species (Thomas and Ougham 2014). It has been correlated with higher NUE in several species including *A. thaliana*, maize and wheat (Spano 2003). Indeed, maintaining a high photosynthetic activity may allow the plant to carry on producing carbohydrate nutrients during late stages of the crop cycle. Further remobilisation to younger organs and seeds may eventually increase the final yield (Habekotté 1993). Examples showing the relationship between the stay-green phenotype and seed yield in several species were reported by Gregersen et al. (2013). However, the stay-green phenotype is not necessarily related to a prolonged photosynthetic activity. Indeed, in many cases, this phenotype translates to an altered chlorophyll catabolism, but



with other senescence processes remaining intact (Thomas and Howarth 2000). Along with the LAI and RUE traits, the stay-green phenotype in rapeseed is expected to be also related to the temporal span of NupE during the reproductive stage (Diepenbrock 2000; Rathke et al., 2006) and may be a source of genetic diversity for enhancement of NUE. For instance, Schulte auf'm Erley et al., (2007) found significant genotypic differences in leaf senescence when comparing six contrasting, N-efficient WOSR accessions in nutrient solution experiments and field conditions.

#### **1.2.4.2. Regulation of senescence associated degradation and transport processes**

As in many other crops, the activity of glutamine synthetase (GS) and nitrate reductase (NR) genes and enzymes have been associated to N transport in rapeseed. For example, Ye et al., (2010) found higher GS and NR activities in N-efficient genotypes under N stress conditions. This was supported by Miro et al., (2010), who found *B. napus* nitrate transporters, and other genes implicated in nitrate regulation, underlying N responsive QTL. Orsel et al. (2014) found sixteen *BnaGLN1* genes coding for cytosolic GS in rapeseed. In addition, two homoeologous genes (*BnSAG12-1* and *BnSAG12-2*) coding for SAG12, a cysteine protease implied in N remobilisation, were observed to reach maximum expression levels at early stages of senescence (Noh and Amasino, 1999). Another example of enhanced senescing genes is the *LEAF SENESCENCE CLONE (LSC)* gene family, coding for GS, cysteine protease or metallothione. In contrast, *Cab* gene expression was found to decrease during senescence (Noh and Amasino, 1999). A recent study identified genes showing differential expression between HN and LN conditions in rapeseed, revealing that genes related to photosynthesis, photorespiration and cell-wall structure are repressed under N starvation, whereas genes related to mitochondrial electron transport and flavonoid synthesis show enhanced expression (Koeslin-Findeklee et al., 2015b).

The polyploid nature of *B. napus* implies the duplication of genes involved in NUE traits within the genome and their organisation into large multigenic families, with possible variations in spatio-temporal gene expression patterns. For instance, the sixteen *BnaGLN1* genes detected by Orsel al. (2014) were organised into five distinct families which were differentially modulated according to N availability. Two genes families were up-regulated under LN conditions and during leaf senescence, while one family was up-regulated under high N conditions. Another family was not impacted by the N nutrition level, but showed higher expression in stems than in leaves. Faes et al. (2015) also demonstrated the differential expression of two sub-groups of genes composing the proline dehydrogenase gene family in rapeseed. These genes, *ProDH1* and *ProDH2*, control the proline catabolism, which is suspected to play a role in the remobilisation of N from old to young leaves. While

the *ProDH1* genes were the most expressed subgroup at the plant level and particularly in pollen and roots, the *ProDH2* genes were characteristically expressed in the vascular tissues of senescing leaves.

### **1.3. Breeding of oilseed rape**

#### **1.3.1. General breeding methods and traits in oilseed rape**

*B. napus* is a facultative outcrossing species. That means that in presence of insects a higher proportion of flowers can be cross-pollinated, while in the absence of insects the self-pollination is dominating form of pollination. Thus, until the early part of this century inbred line varieties dominated the rapeseed production, meaning that genetic diversity was largely addressed to breed for that genetic type by pedigree selection or modifications from it. While seed yield is by far the most important trait seed quality, especially OO-quality (described above) is essential to release a variety on the market. Furthermore, since the predominant usage and economic value of oilseed rape derives from its oil, breeders have focused on improving oil concentration and oil yield. Due to the negative correlation of oil and protein concentration, modern varieties until now have tended towards lower protein concentration. Selection for the sum of oil and protein is therefore an option to simultaneously increase the oil and protein yield (Grami et al., 1977; Arnholdt and Schuster, 1981). Other breeding goals are the specific oil quality, resistance to weeds, insects and diseases as well as winter hardiness (summarized in Snowdon et al., 2006).

In 1995 the first hybrid winter oilseed rape variety was released, and 2004 marked the first time a hybrid variety (Talent) replaced a pure line variety as the most widely cultivated variety in Germany. Whereas in that year more than half of Germany's 1.3 M ha of rapeseed cultivation was planted with hybrid varieties, rapeseed production in Germany today is almost exclusively from hybrid varieties. This strong increase was enabled by two major hybrid systems, namely Male-Sterility Lembke (MSL; NPZ Lembke, Hohenlieth, Germany; Frauen and Paulmann, 1999) and the Ogura cytoplasmatic male sterility system (CMS; INRA, France) derived from *Raphanus sativus* (Ogura 1968). By using breeding techniques incorporating male-sterility systems it was possible for breeders to produce large quantities of F1 hybrid seeds and exploit the heterosis effect, resulting in a yield improvement of around 15% (Snowdon et al., 2006).

Besides their generally better performance and adaptability under abiotic constraints, hybrid cultivars are believed to be more N-efficient (Gehring et al., 2007; Kessel et al., 2012). Koeslin-Findelklee et al. (2014) compared the NUE of eleven inbred lines and seven hybrids over two years in field experiments and determined the superiority of hybrids for both NupE and NutE, resulting in higher overall N-efficiency.

### 1.3.2. Genetic diversity as a prerequisite for breeding

The concentration of breeding efforts in the 1970's, to convert rapeseed production to zero erucic acid and low glucosinolate varieties, led to an extremely intensive selection process. While the erucic acid content is controlled by two co-dominant genes (four alleles), it is assumed that for the glucosinolate content at least 22 genes with moderate to large effects are responsible (Snowdon et al., 2006). Thus, introgression of novel genetic diversity into elite breeding pools requires considerable effort in order to fulfil 00-quality. As a result, a strongly narrowed genetic diversity is used in breeding modern rapeseed (Seyis et al., 2003; Hasan et al., 2006). In classical hybrid crops like maize, sunflower or sugar beet, strongly differentiated heterotic gene pools have been developed to systematically exploit heterosis in hybrid breeding. In rapeseed, the development of distinct genetic pools is difficult due to the comparatively narrow diversity in elite gene pools and the history of inbred line breeding (Snowdon et al., 2015). However, introgressions between the comparatively diverse genetic pools of winter, semi-winter and spring forms may be a first possibility to exploit heterosis.

For instance, Qian et al. (2007) showed high heterosis for seed yield in hybrids between Chinese semi-winter pollinators and spring-type mother lines from Europe and Canada. Improvement of spring type *B. napus* by introgressions of winter-type variability was also assessed by Kebede et al. (2010), who demonstrated the heterosis effect of spring × winter DH lines over their parental lines. To avoid incompatibilities in developmental timing between these potential heterotic groups, Qian et al. (2007) suggested that the genetic material should first be adapted to the local environment before being hybridised. It should be noted that the genetic distance between heterotic groups, commonly used to determine the hybridisation scheme, is often not correlated to the hybrid performance, as offspring from crosses which are too distant can lack important adaptation traits.

Another strategy to widen the global genetic diversity of *B. napus* is the replacement of sub-genomes in new type *B. napus* varieties, by introgressions from related *Brassica* species (Udall et al., 2004; Qian et al., 2007). This can lead to new allelic combinations and new inter-subgenomic heterosis effects. However, crossing between distant heterotic groups or exotic germplasm may also introduce undesirable traits, which must be eliminated to ensure the success of the breeding program.

Synthetic accessions derived from interspecific cross have been used successfully for a long time to improve traits like pathogen and pest resistance (Lühs et al., 2003a, Lühs et al., 2003b). On the other hand, there is just one recent study (Wang et al., 2014) that demonstrated the use of interspecific hybridisation to generate a new-type *B. napus* in the context of NUE improvement.

Older studies (Grami and LaCroix, 1977; Yau and Thurling, 1987) and more recent investigations (Schulte auf'm Erley et al., 2011; Kessel et al., 2012; Koeslin-Findeklee et al., 2014) point to the presence of genetic variation and ongoing cultivar improvement for seed yield at limited N supply. However, most previous studies conducted physiological investigations on a comparatively small number of accessions (Malagoli and Le Deunff, 2014; Le Deunff and Malagoli, 2014; Malagoli et al., 2004; Malagoli et al., 2005b; Rossato, 2001), or used bi-parental populations to study genetic determinants of NUE (Nyikako et al., 2014; Bouchet et al., 2014; Miro, 2010; Gül, 2003). Thus, the genetic variation for NUE-influencing traits remains widely unknown in winter oilseed rape.

#### **1.4. Phenotyping: The bottleneck for breeding progress.**

Besides a genetic variation for NUE, its assessment is a second important concern for breeding towards increased NUE. Although genetic characterisation tools have experienced strong methodological progress within the last decade, phenotyping is still lagging strongly behind and can today be considered as a comparative bottleneck in plant breeding. Thus, the main prerequisites for correct evaluation of performance of genetic material under reduced N nutrition are the precision and quality of the phenotyping, along with the correct estimation of plant growth conditions in different environments. In general, phenotyping methods that enable investigations only at an early developmental stage have to be distinguished from those that consider assessment of genetic variation at the adult plant stage and observe plant development until seed harvest. In the following chapter, advantages and disadvantages of different phenotyping methods will be summarised.

For testing of the accessibility to the complete plant, including the root system, hydroponics experiments in climate chambers are a method of choice. Such experiments ensure a relatively flexible, rapidly and direct sampling of all plant tissues without complex harvesting procedures, thus allowing a fast collection of snap-frozen tissues for transcriptome or metabolome analysis, for example. Moreover, a big advantage of hydroponics is the precise regulation of nutrient supply and vast options for regulation of growth conditions, which can be individually adjusted to the research question and lab utilities. These properties represent a huge advantage if physiological measurements are necessary, especially on the microscale (Conn et al., 2013). For example, Hatzig et al. (2014; 2015) made use of a hydroponic system to investigate metabolic and physiological changes in different *B. napus* genotypes in response to abiotic constraints under controlled conditions.

On the other hand, hydroponic systems provide artificial growth conditions compared to field grown plants and do not represent physical and mechanical circumstances which result in altered root morphology. Furthermore, sterilisation of all technical components and seeds of the hydroponics set-up, along with the need for regular exchange of the nutrient solution to

ensure constant nutrient concentration and avoid occurrence of algae and other microbial contamination, make this kind of phenotyping procedure quite complex and difficult (Arteca et al., 2000; Schlesier et al., 2003).

If plants should be cultivated and investigated in a defined soil instead of hydroponics, Mitscherlich pots have been and are used in plant science for a long period until today (Mitscherlich, 1909; Judd et al., 2015). While hydroponics systems make plant cultivation over the entire life cycle a challenging task, Mitscherlich pot experiments can easily be conducted over a whole growing season. Pot experiments are therefore an important and widely-used method for scientists investigating plant growth under different environments, in reaction to applied treatments or in interaction with other external factors. Environmental factors like biotic and abiotic stresses, soil diversity, temperature and water supply can be controlled and varied, and environmental fluctuations typical for field-based studies can be limited. Consequently pot experiments can be more repeatable and reproducible than field trials. Accordingly, the number of published studies applying pot experiments has increased enormously over the past three decades.

In practical crop breeding, pot experiments often represent an important early step in the identification and implementation of novel plant material carrying interesting phenotypic variation for important agronomic traits. Different kinds of biotic and abiotic stresses can be applied and plant reactions can be investigated under controlled conditions that give insight into the molecular and physiological basis of interesting plant variants. Ideally this can help to identify selection parameters that can speed up the selection process in breeding populations. This is particularly important for crops with a long lifecycle, where early selection can considerably accelerate breeding progress.

Since physiological or molecular parameters often exhibit a lower susceptibility to genotype-by-environment interactions than more complex yield parameters, they are commonly preferred as surrogates for more complex traits during selection (reviewed in Berger 2010). However, after the development of screening techniques in pot experiments, a verification of results under field conditions is essential to ensure the transferability of the controlled phenotyping system (Tavakkoli et al., 2012, Skirycz et al., 2011, Wu et al., 2011). Unfortunately, studies which confirm correlations of complex physiological parameters or yield-associated traits between pot and field experiments are rare. Indeed it has frequently been demonstrated that extrapolation of results from pot experiments to trait expression under natural field conditions is problematic (Passioura 2012, McKersie et al., 1999, Mohamed et al., 2001). There are many putative causes for the low comparability of plant performance under controlled and natural conditions, however the size of the pots used in greenhouse or growth-chamber experiments seems to have the greatest impact. In a meta-analysis, Poorter et al. (2012) found that suppressed development of pot-grown plants is

caused by a negative influence on photosynthesis and shoot growth due to reduced nutrient availability, diminished water holding capacity, altered soil temperature and ultimately a limited root growth in the pots. Passioura (2006) also postulated that the primary disadvantage of small pots is the limited rhizosphere. Additional problems might be caused by differences of the air temperature or quality in a greenhouse compared to the field, a lack of wind and strongly fluctuating pot and soil temperatures caused by strong solar radiation and watering with cold tap water.

### **1.5. Objectives of this study**

NUE in a broader sense has been studied for many decades in several crop species (Fageria and Baligar 2005; Hirel et al., 2007a; Hirel et al., 2007b; Garnett et al. 2009; Xu et al., 2012; Vincourt 2014). However, although many crucial phenotypic characteristics were elucidated in specific cultivars in previous studies, descriptions of key aspects contributing to NUE and its genetic variation in winter oilseed rape have to date been rather rare and of limited scope. To address this deficit, and to make a contribution towards closing the phenotype-genomic gap in this regard, the present study analysed a broad collection of 30 highly diverse winter type *B napus* accessions.

A first experiment aimed to establish a hydroponic system that allows characterisation of the diversity panel for variation in responses to contrasting N supply, four weeks after germination. In a second experiment the genetic material was grown in Mitscherlich pots at divergent nitrogen fertilisation levels, and partitioned into different tissue samples, to gather detailed information on the macro-physiological N responses of the diversity panel at flowering and seed maturity. A third experiment aimed to provide a proof-of-concept that enables plant cultivation over the entire life cycle under controlled-environment conditions, in large containers with a good field-transferability. The final objective was to apply this system to phenotype multiple plant tissues for physiological parameters, providing first insight into the genetic variation for root system traits and N remobilisation in winter oilseed rape.

Overall the investigations described in this thesis focus on the following major issues: 1) Determination of variation for N acquisition during early developmental stages and NupE at flowering; 2) quantification of variation for NutE; 3) determination of the contribution of both uptake and utilisation to total NUE; 4) analysis of interrelationships from 33 phenotypic traits; 5) discovery of winter rapeseed genotypes that greatly differ in senescence behaviour and root system morphology; and 6) identification of potential trait donors for prebreeding programs.

## 2. Material and Methods

### 2.1. Plant material

A highly diverse collection of 30 winter-type *B. napus* accessions (Table 1) from the ERANET-ASSYST diversity panel (Westermeier et al., 2009; Bus et al., 2011) was selected for the investigations based on genetic marker data. The panel included old European oilseed and fodder rape varieties, which are high in glucosinolate and erucic acid content, more recent breeding material, and also two synthetic *B. napus* accessions from Georg August University Göttingen, Germany (Girke et al., 2012a; Girke et al., 2012b; Jesske et al., 2013).

**Table 2:** Seed quality and release period (where known) of investigated winter oilseed rape accessions

Genotype	Erucic acid content	Release Period
Alaska	0	1990-1999
Aragon	0	2004
Beluga	0	2000-2007
Cobra	0	1987
CanxCouDH	0	
Darmor	0	≤ 1984
Expert	0	2000-2007
Jupiter	0	
Librador	0	1980-1989
Libritta	0	1980-1987
Madrigal	0	1990-1999
MSL007c	0	
ONDH5	0	
Pacific	0	2003
Pirola	0	1990-1999
Rapid	0	1990-1999
Savannah	0	2000-2007
Start	0	
Vivol	0	1990-1999
Wotan	0	1990-1999
Kromerska	+	1954-1974
ResynH048	+	
Skziverskij	+	≤ 1980
Olimpiade	+	
Groß Lüsewitzer	+	
Major	+	
Mestnij	+	≤ 1974
Markus	+	
ResynGS4	+	
Dippes	+	

This collection was selected in order to represent a broad range of available genetic diversity of winter type *B. napus*. All seeds were produced at one location in 2012. For Mitscherlich pot and container experiments, all seeds were treated prior to sowing with Elado (Bayer Crop Science, Monheim, Germany).

## **2.2. *In vitro* culture system**

### **2.2.1. Seed sterilisation and germination**

Prior the experiment, 150 to 200 seeds of each accession were placed in a 50 mL beaker with 6% sodium hypochlorite (NaClO) solution for eight minutes and placed on a magnetic stirrer. A drop of detergent was added in order to break the surface tension and avoid floating of seeds on the surface. Afterwards the seeds were rinsed with water until no foam was present. In parallel, 0.2 mL mini-PCR Eppendorf tubes were filled with 1.5% Agar Agar. From each accession 120 seeds representative, similar in seed size were selected and one seed per tube was pressed into the Agar Agar medium. After removing the base of each tube, in order to enable an unlimited root growth, the tubes were placed in a PCR rack and stored in a moist, humidified box for 72 h at 23 degrees to ensure uniform germination (Figure 3).

### **2.2.2. *In vitro* plant cultivation**

Plant *in vitro* cultivation experiment was conducted in climate chambers adjusted to 65% humidity, with a 16°C/12°C and 16/8 h day/night rhythm. From each accession the tubes with the most comparable growth development of the seedlings were selected and placed in a borehole in the lid of 50 mL Falcon tubes (Figure 3). The Falcon tubes were placed in racks above 10 L holding tanks (plastic boxes), with each tank containing one exemplar from each accession. The tanks were grouped according the nitrogen treatment. In each of three identical climate chambers two tanks per N treatment were placed. The Falcon tubes were filled with a nutrient solution containing 25% of the final nutrient concentration, in order to allow the young seedlings to adapt to the nutrient solution for ten days.

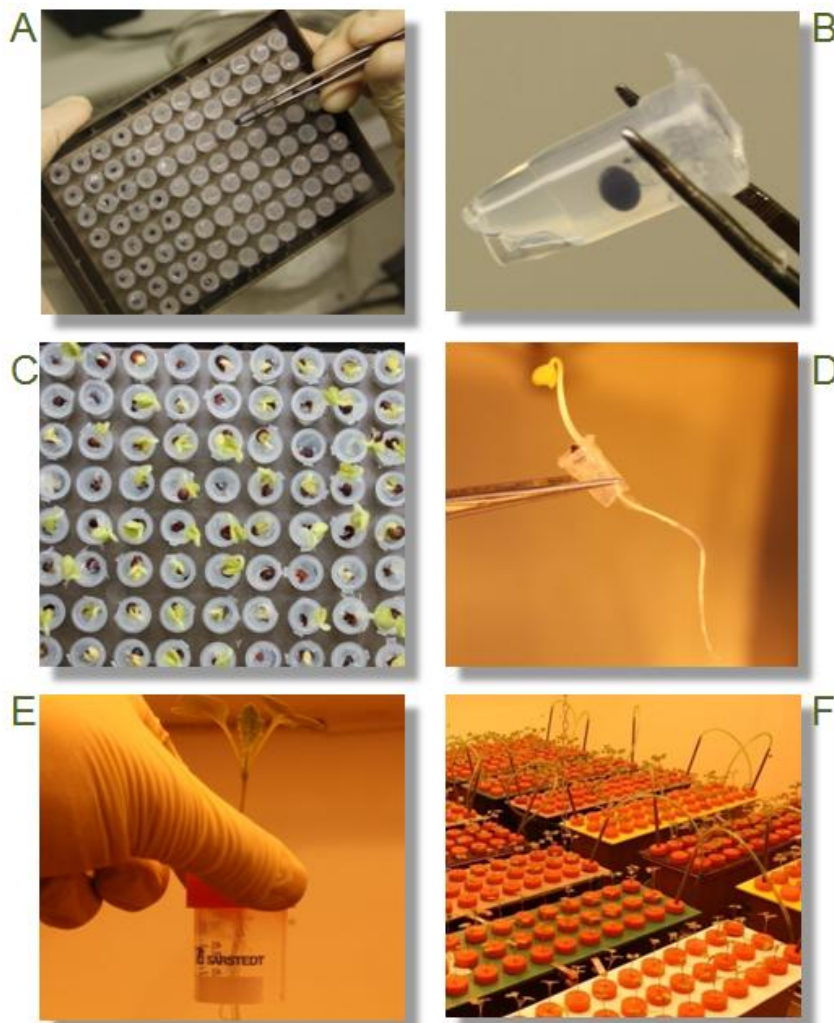
The composition of the nutrient solution contains 2 mM CaCl<sub>2</sub>, 1.5 mM K<sub>2</sub>SO<sub>4</sub>, 1 mM MgSO<sub>4</sub>, 0.25 mM KH<sub>2</sub>PO<sub>4</sub>, 0.1 mM Fe(III)-EDTA, 0.1 mM H<sub>3</sub>BO<sub>4</sub>, 1.0 µM MnSO<sub>4</sub>, 1.0 µM ZnSO<sub>4</sub>, 0.5 µM CuSO<sub>4</sub> und 0.02 µM (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>2</sub>. While the concentration of all nutrient were kept constant the nitrogen concentration differs ten-fold between 5 mM ([2.5 mM (NH<sub>4</sub>)(NO<sub>3</sub>)]) for the high N (HN) and 0.5 mM N ([0.25 mM (NH<sub>4</sub>)(NO<sub>3</sub>)]) for the low N (LN) treatment.

After ten days the intact Falcon tubes were exchanged for Falcon tubes that were cut off at the upper third, to allow the seedling roots to grow into the box filled with 10 L of nutrient solution (at 50% of the final nutrient concentration). Furthermore, since the aeration of the



nutrient solution is an important factor (Smeets et al., 2008), in each tank two air hoses assured the aeration of the nutrient solution. With the beginning of the 14<sup>th</sup> day after sowing (DAS) the nutrient solution was replaced by the fully dosed nutrient concentration, which was subsequently changed every three days.

Before the genotypes of this study were investigated, the system was run one time with one cultivar in order to test for normal nutrition of the plants. Test samples of the shoots were analysed by the state of Hesse agricultural testing laboratory (Landesbetrieb Landwirtschaft Hessen, Kassel) for the content of major nutrients. These data were compared with standard values from the literature (Bergmann, 1983).



**Figure 3:** *In vitro* growth system. A and B: Germination of seeds in Mini-PCR-Eppendorfcaps caps filled with 1.5% Agar Agar. C and D: Seedling three days after sowing. E: Young seedling. F: Hydroponic tanks with nutrient solution and aeration.

### **2.2.3. Harvest of plant material**

For harvest of plant material, plants were transferred at 28 DAS from the nutrient solution to a box with fresh water, in order to wash off all nutrients attached to the root surface. Afterwards plants were separated into root and shoot samples, and carefully dabbed on a paper towel in order to remove the adhering water. Root and shoot samples were dried in an oven for 72 h at 70°C and immediately transferred to desiccators for at least 30 min. Root and shoot dry weights (DW) were measured before the samples were ground. Subsequently the nitrogen content was measured in duplicates according to the Dumas combustion method (Dumas, 1826; Buckee, 1994), using an elemental analyzer (CE Instruments EA 1110, CE Instruments Ltd, Wigan, United Kingdom).

### **2.2.4. Data collection and analysis**

From each accession six biological replicates were analysed per nitrogen fertilisation level (NFL), two in each of three climate chambers. Nitrogen concentration and DM were multiplied to determine the total nitrogen content of both, root and shoot respectively. Since plant growth can vary considerably in early developmental stages, strict rules were applied for elimination of outliers. All data points that drifted more than the 1.5-fold standard deviation from the mean were eliminated from further analysis. Analysis of variance (ANOVA) and Pearson's correlations were calculated using the statistical software R (R core Team, 2013). Two-factorial ANOVA was calculated by a linear model with the R package 'lme4' (Bates et al., 2015a; 2015b). Accessions, NFL and their interaction were considered as fixed effects and replicates as random effects. Least significant difference (LSD) was determined at the 0.05 significance level for each trait and NFL, to determine if the difference between genotypes were significant.

## **2.3. Mitscherlich pot experiment**

### **2.3.1. Plant cultivation in Mitscherlich pots**

Plants were cultivated in 21 cm diameter Mitscherlich pots filled with 4970 g dry matter of a soil:sand mixture (50/50 w/w). Soil properties were determined before mixing with sand (Table 3). All accessions were sown on November 6, 2012, with a preliminary density of eight plants per pot, and thinned to a final density of three plants per pot after vernalisation and before beginning of the spring vegetation.

Basal fertiliser Hakaphos basis 3 (COMPO Expert GmbH, Münster, Germany), containing 0.2 g N, was applied as a pre-sowing dressing. In spring, pots of each accession were split into

two NFL “low N” (LN), with a final nitrogen fertilisation of 0.7 g N, and “high N” (HN), with 2.2 g N, which based on previous experience is a sufficient N supply for unlimited plant growth. The first spring nitrogen application was applied as ammonium nitrate at the beginning of bolting (March 26, 2013) with 0.5 g N for LN and 1.25 g N for HN pots. To avoid temporary over-fertilisation, HN pots received the remaining 0.75 g N as ammonium nitrate three weeks later. Based on parallel container experiments, total nitrogen content at the beginning of the experiment was 9.468 g N per pot and mineralized soil nitrogen content at beginning of spring vegetation was on average 0.354 g N per pot (71.2 mg kg<sup>-1</sup> soil dry matter).

**Table 3:** Soil properties measured prior blending with sand (1/1, w/w) and before additional fertiliser applications. Soil types are classified according to the FAO and World Reference Base classification system.

pH (in 0.01 M CaCl)	6.8
Clay [%]	39.30
Silt [%]	53.88
Sand [%]	6.82
Soil type	silty clay loam
N total [%]	0.381
N min [mg/kg soil]	71.2 <sup>#</sup>
P [mg/100 g soil]	5.8
K [mg/100 g soil]	5.9
Mg [mg/100 g soil]	18.6
C [%]	3.539

<sup>#</sup> based on experience from parallel experiments

Pots were arranged in two blocks for biomass and seed harvest, respectively, and each block was separated into two sub-blocks according to the nitrogen fertilisation level (NFL). Pots were arranged with a space of approximately of 15 cm between each other (Figure 4). Each accession was repeated twice per treatment and harvest. Pots were arranged randomly within each sub-block and repetition, and each sub-block was flanked by border pots planted with a standard accession to avoid neighbor effects. A single treatment with appropriate insecticides and fungicide was applied to prevent damage from pollen beetle and *Sclerotinia* infection. Each pot was watered separately by weighing to maintain a field capacity of 60%. To avoid nutrient loss by leaching, water that leached through a pot was collected and reused for watering the same pot the following day.



**Figure 4:** Mitscherlich pot experiment at flowering time.

### 2.3.2. Biomass harvest

For each accession, in each NFL, the specific onset of flowering was recorded as the number of days after January 1 until emergence of one flower on 50% of the plants in a plot. In order to estimate the nitrogen uptake performance of each accession until the transition to generative development, the biomass of each pot was harvested according to the specific developmental stage at flowering of the main raceme (developmental stage BBCH 67-69). Two pots with three plants each per accession were harvested for each NFL. At each harvest date, the total number of leaves and side branches were counted on all three plants per pot to calculate average numbers per plant. Plants were separated into three segments: a) leaves, b) stems and c) siliques (including flowers). All samples were dried for 72 h at 70°C. The dry weight (DW) of the plant material was measured before the samples were ground and subsequently analysed for nitrogen content according to the Dumas combustion method (Dumas, 1826; Buckee, 1994), using an elemental analyzer (CE Instruments EA 1110, CE Instruments Ltd, Wigan, United Kingdom). Nitrogen concentration and dry matter content were multiplied to determine the total nitrogen content of each harvested plant tissue.

### 2.3.3. Harvest of seeds and plant residues at maturity

Watering was stopped around 36 weeks after sowing, approximately after BBCH 86-88, when plant senescence was so far advanced that no further water uptake could be observed. Subsequently, plants were placed under a roof and allowed to ripen according to the specific developmental stage of each accession. The total aboveground plant material of fully ripened plants was harvested and stored in a greenhouse until further processing. After threshing of seeds the plant residues were separated into stem mass and empty silique walls. Stem and silique samples were ground and analysed for nitrogen content using an elemental analyzer (Vario EL Cube, Elementar Analysensysteme, Hanau, Germany). Seed samples were analysed in duplicate determination by near-infrared reflectance spectroscopy (NIRS; Unity SpectraStar 2500, Brookfield, USA) for water, oil, protein, glucosinolates, sulphur, fatty acids (C18:1, C18:3, and C22:1 content) using standard methods (Tkachuk, 1981; Reinhardt, 1992; Tillmann and Paul, 1998; Tillmann et al., 2000). Seed yield (SY) and seed-related quality data were corrected for 100% dry matter (DM) to allow comparison with data gained from biomass at flowering time.

### 2.3.4. Data collection and analysis

Depending on the research target (agronomic, physiological or economic) the term NUE is defined in different ways (reviewed in Xu et al., 2012). In this study, total nitrogen use efficiency was split into two major sub-traits as suggested by Moll et al. (1982). Nitrogen uptake was determined at the peak of flowering, since oilseed rape drops most of its leaves after flowering. Nitrogen contents of leaves, stems and siliques were summarised by multiplying the respective N concentration of each tissue by its dry weight. Nitrogen uptake efficiency (NupE) was then calculated by division of total plant nitrogen content by nitrogen supplied (Equation 1).

Equation 1:

$$\text{NupE} = \frac{\text{NconcLeavesF} * \text{LeavesMassF} + \text{NconcStemF} * \text{StemMassF} + \text{NconcSiliquesF} * \text{SiliquesMassF}}{\text{N}_{\text{soil}} + \text{N}_{\text{fertilised}}}$$

In this context, the applied definition of NupE takes not only the specific enzymatic processes of N transport over the plasma membrane into account, rather it captures a broader sense of net N acquisition by incorporating all participating effects from root morphology and distribution, over the activity of several N transport systems through to N assimilation and translocation.

Nitrogen utilisation efficiency (NutE) was calculated as the ratio of seed yield (SY) to the amount of nitrogen in the plant during flowering (Equation 2), thus summarising net effects of

subcellular recycling processes including source protein degradation, N remobilisation from source (e.g. leaves, stem and empty silique walls) to sink (mature seeds) and SY production (e.g. yield components as siliques per pot, seeds per silique and 1,000-seed weight). Since N in plant biomass was determined by a destructive method, calculation of complex traits such as NutE require computation of data collected in pots from different blocks. In this study it was assumed that performance of plants of the same accession and treatment was similar in both blocks. Neighbour effects were kept as constant as possible by applying the same randomisation layout in both blocks.

The ratio of SY to supplied nitrogen is defined as the total nitrogen use efficiency (NUE, Equation 3).

Equation 2:

$$\text{NutE} = \frac{\text{SY}}{\text{NconcLeavesF} * \text{LeavesMassF} + \text{NconcStemF} * \text{StemMassF} + \text{NconcSiliquesF} * \text{SiliquesMassF}}$$

Equation 3:

$$\text{NUE} = \frac{\text{SY}}{\text{N}_{\text{soil}} + \text{N}_{\text{fertilised}}}$$

Based on the seed nitrogen content and the nitrogen concentration of plant residues, nitrogen harvest index (NHI) was calculated as described in Equation 4.

Equation 4:

$$\text{NHI} = \frac{\text{SeedNyield}}{\text{NconcStemM} * \text{StemMassM} + \text{NconcSiliquesM} * \text{SiliquesMassM} + \text{SeedNyield}}$$

Analysis of variance (ANOVA) and Pearson's correlations were calculated using the statistical software R (R core Team, 2013). Two-factorial ANOVA was calculated by a linear model with R package 'lme4' (Bates et al., 2015a; Bates et al., 2015b). Accessions, NFL and their interaction were considered as fixed and replicates were considered as random effects. Least significant difference (LSD) was determined at the 0.05 significance level for each trait and NFL. The variety Start, which showed an extremely low seed yield attributed to an inherited trait that limits most siliques to only two seeds, was excluded from statistical analysis for all post-flowering traits. Correlation plots were created with the R packages 'corrplot' (Taiyun, 2013) and 'ggplots2' (Wickham, 2009; Alboukadel, 2014).

## 2.4. Experiments in a container system

To enable winter oilseed rape to be grown to maturity over its entire lifecycle with minimal constriction of roots, a semi-controlled plant growth platform comprising 144 transportable household refuse containers (“wheelie-bins”) with a volume of 120 L and a quadratic planting area of 0.16 m<sup>2</sup> was established. Bins were filled to a depth of 90 cm with a dried soil mixture. A clay-loam soil from the field station of Justus Liebig University Giessen in Rauischholzhausen, Germany, was mixed with sand at a ratio of 1:1. Before each experiment the soils were air-dried during summer for several weeks and crushed to homogenise the soil structure.

All containers were filled with 130 kg soil medium one month prior to sowing in order to allow the substrate to condense, eliminating cracks which might cause inhomogeneous water distribution and potentially influence the plant growth. After filling, the bins were placed in a 180 m<sup>2</sup> greenhouse, with a 4 m high hooped roof made of 200 µ thick, UV A und UV B permeable plastic, located at Rauischholzhausen field station (50°45′N, 8°53′E, 245 m above sea level). Prior to mixing with fertiliser the nutrient composition the soil was analysed using standard procedures (Table 3). The basal fertiliser was only mixed into the topsoil (Table 4).

**Table 4:** Mineral composition of basal fertiliser mixtures used in the container experiment.

Nutrient	Fertiliser [g/container]
N (autumn)	0.56
N (spring)	LN: 0.64 HN: 1.6 + 1.6
P	1.6
K	6.4
S	5.70
Mg	1.54
Ca	0.25
Mn	0.16
Zn	0.32
Cu	0.16
B	0.03

#### **2.4.1. Control of water supply**

For exact measurement of water use and control of water supply to the plant containers, which weigh between 150 and 180 kg when filled, a portable hydraulic hoist incorporating electronic scales was built. This allows each container to be lifted and simultaneously weighed. Water can then be added to retain the soil water capacity (WC) at a predetermined level compared to control containers with no plants. 100% WC was defined to be the amount of water which the soil could hold against gravity after two days. This was measured in a control container which was perforated at the bottom in order to observe water outflow. Containers used for plant cultivation were closed at the bottom and watered to the respective target WC proportional to the 100 % WC container. All containers were weighed twice a week for adjustment a target WC of 60 % until seed maturity.

#### **2.4.2. Plant cultivation in container experiments**

Seeds from the same genotypes as for *in vitro* and pot experiments were planted in container and field trials in the 2012/2013 growing season. Seeds from a single cultivar were direct-sown at a depth of 5 cm in a 3 x 3 grid with 13 cm spacing. The experiment employed a randomised block design comprising 120 experimental containers with two repetitions of 30 cultivars and two N treatments. Each of the 30 genotypes was sown in four separate containers, two with low and two with high NFL. To avoid influences from shading due to repressed growth in plants with lower N applications, the containers with high and low N treatments were blocked in alternate rows. But within N treatment blocks genotypes were randomised. The outer borders of the experiments were flanked with border containers.

After bolting the containers were each enclosed by a nylon net, enabling collection of aborted leaves whilst simultaneously avoiding extensive side branching of the outermost plants in each container. In spring (March 19, 2013) the containers with the low nitrogen (LN) treatment received 0.64 g N each (equivalent to 40 kg N ha<sup>-1</sup> field application), while the containers with the high nitrogen (HN) treatment received two times 1.6 g N with one month apart (equivalent of 100 kg N ha<sup>-1</sup>). Insecticide and fungicide was applied as required.

#### **2.4.3. Field experiments as a reference**

In order to examine the transferability of data from container-grown plants to those grown under field conditions, data from multi-location field experiments (Julia Rudloff, Dissertation University of Göttingen, unpublished) were used. Plot yields of the 30 varieties were measured under two different nitrogen fertilisation levels during the growing season 2012/2013 at Rauischholzhausen (RH, 50°45'N, 8°53'E, 245 m above sea level), Reinshof (RE, 51°29'51.02"N, 9°55'51.45"E, 157 m above sea level), and Rotenkirchen (RO, 51°46'N,



9°50'E, 144 m above sea level), Germany. The plants were grown in 8.75 m<sup>2</sup> (RH) to 18 m<sup>2</sup> (RO) plots in an alpha lattice experimental design, with two replicates of each test cultivar per location and treatment. All cultivars were grown at two NFL, one without N fertilisation (Low nitrogen, LN) and one with fertilisation (High nitrogen, HN). Cumulative N fertilisation was 180 kg N ha<sup>-1</sup> for RH, 177 kg N ha<sup>-1</sup> for RE, and 158 kg N ha<sup>-1</sup> for RO.

#### **2.4.4. Data collection and analysis**

Surrounding each container by nets allowed individual collection of aborted leaves per container at least twice a week. Measurements of DW of collected leaves and root samples were performed directly after drying the samples for 72 h at 70°C. According to the DW data and visual monitoring of degree of senescence, three extreme genotypes were selected for further nitrogen content analyses using an elemental analyzer (Vario EL Cube, Elementar Analysensysteme, Hanau, Germany), with all aborted leaves from each container being clustered into five batches. The groups were labelled according the last day of the respective batch as: June 14, June 23, June 28, July 10, and the respective date of seed harvest. By multiplication of the leaf DW by their respective N concentration, the specific N loss was calculated. N losses and N concentration were afterwards averaged between both replicated containers.

Each container was harvested separately at maturity with harvest date according to the maturity date. Plants were threshed and seed weight was measured separately for the main racemes and the side branches of the middle plant in each container. The main raceme and side branches of the remaining eight plants were harvested as a bulk. Yields of each plant were summed and divided by nine to give the total SY per plant. Stems and empty siliques hulls were weighed separately after threshing and summarised as plant residues. Harvest index (HI) was calculated as the ratio of SY by the complete plant weight, including roots. Root/shoot ratio was defined as the ratio between root DW and aboveground plant mass (plant residues and SY).

Immediately after the harvest of aboveground plant material, each container were lifted into a horizontal position and sprayed with water in order to initiate the outflow of the soil (Figure 5). Later, the complete root apparatus was soaked in water for several hours before being washed under a gentle water flow to remove fine soil particles from the roots. Subsequently the length of the longest root per container was measured, and roots were then dried for 72 h at 70°C until constancy of weight before determination of root dry biomass per container.



**Figure 5:** Process of root phenotyping. Images depict in direction of arrows how roots were washed out of the soil after harvest of aboveground biomass.

Field data of the nitrogen use efficiency field experiment were analysed with Plabstat (version 3A, Utz 2011, <https://plant-breeding.uni-hohenheim.de/software.html>). Other data analysis was performed using the statistical software R (version 2.15.3, R core Team, 2013). Significant differences between control (HN) and treatment (LN) were calculated using Student's *t*-test with a type I error threshold of 0.05. Pearson's correlation tests were performed to compare seed yields of the container experiments with the corresponding field trials. Correlation plots were created with the R package *ggplots2* (Wickham, 2009; Alboukadel, 2014).

### 3. Results

#### 3.1. *In vitro* growth experiments

The pre-experiment analysis of leaf nutrients by Hesse agricultural testing laboratory (Landesbetrieb Landwirtschaft Hessen, Kassel) revealed that the chosen nutrient solution for the hydroponic system is appropriate for oilseed rape cultivation. Comparisons of the analysed values with reference data from Bergmann (1983) confirm that all relevant nutrients are not limiting the plant growth and therefore N will be the only limiting factor in the LN treatment of the main experiment (Appendix 1).

As assumed, the tenfold higher N concentration in HN compared to LN led to an increase of shoot and root biomass as well as to a higher N concentration in tissues. Two-factorial analysis of variance (ANOVA) on each trait revealed highly significant ( $p < 0.001$ ) effects of N concentration and genotype, in nutrient solution, on all shoot and root traits (Table 5). The accession by nitrogen interaction was also highly significant for all traits except for root nitrogen concentration ( $p = 0.0049$ ), shoot N concentration ( $p = 0.0032$ ), root/shoot ratio of DW and root/shoot ratio of N content (both ratios non-significant).

**Table 5:** Phenotypic values of 30 diverse accessions at two nitrogen fertilisation levels.

									LN/HN	Accession	N	Accession x N
Low nitrogen fertilisation (LN)				High nitrogen fertilisation (HN)								
Min	Max	Mean	CoV	Min	Max	Mean	CoV					
NconcShoot [%]	3.30	4.84	4.14	0.09	5.61	7.02	6.25	0.07	0.66	***	***	**
Shoot Mass[mg]	85.90	233.68	157.40	0.22	150.26	382.04	265.65	0.23	0.59	***	***	***
NcontShoot[mg]	2.93	9.67	6.48	0.22	7.32	24.58	16.59	0.24	0.39	***	***	***
NconcRoot [%]	3.15	4.56	3.94	0.08	4.52	5.79	5.11	0.06	0.77	***	***	**
Root Mass[mg]	21.75	56.15	39.95	0.21	31.78	84.72	55.16	0.22	0.72	***	***	***
NcontRoot [mg]	0.48	2.31	1.52	0.26	1.22	4.53	2.82	0.26	0.54	***	***	***
Root/Shoot ratio of DW	0.16	0.32	0.26	0.14	0.17	0.25	0.21	0.12	1.22	***	***	.
Root/shoot ratio of N content	0.16	0.34	0.24	0.21	0.12	0.23	0.17	0.16	1.38	***	***	.

LN/HN gives the relative value of mean at low N (LN) to mean at high N (HN) fertilisation. Level of significance is indicated by ` for  $p < 0.1$ , \* for  $p < 0.01$ , \*\* for  $p < 0.005$  and \*\*\* for  $p < 0.001$ . CoV: Coefficient of variation. Quantity values are given per pot.

##### 3.1.1. Variation for shoot traits

On average the N limitation led to a mass reduction in shoot mass of 108.25 mg, equal to a mean reduction of 41%. With a range of 231.78 mg (150.26 mg to 382.04 mg) the variation

across the diversity panel at HN was bigger than the variation of 147.78 mg (85.9 mg to 233.68 mg) at LN. At HN, cultivars Markus (382.04 mg) and Libritta (380.40 mg) were the genotypes with the highest shoot mass, while Beluga (150.26 mg) and Resyn GöS4 (164.2 mg) exhibited the lowest shoot mass. At LN, cultivars Pacific (233.68 mg) and Pirola (229.68 mg) had the highest shoot mass, while Resyn GöS4 (85.9 mg) and Librador (97.72 mg) exhibited the lowest shoot mass (Figure 6). The rather weak relationship between shoot mass at LN and HN ( $R^2=0.197$ ) underlines the high genotype by N interaction, which was already found by two-factorial ANOVA to be significant at this early growth stage (Table 5).

The shoot N concentration varied across the diversity set at LN from 3.3% (Resyn GöS4) to 4.85% (Alaska) and at HN from 5.61% (Wotan) to 7.02% (Cobra) (Table 6). The low coefficient of determination ( $R^2=0.219$ ) for shoot N concentration between LN and HN, and the ANOVA results (Table 5), both demonstrate the high genotype by nitrogen interaction for this trait. Moreover the lack of correlation between shoot N concentration and shoot N mass illustrates that these are independently inherited traits. The shoot N content was calculated by multiplication of shoot N mass by shoot N concentration.

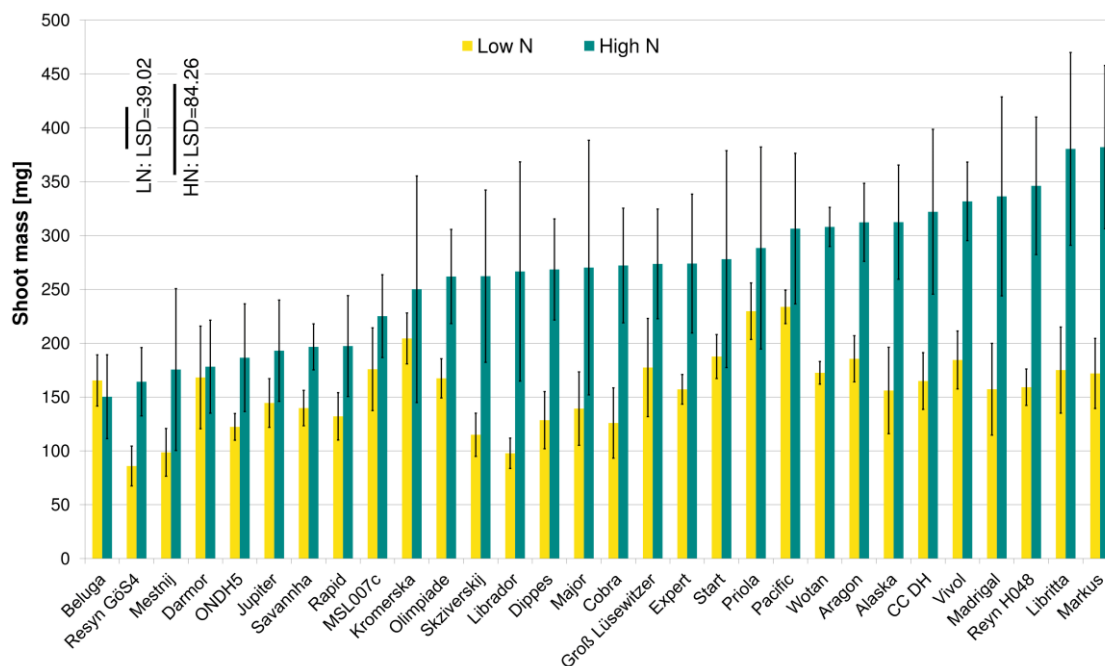
**Table 6:** Mean shoot nitrogen concentrations at low nitrogen and high nitrogen supply.

Genotype	Low N [%]		High N [%]	
	Mean	SD	Mean	SD
Alaska	4.840	0.393	6.696	0.436
Aragon	4.289	0.174	5.856	0.600
Beluga	4.809	0.536	6.674	0.109
Canberra X Courage DH	3.957	0.272	6.580	0.632
Cobra	4.589	0.200	7.019	0.218
Darmor	3.862	0.209	6.376	0.183
Dippes	4.118	0.109	6.324	0.516
Expert	4.359	0.152	6.177	0.492
Groß Lüsewitzer	3.879	0.109	6.057	0.330
Jupiter	4.094	0.454	6.710	0.291
Kromerska	4.170	0.256	5.939	0.338
Librador	4.486	0.301	6.953	0.557
Libritta	3.801	0.222	5.784	0.836
Madrigal	3.740	0.162	6.247	0.631
Major	4.153	0.309	6.549	0.355
Markus	4.332	0.158	6.414	0.484
Mestnij	4.046	0.335	5.647	0.638
MSL007	4.011	0.316	6.692	0.179
Oase x Nugget DH5	3.603	0.265	6.000	1.023
Olimpiade	4.219	0.185	5.821	0.494

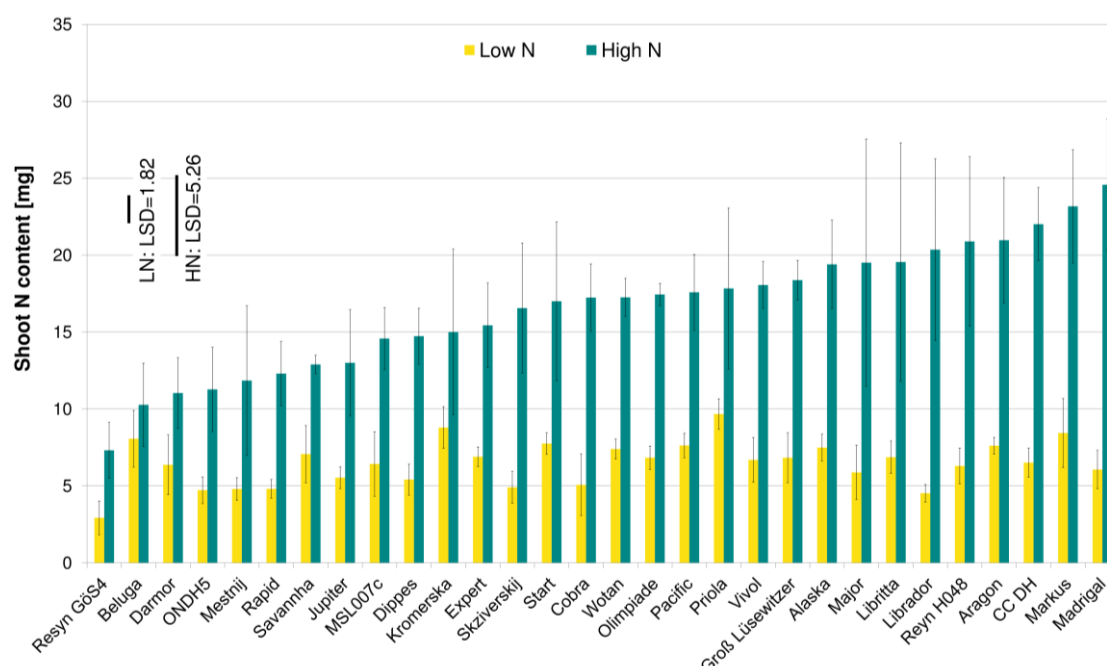
Pacific	3.577	0.315	6.165	0.435
Pirola	4.289	0.239	6.088	0.280
Rapid	3.803	0.193	5.664	0.595
Resyn Gö S4	3.300	0.535	5.690	0.547
Resyn H048	4.241	0.550	5.716	0.577
Savannah	4.636	0.327	6.538	0.633
Skziverskij	4.683	0.475	6.608	0.365
Start	4.173	0.259	6.640	0.493
Vivol	3.762	0.350	6.260	0.964
Wotan	4.446	0.198	5.610	0.340
Least significant difference	0.416		0.527	

Values are presented as means. Standard deviations are indicated as SD. Least significant difference (LSD) for low N (LN) and high N fertilisation (HN) is calculated at the 5% significance value

As indicated in Figure 7, the diversity set contains a huge variation for shoot N content, ranging from 2.93 mg (Resyn GöS4) to 9.67 mg (Pirola) at LN and from 7.31 mg (Resyn GöS4) to 24.57 mg (Madrigal) at HN.



**Figure 6:** Shoot mass of *in vitro* grown plants 28 days after sowing. Data show mean values. Standard deviation is depicted by error bars. Least significant difference (LSD) for low N (LN) and high N fertilisation (HN) is calculated on the 5% significance value.



**Figure 7:** Shoot N content of *in vitro* grown plants 28 days after sowing. Data show mean values. Standard deviation is depicted by error bars. Least significant difference (LSD) for low N (LN) and high N fertilisation (HN) is calculated on the 5% significance value.

A high coefficient of determination between shoot mass and shoot N content (LN:  $R^2=0.77$ ; HN:  $R^2=0.81$ ), along with the much lower coefficient of determination between shoot N concentration and shoot N content (LN:  $R^2=0.11$ ; HN:  $R^2=0.02$ ), indicate that the N content of shoots is predominantly determined by the shoot mass *per se* and not by the N concentration.

### 3.1.2. Variation for root traits

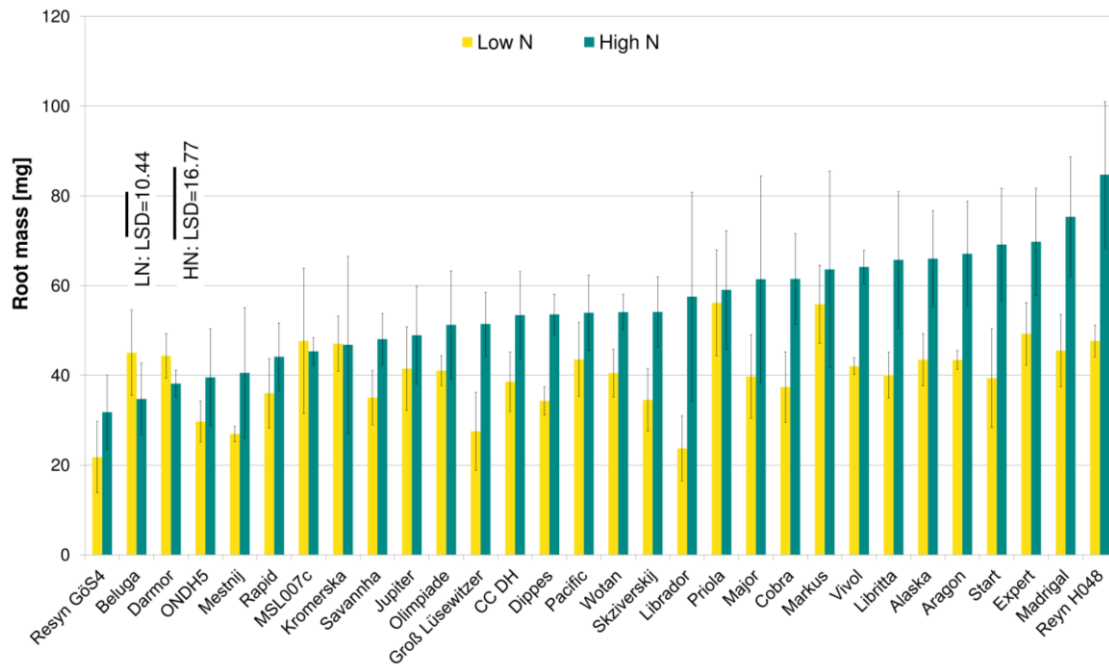
The average reduction of root mass caused by N limitation was 15.21 mg per plant, or 28%. The genotypes Resyn Gö S4 (21.75 mg) and Librador (23.72 mg) had the smallest roots at LN, while Pirola (56.15 mg) and Markus (55.83 mg) exhibited the biggest root system. At HN, Resyn GöS4 (31.78 mg) and Beluga (34.72 mg) showed the smallest roots, while Resyn H048 (84.72 mg) and Madrigal (75.32 mg) had the biggest roots (Figure 8). The genotypes with the lowest root N concentration at both NFL were Resyn GöS4 (LN: 3.15%; HN: 4.52%) and Oase x Nugget DH5 (LN: 3.27%; HN: 4.59%). At LN, cultivars Pirola (4.56%) and Skziverskij (4.35%) exhibited the highest root N concentrations, while Cobra (5.79%) and Major (5.63%) had the highest concentration at HN (Table 7). Root N contents were calculated by multiplication of root mass by root N concentration. However, the correlation between root mass and root N content was in a similar range to that seen in the shoots (LN:

$R^2=0.71$ ; HN:  $R^2=0.79$ ) and the root N concentrations were also correlated to root N content (LN:  $R^2=0.45$ ; HN:  $R^2=0.50$ ) – which was not the case in the shoots. As for shoot N mass, Resyn Gö S4 also had the lowest root N mass (LN: 0.48 mg; HN: 1.22 mg). Cultivars Pirola (2.31 mg) and Resyn H048 (4.53 mg) were the superior genotypes at LN and HN, respective (Figure 9).

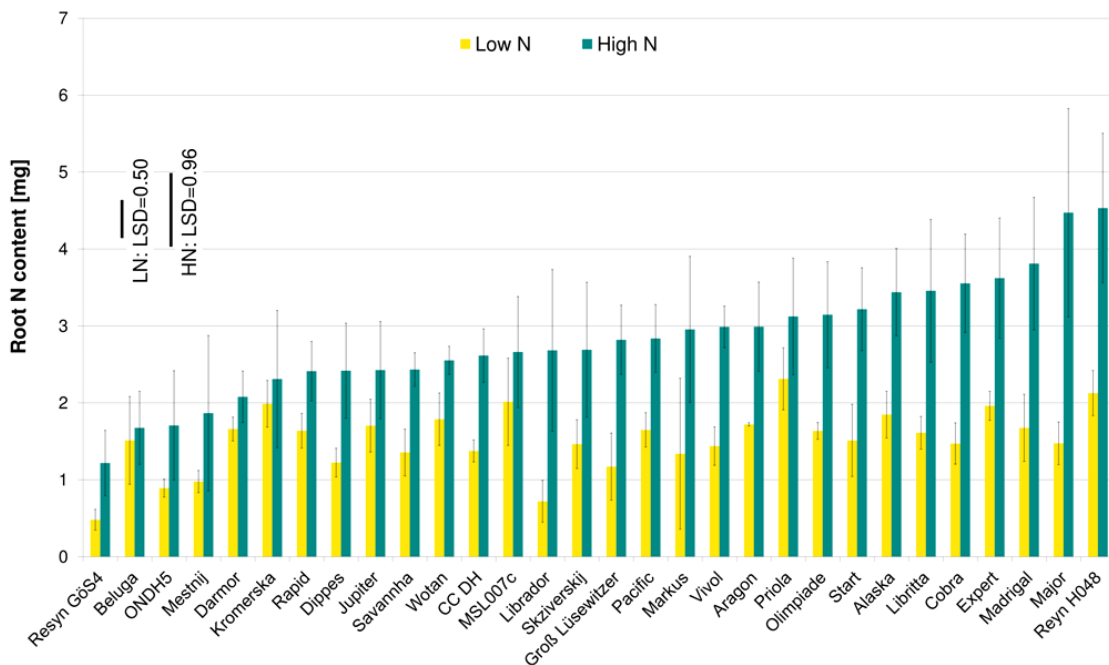
**Table 7:** Mean root nitrogen concentrations at low nitrogen and high nitrogen supply.

Genotype	Low N [%]		High N [%]	
	Mean	SD	Mean	SD
Alaska	4.285	0.077	5.213	0.146
Aragon	3.968	0.175	4.680	0.330
Beluga	3.644	0.223	4.862	0.307
Canberra X Courage DH	3.695	0.113	5.102	0.361
Cobra	3.925	0.174	5.792	0.425
Darmor	4.211	0.282	4.783	0.638
Dippes	3.922	0.164	5.280	0.558
Expert	3.954	0.148	5.047	0.249
Groß Lüsewitzer	4.282	0.206	5.475	0.373
Jupiter	4.132	0.235	4.835	0.493
Kromerska	4.322	0.279	4.958	0.162
Librador	3.544	0.343	5.407	0.272
Libritta	4.022	0.162	5.083	0.140
Madrigal	3.993	0.198	5.184	0.202
Major	3.806	0.305	5.633	0.330
Markus	4.060	0.050	5.053	0.408
Mestnij	3.875	0.609	5.037	0.239
MSL007	3.675	0.186	4.915	0.316
Oase x Nugget DH5	3.269	0.385	4.589	0.276
Olimpiade	3.935	0.236	5.165	0.370
Pacific	3.955	0.192	5.385	0.232
Pirola	4.563	0.179	5.160	0.250
Rapid	3.844	0.485	4.953	0.389
Resyn Gö S4	3.147	0.698	4.516	0.985
Resyn H048	4.193	0.216	5.595	0.352
Savannah	4.136	0.386	5.088	0.303
Skziverskij	4.348	0.121	5.209	0.802
Start	3.857	0.308	5.118	0.305
Vivol	3.739	0.262	5.229	0.343
Wotan	4.042	0.289	5.076	0.182
Least significant difference	0.405		0.508	

Values are presented as means. Standard deviations are indicated as SD. Least significant difference (LSD) for low N (LN) and high N fertilisation (HN) is calculated at the 5% significance value



**Figure 8:** Root mass of *in vitro* grown plants 28 days after sowing. Data show mean values. Standard deviation is depicted by error bars. Least significant difference (LSD) for low N (LN) and high N fertilisation (HN) is calculated at the 5% significance value.

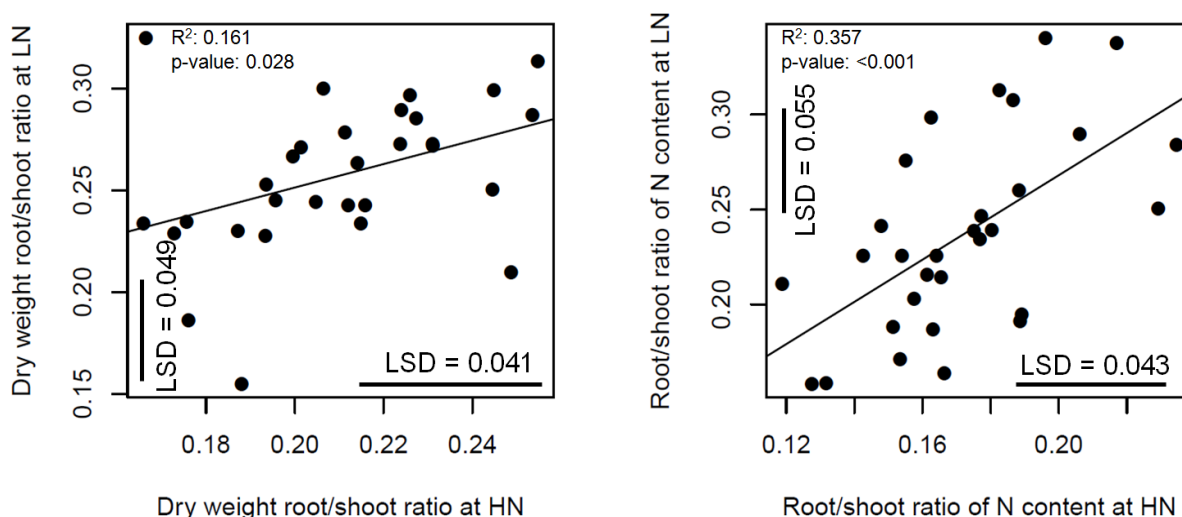


**Figure 9:** Root nitrogen content of *in vitro* grown plants 28 days after sowing. Data show mean values. Standard deviation is depicted by error bars. Least significant difference (LSD) for low N (LN) and high N fertilisation (HN) is calculated at the 5% significance value.



### 3.1.3. Root/Shoot ratio

Calculation of the LN/HN ratio indicates that the average dry matter reduction of shoots (0.59) is more pronounced than in roots (0.72). In other words, the N limitation increased the root/shoot ratio from 0.21 at HN to 0.26 at LN. However, the data show a correlation between shoot and root biomass at LN ( $R^2=0.52$ ) and HN ( $R^2=0.69$ ) and a certain degree of variation for root/shoot ratio. Some genotypes exhibited a rather low root/shoot ratio, for example Groß Lüsewitzer (LN: 0.15; HN: 0.19), Pacific (LN: 0.19; HN: 0.18), and Canberra x Courage DH (LN: 0.23; HN: 0.17). In strong contrast, other genotype showed a much higher root/shoot ratio, for instance Expert (LN: 0.31; HN: 0.25) and Jupiter (LN: 0.29; HN: 0.25). Interestingly there are also genotypes that show contrasting behaviour in root/shoot ratio between LN and HN. While Markus was the genotype with the second lowest value at HN (0.17), its root/shoot ratio increased markedly to 0.32 at LN. Genotype Start reacted in the opposite manner: Whereas the root/shoot ratio of 0.21 placed it among the lowest under LN, it showed the third highest root/shoot ratio (0.25) at HN. Across the entire panel the LN/HN ratio for shoot N concentration (0.66) was lower than for root N concentration (0.77). Together with a LN/HN value of 1.38 for root/shoot ratio of N mass, the data demonstrate that rapeseed plants under N starvation have relatively more N in their roots than in shoots compared to the situation under adequate N nutrition (Table 5, Figure 10).



**Figure 10:** Correlation between root/shoot ratio at high (HN) and low (LN) nitrogen supply for dry weight (left) and N content (right). Least significant difference (LSD) for low N (LN) and high N fertilisation (HN) is calculated on the 5% significance value.

### **3.2. Mitscherlich pot experiment**

#### **3.2.1. Trait variation**

As expected, increasing N fertilisation led to a weight increase in all tissues and N concentrations (Table 8). The delta in nitrogen fertilisation of 1.5 g between LN and HN led to an average SY increase of 20.87 g per pot. Two-factorial analysis of variance (ANOVA) on each trait revealed highly significant ( $p < 0.001$ ) effects of the NFL on all traits besides seed sulphur content, which was significant at  $p = 0.0174$  and C18:3, C22:1 and the ratio of N harvest to supplied N contents (non-significant). Huge genetic variation was also observed among the accessions, with significant accession effects for almost all traits. No significant genotypic effects were seen for N concentration and N content in siliques at maturity. The accession by nitrogen interaction could be only observed to be significant for the seed traits GSL and S content, along with many traits measured at flowering time. For nitrogen harvest index (NHI) no significant effect could be observed.

Within the diversity set flowering time displayed a similar range from 108 to 121 days in LN, and from 108 to 123 days after first of January in HN, respectively. On average, reduced nitrogen fertilisation led to one day earlier flowering, however one accession (Librador) flowered 6 days earlier and one (Resyn-H048) 4 days later under N deprivation, indicating strong genetic differentiation for developmental response to reduced fertilisation. Furthermore, increased fertilisation resulted in a significant increase in average number of leaves (11.6 to 13.3) and side branch development (4.6 to 6.2) prior to flowering.

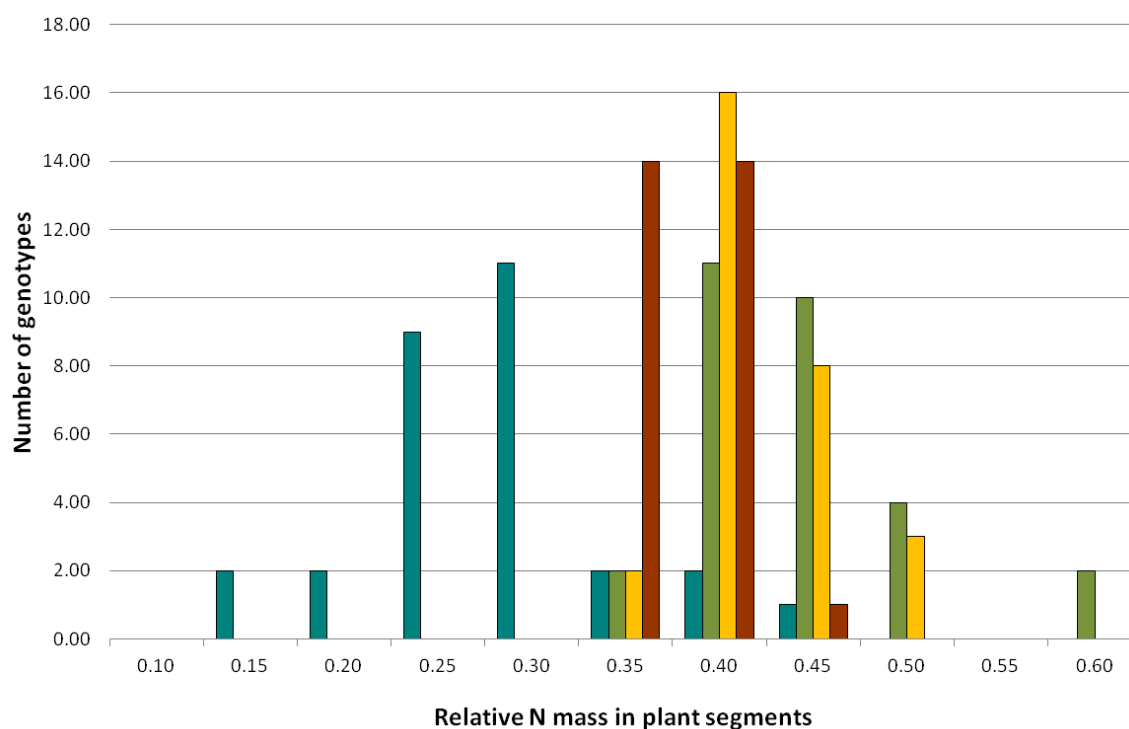
**Table 8:** Phenotypic values of 30 diverse accessions at two nitrogen fertilisation levels for the Mitscherlich pot experiments.

Parameter	Low nitrogen Fertilisation				High nitrogen Fertilisation				LN/HN	Accessions (n)	Accession		Accession x N
	Min	Max	Mean	CoV	Min	Max	Mean	CoV			N		
NoLeaves	9.00	14.67	11.63	0.13	11.00	16.33	13.29	0.11	0.87	30	***	***	*
NoSB	3.33	6.00	4.63	0.14	4.83	8.50	6.16	0.13	0.75	30	***	***	*
DaysAfter0101	108.00	121.00	114.87	0.03	108.00	123.00	115.68	0.03	0.99	28	***	***	*
NconcleavesF [%]	1.64	2.46	2.14	0.09	2.23	3.55	2.94	0.10	0.73	30	***	***	-
LeavesMassF [g]	3.48	9.42	6.72	0.24	12.04	22.82	18.87	0.15	0.36	30	***	***	*
NcontleavesF [g]	0.07	0.20	0.14	0.23	0.37	0.74	0.55	0.17	0.26	30	***	***	*
NconcsiliquesF [%]	2.53	4.41	3.25	0.12	3.45	4.80	3.90	0.08	0.83	30	***	***	-
SiliquesMassF [g]	4.78	11.55	7.92	0.20	10.31	20.65	15.70	0.17	0.50	30	***	***	-
NcontsiliquesF [g]	0.21	0.30	0.25	0.11	0.49	0.75	0.60	0.11	0.42	30	***	***	*
NconcstemF [%]	0.74	1.12	0.88	0.09	1.06	2.09	1.30	0.15	0.68	30	***	***	*
StemMassF [g]	23.46	37.67	29.01	0.12	30.47	65.85	49.76	0.13	0.58	30	***	***	.
NcontstemsF [g]	0.20	0.31	0.25	0.13	0.51	0.74	0.64	0.09	0.40	30	***	***	-
NinbiomassF [g]	0.56	0.74	0.65	0.07	1.62	2.04	1.79	0.05	0.36	30	**	***	-
SeedYield [g]	14.41	23.98	20.26	0.12	31.18	48.06	41.13	0.11	0.49	29	***	***	-
SeedNconc [%]	2.18	3.03	2.51	0.09	2.65	3.36	2.98	0.06	0.84	29	***	***	-
SeedNmass [g]	0.44	0.58	0.50	0.07	0.99	1.43	1.22	0.08	0.41	29	*	***	-
Oilcon [%]	44.58	53.95	49.78	0.04	42.50	50.05	45.92	0.04	1.08	29	***	***	-
OilYield [g]	7.10	12.19	10.10	0.13	13.99	22.90	18.92	0.13	0.53	29	***	***	-
GSL [μmol g <sup>-1</sup> ]	6.63	82.33	36.99	0.80	7.83	67.23	30.42	0.67	1.22	29	***	***	***
S [%]	0.10	0.68	0.32	0.58	0.15	0.55	0.30	0.39	1.07	29	***	*	***
C18:1 [%]	62.65	72.35	67.44	0.03	61.00	70.40	66.15	0.04	1.02	29	***	***	-
C18:3 [%]	8.00	10.55	9.53	0.06	8.38	10.53	9.51	0.05	1.00	29	***	-	-
C22:1 [%]	0.00	22.13	5.68	1.47	0.00	26.88	6.28	1.50	0.91	29	***	-	-
StemMassM [g]	17.65	28.74	22.44	0.13	33.88	49.16	40.17	0.09	0.56	29	***	***	-
NconcstemM [%]	0.21	0.40	0.31	0.15	0.24	0.46	0.35	0.16	0.88	29	*	***	-
NcontstemM [g]	0.05	0.10	0.07	0.19	0.10	0.20	0.14	0.17	0.49	29	*	***	-
PodmassM [g]	16.54	23.42	20.07	0.08	30.49	45.24	38.25	0.10	0.52	29	***	***	-
NconcsiliquesM [%]	0.25	0.46	0.34	0.15	0.41	0.71	0.55	0.13	0.62	29	-	***	-
NcontsiliquesM [g]	0.05	0.10	0.07	0.16	0.15	0.29	0.21	0.17	0.33	29	-	***	-
NupE [%]	49	70	61	0.08	63	80	70	0.05	87	30	**	***	-
NutE [g/g]	23.07	37.93	31.46	0.12	18.37	27.69	23.03	0.10	1.37	29	***	***	-
NUE [g/g]	13.67	22.75	19.22	0.12	12.21	18.82	16.10	0.11	1.19	29	***	***	-
NHI	0.71	0.87	0.80	0.04	0.70	0.87	0.79	0.05	1.01	29	-	-	-
Nharvest Nsupply	0.41	0.55	0.48	0.07	0.39	0.56	0.48	0.08	1.00	29	**	-	-

LN/HN gives the relative value of mean at low N (LN) to mean at high N (HN) fertilisation. Significant differences ( $p < 0.05$ ) between LN and HN are printed in bold type. Level of significance is indicated by . for  $p < 0.1$ , \* for  $p < 0.01$ , \*\* for  $p < 0.005$  and \*\*\* for  $p < 0.001$ . Coefficient of variation (CoV). Indications of quantity are given per pot.

### 3.2.2. Variation for nitrogen uptake

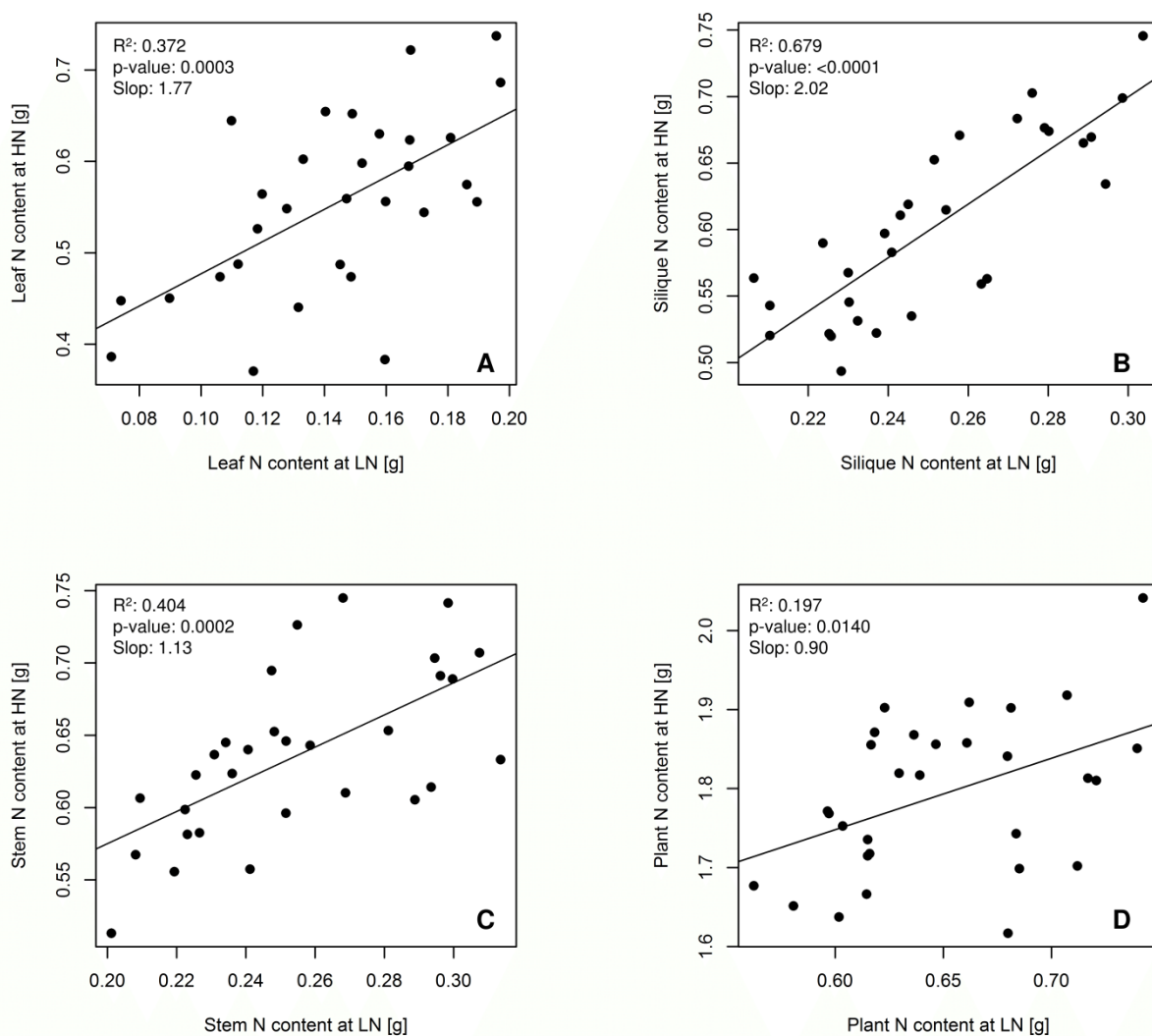
The ability of accessions to acquire nitrogen was determined at developmental stage BBCH 67-69 by analysis of nitrogen concentration in stems, leaves and siliques (including inflorescences). Calculation of LN/HN ratio revealed that average dry matter reduction caused by lowered N fertilisation was more pronounced in leaves (0.36) and siliques (0.50) than in stems (0.58). In contrast, stems showed the strongest reduction in N concentration (0.68), followed by the leaves (0.73) and immature siliques (0.83). However, a high variation was observed across the diversity panel (Table 8). Total N in plant biomass in LN was around 36% of the HN plants. As also depicted in Figure 11, the LN/HN ratio of leaf N content (0.26) indicates that N content of leaves is much more sensitive to lowered N fertilisation than that of siliques (0.42) and stems (0.40). Since the stem weight is much higher than that of leaves and siliques, the amount of stem N is at least as high as in the other tissues, although the N concentration in stems is lower.



**Figure 11** Relative N mass in plant segments at flowering. Diagram depicts the distribution of accessions for relative N mass grown at low N compared to high N fertilisation. Plants were separated into leaves (cyan), flowers with developing siliques (light green), stems (yellow), and the sum of these three categories (brown)

For NupE a variation of 49% to 70% was observed at LN and 63% to 80% at HN. However, a low coefficient of correlation ( $R^2=0.12$ ) between LN and HN for NupE revealed that the ranking of accessions under both NFL is not necessary the same, no significant accession by

N interaction was revealed by the ANOVA (Table 8). Nevertheless, for both NFL, and especially at HN, cultivar Aragon showed a considerably higher NupE than the other accessions. Interestingly, highly significant coefficients of correlation between LN and HN were observed for leaf N content ( $R^2=0.372$ ), stem N content ( $R^2=0.404$ ) and especially silique N content ( $R^2=0.679$ ) but not so pronounced for total plant nitrogen ( $R^2=0.197$ ). This indicates that the higher accession by nitrogen interaction of total N in plant biomass (and, thus, the NupE) can be better explained by the relationship of several tissues to each other than by the accession by nitrogen interaction in any single plant tissue (Figure 12).

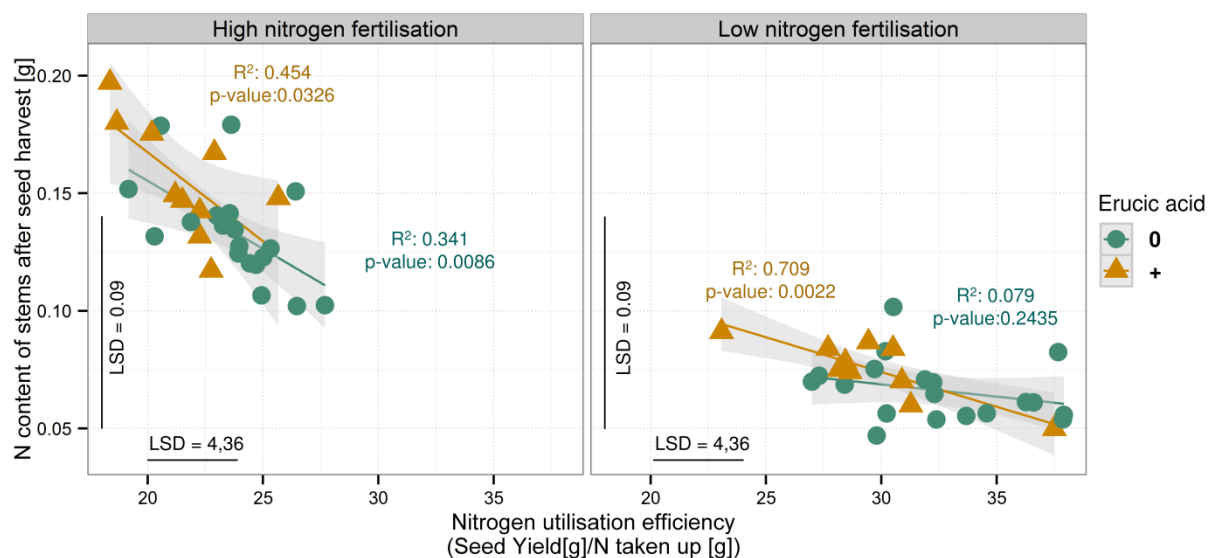


**Figure 12** Accession by nitrogen interaction for leaf N content (A), silique N content (B), stem N content (C) and total plant N content (D) at developmental stage BBCH 67-69.

### 3.2.3. Variation for nitrogen utilisation

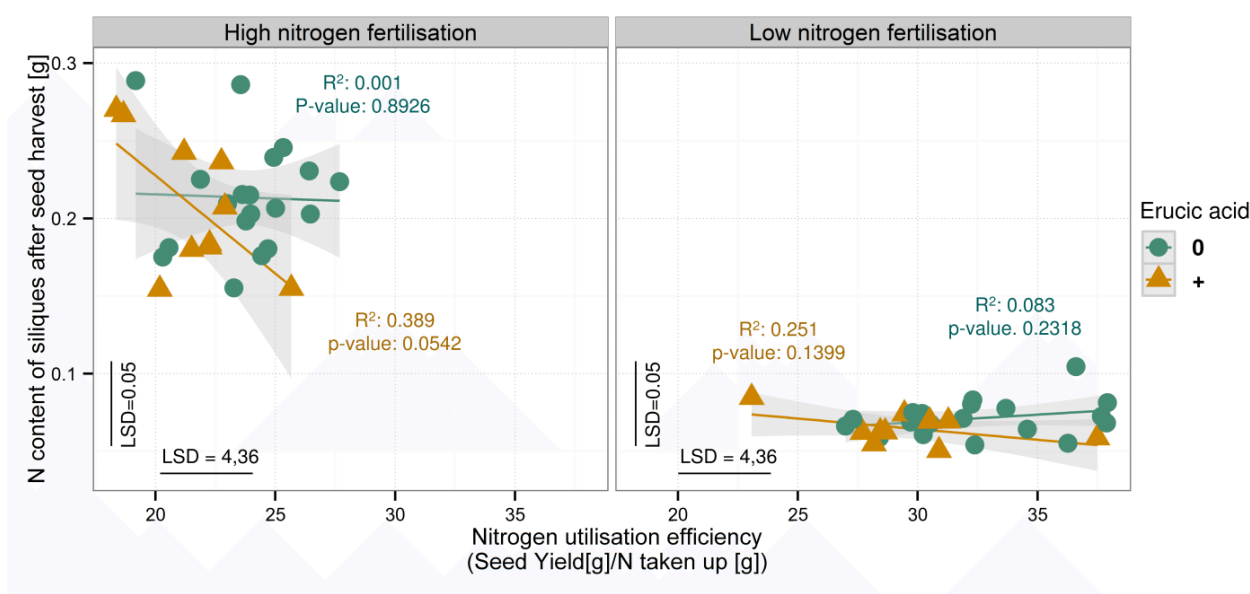
An average NutE of 31.5 g was calculated for the low NFL variant. This was nearly 40% higher than for high NFL, which showed average SY of 23.0 g per gram of uptaken nitrogen. Furthermore, a greater range of variation in NutE was observed across the diversity panel for

LN (14.9 g) than at HN (9.3 g). In contrast the nitrogen harvest index (NHI) showed no difference between HN (0.79) and LN (0.80). Since the diversity set contains older cultivars with high seed erucic acid (C22:1) along with more modern, erucic acid-free cultivars, calculations of coefficient of correlation can be biased and cause spurious correlations (Table 2). Therefore the diversity set was divided for the subsequent data analysis according to the presence of erucic acid. At both NFL the NutE was negatively correlated to N content in stems at maturity for modern (LN:  $R^2=0.079$ ; HN:  $R^2=0.341$ ) and older lines (LN:  $R^2=0.709$ ; HN:  $R^2=0.454$ ) (Figure 13). Correlations between NutE and N content in siliques at maturity were not significant for any NFL or subgroup of accessions. Nevertheless they suggest a trend towards a negative relationship for older accessions at HN (Figure 14).

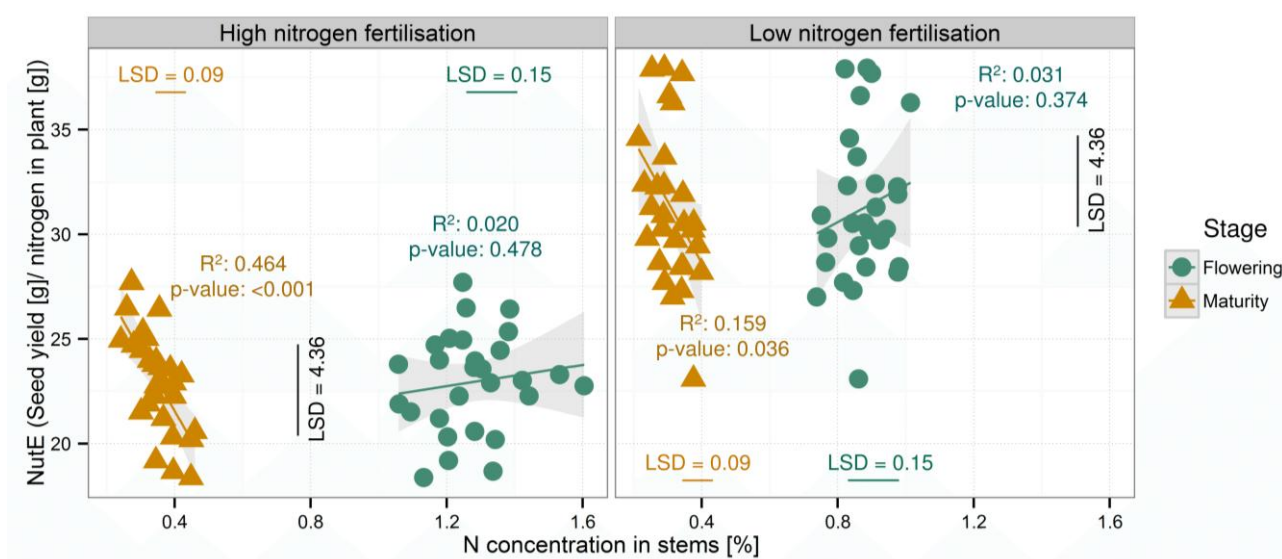


**Figure 13:** Correlation of nitrogen utilisation efficiency (NutE) with nitrogen content in stems after seed harvest at low nitrogen (right) and high nitrogen fertilisation (left). Grey shaded area depicts 95% confidence interval

Correlations between N concentrations and NutE in stems at flowering were not significant but tended to be positive. In contrast, the correlations of N concentration in stems after seed harvest were negatively related to NutE. For HN the coefficient of determination ( $R^2=0.464$ ,  $p<0.001$ ) was much stronger than at LN ( $R^2=0.159$ ,  $p=0.036$ ). Moreover, it was seen that the N concentration in stems at flowering achieved a higher level at HN than for LN, whereas the levels were similar again at maturity. This discrepancy further underlines the positive relationship between NutE and nitrogen remobilisation after flowering (Figure 15).



**Figure 14:** Correlation of nitrogen utilisation efficiency (NutE) with nitrogen mass in siliques after seed harvest at low nitrogen (right) and high nitrogen fertilisation (left). Grey shaded area depicts 95% confidence interval.



**Figure 15:** Correlation of nitrogen utilisation efficiency (NutE) with nitrogen concentration in stems at flowering and maturity at low nitrogen (right) and high nitrogen fertilisation (left). Grey shaded area depicts 95% confidence interval.

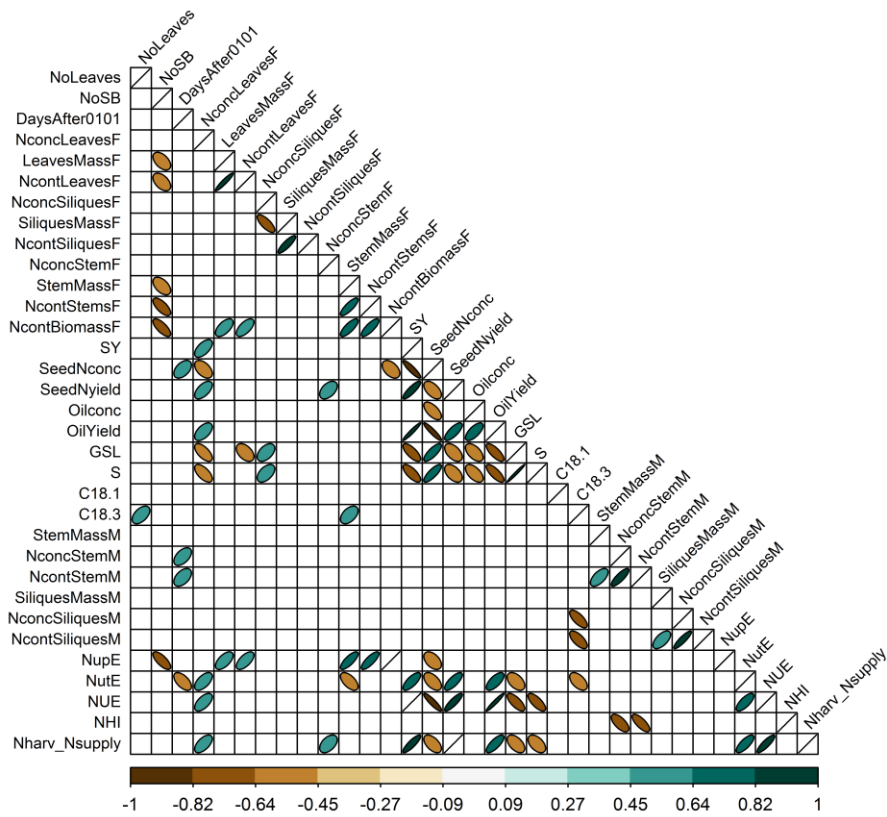
### 3.2.4. Trait interrelationships in Mitscherlich pot experiment

Several significant ( $p < 0.05$ ) correlations were observed in the data from the Mitscherlich pot experiment. Among the modern cultivars of the diversity set, under both NFL the seed N concentration was negatively correlated with SY (Figure 16). Furthermore, N yield showed a strong positive correlation with SY. With the exception of modern cultivars at LN, non-

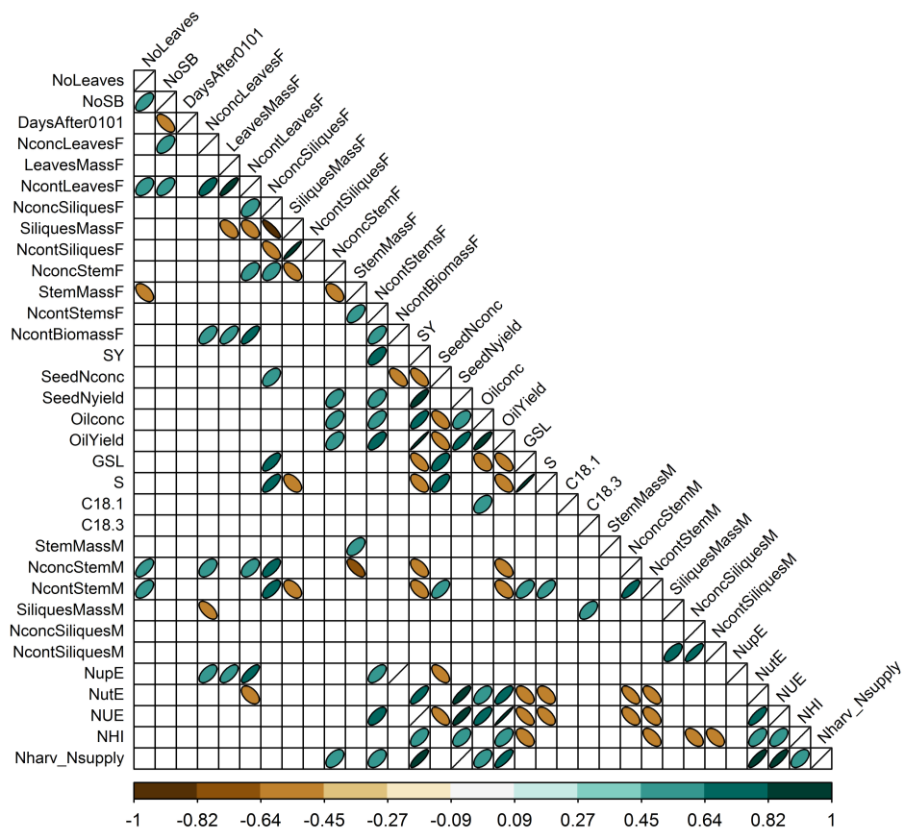
significant correlations were seen between N yield and N concentration. This illustrates that N yield is much more determined by the SY *per se* rather than the N concentration. In contrast, oil yield is positively influenced by both SY and oil content, under both NFL.

At both NFL and for both groups of genotypes, the parameters NoLeaves and NoSB were correlated neither to NutE nor to SY (Figure 16 and 17), indicating that an increase in NoLeaves or NoSB are not beneficial for NUE traits. In contrast, for modern cultivars, negative correlations were observed under LN conditions between the number of side branches to LeavesMassF, NcontLeavesF, StemMassF, NcontStemF and the total N content of plant biomass (and thus the NupE). This is an indication that accessions with increased branching were unable to supply the plant with enough N and, therefore, more pronounced side branching was even a disadvantage.



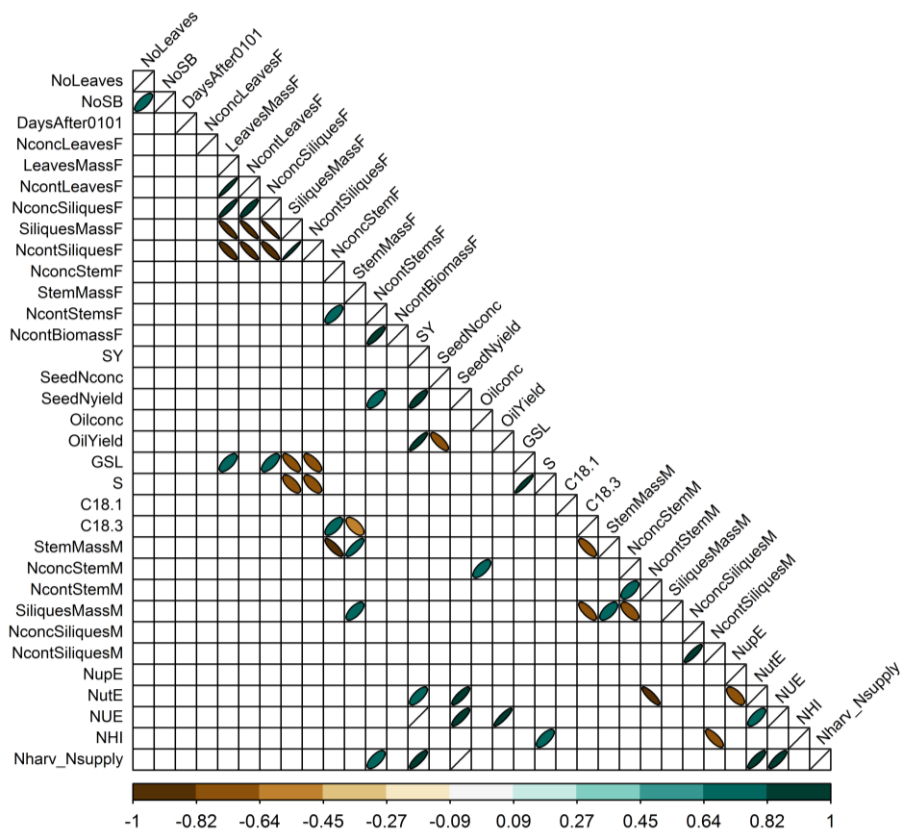


A

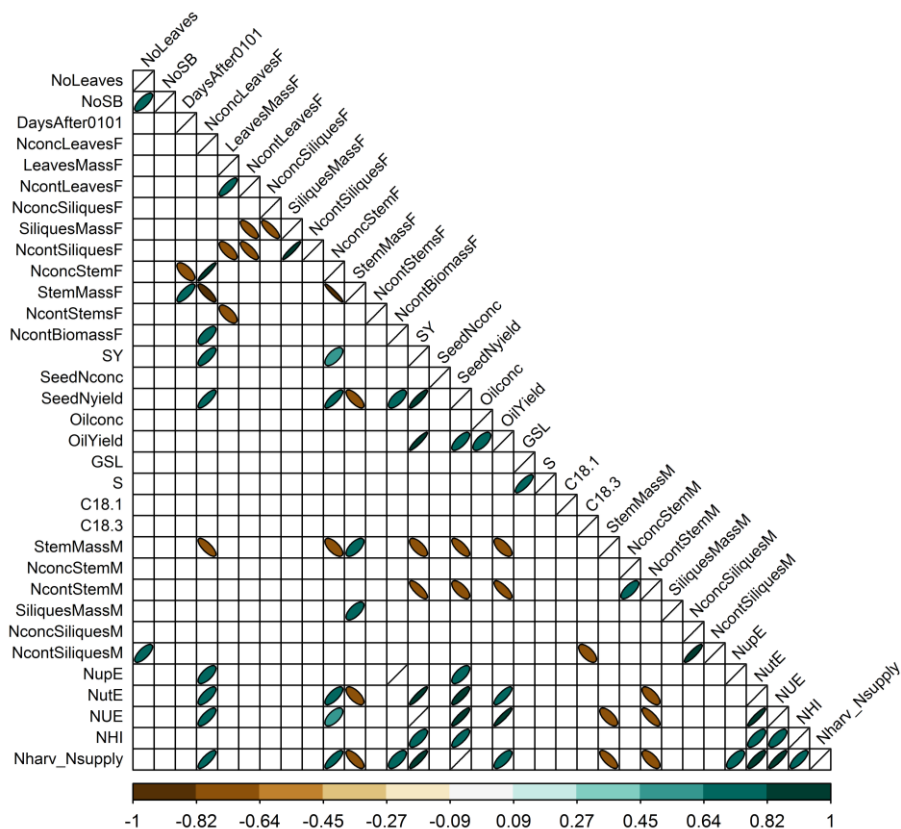


B

**Figure 16:** Pearson coefficients of correlation for traits within the modern variety group (n=19) at (A) low and (B) high nitrogen fertilisation. Colours and shapes of ellipses indicate the strength of correlations. Positive or negative correlations are depicted by the respective direction of each ellipse. Only correlations significant at a confidence level of 95 % are depicted.



A



B

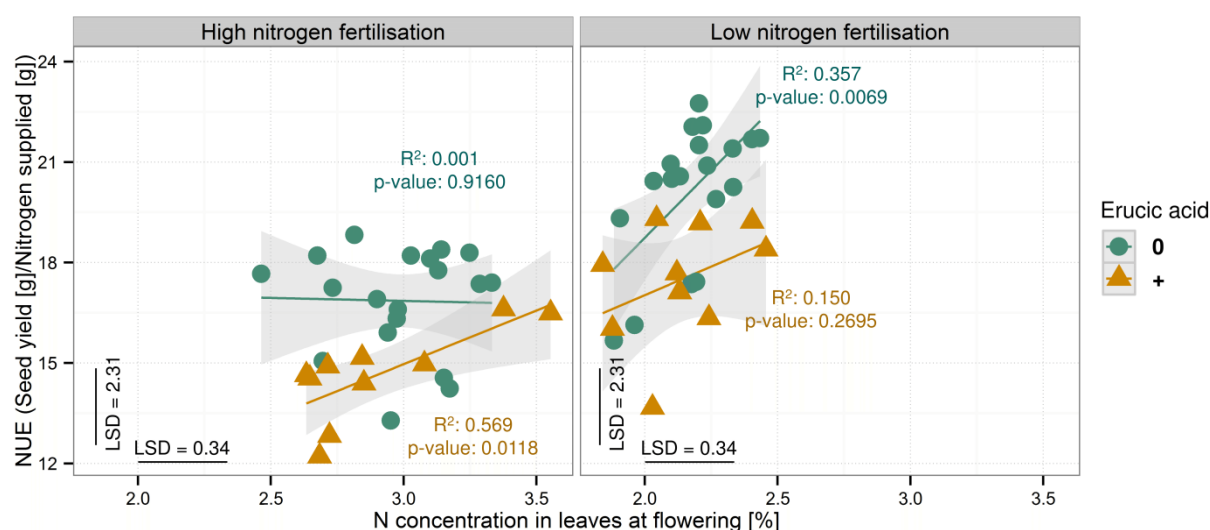
**Figure 17:** Pearson coefficients of correlation for traits within the older variety group (n=10) at (A) low and (B) high nitrogen fertilisation. Colours and shapes of ellipses indicate the strength of correlations. Positive or negative correlations are depicted by the respective direction of each ellipse. Only correlations significant at a confidence level of 95 % are depicted.

**Abbreviations for Figure 16 and 17:**

NoLeaves:	Number of leaves at flowering
NoSB:	Number of side branches
DaysAfter0101:	Days after Jan 1 <sup>st</sup>
NconcLeavesF:	Nitrogen concentration in leaves at flowering
LeavesMassF:	Mass of leaves at flowering
NcontLeavesF:	N content of leaves at flowering
NconcSiliquesF:	Nitrogen concentration in siliques at flowering
SiliquesMassF:	Mass of siliques at flowering
NcontSiliquesF:	N content of siliques at flowering
NconcStemF:	Nitrogen concentration in stems at flowering
StemMassF:	Mass of stems at flowering
NcontStemsF:	N content of stems at flowering
NcontBiomassF:	N content in complete biomass at flowering
SY	Seed yield
SeedNconc:	N concentration of seeds
SeedNyield:	N yield of seeds
Oilconc:	Oil concentration of seeds
OilYield:	Oil yield
GSL:	Glucosinolates
S:	Sulphur
C18:1:	Oleic Acid
C18:3:	Alpha Linolenic acid
C22:1:	Erucic acid
StemMassM:	Mass of stems at maturity
NconcStemM:	Nitrogen concentration in stems at maturity
NcontStemM:	N content of stems at maturity
SiliquesMassM:	Mass of siliques at maturity
NconcSiliquesM:	Nitrogen concentration in siliques at maturity
NcontSiliquesM:	N content of siliques at maturity
NupE:	Nitrogen uptake efficiency
NutE:	Nitrogen utilisation efficiency
NUE:	Nitrogen use efficiency

NHI: Nitrogen harvest index  
 Nharv\_Nsupply: Ratio of N in plant at maturity to N supplied

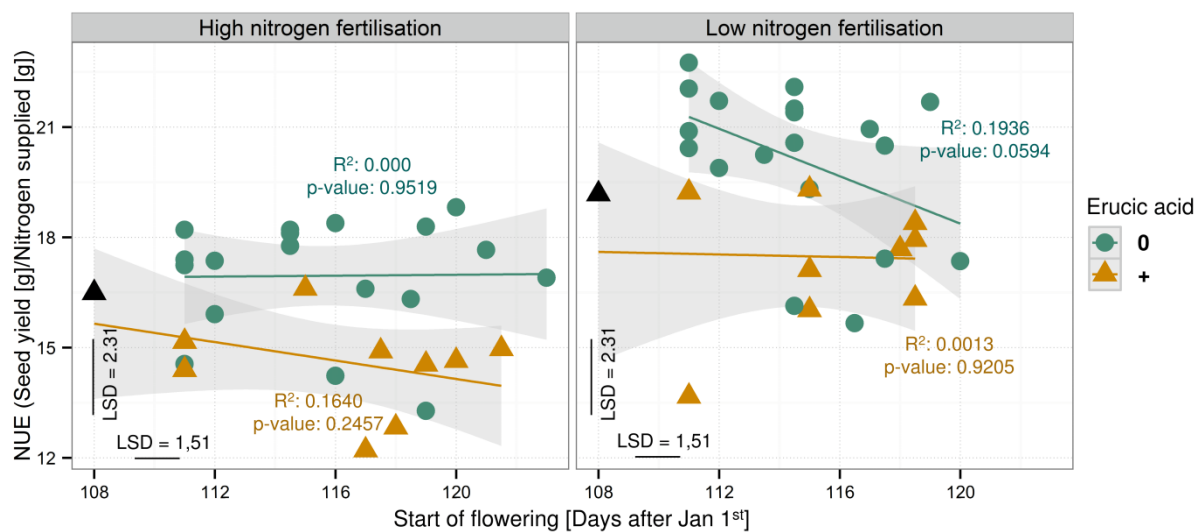
Besides lower yield, observations of several morpho-physiological traits suggest that an N limitation in the LN treatment leads to superior performance of several accessions that show a comparative advantage in dealing with N limitation. At LN, modern varieties showed a stronger correlation of leaf N concentration to oil and seed yield, and therefore also to NutE and NUE (Figure 18). This correlation was not found for the older varieties (Figure 17). Interestingly, at HN the reverse situation was observed. Here, in the older varieties, NUE, NupE, NutE and SY – but not oil yield or concentration – were more strongly correlated with leaf N concentration at flowering, whereas this relationship was seen only for NupE in the modern varieties. At both NFL, the modern types were on average superior to older lines in regard to NUE (Student's t-test:  $p=0.002$  at LN and  $p=0.001$  at HN), however, the leaf N concentration was not significantly different between the two groups at either NFL. Hence, modern varieties appear better at converting increased leaf N concentration into yield, making them more N-efficient than older varieties. In none of the genotype groups a positive relationship between NcontLeavesF and NutE was found. Interestingly, for modern cultivars at HN this relationship was even negative (Figure 16b), suggesting that the N concentration is more relevant than N content.



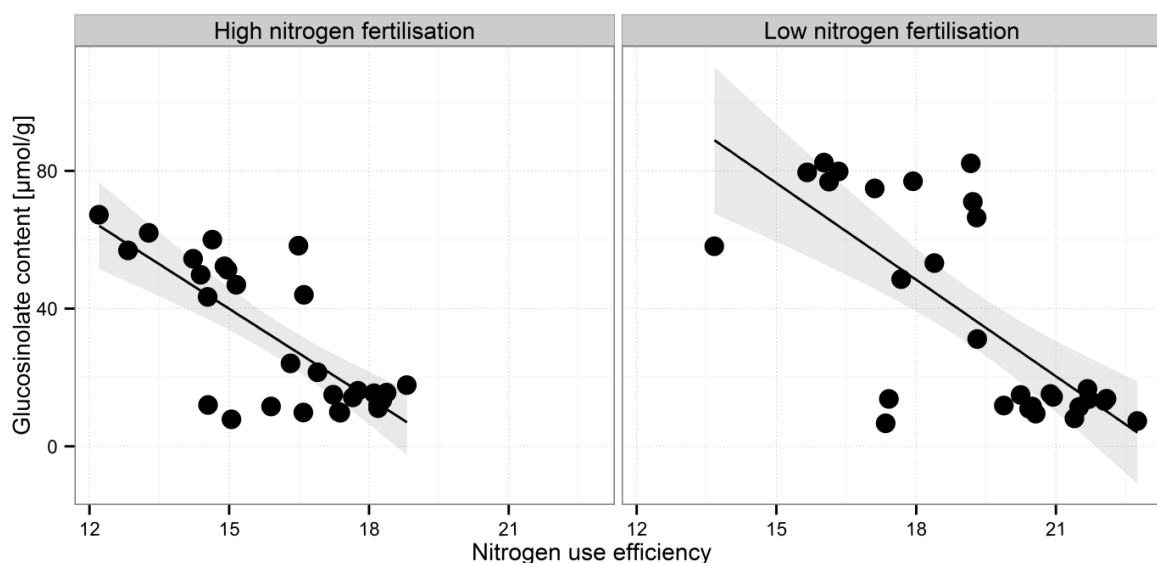
**Figure 18:** Correlation of nitrogen use efficiency (NUE) with N concentration in leaves at flowering at low nitrogen (right) and high nitrogen fertilisation (left). Grey shaded areas depict 95% confidence interval

In modern cultivars, moreover, early flowering at LN tends to be associated with increased overall NUE, whereas at HN the older varieties showed a similar but weaker, non-significant association (Figure 19). Furthermore, flowering time correlated to NutE ( $R^2 = 0.3288$ ,  $p$ -value

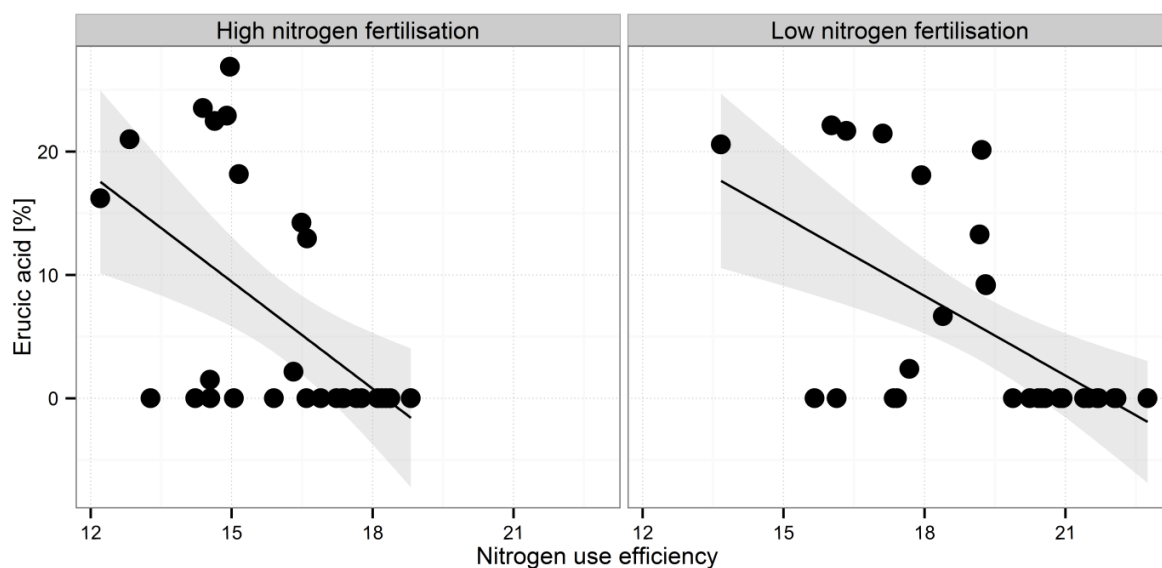
= 0.0103) and to NcontStemM ( $R^2 = 0.2383$ , p-value = 0.0340) in modern cultivars at LN (data not shown). In contrast, for older varieties at LN and modern varieties at HN, no relationship was detected between flowering time and any other trait (Figure 16B and 17A). When the complete diversity set was considered as a whole, significant negative trait interrelationships were also seen between NUE and GSL or erucic acid content, respectively (Figure 20 and 21). This can be regarded as a non-causative correlation, which is rather explained by parallel breeding progress towards seed quality (low erucic acid and GSL content) and yield performance in more recent cultivars (Table 2).



**Figure 19:** Correlation of nitrogen use efficiency (NUE) with flowering time at low nitrogen (right) and high nitrogen fertilisation (left). Grey shaded areas depict 95% confidence interval. Cultivar Olimpiade is marked with black triangles



**Figure 20:** Correlation of glucosinolate content and nitrogen use efficiency for the investigated diversity set (n=29) at low nitrogen (right) and high nitrogen fertilisation (left). Grey shaded areas depict 95% confidence interval.

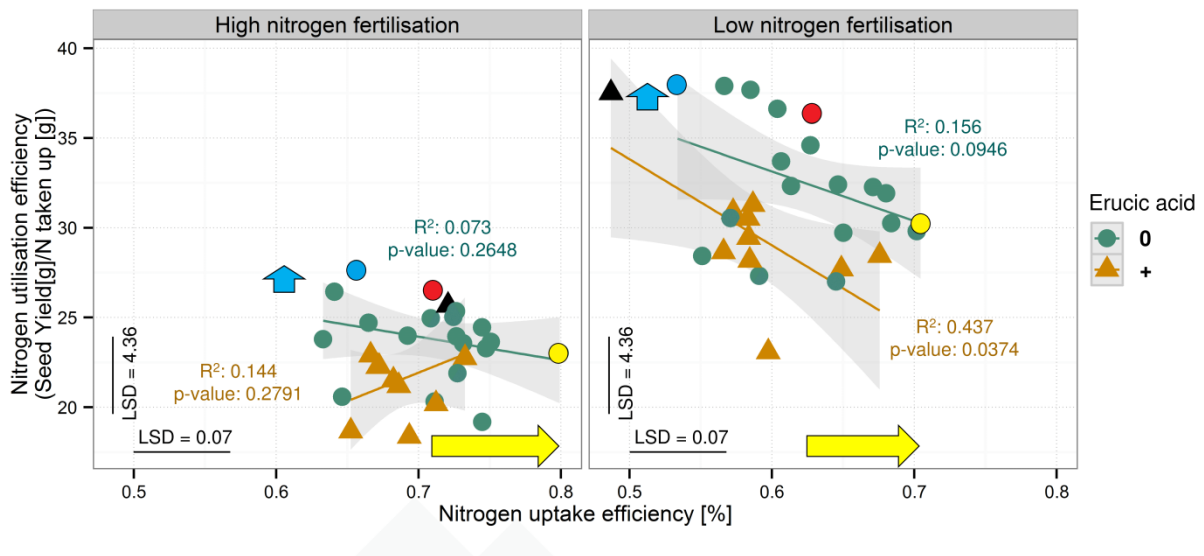


**Figure 21:** Correlation of erucic acid content and nitrogen use efficiency for the investigated diversity set (n=29) at low nitrogen (right) and high nitrogen fertilisation (left). Grey shaded areas depict 95% confidence interval.

### 3.2.5. Relative contributions of NupE and NutE to NUE

Neither under LN, nor HN could a significant correlation be detected between NupE and NutE (correlation at LN for old cultivars is biased by very early flowering cv. Olimpiade as an outlier). This result indicates that these two traits are under completely different genetic control across the investigated diversity panel (Figure 22). Closer investigation of individual accessions reveals that different strategies can confer specific advantages in achieving a high NUE. The accessions with the most extreme differences in NupE and NutE, cultivars Aragon and Madrigal, can each be characterised by the vegetation period in which they deal most inefficiently with available nitrogen. In contrast to all other 29 accessions, Aragon (marked in yellow in Fig. 22) had a superior NupE of 70.4% at LN and 80% at HN, hence it can be considered as a candidate for increased NupE. On the other hand, cultivar Aragon showed only an average NutE. In contrast cultivar Madrigal (marked in blue in Fig. 22) was among the poorest accessions in terms of NupE, but first in NutE at both NFL. Interestingly, these two accessions exhibit no significant difference in absolute SY. This bilateral comparison illustrates that accessions in the same environment can behave quite differently before and after anthesis with regard to nitrogen use. Although Aragon and Madrigal are the best-performing accessions for NupE and NutE, respectively, they are outperformed in overall NUE by two accessions at HN and seven at LN (Figure 22), respectively. Accessions with best NUE show a more balanced contribution of NupE and NutE rather than extreme efficiency for one or the other (Figure 22). Correlations to NUE are much lower for NupE ( $R^2 = 0.13$  for LN and  $R^2 = 0.24$  at HN) than those for NutE ( $R^2 = 0.59$  and  $R^2 = 0.73$  for LN and HN

respectively), indicating that within the diversity panel superiority in NutE was more relevant for total NUE than NupE.



**Figure 22:** Relationship of N utilisation efficiency (NutE) and N uptake efficiency (NupE) at high (left) and low N (right) fertilisation. Within each N level extreme accessions are marked as: Highest NutE, (cultivar Madrigal, blue), highest NupE (cultivar Aragon, yellow), highest total NUE (red). Arrows indicate theoretical potential to improve best NUE accessions for NupE (yellow) and NutE (blue). Cultivar Olimpiade is marked with black triangles

### 3.3. Container experiment

#### 3.3.1. Variation for yield and phenological traits

The remarkable variation in seed yield ( $20.36 \text{ g plant}^{-1}$  for LN and  $20.42 \text{ g plant}^{-1}$  for HN) between the test genotypes was reflected in significant genotypic effects ( $p < 0.001$ ). The two-factorial ANOVA also revealed highly significant effects of the accession on all other determined trait, except for the number of side branches. Whereas for NFL only weak significant effects were seen for plant residues and root length under the different NFL (Table 9). Although the two-factorial ANOVA shows significant effects of the NFL ( $p < 0.001$ ), the average seed yield (LN:  $24.98 \text{ g plant}^{-1}$ ; HN  $26.84 \text{ g plant}^{-1}$ ) is not significantly different between the treatments (Student's t-test:  $p = 0.135$ ), indicating that the soil N levels were not sufficiently low in the LN variant to significantly limit the seed yield (Table 9 and Figure 23). Calculation of the harvest index indicates that the diversity set also contains huge variation for the transformation of plant biomass into seed yield. For example, cultivar Gross Lüsewitzer is higher yielding than genotype Oase x Nugget DH5 (ON DH5) in this experiment, but harvest index of latter accession is much better than the former (Figure 23). Although noteworthy differences in phenology were observed in the genotype panel, the phenology data for number of side branches (Figure 24), number of siliques on the main

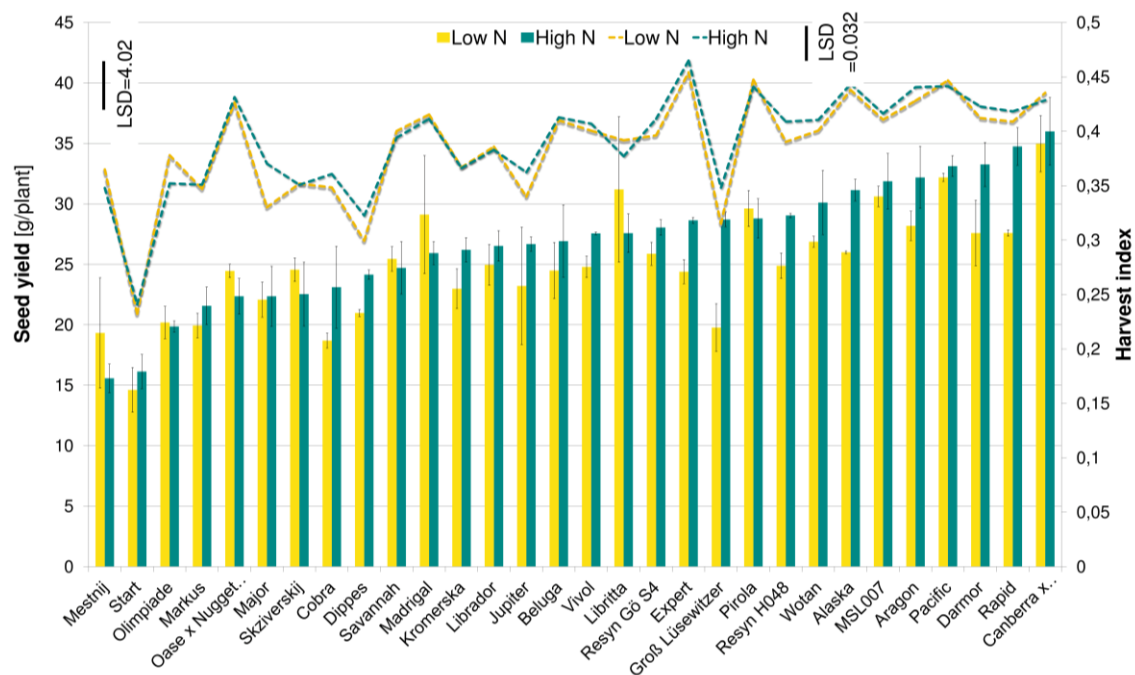
raceme (Figure 25) and start of flowering (Figure 26) all show no correlation of these strongly variant traits to seed yield in the containers, either at HN or at LN. Thus, genetic variation for seed yield could not be attributed to these phenological parameters.

**Table 9:** Phenotypic values of 30 diverse accessions at two nitrogen fertilisation levels.

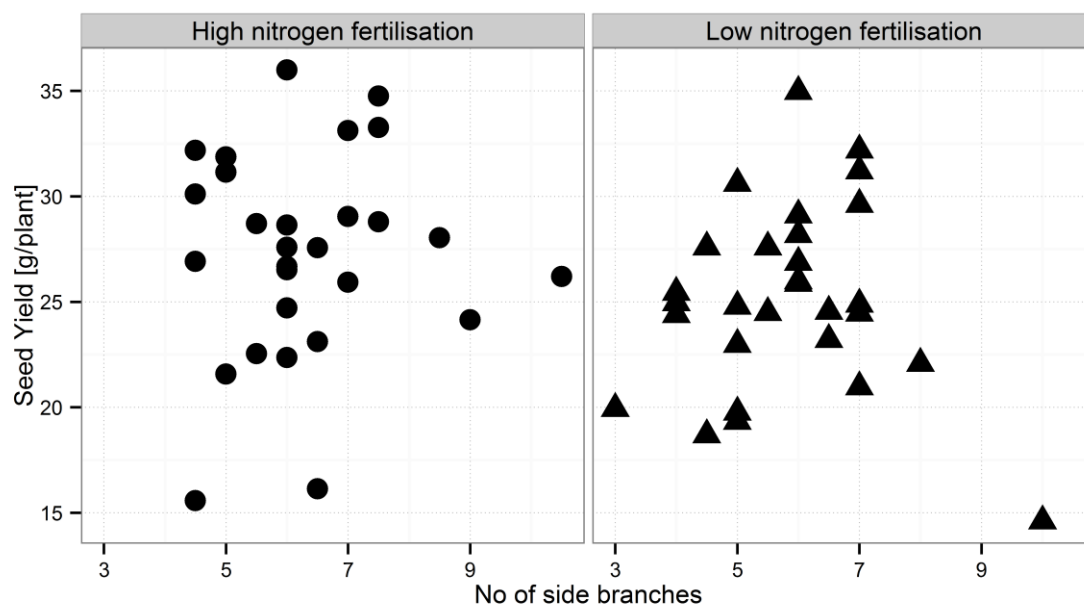
Accession	Low nitrogen fertilisation (LN)				High nitrogen fertilisation (HN)				LN/HN	Accession N	Accession x
	Min	Max	Mean	CoV	Min	Max	Mean	CoV			
<b>Seed yield [g]</b>	14.61	34.97	24.98	0.18	15.57	35.99	26.84	0.18	0.93	***	***
<b>Stem weight [g]</b>	11.60	21.32	16.78	0.14	12.97	23.31	17.57	0.14	0.96	***	.
<b>Siliques weight [g]</b>	18.48	30.46	23.18	0.13	16.90	30.64	24.34	0.14	0.95	**	-
<b>Plant residues [g]</b>	30.08	48.16	39.96	0.12	30.42	52.83	41.91	0.14	0.95	***	*
<b>Root DW [g]</b>	3.65	9.95	6.42	0.27	3.31	14.56	6.87	0.35	0.93	***	-
<b>Harvest index</b>	0.21	0.42	0.35	0.14	0.21	0.43	0.36	0.13	0.99	***	-
<b>Root/shoot ratio</b>	0.07	0.14	0.10	0.22	0.06	0.18	0.10	0.32	0.98	***	-
<b>Root length [cm]</b>	40.00	67.00	55.15	0.12	41.00	89.50	60.90	0.22	0.91	***	**
<b>No siliques MR</b>	55.50	119.00	77.03	0.18	48.00	119.00	77.82	0.18	0.99	***	-
<b>No of SB</b>	3.00	10.00	5.83	0.24	4.50	10.50	6.31	0.22	0.92	-	-
<b>Start of flowering [Days after Jan 1]</b>	110.00	127.50	122.02	0.03	101.00	127.00	121.61	0.04	1.00	***	-

LN/HN gives the relative value of mean at low N (LN) to mean at high N (HN) fertilisation. Level of significance is indicated by . for  $p < 0.1$ , \* for  $p < 0.01$ , \*\* for  $p < 0.005$  and \*\*\* for  $p < 0.001$ . CoV: Coefficient of variation. Quantity values are recorded per container.

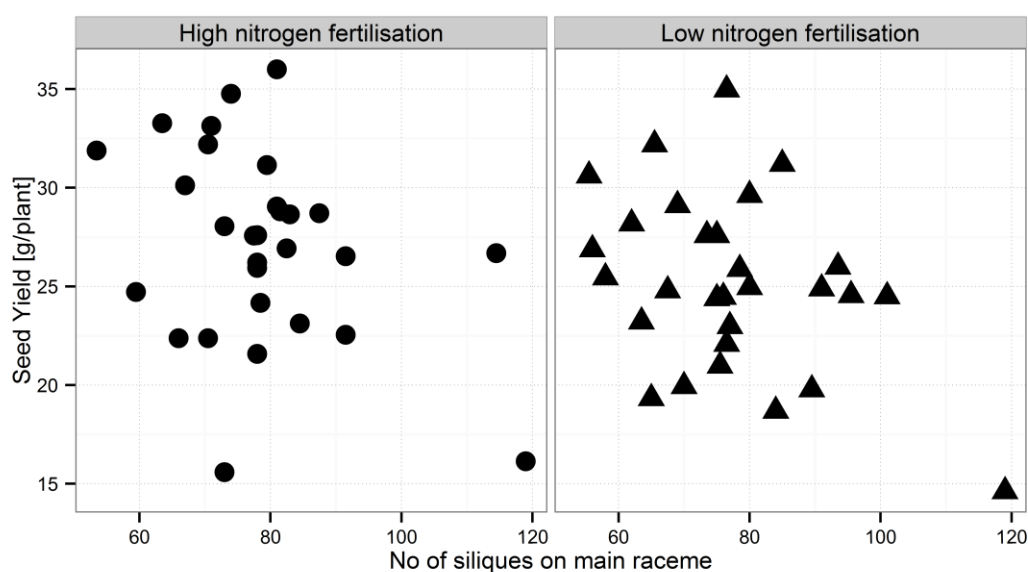




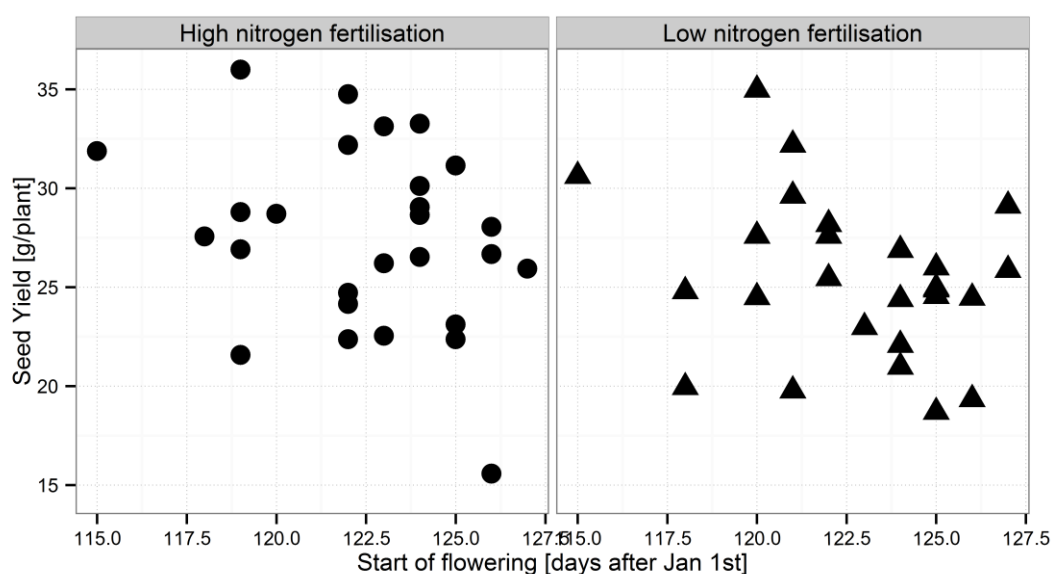
**Figure 23:** Seed yield (columns) and harvest index (doted lines) at maturity. Data represent the average of two replicated container and nine plants within one container. Standard deviation is marked with error bars. Least significant difference (LSD) is calculated on the 5% significance value.



**Figure 24:** Relationship between seed yield in containers and number of side branches at high nitrogen fertilisation (HN, left) and low nitrogen fertilisation (LN, right).



**Figure 25:** Relationship between seed yield in containers and number of siliques on the main raceme at high nitrogen fertilisation (HN, left) and low nitrogen fertilisation (LN, right).



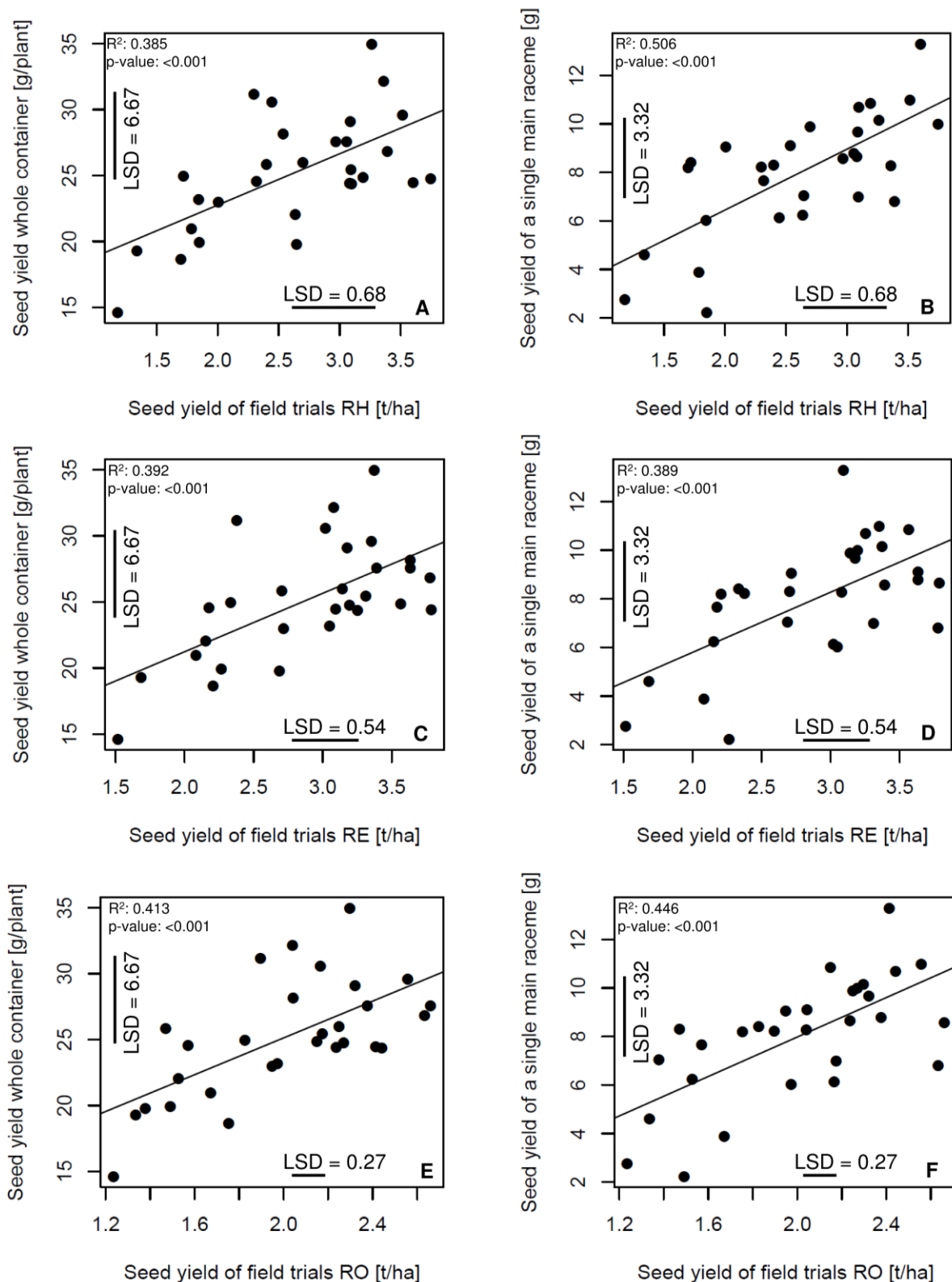
**Figure 26:** Relationship between seed yield in containers and start of flowering at high nitrogen fertilisation (HN, left) and low nitrogen fertilisation (LN, right).

### 3.3.2. Comparisons between field and container trials

In parallel field experiments at three locations (data provided by Julia Rudloff, University of Göttingen), high significant differences (RH  $p = 0.001$ , RE and RO  $p < 0.001$ ) in seed yield between LN and HN were observed. The average seed yield across all three field locations showed a huge variation between genotypes, ranging from 1.310 t/ha to 3.267 t/ha for LN

and from 1.886 t/ha to 4.226 t/ha for HN. Furthermore, average seed yields at the single locations ranged from 2.003 t for RO to 2.887 t for RE at LN and from 2.804 t for RO to 3.894 t for RE at HN.

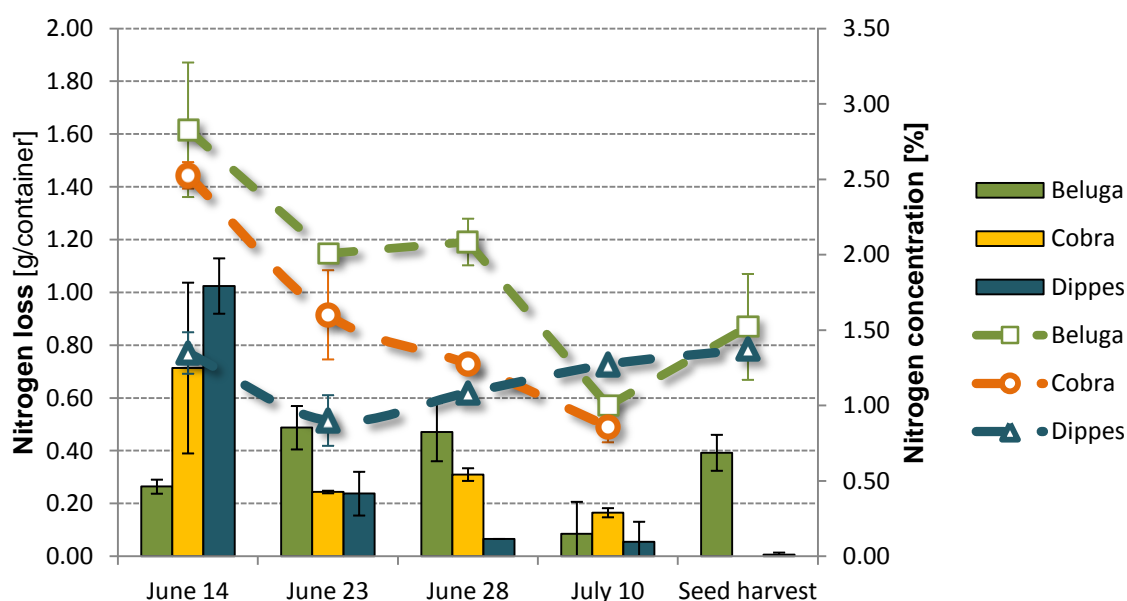
Highly significant ( $p < 0.001$ ) Pearson correlation coefficients were detected between seed yields in the containers and plot yields at the three field locations, at both LN and HN (Figure 27). Comparing the container yields against the average seed yield over all field locations demonstrated a high power to predict average field performance in container experiments. The average seed yields measured in the respective replicated containers showed comparable levels of correlation to the individual field locations for both the LN (RH:  $R^2 = 0.385$ ; RE:  $R^2 = 0.0,392$ ; RO:  $R^2 = 0.413$ ) and HN experiments (RH:  $R^2 = 0.457$ ; RE:  $R^2 = 0.357$ ; RO:  $R^2 = 0.401$ ) (Figure 27). Furthermore, coefficients of correlation to field performance were also consistently high under both N treatments (LN:  $R^2 = 0.450$ ; HN:  $R^2 = 0.432$ ) for average single-plant yields per container. For LN, the use of yield data only from the main raceme of the middle plant in each container slightly improved the correlation to field yield for RH (increase from  $R^2 = 0.385$  to  $R^2 = 0.506$ ) and RO (increase from  $R^2 = 0.413$  to  $R^2 = 0.445$ ), but not for RE ( $R^2 = 0.413$  vs.  $R^2 = 0.389$ ). For HN, on the other hand, no prediction improvement was observed at any location by using the main raceme data (RH:  $R^2 = 0.166$ ; RE:  $R^2 = 0.249$ ; RO:  $R^2 = 0.185$ , not shown).



**Figure 27:** Correlation of seed yield determined in container to field grown plants. Plot diagrams depicting correlations of seed yields for 29 winter oilseed rape accessions from (A,C,E) whole containers, and (B,D,F) main racemes of the middle plants in the containers, to seed yields under low nitrogen fertilisation (LN) from three independent field trials in (A,B) Rauischholzhausen (RH), (C,D), Reinshof (RE) and (E,F) Rotenkirchen (RO). Accession Olimpiade excluded.

### **3.3.3. Assessment of nitrogen losses with aborted leaves**

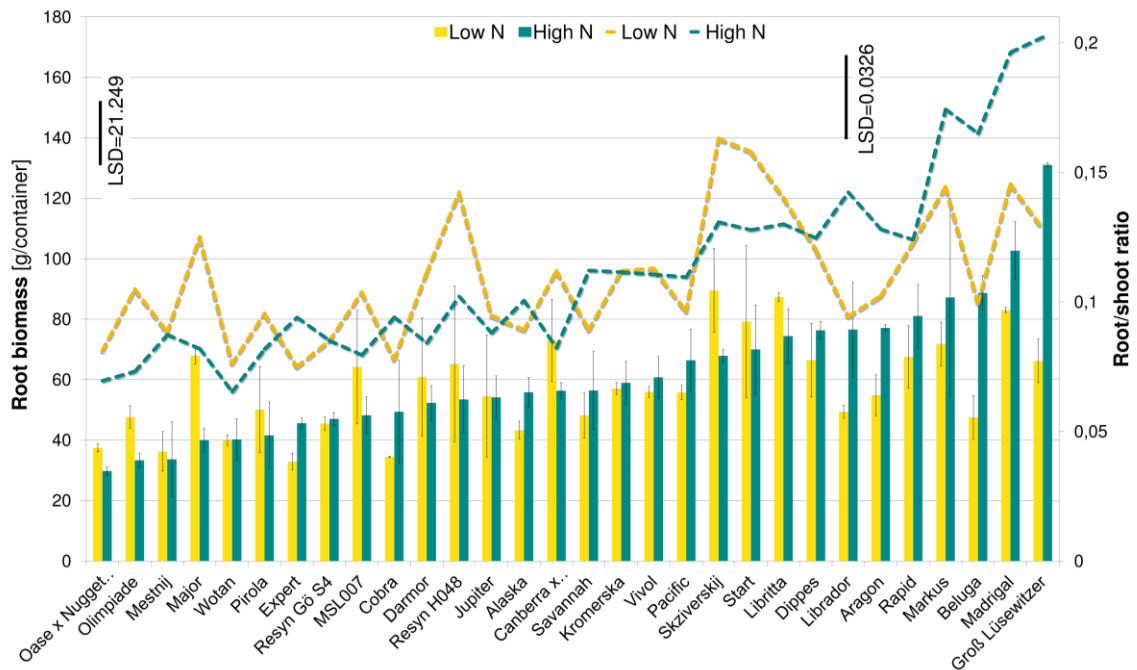
Since yields of the container experiment are in good correlation to yield data from field conditions, the container platform was determined to be suitable for obtaining meaningful data from deeper phenotyping procedures. One highly relevant trait able to be assessed much more exactly in containers than under field conditions is the nitrogen loss via aborted leaves. N loss monitoring was conducted in three extreme genotypes in the low NFL container variant. Depending on the genotype, winter oilseed rape can show different characteristic N loss profiles between flowering and seed harvest. As Figure 28 (right ordinate) indicates, cultivar Dippes had a relatively constant N concentration over all time points, while N concentrations in aborted leaves of cultivars Beluga and Cobra were more than one percent higher in the first period until June 14, but declined continuously over the following weeks. On the other hand, the N concentration in aborted leaves of Beluga was always higher than in those from Cobra and did not decline as fast as Cobra in late June. By multiplication of N concentration with the DW of aborted leaves, N losses were calculated (Figure 28, left ordinate). Although cultivar Dippes had the lowest N concentration due to a higher DW (Appendix 5) it had the highest N losses within the first observation period. However, during all further monitoring periods Dippes showed no further losses and, thus, can be characterized by an early and fast senescence behaviour. By far the lowest N loss until June 14 was seen in cultivar Beluga. In contrast, in the following period (except between June 18 and July 10) Beluga showed the highest N losses. Since it still had noteworthy amounts of leaf N attached to the stem at the day of seed harvest, Beluga can be regarded as a 'stay-green' genotype. By comparing Figure 28 with the SY data depicted in Figure 23, it is obvious that neither a 'stay-green' nor an early 'dry-down' behaviour is advantageous for high overall NUE (measured as SY per unit nitrogen supplied).



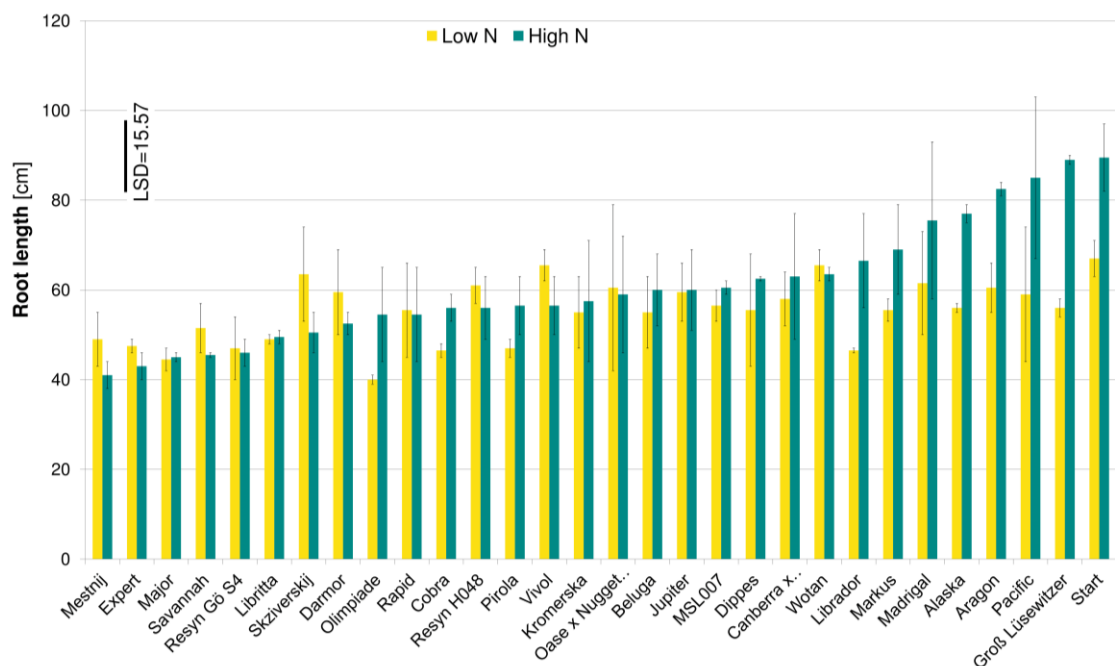
**Figure 28:** Nitrogen loss (left ordinate) and nitrogen concentration (right ordinate) of aborted leaves of three selected genotypes. Dates labelled on abscise represent the last day of the respective collected bulk of aborted leaves. If present, error bars indicate standard deviation between two replicates.

### 3.3.4. Phenotyping of root traits in the container system

Phenotyping of the root system indicated a broad variation between the genotypes in regard to their root length, biomass and morphology. Significant differences in root biomass were observed between the genotypes, with a variation of 56.68 g for LN and 101.3 g for HN, respectively (Figure 29). Significant differences were also seen for the length of the longest root per container, which showed a range of 27.0 cm under LN and 48.5 cm under HN (Figure 30). Cultivar Gross Lüsewitzer exhibited the highest root biomass per container at HN (131.04 g container<sup>-1</sup>), while cultivar Skziverskij had the highest root biomass at LN (89.55 g container<sup>-1</sup>). At HN, Oase x Nugget DH5 had the lowest root biomass (29.79 g container<sup>-1</sup>), while Expert had the lowest biomass (32.85 g container<sup>-1</sup>) at LN. Over all investigated genotypes a rather low coefficient of determination of  $R^2=0.27$  was determined, suggesting that within the gene pool of *B. napus* there is a certain degree of accession by nitrogen interaction for the root system, even though two-factorial ANOVA showed only a low significant interaction ( $p<0.049$ ). The results of this experiment also indicated a significant effect of the accession on the root/shoot ratio at the day of seed harvest. Interestingly, no correlation could be found between the root traits determined *in vitro* and in this experiment.



**Figure 29:** Root biomass (columns, left ordinate) and root/shoot (lines, right ordinate) ratio at day of seed harvest. Data show the mean of two replicates and are scaled on the container level. Standard deviation is marked with error bars. Least significant difference (LSD) is calculated on the 5% significant value.



**Figure 30:** Root length at day of seed harvest. Data show the mean of two replicates and are scaled on the container level. Standard deviation is marked with error bars. Least significant difference (LSD) is calculated on the 5% significant value.

Although root biomass did not correlate to seed yield (LN:  $R^2=0.020$ ; HN:  $R^2=0.010$ ), a significant correlation was detected between root biomass and the aboveground biomass (sum of plant residues after seed harvest). In fact, a stronger correlation was detected at LN ( $R^2=0.558$ , p-value  $<0.001$ ) than at HN ( $R^2=0.245$ , p-value = 0.0054). In contrast to the relationship observed between leaf N concentration at flowering and seed quality traits when the diversity set was divided into older and modern varieties by erucic acid content, this division did not affect the correlation between root biomass and aboveground biomass.

Although LN was not limiting for seed yield, most genotypes differed in root development between the two N treatments (Figure 29), although the extent and direction of the reactions varied strongly. Cultivars Gross Lüsewitzer and Beluga, for example, showed significant 1.97-fold and 1.86-fold increases in root biomass between LN and HN, respectively. The opposite reaction was observed in cultivar Major, which increased its root biomass 1.7-fold under LN conditions.

Comparison of root biomass data gathered at the adult stage in the container system with root biomass weights measured from 28 DAS in the hydroponic system revealed that genotypes behave quite differently depending on the developmental stage and/or the cultivation system. Calculation of the coefficients of determination (LN:  $R^2=0.02$ ; HN  $R^2=0.03$ ) between root weight data from the two contrasting phenotyping systems suggest that data collection on very young plants, from artificial, hydroponic cultivations systems, are not suitable to estimate the performance of a cultivar under field-like growth conditions.

Moreover, images of partially washed roots from container-grown plants (Figure 31) indicate huge differences between genotypes for the penetration of the soil by root branching and fine rooting. For example, cultivars Mestnij, Wotan and Expert produced very low quantities of fine roots, whereas Dippes, Darmor and Gross Lüsewitzer were characterized by very dense soil penetration with fine roots.





**Figure 31:** Different soil penetration of roots in soil profile. Accessions Mestnij (A), Wotan (B), Expert (C), differ drastic from Dippes (D), Darmor (E), Gross Lüsewitzer (F) in their root morphology. Images were taken in the low nitrogen fertilisation treatment.

## **4. Discussion**

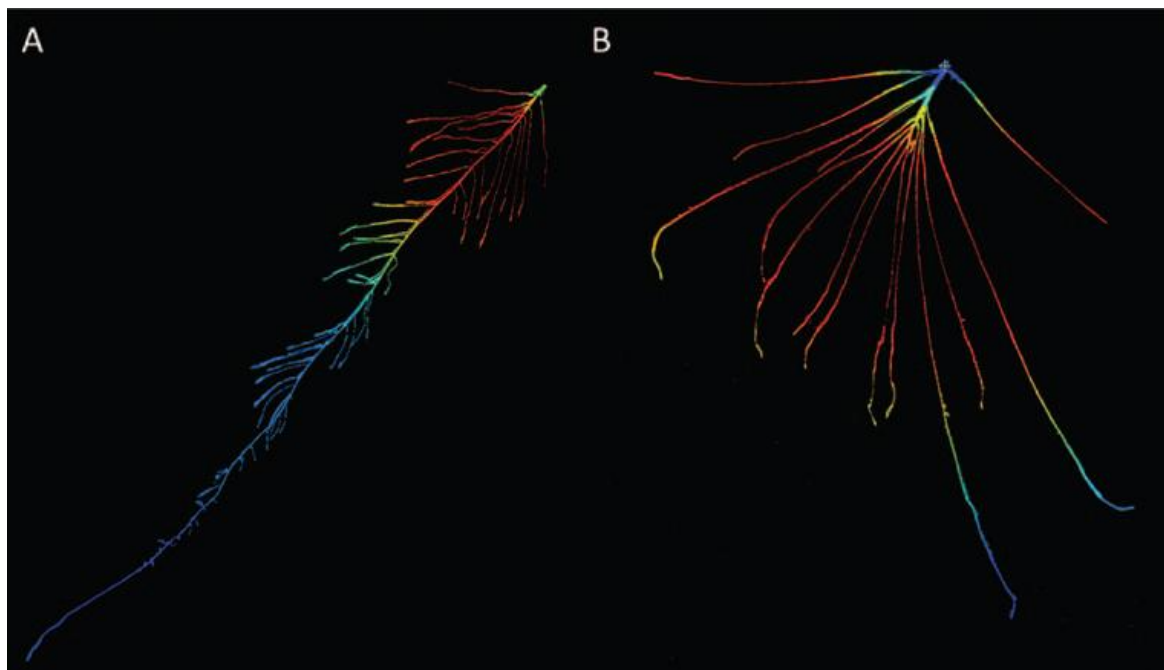
### **4.1. Genetic variation for nitrogen uptake efficiency**

In winter oilseed rape, NupE needs to be investigated primarily at the beginning of the crop cycle, since a quarter to one third of the total N uptake can be accumulated before winter and most of the root system is fully developed before flowering (Barraclough, 1989; Rahman and McClean, 2013; Le Deunff and Malagoli, 2014).

Although it is known that Brassica crops have a higher rate of nitrogen uptake than many cereal and legume crops (Laine et al., 1993; Everaarts, 1993), several studies have reported that NUE correlates stronger to NupE than to NutE under conditions of limiting N fertilisation (Berry et al., 2010; Schulte auf'm Erley et al., 2011; Nyikako et al., 2014). This suggests that future cultivation of oilseed rape with reduced N inputs will increase the relevance of an improved NupE. In other words, the size of the N pool acquired in the aerial biomass, which is later transferable to the seeds during the seed filling period, is in need of further enhancement. The following two sections discuss this issue based on investigations of root variation and in context of net N uptake until flowering, respectively.

#### **4.1.1. Methodological challenges to detect variation of the root system**

In this study the root system was assessed by two distinct approaches: On the one hand a hydroponic-based *in vitro* growth system was used to investigate seedling roots, while on the other large-scale, soil-filled containers were used to assess roots of plants grown through the entire life-cycle under field-like conditions until maturity. Measurements *in vitro*, at early developmental stages, allow detailed investigation of performance, root architecture and distribution without interaction of the root with the soil properties but with exact regulation of nutrient supply (Figure 32). On the other hand, conclusions from hydroponic systems are limited with regard to plant performance at later developmental stages.



**Figure 32:** Extreme phenotypic responses of winter oilseed rape roots to changing conditions (Hatzig et al., 2015)

Winter oilseed rape, as a dicotyledonous plant, develops a long taproot, therefore it is impossible in conventional pot experiments to grow plants to maturity without limitations of root growth. Most studies of oilseed rape root morphology are therefore limited to juvenile growth stages. Despite the importance of the roots for plant development and performance, particularly in regard to nutrient uptake efficiency, the spectrum of variation in adult plant root morphology and root growth responses to nutrient deficiency remains virtually invisible and cannot be effectively addressed by breeders. Field screening for variation in root phenotypic responses to different nitrogen fertilisation regimes is extremely impracticable. Furthermore, the realised root architecture of an adult plant is the complex result of physical, biological and chemical interactions of the plant with the soil. Therefore a standardisation of soil attributes is of high importance to extract genetic influence on root phenotypic responses from the diverse environmental influences.

Recent published methodologies which use indirect measurements to estimate root growth (reviewed in Judd et al., 2015), for example plant DNA quantities in soil (Huang et al., 2013), root electrical capacitance (Dietrich et al., 2013) or digital imaging (Clark et al., 2013) are relatively easy to conduct, but require a calibration of the method and represent only parts of the root system. Furthermore, those do not provide an overall picture of the exact root volume or architecture. More recently, X-ray CT technologies for 3D imaging have been suggested as a promising strategy to quantify the root mass and surface by non-destructive measurements (Metzner et al., 2015). However, those technologies are until now limited

either to earlier developmental stages, or complicated by insufficient contrast between fine roots and soil medium.

The container system described in the present study provides a homogeneous root environment that simulates field growth conditions, whilst standardising many of the variables which would be encountered in field phenotyping. Furthermore, it is considerably easier to extract and wash roots from containers than from field plots, enabling an unprecedented overall image of the root morphology, although the exact three-dimensional structure of the root architecture is disrupted during root washing. On the other hand, the container system is very time and labour intensive and thus has limitations when large numbers of genotypes need to be screened.

In line with earlier studies (Kamh et al., 2005; Schulte auf'm Erley et al., 2007), the diversity set investigated in this study was confirmed to carry considerable genetic variation for root traits. However, no correlation was found between root DW in the *in vitro* and container systems, indicating either a specific behaviour of the genotypes at different developmental stages, or that *in vitro* measurements from seedlings are not useful for predicting adult plant root variation. For example, cultivar Gross Lüsewitzer was among the genotypes with the smallest root system during *in vitro* phenotyping, but had the highest root biomass in the HN treatment in the container system. In contrast, Resyn H048 showed the highest root biomass in the *in vitro* phenotyping experiment but produced less than the average root biomass of all accessions, in both HN and LN, in the container system (Figure 8, Figure 29).

#### **4.1.2. Adaptation of the root system to contrasting nitrogen supply**

The finding that the root/shoot ratio in most genotypes increases with reduced N supply (Table 5 and Figure 10) indicates their ability to adapt to LN conditions. Obviously the plants shift their focus of growth towards tissues that increase the acquisition of nitrogen. The ten-fold reduction of N supply in the LN treatment of the hydroponic system represents a drastic N starvation, which even with stricter fertiliser legislation is not realistic under field conditions. Nevertheless, the results demonstrate that the root system might deserve more attention when N fertilisation is decreased. This finding is in line with earlier findings that demonstrate increased relevance of N uptake under limiting N fertilisation (Berry et al., 2010; Schulte auf'm Erley et al., 2011; Nyikako et al., 2014).

While genotypes can show noteworthy differences in their root/shoot ratio at earlier developmental stages, it was already observed in previous studies that the root/shoot ratio of vigorous genotypes does not always differ from that of non-vigorous genotypes at harvest (Liao et al., 2004; Liao et al., 2006). However, root DW determination at maturity in the container experiment led to an identification of genotypes with noteworthy positive (cultivars Beluga and Groß Lüsewitzer) or negative (cultivar Major) responses in terms of root biomass



to increased NFL, even when their aboveground biomass or yield showed no response to variable N levels. This observation of root biomass response, without a significant response in the remainder of the plant (Figures 23 and 29), reflects the role of N both as a nutrient and as a signalling molecule (Nacry et al., 2013). It is well known that nitrate transporters have dual role: they are not only responsible for nitrate uptake, but also act as signal sensors (Little et al., 2005; Remans et al., 2006a; Remans et al., 2006b). Zhang and Forde (1998; 2000) suggested that the N status of a plant has an effect on root architecture via hormonal regulation. It is apparent that soil N concentration triggers an N-signal cascade that lead to root morphological switches (Leblanc et al., 2013).

At the end of this study it remains unclear if a bigger root system is advantageous for NUE. As detailed above, the N uptake depends on morphological and enzymatic traits. N uptake in later developmental stages was not subject of this study, but might mask the effect of the root size on NupE. For this reason the net effect of a bigger root system cannot be separated from other effects, and requires further studies in which the activity of nitrate and ammonium transport processes are determined. In principle, a bigger root system is not necessarily indicative for a higher NupE. The increase in root/shoot ratio at reduced N supply found in the hydroponic experiment (Figure 10) is an indicator for a relative shift of metabolic activities towards the root system. As root growth and maintenance is costly in energy, the root architecture is a lever to optimize the balance between nitrogen absorption ability and metabolic costs (Lynch 2014). From this perspective, an increased root biomass can incur excess plant internal resources, creating a trade-off to NUE. From this point of view, increasing the root surface area by enhancing fine root density has been considered as one possible strategy in other crops (White et al., 2013) such as maize (Wiesler and Horst 1994), faba beans (Kage, 1997) or Kentucky Bluegrass (Sullivan et al., 2000). Washing the roots of plants grown to maturity in the container system enabled, for the first time, the discovery and identification of the huge variation in root architecture and fine roots that are present in genetically diverse winter type *B. napus* accessions (Figure 31).

#### **4.1.3. Developmental stage specific determination by destructive measurements**

In order to compare the ability of different genotypes to acquire N, by destructive N analysis in different plant segments, it is inevitably to decide upon a single developmental stage in order to keep a manageable number of pots. In this study, plant biomass harvest was conducted during flowering of the main raceme (BBCH67-69), since by far the most nitrogen is taken up prior to flowering (Malagoli and Le Deunff, 2014) and increasing amounts of N might be subsequently lost during leaf senescence. At this point, however, it should be mentioned that Wiesler et al. (2001) questioned the notion that N uptake after flowering is negligible in rapeseed, since they found seed yield to be correlated to N uptake during

reproductive growth. This was confirmed by other studies performed in the field which showed that post-flowering NupE had a significant effect on total NUE and was an important phase to discriminate varieties in terms of yield (Berry et al., 2010; Schulte auf'm Erley et al., 2011; Ulas et al., 2013). More precisely, Malagoli et al. (2005a) showed that 30% of the plant total N of a genotype grown in field conditions was absorbed during seed filling, even though this represented only 27% of the total seed N.

#### **4.1.4. Nitrogen uptake until flowering in light of breeding progress**

Several studies reported about genetic variation for NupE (Balint et al., 2008; Balint and Rengel, 2008; Kessel et al., 2012; Lee et al., 2015). In this study, detailed partitioning of tissues revealed that the genotype by NFL interaction was more strongly influenced by the relationship between leaf, stem and silique biomass than by the individual reactions of those tissues. Since fertiliser inputs rose in Europe until the 1980s and stayed constantly high for many further years (Lassaletta et al., 2014), one might expect that older varieties should be better adapted to low NFL than more recent varieties. In contrast to this assumption, however, the results of this study indicate that this does not necessarily hold true, with both older and modern varieties being identified among the varieties with most efficient N uptake. Indeed the best-performing variety by far in terms of NupE (Aragon) was released in 2004 and is thus among the very youngest commercial accessions in the diversity panel. Although simple, easily-measured selection parameters would be beneficial for breeding towards increased NUE, it must be considered that NUE is a multi-faceted trait complex that probably cannot be described with single parameters. The data in this study reveal that the majority of vegetative parameters are not at all associated to NUE traits or SY (Figure 16 and 17). It is therefore worth mentioning that leaf N concentration correlates more strongly to NUE, and to both seed and oil yield, than the total N in biomass at flowering. This is particularly the case under LN in modern varieties. The relationship of high leaf N content to NUE at low NFL ( $R^2=0.357$ ;  $p=0.0069$ ), putatively contributing to maintenance of photosynthesis, may therefore be an important selection criteria for breeders aiming to improve NUE.

### **4.2. Genetic variation for nitrogen utilisation efficiency**

#### **4.2.1. Post-anthesis source-sink relationship**

Flowering is the paroxysm of the change from vegetative to reproductive stage. This phase is characterised by a massive change in source-sink relationships. During flowering, the vegetative plant segments switch from sink to source organs, with associated modifications of N fluxes from older to younger tissues and reproductive organs (Le Deunff and Malagoli,

2014). Labelling studies in *Arabidopsis thaliana* (Taylor et al., 2010) and *B. napus* (Rossato et al., 2001; Malagoli et al., 2005a) showed that the N accumulated in the seeds at harvest originates mainly from the degradation of proteins in vegetative plant segments. In detailed studies with labelled nitrogen isotopes, Rossato et al. (2001) and Malagoli et al. (2005a; 2005b) demonstrated clearly that N remobilisation already begins before flowering, by sequential senescence of older leaves in nodes beneath the elongating stems and subsequently younger, upper leaf stages. During this developmental stage N uptake can still be dominant. After flowering, when plant tissues switch from vegetative to generative development, senescence leads to N remobilisation from leaves to stems, siliques and finally, in case of complete remobilisation, to seeds. Since oilseed rape aborts nearly all of its leaves prior to seed maturity, remobilisation ability is particularly crucial to NUE. Several studies reported that insufficient remobilisation is the bottleneck towards an increased NUE in oilseed rape compared to other crops (Malagoli et al., 2005b; Tilsner et al., 2005; Gombert et al., 2006).

#### **4.2.2. Nitrogen utilisation efficiency in the context of senescence**

Incomplete protein hydrolysis in the source organs can lead to noteworthy N losses through fallen leaves (Aufhammer et al., 1994; Diepenbrock 2000; Rossato et al., 2001; Malagoli et al., 2005a; Ulas et al., 2013; Avice and Etienne, 2014). Leaf senescence is therefore a critical stage at the crossroad between improvement of N uptake and N remobilisation in the reproductive organs (Schulte auf'm Erley et al., 2007). Avice and Etienne (2014) pointed out the difficulty to precisely determine the advancement of the senescence process. While direct measurement of leaf chlorophyll content is a commonly used method, several other biomarkers have been tested for evaluation of leaf senescence. For instance, Schulte auf'm Erley et al. (2007) showed a good correlation between SPAD (chlorophyll) values and photosynthetic rate. Gombert et al. (2006) developed a molecular method based on the kinetic expression of two genes that are up-regulated (*SENESCENCE ASSOCIATED GENE 12*; *SAG12*) or down-regulated (*CHLOROPHYLL a/b-BINDING PROTEIN*; *Cab*) during leaf senescence. The inversion of the relative expression level of these two genes defines the onset of leaf senescence. The higher NupE and lower NutE at HN than at LN provides evidence for noteworthy post-flowering N losses prior to maturity. The two possible explanations for this N loss are 1) non-remobilised N from aborted leaves, and 2) non-remobilised N in stems and siliques (i.e. harvest residues). Leaf N losses were not quantified in the Mitscherlich pot experiment. However, since the ratio of average N in the total plant at harvest to N in the total plant at flowering time is higher at LN (97%) than at HN (84%), but NHI was found to be similar in both NFL, it can be concluded that differences in

NutE efficiency between NFL are rather explained by differences in leaf N remobilisation than by insufficient remobilisation of stem and silique N. N analysis of nitrogen concentration on three accessions in the container system revealed higher N concentration of aborted leaves, especially in the first periods after flowering, than those found in stems and siliques after seed harvest in Mitscherlich pots. These findings are in line with previous studies. For example, Hocking et al. (1997) found higher N concentrations in aborted leaves than in stems at maturity and concluded that N remobilisation from leaves was incomplete. Additionally, the strong variations observed in the profile of N losses (exemplified by the strongly differentiating cultivars Cobra, Beluga and Dippes) demonstrate that winter oilseed rape can behave quite differently in terms of the start, degree and duration of senescence (Figure 28).

Grafting experiments revealed homeostasis of biologically active cytokinins as the predominant leaf-inherent aspect for genetic variation in N limitation-induced leaf senescence (Koeslin-Findeklee et al., 2015a). In particular, positive correlations were found between delayed leaf senescence and N efficiency Wiesler et al. (2001). In addition, the most N-efficient cultivar in that study showed better photosynthetic capacities at the end of flowering. However, one has to bear in mind that, in contrast to other crop species such as cereals, photosynthesis activity in the siliques increases strongly after flowering in rapeseed, making the relative contribution of leaf photosynthetic activity less important (Gammelvind et al., 1996). Although in the present study the cultivar Beluga, which showed stay-green attributes, was higher yielding than the early and fast-senescing cultivar Dippes, its performance nevertheless lay only around the middle of the investigated diversity set. From this perspective the advantage of the stay-green character in relation to SY and NUE has to be questioned. Besides, a late N remobilisation may lead to greater N losses through the combination of leaf loss and high N/C ratio in stems at harvest (Kaiser et al., 1998; Baggs et al., 2000). In another study, NUE superiority under N limitation was found to be mainly related to the NRE and not to delayed leaf senescence (Koeslin-Findeklee et al., 2014). Similarly, in other crops like rice and wheat, no significant or even negative correlations were observed between SY and stay-green behavior (see Gregerson et al., 2013 and references therein).

Further investigations are inevitably needed to fully evaluate the role of senescence behaviour and NutE. Since collection of aborted leaves is definitely not feasible in large scale experiments, especially not for breeding programs, senescence markers that can be assessed via non-destructive methods are essential. Recently, it was demonstrated that cellular structural changes associated with leaf senescence can be monitored by non-invasive <sup>1</sup>H-NMR relaxometry (Musse et al., 2013; Sorin et al., 2015). Capture of NMR signals associated with chloroplast dismantling represents a potentially very precise method



to measure leaf senescence (Sorin et al., 2015). These techniques might be promising methods to gather more data on the relationship of senescence and NUE in different environments.

#### **4.2.3. Nitrogen remobilisation from stems and siliques**

According to Malagoli et al. (2005b), optimizing the NRE from vegetative to reproductive tissue could improve seed yield by 15%. In this case, the NRE could be enhanced by improvement of synchrony between the N source availability and the N demand in the seeds. In rapeseed, the stems have been described as N-storage buffer organs that could compensate for this desynchrony (Hocking et al., 1997; Rossato et al., 2001; Malagoli et al., 2005a). According to Girondé et al. (2015), a high amount of N in the stems at the beginning of the reproductive stage is characteristic for high NRE, as is a better N remobilization from the stems to the seeds. In the present study, a certain variation for N concentration in plant residues was observed at both NFL. Although, stems N concentrations at flowering were not significantly correlated to NutE, they tended to have a positive relationship. In contrast, a negative relationship between stem N concentration and NutE at maturity was confirmed. The disparity between genotypes with regard to their NutE and the ratio of supplied to harvested N is partly explainable by low N mass in plant residues (stems and siliques). These findings are supported by the observed genetic variation in N stem remobilisation in rapeseed. Nevertheless, even when N losses in plant residues do not correlate to NutE or NUE (Berry et al., 2010; Girondé et al., 2015), a low N/C ratio in stems and empty siliques of mature plants is beneficial for the environmental balance of oilseed rape production, since a high N/C ratio is suggested to be a major reason for postharvest N<sub>2</sub>O emissions. At this stage these emissions depend more on the final N/C ratio of plant residues than on N fertiliser inputs (Kaiser et al., 1998; Walter et al., 2014).

#### **4.2.4. Nitrogen utilisation efficiency in the context of flowering time**

Considering that the different parts of the plant (main raceme vs. side branches) enter the generative phase sequentially, multiple overlapping source-sink situations are possible in rapeseed. This desynchronisation between N source availability of old leaves and N requirements on developing siliques make the flowering and early post-flowering period a critical stage. As already stressed by Jung and Müller (2009) and Cockram et al. (2007), flowering time is an important aspect to consider in terms of SY. One might argue that an earlier switch from the vegetative to generative phase, and therewith an earlier begin of senescence processes, can stretch the remobilisation phase, mobilise more nutrients from

source to sink organs and thus improve the NutE. Indeed, especially under LN among the group of modern varieties, it was observed in this study that early flowering was associated with higher NutE and low N residues in stems after harvest (Figure 16). Since these relationships could not be observed under high N, this observation suggests a potential to increase NUE in low input environments by modifying flowering time, thus negating the desynchronisation of nitrogen remobilisation in oilseed rape (Malagoli et al., 2005a; Malagoli et al., 2005b). However, breeding for early flowering is limited in winter oilseed rape by negative effects on pollination through cold night temperatures, hence, a very precise fine-tuning of flowering time is necessary. This is particularly relevant in light of the significant accession by N interaction observed for flowering time, meaning that the ranking of genotypes can change under different NFL. Recent publications (Schiessl et al., 2014; Jung and Müller, 2009) described considerable genetic variation for fine manipulation of flowering-time regulation. Targeted breeding through gene-based selection might help breeders to modify flowering time to more exactly fit N remobilisation patterns.

#### **4.2.5. Seed yield: A good indicator for nitrogen use efficiency in rapeseed?**

Improving seed yield under low N nutrition level is a key step towards improvement of NUE (Good et al., 2004), so that yield estimation under different N regimes can be used as an indicator of the global NUE. In general, yield saturation is expected when fertilisation is increased to an optimal level. In accordance with this well-known phenomenon, an average decrease in NUE of between 26.47 and 18.12 g SY per gram N supplied was observed, while NutE decreased from 31.0% to 22.75%. Especially at limited NFL, the most modern accessions (except for very early flowering cv. Olimpiade) seemed to take up and utilize N much more efficiently than the majority of the older accessions.

Furthermore, the data indicate an increased relevance of both NupE and NutE for total NUE with increased NFL. The NutE correlates more strongly to total NUE (LN:  $R^2=0.59$ ; HN:  $R^2=0.73$ ) than NupE ( $R^2=0.13$  for LN and  $R^2=0.24$  at HN) does. These results are in line with previous findings from Svecnjak and Rengel (2006), who also conducted a pot experiment with four spring-type oilseed rape accessions and demonstrated that genetic differences in NUE were not explained by differences in NupE. Given that root traits are an important aspect to consider for N acquisition, that the roots can be strongly influenced by the reduced soil volumes and higher soil temperatures of pot experiments, and because the leaching N was captured and returned back to the Mitscherlich pot, the contribution of NupE and NutE to overall NUE must be assessed with particular care and need to be evaluated in larger containers.

As discussed before, remobilised N is the predominant source of seed N content (Gombert et al., 2006; Malagoli et al., 2005a; Rossato et al., 2001; Hocking et al., 1997). Efficient remobilization requires both a source (i.e. leaves, stems and siliques) that provides enough N and a sink (seeds) ready to receive the mobilized N. Investigation of phloem sap indicated that amino acids, as the primary N transport form, are high in oilseed rape, and certainly not below the levels in other crops. This suggests that source organs are able to degrade proteins efficiently and make N available. Hence oilseed rape appears to be rather sink-limited (Tilsner et al., 2005). The capacity for enhancement of NUE by two main meta-factors, namely SY and seed protein concentration, are discussed in the following sections.

#### **4.2.5.1. NHI as an indicator for NutE?**

To achieve a low N-balance surplus, the harvest index (HI; defined as the ratio of seed weight to total aboveground plant dry matter) and the nitrogen harvest index (NHI; defined as the ratio of seed N yield to total plant N amount at harvest), provide two estimators of the capacity to mobilise the N and C assimilates to produce seeds. Indeed, several studies showed significant correlations between the HI and NutE (Berry et al., 2010; Schulte auf'm Erley et al., 2011; Nyikako et al., 2014), demonstrating that increasing the HI would increase the sink capacity for N and C.

In the present Mitscherlich pot experiment, besides the parameter NutE (Equation 3, which describes the ability of the plant to generate seeds with the N that is taken up until flowering, the parameter NHI (Equation 4) characterises the ability to mobilise N from stems and silique walls into seed protein prior to maturity and harvest. The observed respective average NHI of 0.80 (LN) and 0.79 (HN) correspond to NHI of 0.75 reported by Svecnjak and Rengel (2006), but exceed values reported by Malagoli et al. (2005a) and the 0.57-0.67 by Chamorro et al. (2002). However, HI and NHI were the only traits that were not significantly affected by the factors accession and NFL, nor their interaction, in the Mitscherlich pot experiment. This means that whatever the seed yield of an accession, the plant residues always showed a similar relationship. Although HI or NHI were found to be not associated to NUE in this study, results from Miersch (2015) suggest that adoption of hybrids with a semi-dwarf growth habit might be a potential strategy to increase NUE in oilseed rape. That study compared 75 semi-dwarf hybrids and 75 normal hybrids, all derived from sister double-haploid (DH) lines crossed with the same maternal tester, at two locations and two nitrogen fertilisation levels. The results revealed higher seed yields of the semi-dwarf hybrids under low N and similar seed yields at high N supply. In fact, the lower straw yield of the semi-dwarf hybrids led to an improved harvest index at both N levels. However, these indicators are complex variables acquired at the end of the crop cycle, meaning that additional processes linked to global life-

cycle traits (e.g. plant growth or stress resistance) will impact their implementation for estimation of the global NUE. Further research approaches should address whether plant biomass can be reduced during the vegetative developmental stages without other negative impacts on growth.

#### **4.2.5.2. Seed quality traits in the context of a high NutE**

Numerous authors have found that metabolic competition for carbohydrates leads to a negative correlation of seed oil and protein content in seeds of oilseed rape (Grami and LaCroix, 1977; Grami and Stefansson, 1977; Gül, 2003; Zhao et al., 2006). Successful breeding for enhanced oil yield during the last few decades can probably explain why enhanced seed yield *per se* appears to be related to higher oil concentration, whereas both are negatively correlated to seed N concentration. However, this relationship is unlikely to represent a penalty for a reduced N balance surplus. The strong and significant correlation of SY and oil content with N yield, and the contrasting strong negative relationship between seed N concentration and N yield, underlines the assumption that yield *per se* is the much stronger determinant for N yield than seed N concentration (Figure 16 and 17). This observation was found to be even more pronounced when only modern varieties were considered. Since older and less adapted accessions have not generally been subjected to strong selection towards increased oil yield, they tend to contain higher seed N concentrations. On the other hand, since those accessions are not as high yielding as modern varieties, the total N yield (the parameter of most important to reduce the N balance surplus) is mainly inferior to that of modern varieties. In other words, in agreement with Schulte auf'm Erley et al. (2011) and Koeslin-Findeklee et al. (2014), the data from a diversity panel suggest that high seed protein content is not advantageous for simultaneously achieving a high NUE and a reduced N balance surplus in oilseed rape. On the other hand, by selecting accessions that exhibit a higher protein concentration without affecting oil yield (Wittkop et al., 2009) it should also be possible to breed varieties with a more desirable combination of oil yield and N concentration.

## **5. Conclusions for further breeding and prebreeding programs**

For improvement of NUE, plant breeders will be forced to select for more efficient varieties in both the short and long term. Breeding for N-efficient rapeseed varieties is a key target to improve the economic and environmental competitiveness of the crop. Based on the results of this study several conclusions can be drawn for implementation of genetic diversity during the breeding process..

### **5.1. Implementation of genetic variation**

To improve NUE by breeding, genetic variation for appropriate target traits is essential. Narrow elite gene pools of modern crops generally exhibit reduced genetic variation due to extensive selection for adaptation, quality and resistance traits (Dawson et al., 2008; Qian et al., 2014). In recent decades, since the onset of the so-called “green revolution”, crop breeders have generally focused on selection of varieties that generate high yields under high nutrient input. Arguably, this could be expected to reduce diversity for nutrient use efficiency, because efficient varieties have no obvious selective advantage under optimal nutrient supply. Conversely, older varieties that were not subjected to strong selection progress through high-N environments might conceivably be more adapted to LN environments (Brancourt-Hulmel et al., 2005). However, the negative correlation of erucic acid and glucosinolates (Figure 20 and 21) to SY and NUE observed in this study contradicts the notion of indirect selection against high NUE in winter oilseed rape. On the contrary: the group of modern varieties appears to outperform older cultivars for important NUE traits at both NFL. Up to this point it can be concluded that no useful variation to increase NUE exists among the older accessions investigated in this study. However, this ignores the fact that this is an endpoint analysis of NupE and NutE. Considering that N uptake and utilisation are both large trait complexes with dozen of sub-traits, one can have reasonable confidence that single positive effects of one accession on NupE (for example root morphology) have been negated or even suppressed by negative impacts of other traits (for example enzyme activity) and, thus were not reflected in a positive net effect. Indeed, this study presented for the first time phenotypic characteristics of winter oilseed rape accessions that might be worth reintroducing into elite gene pools of modern breeding programs. Prominent examples include the extraordinary root morphology of cultivar Gross Lüsewitzer, the inverse root growth response to increasing NFL of cultivar Major, and the strong NupE prior to flowering in cultivar Aragon. These specific trait donors, and other accessions with superior NUE, could be used to systematically reintroduce genetic diversity into elite material, providing promise to enhance the NUE performance of future varieties (Snowdon et al., 2015).

However, in doing so important questions arise with regard to how an effective selection strategy can be designed.

## 5.2. Selection for NUE traits

The breeding strategy depends on the stress level of the target environment and the loss of yield between the selection and the target environments. Breeders attempt to manage their selection environments to simulate common agricultural practice as closely as possible. In rapeseed, studies reporting interactions between genotypes and the N regime remain scarce (Gül 2003; Miro 2010; Bouchet et al., 2014). Accession by nitrogen interaction was also not significant for seed yield and quality-related traits in the present study, and superior accessions under HN were in most cases also the most efficient under LN. From this point of view, indirect selection in a HN environment is possible (Cormier et al., 2013) and has apparently occurred over the last decades. However, since other studies found significant quantitative trait locus (QTL) by N interactions for most yield and NUE traits (Miro, 2010) and breeding progress might further increase by direct selection in LN target environments (as demonstrated for wheat by Brancourt-Hulmel et al., 2005), it will be advisable in future to fertilise breeding nurseries with reduced NFL.

Destructive phenotyping at different developmental stages, as performed in this study, can help to identify potential genotypes and help to combine these desirable factors in elite varieties. This approach is extremely time-consuming and cost intensive, however, and hence not feasible for analysing thousands of breeding lines in a commercial breeding programme. Root phenotyping is similarly time-consuming, and even more difficult to develop (Postma et al., 2014). Rhizotrons with cameras, cultivation on transparent matrices, X-ray or MRI methods are emerging possibilities to explore roots traits in 2D or 3D, but such techniques remain costly and are complicated to calibrate and are a long way from the high-throughput scale which is required for screening of breeding populations (Metzner et al., 2015). Even more importantly, since winter oilseed rape requires a vernalisation period and has a long life cycle, most of the phenotyping technologies are not able to be carried out at the adult stage and/or are not economically viable.

Efficient methods and platforms to screen large breeding populations for meaningful variation at early stages of development would be immensely valuable to accelerate breeding programs, not only needed for root traits but also for aboveground plant behaviour. However, data which are collected at the early developmental stages can be insufficient and misleading. This study demonstrated that there is no consistency between phenotypic data of complex traits measured in the early developmental stage (*in vitro*) and the adult stage. This finding is in line with the study of Balint and Rengel (2008), who also found no correlations

between the N-efficiency rankings of 12 rapeseed varieties between the vegetative and the reproductive stages. Moreover it was observed that Madrigal was superior for shoot N content 28 DAS in the *in vitro* experiment (Figure 7) but finally was among the accessions with the lowest NupE until flowering (Figure 22), indicating the developmental and/or environmental specific behaviour of the accessions. Nevertheless, the complete genetic potential cannot be determined by measurement of seed yield alone; for example this would have overlooked the specific N-uptake superiority of cultivar Aragon or several other candidates identified in the present study. This strategy – to decipher complex traits into simpler variables that can be assessed throughout the crop cycle – was already proposed by Thurling (1991). Hence, the challenge is to gather as much information about genotype behaviour throughout the vegetation period, until maturity, in multi-environment field trials, but without the necessity to phenotype very large populations. This underscores the potential value of use of genome-based performance prediction (Snowdon et al., 2015) coupled to high throughput field phenotyping to pre-screen for potential genome-wide variation associated to NupE, NutE or root traits (Cormier et al., 2013; Würschum et al., 2014). By estimation of the collective effect of thousand of sequence variants on complex phenotypes, this strategy potentially enables *in silico* pre-selection of candidates with high predicted NUE. Consequently, the numbers of breeding materials that need to be phenotyped in complex assays or field trials can be drastically reduced, improving prospects for accurate selection at the top level of available NUE variation (Heslot et al., 2012; Technow et al., 2015). Incorporation of crop growth models into genomic prediction models, based on data from physiological traits measured in different target environments (Hammer et al., 2006; Schulz-Streeck et al., 2013; Technow et al., 2015), can potentially even further improve their predictive ability. As nutrient use efficiency and other major complex traits gain in importance, genomics-assisted breeding strategies are predestined to play a major role in future breeding success.

## 6. Summary

Rapeseed, (*Brassica napus* L.) is a crop of major economic importance, mainly grown for its oil-rich seeds. These are used for human consumption as well as for industrial purposes, and the seedcake serves as an important protein source in livestock feeding. It is the third most important oil crop in the world, behind soybean and palm oil. However, rapeseed production requires a relative high mineral nitrogen (N) inputs. For this reason an enhanced nitrogen use efficiency (NUE) has become a major aim in rapeseed production in order to ensure a sustainable agricultural production, particularly in association with the reduced release of nitrogen-derived greenhouse gases from soils and nitrate contamination of waterways. The use of genetic variation to breed more efficient varieties is seen a promising option to improve agricultural sustainability, but first requires detection of suitable variants by appropriate phenotyping procedures. To address this issue, this study assessed 30 diverse winter oilseed rape accessions under contrasting nitrogen fertilisation levels for physiological traits at several developmental stages, covering the entire vegetation period from 28 days after sowing until seed harvest and comparing detailed results from multiple experiments.

An *in vitro* hydroponic growth system was used to screen the material at a very early stage with regard to nitrogen acquisition. Besides a variation in shoot N content of more than 60%, and root N content of more than 70%, a higher root-shoot ratio under limiting N was determined. Moreover, cross-correlations of 33 physiological traits associated with N uptake or utilisation efficiency revealed considerable variation for NUE parameters, including positive effects of early flowering and high leaf N concentration on enhanced N utilisation under low N input. Furthermore, seed yield *per se* was found to be more important than the seed N concentration for simultaneously achieving both high N utilisation and reduced N balance surplus.

Additionally, a plant growth system comprising 120 large, 90 cm deep containers was established to allow phenotyping of the root system at an adult developmental stage. Comparison of seed yield from container grown plants to three field locations revealed a good transferability of phenotypic data from the semi-controlled environment to field conditions, encouraging use of this system for further physiological studies of N-mediated genotype responses.

Finally, phenotyping of the root system provided new insights into genetic variation for root biomass and degree of fine rooting, as well as the response to divergent nitrogen fertilisation levels. Furthermore, huge variation was seen for nitrogen losses with aborted leaves. Collectively, the results of this study suggest a considerable scope for further NUE improvement in oilseed rape by targeted combination of contributing factors in new, high-yielding varieties.



## 7. Zusammenfassung

Raps (*Brassica napus* L.) ist eine ökonomisch bedeutende Nutzpflanze, die vorwiegend wegen ihres hohen Samenölgehaltes angebaut wird. Dieses wird für die Humanernährung aber auch für industrielle Verwendungen genutzt. Gleichzeitig stellt der Rapskuchen eine hochwertige Proteinquelle in der Tierernährung da. Neben der Sojabohne und der Ölpalme ist Raps weltweit die dritt wichtigste Ölpflanze. Allerdings benötigt Raps eine relative hohe mineralische Stickstoffdüngung, weshalb eine gesteigerte Stickstoffnutzungseffizienz, besonders vor dem Hintergrund Stickstoff assoziierter Treibhausgasemissionen aus dem Boden und Nitrat-Verunreinigungen von Gewässern, eine zunehmend wichtigere Ziel in der Rapsproduktion darstellt. Hierzu wird die Nutzung genetischer Variation im Rahmen der Züchtung noch effizienterer Sorten als ein aussichtsreicher Weg zur Steigerung der landwirtschaftlichen Nachhaltigkeit gesehen, erfordert aber zunächst die Detektion der genetischen Variation durch geeignete Phänotypisierungsverfahren. Deshalb wurden in dieser Studie 30 diverse Winterraps Akzessionen unter kontrastierender Stickstoffdüngung zu unterschiedlichen Entwicklungsstadien über die gesamte Vegetation von 28 Tage nach der Aussaat bis zur Kornreife hinsichtlich physiologischer Merkmale untersucht und die Ergebnisse der verschiedenen Experimente verglichen.

Ein *in vitro* Wasserkultursystem diente dem Screening des Materials zu einem sehr frühen Entwicklungsstadium hinsichtlich der Stickstoffaneignung. Neben einer Variation für die N-Gehalte von mehr als 60% im Spross und mehr als 70% in den Wurzeln, wurde ein höheres Wurzel-Spross-Verhältnis unter limitierender N-Versorgung festgestellt.

Zusätzlich zeigten die Kreuzkorrelationen von 33 physiologischen Merkmalen, die mit N-Aufnahme- oder Verwertungseffizienz assoziiert sind, eine beachtliche Variation für NUE-Parameter; einschließlich positiver Effekte einer frühen Blüte und einer hohen Blatt-N-Konzentration auf eine erhöhte NUE bei geringer N-Düngung. Darüber hinaus wurde ermittelt, dass der Kornertrag *per se* wichtiger war als die N-Konzentration im Korn, um eine hohe N-Verwertung und auch einen reduzierten N-Bilanzüberschuss gleichzeitig zu erreichen.

Zusätzlich wurde eine Kultivierungsplattform mit 120 großen, 90cm tiefen Container etabliert, die auch die Phänotypisierung des Wurzelsystems im erwachsenden Stadium ermöglicht. Ein Vergleich zwischen der Kornerträge des Containersystems mit Feldversuchen von drei Orten zeigte eine gute Übertragbarkeit der phänotypischen Daten von der partiell kontrollierten Umwelt auf Feldbedingungen, was eine Nutzung des Systems für weitere physiologische Studien bezüglich N-vermittelter Reaktionen von Genotypen ermöglicht.

Schließlich erbrachte die Phänotypisierung des Wurzelsystems neue Einblicke in die genetische Variation der Wurzelbiomasse, den Grad der Feinbewurzelung als auch der

Reaktion auf eine unterschiedliche N-Düngung. Außerdem wurde eine große Variation für N-Verluste mit abgeworfenen Blättern beobachtet.

Zusammenbetrachtet legen die Ergebnisse nahe, dass es durch Kombination der beeinflussenden Faktoren in neue, hocheertragsreiche Sorten, einen beachtlichen Spielraum zur Verbesserung der NUE in Winterraps gibt.

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**Appendix 1:** Comparison of analysed leaf nutrients of *in vitro* grown plants with reference value according to Bergmann (1983).

	<i>In vitro</i> (DW)	Bergmann*	Units
Nitrogen	5.86	4-5	%
Calcium	2.79	1-2	%
Phosphorus	0.79	0.35-0.70	%
Magnesium	0.45	0.25-0.40	%
Sodium	0.01		%
Potassium	6.04	2.80-5.00	%
Sulfur	1.01		%
Boron	415	30-60	mg/kg
Copper	5.55	5-12	mg/kg
Manganese	63.2	30-150	mg/kg
Molybdenum	3.27	0.40-1.00	mg/kg
Zinc	38.9	25-70	mg/kg

\*values refer to oilseed rape plants with a height of 30-50 cm and fully developed leaves.

**Appendix 2a:** Means and standard deviation (SD) of phenotypic data collected during the *in vitro* growth experiment at low nitrogen supply.

Accession	NconcRoot		Root mass		NcontRoot		NconcShoot		Shoot mass		NcontShoot	
	[%]		[mg]		[mg]		[%]		[mg]		[mg]	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Alaska	4.285	0.077	43.460	5.795	1.848	0.304	4.840	0.393	156.050	40.151	7.491	0.878
Aragon	3.968	0.175	43.420	2.103	1.720	0.021	4.289	0.174	185.480	21.449	7.616	0.548
Beluga	3.644	0.223	45.020	9.518	1.512	0.570	4.809	0.536	165.360	23.757	8.071	1.840
Canberra X Courage DH	3.695	0.113	38.580	6.609	1.375	0.144	3.957	0.272	164.840	26.311	6.517	0.948
Cobra	3.925	0.174	37.380	7.832	1.471	0.269	4.589	0.200	125.840	32.633	5.072	1.999
Darmor	4.211	0.282	44.360	4.934	1.660	0.155	3.862	0.209	168.167	47.659	6.373	1.943
Dippes	3.922	0.164	34.300	3.120	1.223	0.187	4.118	0.109	128.480	26.546	5.415	1.009
Expert	3.954	0.148	49.240	6.960	1.962	0.189	4.359	0.152	157.120	13.768	6.899	0.636
Groß Lüsewitzer	4.282	0.206	27.520	8.648	1.172	0.436	3.879	0.109	177.360	45.601	6.833	1.612
Jupiter	4.132	0.235	41.500	9.290	1.703	0.344	4.094	0.454	144.440	22.607	5.537	0.698
Kromerska	4.322	0.279	47.060	6.160	1.988	0.303	4.170	0.256	204.400	23.639	8.797	1.363
Librador	3.544	0.343	23.720	7.223	0.720	0.272	4.486	0.301	97.720	14.099	4.527	0.576
Libritta	4.022	0.162	40.060	5.101	1.611	0.213	3.801	0.222	174.980	39.975	6.869	1.057
Madrigal	3.993	0.198	45.500	8.015	1.675	0.435	3.740	0.162	157.200	42.596	6.065	1.246
Major	3.806	0.305	39.740	9.296	1.475	0.277	4.153	0.309	139.220	34.097	5.881	1.770
Markus	4.060	0.050	55.833	8.712	1.338	0.980	4.332	0.158	171.860	32.523	8.442	2.241
Mestnij	3.875	0.609	26.920	1.686	0.977	0.145	4.046	0.335	98.580	22.164	4.804	0.740
MSL007	3.675	0.186	47.683	16.185	2.014	0.566	4.011	0.316	175.780	38.410	6.432	2.097
Oase x Nugget DH5	3.269	0.385	29.680	4.555	0.891	0.116	3.603	0.265	122.260	12.382	4.725	0.867
Olimpiade	3.935	0.236	41.050	3.310	1.635	0.109	4.219	0.185	167.340	18.181	6.835	0.764
Pacific	3.955	0.192	43.540	8.215	1.648	0.223	3.577	0.315	233.680	15.527	7.631	0.794
Pirola	4.563	0.179	56.150	11.764	2.312	0.403	4.289	0.239	229.680	26.257	9.674	0.994
Rapid	3.844	0.485	36.020	7.699	1.638	0.226	3.803	0.193	132.040	21.975	4.813	0.611
Resyn Gö S4	3.147	0.698	21.750	7.936	0.481	0.134	3.300	0.535	85.900	18.474	2.931	1.093
Resyn H048	4.193	0.216	47.650	3.517	2.127	0.293	4.241	0.550	159.100	16.887	6.301	1.160
Savannah	4.136	0.386	35.020	6.026	1.355	0.305	4.636	0.327	139.725	16.426	7.072	1.856
Skziverskij	4.348	0.121	34.517	6.957	1.464	0.316	4.683	0.475	114.920	20.036	4.906	1.037
Start	3.857	0.308	39.360	10.983	1.511	0.469	4.173	0.259	187.560	20.402	7.754	0.697
Vivol	3.739	0.262	42.000	1.826	1.438	0.250	3.762	0.350	184.400	26.880	6.697	1.452
Wotan	4.042	0.289	40.480	5.303	1.788	0.340	4.446	0.198	172.460	10.502	7.400	0.649

Data represent the mean values of six replicates after excluding outliers as described in material and methods. Nitrogen concentration of roots (NconcRoot), nitrogen concentration of shoots (NconcShoot), nitrogen content Roots (NcontRoot), nitrogen content shoot (NcontShoot).

**Appendix 2b:** Means and standard deviation (SD) of phenotypic data collected during the *in vitro* growth experiment at high nitrogen supply.

Accession	NconcRoot		RootMass		NcontRoot		NconcShoot		ShootMass		NcontShoot	
	[%]		[mg]		[mg]		[%]		[mg]		[mg]	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Alaska	5.213	0.146	66.000	10.668	3.439	0.567	6.696	0.436	312.380	53.020	19.402	2.868
Aragon	4.680	0.330	67.080	11.693	2.990	0.580	5.856	0.600	312.200	36.235	20.971	4.088
Beluga	4.862	0.307	34.720	8.008	1.675	0.476	6.674	0.109	150.260	38.926	10.273	2.711
Canberra X Courage DH	5.102	0.361	53.420	9.691	2.615	0.348	6.580	0.632	322.020	76.559	22.015	2.376
Cobra	5.792	0.425	61.480	10.076	3.554	0.639	7.019	0.218	272.120	53.221	17.243	2.191
Darmor	4.783	0.638	38.140	2.998	2.079	0.334	6.376	0.183	178.160	43.171	11.038	2.296
Dippes	5.280	0.558	53.575	4.508	2.418	0.618	6.324	0.516	268.440	46.945	14.735	1.820
Expert	5.047	0.249	69.767	11.960	3.622	0.781	6.177	0.492	273.933	64.459	15.439	2.750
Groß Lüsewitzer	5.475	0.373	51.440	7.081	2.820	0.450	6.057	0.330	273.620	50.937	18.378	1.285
Jupiter	4.835	0.493	48.920	10.844	2.426	0.631	6.710	0.291	193.000	47.022	13.003	3.449
Kromerska	4.958	0.162	46.800	19.720	2.310	0.892	5.939	0.338	250.067	105.187	15.007	5.382
Librador	5.407	0.272	57.533	23.240	2.682	1.051	6.953	0.557	266.567	101.794	20.359	5.902
Libritta	5.083	0.140	65.740	15.195	3.457	0.927	5.784	0.836	380.400	89.522	19.552	7.752
Madrigal	5.184	0.202	75.320	13.368	3.810	0.860	6.247	0.631	336.300	92.372	24.577	4.263
Major	5.633	0.330	61.420	22.986	4.473	1.354	6.549	0.355	270.200	118.218	19.514	8.030
Markus	5.053	0.408	63.600	21.850	2.955	0.949	6.414	0.484	382.040	75.805	23.174	3.681
Mestnij	5.037	0.239	40.540	14.506	1.867	1.008	5.647	0.638	175.480	75.170	11.849	4.861
MSL007	4.915	0.316	45.333	3.021	2.661	0.720	6.692	0.179	225.075	38.450	14.579	2.019
Oase x Nugget DH5	4.589	0.276	39.540	10.778	1.707	0.710	6.000	1.023	186.500	50.013	11.278	2.733
Olimpiade	5.165	0.370	51.240	12.051	3.146	0.691	5.821	0.494	261.900	43.724	17.445	0.723
Pacific	5.385	0.232	53.940	8.348	2.837	0.440	6.165	0.435	306.383	69.926	17.587	2.467
Pirola	5.160	0.250	59.033	13.201	3.123	0.758	6.088	0.280	288.350	93.821	17.838	5.228
Rapid	4.953	0.389	44.140	7.514	2.412	0.387	5.664	0.595	197.283	46.874	12.300	2.087
Resyn Gö S4	4.516	0.985	31.780	8.183	1.218	0.423	5.690	0.547	164.200	31.713	7.319	1.806
Resyn H048	5.595	0.352	84.720	16.294	4.533	0.971	5.716	0.577	346.100	63.875	20.891	5.504
Savannah	5.088	0.303	48.060	5.710	2.433	0.218	6.538	0.633	196.560	21.355	12.898	0.599
Skziverskij	5.209	0.802	54.120	7.814	2.690	0.877	6.608	0.365	262.200	79.988	16.558	4.227
Start	5.118	0.305	69.140	12.490	3.217	0.539	6.640	0.493	278.040	100.701	17.007	5.155
Vivol	5.229	0.343	64.140	3.695	2.988	0.272	6.260	0.964	331.700	36.456	18.060	1.521
Wotan	5.076	0.182	54.080	3.900	2.553	0.182	5.610	0.340	307.960	18.144	17.260	1.236

Data represent the mean values of six replicates after excluding outliers as described in material and methods. Nitrogen concentration of roots (NconcRoot), nitrogen concentration of shoots (NconcShoot), nitrogen content Roots (NcontRoot), nitrogen content shoot (NcontShoot).

**Appendix 3a:** Means of phenotypic data collected during the Mitscherlich pot experiment at low nitrogen supply.

Accession	NoLeaves	NoSB	DaysAfter01	Nconc LeavesF [%]	Leaves MassF [g]	Ncont LeavesF [g]	Nconc SiliquesF [%]	Siliques massF [g]	Ncont SiliquesF [g]	Nconc StemF [%]	Stem massF [g]	Ncont StemsF [g]	NcontBio massF [g]
Alaska	9.167	4.000	119.000	2.405	5.465	0.132	3.352	8.730	0.291	0.977	30.630	0.295	0.717
Aragon	11.333	3.833	117.000	2.098	9.420	0.196	3.243	7.380	0.239	0.893	34.570	0.308	0.742
Beluga	12.167	3.833	114.500	2.204	8.980	0.197	3.589	5.865	0.210	0.976	30.855	0.300	0.707
Cobra	14.667	5.167	116.500	1.884	7.085	0.133	4.099	5.570	0.228	0.884	24.730	0.219	0.581
CxCDH	12.500	4.833	111.000	2.034	7.760	0.158	3.411	8.390	0.280	0.858	23.455	0.201	0.639
Darmor	13.000	4.333	115.000	1.907	7.790	0.149	3.361	7.235	0.243	0.926	31.745	0.294	0.685
Dippes	13.667	6.000	115.000	1.878	7.745	0.147	3.557	6.290	0.224	0.766	29.565	0.226	0.597
Expert	9.833	4.000	111.000	2.181	6.980	0.152	3.537	7.815	0.276	0.867	23.975	0.208	0.636
GroßL	11.333	3.333	118.500	1.842	9.350	0.172	3.822	6.035	0.230	0.818	34.380	0.281	0.684
Jupiter	9.167	4.667	114.500	1.962	5.590	0.110	3.173	8.050	0.254	0.846	30.575	0.259	0.623
Kromerska	9.833	4.167	118.000	2.121	3.495	0.074	2.529	11.420	0.289	0.752	32.010	0.241	0.604
Librador	13.500	4.667	117.500	2.194	6.590	0.145	3.117	7.430	0.230	0.879	25.765	0.227	0.602
Libritta	13.167	4.833	120.000	2.176	7.345	0.160	3.060	9.145	0.279	0.739	32.760	0.241	0.680
Madrigal	12.333	5.167	113.500	2.334	4.810	0.112	3.033	7.955	0.241	0.887	23.655	0.209	0.562
Major	11.500	5.000	111.000	2.405	7.815	0.186	3.381	7.010	0.237	0.981	29.645	0.289	0.712
Markus	12.000	4.333	115.000	2.132	7.885	0.167	3.325	6.775	0.225	0.864	25.695	0.222	0.615
Mestnij	9.667	3.833	111.000	2.029	4.460	0.090	2.629	11.550	0.304	0.862	27.360	0.236	0.630
MSL007c	10.167	3.667	117.500	2.102	7.605	0.160	2.922	9.040	0.265	0.944	31.555	0.296	0.721
Olimpiade	9.000	4.167	108.000	2.209	6.350	0.140	3.030	7.450	0.226	1.120	26.405	0.314	0.680
ONDH5	11.333	5.333	114.500	2.219	5.775	0.128	2.871	8.985	0.258	0.900	25.705	0.231	0.617
Pacific	12.167	5.167	112.000	2.269	6.570	0.149	3.169	8.370	0.263	0.829	28.270	0.234	0.647
Pirola	13.000	4.500	114.500	2.133	8.940	0.189	3.066	9.695	0.299	0.771	32.575	0.252	0.740
Rapid	14.500	5.167	114.500	2.331	7.195	0.168	3.118	6.620	0.206	0.822	27.235	0.223	0.597
ResynGS4	11.833	5.167	118.500	2.242	5.220	0.117	3.329	7.555	0.252	0.978	25.095	0.247	0.616
ResynH048	12.667	5.667	118.500	2.457	4.840	0.118	3.064	8.005	0.245	0.913	28.270	0.255	0.618
Savannah	11.500	5.167	111.000	2.205	7.580	0.168	3.332	7.420	0.246	1.014	24.465	0.248	0.662
Skziverskij	11.500	5.000	115.000	2.045	3.475	0.071	2.542	11.105	0.294	0.845	29.930	0.252	0.615
Start	10.833	4.833	121.000	1.644	6.520	0.106	4.407	4.775	0.210	0.789	37.665	0.298	0.615
Vivol	11.000	4.167	111.000	2.236	8.120	0.181	3.531	6.590	0.232	0.911	29.525	0.268	0.681
Wotan	10.417	5.000	112.000	2.434	4.915	0.120	2.998	9.200	0.272	0.835	32.190	0.269	0.661

Data represent the mean values of two replicates.

**Appendix 3a (continued):** Means of phenotypic data collected during the Mitscherlich pot experiment at low nitrogen supply.

Accession	Seed Yield [g]	Seed Nconc [%]	Seed Nyield [g]	Oilconc [%]	OilYield [g]	GSL [μmol/g]	S [%]	C18:1 [%]	C18:3 [%]	C22:1 [%]
Alaska	22.851	2.380	0.544	47.725	10.905	16.65	0.20	64.48	8.98	0.00
Aragon	22.073	2.232	0.493	50.625	11.173	14.33	0.20	68.48	9.45	0.00
Beluga	22.662	2.392	0.541	50.800	11.528	11.38	0.18	66.15	9.83	0.00
Cobra	16.510	2.872	0.473	44.575	7.364	79.48	0.68	62.65	9.75	0.00
CxCDH	21.531	2.348	0.506	47.700	10.270	10.85	0.18	67.60	8.90	0.00
Darmor	20.359	2.392	0.487	52.775	10.734	31.10	0.30	69.43	9.75	9.15
Dippes	16.890	2.628	0.443	50.000	8.451	82.33	0.58	69.13	9.13	22.13
Expert	23.237	2.284	0.530	48.550	11.281	13.13	0.13	66.65	8.00	0.00
GroßL	18.905	2.744	0.519	48.775	9.219	76.90	0.55	68.15	9.10	18.08
Jupiter	17.004	2.704	0.460	47.500	8.079	76.80	0.60	68.05	9.05	0.00
Kromerska	18.635	2.672	0.498	48.350	9.005	48.48	0.40	67.40	9.43	2.38
Librador	18.359	2.628	0.482	49.300	9.051	13.70	0.23	66.35	9.83	0.00
Libritta	18.287	2.516	0.460	49.475	9.047	6.63	0.13	66.15	10.38	0.00
Madrigal	21.338	2.456	0.524	50.800	10.845	14.90	0.18	65.75	9.13	0.00
Major	20.260	2.400	0.486	53.950	10.936	70.95	0.50	70.95	9.98	20.13
Markus	18.038	2.724	0.491	52.775	9.522	74.80	0.50	72.35	9.53	21.45
Mestnij	14.407	3.028	0.435	49.200	7.103	58.03	0.40	69.30	10.55	20.58
MSL007c	21.601	2.412	0.521	49.875	10.774	11.55	0.10	67.00	9.80	0.00
Olimpiade	20.213	2.884	0.583	46.625	9.422	82.13	0.60	66.55	10.50	13.28
ONDH5	23.284	2.344	0.544	51.675	12.046	13.80	0.20	66.15	9.00	0.00
Pacific	20.959	2.280	0.477	51.900	10.899	11.83	0.13	64.55	9.43	0.00
Pirola	21.683	2.352	0.510	49.575	10.749	9.45	0.15	63.10	9.93	0.00
Rapid	22.555	2.356	0.531	48.500	10.936	8.05	0.18	68.53	9.55	0.00
ResynGS4	17.224	2.672	0.460	53.000	9.128	79.78	0.58	67.65	10.53	21.68
ResynH048	19.388	2.696	0.519	48.175	9.358	53.15	0.45	70.98	9.18	6.65
Savannah	23.977	2.180	0.523	50.850	12.192	7.33	0.15	65.25	9.50	0.00
Skziverskij	20.346	2.684	0.548	49.350	10.034	66.38	0.53	69.78	8.98	9.25
Start	10.716	3.300	0.354	40.175	4.305	40.95	0.40	64.13	8.45	0.00
Vivol	22.012	2.288	0.504	52.150	11.474	15.15	0.18	68.75	10.05	0.00
Wotan	22.881	2.304	0.526	49.100	11.250	13.65	0.18	68.43	9.33	0.00

Data represent the mean values of two replicates.

Seed yield (SY) is normalised to 100% DM

**Appendix 3a (continued):** Means of phenotypic data collected during the Mitscherlich pot experiment at low nitrogen supply.

<b>Accession</b>	<b>Stem MassM [g]</b>	<b>Nconc StemM [%]</b>	<b>Ncont StemM [g]</b>	<b>Siliques massM [g]</b>	<b>Nconc SiliquesM [%]</b>	<b>Ncont SiliquesM [g]</b>	<b>NupE [%]</b>	<b>NutE [g/g]</b>	<b>NUE [g/g]</b>	<b>NHI [g/g]</b>	<b>Nharv_ Nsupply</b>
Alaska	20.740	0.345	0.071	18.095	0.392	0.071	0.680	31.906	21.680	0.793	0.516
Aragon	22.120	0.374	0.083	20.090	0.370	0.074	0.704	30.195	20.942	0.758	0.468
Beluga	25.555	0.270	0.070	23.415	0.343	0.080	0.671	32.260	21.501	0.784	0.513
Cobra	19.930	0.345	0.069	17.810	0.330	0.059	0.551	28.419	15.664	0.788	0.449
CxCDH	18.885	0.291	0.055	19.280	0.402	0.078	0.607	33.689	20.427	0.845	0.480
Darmor	23.020	0.323	0.075	19.515	0.356	0.069	0.650	29.712	19.316	0.772	0.462
Dippes	26.790	0.276	0.074	22.740	0.275	0.062	0.566	28.647	16.024	0.807	0.420
Expert	20.000	0.305	0.061	22.715	0.460	0.104	0.604	36.615	22.047	0.762	0.503
GroßL	28.740	0.291	0.084	21.785	0.284	0.062	0.649	27.695	17.936	0.824	0.492
Jupiter	21.240	0.341	0.072	19.300	0.365	0.070	0.591	27.309	16.133	0.763	0.436
Kromerska	24.425	0.288	0.070	20.355	0.248	0.050	0.573	30.891	17.680	0.805	0.472
Librador	28.415	0.377	0.102	20.165	0.335	0.068	0.571	30.531	17.418	0.741	0.458
Libritta	22.145	0.315	0.070	20.105	0.327	0.066	0.645	26.999	17.351	0.773	0.436
Madrigal	19.005	0.290	0.056	20.810	0.390	0.081	0.534	37.927	20.245	0.792	0.497
Major	22.900	0.341	0.078	20.890	0.299	0.063	0.676	28.442	19.222	0.776	0.461
Markus	22.290	0.389	0.087	19.390	0.378	0.074	0.584	29.444	17.114	0.797	0.466
Mestnij	23.560	0.376	0.091	18.165	0.463	0.085	0.597	23.075	13.669	0.714	0.413
MSL007c	20.000	0.285	0.056	20.495	0.295	0.061	0.684	30.241	20.494	0.817	0.494
Olimpiade	17.650	0.287	0.050	20.270	0.289	0.059	0.487	37.502	19.177	0.843	0.553
ONDH5	24.315	0.343	0.082	20.160	0.356	0.072	0.585	37.675	22.091	0.779	0.516
Pacific	22.625	0.289	0.065	18.735	0.444	0.083	0.614	32.313	19.885	0.762	0.452
Pirola	19.865	0.240	0.047	21.175	0.351	0.075	0.702	29.804	20.572	0.807	0.484
Rapid	21.225	0.254	0.054	20.515	0.334	0.068	0.567	37.887	21.399	0.861	0.504
ResynGS4	18.820	0.399	0.075	16.535	0.332	0.055	0.585	28.179	16.341	0.874	0.437
ResynH048	23.680	0.253	0.060	20.140	0.347	0.069	0.587	31.280	18.395	0.800	0.493
Savannah	19.505	0.315	0.061	18.570	0.297	0.055	0.628	36.279	22.749	0.818	0.496
Skziverskij	23.975	0.348	0.084	21.810	0.314	0.069	0.583	30.510	19.304	0.783	0.520
Start	34.810	0.212	0.074	20.190	0.446	0.091	0.584	17.464	10.167	0.684	0.336
Vivol	22.905	0.231	0.054	18.360	0.295	0.054	0.647	32.397	20.884	0.824	0.478
Wotan	26.295	0.215	0.056	20.620	0.311	0.064	0.627	34.582	21.708	0.813	0.499

Data represent the mean values of two replicates.

**Appendix 3b:** Means of phenotypic data collected during the Mitscherlich pot experiment at high nitrogen supply.

Accession	NoLeaves	NoSB	DaysAfter01	Nconc LeavesF [%]	Leaves MassF [g]	Ncont LeavesF [g]	Nconc SiliquesF [%]	Siliques massF [g]	Ncont SiliquesF [g]	Nconc StemF [%]	Stem massF [g]	Ncont StemsF [g]	NcontBio massF [g]
Alaska	11.000	4.833	120.000	2.815	15.625	0.440	3.567	18.805	0.669	1.258	55.890	0.703	1.813
Aragon	13.833	5.667	119.000	3.249	22.585	0.737	4.074	14.655	0.597	1.423	49.730	0.707	2.041
Beluga	15.167	6.667	114.500	3.131	21.940	0.686	3.853	14.235	0.543	1.281	54.170	0.689	1.918
Cobra	16.000	6.167	119.000	2.952	20.395	0.602	4.801	10.305	0.493	1.283	43.295	0.556	1.651
CxCDH	14.500	7.167	111.000	3.153	19.985	0.630	3.514	19.285	0.674	1.203	42.735	0.513	1.817
Darmor	11.500	5.167	118.500	2.975	15.935	0.474	3.707	17.055	0.611	1.167	52.675	0.614	1.698
Dippes	16.333	8.500	118.000	2.721	20.540	0.559	4.123	14.330	0.590	1.133	55.030	0.622	1.771
Expert	13.667	5.667	111.000	2.733	21.905	0.598	3.951	17.770	0.703	1.304	43.740	0.567	1.868
GroßL	11.000	5.000	120.000	2.634	20.675	0.544	3.988	13.675	0.545	1.095	59.530	0.653	1.743
Jupiter	12.000	6.000	116.000	3.175	20.260	0.644	4.184	14.710	0.615	1.206	53.360	0.643	1.902
Kromerska	13.833	6.000	119.000	2.647	16.900	0.447	3.447	19.380	0.665	1.179	54.535	0.640	1.752
Librador	14.500	5.333	123.000	2.900	16.785	0.487	4.032	14.110	0.568	1.386	42.015	0.582	1.637
Libritta	12.333	5.833		2.696	14.245	0.383	3.514	19.300	0.676	1.059	52.650	0.557	1.617
Madrigal	14.000	6.167	114.500	2.676	18.265	0.487	3.665	15.955	0.583	1.248	48.605	0.606	1.677
Major	11.833	5.333	111.000	2.844	19.815	0.574	3.747	14.400	0.522	1.330	45.960	0.605	1.702
Markus	13.167	5.333	117.500	2.715	21.870	0.595	3.906	13.455	0.522	1.237	48.530	0.599	1.715
Mestnij	11.500	5.833	111.000	2.851	15.835	0.450	3.615	20.650	0.746	1.344	46.395	0.623	1.819
MSL007c	11.667	5.833	121.000	2.465	22.820	0.556	3.782	15.180	0.563	1.247	55.570	0.691	1.810
Olimpiade	11.667	6.500	108.000	3.553	18.410	0.654	4.250	12.110	0.520	2.091	30.470	0.633	1.841
ONDH5	12.833	6.333	116.000	3.142	17.465	0.548	3.823	17.695	0.671	1.382	45.980	0.636	1.855
Pacific	14.500	7.667	112.000	3.287	19.840	0.652	3.939	14.195	0.559	1.284	50.190	0.645	1.856
Pirola	14.167	6.667	114.500	3.100	18.185	0.556	3.573	19.615	0.699	1.209	49.465	0.596	1.851
Rapid	16.167	7.000	117.000	2.979	20.905	0.623	3.769	14.960	0.563	1.179	49.270	0.581	1.768
ResynGS4	11.167	6.333	121.500	3.079	12.035	0.370	4.033	16.230	0.652	1.443	48.140	0.695	1.717
ResynH048	13.667	7.000	115.000	3.376	15.545	0.526	4.018	15.480	0.619	1.605	45.415	0.726	1.871
Savannah	13.500	6.833	111.000	3.333	21.590	0.722	4.268	12.535	0.535	1.533	42.605	0.652	1.909
Skziverskij	12.333	5.500	117.000	2.684	14.400	0.386	3.703	17.180	0.634	1.337	48.270	0.646	1.666
Start	14.500	6.167		2.225	21.360	0.473	4.519	11.485	0.520	1.126	65.845	0.741	1.735
Vivol	13.333	6.167	111.000	3.028	20.710	0.626	3.827	13.905	0.531	1.358	55.020	0.745	1.902
Wotan	13.000	6.167	112.000	2.941	19.180	0.564	3.763	18.455	0.683	1.060	57.565	0.610	1.858

Data represent the mean values of two replicates.



**Appendix 3b (continued):** Means of phenotypic data collected during the Mitscherlich pot experiment at high nitrogen supply.

<b>Accession</b>	<b>SeedYield</b> [g]	<b>SeedNconc</b> [%]	<b>SeedNyield</b> [g]	<b>Oilconc</b> [%]	<b>OilYield</b> [g]	<b>GSL</b> [μmol/g]	<b>S</b> [%]	<b>C18:1</b> [%]	<b>C18:3</b> [%]	<b>C22:1</b> [%]
Alaska	48.060	2.736	1.314	44.500	21.389	17.70	0.25	62.10	9.53	0.00
Aragon	46.710	2.652	1.239	47.550	22.199	13.35	0.23	68.23	9.40	0.00
Beluga	45.373	2.948	1.336	45.725	20.779	16.08	0.28	62.95	9.70	0.00
Cobra	33.911	3.220	1.092	42.500	14.412	61.95	0.53	62.65	9.08	0.00
CxCDH	37.163	2.872	1.066	43.625	16.189	11.95	0.20	66.35	8.38	0.00
Darmor	41.679	2.904	1.210	46.175	19.267	24.03	0.28	65.35	8.88	2.15
Dippes	32.770	3.080	0.989	44.700	14.925	56.83	0.45	67.00	8.93	21.00
Expert	44.025	2.840	1.254	45.850	20.163	14.93	0.20	65.13	9.18	0.00
GroßL	37.398	3.000	1.121	46.725	17.493	59.95	0.38	69.13	9.48	22.48
Jupiter	36.348	3.088	1.122	43.550	15.828	54.40	0.45	64.95	9.63	0.00
Kromerska	37.137	3.220	1.184	43.850	16.386	43.33	0.38	67.03	9.48	1.50
Librador	43.164	3.308	1.432	44.225	19.055	21.45	0.30	64.70	10.33	0.00
Libritta	38.442	2.924	1.124	44.875	17.250	7.83	0.15	64.40	10.35	0.00
Madrigal	46.487	2.896	1.345	46.400	21.601	12.03	0.20	64.35	8.98	0.00
Major	38.716	2.916	1.129	49.025	18.980	46.85	0.35	70.08	9.88	18.18
Markus	38.057	3.104	1.180	50.050	19.052	52.23	0.40	70.40	9.00	22.90
Mestnij	36.761	3.332	1.221	47.950	17.649	49.75	0.38	69.95	10.30	23.53
MSL007c	45.093	2.872	1.290	46.650	21.072	14.18	0.20	64.58	10.23	0.00
Olimpiade	42.112	3.280	1.380	44.650	18.821	58.20	0.48	70.20	9.75	14.23
ONDH5	46.949	2.764	1.295	48.725	22.897	15.53	0.23	66.33	9.45	0.00
Pacific	44.329	2.820	1.250	46.925	20.801	9.95	0.15	61.00	9.78	0.00
Pirola	46.259	2.812	1.301	45.650	21.113	15.35	0.20	64.53	9.58	0.00
Rapid	42.386	2.852	1.208	43.900	18.615	9.78	0.15	64.05	9.23	0.00
ResynGS4	38.224	3.096	1.184	49.500	18.917	51.23	0.40	67.58	10.53	26.88
ResynH048	42.414	3.044	1.291	47.275	20.044	43.93	0.38	68.98	9.65	12.95
Savannah	44.417	2.868	1.274	45.550	20.235	9.73	0.20	63.95	9.20	0.00
Skziverskij	31.181	3.360	1.050	44.900	13.995	67.23	0.55	70.10	8.83	16.20
Start	25.461	3.628	0.924	38.800	9.876	42.20	0.40	63.05	9.18	0.00
Vivol	46.483	2.800	1.302	47.925	22.276	11.03	0.20	69.25	9.78	0.00
Wotan	40.621	2.864	1.164	42.650	17.326	11.53	0.20	63.10	9.40	0.00

Data represent the mean values of two replicates.

Seed yield (SY) is normalised to 100% DM

**Appendix 3b (continued):** Means of phenotypic data collected during the Mitscherlich pot experiment at high nitrogen supply.

Accession	Stem MassM [g]	Nconc StemM [%]	Ncont StemM [g]	Siliques massM [g]	Nconc SiliquesM [%]	Ncont SiliquesM [g]	NupE [%]	NutE [g/g]	NUE [g/g]	NHI [g/g]	Nharv_ Nsupply
Alaska	39.415	0.260	0.102	34.600	0.588	0.203	0.710	26.473	18.818	0.811	0.515
Aragon	39.040	0.359	0.140	39.220	0.534	0.209	0.799	23.009	18.289	0.780	0.485
Beluga	49.100	0.358	0.179	36.830	0.587	0.215	0.751	23.629	17.765	0.772	0.523
Cobra	38.810	0.460	0.179	34.625	0.523	0.181	0.647	20.573	13.277	0.752	0.428
CxCDH	34.950	0.394	0.132	34.860	0.513	0.175	0.711	20.310	14.551	0.771	0.417
Darmor	42.620	0.280	0.119	34.525	0.523	0.180	0.665	24.703	16.319	0.801	0.474
Dippes	44.170	0.448	0.197	45.235	0.604	0.270	0.694	18.367	12.831	0.729	0.387
Expert	36.885	0.387	0.141	42.330	0.669	0.286	0.731	23.560	17.238	0.744	0.491
GroßL	49.155	0.301	0.147	43.545	0.412	0.180	0.682	21.506	14.643	0.774	0.439
Jupiter	43.915	0.345	0.152	40.675	0.709	0.289	0.745	19.178	14.232	0.719	0.439
Kromerska	40.965	0.367	0.149	43.610	0.558	0.243	0.686	21.194	14.541	0.839	0.464
Librador	42.035	0.355	0.151	41.815	0.530	0.230	0.641	26.422	16.901	0.795	0.561
Libritta	39.340	0.340	0.135	44.185	0.449	0.198	0.633	23.780	15.052	0.772	0.440
Madrigal	37.765	0.274	0.102	39.390	0.568	0.224	0.657	27.692	18.202	0.805	0.526
Major	42.330	0.399	0.167	37.510	0.553	0.207	0.666	22.896	15.159	0.752	0.442
Markus	38.320	0.343	0.132	36.310	0.504	0.184	0.671	22.256	14.901	0.837	0.462
Mestnij	39.660	0.449	0.175	34.275	0.452	0.154	0.712	20.181	14.394	0.787	0.478
MSL007c	43.905	0.242	0.106	44.160	0.541	0.239	0.709	24.941	17.656	0.858	0.505
Olimpiade	36.180	0.411	0.148	35.725	0.438	0.155	0.721	25.670	16.489	0.863	0.541
ONDH5	41.180	0.307	0.126	38.050	0.652	0.245	0.726	25.338	18.382	0.777	0.507
Pacific	35.910	0.346	0.124	35.225	0.610	0.215	0.727	23.937	17.357	0.787	0.489
Pirola	38.215	0.318	0.123	39.310	0.526	0.206	0.725	25.021	18.112	0.799	0.509
Rapid	38.950	0.327	0.127	40.845	0.495	0.203	0.692	23.980	16.596	0.786	0.473
ResynGS4	35.700	0.398	0.142	33.780	0.538	0.181	0.672	22.261	14.966	0.785	0.464
ResynH048	33.880	0.346	0.117	39.070	0.609	0.236	0.733	22.753	16.607	0.785	0.506
Savannah	35.680	0.421	0.136	30.490	0.507	0.155	0.748	23.280	17.391	0.866	0.499
Skziverskij	44.665	0.396	0.180	36.635	0.705	0.267	0.652	18.664	12.209	0.703	0.411
Start	64.210	0.344	0.220	37.940	0.516	0.196	0.679	14.670	9.969	0.690	0.362
Vivol	39.550	0.304	0.120	35.355	0.493	0.176	0.745	24.442	18.200	0.816	0.510
Wotan	42.500	0.322	0.138	37.165	0.603	0.225	0.727	21.883	15.905	0.762	0.456

Data represent the mean values of two replicates.

**Appendix 4a:** Means and standard deviation (SD) of phenotypic data collected during the container growth experiment at low nitrogen supply

Accession	SeedYield		Stem weight		Siliques hulls		Plant residues		Root biomass		Harvest index	
	[g/plant]		[g/plant]		[g/plant]		[g/plant]		[g/plant]			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Alaska	25.992	0.116	14.082	0.138	20.021	0.158	34.103	0.020	4.813	0.324	0.400	0.001
Aragon	28.181	1.210	14.530	0.210	23.898	0.775	38.428	0.565	6.101	0.753	0.387	0.011
Beluga	24.486	2.322	15.184	0.546	19.771	2.722	34.955	3.268	5.282	0.796	0.378	0.001
Canberra X Courage DH	34.968	2.326	17.665	0.325	27.913	4.961	45.578	5.286	8.108	1.508	0.396	0.014
Cobra	18.688	0.610	15.626	1.494	20.526	0.742	36.152	2.237	3.829	0.029	0.319	0.005
Darmor	27.587	2.696	16.940	2.730	22.628	1.081	39.568	1.649	6.766	2.161	0.373	0.004
Dippes	20.975	0.288	20.819	2.375	27.337	0.998	48.156	1.377	7.386	1.341	0.275	0.012
Expert	24.391	0.996	11.599	2.336	18.484	0.522	30.084	1.814	3.652	0.312	0.420	0.001
Groß Lüsewitzer	19.775	1.963	21.321	1.708	22.876	0.128	44.196	1.836	7.357	0.802	0.278	0.030
Jupiter	23.211	4.858	17.289	4.339	28.348	3.650	45.637	7.989	6.061	2.234	0.309	0.003
Kromerska	22.979	1.635	16.313	0.917	24.118	3.996	40.431	3.079	6.339	0.216	0.329	0.000
Librador	24.953	1.692	16.971	0.833	23.088	1.032	40.059	0.199	5.484	0.229	0.354	0.013
Libritta	31.196	6.022	17.435	0.011	30.462	4.924	47.897	4.913	9.706	0.165	0.348	0.024
Madrigal	29.113	4.880	18.040	0.533	24.061	3.284	42.101	3.817	9.223	0.093	0.360	0.021
Major	22.077	1.453	18.799	1.913	26.678	0.020	45.477	1.893	7.555	0.308	0.294	0.005
Markus	19.938	1.010	18.918	0.399	21.531	0.455	40.449	0.854	7.983	0.804	0.291	0.003
Mestnij	19.327	4.562	14.168	2.718	18.844	4.984	33.012	7.702	4.027	0.727	0.342	0.002
MSL007	30.611	0.845	17.944	0.103	26.943	2.726	44.886	2.624	7.135	2.083	0.371	0.015
Oase x Nugget DH5	24.455	0.557	14.508	0.735	18.478	0.515	32.985	0.220	4.159	0.148	0.397	0.003
Olimpiade	20.188	1.335	13.739	0.056	21.491	0.957	35.230	1.014	5.293	0.411	0.332	0.007
Pacific	32.186	0.343	15.507	0.094	24.476	1.295	39.983	1.201	6.191	0.263	0.411	0.007
Pirola	29.618	1.490	14.920	2.465	20.893	0.106	35.813	2.359	5.568	1.583	0.418	0.011
Rapid	27.588	0.260	17.291	0.519	23.253	0.727	40.544	0.207	7.500	1.143	0.365	0.002
Resyn Gö S4	25.872	0.955	15.813	1.278	24.484	2.562	40.297	1.284	5.053	0.238	0.363	0.001
Resyn H048	24.880	1.053	14.141	2.538	19.311	2.284	33.453	4.821	7.244	2.856	0.384	0.035
Savannah	25.449	1.012	18.166	2.407	22.023	1.494	40.189	3.901	5.350	0.828	0.360	0.015
Skziverskij	24.554	0.976	20.246	0.436	24.469	1.853	44.716	1.417	9.949	1.541	0.310	0.009
Start	14.613	1.846	20.882	0.528	26.570	0.794	47.452	1.322	8.803	2.801	0.205	0.009
Vivol	24.786	0.887	16.274	0.573	20.866	2.472	37.140	1.900	6.218	0.200	0.364	0.003
Wotan	26.870	0.449	18.326	1.576	21.475	2.797	39.801	4.374	4.437	0.189	0.379	0.018

Data represent the mean values of two replicates. Seed yield (SY) is normalised to 91% DM

**Appendix 4a (continued):** Means and standard deviation (SD) of phenotypic data collected during the container growth experiment at low nitrogen supply.

Accession	Root/shoot ratio		Root length [cm]		No pods MR		No. of SB		Start of flowering [Days after Jan 1]	
	Mean	SD	Means	SD	Mean	SD	Mean	SD	Means	SD
Alaska	0.080	0.005	56.00	1.00	93.50	14.50	6.00	0.00	125.00	1.00
Aragon	0.092	0.014	60.50	5.50	62.00	0.00	6.00	2.00	122.50	0.50
Beluga	0.088	0.005	55.00	8.00	101.00	0.00	5.50	0.50	119.00	1.00
Canberra X Courage DH	0.100	0.009	58.00	6.00	76.50	2.50	6.00	0.00	119.00	2.00
Cobra	0.070	0.004	46.50	1.50	84.00	3.00	4.50	0.50	125.00	0.00
Darmor	0.099	0.026	59.50	9.50	75.00	2.00	4.50	0.50	123.50	2.50
Dippes	0.107	0.018	55.50	12.50	75.50	1.50	7.00	0.00	124.00	0.00
Expert	0.068	0.009	47.50	1.50	75.00	3.00	4.00	0.00	120.00	1.00
Groß Lüsewitzer	0.115	0.013	56.00	2.00	89.50	7.50	5.00	0.00	126.00	0.00
Jupiter	0.085	0.017	59.50	6.50	63.50	2.50	6.50	0.50	122.50	1.50
Kromerska	0.100	0.004	55.00	8.00	77.00	1.00	5.00	1.00	124.00	1.00
Librador	0.084	0.001	46.50	0.50	80.00	6.00	4.00	0.00		
Libritta	0.125	0.015	49.00	1.00	85.00	8.00	7.00	0.00	127.00	0.00
Madrigal	0.131	0.015	61.50	11.50	69.00	8.00	6.00	0.00	122.00	0.00
Major	0.112	0.001	44.50	2.50	76.50	6.50	8.00	0.00	118.50	0.50
Markus	0.132	0.009	55.50	2.50	70.00	13.00	3.00	0.00	126.00	0.00
Mestnij	0.078	0.004	49.00	6.00	65.00	8.00	5.00	0.00	115.00	0.00
MSL007	0.093	0.023	56.50	3.50	55.50	1.50	5.00	2.00	125.00	0.00
Oase x Nugget DH5	0.072	0.002	60.50	18.50	76.00	2.00	7.00	0.00	122.00	1.00
Olimpiade	0.095	0.003	40.00	1.00					110.00	0.00
Pacific	0.086	0.005	59.00	15.00	65.50	2.50	7.00	0.00	122.50	0.50
Pirola	0.084	0.019	47.00	2.00	80.00	7.00	7.00	0.00	119.00	2.00
Rapid	0.110	0.017	55.50	10.50	73.50	2.50	5.50	1.50	121.50	0.50
Resyn Gö S4	0.076	0.001	47.00	7.00	78.50	6.50	6.00	1.00	127.50	0.50
Resyn H048	0.120	0.037	61.00	4.00	91.00	3.00	7.00	2.00	124.00	1.00
Savannah	0.081	0.007	51.50	5.50	58.00	0.00	4.00	0.00	121.50	0.50
Skziverskij	0.145	0.027	63.50	10.50	95.50	1.50	6.50	1.50	123.00	0.00
Start	0.140	0.038	67.00	4.00	119.00	21.00	10.00	2.00		
Vivol	0.100	0.001	65.50	3.50	67.50	8.50	5.00	0.00	118.00	0.00
Wotan	0.067	0.008	65.50	3.50	56.00	4.00	6.00	1.00	123.50	0.50

Data represent the mean values of two replicates.

**Appendix 4b:** Means and standard deviation (SD) of phenotypic data collected during the container growth experiment at high nitrogen supply

Accession	SeedYield		Stem weight		Siliques hulls		Plant residues		Root biomass		Harvest index	
	[g/plant]		[g/plant]		[g/plant]		[g/plant]		[g/plant]			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Alaska	31.138	0.894	16.259	1.011	22.406	0.764	38.665	0.248	6.203	0.545	0.410	0.011
Aragon	32.183	2.558	17.244	1.198	25.234	2.461	42.478	3.658	8.570	0.120	0.386	0.002
Beluga	26.913	2.993	16.618	0.162	22.848	2.779	39.465	2.941	9.851	0.640	0.352	0.009
Canberra X Courage DH	35.991	2.790	20.668	0.427	28.564	2.262	49.233	2.689	6.261	0.293	0.393	0.008
Cobra	23.107	3.382	18.027	0.558	22.582	3.778	40.609	4.336	5.487	1.885	0.334	0.003
Darmor	33.261	1.823	18.569	3.524	26.807	1.524	45.376	5.048	5.813	0.632	0.395	0.034
Dippes	24.146	0.385	21.240	1.594	30.644	2.239	51.884	0.645	8.479	0.319	0.286	0.002
Expert	28.637	0.248	12.973	2.047	19.676	0.514	32.649	2.561	5.065	0.188	0.432	0.018
Groß Lüsewitzer	28.704	0.603	23.310	0.582	29.519	1.968	52.829	1.386	14.562	0.077	0.299	0.000
Jupiter	26.675	0.584	19.353	1.734	29.666	0.956	49.019	0.778	6.016	0.786	0.327	0.011
Kromerska	26.198	1.012	18.853	2.609	23.500	0.810	42.353	3.419	6.548	0.788	0.349	0.021
Librador	26.522	1.258	17.486	0.028	24.033	0.627	41.519	0.599	8.508	1.737	0.347	0.021
Libritta	27.576	1.601	16.278	0.006	28.356	0.890	44.634	0.896	8.268	0.996	0.342	0.013
Madrigal	25.926	0.942	16.108	1.108	22.351	1.484	38.459	0.376	11.408	1.063	0.342	0.015
Major	22.357	2.483	19.396	2.888	24.059	15.928	43.454	18.816	4.440	0.430	0.340	0.070
Markus	21.566	1.557	17.954	1.325	21.986	0.131	39.939	1.456	9.689	3.647	0.304	0.007
Mestnij	15.572	1.180	13.333	0.159	17.551	1.343	30.884	1.502	3.738	1.379	0.310	0.002
MSL007	31.871	2.292	18.702	4.079	25.388	1.318	44.089	2.762	5.357	0.673	0.392	0.001
Oase x Nugget DH5	22.356	1.477	13.518	0.114	16.899	1.697	30.416	1.584	3.307	0.156	0.398	0.003
Olimpiade	19.858	0.453	14.864	0.736	22.029	0.053	36.894	0.789	3.703	0.247	0.328	0.008
Pacific	33.120	0.864	16.538	2.044	25.812	2.384	42.349	0.341	7.376	1.143	0.400	0.010
Pirola	28.792	1.631	14.314	0.909	21.156	0.757	35.469	0.152	4.621	1.228	0.418	0.007
Rapid	34.745	1.557	19.249	0.259	28.718	1.776	47.967	2.034	9.006	1.158	0.379	0.007
Resyn Gö S4	28.041	0.638	14.852	0.949	25.777	0.341	40.629	1.290	5.224	0.231	0.380	0.002
Resyn H048	29.040	0.174	15.328	1.162	23.036	0.546	38.364	1.709	5.939	1.236	0.397	0.014
Savannah	24.706	2.169	17.618	0.998	20.584	0.081	38.203	0.917	6.268	1.429	0.357	0.032
Skziverskij	22.535	2.631	17.997	1.432	24.300	0.034	42.297	1.466	7.542	0.218	0.310	0.020
Start	16.134	1.409	21.864	0.116	29.792	0.468	51.656	0.353	7.775	1.628	0.213	0.009
Vivol	27.560	0.104	18.237	0.346	22.482	0.712	40.719	0.366	6.753	0.760	0.367	0.006
Wotan	30.106	2.649	20.401	0.651	24.526	1.269	44.926	1.920	4.469	0.747	0.378	0.008

Data represent the mean values of two replicates. Seed yield (SY) is normalised to 91% DM

**Appendix 4b (continued):** Means and standard deviation (SD) of phenotypic data collected during the container experiment at high nitrogen supply

Accession	Root/shoot ratio		Root length [cm]		No pods MR		No. of SB		Start of flowering [Days after Jan 1]	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Alaska	0.089	0.009	77.00	2.00	79.50	3.50	5.00	0.00	125.00	1.00
Aragon	0.116	0.011	82.50	1.50	70.50	3.50	4.50	0.50	121.00	0.00
Beluga	0.149	0.004	60.00	8.00	82.50	10.50	4.50	0.50	120.00	0.00
Canberra X Courage DH	0.074	0.008	63.00	14.00	81.00	12.00	6.00	0.00	120.00	0.00
Cobra	0.084	0.019	56.00	3.00	84.50	5.50	6.50	1.50	125.00	0.00
Darmor	0.074	0.011	52.50	2.50	63.50	5.50	7.50	1.50	120.00	2.00
Dippes	0.112	0.006	62.50	0.50	78.50	1.50	9.00	0.00	124.00	0.00
Expert	0.083	0.006	43.00	3.00	83.00	10.00	6.00	0.00	120.50	1.50
Groß Lüsewitzer	0.179	0.003	89.00	1.00	87.50	4.50	5.50	0.50		
Jupiter	0.079	0.010	60.00	9.00	114.50	47.50	6.00	0.00	122.50	0.50
Kromerska	0.096	0.015	57.50	13.50	78.00	11.00	10.50	4.50	124.50	1.50
Librador	0.125	0.027	66.50	10.50	91.50	0.50	6.00	0.00		
Libritta	0.114	0.013	49.50	1.50	78.00	3.00	6.00	1.00	127.00	0.00
Madrigal	0.177	0.018	75.50	17.50	78.00	4.00	7.00	0.00	124.00	0.00
Major	0.073	0.017	45.00	1.00	66.00	3.00	6.00	0.00	118.00	0.00
Markus	0.155	0.052	69.00	10.00	78.00	8.00	5.00	0.00	126.00	0.00
Mestnij	0.079	0.025	41.00	3.00	73.00	14.00	4.50	0.50	115.00	0.00
MSL007	0.070	0.004	60.50	1.50	53.50	9.50	5.00	0.00	125.50	0.50
Oase x Nugget DH5	0.063	0.001	59.00	13.00	70.50	0.50	6.00	1.00	123.50	1.50
Olimpiade	0.065	0.005	54.50	10.50	48.00	2.00			101.00	9.00
Pacific	0.098	0.017	85.00	18.00	71.00	3.00	7.00	0.00	120.50	0.50
Pirola	0.072	0.017	56.50	6.50	81.50	4.50	7.50	0.50	121.00	0.00
Rapid	0.110	0.019	54.50	10.50	74.00	8.00	7.50	0.50	121.50	0.50
Resyn Gö S4	0.076	0.001	46.00	3.00	73.00	2.00	8.50	0.50	125.00	0.00
Resyn H048	0.088	0.016	56.00	7.00	81.00	5.00	7.00	1.00	124.50	0.50
Savannah	0.100	0.025	45.50	0.50	59.50	1.50	6.00	0.00	122.00	2.00
Skziverskij	0.117	0.011	50.50	4.50	91.50	4.50	5.50	0.50	124.50	0.50
Start	0.114	0.021	89.50	7.50	119.00	2.00	6.50	0.50		
Vivol	0.099	0.011	56.50	6.50	77.50	11.50	6.50	0.50	118.00	0.00
Wotan	0.059	0.006	63.50	1.50	67.00	5.00	4.50	1.50	124.00	1.00

Data represent the mean values of two replicates.

## Appendix 5: Nitrogen concentration, leaf dry weight and nitrogen losses with aborted leaves

Accession	Rep	June 14	June 23	June 28	July 10	Seed harvest
<b>N concentration [%]</b>						
Beluga	1	3.14	2.02	1.98	1.00	1.77
Beluga	2	2.51	1.99	2.19		1.27
Cobra	1	2.59	1.81	1.24	0.79	
Cobra	2	2.46	1.39	1.31	0.94	
Dippes	1	1.45	1.02		1.27	
Dippes	2	1.25	0.78	1.09		1.37
<b>Leaf dry weight [g/container]</b>						
Beluga	1	9.00	26.97	19.89	17.13	24.90
Beluga	2	9.76	21.53	25.01	22.17	27.04
Cobra	1	18.73	13.66	23.61	22.58	
Cobra	2	38.24	17.27	24.98	16.39	
Dippes	1	75.97	29.05	9.76	10.62	
Dippes	2	75.94	22.89	6.09	1.80	0.87
<b>N loss [g/container]</b>						
Beluga	1	0.28	0.55	0.39	0.17	0.44
Beluga	2	0.25	0.43	0.55	0.00	0.34
Cobra	1	0.48	0.25	0.29	0.18	0.00
Cobra	2	0.94	0.24	0.33	0.15	0.00
Dippes	1	1.10	0.30		0.11	0.00
Dippes	2	0.95	0.18	0.07	0.00	0.01

Data refer to the low nitrogen treated container.

## Declaration

I declare: this dissertation submitted is a work of my own, written without any illegitimate help by any third party and only with materials indicated in the dissertation. I have indicated in the text where I have used texts from already published sources, either word for word or in substance, and where I have made statements based on oral information given to me. At any time during the investigations carried out by me and described in the dissertation, I followed the principles of good scientific practice as defined in the Statutes of the Justus Liebig University Giessen for the Safeguarding of Good Scientific Practice

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Place, Date/Ort, Datum

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Signature/Unterschrift