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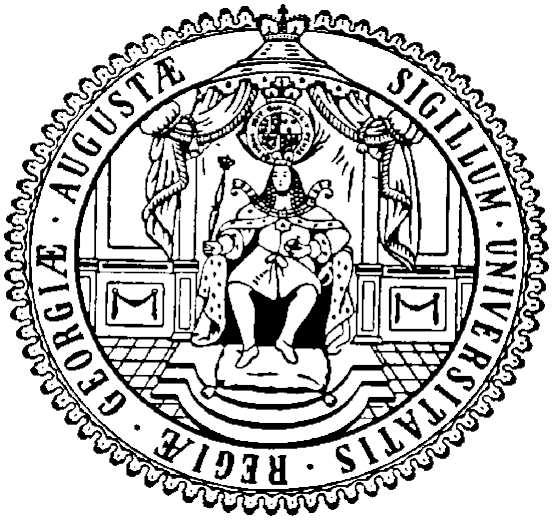
Nitrogen efficiency in semi-dwarf and normal hybrids of oilseed rape

Sebastian Miersch



Göttingen, May 2014

Nitrogen efficiency in semi-dwarf and normal hybrids of oilseed rape

Dissertation

to obtain the Ph.D. degree

in the Faculty of Agricultural Sciences,

Georg-August-Universität Göttingen

presented by

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born in Hoyerswerda

Göttingen, May 2014

D7

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Date of dissertation: 24.07.2014

Dedicated to my parents and grandparents.

Acknowledgements

The author gratefully acknowledge funding from the European Community financial participation under the Seventh Framework Programme for Research, Technological Development and Demonstration Activities, for the Integrated Project NUE-CROPS FP7-CP-IP 222645. The views expressed in this publication are the sole responsibility of the author and do not necessarily reflect the views of the European Commission. Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use which might be made of the information contained herein.

I like to thank KWS SAAT AG, especially Frank Breuer, Andreas Gertz and Frank Weber for producing DH lines and testcross hybrids as well as for providing the marker data. Their excellent support in conducting the field experiments is gratefully acknowledged.

Thanks to Wolfgang Ecke for his assistance in the construction of the linkage map.

A special thanks goes to Professor Heiko C. Becker for giving me the opportunity to work on this multifaceted project. I am very grateful for his continuous scientific advises, the high degree of confidence as well as the encouragement during the last four years.

Last, but not least, I want to thank my family and friends for their constant support throughout this exciting and challenging period.

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Aim of the study

The cultivation of winter oilseed rape is characterised by high rates of nitrogen fertilization. For economical, environmental and legislative reasons it is aspired to reduce nitrogen fertilization in oilseed rape cultivation. The development of oilseed rape cultivars with an improved nitrogen efficiency is an important factor in this context.

The major research objectives in the present study were:

- to investigate the effect of the dwarf gene *Bzh* on various physiological parameters
- to compare the agronomic performance of semi-dwarf and normal type oilseed rape hybrids at low and high nitrogen supply
- to analyse factors influencing nitrogen use efficiency
- to compare nitrogen use efficiency of semi-dwarf and normal type hybrids at low and high nitrogen supply
- to analyse genetic mechanisms controlling agronomic and nitrogen related traits

The present study is subdivided into four chapters. Chapter 1 gives an general introduction to the relevance of oilseed rape, the meaning of nitrogen use efficiency and the concept of QTL mapping. Chapter 2 contains the comparison of agronomic traits among semi-dwarf and normal type oilseed rape hybrids based on three-year field trials at low and high N supply. Chapter 3 focus on parameters influencing nitrogen use efficiency in semi-dwarf and normal type hybrids at low and high nitrogen supply. Chapter 4 comprises the detection of genetic mechanisms influencing agronomic and nitrogen related traits.

1 Introduction

1.1 Agronomic relevance of *Brassica napus* L.

Winter oilseed rape (*Brassica napus* L.) is the most grown oilseed crop in temperate regions. In 2013 the cultivated area for winter and spring types of oilseed rape and turnip in the EU-27 was about 6.73 million ha. With a cultivated area of 1.47 million ha in 2013, Germany was the most important producer of oilseed rape in the EU followed by France (1.44 million ha) and Poland (0.92 million ha) (Eurostat, 2014). Other important producing countries (mostly spring types) are China, Canada and Australia.

Most oilseed rape cultivars (80 % in 2013) grown in Germany are hybrid varieties. Hybrid varieties are characterized by a high yield potential, a better yield stability and a more vigorous plant development compared to open pollinating varieties. Hybrids are generated by crossing two genetically different, homozygous parental lines. The resulting hybrid (F_1) is more vigorous and higher yielding compared to the parental lines. This genetic phenomenom is known as hybrid vigor or 'heterosis' (Shull, 1948).

Botanically *Brassica napus* L. belongs to the family *Brasscicaceae* with about 3.700 known species. It is the result of a spontaneous hybridisation event between the two species *Brassica rapa* L. (AA, 2n=2x=20) and *Brassica oleracea* L. (CC, 2n=2x=18) resulting in the allopolyploid hybrid *Brasscia napus* L. (AACC, 2n=4x=38). The genetic relationship among different Brassica species was first demonstrated with the 'Triangle of U' (Nagaharu, 1935).

Oilseed rape generated by crossing the diploid species *Brassica rapa* L. and *Brassica oleracea* L. is called 'resythesized' oilseed rape. Resynthesized genotypes are valuable to extend the genetic diversity within the breeding material by introducing alternative forms of genes (alleles) (Girke, 2002).

1.2 Nitrogen use efficiency

There is no generally accepted definition for nitrogen use efficiency. Nitrogen use efficiency in its broadest sense indicates how effectively a plant is able to capture and utilize nitrogen to produce biomass or grain yield (Hawkesford and Barraclough, 2011). In the present

study nitrogen use efficiency (NUE) is defined as seed production per unit nitrogen (N) available in the soil, according to Moll et al. (1982).

The use of nitrogen involves several steps including uptake, assimilation, translocation and remobilization (Good et al., 2004). Each of these steps can be subdivided into many specific physiological traits and processes, encoded by multiple genes and regulatory processes (Hawkesford and Barraclough, 2011). In this study nitrogen use efficiency is split into two primary components: (1) the efficiency of absorption (N uptake efficiency) and (2) the efficiency with which the absorbed N is utilized to produce seeds (N utilization efficiency) (Moll et al., 1982).

1.2.1 Nitrogen uptake

Soil nitrogen availability can fluctuate greatly in both space and time due to factors such as precipitation, temperature, wind, soil type and pH. Nitrogen uptake occurs at the root level and depends on root architecture, root functions in terms of transporters and exudates and often the presence of symbiotic associations such as mycorrhiza (Hawkesford and Barraclough, 2011).

A well developed shallow root system will be ideal for the uptake of fertilizer applications. Deeper roots are important when surface nutrients are depleted or restricted nutrient uptake due to limited surface water. In the case of high water supply deeper roots can intercept nutrients that would be potentially leached from the soil profile. The experimental assessment of size and architecture of a root system is very laborious under field conditions.

Besides root architecture transmembrane transport of nitrogen is another important factor for nitrogen uptake. The nitrogen sources taken up by higher plants are nitrate or ammonium as inorganic nitrogen sources and amino acids under particular conditions of soil composition (Masclaux-Daubresse et al., 2010). Nitrogen assimilation requires the reduction of nitrate to ammonium, followed by ammonium assimilation into amino acids.

Ammonium is taken up by different ammonium transport proteins (AMT) (Andrade and Einsle, 2007). If nitrogen is not limiting or high ammonium concentrations in the cell needs to be avoided due to toxicity, AMT-mediated transport is blocked. Nitrate is taken up by two different transport systems. They act co-ordinately to take up nitrate from the soil solution and distribute it within the whole plant (Masclaux-Daubresse et al., 2010). At high external nitrate concentration a low affinity transport system (LATS) is operating mediated by members of the NRT1 gene family. At low external nitrate concentration nitrate is taken up by a high affinity transport system (HATS) controlled by members of the NRT2 family. In Arabidopsis 53 genes were identified to belong to the NRT1 family and seven genes belonging to the NRT2 family (Masclaux-Daubresse et al.,

2010). Depending on the species, nitrogen uptake could be negatively regulated or even in some cases totally inhibited during seed production. For example, in oilseed rape, the transition between the vegetative and reproductive phases is characterized by a drastic decrease of HATS and HATS+LATS activities (Malagoli et al., 2005).

Another important characteristic for nitrogen uptake efficiency is the availability of adequate sinks to store accumulated nitrogen (Hawkesford and Barraclough, 2011). Adequate sinks will prevent negative feedback regulation on the initial uptake processes and provide important remobilizable storage.

Due to the variety of factors involved into nitrogen uptake and their complexity it is practically not feasible to determine all of them in a single experiment. Therefore the most simple approach is to determine the amount of nitrogen taken up by a plant. This integrates the separate features of root architecture and root functions contributing to this trait (Hawkesford and Barraclough, 2011).

1.2.2 Nitrogen utilization

Nitrogen utilization efficiency is defined as unit of harvestable product (seed) per unit of nitrogen taken up, according to Moll et al. (1982). A key attribute to improve nitrogen utilization efficiency in oilseed rape is N remobilization. Nitrogen uptake after flowering is generally insufficient for the high N demand of seeds during the reproductive period (Masclaux-Daubresse et al., 2010). Therefore, oilseed rape remobilize N from senescing leaves, roots, stems and pod walls during the reproductive period (Rathke et al., 2006; Gombert et al., 2010). Leaf proteins and in particular photosynthetic proteins of plastids are extensively degraded during senescence, providing an enormous source of nitrogen (Masclaux-Daubresse et al., 2010).

At high nitrogen supply about 70 % of seed nitrogen was remobilized from stems, leaves and pod walls of oilseed rape (Gombert et al., 2010; Malagoli et al., 2005). The contribution of remobilized nitrogen to pod nitrogen content in rice, wheat or maize grain nitrogen content is cultivar dependent, varying from 50 to 90 % (Masclaux-Daubresse et al., 2010). At higher nitrogen supplies leaf senescence in oilseed rape is delayed compared to low nitrogen supply. According to Schulte auf'm Erley et al. (2007) delayed leaf senescence during grain filling enhance nitrogen use efficiency. Thus, nitrogen uptake and nitrogen remobilization is possibly delayed, too (Gombert et al., 2010).

There is evidence that plants share common nitrogen remobilization mechanisms whether they are monocotyledonous, dicotyledonous, C3 or C4 photosynthesis types (Masclaux-Daubresse et al., 2010). Grain nitrogen accumulation usually appears to be regulated by the nitrogen source. Nitrogen remobilization is also environment dependent and favoured under limiting nitrate supplies.

1.3 QTL mapping

The appearance of a plant with all its observable characteristics or traits is called 'phenotype'. A phenotype is determined by the expression of its genes, environmental effects and the interactions between genes and environment. Phenotypic traits controlled by one or only a few genes are often called 'Mendelian traits' and generate a limited number of expression levels.

In contrast to Mendelian traits, quantitative/complex traits are affected by multiple genes usually with small effects (Doerge 2002). Quantitative traits exhibit a continuous variation such as plant height, seed yield or oil content. Genes or chromosomal regions determining a quantitative trait are called quantitative trait loci (QTL) (Geldermann, 1975).

The process of identifying and localizing QTL along chromosomes is usually refered to as QTL mapping. Several parameters such as heritability of a trait, number of QTL affecting the trait, epistasis or interaction between QTL, genome size, marker density and variation due to type and size of a mapping population need to be considered for QTL mapping (Doerge, 2002).

One approach to conduct a QTL mapping study is to cross two homozygous parental lines to produce F_1 lines which are heterozygous at all markers and QTL where the two parents differ (Doerge, 2002). Based on the F_1 lines a mapping population, segregating for the trait of interest is generated. Common designs for mapping populations are F_2 intercross, backcross, recombinant inbred lines or F_1 derived doubled haploid lines (DH).

In oilseed rape DH lines are generated from microspores isolated from anthers of maturing buds (Möllers et al., 1994). The microspores carry a haploid (1x) set of chromosomes. Following a colchicine treatment of microspores, the chromosomes are doubled and diploid plants (doubled haploid) are generated.

For sophisticated methods of QTL mapping such as interval mapping (IM) or composite interval mapping (CIM) it is necessary to create a genetic map. A genetic map provides a genetic representation of the chromosomes (linkage groups) on which previously identified polymorphic DNA markers are placed (Doerge, 2002). The markers are placed on the map according their genetic distance using a map function (such as the Haldane or Kosambi map functions). The accuracy of locating QTL is limited by the number of recombinants, gained from observing the genotypic states of the markers. These observed recombinants can be limited by both small sample size (genotypes) and small number of markers.

A common method for QTL mapping is composite interval mapping (CIM) described by Zeng (1994). Composite interval mapping combines the interval mapping approach (Lander and Botstein, 1989) with linear regression, including additional markers to the neighbouring pair of linked markers (Zeng, 1994). When positions for QTL are estimated,

the resulting maximum or peak has to surpass a specified significance threshold. The estimation of the thresholds is most commonly performed by including permutation tests (Churchill and Doerge, 1994). These tests involve a certain number of repeats (e.g. 500 or 1000) where the false positive marker-trait associations are estimated by randomisation of the phenotypic data with simultaneously constant marker values of the genotypes (Hackett, 2002).

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2 Agronomic performance of semi-dwarf and normal type winter oilseed rape hybrids

2.1 Introduction

Oilseed rape (*Brassica napus* L.) is the most important oilcrop in Europe. The cultivated area in Germany increased rapidly in the last decade to 1.47 million ha in the year 2013 (Eurostat, 2014). The economy of winter oilseed rape cultivation in Germany is primarily determined by seed yield and less by oil content (Rathke et al., 2005). One possibility to increase seed yield is to influence the distribution of assimilates within a plant. By reducing the vegetative biomass without reducing the vitality of a plant, more assimilates can be transferred into the seeds. In the end this would result in an increase of harvest index.

A reduction of vegetative biomass combined with an increase in seed yield and harvest index was already achieved in wheat and rice in the mid 20th century (Hedden, 2003). The shorter wheat and rice varieties were the prototypes of today's modern cultivars. These so called 'semi-dwarf' wheat and rice varieties were characterised by short strong stems resulting in an improved lodging resistance. In combination with high amounts of nitrogen fertilizer these new varieties led to impressive yield increases generally referred to as 'Green Revolution'. The dwarfism in both crops is caused by mutations in genes controlling the signaling pathway or biosynthesis of gibberellic acid (GA) (Hedden, 2003). Gibberellic acids form a large family of tetracyclic diterpenoid plant hormones affecting plant growth and development in higher plants. Some of the traits influenced by GA are stem and root extension, leaf expansion, seed germination and floral induction (Sun and Gubler, 2004). Two different forms of GA mutants with similar phenotypes are known. The dwarfism of GA-deficient mutants is caused by a mutated gene in the biosynthesis pathway of GA resulting in GA deficiency. By application of bioactive GAs the normal height can be restored. GA-insensitive mutants do not respond to exogenous GA treatment because they encode a mutant form of DELLA proteins. DELLA proteins affect various aspects of

plant growth by inhibiting GA-dependent pathways. In a normal GA signaling pathway DELLA proteins are degraded. But through gain-of-function mutations in the N-terminus or loss-of-function mutations in the C-terminus the proteins are resistant to GA induced degredation and GA signaling is constitutively blocked (Peng et al., 1999).

In *Brassica napus* (L.) dwarf mutants were used in physiological studies by Williams and Hill (1986) and Zanewich et al. (1991). A resynthesized *B. napus* line was developed by crossing the dwarf *Brassica rapa* stock described by Zanewich et al. (1991) with a non-dwarf *Brassica oleracea* stock (Muangprom et al., 2006). The dwarf *Brassica rapa* stock carried the dwarf allele of the *BrGAI* gene, which is a homolog of *rga* in Arabidopsis, *rht* in wheat or *d8* in maize. Two parents of a commercial canola hybrid were backcrossed with the resynthesized line to generate dwarf, semi-dwarf and normal hybrids. No significant differences in seed yield were observed between the three different hybrids. Due to damages by flee beetles the dwarf and semi-dwarf hyrids had a higher percentage of aborted seeds. At optimal growing conditions semi-dwarf hybrids might have higher seed yield than normal hybrids according to Muangprom et al. (2006).

Foisset et al. (1995) obtained a dwarf mutant by chemical mutagenesis of seeds from the oilseed rape cultivar 'Primor' at INRA / France. Genetic analysis of the dwarf mutant indicated that the dwarfism is controlled by a single gene named *bzh* with additive effect. Normal growing genotypes are homogzygous *Bzh/Bzh*, dwarf genotypes are *bzh/bzh* and semi-dwarfs are heterozygous *Bzh/bzh*. The mutated allele was transferred to different oilseed rape lines to test the agronomic performance of dwarf and semi-dwarf progenies. The authors reported a positive effect on lodging resistance and yield performance without giving further details.

A Chinese research group generated a dwarf mutant by treating seeds with neutron bombardment and chemical mutagens (Wang et al., 2004). The dwarfism in the mutant was caused by a single gene with additive effect called *ndf-1* which is discussed to be allelic to *bzh*. Other dwarf lines were generated by introducing the dwarf trait into several elite accessions. They were used to produce ten different semi-dwarf hybrids which were tested in field trials for two years. According to Wang et al. (2004) semi-dwarf hybrids showed good agronomic performance with higher seed yield and higher thousand seed weight compared to the normal parents. Later it was shown that the *ndf-1* mutant is a gibberellin insensitive dwarf (Li et al., 2011). Another GA insensitive dwarf mutant was obtained by treating seeds from the Chinese inbred line 'T6' with chemical mutagens (Zeng et al., 2011). The dwarfism was controlled by a single recessive gene (*bnaC.dwf*) mapped to linkage group N18.

Several semi-dwarf hybrid varieties using the *bzh* gene described by Foisset et al. (1995) have been released to the European market. The plants are shorter and branching begins

closer to the stem base compared to normal types. Thus lodging resistance is improved which is of importance in oilseed rape cultivation (Sieling and Kage, 2008). Further benefits are reduced damages when applying plant protection agents at flowering stage, a more uniform maturation and a swifter seed harvest due to less straw.

The aim of the present experiment was to explore the effect of the dwarf gene *Bzh* described by Foisset et al. (1995), in a segregating winter oilseed rape population of semi-dwarf and normal hybrids. It was investigated whether the semi-dwarf growth type has positive or negative effects on agronomic traits, especially on seed yield. Furthermore the response of both growth types to different nitrogen supply levels was analysed. Special attention was paid to the question if the semi-dwarf growth type is beneficial at nitrogen deficient conditions.

2.2 Materials and Methods

2.2.1 Plant Material and Experimental Design

A doubled-haploid (DH) population (242 lines) derived from the cross 'Alesi-*bzh* x H30' was produced. The 'Alesi-*bzh*' parent is a dwarf isogenic line derived from the German winter oilseed cultivar 'Alesi' backcrossed (BC4) to the *bzh* dwarf mutant described by Foisset et al. (1995). 'H30' is a normal type, resynthesized rapeseed line originating from a cross of *Brasscia rapa* ssp. *chinensis* with *Brasscica napus* ssp. *napus* var. *pabularia* with moderate winterhardiness (Girke, 2002). The DH population is segregating into dwarf and tall growing DH lines. All DH lines were crossed with a normal type, male sterile tester (canola quality) using the MSL ('Männliche Sterilität Lembke') hybrid system (Paulmann and Frauen, 1998). The resulting testcross hybrids (TC) segregate into semi-dwarf and normal types. In the season 2010/11 150 hybrids (75 semi-dwarf and 75 normal types) were selected based on the availability of seeds and evaluated in two adjacent field experiments.

In season 2010/11 150 TC (75 semi-dwarfs and 75 normal types) were selected based on the availability of seeds and evaluated in two adjacent experiments. One experiment was harvested at the end of flowering (EOF) (BBCH 69, Lancashire et al. (1991)) and the second experiment at seed maturity (BBCH 89). Experiments were conducted at two sites near Einbeck (Ei) (brunic aerosol) and near Göttingen (Go) (alluvial meadow soil) in Central Germany. The plot sizes were 18 m^2 in Einbeck and 11.25 m^2 in Göttingen with plant densities of 50 and 90 plants/m^2, respectively. Both experiments were evaluated without nitrogen fertilization (low nitrogen supply). Soil mineral nitrogen determined at the beginning of plant growth in spring was 35 kg N/ha in Einbeck (Ei11) and 27 kg N/ha in Göttingen (Go11). All other agronomic treatments (except fertilization) were done according to recommendations for oilseed rape cultivation in Germany. Growth types were interlaced within each block by alternately growing one strip of semi-dwarfs and one strip of normal types. Experiments were designed as split-plot with growth type as main plot factor and genotypes as subplot factor. The experimental design was identical for both environments.

In season 2011/12 experiments from season 2010/11 were repeated using 108 TC (54 semi-dwarfs and 54 normal types). The 108 TC were selected based on erucic acid content and seed yield in season 2010/11. For each growth type 27 TC with erucic acid (14 highest seed yield, 13 lowest seed yield) and the 27 TC without erucic acid (14 highest seed yield, 13 lowest seed yield) were selected. Hybrids were evaluated in two identical experiments at both sites (Göttingen and Einbeck). One experiment was harvested at the end of flowering (BBCH 69) and the second experiment at seed maturity (BBCH 89). Both experiments were conducted at two nitrogen levels: without nitrogen fertilization

(low nitrogen supply) and fertilized with 177 kg N/ha (high nitrogen supply). Mineral nitrogen in the soil was determined at the beginning of plant growth in spring in Einbeck (27 kg N/ha) and Göttingen (27 kg N/ha). Both growth types were interlaced within each block and arranged in split-plot design. Genotypes within growth types were randomized in lattice design.

In season 2012/13 the 108 TC (54 semi-dwarfs and 54 normal types) were again tested at two locations (Einbeck and Göttingen). In season 2012/13 all plots were harvested at seed maturity only. Field trials were conducted at two nitrogen levels: without nitrogen fertilization (low nitrogen supply) and fertilized with 177 kg N/ha (high nitrogen supply). Mineral nitrogen in the soil at the beginning of plant growth in spring was determined in Göttingen (24 kg N/ha) only. Genotypes within growth types were randomized in lattice design. Both growth types were interlaced within each block and arranged in split-plot design.

Each season-site combination is considered as one environment and is addressed by location abbreviation and harvest year, such as Ei11 for Einbeck in the season 2010/2011.

2.2.2 Evaluated traits and Measurements

In all trials begin of flowering (days from 1st of January, BBCH 61) and plant height (in cm at BBCH 89) were recorded. Seed yield at maturity was determined with a plot combine harvester. Due to a heavy hail storm seed yield could not be determined in Einbeck 2012. The straw of every plot was collected in a canvas tissue attached to the back of the harvester and weighted (except in Einbeck 2011). All plants in a plot were cut right above soil level. A straw subsample including stems and pods was taken, weighted and dried at 60 °C for 48 h in a drying oven. Subsequently the dried sample was weighted to calculate straw dry matter content. Thousand seed weight (TSW) was measured on a representative sample of 500 seeds. Oil, protein and moisture content in % of seed dry mater were determined with a NIRS monochromator model 6500 (NIRSystems, Inc., Silversprings, MD, USA) using calibrations (raps2011.eqa, raps2012.qa, raps2013.eqa) provided by VDLUFA Qualitätssicherung NIRS GmbH.

2.2.3 Statistical analysis

Analysis of variance (ANOVA), heritabilities (h^2) and Spearman's rank correlation coefficients were calculated using PLABSTAT software (Version 3B) (Utz, 2001).
ANOVA within each nitrogen level for a single environment was calculated using the following model for a split-plot design:

$$Y_{jkl} = \mu + b_j + t_k + \epsilon_{jk} + g_{kl} + \epsilon_{jkl} \tag{2.1}$$

in which Y_{jkl} is the observation of a genotype (l) within growth type (k) in block (j). Whole plot error is denoted by (ϵ_{jk}) and the sub-plot error is called (ϵ_{jkl}). The general mean is designated by μ. The factors block and genotype were considered as random.
To include the effect of different environments (year-location combination) into the ANOVA, following model for a split-plot design was used:

$$\begin{aligned} Y_{ijkl} = \mu + e_i + t_k + b_{ij} + et_{ik} + \epsilon_{ijk} \\ + g_{kl} + eg_{ikl} + \epsilon_{ijkl} \end{aligned} \tag{2.2}$$

in which Y_{ijkl} is the observation of a genotype (l) within the growth type (k) in block (j) within environment (i). Interaction effects between growth type and environment are denoted by et_{ik} and interactions between environment and genotype are called eg_{ikl}. Whole plot error is denoted by (ϵ_{ijk}) and the sub-plot error is denoted by (ϵ_{ijkl}). The factors environment, block and genotype were considered as random.
To test the effects of nitrogen levels following model for a split-split-plot design was used:

$$\begin{aligned} Y_{ijklo} = \mu + e_i + n_o + b_{ijo} + en_{io} + \epsilon_{ijo} \\ + t_k + et_{ik} + tn_{ko} + etn_{iko} + \epsilon_{ijko} \\ + g_{kl} + eg_{ikl} + gn_{klo} + egn_{iklo} + \epsilon_{ijklo} \end{aligned} \tag{2.3}$$

in which Y_{ijklo} is the observation of a genotype (l) within growth type (k) in block (j) within nitrogen level (o) at environment (i). Interaction effects were tested for environment and nitrogen level (en_{io}), environment and growth type (et_{ik}), environment and genotype (eg_{ikl}), growth type and nitrogen level (tn_{ko}) and genotype by nitrogen level (gn_{klo}). Threefold interactions were testes for environment by genotype by nitrogen level (egn_{iklo}) and environment by growth type by nitrogen level (etn_{iko}). Whole plot error is denoted by ϵ_{ijo}, sub-plot error is denoted by ϵ_{ijko} and the sub-sub-plot error is denoted by ϵ_{ijklo}. The factors environment, block and genotype were considered as random.

Broad sense heritability (h^2) was estimated as follows:

$$h^2 = \frac{\hat{\sigma}^2_G}{\hat{\sigma}^2_G + \frac{\hat{\sigma}^2_{GE}}{E}}$$

where $\hat{\sigma}^2_G$ and $\hat{\sigma}^2_{GE}$ are variances of genotypes and genotypes by environment respectively; E refers to number of environments.

Violin plots as described by Hintze and Nelson (1998) were generated using R software package.

2.3 Results

2.3.1 Agronomic performance at low nitrogen supply

At low nitrogen supply both growth types showed a significant difference in plant height across all environments (Tab.2.1). The average difference in plant height was about 28

Table 2.1: Mean values and significances for agronomic traits at low nitrogen supply (108 genotypes, six environments)

Trait		Ei11	Go11	Ei12	Go12	Ei13	Go13	Mean
Begin of flowering	Nt	110	110	104	115	121	125	114
(days)	Sd	110 ns	110 ns	104 ns	114 *	121 ns	124 +	113 ns
Plant height	Nt	126	119	108	90	143	122	118
(cm)	Sd	100 *	92 **	72 **	70 **	110 **	93 *	90 **
Seed yield	Nt	17.0	12.9	-	10.5	22.5	30.7	18.3
(dt/ha)	Sd	18.0 *	17.0 *	-	12.8 **	22.4 ns	33.9 **	20.7 *
Straw yield	Nt	-	40.1	18.9	22.9	46.4	36.4	32.9
(dt/ha)	Sd	-	34.9 **	17.7 ns	19.8 **	36.1 **	32.0 +	28.1 *
Harvest index	Nt	-	0.25	-	0.32	0.33	0.46	0.34
	Sd	-	0.33 **	-	0.39 **	0.38 **	0.52 ns	0.41**
Total biomass	Nt	-	51.9	-	32.5	58.5	65.9	55.6
(dt/ha)	Sd	-	53.0 ns	-	33.3 ns	68.9 **	67.1 ns	52.2 ns
TSW	Nt	4.65	4.81	4.63	4.82	4.41	4.69	4.67
(g)	Sd	4.79 +	4.70 ns	4.52 ns	4.66 **	4.20 **	4.42 **	4.55 ns
Oil content	Nt	52.3	49.5	48.2	49.2	50.0	50.7	50.0
(%)	Sd	51.6 **	49.2 **	47.9 ns	49.4 ns	49.2 ns	50.4 ns	49.6 ns
Oil yield	Nt	7.6	6.4	-	5.1	11.2	15.5	9.2
(dt/ha)	Sd	8.8 ns	8.4 *	-	6.3 **	11.0 ns	17.1 **	10.3 *
Protein content	Nt	16.9	19.3	20.2	19.0	17.9	17.4	18.4
(%)	Sd	17.02 ns	19.2 ns	20.0 ns	18.5 **	17.9 ns	17.0 **	18.3 ns

,*,+ Significant difference at 0.01, 0.05, 0.10 probability level, respectively; ns non-significant (F-test from ANOVA); **Ei Einbeck; **Go** Göttingen; **Sd** semi-dwarf; **Nt** normal type; **TSW** thousand seed weight

cm between growth types. In Göttingen 2012 normal types reached an average height of 90 cm only and the difference between both types was only 20 cm. Differences in begin

of flowering among both growth types were not significant across all environments (Tab. 2.2). Semi-dwarf hybrids revealed a significantly higher seed yield compared to normal types types on average across five environments with low nitrogen supply (Tab.2.1). In Einbeck 2012/13 seed yields of both growth types were nearly on the same level. The distributions of genotypes for seed yield were quite similar in 2010/11 and 2011/12 (Fig.2.1). In 2013 seed yields of normal genotypes were distributed in a wider range compared to

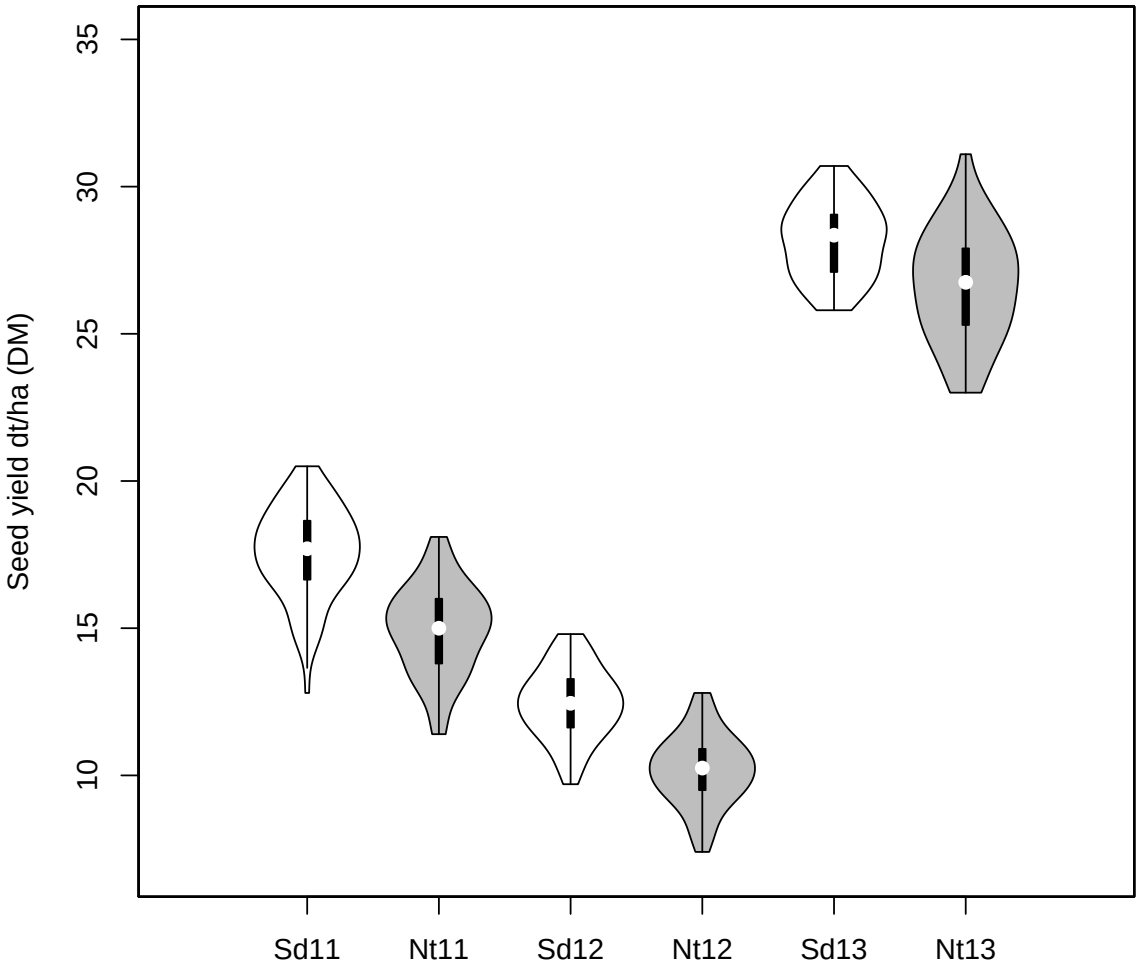

Figure 2.1: Violinplot of semi-dwarf (Sd) and normal type (Nt) hybrids for seed yield across three seasons at low nitrogen supply.The plot is a combination of a box plot and a kernel density plot.

the semi-dwarfs. In that season the highest yielding genotype was a normal type, while in earlier years a semi-dwarf had the highest yield (Fig.2.1). Semi-dwarfs had signifi-

cantly less straw than normal types in all environments except Einbeck 2012 (Tab.2.1). On average across five environments semi-dwarfs produced about 6 dt/ha less straw than normal types. This resulted in a significantly higher harvest index of semi-dwarfs (0.41) compared to normal types (0.34). The highest harvest indexes of normal types (0.46) and semi-dwarfs (0.52) were observed in Göttingen 2013 (Tab. 2.1).
The total biomass as sum of seed yield and straw yield, revealed no significant difference between growth types across all environments. In Einbeck 2013 semi-dwarf types had significantly higher biomass yields than normal types. Both growth types differed significantly in thousand seed weight (TSW). In all environments, except Einbeck 2011, semi-dwarfs had smaller seeds than normal types resulting in a lower TSW. Yet, in Go11 and Ei12 this difference was not significant (Tab. 2.1). Semi-dwarfs had a slightly lower oil content than normal types. A statistical significant difference in oil content among growth types was only observed in season 2010/11.
Differences in oil yield were mainly influenced by differences in seed yield due to relatively small differences in oil content among growth types. Significant differences in oil yield were only observed when seed yield differed between the growth types. In most environments no clear difference in protein content could be observed between growth types. Only in Go12 and Go13 semi-dwarf types showed a significantly lower protein content of about 0.5% compared to normal types (Tab.2.1).

Analysis of variance for low nitrogen environments revealed significant differences in plant height, seed yield, straw yield, harvest index and oil yield between growth types (Tab.2.2). Begin of flowering, total biomass at maturity, TSW, oil and protein content were not significantly affected by the growth type. Significant genotypic variation was detected for all traits except for straw yield and total biomass. All traits were significantly affected by genotype by environment interactions. Heritabilities ranged from 0.17 (straw yield) up to 0.87 (oil content). For biomass no heritability was calculated due to a negative variance component for genotypes (Tab.2.2).

Table 2.2: Variance components of agronomic traits at low nitrogen supply in 2010/11, 2011/12 and 2012/13 (108 genotypes)

Source	**E**	**T**	**G**	**T**×**E**	**G**×**E**	h^2
Degrees of freedom	5	1	106	5	530	
Begin of flowering (days)	60.71^{**}	0^{ns}	0.23^{**}	0.09^{**}	0.29^{**}	0.77
Plant height (cm)	274.85^{**}	402.74^{**}	4.20^{**}	16.07^{**}	7.41^{**}	0.54
Seed yield (dt/ha)[a]	66.04^{**}	2.70^{*}	0.78^{**}	1.09^{**}	0.83^{**}	0.54
Straw yield (dt/ha)[a]	92.34^{*}	10.56^{*}	0.65^{ns}	5.39^{**}	5.86^{**}	0.17
Biomass (dt/ha)[b]	228.07^{**}	2.96^{ns}	0^{ns}	10.52^{**}	11.53^{**}	0
Harvest index[b]	0.68^{**}	0.23^{**}	0.02^{**}	0.005^{ns}	0.03^{**}	0.46
Thousand seed weight(g)	0.03^{**}	0.01^{ns}	0.02^{**}	0.01^{**}	0.01^{**}	0.83
Oil content(%)	1.67^{**}	0.04^{ns}	0.81^{**}	0.04^{+}	0.19^{**}	0.87
Oil yield (dt/ha)[a]	17.02^{**}	0.58^{*}	0.25^{**}	0.30^{**}	0.21^{**}	0.59
Protein content (%)	1.47^{**}	0.01^{ns}	0.07^{**}	0.03^{**}	0.06^{**}	0.59

$^{**,*,+}$ Significant difference at 0.01, 0.05, 0.10 probability level, respectively; ns non-significant (F-test from ANOVA); negative variance components were set to zero; **E** environment; **T** growth type; **G** genotype within growth type; h^2 heritability within growth types; **Biomass** seed yield + straw yield;

[a] values for five environments;

[b] values for four environments

2.3.2 Agronomic performance at high nitrogen supply

At high nitrogen supply both growth types started flowering at the same time or by one day delay (Go13) (Tab.2.3). In the season 2011/12 genotypes in Göttingen started flowering 11 days later than in Einbeck. Average height of semi-dwarfs across all environments was 88 cm with large variation between environments. Normal types had an average height of 124 cm and were on average about 36 cm taller compared to semi-dwarf types.

Table 2.3: Mean values and significance levels for agronomic traits at high nitrogen supply (108 genotypes, four environments)

Trait		Ei12	Go12	Ei13	Go13	Mean
Begin of flowering	Nt	104	115	122	126	117
(days)	Sd	104 ns	115 ns	122 ns	125 **	117 ns
Plant height	Nt	129	91	147	128	124
(cm)	Sd	78 **	67 *	115 **	92 **	88 **
Seed yield	Nt	-	26.4	28.8	37.8	31.0
(dt/ha)	Sd	-	29.7 **	27.5 **	37.6 ns	31.6 ns
Straw yield	Nt	33.4	39.4	54.8	41.2	42.2
(dt/ha)	Sd	30.1 **	32.1 **	44.5 **	34.6 **	35.4 *
Biomass	Nt	-	65.8	83.3	79.0	76.0
(dt/ha)	Sd	-	61.8 **	72.1 **	72.2 **	68.7 +
Harvest index	Nt	-	0.40	0.34	0.48	0.41
	Sd	-	0.48 **	0.38 **	0.52 ns	0.46 *
TSW	Nt	4.09	4.41	5.23	4.97	4.57
(g)	Sd	4.17 *	4.13 **	4.54 ns	4.75 **	4.40 ns
Oil content	Nt	49.0	47.9	47.4	47.6	48.0
(%)	Sd	47.6 **	46.7 **	46.8 +	46.9 **	47.0 **
Oil yield	Nt	-	12.6	13.7	18.0	14.8
(dt/ha)	Sd	-	13.8 **	12.9 **	17.6 ns	14.8 ns
Protein content	Nt	19.5	20.7	20.7	20.8	20.4
(%)	Sd	20.3 **	21.2 +	20.8 *	20.8 ns	20.7 ns

,*,+ Significant difference at 0.01, 0.05, 0.10 probability level, respectively; ns non-significant (F-test from ANOVA); **Ei Einbeck; **Go** Göttingen; **Sd** semi-dwarf; **Nt** normal type; **TSW** thousand seed weight

Lodging of normal types was observed in Einbeck 2013. Semi-dwarfs were not affected

by lodging (data not shown). Significant differences in seed yield among growth types were observed in Göttingen 2012 and Einbeck 2013 (Tab.2.3). Seed yield in Göttingen 2013 was similar for both types. The distribution of genotypes for seed yield in 2012 was similar for both growth types (Fig.2.2). In 2012 the highest yielding genotypes were semi-dwarfs while in 2013 normal types produced the highest seed yield. In 2013 seed yields of normal genotypes were distributed in a wider range compared to semi-dwarfs (Fig.2.2).

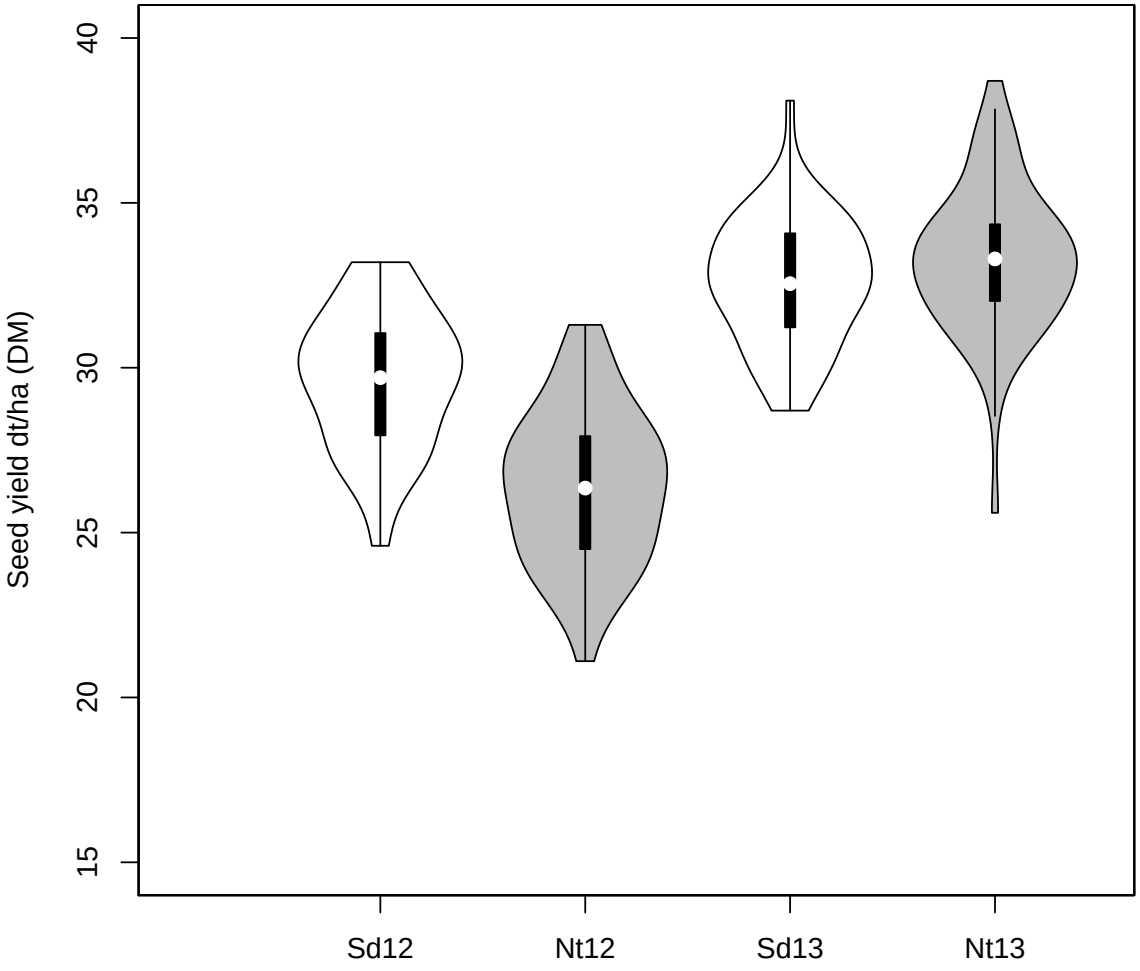

Figure 2.2: Violinplot of semi-dwarf (Sd) and normal type (Nt) hybrids for seed yield across two seasons at high nitrogen supply. The plot is a combination of a box plot and a kernel density plot.

In all environments semi-dwarf types formed significantly less straw than normal types

(Tab.2.3). In combination with seed yield this resulted in a higher harvest index of semi-dwarfs compared to normal types. Thousand seed weight of semi-dwarf types was significantly lower compared to normal types in Göttingen 2012 and 2013. In Einbeck 2012 semi-dwarfs had significantly higher thousand seed weight. Oil content of semi-dwarfs was significantly reduced by 1% on average across all environments compared to normal types. Semi-dwarf types showed a significantly higher protein content in Einbeck 2012,2013 and Göttingen 2012. In Göttingen 2013 both growth types had same mean values for protein content (Tab.2.3).

The environments differed significantly for all traits (Tab.2.4). Significant differences between growth types were detected for plant height, straw yield, total biomass yield, harvest index and oil content. Seed yield and oil yield were not significantly different among growth types. Significant genotypic variations were observed for all traits. Interaction effects of growth type by environment and genotype by environment influenced all traits significantly. Heritabilities (h^2) ranged from 0.27 for seed yield to 0.83 for thousand seed weight (Tab.2.4).

Table 2.4: Variance components of agronomic traits at high nitrogen supply in 2011/12 and 2012/13 (108 genotypes)

Source	**E**	**T**	**G**	**T**×**E**	**G**×**E**	$\boldsymbol{h^2}$
Degrees of freedom	3	1	106	3	318	
Begin of flowering (days)	92.80^{**}	0^{ns}	0.18^{**}	0.16^{**}	0.44^{**}	0.56
Plant height (cm)	456.81^{**}	622.62^{**}	3.74^{**}	59.63^{**}	5.29^{**}	0.54
Seed yield (dt/ha)[a]	30.88^{**}	0^{ns}	0.73^{*}	2.08^{+}	2.10^{**}	0.27
Straw yield (dt/ha)	57.36^{**}	22.18^{*}	6.01^{**}	3.86^{**}	5.50^{**}	0.66
Biomass (dt/ha)[a]	54.44^{**}	24.30^{+}	10.22^{**}	6.34^{**}	6.36^{**}	0.59
Harvest index[a]	0.45^{**}	0.14^{*}	0.37^{**}	2.97^{+}	0.04^{**}	0.65
Thousand seed weight (g)	0.12^{**}	0.01^{ns}	0.02^{**}	0.01^{**}	0.01^{**}	0.83
Oil content (%)	0.22^{*}	0.51^{**}	0.42^{**}	0.06^{*}	0.20^{**}	0.73
Oil yield (dt/ha)[a]	6.82^{**}	0^{ns}	0.28^{**}	0.43^{+}	0.46^{**}	0.40
Protein content (%)	0.21^{**}	0.03^{ns}	0.03^{**}	0.08^{**}	0.05^{*}	0.33

$^{**,*,+}$ Significant difference at 0.01, 0.05, 0.10 probability level, respectively; ns non-significant (F-test from ANOVA); negative variance components were set zero; **E** environment; **T** growth type; **G** genotype within growth type; $\boldsymbol{h^2}$ heritability within growth types; **Biomass** seed yield + straw yield;
[a] values for three environments

2.3.3 Comparison of agronomic performance at low and high nitrogen supply

Nitrogen fertilization increased seed yield at all environments (Tab.2.5). Without nitrogen fertilization semi-dwarf hybrids produced between 43 % (Go12) and 91 % (Go13) of the seed yield achieved at fertilized conditions. Normal types produced between 40 % (Go12) and 82 % (Go13) of the seed yield achieved with nitrogen fertilization. In general seed yields without nitrogen fertilization in Go12 were rather low while in Go13 seed yields proved to be very high compared to other environments without nitrogen fertilization. The mean seed yield of semi-dwarf hybrids without nitrogen fertilization was reduced by 28 % compared to the fertilized treatment. Normal types responded to nitrogen deficiency on average with a yield reduction of 33 % compared to the fertilized treatment. The mean difference in seed yield of 5 % between growth types across environments, proved to be non-significant (Tab.2.5).

Table 2.5: Seed yield, straw yield and their coefficients at low (N0) and high (N1) nitrogen supply in 2011/12 and 2012/13 (108 genotypes)

		Low nitrogen			**High nitrogen**			**Coefficient N0:N1**		
		Nt	**Sd**		**Nt**	**Sd**		**Nt**	**Sd**	
Seed yield	Go12	10.4	12.8	**	26.4	29.7	**	0.40	0.43	+
(dt/ha)	Ei13	22.5	22.4	ns	28.5	27.5	+	0.79	0.82	*
	Go13	30.7	33.9	**	37.8	37.6	ns	0.82	0.91	ns
	Mean	21.2	23.0	ns	30.9	31.6	ns	0.67	0.72	ns
Straw yield	Ei12	18.9	17.7	ns	33.4	30.1	**	0.58	0.60	ns
(dt/ha)	Go12	22.9	19.8	**	39.4	32.1	**	0.58	0.62	+
	Ei13	46.4	36.1	**	54.8	44.5	**	0.85	0.83	ns
	Go13	36.4	32.0	+	41.2	34.6	**	0.89	0.94	+
	Mean	31.1	26.4	+	42.2	35.4	*	0.73	0.75	ns

,*,+ Significant difference at 0.01, 0.05, 0.10 probability level, respectively; ns non-significant (F-test from ANOVA); **Nt normal types; **Sd** semi-dwarfs; **Ei** Einbeck; **Go** Göttingen; all traits based on dry matter

Growth types differed significantly in straw yield at both nitrogen levels (Tab.2.5). At low nitrogen supply semi-dwarf types produced between 60 % (Ei12) and 94 % (Go13) of straw compared to high nitrogen supply. Normal types produced between 58 % (Ei12, Go12) and 89 % (Ei13) of straw compared to high nitrogen supply. Mean values for straw yield across all environments revealed no significant difference in the reduction of straw yield among growth types. In general it seems that seed yield responded stronger

to nitrogen deficiency than straw yield.

Seed yield of semi-dwarfs at low and high nitrogen supply is correlated by r = 0.54 (Tab.2.6, Fig.2.3). Seed yield of normal types at low and high nitrogen is correlated by r = 0.55. Coefficients of correlation for single traits differ between growth types.

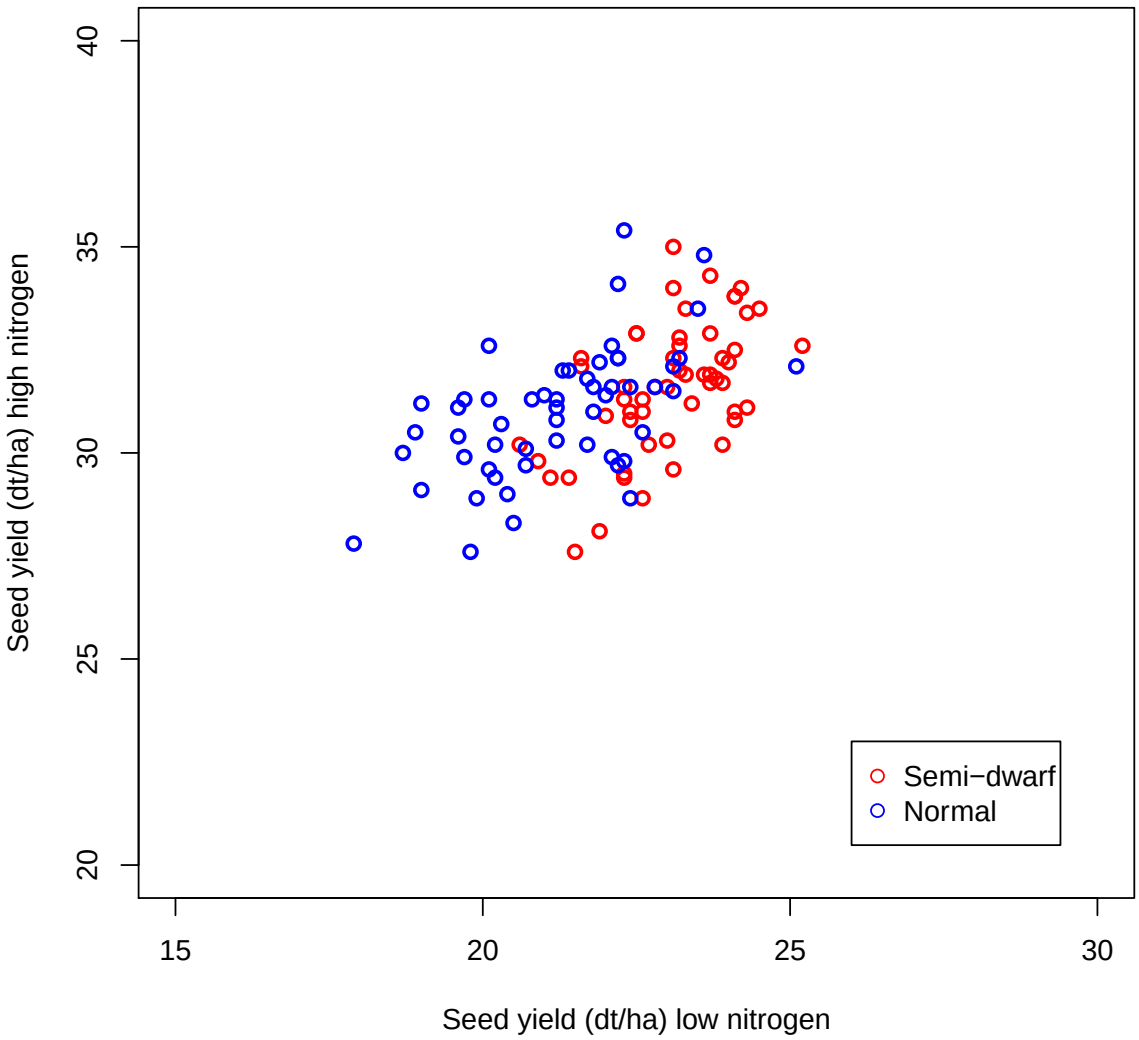

Figure 2.3: Mean seed yields across three environments (Go12, Ei13, Go13) at low and high nitrogen supply (108 genotypes)

At low nitrogen supply seed yield of semi-dwarf hybrids was significantly negatively correlated to begin of flowering and thousand seed weight (Tab.2.7). Normal type hybrids also showed negative correlation but without significance. Significant positive correlations were observed for seed yield and harvest index as well as for seed yield and oil yield. Straw yield and plant height showed no correlation with seed yield in both types. Harvest index in both growth types was significantly negatively correlated with straw yield. Protein content in the semi-dwarfs as well as in the normal types was significantly negatively correlated with seed yield and oil content.

Seed yield of semi-dwarfs at high nitrogen supply was significantly positive correlated to plant height, straw yield and oil content (Tab.2.7). No significant correlation was observed between harvest index and seed yield. Seed yield of normal types was significantly correlated to harvest index ($r = 0.55$). Normal types showed no significant correlation of plant height, straw yield and oil content with seed yield. In both types a significant negative correlation of straw yield and harvest index has been observed. Protein content in both types was negatively correlated with oil content (Tab.2.7).

The ANOVA including both nitrogen treatments revealed highly significant differences among environments for all traits except oil content (Tab.2.8). Significant differences between nitrogen fertilization levels were observed for begin of flowering, straw yield, oil content and protein content only. Seed yield revealed no significant difference between the two fertilization levels. Both growth types differed significantly for plant height, straw yield, harvest index, TSW and oil content. A significant genotypic variation was observed for all traits. Significant interactions between nitrogen fertilization and environment were observed for all traits except straw yield. Interactions between growth type and environment were significant for all traits. Significant interactions between growth type and nitrogen fertilization were observed for plant height, straw yield, oil content and protein content. Interactions of genotypes with fertilization level were observed for straw yield, total biomass and TSW.

Table 2.6: Mean values, range and rank correlations for agronomic traits between low and high nitrogen supply (108 genotypes, four environments)

Trait		low nitrogen			high nitrogen			$r_{N0:N1}$
		Min	Max	Mean	Min	Max	Mean	
Begin of flowering	Nt	115	117	116	116	118	117	0.77 **
(days)	Sd	114	117	116 ns	115	118	117 ns	0.86 **
Plant height	Nt	109	120	116	119	130	124	0.42 **
(cm)	Sd	80	95	86 **	81	96	88 **	0.79 **
Seed yield[a]	Nt	17.9	25.1	21.2	27.6	35.4	30.9	0.55 **
(dt/ha)	Sd	20.6	25.2	23.0 ns	27.6	35.0	31.6 ns	0.54 **
Straw yield	Nt	26.6	36.8	31.1	37.1	49.1	42.2	0.33 *
(dt/ha)	Sd	22.8	30.8	26.4 +	29.0	45.2	35.4 *	0.65 **
Biomass[a]	Nt	49.1	62.7	56.4	68.8	85.5	76.0	0.39 **
(dt/ha)	Sd	44.6	58.3	52.3 ns	60.8	81.5	68.7 +	0.50 **
Harvest index	Nt	0.32	0.42	0.37	0.36	0.46	0.41	0.49 **
	Sd	0.37	0.48	0.43 **	0.36	0.52	0.46 *	0.65 **
TSW	Nt	4.15	5.05	4.64	4.26	5.06	4.57	0.49 **
(g)	Sd	4.00	4.87	4.45 **	4.02	4.82	4.40 ns	0.89 **
Oil content	Nt	47.6	51.4	49.5	46.5	49.7	48.0	0.76 **
(%)	Sd	47.4	51.1	49.2 ns	45.4	48.1	47.0 **	0.84 **
Oil yield[a]	Nt	9.1	12.2	10.6	13.3	17.2	14.8	0.62 **
(dt/ha)	Sd	9.9	12.9	11.5 ns	12.4	16.4	14.8 ns	0.57 **
Protein content	Nt	17.9	19.2	18.6	19.8	21.3	20.4	0.32 *
(%)	Sd	17.4	19.2	18.3 ns	20.1	21.3	20.7 ns	0.53 **

,*,+ Significant difference at 0.01, 0.05, 0.10 probability level, respectively; ns non-significant (F-test from ANOVA); $r_{N0:N1}$ Spearman's rank correlation between low and high nitrogen supply; **Nt normal types; **Sd** semi-dwarfs; **Biomass** seed yield + straw yield; **TSW** thousand seed weight; all traits based on dry matter; (a) values across three environments (Go12, Ei13 and Go13)

Table 2.7: Rank correlations for agronomic traits across four environments (Ei12, Go12, Ei13,Go13) at low and high nitrogen supply

Low nitrogen supply		BFL	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(2) Plant height	Nt	0.35^{**}							
	Sd	0.43^{**}							
(3) Seed yield [a]	Nt	-0.04^{ns}	-0.11^{ns}						
	Sd	-0.30^{*}	0.02^{ns}						
(4) Straw yield	Nt	0.37^{**}	0.31^{*}	0.05^{ns}					
	Sd	0.25^{ns}	0.33^{*}	0.09^{ns}					
(5) Harvest index [a]	Nt	-0.35^{*}	-0.40^{**}	0.68^{**}	-0.53^{**}				
	Sd	-0.37^{**}	-0.16^{ns}	0.63^{**}	-0.52^{**}				
(6) TSW	Nt	0.07^{ns}	-0.18^{ns}	-0.26^{ns}	-0.11^{ns}	-0.06^{ns}			
	Sd	0.12^{ns}	0.11^{ns}	-0.30^{*}	0.09^{ns}	-0.27^{ns}			
(7) Oil content	Nt	-0.07^{ns}	-0.13^{ns}	0.13^{ns}	-0.03^{ns}	0.06^{ns}	-0.17^{ns}		
	Sd	-0.18^{ns}	-0.15^{ns}	0.21^{ns}	-0.21^{ns}	0.32^{*}	-0.20^{ns}		
(8) Oil yield[a]	Nt	-0.08^{ns}	-0.12^{ns}	0.93^{**}	0.03^{ns}	0.65^{**}	-0.32^{*}	0.44^{**}	
	Sd	-0.34^{*}	-0.05^{ns}	0.92^{**}	-0.03^{ns}	0.67^{**}	-0.31^{ns}	0.53^{**}	
(9) Protein content	Nt	0.14^{ns}	-0.04^{ns}	-0.36^{**}	0.03^{ns}	-0.30^{*}	0.18^{ns}	-0.44^{**}	-0.49^{**}
	Sd	0.30^{*}	0.25^{ns}	-0.40^{**}	0^{ns}	-0.26^{ns}	0.41^{**}	-0.47^{**}	-0.52^{**}
High nitrogen supply		BFL	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(2) Plant height	Nt	0.15^{ns}							
	Sd	0.52^{**}							
(3) Seed yield [a]	Nt	0.15^{ns}	-0.20^{ns}						
	Sd	0.09^{ns}	0.41^{**}						
(4) Straw yield	Nt	0.12^{ns}	0.07^{ns}	0.11^{ns}					
	Sd	0.49^{**}	0.71^{**}	0.33^{*}					
(5) Harvest index [a]	Nt	-0.02^{ns}	-0.20^{ns}	0.50^{**}	-0.71^{**}				
	Sd	-0.38^{**}	-0.39^{**}	0.24^{ns}	-0.77^{**}				
(6) TSW	Nt	0^{ns}	0.19^{ns}	-0.21^{ns}	-0.05^{ns}	-0.16^{ns}			
	Sd	0^{ns}	0.01^{ns}	-0.07^{ns}	0.28^{*}	-0.26^{ns}			
(7) Oil content	Nt	0.23^{ns}	0.04^{ns}	0.13^{ns}	-0.05^{ns}	0.13^{ns}	-0.10^{ns}		
	Sd	-0.16^{ns}	-0.05^{ns}	0.29^{*}	-0.08^{ns}	0.21^{ns}	0.10^{ns}		
(8) Oil yield [a]	Nt	0.19^{ns}	-0.21^{ns}	0.92^{**}	0.03^{ns}	0.53^{**}	-0.27^{*}	0.44^{**}	
	Sd	0.06^{ns}	0.35^{**}	0.95^{**}	0.30^{*}	0.24^{ns}	-0.01^{ns}	0.52^{**}	
(9) Protein content	Nt	-0.05^{ns}	0.08^{ns}	-0.03^{ns}	0.23^{ns}	-0.29^{*}	0.10^{ns}	-0.31^{*}	-0.08^{ns}
	Sd	0.22^{ns}	0.03^{ns}	-0.54^{**}	0.14^{ns}	-0.37^{**}	0.12^{ns}	-0.49^{**}	-0.59^{**}

,*,+ Significant at 0.01, 0.05, 0.10 probability level, respectively; ns non-significant; **Nt normal types; **Sd** semi-dwarfs; **BFL** begin of flowering; **TSW** thousand seed weight; (a) values across three environments (Go12, Ei13 and Go13)

Table 2.8: Variance components (Var.cp.) for a split-split-plot model with 108 genotypes (54 semi-dwarf, 54 normal types) across four environments at low or high nitrogen supply

Source	**Environment(E)**		**Nitrogen(N)**		**Growth type(T)**		**Genotype(G)**		**E×N**	
	Df	Var.cp.	Df	Var.cp.	Df	Var.cp.	Df	Var.cp.	Df	Var.cp.
BFL (days)	3	88.83 **	1	0.15^{+}	1	0 ^{ns}	106	0.19 **	3	0.13 **
Plant height (cm)	3	428.52 **	1	7.26^{ns}	1	522.90 **	106	3.70 **	3	18.00 **
Seed yield (dt/ha)[a]	2	59.08 **	1	34.91^{ns}	1	0.29 ^{ns}	106	0.76 **	2	19.98 **
Straw yield (dt/ha)	3	78.78 **	1	46.93*	1	15.33 *	106	3.90 **	3	6.46 ^{ns}
Biomass (dt/ha)[a]	2	168.90 **	1	140.02^{ns}	1	12.94 ^{ns}	106	5.37 **	2	64.79 **
Harvest index[a]	2	0.005 **	1	0	1	0.002*	106	0.0003**	2	0.0010**
TSW (g)	3	0.02 **	1	0	1	0.01 *	106	0.02 **	3	0.11 **
Oil content (%)	3	0.07 ^{ns}	1	1.46^{+}	1	0.21 **	106	0.61 **	3	1.04 **
Oil yield (dt/ha)[a]	2	14.71 **	1	5.02^{ns}	1	0	106	0.28 **	2	5.49 **
Protein content (%)	3	0.21 **	1	1.89^{+}	1	0	106	0.04 **	3	1.35 **

Source	**T×E**		**T×N**		**G×N**		**G×E**		h^2
	Df	Var.cp.	Df	Var.cp.	Df	Var.cp.	Df	Var.cp.	
BFL (days)	3	0.14 **	1	0.02^{ns}	106	0 ^{ns}	318	0.32 **	0.67
Plant height (cm)	3	39.13 **	1	7.49^{ns}	106	0.25 ^{ns}	318	5.60 **	0.62
Seed yield (dt/ha)[a]	2	1.35 **	1	0 ^{ns}	106	0 ^{ns}	212	1.05 **	0.48
Straw yield (dt/ha)	3	5.37 **	1	0.88^{+}	106	0.57 $^{+}$	318	2.48 **	0.73
Biomass (dt/ha)[a]	2	9.84 **	1	2.05^{ns}	106	1.34 $^{+}$	106	4.32 **	0.60
Harvest index[a]	2	0.0001**	1	0	106	0	212	0	0.74
TSW (g)	3	0.01 **	1	0	106	0.001^{+}	318	0.005 **	0.90
Oil content(%)	3	0.01 $^{+}$	1	0.11^{+}	106	0	318	0.08 **	0.88
Oil yield (dt/ha)[a]	2	0.33 **	1	0.07^{ns}	106	0	212	0.26 **	0.57
Protein content (%)	3	0.03 **	1	0.07^{+}	106	0	318	0.02 *	0.54

**,*,$^{+}$ Significant at 0.01, 0.05, 0.10 probability level respectively, ns nonsignificant (F-test from ANOVA); **Df** degrees of freedom ;**Var.cp.** variance component, negative variance components were set to zero; h^2 heritability within growth types; **BFL** begin of flowering; **Biomass** seed yield + straw yield; **TSW** thousand seed weight

2.4 Discussion

In the present study the agronomic performance of semi-dwarf and normal type winter oilseed rape hybrids was compared at two contrasting nitrogen supply regimes. In Einbeck 2013 semi-dwarfs revealed a clear superiority in lodging resistance compared to normal types at high nitrogen supply (data not shown). This is in accordance with observations from Wang et al. (2004), who argued that the different branching structure of semi-dwarfs, with branches starting at lower stem sections, lead to a more even weight distribution throughout the plant stem. Genotypes within growth types varied significantly in plant height. But no semi-dwarf was taller than the shortest normal type and vice versa. Plant height of both growth types showed no significant difference between nitrogen supply levels. The average height of semi-dwarfs differed only by 2 cm between low and high nitrogen supply (Tab. 2.6).

At low nitrogen supply seed yield of semi-dwarf hybrids was significantly higher (2.4 dt/ha) compared to normal hybrids (Tab. 2.1). The different performance in individual environments (no difference in Einbeck 2013) is expressed as significant growth types by environment interaction (Tab. 2.2). At high nitrogen supply both growth types produced similar seed yields with a non-significant difference of 0.2 dt/ha. In Göttingen 2012 plants were relatively short compared to other environments and semi-dwarf hybrids had a significantly higher seed yield than normal types (Tab. 2.3). In Einbeck 2013 they yielded less than normal types and in Göttingen 2013, no significant difference in seed yield was observed among growth types at high nitrogen supply. The yield advantage of normal types in Einbeck 2013 (high nitrogen supply) would have been probably greater without lodging. The ANOVA for high nitrogen supply revealed a significant effect of growth type by environment and genotype by environment interactions on seed yield, resulting in a low heritability of $h^2 = 0.27$ (Tab. 2.4). To draw a meaningful conclusion about the effect of the dwarf gene on seed yield at conditions of high nitrogen supply more environments need to be tested. The comparison of seed yields at low and high nitrogen supply give the impression that normal types respond to nitrogen deficiency by a stronger reduction in seed yield than semi-dwarf types (Tab. 2.5). But results from ANOVA across both nitrogen levels revealed no significant interaction for growth type and nitrogen level (Tab. 2.8). The factors environment and nitrogen level had by far the greatest effect on seed yield (Tab. 2.8). Nevertheless the effect of nitrogen supply was not significant due to a significant environment by nitrogen supply level interaction which indicates that nitrogen fertilization had different effects in single environments. Especially the difference in seed yield between low and high nitrogen supply in Göttingen 2013 was relatively small compared to other environments. This might have been caused by high rates of nitrogen mineralization in this environment resulting in a high nitrogen supply from the soil even in

the unfertilized experiments. Genotypes within growth types differed significantly in seed yield but revealed no interaction with nitrogen supply. Thus the ranking of genotypes for seed yield should be the same at both nitrogen supply levels and high yielding genotypes at low nitrogen supply are also superior at high nitrogen supply. However, rank correlations for seed yield between low and high nitrogen supply are rather low for both growth types (semi-dwarf r=0.54, normal type r=0.55)(Tab. 2.6). The rank shift in seed yield between low and high nitrogen levels could have been caused by experimental errors. Straw yield of semi-dwarfs at low nitrogen supply was significantly reduced compared to normal types (Tab.2.1). The relative differences in straw yield between growth types varied among environments resulting in a significant environment by growth type interaction effect (Tab. 2.2). Genotypic variation for straw yield was non-significant but genotype by environment interaction was highly significant (Tab.2.2). Heritability for straw yield at low nitrogen supply was low ($h^2 = 0.17$) due to high experimental errors. It is often observed that experimental errors at suboptimal conditions are higher compared to optimal conditions and heritabilities are reduced. At high nitrogen supply straw yield of semi-dwarf hybrids was significantly reduced compared to normal types. Interaction between growth type and environment was significant as already observed at low nitrogen supply. Growth types and genotypes within the growth types seem to respond differently in straw yield at different nitrogen levels indicated by a significant growth type by nitrogen supply or genotype by nitrogen supply interaction, respectively (Tab. 2.8). A significant difference in straw yield between low and high nitrogen supply was also observed by Kessel et al. (2012). The authors reported no significant interaction between genotype and environment for straw yield.

Due to higher or similar seed yields and less straw yield, harvest index of semi-dwarf hybrids was significantly improved at both nitrogen levels compared to normal hybrids (Tab.2.1, Tab. 2.3). This result is contradictory to conclusions by Sieling and Kage (2008). The harvest indexes of normal hybrids at low nitrogen supply (0.34) and high nitrogen supply (0.41) in the present experiment are in accordance with results from Kessel et al. (2012), describing a harvest index of 0.42 (high nitrogen supply) and 0.36 (low nitrogen supply) for normal hybrid varieties. Correlation coefficient of semi-dwarfs for seed yield and harvest index at high nitrogen supply was only 0.24 (Tab.2.7). Normal types showed a significant correlation of 0.50. By comparing total biomass yields of both growth types at maturity it becomes obvious that the improved harvest index of semi-dwarfs is mostly based on a reduction in straw yield. Therefore the reduction of vegetative biomass (straw) does not seem to result in an improved seed yield at high nitrogen supply. On the other hand the semi-dwarf growth type revealed no disadvantage in seed yield at high nitrogen supply either and still possesses other benefits like a swifter harvest and improved lodging

resistance.

Wang et al. (2004) conducted field experiments over two years with ten semi-dwarf hybrids using the *ndf1* dwarf gene which is probably allelic to *bzh*. The authors described a good agronomic performance of semi-dwarf hybrids based on more seeds per pod and higher thousand seed weight than the normal parent. The number of pods per plant was reported to be lower compared to conventional cultivars (Wang et al., 2004). In the present experiment, thousand seed weight of semi-dwarfs was slightly lower at low and high nitrogen supply compared to normal types, but without significance at high nitrogen supply (Tab. 2.6). Considering the higher or similar seed yield of semi-dwarfs this could indicate that number of seeds per pod or number of pods is increased compared to normal types.

Protein content was significantly affected by nitrogen supply (Tab. 2.8) with higher values at high nitrogen supply compared to low nitrogen supply (Tab. 2.6). This oberservation is in accordance with other results published (Brennan et al., 2000; Rathke et al., 2005). It is known that protein and oil content are negatively correlated due to their competition for carbon compounds during carbohydrate metabolism (Bhatia and Rabson, 1976). Proteins have a lower carbohydrate content and an increased N supply intensifies the protein synthesis at the expense of fatty acid synthesis and thus reducing the oil content in seeds (Bhatia and Rabson, 1976; Rathke et al., 2005). The negative correlation of protein content and oil content had been observed at both nitrogen levels (Tab. 2.7). As a consequence of this competition oil content at high nitrogen supply was significantly reduced compared to low nitrogen supply (Tab.2.6, 2.8). Oil content of normal types at high nitrogen supply was significantly lower compared to semi-dwarfs (Tab. 2.6). At low nitrogen supply no significant difference in oil content was detected among growth types. The reason for variation in oil content between growth types at high nitrogen supply is unknown.

Based on the results of the present study, semi-dwarf hybrids seem to have a superiority in seed yield at nitrogen deficient conditions compared to normal types. At high nitrogen supply no significant difference in seed yield had been observed between both growth types but contrary results at single environments demand for further testing at more environments with high nitrogen supply. Other agronomic traits were not negatively affected by the dwarf gene *Bzh*. Therefore the semi-dwarf growth type is an interesting option for rapeseed cultivation, especially for cropping systems with low nitrogen input.

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3 Nitrogen use efficiency of semi-dwarf and normal type hybrids

3.1 Introduction

Germany produced about 3.41 million tons of oilseed rape oil in 2012 (UFOP, 2012). Most of the oil is used for the production of biofuels and technical oils. In 2009 the European Union (EU) introduced the Renewable Energy Directive (RED 2009/28/EG) with the objective to increase the use of biofuels. To meet the new regulatory standards only biofuels with a reduction of 35 % in greenhouse gas (GHG) emission compared to conventional fossil fuels are allowed. This percentage will increased to at least 50% by 2017.

The vast majority of GHG emissions from biofuels based on oilseed rape oil are caused by cultivation and processing of oilseed rape. More than 82 % of GHG emissions in the cultivation of winter oilseed rape is caused by N-fertilization (42 %) and N_2O emissions in the field (40.8 %) (Schiemenz and Gurgel, 2013).

In field trials in Northern Germany Schiemenz and Gurgel (2013) investigated the effect of a reduction in GHG emissions to 50 % compared to fossil fuels on the cultivation of winter oilseed rape. In a typical crop rotation winter wheat is followed by oilseed rape. For a oilseed rape yield of 50 dt/ha 247 kg N/ha are required. To meet future EU regulations nitrogen fertilization would have to be reduced to 83 kg N/ha. As a consequence oilseed rape yield would decrease to 38 dt/ha (Schiemenz and Gurgel, 2013). Although it is unlikely that farmers respond to the new regulations only by reducing nitrogen fertilization, the scenario illustrates the future challenge of maintaining current seed yields while using less mineral nitrogen fertilizer than today. This challenge could be met by the development of winter oilseed rape cultivars with an improved nitrogen use efficiency. In the present study nitrogen use efficiency is defined as seed production per unit nitrogen available in the soil. According to Moll et al. (1982) it is subdivided into the proportion of available nitrogen that is taken up by the plant (nitrogen uptake efficiency) and the efficiency with which the absorbed nitrogen is utilized to produce seeds (nitrogen utilization efficiency).

Genetic variation for nitrogen uptake efficiency and nitrogen utilization efficiency between European winter oilseed rape cultivars and breeding lines was detected in several studies (Berry et al., 2010; Kessel et al., 2012; Schulte auf'm Erley et al., 2011). At low nitrogen conditions nitrogen uptake proved to be more important for seed yield than nitrogen utilization. With increasing nitrogen supply nitrogen utilization become more important (Berry et al., 2010; Kessel et al., 2012). Interactions between genotype and nitrogen level have been observed as well. That means that genotypes selected for high yield at high nitrogen conditions might not be high yielding under low nitrogen conditions (Berry et al., 2010; Kessel et al., 2012).

The objective of this study was to compare the nitrogen use efficiency of semi-dwarf and normal-type winter oilseed rape hybrids. Semi-dwarf types are of special interest, because they might need less nitrogen per unit seed yield than normal types, due to the reduced formation of vegetative biomass. To evaluate the response to nitrogen deficiency, genotypes were tested at low and high nitrogen conditions. Furthermore, parameters influencing nitrogen use efficiency at low and high nitrogen supply were investigated.

3.2 Materials and Methods

3.2.1 Plant Material and Experimental Design

A doubled-haploid (DH) population (242 lines) derived from the cross 'Alesi-*bzh* x H30' was produced. The 'Alesi-*bzh*' parent is a dwarf isogenic line derived from the German winter oilseed cultivar 'Alesi' backcrossed (BC4) to the *bzh* dwarf mutant described by Foisset et al. (1995). 'H30' is a normal type, resynthesized rapeseed line originating from a cross of *Brasscia rapa* ssp. *chinensis* with *Brasscica napus* ssp. *napus* var. *pabularia* with moderate winterhardiness (Girke, 2002). The DH population is segregating into dwarf and tall growing DH lines. All DH lines were crossed with a normal type, male sterile tester (canola quality) using the MSL ('Männliche Sterilität Lembke') hybrid system (Paulmann and Frauen, 1998). The resulting testcross hybrids (TC) segregate into semi-dwarf and normal types.

In season 2010/11 150 TC (75 semi-dwarfs and 75 normal types) were selected based on the availability of seeds and evaluated in two adjacent experiments. One experiment was harvested at the end of flowering (EOF) (BBCH 69, Lancashire et al. (1991)) and the second experiment at seed maturity (BBCH 89). Experiments were conducted at two sites near Einbeck (Ei) (brunic aerosol) and near Göttingen (Go) (alluvial meadow soil) in Central Germany. The plot sizes were 18 m^2 in Einbeck and 11.25 m^2 in Göttingen with plant densities of 50 and 90 plants/m^2, respectively. Both experiments were evaluated without nitrogen fertilization (low nitrogen supply). Soil mineral nitrogen determined at

the beginning of plant growth in spring was 35 kg N/ha in Einbeck (Ei11) and 27 kg N/ha in Göttingen (Go11). All other agronomic treatments (except fertilization) were done according to recommendations for oilseed rape cultivation in Germany. Growth types were interlaced within each block by alternately growing one strip of semi-dwarfs and one strip of normal types. Experiments were designed as split-plot with growth type as main plot factor and genotypes as subplot factor. The experimental design was identical for both environments.

In season 2011/12 experiments from season 2010/11 were repeated using 108 TC (54 semi-dwarfs and 54 normal types). The 108 TC were selected based on erucic acid content and seed yield in season 2010/11. For each growth type 27 TC with erucic acid (14 highest seed yield, 13 lowest seed yield) and the 27 TC without erucic acid (14 highest seed yield, 13 lowest seed yield) were selected. Hybrids were evaluated in two identical experiments at both sites (Göttingen and Einbeck). One experiment was harvested at the end of flowering (BBCH 69) and the second experiment at seed maturity (BBCH 89). Both experiments were conducted at two nitrogen levels: without nitrogen fertilization (low nitrogen supply) and fertilized with 177 kg N/ha (high nitrogen). Mineral nitrogen in the soil was determined at the beginning of plant growth in spring in Einbeck (27 kg N/ha) and Göttingen (27 kg N/ha). Both growth types were interlaced within each block and arranged in split-plot design. Genotypes within growth types were randomized in lattice design.

Each season-site combination is considered as one environment and is addressed by location abbreviation and harvest year, such as Ei11 for Einbeck in the season 2010/2011.

3.2.2 Evaluated traits and Measurements

The following measurements and calculations were done for every single plot in the experiments. Begin of flowering in days from 1st of January was recorded at BBCH 61, when 10 % of the plants flowered. Plant height in cm was measured at grain maturity (BBCH 89). Biomass yield was determined in the first experiment by harvesting whole plots with a combine harvester at EOF. The plots were cut right above soil level and biomass yield was weighted. A subsample of fresh plant material of every plot was weighed and dried at 105 °C for 72 h in a dry oven. Dried biomass samples was weighed again to calculate dry matter content in the biomass. The dried biomass samples were milled (particle sizes of about 0.2 mm) for nitrogen analysis.

In the second experiment seed yield was determined with a combine harvester at maturity (BBCH 89). At the same time the straw of every plot was collected in a canvas tissue attached to the back of the combine harvester and weighted (except in Einbeck 2011). Therefore all plants in a plot were cut right above soil level by the harvester. A straw

subsample including stems and pod walls was taken, weighed and dried at 60 °C for 48 h in a dry oven. The dried subsample was weighted to calculate straw dry matter content and subsequently milled (particle sizes of 0.2 mm). Due to a heavy hail storm in Einbeck 2012, seed yield could not be determined at this location.

Nitrogen content was determined by analysing approximately 16 mg grinded material by the Dumas based combustion method using a CN Elemental analyser, type vario EL (Elementar Analysensysteme GmbH) as described in the manual (Anonymous 1992). Thousand seed weight (TSW) was measured on a representative sample of 500 seeds. Oil, protein and moisture content in % at seed dry matter basis were determined with a NIRS monochromator model 6500 (NIRSystems, Inc., Silversprings, MD, USA) using calibrations (raps2010.eqa, raps2011.eqa, raps2012.eqa) provided by VDL-UFA Qualitätssicherung NIRS GmbH. Nitrogen yield in kg N/ha at end of flowering was calculated on basis of biomass dry matter yield and nitrogen content in the biomass. Nitrogen yield at grain maturity was calculated as the sum of nitrogen in the straw and nitrogen in the seeds. Nitrogen yield of the straw (kg N/ha) was calculated on basis of straw dry matter yield and nitrogen content in the straw. Nitrogen yield in the grain (kg N/ha) was calculated on the basis of seed dry matter yield (kg/ha) and nitrogen content in the seeds (%), obtained by dividing the NIRS determined protein content in the seeds (%) by the factor 6.25. Nitrogen harvest index was calculated dividing nitrogen in the seeds by total nitrogen yield at grain maturity.
Nitrogen use efficiency is defined as seed production per unit of nitrogen available in the soil (Moll et al., 1982). According to (Moll et al., 1982) nitrogen use efficiency, nitrogen uptake efficiency and nitrogen utilization efficiency were calculated using the following equations:

Nitrogen use efficiency = Seed yield (kg/ha) / total nitrogen supply (kg N/ha)

Nitrogen uptake efficiency = Nitrogen fixed in the plant at maturity (kg N/ha) / total nitrogen supply (kg N/ha)

Nitrogen utilization efficiency = Seed yield (kg/ha)/ Nitrogen fixed in the plant at maturity (kg N/ha)

Nitrogen supply at the unfertilized treatment was determined in each replication by the highest amount of total nitrogen taken up by a genotype at maturity. The nitrogen supply at fertilised treatment was determined by the mean value of nitrogen supply for both unfertilized replications plus the amount of nitrogen fertilised. The total amount

of nitrogen fixed in a plant at maturity is the sum of nitrogen in straw and nitrogen in seeds. The relative contributions of nitrogen uptake and nitrogen utilization efficiency to variation in overall nitrogen use efficiency were calculated according to Moll et al. (1982).

3.2.3 Statistical analysis

Analysis of variance (ANOVA), heritabilities (h^2) and Spearman's rank correlation coefficients were calculated using PLABSTAT software (Version 3B) (Utz 2011).
ANOVA within each N-level for a single environment was done using the following model for a split-plot design:

$$Y_{jkl} = \mu + b_j + t_k + \epsilon_{jk} + g_{kl} + \epsilon_{jkl} \tag{3.1}$$

in which Y_{jkl} is the observation of a genotype (g_l) within growth type (t_k) in block (b_j). The effect of the genotype within growth type is denoted by (g_{kl}). Whole plot error is denoted by ϵ_{jk} and the sub-plot error is called ϵ_{jkl}. The general mean is designated by μ. The factors block and genotype were considered as random.
To include the effect of different environments (year-location combination) into the ANOVA, following model for a split-plot design was used:

$$\begin{aligned} Y_{ijkl} = \mu + e_i + t_k + b_{ij} + et_{ik} + \epsilon_{ijk} \\ + g_{kl} + eg_{ikl} + \epsilon_{ijkl} \end{aligned} \tag{3.2}$$

in which Y_{ijkl} is the observation of a genotype (g_l) within the growth type (t_k) in block (b_j) within environment (e_i). Interaction effects between growth type and environment are denoted by et_{ik} and interactions between environment and genotype are called eg_{ikl}. Whole plot error is denoted by ϵ_{ijk} and the sub-plot error is denoted by ϵ_{ijkl}. The general mean is denoted by μ. The factors environment, block and genotype were considered as random.

To test the effects of nitrogen levels, following model for a split-split-plot design was used:

$$\begin{aligned} Y_{ijklo} = \mu + e_i + n_o + b_{ijo} + en_{io} + \epsilon_{ijo} \\ + t_k + et_{ik} + tn_{ko} + etn_{iko} + \epsilon_{ijko} \\ + g_{kl} + eg_{ikl} + gn_{klo} + egn_{iklo} + \epsilon_{ijklo} \end{aligned} \tag{3.3}$$

in which Y_{ijklo} is the observation of a genotype (g_l) within the growth type (t_k) in block (b_j) within nitrogen level (n_o) at environment (e_i). Interaction effects were tested for environment and nitrogen level (en_{io}), environment and growth type (et_{ik}), environment and genotype (eg_{ikl}), growth type and nitrogen level (tn_{ko}) and genotype by nitrogen level (gn_{klo}). Threefold interactions were testes for environment by genotype by nitrogen level (egn_{iklo}) and environment by growth type by nitrogen level (etn_{iko}). Whole plot error is denoted by ϵ_{ijo}, sub-plot error is denoted by ϵ_{ijko} and the sub-sub-plot error is denoted by ϵ_{ijklo}. The factors environment, block and genotype were considered as random.

Broad sense heritability (h^2) was estimated as follows:

$$h^2 = \frac{\hat{\sigma}^2_G}{\hat{\sigma}^2_G + \frac{\hat{\sigma}^2_{GE}}{E}}$$

where $\hat{\sigma}^2_G$ and $\hat{\sigma}^2_{GE}$ are variances of genotypes and genotypes by environment respectively; E refers to number of environments.

3.3 Results

3.3.1 Semi-dwarf and normal type hybrids at low nitrogen supply

At low nitrogen supply semi-dwarf hybrids produced significantly less biomass by end of flowering compared to normal bybrids (Tab.3.1, Tab. 3.2). The ANOVA for biomass yield EOF at low nitrogen supply detected no significant variation between genotypes within growth types, but a highly significant interaction of growth type and genotype with the factor environment (Tab. 3.2).

Both growth types took up comparable quantities of nitrogen (Nitrogen yield EOF) at low nitrogen supply by end of flowering (Tab. 3.1). The largest difference between both types of about 6 kg N/ha was observed in Go11. The ANOVA did not reveal a significant variation in nitrogen yield EOF among growth types or genotypes within growth types (Tab.3.2). Interaction effects of growth type by environment and genotype by environment were significant. Due to similar amounts of nitrogen taken up until end of flowering and less biomass, semi-dwarfs had higher nitrogen contents in the biomass, compared to the normal types (Tab.3.1). No significant variation in nitrogen content EOF had been detected among the genotypes at low nitrogen supply (Tab.3.2).

At maturity semi-dwarf types formed significantly less straw compared to normal types (Tab.3.1). The single genotypes showed strong interaction effects for straw yield with the environments, resulting in a low heritability of 0.06 (Tab.3.2). No significant variation in nitrogen content of straw was observed between the two growth types (Tab.3.1). Genotypes within growth types differed significantly in nitrogen content of straw and showed also a significant interaction with environments (Tab.3.2). Due to lower straw yield and similar nitrogen content of straw semi-dwarf types contained about 1.5 kg/ha less nitrogen in straw than normal types (Tab.3.1).

The mean seed yield of semi-dwarf types at low nitrogen supply was 2.4 dt/ha higher compared to normal types (Tab.3.1). Nevertheless, the effect was not significant (Tab.3.2). Genotypes within growth types showed significant variation for nitrogen yield in seeds but no significant difference between growth types. Total nitrogen yield at maturity was calculated only for Go11 and Go12. The difference of about 7 kg N/ha among growth types was not significant (Tab.3.1). In Go11 both growth types fixed less nitrogen in biomass at maturity compared to end of flowering. In Go12 the amount of nitrogen at maturity was higher compared to end of flowering. Semi-dwarf hybrids had a higher nitrogen harvest index due to less nitrogen in straw and more nitrogen in seeds (Tab.3.1).

Table 3.1: Performance of semi-dwarf (Sd) and normal type (Nt) hybrids at low nitrogen supply in 2010/11 and 2011/12 (108 genotypes, four environments)

Trait		**Ei11**		**Go11**		**Ei12**		**Go12**		**Mean**	
Beginn of flowering	Nt	110		110		104		115		110	
(days)	Sd	110	ns	110	ns	104	ns	114	*	110	ns
Plant height	Nt	126		119		108		90		110.8	
(cm)	Sd	100	**	92	**	72	**	70	**	83.5	**
Biomass EOF	Nt	36.7		59.3		17.1		17.1		32.6	
(dt/ha)	Sd	34.1	*	48.3	ns	12.2	**	14.5	**	27.3	+
Seed yield	Nt	17.0		12.9		-		10.5		13.5	
(dt/ha)	Sd	18.0	ns	17.0	*	-		12.8	**	15.9	ns
Straw yield	Nt	-		40.1		18.9		22.9		27.3	
(dt/ha)	Sd	-		34.9	**	17.7	ns	19.8	**	24.2	+
Nitrogen content EOF	Nt	1.66		1.53		1.77		1.60		1.64	
(%)	Sd	1.81	+	1.75	ns	2.11	**	1.86	**	1.89	**
Nitrogen content straw	Nt	-		0.50		0.61		0.51		0.54	
(%)	Sd	-		0.53	**	0.60	ns	0.50	ns	0.54	ns
Nitrogen content seed	Nt	2.70		3.09		3.17		3.04		3.01	
(%)	Sd	2.72	+	3.07	ns	3.26	ns	2.95	**	2.99	ns
Nitrogen yield EOF	Nt	61.1		90.3		26.7		27.3		52.2	
(kg N/ha)	Sd	62.4	ns	84.2	**	29.1	**	27.4	ns	49.9	ns
Nitrogen yield straw	Nt	-		20.5		13.7		11.7		14.5	
(kg N/ha)	Sd	-		18.7	*	7.9	+	9.8	**	12.9	**
Nitrogen yield seed	Nt	46.0		39.9		-		31.7		39.2	
(kg N/ha)	Sd	48.8	ns	52.1	*	-		37.7	*	46.2	ns
Nitrogen yield total	Nt	-		60.6		-		43.4		52.0	
(kg N/ha)	Sd	-		70.9	+	-		47.4	*	59.2	ns
Nitrogen harvest index	Nt	-		0.66		-		0.73		0.70	
	Sd	-		0.74	**	-		0.79	*	0.77	**

,*,+ Significant difference at 0.01, 0.05, 0.10 probability level, respectively; ns non-significant (F-test from ANOVA); **Ei Einbeck; **Go** Göttingen; **Sd** semi-dwarf; **Nt** normal type; **EOF** end of flowering

Table 3.2: Variance components of agronomic and nitrogen related traits at low nitrogen supply in 2010/11 and 2011/12 (108 genotypes)

Source	**E**	**T**	**G**	**T×E**	**G×E**	h^2
Degrees of freedom	3	1	106	3	318	
Begin of flowering (days)	19.08 **	0	0.28 **	0	0.27 **	0.71
Plant height (cm)	222.97 **	367.18 **	3.98 **	24.18 **	4.78 **	0.42
Biomass EOF (dt/ha)	342.96 **	12.08 $^{+}$	0.69 ^{ns}	7.61 **	7.96 **	0.15
Seed yield (dt/ha)[a]	8.64 **	2.56 ^{ns}	0.83 **	0.94 ^{ns}	1.00 **	0.47
Straw yield (dt/ha)[b]	91.97 $^{+}$	4.31 $^{+}$	0.36 ^{ns}	1.73 **	4.96 **	0.06
Nitrogen content EOF (%)	0.01 **	0.03 **	0.001 ^{ns}	0.003 **	0.003 ^{ns}	0.09
Nitrogen content straw (%)[b]	0.002^{ns}	0	0.0003^{+}	0.0003**	0.0007**	0.22
Nitrogen content seed (%)	0.04 **	0.0001^{ns}	0.002 **	0.001 **	0.001 **	0.51
Nitrogen yield EOF (kg N/ha)	838.17 **	0.92 ^{ns}	1.70 ^{ns}	5.64 **	22.71 **	0.08
Nitrogen yield straw (kg N/ha)[b]	21.34 $^{+}$	1.15 **	0.10 ^{ns}	0.06 ^{ns}	2.10 **	0.04
Nitrogen yield seed (kg N/ha)[a]	47.40 **	20.50 ^{ns}	5.45 **	9.00 ^{ns}	7.99 **	0.42
Nitrogen yield total (kg N/ha)[c]	199.61 *	20.70 ^{ns}	7.29 $^{+}$	8.04 $^{+}$	21.46 **	0.22
Nitrogen harvest index [c]	0.17 $^{+}$	0.23 **	0.02 $^{+}$	0	0.01 ^{ns}	0.26

$^{**,*,+}$ Significant difference at 0.01, 0.05, 0.10 probability level, respectively; ns non-significant (F-test from ANOVA); negative variance components were set to zero; **E** environment; **T** growth type; **G** genotype within growth type; h^2 heritability within growth types; **EOF** end of flowering;
[a] values for three environments (Ei11, Go11, Go12)
[b] values for three environments (Go11, Ei12, Go12)
[c] values for two environments (Go11, Go12)

3.3.2 Semi-dwarf and normal type hybrids at high nitrogen supply

At high nitrogen supply both growth types differed in biomass yield at EOF in average by about 9 dt/ha (Tab.3.3). This difference proved to be non-significant. But interaction effects of growth types and environment were significant (Tab.3.4).

Results for nitrogen yield EOF in high nitrogen environments were contradictory (Tab.3.3). In Einbeck 2012 normal hybrids took up nearly 20 kg N/ha more than semi-dwarf hybrids. In Göttingen 2012 semi-dwarf hybrids took up about 5 kg N/ha more compared to normal hybrids. The difference in average nitrogen yield between both environments was up to 60 kg N/ha (Tab.3.3). Therefore the ANOVA revealed a significant growth type by environment interaction but no significant variation between growth types (Tab. 3.4). Variation in nitrogen yield EOF between genotypes was also significant. Semi-dwarfs had significantly higher nitrogen contents in the biomass EOF (Tab.3.3). At maturity semi-dwarf hybrids formed on average 5.3 dt/ha less straw compared to normal hybrids. The ANOVA revealed this difference between growth types as non-significant.

Table 3.3: Performance of semi-dwarf (Sd) and normal type (Nt) at high nitrogen supply in 2011/12 (108 genotypes, two environments)

Trait		**Einbeck**		**Göttingen**		**Mean**	
Beginn of flowering	Nt	104		115		109	
(days)	Sd	104	ns	115	ns	109	ns
Plant height	Nt	129		91		109.9	
(cm)	Sd	78	**	67	*	72.8	ns
Biomass EOF	Nt	45.0		42.4		43.7	
(dt/ha)	Sd	30.5	**	38.5	ns	34.5	ns
Seed yield	Nt	-		26.4		26.4	
(dt/ha)	Sd	-		29.7	**	29.7	**
Straw yield	Nt	33.4		39.4		36.4	
(dt/ha)	Sd	30.1	**	32.1	**	31.1	ns
Nitrogen content EOF	Nt	2.38		3.35		2.86	
(%)	Sd	2.83	**	3.81	**	3.32	**
Nitrogen content straw	Nt	0.50		0.78		0.64	
(%)	Sd	0.56	**	0.85	**	0.7	**
Nitrogen content seed	Nt	3.12		3.31	+	3.21	
(%)	Sd	3.24	**	3.39	**	3.31	+
Nitrogen yield EOF	Nt	106.7		141.3		124.0	
(kg N/ha)	Sd	86.0	*	146.4	ns	116.2	ns
Nitrogen yield straw	Nt	16.5		30.7		23.6	
(kg N/ha)	Sd	16.8	ns	27.4	**	22.1	ns
Nitrogen yield seed	Nt	-		87.3		87.3	
(kg N/ha)	Sd	-		100.3	**	100.3	**
Nitrogen yield total	Nt	-		118.0		118.0	
(kg N/ha)	Sd	-		127.7	**	127.7	**
Nitrogen harvest index	Nt	-		0.74		0.74	
	Sd	-		0.79	**	0.79	**

,*,+ Significant difference at 0.01, 0.05, 0.10 probability level, respectively; ns non-significant (F-test from ANOVA); **Sd semi-dwarf; **Nt** normal type; **EOF** end of flowering

Nitrogen content of straw varied significantly among environments, growth types and genotypes within growth types (Tab.3.4). The amount of nitrogen fixed in the straw showed no significant variation between growth types and genotypes, but significant interactions of both factors with environments.

Seed yield at high nitrogen levels could only be measured in Göttingen 2012. It revealed a significantly higher seed yield of semi-dwarfs compared to normal hybrids (Tab.3.3). Due to higher seed yields and higher nitrogen content of seeds semi-dwarf hybrids had a significantly higher nitrogen yield of seeds (Tab.3.3). Total nitrogen yield at maturity revealed a significant difference between growth types of about 10 kg N/ha in Göttingen 2012. Compared to the values for nitrogen yield EOF in Göttingen 2012 this is a reduction of about 20 kg N/ha from the end of flowering to maturity in both growth types (Tab.3.3).

Table 3.4: Variance components of agronomic and nitrogen related traits at high nitrogen supply in 2011/12 (108 genotypes)

Source	**E**	**T**	**G**	**T×E**	**G×E**	h^2
Degrees of freedom	1	1	106	1	106	
Begin of flowering (days)	56.83^{**}	0	0.34^{**}	0.16^{*}	0.52^{**}	0.49
Plant height (cm)	290.52^{**}	599.63^{ns}	3.74^{*}	172.80^{**}	7.43^{**}	0.36
Biomass EOF (dt/ha)	0	28.26^{ns}	2.14^{*}	27.43^{**}	1.20^{ns}	0.30
Seed yield (dt/ha)[a]	-	5.26^{**}	2.66^{**}		-	0.55
Straw yield (dt/ha)	5.55^{ns}	11.82^{ns}	2.96^{**}	3.77^{**}	1.87^{*}	0.44
Nitrogen content EOF (%)	46.50^{*}	10.34^{**}	0	0	0.86^{**}	-
Nitrogen content straw (%)	4.03^{**}	0.22^{**}	0.10^{*}	0	0.22^{ns}	0.32
Nitrogen content seed (%)	1.31^{*}	0.50^{+}	0.13^{**}	0.03^{ns}	0.14^{**}	0.40
Nitrogen yield EOF (kg N/ha)	1096.46^{*}	0	16.47^{+}	162.54^{**}	0.67^{ns}	0.25
Nitrogen yield straw (kg N/ha)	73.39^{*}	0	1.72^{ns}	3.18^{**}	7.32^{**}	0.22
Nitrogen yield seed (kg N/ha)[a]	-	84.67^{**}	30.99^{**}	-	-	0.56
Nitrogen yield total (kg N/ha)[a]	-	46.07^{**}	57.63^{**}	-	-	0.59
Nitrogen harvest index [a]	-	0.11^{**}	0.07^{**}		-	0.72

$^{**,*,+}$ Significant difference at 0.01, 0.05, 0.10 probability level, respectively; ns non-significant (F-test from ANOVA); negative variance components were set to zero; **E** environment; **T** growth type; **G** genotype within growth type; h^2 heritability within growth types; **EOF** end of flowering; [a] values for Göttingen 2011/12 only

3.3.3 Nitrogen uptake and nitrogen utilization

At low nitrogen supply in the first experiment (harvest EOF) nitrogen uptake (kg N/ha) and nitrogen uptake efficiency were non-significantly different between growth types or genotypes within growth types (Tab. 3.5, Tab. 3.6). Nitrogen uptake and nitrogen uptake efficiency were significantly influenced by interactions with environments. At high nitrogen supply genotypes differed significantly in nitrogen uptake and nitrogen uptake efficiency. Both traits were significantly affected by interactions of environments with growth type.

Table 3.5: Nitrogen uptake and nitrogen uptake efficiency EOF at low and high nitrogen supply (108 genotypes, Einbeck and Göttingen 2010/11 and 2011/12)

		Semi-dwarf			Normal type		
Trait	**Env.**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**
Low nitrogen supply							
Nitrogen uptake EOF (kg N/ha)	Ei11	43.4	86.3	62.4	43.9	96.3	61.1
	Go11	55.1	114.0	84.2	66.0	117.3	90.3
	Ei12	17.0	38.8	25.7	22.0	39.4	30.1
	Go12	13.2	36.3	27.4	21.0	32.7	27.3
	Mean			49.9			52.2
Nitrogen uptake efficiency EOF	Ei11	0.42	0.82	0.59	0.42	0.92	0.58
	Go11	0.38	0.78	0.58	0.45	0.79	0.62
	Ei12	0.36	0.79	0.53	0.45	0.82	0.63
	Go12	0.33	0.85	0.65	0.50	0.78	0.65
	Mean			0.59			0.62
High nitrogen supply							
Nitrogen uptake EOF (kg N/ha)	Ei12	66.8	120.2	85.9	78.1	156.1	106.7
	Go12	126.1	186.7	146.4	126.9	163.0	141.3
	Mean			116.2			124.0
Nitrogen uptake efficiency EOF	Ei12	0.30	0.53	0.38	0.35	0.69	0.47
	Go12	0.58	0.85	0.67	0.58	0.74	0.64
	Mean			0.52			0.56

Env environment; **Ei** Einbeck; **Go** Göttingen; **EOF** end of flowering

Table 3.6: Variance components for nitrogen uptake and nitrogen uptake efficiency EOF at low and high nitrogen supply (108 genotypes, Einbeck and Göttingen 2010/11 and 2011/12)

Source	E	T	G	T×E	G×E	h^2
Low nitrogen supply						
Degrees of freedom	3	1	106	3	318	
Nitrogen uptake EOF	838.17^{**}	0.92^{ns}	1.70^{ns}	5.64^{**}	22.71^{**}	0.08
Nitrogen uptake efficiency EOF	0	0.01^{ns}	0.02^{ns}	0.11^{**}	0.27^{**}	0.08
High nitrogen supply						
Degrees of freedom	1	1	106	1	106	
Nitrogen uptake EOF	1096.46^{*}	0	16.47^{+}	162.55^{**}	0.6^{ns}	0.25
Nitrogen uptake efficiency EOF	2.55^{*}	0	0.03^{+}	0.32^{**}	0.002^{ns}	0.25

$^{**},^{*},^{+}$ Significant difference at 0.01, 0.05, 0.10 probability level, respectively; ns non-significant (F-test from ANOVA); negative variance components were set to zero; **E** environment; **T** growth type; **G** genotype within growth type; h^2 heritability within growth types; **EOF** end of flowering

For the estimation of nitrogen uptake and nitrogen utilization parameters at maturity data from Go11 and Go12 was analysed. The means for nitrogen uptake at low nitrogen supply differed non-significantly between growth types (Tab. 3.7, 3.8). At high nitrogen supply semi-dwarf hybrids took up significantly more nitrogen than normal hybrids. Nitrogen uptake efficiency was significantly different between growth types at both nitrogen levels with higher values for semi-dwarfs. Nitrogen utilization efficiency of semi-dwarfs was also significantly higher at both nitrogen levels compared to normal hybrids. Semi-dwarf hybrids proved to be superior in nitrogen use efficiency at both nitrogen levels, compared to normal hybrids. (Tab. 3.7, 3.8)

The analysis of relative contributions of nitrogen uptake efficiency and nitrogen utilization efficiency to nitrogen use efficiency revealed that in both growth types, and at both nitrogen levels, nitrogen uptake efficiency contributes largely to variation in nitrogen use efficiency (Tab.3.9). Nitrogen utilization efficiency contributed more to nitrogen use efficiency in the semi-dwarf hybrids compared to normal hybrids (Tab.3.9).

Table 3.7: Parameters of nitrogen use efficiency (maturity) at low and high nitrogen supply, according to Moll et al. (1982)(108 genotypes, Göttingen 2010/11 and 2011/12)

		Semi-dwarf			Normal type		
Trait	**Env.**	**Min.**	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**
Low nitrogen supply							
Nitrogen uptake	Go11	49.0	88.7	70.9	40.7	98.0	60.6
(kg N/ha)	Go12	41.8	68.6	47.4	37.5	69.9	43.4
	Mean			59.2			52.0
Nitrogen uptake	Go11	0.51	0.93	0.75	0.44	0.98	0.64
efficiency	Go12	0.60	0.94	0.82	0.52	0.88	0.75
	Mean			0.78			0.70
Nitrogen utilization	Go11	19.6	26.5	24.0	17.2	24.8	21.5
efficiency	Go12	23.1	28.2	26.9	20.2	24.6	24.1
	Mean			25.5			22.8
Nitrogen use	Go11	11.4	23.0	18.1	9.6	19.5	13.8
efficiency	Go12	17.1	26.3	22.0	13.2	22.7	18.1
	Mean			20.1			15.9

		Semi-dwarf			Normal type		
Trait	**Env.**	**Min.**	**Max.**	**Mean**	**Min.**	**Max.**	**Mean**
High nitrogen supply							
Nitrogen uptake (kg N/ha)	Go12	110.3	160.4	127.7	94.2	148.5	118.0
Nitrogen uptake efficiency	Go12	0.67	0.97	0.77	0.57	0.90	0.72
Nitrogen utilization efficiency	Go12	18.8	25.5	23.3	20.4	24.8	22.2
Nitrogen use efficiency	Go12	10.3	13.6	12.6	6.3	13.8	11.2

Env environment; **Ei** Einbeck; **Go** Göttingen

Table 3.8: Variance components of parameters for nitrogen use efficiency (maturity) at low and high nitrogen supply (108 genotypes, Göttingen 2010/11 and 2011/12)

Source	E	T	G	T×E	G×E	h^2
Low nitrogen supply						
Degrees of freedom	1	1	106	1	106	
Nitrogen uptake (kg N/ha)	199.61^{*}	20.70^{ns}	7.29^{+}	8.04^{+}	21.46^{**}	0.22
Nitrogen uptake efficiency	0.39^{**}	0.38^{+}	0.13^{*}	0.02^{ns}	0.27^{**}	0.29
Nitrogen utilization efficiency	3.44^{*}	3.50^{**}	0.34^{*}	0	0.34^{+}	0.32
Nitrogen use efficiency	8.38^{**}	8.55^{**}	1.48^{**}	0	1.68^{**}	0.42
High nitrogen supply						
Degrees of freedom		1	106			
Nitrogen uptake (kg N/ha)	-	22.13^{**}	57.63^{**}	-	-	0.59
Nitrogen uptake efficiency	-	0.08^{**}	0.10^{**}	-	-	0.59
Nitrogen utilization efficiency	-	0.32^{*}	0.80^{**}	-	-	0.64
Nitrogen use efficiency	-	0.95^{**}	0.48^{**}	-	-	0.55

$^{**},^{*},^{+}$ Significant difference at 0.01, 0.05, 0.10 probability level, respectively; ns non-significant (F-test from ANOVA); negative variance components were set to zero; **E** environment; **T** growth type; **G** genotype within growth type; h^2 heritability within growth types

Table 3.9: Relative contribution of nitrogen uptake and nitrogen utilization efficiency to total genetic variation in nitrogen use efficiency, according to Moll et al. (1982)

Growth type	Low nitrogen supply		High nitrogen supply	
	Uptake efficiency	Utilization efficiency	Uptake efficiency	Utilization efficiency
Göttingen 2010/11				
Normal type	0.90	0.10	-	-
Semi-dwarf	0.75	0.25	-	-
Göttingen 2011/12				
Normal type	0.72	0.28	0.87	0.13
Semi-dwarf	0.72	0.28	0.74	0.26

The proportion of nitrogen uptake and nitrogen utilization at low nitrogen supply is visualized in Fig. 3.1. The highest yielding genotypes within growth types are characterized by high amounts of nitrogen uptake and/or high nitrogen utilization efficiencies (Fig. 3.1). The relationship between nitrogen uptake and nitrogen utilization at high

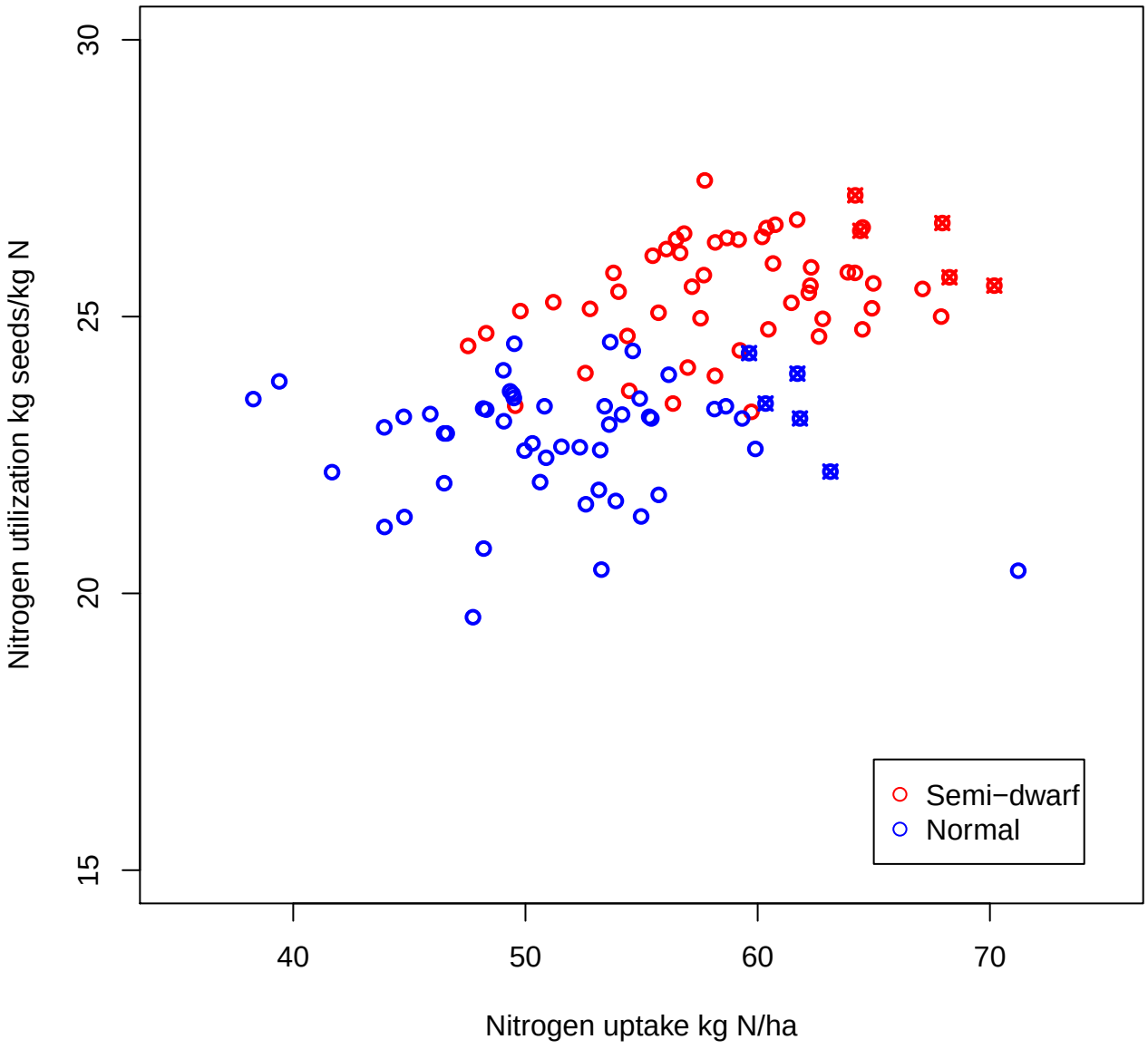

Figure 3.1: Nitrogen uptake and nitrogen utilization efficiency of 108 genotypes at low nitrogen supply (Göttingen 2010/11 and 2011/12). The five genotypes with the highest seed yields in each group, are marked with crosses

nitrogen supply revealed a similar pattern. Genotypes with the highest seed yield in both

growth types are characterized by high amounts of nitrogen uptake and/or high nitrogen utilization efficiencies (Fig. 3.2).

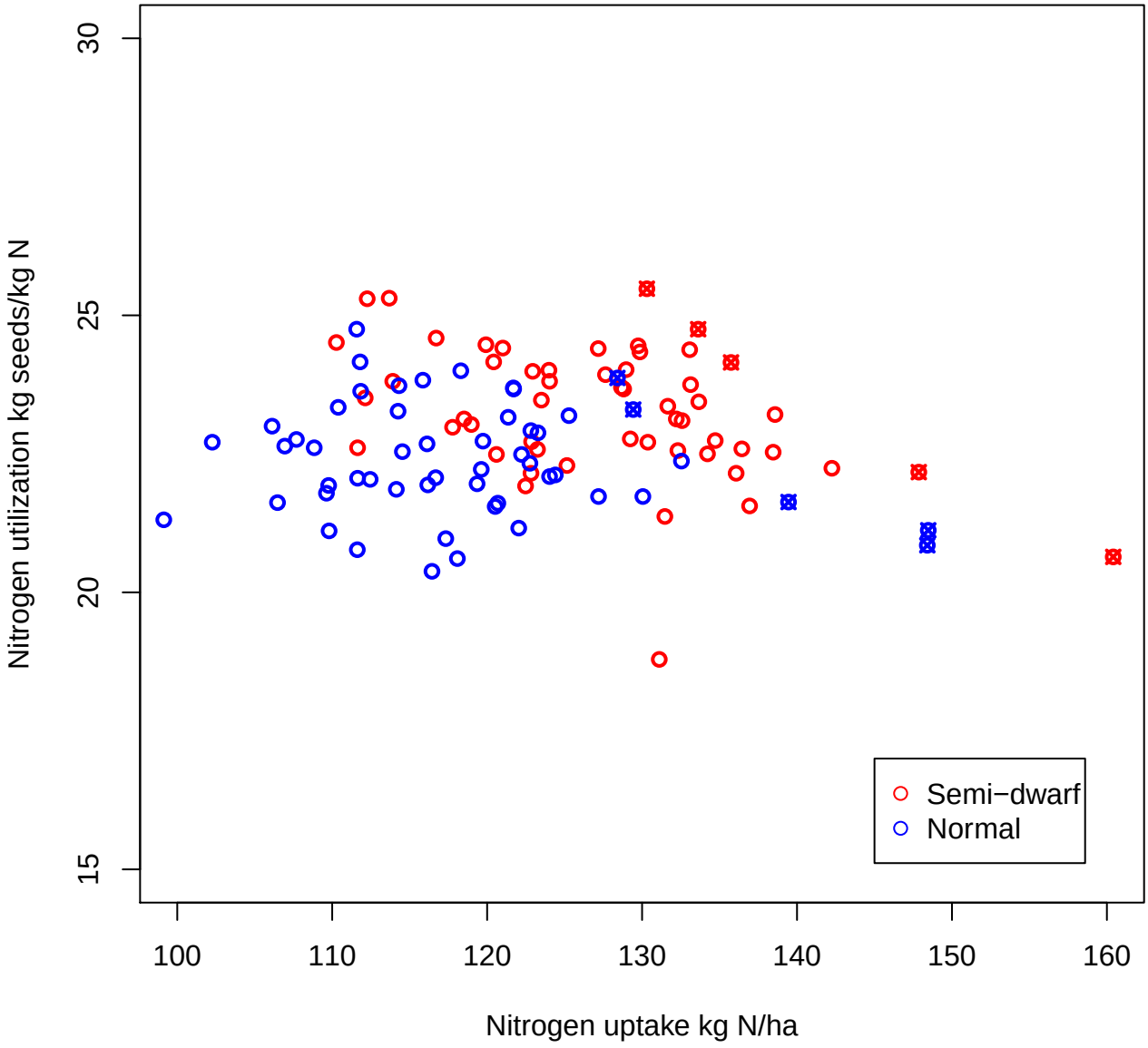

Figure 3.2: Nitrogen uptake and nitrogen utilization efficiency of 108 genotypes at high nitrogen supply (Einbeck and Göttingen 2011/12). The five genotypes with the highest seed yields in each group, are marked with crosses

3.3.4 Comparison of low and high nitrogen supply

ANOVA across nitrogen levels revealed a significant effect of nitrogen fertilization on BFL, Biomass EOF, seed yield, straw yield, nitrogen yield of seeds and the total amount of nitrogen at maturity (Nitrogen yield total) (Tab. 3.10). Nitrogen uptake efficiency and nitrogen utilization efficiency differed significantly between nitrogen levels. No effect of nitrogen fertilization was detected for plant height, nitrogen content of straw, nitrogen content of seeds and nitrogen harvest index. Growth types differed significantly in seed yield and responded significantly different to nitrogen fertilization (TN interaction).

Genotypic variation was detected for all traits except nitrogen content EOF (Tab. 3.10). A significant interaction of genotypes and nitrogen level was observed for seed yield, nitrogen yield of seeds, total amount of nitrogen fixed at maturity (Nitrogen yield total), nitrogen harvest index, nitrogen uptake efficiency and nitrogen utilization efficiency. All traits were significantly affected by genotype by environment interactions, except nitrogen yield EOF. Heritabilities ranged from 0.27 (Nitrogen yield EOF) to 0.77 (Nitrogen utilization efficiency). At both nitrogen levels seed yield of semi-dwarf and normal type hybrids was positively correlation with nitrogen uptake and nitrogen uptake efficiency (Tab. 3.11). Nitrogen utilization efficiency was not correlated with seed yield of semi-dwarf hybrids but showed a weak significant positive correlation with seed yield of normal hybrids at both nitrogen levels . The amount of nitrogen fixed by the end of flowering was not correlated with seed yield. At low nitrogen supply straw yield was positively correlated to seed yield. Nitrogen utilization efficiency of normal hybrids correlated positively with seed yield at low nitrogen supply. Nitrogen utilization efficiency was negatively correlated to nitrogen content of straw at both nitrogen levels.

Correlations for individual traits at low and high nitrogen supply were rather low (Tab. 3.12). Seed yield of normal hybrids was correlated with $r = 0.60$ between low and high nitrogen supply. Seed yield of semi-dwarfs was correlated with $r = 0.46$. No correlation among the two nitrogen levels was observed for nitrogen content EOF, nitrogen content straw and nitrogen yield EOF.

Table 3.10: Variance components of agronomic traits and nitrogen affected traits at low and high nitrogen supply (108 genotypes, Einbeck and Göttingen 2011/12)

Source	E	N	T	G	E×N
Degrees of freedom	1	1	1	106	1
Beginn of flowering (days)	56.83 **	0.02**	0	0.29**	0
Plant height (cm)	146.88 **	0	475.65^{ns}	4.32**	48.59**
Biomass EOF (dt/ha)	0	284.93*	15.80^{ns}	0.91*	0
Seed yield (dt/ha)[a]	-	134.71**	3.84**	1.66**	-
Straw yield (dt/ha)	0.57 ^{ns}	96.72*	5.75^{ns}	2.11**	0
Nitrogen content EOF (%)	7.39 *	60.97^{ns}	7.41**	0.06^{ns}	33.43*
Nitrogen content straw (%)	0.43 **	0	0.04**	0.06**	3.65**
Nitrogen content seed (%)	0.01 ^{ns}	0	0	0.08**	3.58**
Nitrogen yield EOF (kg N/ha)	268.88 *	3985.42^{ns}	0	5.61^{+}	563.93*
Nitrogen yield straw (kg N/ha)	16.67 $^{+}$	53.66^{ns}	0.37^{ns}	1.29**	34.30^{+}
Nitrogen yield seed (kg N/ha)[a]	-	1747.70**	44.73**	15.78**	-
Nitrogen yield total (kg N/ha)[a]	-	2995.64*	23.23**	23.67**	-
Nitrogen harvest index[a]	-	0	0.15**	0.06**	-
Nitrogen uptake efficiency[a]	-	3.41*	0.15**	0.15**	-
Nitrogen utilization efficiency[a]	-	3.42^{+}	1.64**	0.92**	-

Source	E×T	T×N	G×N	G×E	h^2
Degrees of freedom	1	1	106	106	
Beginn of flowering (days)	0.08 **	0.04^{ns}	0	0.49**	0.51
Plant height (cm)	118.38 **	13.60^{ns}	0.97^{ns}	4.64**	0.53
Biomass EOF (dt/ha)	10.51 **	3.11^{ns}	0.65^{ns}	1.34**	0.30
Seed yield (dt/ha)[a]	-	0.21**	0.45*	-	0.73
Straw yield (dt/ha)	2.09 **	2.15^{ns}	0.22^{ns}	0.58^{+}	0.58
Nitrogen content EOF (%)	0.002^{ns}	0.47*	0	0.36*	0.08
Nitrogen content straw (%)	0	0.16**	0.04^{ns}	0.08**	0.40
Nitrogen content seed (%)	0.07 **	0.61**	0	0.06*	0.39
Nitrogen yield EOF (kg N/ha)	57.47 **	0	4.75^{ns}	2.92^{ns}	0.27
Nitrogen yield straw (kg N/ha)	1.28 **	0	0	2.13**	0.41
Nitrogen yield seed (kg N/ha)[a]	-	12.53**	7.64**	-	0.70
Nitrogen yield total (kg N/ha)[a]	-	7.71**	19.00**	-	0.67
Nitrogen harvest index[a]	-	0.62*	0.02**	-	0.74
Nitrogen uptake efficiency[a]	-	0.02*	0.09**	-	0.71
Nitrogen utilization efficiency[a]	-	0.95**	0.27**	-	0.77

**,*,$^{+}$ Significant difference at 0.01, 0.05, 0.10 probability level, respectively; ns non-significant (F-test from ANOVA); negative variance components were set to zero; **E** environment; **N** nitrogen supply level; **T** growth type; **G** genotype within growth type; $\boldsymbol{h}^2$ heritability within growth types; **EOF** end of flowering;
[a] values for Göttingen 2011/12 only

Table 3.11: Spearman's rank correlations of agronomic and nitrogen affected traits at low and high nitrogen supply

Low nitrogen supply [a]		Seed yield	(2)	(3)	(4)	(5)	(6)	(7)
(2) Straw yield	Sd	0.47^{**}						
	Nt	0.57^{**}						
(3) Nitrogen total EOF	Sd	0.17^{ns}	0.22^{ns}					
	Nt	0.15^{ns}	0.28^{*}					
(4) Nitrogen seed	Sd	0.64^{**}	0.73^{**}	0.28^{*}				
	Nt	0.99^{**}	0.57^{**}	0.19^{ns}				
(5) Nitrogen straw	Sd	0.64^{**}	0.60^{**}	0.16^{ns}	0.35^{**}			
	Nt	0.50^{**}	0.81^{**}	0.27^{*}	0.51^{**}			
(6) Nitrogen uptake	Sd	0.75^{**}	0.82^{**}	0.29^{*}	0.94^{**}	0.61^{**}		
	Nt	0.92^{**}	0.75^{**}	0.25^{ns}	0.93^{**}	0.77^{**}		
(7) Nitrogen utilization	Sd	0.06^{ns}	0.30^{*}	0.10^{ns}	0.53^{**}	-0.38^{**}	0.33^{*}	
efficiency	Nt	0.40^{**}	-0.24^{ns}	-0.27^{*}	0.35^{**}	-0.47^{**}	0.09^{ns}	
(8) Nitrogen uptake	Sd	0.73^{**}	0.80^{**}	0.22^{ns}	0.94^{**}	0.56^{**}	0.98^{**}	0.35^{**}
efficiency	Nt	0.92^{**}	0.72^{**}	0.18^{ns}	0.93^{**}	0.72^{**}	0.98^{**}	0.12^{ns}
High nitrogen supply [b]		Seed yield	(2)	(3)	(4)	(5)	(6)	(7)
(2) Straw yield	Sd	0.17^{ns}						
	Nt	-0.15^{ns}						
(3) Nitrogen total EOF	Sd	0.14^{ns}	-0.03^{ns}					
	Nt	0.09^{ns}	0.03^{ns}					
(4)Nitrogen seed	Sd	0.96^{**}	0.17^{ns}	0.08^{ns}				
	Nt	0.97^{**}	-0.11^{ns}	0.06^{ns}				
(5) Nitrogen straw	Sd	0.19^{ns}	0.75^{**}	-0.04^{ns}	0.22^{ns}			
	Nt	0.11^{ns}	0.67^{**}	-0.05^{ns}	0.18^{ns}			
(6) Nitrogen uptake	Sd	0.77^{**}	0.53^{**}	0.09^{ns}	0.81^{**}	0.71^{**}		
	Nt	0.85^{**}	0.17^{ns}	0.02^{ns}	0.90^{**}	0.54^{**}		
(7) Nitrogen utilization	Sd	0.22^{ns}	-0.61^{**}	0.20^{ns}	0.11^{ns}	-0.85^{**}	-0.39^{**}	
efficiency	Nt	0.35^{*}	-0.62^{**}	0.10^{ns}	0.22^{ns}	-0.81^{**}	-0.11^{ns}	
(8) Nitrogen uptake	Sd	0.78^{**}	0.54^{**}	0.09^{ns}	0.81^{**}	0.70^{**}	1.00^{**}	-0.37^{**}
efficiency	Nt	0.86^{**}	0.14^{ns}	0.02^{ns}	0.91^{**}	0.51^{**}	1.00^{**}	-0.08^{ns}

,*,+ Significant difference at 0.01, 0.05, 0.10 probability level respectively; ns non-significant; **Sd semi-dwarfs; **Nt** normal types; **a** Göttingen 2010/11 and 2011/12; **b** Göttingen 2011/12; **EOF** end of flowering

Table 3.12: Mean values of agronomic and nitrogen related traits at low and high nitrogen supply. Spearman's rank correlations between the two nitrogen levels (108 genotypes, Göttingen 2011/12)

Trait		**Low nitrogen supply**		**High nitrogen supply**		**Correlation coefficient**
Beginn of flowering	Nt	109		109		0.59 **
(days)	Sd	109	ns	109	ns	0.66 **
Plant height	Nt	99		110		0.44 **
(cm)	Sd	71	ns	73	ns	0.24 ns
Biomass EOF	Nt	17.1		43.7		0.36 **
(dt/ha)	Sd	13.3	ns	34.5	ns	0.39 **
Seed yield	Nt	10.5		26.4		0.60 **
(dt/ha)	Sd	12.8	**	29.7	**	0.46 **
Straw yield	Nt	11.5		36.4		0.28 *
(dt/ha)	Sd	10.1	ns	31.1	ns	0.47 **
Nitrogen content EOF	Nt	1.68		2.86		0.19 ns
(%)	Sd	2.00	**	3.32	**	0.20 ns
Nitrogen content straw	Nt	0.56		0.64		0.24 ns
(%)	Sd	0.55	+	0.70	**	0.18 ns
Nitrogen content seed	Nt	3.13		3.21		0.41 **
(%)	Sd	3.08	ns	3.31	+	0.35 **
Nitrogen yield EOF	Nt	28.7		124.0		0.07 ns
(kg N/ha)	Sd	26.6	ns	116.2	ns	0.15 ns
Nitrogen yield straw	Nt	11.5		23.6		0.31 *
(kg N/ha)	Sd	10.1	ns	22.1	ns	0.30 *
Nitrogen yield seed	Nt	31.7		87.3		0.53 **
(kg N/ha)	Sd	37.7	*	100.3	**	0.46 **
Nitrogen yield total	Nt	43.3		118.0		0.47 **
(kg N/ha)	Sd	47.4	*	127.7	**	0.33 *
Nitrogen harvest index	Nt	0.73		0.74		0.40 **
	Sd	0.79	*	0.79	**	0.42 **
Nitrogen uptake efficiency	Nt	0.75		0.50		0.47**
	Sd	0.82	+	0.54	**	0.36**
Nitrogen utilization efficiency	Nt	24.09		22.24		0.51**
	Sd	26.89	**	23.26	*	0.46**

,*,+ Significant difference at 0.01, 0.05, 0.10 probability level, respectively; ns non-significant; **EOF end of flowering

3.4 Discussion

Nitrogen use efficiency can be subdivided into nitrogen uptake efficiency and nitrogen utilization efficiency. Nitrogen uptake efficiency was calculated as the ratio of total nitrogen taken up by a genotype at maturity and available nitrogen. The only source of nitrogen for plants at low nitrogen conditions is the so called 'mineralized nitrogen'. Mineralized nitrogen is the results of organic matter decomposition by soil microbes. The intensity of nitrogen mineralization strongly depends on temperature, moisture and aeriation in the soil as well as on the amount of organic matter. Therefore an exact quantification of available nitrogen in the soil is quite difficult. In the present experiment the available amount of nitrogen was defined individually for each replication within each environment as the highest amount of nitrogen taken up by a genotype at end of flowering or maturity, respectively.

Variation in nitrogen at the end of flowering and maturity

At low nitrogen supply in Göttingen 2011, the total nitrogen uptake at maturity was lower than the nitrogen uptake at end of flowering (Tab. 3.1). The average difference in the nitrogen uptake of genotypes between the two experiments (harvest EOF or maturity) was 30 kg N/ha for normal types and 13 kg N/ha for semi-dwarf types. The same pattern was observed at high nitrogen supply in Göttingen 2012 with a difference in total nitrogen uptake between the two harvest dates of about 20 kg N/ha for both types (Tab. 3.3).

Oilseed rape looses its leaves between end of flowering and maturity. The decline in total nitrogen in Göttingen 2011 and Göttingen 2012 could have been caused by nitrogen loss through leaf shedding. Ulas et al. (2013) reported nitrogen loss rates through leaf shedding of 3.7 kg N/ha at N0 (no nitrogen fertilization) and 13.4 kg N/ha at N240 (240 kg N/ha fertilized). Gombert et al. (2010) reported that leaves and stems of oilseed rape are the major storage organs for nitrogen until flowering stage. As soon as reproductive organs develop, the nitrogen stored in leaves and stems is remobilized and pods become the most important nitrogen sink in the plant. The first basal leaves were dropped before pod development. Thus stems acted as a nitrogen buffer and became the main sink of remobilized nitrogen from leaves. However the proportion of remobilized nitrogen allocated to the stem decreased with increasing nitrogen supply. The nitrogen concentration of dropped leaves increased with rising nitrogen level (Gombert et al., 2010). An increase in nitrogen concentration of dropped leaves with rising nitrogen supply was also observed by Kessel (2000). Gombert et al. (2010) assumed a sink limitation for remobilized nitrogen from leaves, causing higher nitrogen concentrations in dropped leafs. Furthermore the authors concluded that the time course of nitrogen uptake seems to have a stronger impact on the efficiency of nitrogen remobilization than the overall nitrogen supply. This

conclusion is confirmed when comparing the present observations in Göttingen 2012 at low and high nitrogen supply. At low nitrogen supply (Go12) both growth types took up about 27 kg/ha of mineralized nitrogen by end of flowering and continued nitrogen uptake after flowering (Tab. 3.1). At high nitrogen supply both growth types took up already 140 kg N/ha by end of flowering but lost about 20 kg N/ha until maturity (Tab. 3.3).

Nitrogen uptake and nitrogen uptake efficiency

At harvest EOF nitrogen uptake and nitrogen uptake efficiency showed no significant difference between the growth types at low and high nitrogen supply (Tab. 3.6). But both traits were significantly influenced by interactions between growth type and environment at both nitrogen levels. Results from the harvest at maturity revealed that semi-dwarfs had a significantly higher nitrogen uptake efficiency than normal types at low and high nitrogen supply. Nitrogen uptake proved to be non-significantly different between growth types at low nitrogen supply but significantly different at high nitrogen supply (Tab. 3.8). Based on the observations for harvest at EOF the positive effect of the semi-dwarf type on nitrogen uptake and nitrogen uptake efficiency at maturity could be a result of less nitrogen loss through leaf shedding compared to normal types. For a reliable conclusion more environments need to be tested due to the strong interaction effects of growth type with environments.

Heritabilities for nitrogen uptake and nitrogen uptake efficiency at low and high nitrogen supply were low at both harvest dates due to significant genotype by environment interactions and high experimental errors. Heritabilities at high nitrogen supply are probably overestimated due to only one environment.

Nitrogen uptake efficiency at maturity in the semi-dwarf types correlated with seed yield at low nitrogen supply ($r = 0.73$) and high nitrogen supply ($r = 0.78$). The same was true for normal types with $r = 0.92$ at low nitrogen and $r = 0.86$ at high nitrogen supply (Tab. 3.11). Schulte auf'm Erley et al. (2011) analysed a set of 17 winter oilseed rape lines and hybrids and found significant correlations of nitrogen uptake with seed yield of $r = 0.78$ at low nitrogen supply and $r = 0.65$ at high nitrogen supply. In the interpretation of correlation coefficients for nitrogen uptake, nitrogen uptake efficiency and nitrogen utilization efficiency with seed yield, it shall be considered that they could be overestimated, due to use of seed yield in the estimation of all three components.

Nitrogen utilization efficiency and nitrogen use efficiency

Semi-dwarf hybrids revealed a significantly higher nitrogen utilization efficiency at low and high nitrogen supply compared to normal types (Tab. 3.7, 3.8). The difference in nitrogen utilization efficiency between growth types was much more pronounced at low than at

high nitrogen supply. Seed yield of normal types was positively correlated with nitrogen utilization efficiency at low ($r = 0.40$) and high nitrogen supply ($r = 0.35$) (Tab.3.11). Nitrogen utilization efficiency in the semi-dwarfs was not significantly correlated with seed yield. The average nitrogen utilization efficiency of 22.8 kg seeds/kg N (Tab. 3.7) for normal type hybrids at low nitrogen supply in the present study is in accordance with results from Berry et al. (2010), reporting nitrogen utilization efficiencies of 19.5 kg seed/ kg N to 23.1 kg seeds/kg N for normal hybrids at low nitrogen supply. At both nitrogen levels, nitrogen utilization efficiency was significantly negatively correlated with the amount of nitrogen of straw. This means that genotypes with less straw and/or lower nitrogen contents in the straw are favorable in terms of nitrogen utilization efficiency (Tab. 3.11). A significant genotypic variation for nitrogen utilization efficiency was observed in both growth types and at both nitrogen levels (Tab. 3.8).

Semi-dwarfs proved to have a significantly higher nitrogen use efficiency at low and high nitrogen supply (Tab. 3.7). The relative contributions of nitrogen uptake efficiency and nitrogen utilization efficiency to the genetic variation in nitrogen use efficiency were calculated according to Moll et al. (1982). At both nitrogen levels nitrogen uptake efficiency was the most important parameter for genetic variation in nitrogen use efficiency (Tab. 3.9). These results are contrary to other studies for nitrogen use efficiency in winter oilseed rape where nitrogen utilization efficiency was more important with increased nitrogen supply (Kessel, 2000; Schulte auf'm Erley et al., 2011). Nitrogen uptake efficiency and nitrogen utilization efficiency at low nitrogen supply were correlated by $r = 0.35$ in the semi-dwarf types and $r = 0.12$ in normal types.

Selection for nitrogen use efficiency

Schulte auf'm Erley et al. (2011) resumed, when nitrogen is the limiting factor and when nitrogen taken up is already used at maximum efficiency, additional N is of overwhelming importance for seed yield. This finding could be confirmed in the present study because high yielding genotypes at low nitrogen supply are among the ones with highest nitrogen uptake rates (Fig. 3.1). Based on the results of this study a breeder should select primarily for high nitrogen uptake efficiency to gain genotypes with superior seed yield at low nitrogen supply. The selection should be conducted at low nitrogen conditions due to the low correlation for nitrogen uptake efficiency between low and high nitrogen supply (Tab. 3.12). Determination of nitrogen uptake efficiency is quite laborious especially in larger scales. Due to the correlation with nitrogen uptake efficiency, seed yield is a parameter to select for high nitrogen uptake rates and efficiencies at low nitrogen conditions.

However, nitrogen use efficiency is the combination of nitrogen uptake and nitrogen utilization efficiency. Thus, seed yield represents the combination of these two separate traits.

Small improvements or even negative trends in one trait (most likely nitrogen uptake), may be hidden by gains in the other (utilization efficiency) (Hawkesford and Barraclough, 2011). Genotypes with lower nitrogen uptake rates can still achieve high seed yields by having a higher nitrogen utilization efficiency (Fig. 3.2). Therefore, selecting for yield alone may not select for optimal nitrogen uptake or utilization characteristics (Hawkesford and Barraclough, 2011). Based on the low (low nitrogen) or negative phenotypic correlation (high nitrogen) between nitrogen uptake efficiency and nitrogen utilization efficiency a selection for genotypes with high nitrogen uptake efficiency and high nitrogen utilization efficiency seems to be challenging (Tab. 3.11). The semi-dwarf growth type could be a solution to this problem. Due to less straw and less nitrogen fixed in the straw semi-dwarfs had a higher nitrogen utilization efficiency compared to normal types. This seems to be of special importance at nitrogen deficient conditions where semi-dwarf types had significantly higher seed yields than normal types.

This experiment has shown that nitrogen uptake efficiency is significantly correlated to seed yield at low and high nitrogen supply and confirmed results from other studies in winter oilseed rape (Berry et al., 2010; Schulte auf'm Erley et al., 2011; Kessel et al., 2012). At low and high nitrogen supply levels nitrogen uptake efficiency was more important to nitrogen use efficiency than nitrogen utilization efficiency. This is in clear contrast to results from Kessel et al. (2012). A significant genetic variation with strong interactions of genotype with environments was detected for nitrogen uptake efficiency and nitrogen utilization efficiency. The amount of nitrogen fixed by a genotype at end of flowering proved to be not correlated with seed yield. This confirms observations from Berry et al. (2010).

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4 QTL mapping in semi-dwarf and normal type oilseed rape hybrids

4.1 Introduction

Many agronomic traits are quantitatively controlled by several genes with different effects. The use of molecular marker for quantitative trait locus (QTL) analysis proved to be a powerful genetic approach to dissect complex traits (Paran and Zamir, 2003). One of the most complex traits is seed yield. Shi et al. (2009) identified more than 400 genomic regions directly or indirectly related to seed yield in oilseed rape. Forty-seven of these QTL were relevant for seed yield. It was demonstrated that many QTL for seed yield and yield related traits clustered into unique QTL with pleiotropic effect. However, only a few QTL studies in oilseed rape were conducted at different nitrogen levels (Gül, 2003; Miro, 2010).

The aim of this experiment is to detect genetic mechanisms controlling agronomic and nitrogen related traits at low and high nitrogen supply.

4.2 Materials and Methods

4.2.1 Plant material and Experimental Design

A doubled-haploid (DH) population (242 DH lines) derived from the cross 'Alesi-*bzh*' x 'H30' was produced. 'Alesi-*bzh*' is a dwarf isogenic line derived from the German winter oilseed cultivar 'Alesi' backcrossed (BC4) to the *bzh* dwarf mutant described by (Foisset et al., 1995). 'H30' is a normal type, resynthesized rapeseed line originating from a cross of *Brassica rapa* ssp.*chinensis* with *Brassica napus* ssp. *napus* var. *pabularia* with moderate winterhardiness (Girke, 2002). The DH population is segregating into dwarf and normal type lines. Furthermore, DH lines segregate for erucic acid content due to one erucic acid gene of the resynthesized parent 'H30'. All DH lines were crossed with a normal type, male sterile tester (canola quality) using the MSL ('Männliche Sterilität Lembke') hybrid system (Paulmann and Frauen, 1998). The resulting testcross hybrids (TC) segregate

into semi-dwarf and normal types.

In season 2010/11 150 TC (75 semi-dwarfs and 75 normal types) were selected based on the availability of seeds and evaluated in two adjacent experiments. One experiment was harvested at the end of flowering (EOF) (BBCH 69, Lancashire et al. (1991)) and the second experiment at seed maturity (BBCH 89). Experiments were conducted at two sites near Einbeck (Ei) (brunic aerosol) and near Göttingen (Go) (alluvial meadow soil) in Central Germany. The plot sizes were 18 m^2 in Einbeck and 11.25 m^2 in Göttingen with plant densities of 50 and 90 plants/m^2, respectively. Both experiments were evaluated at low nitrogen (N0) supply, without additional nitrogen fertilization. Soil mineral nitrogen determined at the beginning of plant growth in spring was 35 kg N/ha in Einbeck (Ei11) and 27 kg N/ha in Göttingen (Go11). All other agronomic treatments (except fertilization) were done according to recommendations for oilseed rape cultivation in Germany. Growth types were interlaced within each block by alternately growing one strip of semi-dwarfs and one strip of normal types. Experiments were designed as split-plot with growth type as main plot factor and genotypes as subplot factor. The experimental design was identical for both environments.

In season 2011/12 experiments from season 2010/11 were repeated using 108 TC (54 semi-dwarfs and 54 normal types). The 108 TC were selected based on erucic acid content and seed yield in season 2010/11. For each growth type 27 TC with erucic acid (14 highest seed yield, 13 lowest seed yield) and the 27 TC without erucic acid (14 highest seed yield, 13 lowest seed yield) were selected. Hybrids were evaluated in two identical experiments at both sites (Göttingen and Einbeck). One experiment was harvested at the end of flowering (BBCH 69) and the second experiment at seed maturity (BBCH 89). Both experiments were conducted with two levels of nitrogen supply: low nitrogen supply (without nitrogen fertilization) and high nitrogen supply (fertilized with 177 kg N/ha). Mineral nitrogen in the soil was determined at the beginning of plant growth in spring in Einbeck (27 kg N/ha) and Göttingen (27 kg N/ha). Both growth types were interlaced within each block and arranged in split-plot design. Genotypes within growth types were randomized in lattice design.

In season 2012/13 the 108 TC (54 semi-dwarfs and 54 normal types) were tested at two locations (Einbeck and Göttingen). In season 2012/13 all plots were harvested at seed maturity only. Field trials were conducted at two levels of nitrogen supply: low nitrogen supply (without nitrogen fertilization) and high nitrogen supply (fertilized with 177 kg N/ha). Mineral nitrogen in the soil at the beginning of plant growth in spring was determined in Göttingen (24 kg N/ha) only. Genotypes within growth types were randomized in lattice design. Both growth types were interlaced within each block and arranged in split-plot design.

Each season-site combination is considered as one environment and is addressed by location abbreviation and harvest year, such as Ei11 for Einbeck in the season 2010/2011.

4.2.2 Genetic markers and linkage map

In total 139 DH lines (out of 150) used to produce TC were genotyped. In season 2011/12 and 2012/13 genotypic information was available for 100 (out of the 108 DH lines) used for TC production.

Genotyping was conducted using simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) marker. The SSR marker (primer) labeled with prefix 'BRAS' and 'CB' were developed by Celera AgGen Brassica Consortium (Piquemal et al., 2005). SSR primer named with prefix 'MR' were developed by the Georg-August-Universität Göttingen (Uzunova and Ecke, 1999). The SNP marker are coded with prefix 'ra'. Complete marker data was provided by KWS SAAT AG.

The genome of *Brassica napus* contains homologous regions in the A and C genome, due to the amphidiploid structure of *Brassica napus* (Schranz et al., 2006). Therefore, some SSR primer pairs amplify more than one locus. These primer pairs are labelled with an additional letter at the end to distinguish different loci.

Genetic linkage map was constructed with MAPMAKER / EXP 3.0b (Lander et al., 1987). A pearl-script that automates the mapping process by controlling MAPMAKER / EXP 3.0b was used (Ecke, personal communication). Recombination frequencies were converted by Kosambi mapping function into genetic distances given in centi Morgan (cM) (Kosambi, 1943). A subset of markers was used to construct a 'high fidelity' map with marker orders supported by a minimum LOD of 3.0 and a maximum genetic distance of 35 cM between adjacent markers on a linkage group (LG). Remaining markers were fitted into the high fidelity map by 'order' and 'try' command. A χ^2 test (P=0.05) was used to check for deviations from Mendelian segregation ratios (1:1 or 3:1) at each marker locus. Linkage groups were named according to the AC nomenclature (Choi et al., 2007). Linkage groups A01 - A10 correspond to the A-genome chromosomes derived from *Brassica rapa* and C01 - C09 correspond to the C-genome chromosomes from *Brassica oleracea*.

Linkage groups with marker names and position in cM were drawn using MapChart 2.2 (Voorrips, 2002).

4.2.3 Statistical analysis

Analyses of variance (ANOVA) and heritabilities (h^2) were calculated using PLABSTAT software (Version 3B) (Utz, 2001).

ANOVA within each N-level was performed across environments using the following model:

$$Y_{ijk} = \mu + e_i + b_{ij} + g_k + eg_{ik} + \epsilon_{ijk} \tag{4.1}$$

where Y_{ijk} is the observation of a genotype (k) at environment (i) in block (j)within environment (i). The interaction effect of environment and genotype is denoted by eg_{ik}. The error is denoted by (ϵ_{ijk}). The general mean is designated by μ. The factors environment, block and genotype were considered as random.

4.2.4 QTL mapping

Location of putative QTL and estimation of their genetic effects was calculated by composite interval mapping (CIM) (Zeng, 1994) implemented in Windows QTL Cartographer 2.5 (Wang et al., 2006). According to Cowen (1988) it is appropriate to use an additive genetic model to analyse TC progenies. According to Schön et al. (1994) the corresponding model can be written as:

$$Y_i = \mu_{P1} + k_j a + \epsilon_j \tag{4.2}$$

where Y_i is the phenotypic trait value of TC line j, μ_{P1} mean phenotypic trait value of TC carrying the allele from parent 1 ('Alesi-*bzh*') at the QTL, k_j indicator variable depending on the parental genotype at the QTL: 0 if parental DH line carries allele from parent 1 or 1 if parental DH line carries allele from parent 2 ('H30'). The average effect of substituting allele from P1 by allele of P2 at the QTL is designated with a. The variation of the trait not controlled by the QTL is indicated by ϵ_j.

The software Windows QTL Carthographer 2.5 was used for QTL mapping (Wang et al., 2006). The CIM procedure use markers as cofactors to eliminate effects of other QTL located in other regions of the same chromosome that harbours a putative QTL (Rifkin, 2012). Including cofactors reduce the confidence interval of the putative QTL position and increase the power of QTL detection. Default values of 5 control markers and 10 cM for window size were used as parameters for 'Model-6'. The window size will exclude a region of 10 cM on either side of the markers flanking the test site The flanking regions are tightly linked to the testing site and would eliminate the signal from the test site itself. The threshold LOD score for detecting a significant QTL was determined

individually for every trait by permutation analysis (500 permutations). The confidence interval of a QTL is determined by the interval in which the LOD decreases by 1.0 relative to the maximum LOD score (most likely position of a QTL). QTL on one linkage group with overlapping confidence intervals between years and/or traits were considered as the same QTL.

QTL mapping was performed with mean values for 17 traits from individual environments and means across environments for each nitrogen level. The traits analysed were described in detail in chapter 2 and chapter 3.

4.3 Results

4.3.1 Genetic linkage map

A total of 541 (432 SNP, 109 SSR) polymorphic marker loci were identified in the 'Alesi-*bzh* x H30' population. A proportion of 55 markers (39 SNP, 16 SSR) showed a significant deviation (P=0.05) from the expected segregation ratio. The final linkage map includes 437 markers (392 SNP, 45 SSR) distributed on 22 linkage groups (LGs) with a total map length of 1301 cM (Kosambi). The number and density of markers differed considerably between the LGs, ranging from 2 markers on LG 22 up to 52 on LG C06 (Tab. 4.1). In total 157 marker loci co-segregated with other markers on the map. Twenty-one LGs could be assigned to the 19 chromosomes of *Brassica napus*. Two linkage groups (LG C04 and LG C05) are split into two subgroups. The full linkage map is shown in the appendix A.

The linkage groups $A01 - A10$ represent the A-genome (*Brassica rapa*), while LG $C01 - C09$ belong to the C-genome (*Brassica oleracea*) (Choi et al., 2007). The small LG 22 with two markers only, could not be assigned to any of the 19 chromosomes of *Brassica napus*. The SNP marker (SNP-bzhrga1) inside the dwarf gene *Bzh* was mapped to the distal part of LG A06 (3 cM). This is in accordance with other linkage maps (Breuer personal communication).

Table 4.1: Genetic linkage map with length and number of markers per linkage group

Linkage group	Length [cM]	Number of marker	Linkage group	Length [cM]	Number of marker
A01	75.3	25	C01	71.9	28
A02	44.1	12	C02	8.0	4
A03	135.3	36	C03	79.2	22
A04	27.1	8	C04a	29.8	4
A05	98.1	29	C04b	63.0	15
A06	90.8	32	C05a	44.4	15
A07	70.4	38	C05b	13.6	6
A08	19.7	11	C06	110.9	52
A09	80.7	18	C07	54.5	22
A10	59.7	12	C08	38.4	15
LG22	6.9	2	C09	79.1	20

centi Morgan (cM)

4.3.2 QTL at low nitrogen supply

QTL at individual environments with low nitrogen supply

Analysis of variance (ANOVA) revealed significant genotypic variation for all traits, except protein content, nitrogen uptake EOF and nitrogen uptake efficiency EOF (Tab. 4.2). Significant genotype by environment interactions were detected for all traits, except nitrogen harvest index. Heritabilities ranged from 0.11 (Nitrogen uptake EOF) to 0.74 (Nitrogen utilization and Nitrogen use efficiency). The heritability for seed yield was rather low ($h^2 = 0.20$).

Six QTL in four individual environments were detected for begin of flowering. The high-

Table 4.2: Variance components and heritabilities at low nitrogen supply (six environments, 100 genotypes)

Source	E	B	G	G×E	h^2
Df	5	6	99	495	
Begin of flowering (days)	60.52 **	0.02 **	0.13 **	0.44 **	0.56
Plant height (cm)	147.48 **	0.26 +	72.82 **	153.30 **	0.72
Seed yield (dt/ha)[a]	64.64 **	0.32 **	0.26 +	3.21 **	0.20
Straw yield (dt/ha)[a]	90.60 *	20.38 **	4.29 **	10.74 **	0.51
Harvest index [b]	0.67 **	0.03 **	0.05 **	0.11 **	0.57
Thousand seed weight (g)	0.03 **	0.01 **	0.01 **	0.03 **	0.60
Oil content (%)	1.70 **	0.15 **	0.39 **	0.60 **	0.67
Protein content (%)	1.49 **	0.05 **	0.01 ns	0.14 **	0.14
Nitrogen uptake EOF (kg N/ha) [b]	828.28 **	4.62 **	2.51 ns	25.20 **	0.11
Nitrogen uptake maturity (kg N/ha) [a]	198.23 *	14.82 **	18.04 **	28.31 **	0.38
Nitrogen content straw (%) [c]	0.26 *	0.03 **	0	0.04 *	-
Nitrogen harvest index [d]	0.17 +	0.03 **	0.13 **	0.01 ns	0.73
Nitrogen uptake efficiency EOF [b]	0.05 ns	0.15 **	0.03 ns	0.31 **	0.13
Nitrogen uptake efficiency [d]	0.40 **	0	0.33 **	0.31 **	0.49
Nitrogen utilization efficiency [d]	350.66 *	34.11 **	209.98 **	31.11 *	0.74
Nitrogen use efficiency [d]	862.95 **	7.88 +	594.54 **	169.93 **	0.74

,*,+ Significant difference at 0.01, 0.05, 0.10 probability level respectively; ns non-significant (F-test from ANOVA), negative variance components were set to zero, **E environment, **B** block within environment,**G** genotype, h^2 heritability, **EOF** end of flowering,
[a] five environments,
[b] four environments,
[c] three environments,
[d] two environments

est proportion of explained phenotypic variance was detected in Göttingen 2013 (78 %) (Tab. 4.3). At the QTL on A07, C06 and C07 the allele from 'Alesi-*bzh*' delayed begin of flowering. At the other three QTL the allele had the opposite effect.

Table 4.3: Putative QTL for agronomic traits at low nitrogen supply (six environments, 100 genotypes)

Env.	QTL	LG	CI [cM]	Pos.[cM]	LOD	Effect	R^2
Begin of flowering [days]							
Ei13	*BFL-A01*	A01	19.4 - 36.1	32.5	4.32	0.35	0.14
Ei13	*BFL-A03*	A03	62.3 - 74.4	68.8	3.15	0.28	0.09
Go13	*BFL-A06*	A06	1.4 - 5.2	3.0	39.24	0.53	0.78
Go11	*BFL-A07*	A07	65.0 - 69.3	68.3	4.1	-0.25	0.10
Ei13	*BFL-C06*	C06	54.0 - 72.7	64.9	3.23	-0.29	0.10
Ei12	*BFL-C07*	C07	0.0 - 7.0	1.0	5.36	-0.56	0.18
Plant height [cm]							
Ei11	*PH-A06*	A06	0.0 - 2.6	1.0	54.0	10.30	0.79
Go12	*PH-A06*	A06	1.9 - 5.2	3.0	72.0	9.93	0.93
Ei12	*PH-A06*	A06	1.4 - 5.2	3.0	65.4	18.75	0.90
Go13	*PH-A06*	A06	1.4 - 5.2	3.0	87.0	14.90	0.93
Ei13	*PH-A06*	A06	3.5 - 9.0	5.0	48.2	16.66	0.87
Ei12	*PH-C03*	C03	41.0 - 62.4	51.3	3.6	-1.91	0.01
Ei13	*PH-C03*	C03	59.4 - 78.1	71.1	3.0	-2.63	0.02
Ei12	*PH-A03*	A03	93.2 - 114.6	102.5	3.4	1.99	0.01
Seed yield [dt/ha] [a]							
Ei11	*Seed-A06*	A06	2.6 - 36.3	20.0	3.4	-0.57	0.09
Go11	*Seed-A06*	A06	0.0 - 2.1	1.0	26.1	-2.39	0.54
Go12	*Seed-A06*	A06	1.3 - 10.1	3.0	19.8	-1.20	0.48
Go13	*Seed-A06*	A06	1.0 - 12.2	3.0	11.2	-1.57	0.33
Ei11	*Seed-C07*	C07	40.2 - 51.0	47.1	2.9	0.51	0.08
Go11	*Seed-C09*	C09	47.4 - 55.8	48.7	4.2	-0.76	0.05
Straw yield [dt/ha] [a]							
Go11	*Straw-A06*	A06	0.0 - 2.3	0.0	4.2	-0.02	0.12
Go12	*Straw-A06*	A06	0.0 - 12.2	0.0	9.4	1.57	0.26
Ei13	*Straw-A06*	A06	2.8 - 14.6	7.0	18.5	0.51	0.53
Go13	*Straw-A06*	A06	0.0 - 2.9	0.0	11.3	2.76	0.36
Harvest index [b]							
Go12	*HI-A06*	A06	3.0 - 8.1	4.0	25.5	-0.04	0.53
Ei13	*HI-A06*	A06	3.0 - 16.0	8.0	15.7	-0.03	0.46
Go13	*HI-A06*	A06	0.7 - 3.8	2.0	21.4	-0.03	0.52
Ei13	*HI-A07*	A07	46.2 - 56.3	50.7	4.5	-0.02	0.09
Go13	*HI-A07*	A07	55.7 - 65.3	55.8	3.5	-0.01	0.06

Env environment, **LG** linkage group, **CI** LOD-1 confidence intervals in centi Morgan (cM), **Pos** position with highest LOD, **LOD** maximum LOD score, **Effect** effect of substituting allele of P1 by allel of P2 in the TC, $\mathbf{R}^2$ proportion of phenotypic variance explained by the QTL,

[a] five environments,

[b] three environments

Two QTL were detected. The QTL *PH-A06* was detected in five environments in an interval from 0.0 cM to 9.0 cM at the distal part of LG A06. The phenotypic variance for plant height by *PH-A06* ranged from 79 % to 93 %. Confidence intervals overlapped among all five environments. The second QTL *PH-C03* was detected in Einbeck only.
Three putative QTL were discovered for seed yield on LG A06, C07 and C09. The QTL on LG A06 was detected in four environments with overlapping confidence intervals. The QTL *Seed-A06* explained between 9 % and 54 % of phenotypic variation in seed yield. For the QTL *Seed-A06* and *Seed-C09* the 'Alesi-*bzh*' allele increased seed yield while it had a decreasing effect at *Seed-C07*.
One QTL for straw yield was detected in four individual environments. In Göttingen 2011 the 'Alesi-*bzh*' allele increased straw yield, while it had an decreasing effect in the other environments. Two QTL on LG A06 and A07 were identified for harvest index. At both QTL the 'Alesi-*bzh*' allele had an increasing effect on harvest index. The QTL *HI-A07* was detected only detected in the year 2013 and accounted for 6 % of phenotypic variation in Einbeck 2013 and 9 % in Göttingen 2013.
Five QTL were detected for thousand seed weight (TSW) (Tab. 4.4). The QTL on LG A01 (*TSW-A01*) explained between 7 % and 12 % of phenotypic variation in thousand seed weight. The QTL on A06 (*TSW-A06*) was detected in three environments and had the strongest effect on TSW. The QTL LG A07 was only detected in Einbeck 2013 and Göttingen 2013. The fifth QTL (*TSW-C01*) was located on LG C01 and explained 7 % to 15 % of variation in TSW. The QTL on LG C04b was only detected in the year 2011 (Tab. 4.4). Three QTL were detected for oil content. The allele from 'H30' at QTL *Oil%-A08* increased oil content by nearly 1 %. The effect of this QTL accounted for 43 % to 57% of the phenotypic variation in oil content. The QTL on LG A05 was detected in Einbeck 2012 only. The QTL on LG A06 was identified in the year 2011 only.

Most of the traits in Table 4.5 were only determined in Göttingen 2011 and 2012. Two QTL for nitrogen uptake EOF were detected on LG A06 and C06 (Tab. 4.5). Nitrogen uptake maturity was influenced by two QTL on LG C06. Both QTL were detected in Göttingen 2012 only. Three QTL were identified for the difference in nitrogen uptake between maturity and end of flowering. Two QTL were detected for nitrogen content of straw in Göttingen 2011 and Einbeck 2012. Nitrogen harvest index, nitrogen uptake efficiency EOF, Nitrogen uptake efficiency and nitrogen utilization efficiency were all influenced by a QTL on LG A06 (Tab. 4.5). Confidence intervals for nitrogen harvest index and nitrogen uptake efficiency overlapped between Göttingen 2011 and 2012. Four QTL were detected for nitrogen use efficiency (NUE). The allele from 'Alesi-*bzh*' increased NUE at QTL *NUE-A06* and *NUE-C09* while it decreased NUE at the other two QTL.

Table 4.4: Putative QTL for thousand seed weight, oil content and protein content at low nitrogen supply (six environments, 100 genotypes)

Env.	QTL	LG	CI [cM]	Pos.[cM]	LOD	Effect	R^2
Thousand seed weight [g]							
Ei12	*TSW-A01*	A01	0.0 - 9.8	0.0	4.0	0.08	0.12
Go12	*TSW-A01*	A01	0.0 - 8.3	0.0	3.7	0.07	0.09
Ei13	*TSW-A01*	A01	1.1 - 22.9	9.8	3.2	0.06	0.07
Go13	*TSW-A01*	A01	8.3 - 23.0	14.1	5.0	0.07	0.09
Go12	*TSW-A06*	A06	0.0 - 15.2	3.0	6.0	0.09	0.15
Ei13	*TSW-A06*	A06	1.0 - 12.2	3.0	11.0	0.11	0.27
Go13	*TSW-A06*	A06	1.1 - 13.1	3.0	16.0	0.14	0.36
Ei13	*TSW-A07*	A07	58.3 - 69.3	65.3	6.2	0.08	0.13
Go13	*TSW-A07*	A07	58.3 - 69.3	66.3	5.1	0.07	0.09
Go11	*TSW-C01*	C01	40.2 - 60.9	40.8	3.1	0.08	0.07
Ei12	*TSW-C01*	C01	40.7 - 64.3	48.8	2.8	0.07	0.09
Go12	*TSW-C01*	C01	46.4 - 64.9	50.9	4.3	0.08	0.11
Ei13	*TSW-C01*	C01	45.4 - 58.9	50.8	6.1	0.08	0.13
Go13	*TSW-C01*	C01	47.6 - 57.4	50.9	8.2	0.13	0.15
Ei11	*TSW-C04b*	C04b	3.0 - 20.5	3.6	2.9	-0.09	0.06
Go11	*TSW-C04b*	C04b	0.0 - 3.3	2.0	4.3	-0.11	0.10
Oil content [%]							
Ei12	*Oil%-A05*	A05	0.0 - 7.0	3.7	3.6	0.44	0.13
Ei11	*Oil%-A06*	A06	0.0 - 2.5	1.0	5.8	0.32	0.06
Go11	*Oil%-A06*	A06	0.0 - 36.3	3.0	3.5	0.26	0.05
Ei11	*Oil%-A08*	A08	0.0 - 4.2	0.0	29.8	0.97	0.52
Go11	*Oil%-A08*	A08	0.0 - 4.2	2.0	20.6	0.74	0.43
Go12	*Oil%-A08*	A08	1.8 - 6.6	4.0	22.4	0.85	0.55
Ei13	*Oil%-A08*	A08	2.9 - 7.8	4.8	15.5	0.99	0.44
Go13	*Oil%-A08*	A08	3.1 - 8.3	4.8	26.1	0.96	0.57
Protein content [%]							
Go12	*Pro%-A06*	A06	0.7 - 10.2	3.0	11.8	0.28	0.31
Go13	*Pro%-A06*	A06	5.6 - 18.0	10.0	9.3	0.32	0.37
Ei11	*Pro%-A08*	A08	0.0 - 17.8	16.8	2.8	-0.13	0.06

Env environment, **LG** linkage group, **CI** LOD-1 confidence intervals in centi Morgan (cM), **Pos** position with highest LOD, **LOD** maximum LOD score, **Effect** effect of substituting allele of P1 by allel of P2 in the TC, $\mathbf{R}^2$ proportion of phenotypic variance explained by the QTL

Table 4.5: Putative QTL for nitrogen related traits at low nitrogen supply (four environments, 100 genotypes)

Env.	QTL	LG	CI[cM]	Pos.[cM]	LOD	Effect	R^2
Nitrogen uptake EOF [kg N/ha]							
Go11	*NkgEOF-A06*	A06	0.0 - 2.6	1.0	3.5	3.90	0.10
Ei12	*NkgEOF-A06*	A06	0.0 - 22.4	11.0	6.5	2.54	0.27
Ei12	*NkgEOF-C06*	C06	78.8 - 85.4	79.7	3.2	1.51	0.10
Nitrogen uptake maturity [kg N/ha] [a]							
Go12	*NkgMat-C06*	C06	0.8 - 11.6	3.0	7.4	-2.07	0.21
Go12	*NkgMat-C06*	C06	64.5 - 70.2	67.5	4.8	1.63	0.13
Nitrogen uptake difference maturity - EOF [kg N/ha] [a]							
Go11	*Ndiff-A06*	A06	0.0 - 2.0	1.0	14.7	-9.30	0.34
Go12	*Ndiff-A06*	A06	0.9 - 10.4	3.0	18.0	-0.14	0.48
Go11	*Ndiff-C19*	C19	71.0 - 75.4	73.7	3.3	3.91	0.06
Nitrogen content straw [%] [b]							
Go11	*N%straw-A06*	A06	0.0 - 2.9	0.0	4.2	-0.02	0.12
Ei12	*N%straw-A09*	A09	4.5 - 14.9	11.9	3.2	-0.03	0.12
Nitrogen harvest index [a]							
Go11	*NHI-A06*	A06	0.0 - 2.1	0.0	21.2	-0.04	0.46
Go12	*NHI-A06*	A06	0.9 - 9.0	3.0	17.4	-0.03	0.47
Nitrogen uptake efficiency EOF							
Go11	*NupEOF-A06*	A06	0.0 - 2.6	0.0	3.54	0.03	0.09
Nitrogen uptake efficiency [a]							
Go11	*Nup-A06*	A06	0.0 - 2.0	1.0	11.5	-0.06	0.28
Go12	*Nup-A06*	A06	0.8 - 11.8	3.0	7.5	-0.04	0.22
Nitrogen utilization efficiency [a]							
Go12	*Nut-A06*	A06	0.9 - 8.5	3.0	19.1	-1.40	0.48
Nitrogen use efficiency [a]							
Go11	*NUE-A06*	A06	0.0 - 2.2	1.0	25.1	-2.46	0.51
Go11	*NUE-C06*	C06	65.9 - 79.3	67.5	3.0	0.67	0.04
Go12	*NUE-C08*	C08	13.3 - 22.5	19.3	3.5	1.54	0.13
Go11	*NUE-C09*	C09	20.3 - 55.7	48.7	3.1	-0.69	0.04

Env environment, **LG** linkage group, **CI** LOD-1 confidence intervals in centi Morgan (cM), **Pos** position with highest LOD, **LOD** maximum LOD score, **Effect** effect of substituting allele of P1 by allel of P2 in the TC, $\mathbf{R}^2$ proportion of phenotypic variance explained by the QTL, **EOF** end of flowering,

[a] two environments,

[b] three environments

QTL across environments with low nitrogen supply

The analysis of QTL across environments with low nitrogen supply identified three QTL for begin of flowering on LG A01, A07 and C06 (Tab. 4.6). The 'Alesi-*bzh*' allele at *BFL-A07* and *BFL-C06* delayed begin of flowering. At *BFL-A01* it had the opposite effect. All three QTL together explained 39 % of phenotypic variance in begin of flowering.
Plant height was affected by two QTL on LG A03 and A06. The QTL *A06* explained the majority (93 %) of phenotypic variation in plant height.

Table 4.6: Putative QTL for agronomic traits across six environments with low nitrogen supply (100 genotypes)

QTL	LG	CI [cM]	Pos.[cM]	LOD	Effect	R^2
Begin of flowering [days]						
BFL-A01	A01	27.3 - 39.9	31.5	4.7	0.23	0.16
BFL-A07	A07	24.3 - 50.1	37.8	2.8	-0.18	0.08
BFL-C06	C06	60.9 - 72.8	64.9	4.6	-0.23	0.15
Plant heigth [cm]						
PH-A03	A03	93.0 - 113.1	102.5	3.3	1.10	0.01
PH-A06	A06	1.4 - 5.2	3.0	78.9	14.41	0.93
Seed yield [dt/ha] [a]						
Seed-A06	A06	1.2 - 11.4	5.0	20.1	-1.03	0.45
Seed-A07	A07	51.4 - 65.3	57.8	4.7	-0.42	0.07
Seed-A10	A10	24.0 - 39.9	31.5	4.6	-0.40	0.07
Seed-C06	C06	54.0 - 74.2	65.9	3.4	0.34	0.05
Straw yield [dt/ha] [a]						
Straw-A02	A02	14.4 - 38.7	25.5	3.2	0.68	0.04
Straw-A06	A06	0.0 - 4.0	0.0	24.8	2.39	0.56
Straw-C07	C07	30.1 - 38.0	34.5	3.5	-0.70	0.05
Harvest index [b]						
HI-A06	A06	0.8 - 3.1	2.0	39.7	-0.04	0.74
HI-A07	A07	52.3 - 62.5	55.8	3.9	-0.01	0.03
HI-A08	A08	0.0 - 12.6	4.8	3.5	0.01	0.03
HI-C06	C06	50.3 - 63.3	55.7	4.9	0.01	0.04

Env environment, **LG** linkage group, **CI** LOD-1 confidence intervals in centi Morgan (cM), **Pos** position with highest LOD, **LOD** maximum LOD score, **Effect** effect of substituting allele of P1 by allel of P2 in the TC, R^2 proportion of phenotypic variance explained by the QTL,
[a] five environments,
[b] four environments

Four QTL were detected for seed yield. The 'Alesi-*bzh*' allele had an increasing effect at the QTL *Seed-A06*, *Seed-A07* and *Seed-A10*. The QTL *Seed-A06* accounted for 45 % of variance in seed yield. The other three QTL explained 19 % of phenotypic variance in seed yield.
Straw yield was influenced by three QTL on LG A02, A06 and C07. The three QTL explained 65 % of phenotypic variance in straw yield. The 'Alesi-*bzh*' allele decreased straw yield at *Straw-A02* and *A06* while it had an increasing effect at *Straw-C07*.
Four QTL were detected for harvest index. The QTL on LG A06 explained most (74 %) of the phenotypic variance in harvest index. The other three QTL accounted for 10 % of phenotypic variance together.

Thousand seed weight was influenced by four QTL on LG A01, A06, C01 and C02 (Tab. 4.7). These QTL explained 54 % of phenotypic variance in thousand seed weight together.

Table 4.7: Putative QTL for thousand seed weight, oil yield, oil content and protein content across six environments with low nitrogen supply (100 genotypes)

QTL	LG	CI [cM]	Pos.[cM]	LOD	Effect	R^2
Thousand seed weight [g]						
TSW-A01	A01	0.0 - 12.1	4.0	4.3	0.06	0.10
TSW-A06	A06	0.9 - 18.2	5.0	5.8	0.07	0.15
TSW-C01	C01	47.4 - 60.5	52.9	7.4	0.08	0.20
TSW-C02	C02	0.0 - 6.4	3.0	4.0	-0.06	0.09
Oil content [%]						
Oil%-A01	A01	60.5 - 74.0	66.0	2.8	-0.16	0.03
Oil%-A06	A06	0.0 - 2.0	0.0	6.9	0.27	0.08
Oil%-A08	A08	3.0 - 7.1	4.9	27.5	0.79	0.66
Oil%-C01	C01	35.7 - 49.5	41.8	4.0	0.21	0.04
Protein content [%]						
Pro%-A01	A01	61.0 - 74.0	70.0	2.9	0.11	0.09
Pro%-A03	A03	121.3 - 133.3	131.3	4.5	0.14	0.14

Env environment, **LG** linkage group, **CI** LOD-1 confidence intervals in centi Morgan (cM), **Pos** position with highest LOD, **LOD** maximum LOD score, **Effect** effect of substituting allele of P1 by allel of P2 in the TC, $\mathbf{R}^2$ proportion of phenotypic variance explained by the QTL

Oil content was influenced by four QTL on LG A01, A06, A08 and C01. The QTL *Oil%-A08* accounted for 66 % of phenotypic variance in oil content. The allele of 'H30' increased oil content by 0.79 %.
Two QTL on LG A01 and A03 were detected for protein content. Both QTL accounted for 23 % of phenotypic variance.

No QTL was detected for nitrogen uptake EOF (Tab. 4.8). One QTL on LG A06 was identified for nitrogen uptake at maturity. The 'Alesi-*bzh*' allele increased N uptake maturity by about 4 kg N/ha. Nitrogen content of straw was influenced by a QTL on LG C01. Three QTL were detected for nitrogen harvest index. The QTL *NHI-A06* accounted for 60 % of variance in nitrogen harvest index with an increasing effect of the allele from 'Alesi-*bzh*'. One QTL on LG A05 was detected for nitrogen uptake efficiency EOF. A QTL on LG A06 explained most of the phenotypic variance in nitrogen uptake efficiency, nitrogen utilization efficiency and nitrogen use efficiency. Two other QTL with minor effects on nitrogen utilization efficiency were detected on LG C01 and C06. Nitrogen use efficiency was influenced by a second QTL on LG A07. The allele of 'Alesi-*bzh*' increased nitrogen use efficiency at both QTL.

Table 4.8: Putative QTL for nitrogen related traits across four environments with low nitrogen supply (100 genotypes)

QTL	LG	CI [cM]	Pos.[cM]	LOD	Effect	R^2
Nitrogen uptake EOF [kg N/ha]						
no QTL						
Nitrogen uptake maturity [kg N/ha] [a]						
NkgMat-A06	A06	1.7 - 16.9	9.0	9.6	-4.19	0.36
Nitrogen uptake difference maturity - EOF [kg N/ha] [a]						
Ndiff-A06	A06	1.2 - 9.2	3.0	10.8	-5.08	0.31
Ndiff-C04a	C04a	18.3 - 25.6	20.4	3.2	3.39	0.07
Nitrogen content straw [%] [b]						
N%straw-C01	C01	15.5 - 30.0	23.6	5.0	0.02	0.20
Nitrogen harvest index [a]						
NHI-A06	A06	1.1 - 10.5	5.0	25.4	-0.03	0.60
NHI-A08	A08	0 - 17.0	4.8	2.5	0.01	0.03
NHI-C06	C06	60.7 - 67.7	63.3	7.4	0.02	0.11
Nitrogen uptake efficiency EOF						
NupEOF-A05	A05	43.6 - 65.0	56.5	3.0	-0.15	0.11
Nitrogen uptake efficiency [a]						
Nup-A06	A06	1.1 - 13.4	3.0	10.3	-0.05	0.30
Nitrogen utilization efficiency [a]						
Nut-A06	A06	1.5 - 9.4	3.0	28.6	-1.43	0.64
Nut-C01	C01	34.4 - 50.8	40.8	2.9	-0.33	0.03
Nut-C06	C06	71.0 - 78.5	71.5	5.4	0.45	0.07
Nitrogen use efficiency [a]						
NUE-A06	A06	0.5 - 14.8	3.0	4.7	-0.94	0.16
NUE-A07	A07	51.0 - 65.3	55.8	2.85	-0.76	0.09

Env environment, **LG** linkage group, **CI** LOD-1 confidence intervals in centi Morgan (cM), **Pos** position with highest LOD, **LOD** maximum LOD score, **Effect** effect of substituting allele of P1 by allel of P2 in the TC, $\mathbf{R^2}$ proportion of phenotypic variance explained by the QTL, **EOF** end of flowering,
[a] two environments,
[b] three environments

4.3.3 QTL at high nitrogen supply

QTL at individual environments with high nitrogen supply

Analysis of variance for high nitrogen supply in 2011/12 and 2012/13 revealed significant variation between genotypes for all traits, except seed yield, nitrogen uptake EOF and nitrogen uptake efficiency EOF (Tab. 4.9). All traits were significantly affected by interactions of genotypes with environments. Heritabilities ranged from 0.16 (seed yield) to 0.97 (plant height). Heritabilities for traits determined only in Göttingen 2012 are probably overestimated due to one environment. Significant genotype by environment interactions were observed for all traits.

Table 4.9: Variance components and heritabilities at high nitrogen supply (four environments, 100 genotypes)

Source	E	B	G	GE	h^2
Df	3	4	99	297	
Begin of flowering (days)	92.25 **	0.02 **	0.18 **	0.05 **	0.53
Plant height (cm)	450.42 **	0.28 *	317.30 **	35.88 **	0.97
Seed yield (dt/ha) [a]	30.74 **	0.07 ns	0.47 ns	3.24 **	0.16
Straw yield (dt/ha) [a]	55.99 **	3.51 **	18.33 **	7.47 **	0.84
Harvest index [a]	0.45 **	0.03 **	0.11 **	0.06 **	0.81
Thousand seed weight (g)	0.11 **	0.01 **	0.03 **	0.02 **	0.81
Oil content (%)	0.22 *	0.07 **	0.65 **	0.24 **	0.79
Protein content (%)	0.19 **	0.02 **	0.04 **	0.09 **	0.37
Nitrogen uptake EOF (kg N/ha) [b]	1113.99 *	76.87 **	0	78.76 **	-
Nitrogen uptake maturity (kg N/ha) [c]	-	7.55 **	89.16 **	-	0.72
Nitrogen content straw (%) [b]	3.90 **	0.04 **	0.22 **	0.22 **	0.50
Nitrogen harvest index [c]	-	0.03 **	0.13 **	-	0.78
Nitrogen uptake efficiency EOF [b]	2.63 *	0.16 **	0	0.16 **	-
Nitrogen uptake efficiency [c]	-	0.01 **	0.15 **	-	0.68
Nitrogen utilization efficiency [c]	-	0.27 **	1.00 **	-	0.68
Nitrogen use efficiency [c]	-	0	0.97 **	-	0.72

,*,+ Significant difference at 0.01, 0.05, 0.10 probability level respectively; ns non-significant (F-test from ANOVA), negative variance components were set to zero, **E environment, **B** block within environment,**G** genotype, h^2 heritability, **EOF** end of flowering,
[a] three environments,
[b] two environments,
[c] Göttingen 2012 only

At high nitrogen supply seven QTL influencing begin of flowering were identified in four different environments (Tab. 4.10). Most of them explained around 10 % of the phenotypic variance in begin of flowering.

Table 4.10: Putative QTL for agronomic traits at high nitrogen supply (four environments, 100 genotypes)

Env.	QTL	LG	CI [cM]	Pos.[cM]	LOD	Effect	R^2
Begin of flowering [days]							
Ei12	*BFL-C01*	C01	37.2 - 50.8	42.8	3.45	0.48	0.11
Ei12	*BFL-C06*	C06	58.1 - 67.0	61.3	3.9	-0.49	0.12
Ei12	*BFL-C07*	C07	0.0 - 11.6	4.0	3.1	-0.48	0.11
Go12	*BFL-C07*	C07	38.3 - 43.7	40.7	3.22	-0.18	0.10
Ei13	*BFL-A01*	A01	44.8 - 56.0	50.4	3.16	0.28	0.11
Ei13	*BFL-A09*	A09	27.6 - 51.0	39.0	2.94	0.29	0.12
Go13	*BFL-A06*	A06	11.2 - 19.9	15.0	39.0	0.51	0.81
Plant heigth [cm]							
Ei12	*PH-A06*	A06	1.4 - 5.2	3.0	73.6	25.37	0.94
Go12	*PH-A06*	A06	1.0 - 5.3	3.0	87.2	12.06	0.94
Ei13	*PH-A06*	A06	1.4 - 5.2	3.0	55.5	16.06	0.89
Go13	*PH-A06*	A06	1.4 - 5.2	3.0	102.6	18.39	0.95
Ei12	*PH-C03*	C03	52.5 - 78.1	71.1	3.5	-2.15	0.01
Seed yield [dt/ha] [a]							
Go12	*Seed-A06*	A06	0.2 - 3.0	3.0	15.1	-1.82	0.42
Ei13	*Seed-A06*	A06	0.0 - 30.2	12.0	3.0	0.08	0.14
Straw yield [dt/ha]							
Ei12	*Straw-A06*	A06	1.3 - 13.3	4.0	11.1	1.71	0.34
Go12	*Straw-A06*	A06	0.2 - 3.0	2.0	17.5	3.91	0.50
Ei13	*Straw-A06*	A06	0.9 - 12.4	6.0	21.0	0.61	0.65
Go13	*Straw-A06*	A06	0.0 - 2.5	2.0	11.8	0.36	0.32
Harvest index [a]							
Go12	*HI-A06*	A06	0.8 - 6.0	2.0	24.2	-0.04	0.60
Ei13	*HI-A06*	A06	0.0 - 10.2	3.0	9.8	-0.02	0.30
Go13	*HI-A06*	A06	0.0 - 2.5	2.0	11.6	-0.02	0.33

Env environment, **LG** linkage group, **CI** LOD-1 confidence intervals in centi Morgan (cM), **Pos** position with highest LOD, **LOD** maximum LOD score, **Effect** effect of substituting allele of P1 by allel of P2 in the TC, $\mathbf{R}^2$ proportion of phenotypic variance explained by the QTL,
[a] three environments

The QTL *BFL-A06* detected in Göttingen 2013 explained 81 % of the variance for begin of flowering (Tab. 4.11). Confidence intervals of QTL on same LG were not overlapping.

Plant height was significantly affected by two QTL on LG A06 and C03. The QTL *PH-A06* was detected in four environments in overlapping intervals between 1.0 and 5.3 cM.

The explained phenotypic variance in plant height by this QTL ranged from 89 % (Ei13) to 95 % (Go13). The QTL *PH-C03* was identified in Einbeck 2012 only.
One putative QTL for seed yield was identified in Göttingen 2012 and Einbeck 2013. Confidence intervals for the QTL overlapped between environments. In Göttingen 2012 the allele from 'Alesi-*bzh*' increased seed yield while it decreased seed yield in Einbeck 2013.
A QTL on LG A06 significantly influenced straw yield at four environments (Tab. 4.10). Confidence intervals overlapped between environments ranging from 0 cM to 13.3 cM. The explained phenotypic variance ranged from 34 % (Ei12) to 65 % (Go13). The 'Alesi-*bzh*' allele reduced straw yield in all environments. One QTL on LG A06 was detected

Table 4.11: Putative QTL for thousand seed weight, oil content and protein content at high nitrogen supply (four environments, 100 genotypes)

Env.	QTL	LG	CI [cM]	Pos.[cM]	LOD	Effect	R^2
Thousand seed weight [g]							
Ei13	*TSW-A01*	A01	2.6 - 23.0	13.1	3.3	0.07	0.07
Ei13	*TSW-A06*	A06	0.6 - 8.7	3.0	13.6	0.16	0.36
Go13	*TSW-A06*	A06	0.4 - 19.6	8.0	8.9	0.14	0.25
Ei13	*TSW-C01*	C01	40.8 - 68.8	57.9	3.2	0.07	0.08
Go13	*TSW-C01*	C01	47.3 - 61.0	53.9	8.2	0.13	0.22
Oil content [%]							
Ei12	*Oil%-A06*	A06	0.0 - 3.0	2.0	20.0	0.71	0.37
Go12	*Oil%-A06*	A06	0.9 - 10.1	4.0	16.5	0.70	0.31
Ei13	*Oil%-A06*	A06	0.0 - 13.8	1.0	4.4	0.40	0.16
Go13	*Oil%-A06*	A06	0.0 - 14.9	1.0	4.2	0.33	0.07
Ei12	*Oil%-A08*	A08	1.4 - 17.0	4.0	17.4	0.65	0.30
Go12	*Oil%-A08*	A08	7.4 - 10.8	7.8	21.7	0.82	0.43
Go13	*Oil%-A08*	A08	3.0 - 7.2	4.8	21.2	0.88	0.51
Protein content [%]							
Ei12	*Pro%-A06*	A06	0.0 - 12.9	1.0	14.2	-0.36	0.39
Go12	*Pro%-A06*	A06	3.4 - 18.9	8.0	8.7	-0.31	0.31

Env environment, **LG** linkage group, **CI** LOD-1 confidence intervals in centi Morgan (cM), **Pos** position with highest LOD, **LOD** maximum LOD score, **Effect** effect of substituting allele of P1 by allel of P2 in the TC, $\mathbf{R^2}$ proportion of phenotypic variance explained by the QTL

for harvest index in three environments. In Göttingen 2012 *HI-A06* accounted for 60 % of phenotypic variance in harvest index. In Einbeck 2013 and Göttingen 2013 the QTL explained about one third of the variance in harvest index. The allele from 'Alesi-*bzh*' increased the harvest index in all three environments (Tab. 4.10).

Five QTL in four individual environments were identified for thousand seed weight (Tab. 4.11). The allele from 'Alesi-*bzh*' reduced TSW at all QTL. Two QTL on LG A06 and A08 were detected for oil content. Confidence intervals for both QTL overlapped between individual environments. The QTL on A06 explained between 7% and 37 % of the phenotypic variance in oil content. The 'Alesi-*bzh*' allele reduced oil content at the QTL on A06 in a range from 0.33 % to 0.71 %. The explained phenotypic variance in oil content of QTL *Oil%-A08* ranged 30% to 51 %. The 'Alesi-*bzh*' allele reduced oil content in a range from 0.65 % to 0.88 %.
Protein content was affected by a QTL on LG A06. The QTL accounted for 31 % (Go12) or 39 % (Ei12) of phenotypic variance in protein content. The 'Alesi-*bzh*' allele increased protein content in both environments.

Four QTL were detected for nitrogen uptake EOF (Tab. 4.12). The effect OF QTL on LG A06 had different directions in the single environments.
One QTL was detected for nitrogen uptake maturity on LG A06. It explained 23 % of phenotypic variance in the trait. Two QTL were identified for the difference in nitrogen uptake between maturity and end of flowering. Together they accounted for 21 % of phenotypic variance.
Nitrogen content of straw was influenced by two QTL on LG A06 and C06. Confidence intervals of both QTL overlapped between environments. The QTL on LG A06 explained about one third of the phenotypic variance in nitrogen content of straw. The QTL on C06 explained about 8 %. Nitrogen harvest index, nitrogen uptake efficiency EOF and nitrogen utilization efficiency were all affected by two QTL on LG A06 and LG C04b. Confidence intervals of QTL overlapped between traits and among environments.
For nitrogen uptake efficiency a single QTL was detected on LG A06 (Tab. 4.12). Three QTL on LG A03 and A06 were identified for nitrogen use efficiency. For one QTL on LG A06 the 'Alesi-*bzh*' allele increased nitrogen use efficiency while it had an decreasing effect at the second QTL on A06. All three QTL explained 59 % of phenotypic variance in nitrogen use efficiency.

Table 4.12: Putative QTL for nitrogen related traits at high nitrogen supply (two environments, 100 genotypes)

Env.	QTL	LG	CI [cM]	Pos.[cM]	LOD	Effect	R^2
Nitrogen uptake EOF [kg N/ha]							
Ei12	*NkgEOF-A06*	A06	3.8 - 14.8	8.0	16.1	10.43	0.52
Go12	*NkgEOF-A06*	A06	0.1 - 30.8	15.0	3.0	-4.02	0.15
Ei12	*NkgEOF-C04a*	C04a	0.0 - 18.1	6.0	2.9	3.87	0.07
Go12	*NkgEOF-C08*	C08	10.9 - 28.1	19.3	3.4	-3.54	0.11
Nitrogen uptake maturity [kg N/ha] [a]							
Go12	*NkgMat-A06*	A06	1.0 - 8.7	3.0	7.45	-5.37	0.23
Nitrogen uptake difference maturity - EOF [kg N/ha] [a]							
Go12	*Ndiff-C06*	C06	60.4 - 67.5	63.9	2.9	4.29	0.09
Go12	*Ndiff-C08*	C08	12.5 - 23.0	17.1	3.7	4.93	0.12
Nitrogen content straw [%]							
Ei12	*N%straw-A06*	A06	1.3 - 12.2	3.0	11.4	-0.03	0.34
Go12	*N%straw-A06*	A06	4.1 - 26.2	17.0	7.0	-0.06	0.31
Ei12	*N%straw-C06*	C06	32.8 - 47.3	42.4	3.3	0.02	0.08
Go12	*N%straw-C06*	C06	48.5 - 69.3	60.3	2.7	0.03	0.07
Nitrogen harvest index [a]							
Go12	*NHI-A06*	A06	0.0 - 2.0	0.0	11.7	-0.02	0.34
Go12	*NHI-C04b*	C04b	15.5 - 39.5	23.3	3.5	-0.01	0.08
Nitrogen uptake efficiency EOF							
Ei12	*NupEOF-A06*	A06	3.9 - 14.4	8.0	16.3	0.05	0.51
Ei12	*NupEOF-C04a*	C04a	0.0 - 18.0	6.0	3.5	0.02	0.09
Nitrogen uptake efficiency [a]							
Go12	*Nup-A06*	A06	0.8 - 8.3	3.0	7.8	-0.02	0.23
Nitrogen utilization efficiency [a]							
Go12	*Nut-A06*	A06	0.0- 20.9	3.0	7.8	-0.02	0.23
Go12	*Nut-C04b*	C04b	10.5 - 21.2	14.5	2.7	0.01	0.07
Nitrogen use efficiency [a]							
Go12	*NUE-A03*	A03	66.8 - 87.1	71.8	3.72	-0.34	0.08
Go12	*NUE-A06*	A06	0.7 - 10.6	3.0	7.77	-0.77	0.42
Go12	*NUE-A06*	A06	42.7 - 57.6	49.9	4.07	0.35	0.09

Env environment, **LG** linkage group, **CI** LOD-1 confidence intervals in centi Morgan (cM), **Pos** position with highest LOD, **LOD** maximum LOD score, **Effect** effect of substituting allele of P1 by allel of P2 in the TC, $\mathbf{R^2}$ proportion of phenotypic variance explained by the QTL, **EOF** end of flowering,
[a] Göttingen 2012 only

QTL across environments with high nitrogen supply

Begin of flowering at high nitrogen supply was affected by four QTL on LG A02, A05, A06 and C07 (Tab. 4.13). Together they explained 42 % of phenotypic variance.
Two QTL on LG A06 and A07 were detected for plant height. The QTL *PH-A06* explained 92 % of phenotypic variance in plant height. The allele from 'Alesi-*bzh*' reduced plant height by about 18 cm.
Seed yield was affected by two QTL on LG A06 and C02. The 'Alesi-*bzh*' allele had an increasing effect on seed yield at both QTL. Straw yield was affected by one QTL on LG A06 with an explained phenotypic variance of 74 %.
Two QTL on LG A06 and A07 were detected for harvest index. Together they accounted for 73 % of phenotpypic variation in harvest index.

Table 4.13: Putative QTL for begin of flowering, plant height, seed yield, straw yield and harvest index across four environments with high nitrogen supply (100 genotypes)

QTL	LG	CI [cM]	Pos.[cM]	LOD	Effect	R^2
Begin of flowering [days]						
BFL-A02	A02	13.2 - 35.7	23.5	2.82	0.16	0.08
BFL-A05	A05	82.0 - 95.8	91.9	2.88	0.17	0.09
BFL-A06	A01	40.3 - 49.7	47.4	5.51	0.24	0.16
BFL-C07	C07	0 - 11.3	4.0	2.92	-0.18	0.09
Plant heigth [cm]						
PH-A06	A06	1.4 - 5.2	3.0	88.1	17.90	0.92
PH-C07	C07	22.1 - 30.1	22.5	2.7	-0.85	0.002
Seed yield [dt/ha] [a]						
Seed-A06	A06	0.7 - 8.6	3.0	13.3	-1.21	0.39
Seed-C02	C02	75.5 - 54.5	50.7	5.1	-0.65	0.11
Straw yield [dt/ha]						
Straw-A06	A06	4.3 - 12.3	7.0	23.7	4.00	0.74
Harvest index [a]						
HI-A06	A06	0.8 - 4.0	2.0	28.7	-0.04	0.67
HI-A07	A07	50.6 - 61.1	55.7	4.3	-0.01	0.06

Env environment, **LG** linkage group, **CI** LOD-1 confidence intervals in centi Morgan (cM), **Pos** position with highest LOD, **LOD** maximum LOD score, **Effect** effect of substituting allele of P1 by allel of P2 in the TC, $\mathbf{R^2}$ proportion of phenotypic variance explained by the QTL,
[a] three environments

Three QTL were detected for thousand seed weight (Tab. 4.14). The allele of 'Alesi-*bzh*' reduced seed yield at all three QTL. Oil content was influenced by four QTL on different linkage groups. The QTL *Oil%-A08* accounted for 42 % of variation in oil content with an increasing effect of 0.59 % by the allele of 'H30'. The QTL on LG A06 explained another 34 % of phenotypic variation in oil content. Two QTL for protein content with opposite effects were detected on LG A03 and A06 .

Nitrogen uptake EOF and nitrogen uptake maturity were both affected by a QTL on LG A06 (Tab. 4.15). The allele of 'Alesi-*bzh*' decreased nitrogen uptake EOF and increased nitrogen uptake maturity. Two QTL on LG C06 and C08 were detected for the difference in nitrogen uptake. The nitrogen content of straw was affected by three QTL on LG A06, C04b and C06. Together they accounted for 59 % of phenotypic variance. Two QTL on LG A06 and C04b were identified for nitrogen harvest index.
Nitrogen uptake efficiency at EOF was influenced by two QTL on LG A06 and C08.
Nitrogen uptake efficiency was affected by two QTL on LG A06 and C04b with opposite effects. Two QTL were detected for nitrogen utilization efficiency. The allele of 'Alesi-*bzh*' increased nitrogen utilization efficiency at both QTL. Three QTL on two LG were identified for nitrogen use efficiency. The two QTL on LG A06 had opposite effects.

Table 4.14: Putative QTL for thousand seed weight, oil yield, oil content and protein content across four environments with high nitrogen supply (100 genotypes)

QTL	LG	CI [cM]	Pos.[cM]	LOD	Effect	R^2
Thousand seed weight [g]						
TSW-A06	A06	0 - 13.0	1.0	8.0	0.08	0.21
TWS-A07	A07	54.0 - 69.3	60.8	4.6	0.06	0.12
TSW-C01	C01	42.7 - 64.6	54.9	4.6	0.06	0.13
Oil content [%]						
Oil%-A06	A06	0.7 - 2.9	2.0	20.1	0.55	0.34
Oil%-A08	A08	1.7 - 4.8	4.0	22.5	0.59	0.42
Oil%-A09	A09	6.9 - 20.9	15.2	2.7	-0.16	0.03
Oil%-C01	C01	42.9 - 60.4	48.8	3.7	0.21	0.05
Protein content [%]						
Pro%-A03	A03	125.2 - 133.3	132.3	4.6	0.13	0.14
Pro%-A06	A06	0 - 3.0	2.0	8.5	-0.17	0.25

Env environment, **LG** linkage group, **CI** LOD-1 confidence intervals in centi Morgan (cM), **Pos** position with highest LOD, **LOD** maximum LOD score, **Effect** effect of substituting allele of P1 by allel of P2 in the TC, $\mathbf{R}^2$ proportion of phenotypic variance explained by the QTL

Table 4.15: Putative QTL for nitrogen affected traits across two environments with high nitrogen supply (100 genotypes)

QTL	LG	CI [cM]	Pos.[cM]	LOD	Effect	R^2
Nitrogen uptake EOF [kg N/ha]						
NkgEOF-A06	A06	0.5- 23.1	12.0	4.7	3.87	0.21
Nitrogen uptake maturity [kg N/ha] [a]						
NkgMat-A06	A06	1.0 - 8.7	3.0	7.45	-5.37	0.23
Nitrogen uptake difference maturity - EOF [kg N/ha] [a]						
Ndiff-C06	C06	60.4 - 67.5	63.9	2.9	4.29	0.09
Ndiff-C08	C08	12.5 - 23.0	17.1	3.7	4.93	0.12
Nitrogen content straw [%]						
N%straw-A06	A06	3.6 - 19.0	10	11.1	-0.04	0.38
N%straw-C04b	C04b	13.6 - 18.9	14.5	5.2	0.04	0.13
N%straw-C06	C06	34.1 - 54.6	45.4	3.1	0.02	0.08
Nitrogen harvest index [a]						
NHI-A06	A06	0 - 9.2	0.0	11.7	-0.02	0.34
NHI-C04b	C04b	15.5 - 39.5	23.3	3.5	-0.01	0.08
Nitrogen uptake efficiency EOF						
NupEOF-A06	A06	0 - 24.5	13.0	4.0	0.02	0.19
NupEOF-C08	C08	9.2 - 28.6	19.3	2.6	-0.01	0.08
Nitrogen uptake efficiency [a]						
Nup-A06	A06	0.8 - 8.3	3.0	7.8	-0.02	0.23
Nup-C04a	C04a	9.5 - 21.4	14.5	2.7	0.01	0.07
Nitrogen utilization efficiency [a]						
Nut-A06	A06	0 - 20.9	0.0	4.2	-0.47	0.14
Nut-C04b	C04b	10.5 - 21.1	14.5	2.7	-0.41	0.08
Nitrogen use efficiency [a]						
NUE-A03	A03	66.8 - 87.1	71.8	3.7	-0.34	0.08
NUE-A06	A06	0.9 - 10.2	3.1	13.6	-0.78	0.40
NUE-A06	A06	42.2 - 57.0	50.0	4.00	0.38	0.06

Env environment, **LG** linkage group, **CI** LOD-1 confidence intervals in centi Morgan (cM), **Pos** position with highest LOD, **LOD** maximum LOD score, **Effect** effect of substituting allele of P1 by allel of P2 in the TC, $\mathbf{R}^2$ proportion of phenotypic variance explained by the QTL, **EOF** end of flowering,
[a] Göttingen 2012 only

4.3.4 Discussion

Linkage map

For the construction of the linkage map 139 DH-lines were genotyped with 541 polymorphic SSR and SNP markers. Fifty-five marker loci differed significantly from the expected segregation ratio. The occurrence of marker loci with disturbed segregation is common in microspore-derived DH populations of *Brassica napus* and other plant species (Foisset and Delourme, 1996). A total of 437 markers were assembled to 22 linkage groups. Based on alignment with other linkage maps for *Brassica napus* and informations about the physical position of SNP markers, 21 LG could be assigned to the 19 chromosomes of *Brassica napus*. Linkage group C04 and C05 were split into two parts and LG 22 could not be assigned to any chromosome. The reduction of the LOD threshold to 2.5 and an increase of the maximum distance between markers to 40 cM did not lead to better results. Some LGs contain relatively large gaps between markers. For example on LG A06 the markers linked to the dwarf gene (SNP-bzh and SNP-bzhrga1) on the distal part of LG A06 are about 33 cM away from the next linked marker. This incomplete marker-coverage may be caused by differences in recombination frequencies in different parts of the genome. Using more marker for genotyping of DH lines might 'fill' these gaps with additional marker.

The use of more DH-lines would reduce the number of co-segregating markers. The total map length of 1301 cM (Kosambi) is relatively short, compared to other *Brassica napus* maps. Delourme et al. (2013) generated an integrated *Brassica napus* linkage map based on four populations. The integrated map comprises 7367 marker loci and covers a total genetic length of 2250 cM. In comparison to those maps the map in the present experiment covers about 58 % of the *Brassica napus* genome.

QTL mapping

The phenotypic data in the present study were obtained from testcross hybrids (TC). The testcrosses carry only one allele from the DH lines in combination with another, unknown allele from the tester. Therefore, genetic effects of QTL detected in testcrosses are always confounded by interactions with tester alleles (Schön et al., 1994). If a tester allele is completely dominant over the alleles carried by the DH lines, no QTL will be detected. The chances of detecting a QTL are a function of the genetic variance generated by the cross itself and the interaction with the tester alleles (Schön et al., 1994). Furthermore the detection of QTL is influenced by the size of the mapping population, heritability of a trait, number of loci affecting the trait, genome size and marker density. The estimation of the QTL effect on the variation in a trait is biased by low heritabilities and small population sizes (Beavis, 1998). With decreasing population size the overestimation of

QTL effects increases. The detected QTL for each trait represent the minimal number of genes determining the trait. A QTL is detected as significant if the LOD score exceeds a significance threshold. The threshold represent a 5 % probability of detecting a false positive QTL (type I error) (Rifkin, 2012). Thus, more QTL can be detected by reducing the threshold but this would also increase the type I error rate. The number of undetected QTL influences the proportion of phenotypic variance explained (R^2) by all QTL for an individual trait. The higher the proportion of detected QTL influencing a trait, the higher the explained phenotypic variance for the trait. In the present study values of R^2 across environments ranged from 11 % for nitrogen uptake efficiency EOF at low nitrogen supply (Tab. 4.8) to 93 % for plant hight at low nitrogen supply (Tab. 4.6). Most of the QTL explained only small proportion of the phenotypic variance. Only the QTL at the distal part of LG A06 contributed more than 20 % to the phenotypic variance of some traits.

In individual environments with low nitrogen supply 34 QTL were detected for 17 traits (Tab. 4.3-4.5). At high nitrogen supply 36 QTL were identified for 17 traits (Tab. 4.10-Tab. 4.12). Some of these QTL were detected in one or two environments only. A few QTL were expressed in all environments. Differences in the expression of QTL among environments is common and can be explained by QTL by environment interactions. Shi et al. (2009) identified 401 consensus QTL for nine traits in two populations tested at ten environments. Nearly 80 % of the 401 consensus QTL were identified in one or two environments, and no consensus QTL, except one for flowering time appeared in all environments. The presence of significant genotype by environment interactions for all traits at low and high nitrogen supply (Tab. 4.2, Tab. 4.9) indicates the presence of QTL x environment interaction as well. Interactions between QTL and environment can be tested using a two-factorial ANOVA model with marker genotype (closest marker to the QTL) and environment as factors. Other reasons for environmental differences in the expression of QTL may be a high residual error, reducing the heritability of a trait. The size of the mapping population can also influence the power of detecting a QTL.

The most important characteristic of testcross hybrids in this study is the segregation into semi-dwarf and normal growth types. The segregation is caused by a single dwarf gene *Bzh* with two alleles (*bzh* or *Bzh*) with an additive effect (Foisset et al., 1995). Semi-dwarf hybrids have the allelic composition *bzh/Bzh* and normal type hybrids *Bzh/Bzh*. The SNP marker (bzhrga1) inside the dwarf gene *Bzh* was mapped to a position on the distal part (3 cM) of LG A06 (Appendix A).

QTL for agronomic traits

Seed yield is the most important trait in oilseed rape breeding. Seed yield is a com-

plex trait and reflects the interaction of the environment with growth and development processes throughout the life cycle of a plant (Shi et al., 2009). Several QTL studies in different crops suggest that QTL affecting crop yield are involved in diverse pathways, such as flowering time, plant height, seed number, seed weight and straw yield. Shi et al. (2009) identified 55 QTL for seed yield in oilseed rape and 47 of the 55 QTL co-localized with other yield associated traits.

In the present study seven QTL affecting seed yield at low and/or high N supply were identified (Tab. 4.6, Tab. 4.13). Two QTL on LG A06 and A07 controlled seed yield as well as other yield related traits.

The QTL at the distal part of LG A06 explained 45 % of the phenotypic variation in seed yield at low nitrogen supply (Tab. 4.6) and 46 % of phenotypic variance in seed yield at high N supply (Tab. 4.13). A substitution of the 'Alesi-*bzh*' allele would reduce seed yield by 1.0 dt/ha at low nitrogen and by 1.3 dt/ha at high nitrogen supply. Nevertheless, seed yield of semi-dwarf hybrids at high nitrogen supply was not significantly higher compared to normal types (see chapter 2). This might be caused by negative effects of other, undetected QTL for seed yield at high nitrogen supply.

In addition to seed yield a QTL on LG A06 affected plant height, straw yield, harvest index and thousand seed weight at both nitrogen levels. A QTL at the distal part of LG A06 was also identified for begin of flowering at low nitrogen supply. Based on the position of the QTL on LG A06 and the co-localization of the dwarf locus in the same genomic region, it is most likely that all traits were affected by the dwarf gene *Bzh* itself. The pleiotropic effect of the *Bzh* gene seems to be reasonable because the mutated dwarf allele *bzh* manipulates the signaling pathway of gibberellic acid in plants (Li et al., 2011). Gibberellic acid is a growth hormone in plants and influences many traits such as stem and root extension, leaf expansion, seed germination and floral induction (Sun and Gubler, 2004).

The 'Alesi-*bzh*' allele had an opposite effect at the QTL on LG A06 detected at high nitrogen supply in Einbeck 2013 (Tab. 4.10). The confidence interval for *Seed-A06* was much larger compared to other environments. It might be that this QTL represent a second genomic region on LG A06 influencing seed yield. Ding et al. (2012) conducted a QTL study for seed yield at low and normal phosphorus levels. At low phosphorus level the authors identified a QTL for seed yield on LG A06 in an interval of 30 to 38 cM. This QTL accounted for 11 % to 13 % of the variation in seed yield. The same QTL influenced pod number and branch number at low phosphorus supply.

A second genomic region with effects on seed yield and several other traits was identified on LG A07 at low nitrogen supply. The QTL explained 7 % of the phenotypic variance (0.42 dt/ha) in seed yield across all environments with low nitrogen supply (Tab. 4.6).

The QTL on LG A07 was also identified for harvest index and nitrogen use efficiency (Tab. 4.6, Tab. 4.8). The same QTL (overlapping confidence intervals) was identified for thousand seed weight in Einbeck and Göttingen 2013 (Tab. 4.4). QTL for seed yield and thousand seed weight on A07 were identified by Quijada et al. (2006) as well. Additional QTL for seed yield were detected on LG A10 and C06 at low nitrogen supply (Tab. 4.6).

Three QTL explained 71 % of phenotypic variation in straw yield at low nitrogen supply (Tab. 4.6. The allele of parent 1 ('Alesi-*bzh*') reduced straw yield at the QTL *Straw-A02* and *Straw-A06* by 0.7 or 2.4 dt/ha, respectively. For QTL *Straw-C07* the allele from parent 1 increased straw yield by 0.7 dt/ha. At high nitrogen supply only the QTL *Straw-A06* was detected (Tab. 4.13). The higher number of QTL detected for straw yield at low nitrogen compared to high nitrogen supply might be caused by a better differentiation among genotypes at nitrogen deficient conditions (higher stress level). The QTL for straw yield detected by Miro (2010) were not identified in this experiment.

Harvest index at low and high nitrogen supply was significantly influenced by QTL on LG A06 and A07 (Tab.4.6, Tab. 4.13). Miro (2010) identified two QTL for harvest index on Chromosome A07. One QTL was detected at low and high nitrogen supply levels, the other only at high nitrogen supply. In the present experiment two additional QTL for harvest index were identified at low nitrogen supply on LG A08 and C06 (Tab. 4.6). Together they accounted for 7 % of phenotypic variation in harvest index at low nitrogen supply. A study by Radoev et al. (2008) identified QTL for harvest index on LGs 5, 6, 10, 12, 13, 17 and 19.

Five genomic regions influenced thousand seed weight at low and high nitrogen supply (Tab. 4.7, Tab. 4.14). The QTL on LG A06 and LG C01 were identified at both nitrogen levels. The 'Alesi-*bzh*' allele at QTL *TSW-A06* caused a reduction in thousand seed weight and explained 15 % (low nitrogen) or 21 % (high nitrogen) of the trait variance. The QTL *TSW-C01* accounted for 20 % (low nitrogen) or 13 % (high nitrogen) of variance in thousand seed weight with an decreasing effect of the 'Alesi-*bzh*' allele. Fan et al. (2010) identified three QTL for thousand seed weight on LG A01 and A07. The QTL on LG A01 explained 9 % of the variance in thousand seed weight. One QTL on LG A07 explained between 13 and 18 % of phenotypic variance and the other other QTL between 5 and 21 %. In the present experiment a QTL for thousand seed weight on LG A01 (R^2 = 10 %) was detected at low nitrogen supply (Tab. 4.6). At high nitrogen supply a QTL for thousand seed weight (R^2 = 12 %) was identified on A07 (Tab. 4.14).

The DH lines used for TC production segregated for erucic acid content due to the use of the parental line 'H30' (high erucic acid content). Marker assisted selection for low erucic content was only successful for one of the two loci influencing erucic acid content (Breuer personal communication). Therefore the QTL for oil content on LG A08 (Tab. 4.7, Tab.

4.14) most likely corresponds to the erucic acid gene on A08 described by Jourdren et al. (1996). A QTL for oil content at low and high nitrogen supply was also identified on LG A06 in vicinity to the dwarf locus. The 'Alesi-*bzh*' allele reduced oil content at the QTL *Oil%-A06* at both nitrogen levels with a stronger effect at high nitrogen supply. This is in accordance with results in chapter 3. The reason for a reduction of oil content by the 'Alesi-*bzh*' allele is not clear. Oil content and protein content are negatively correlated. Therefore the QTL on LG A01 (low nitrogen supply) and LG A06 (high nitrogen supply) affecting oil content had a opposite effect on protein content (Tab. 4.7, Tab. 4.14).

QTL for nitrogen related traits

Nitrogen uptake efficiency at maturity was affected by a QTL on LG A06. At low nitrogen supply this QTL accounted for 30 % of variance (Tab. 4.8). At high nitrogen supply the QTL explained 23 % of phenotypic variance in nitrogen uptake efficiency (Tab. 4.15). In the present study none of the QTL for nitrogen uptake efficiency reported by Miro (2010) (chromosomes A01, A04, A07, C05, C06 and C07) was detected. The fact that maximal 30 % of variance for nitrogen uptake efficiency was explained indicates that several other QTL are not detected.

Nitrogen utilization efficiency at low nitrogen supply (mean across two environments) was affected by three QTL on LG A06, C01 and C06 (Tab. 4.8). All three QTL explained 74 % of the phenotypic variance for nitrogen utilization at low nitrogen supply. The 'Alesi-*bzh*' allele increased nitrogen utilization efficiency at QTL *Nut-A06* and *Nut-C01* while it had a decreasing effect on *Nut-C06*. The higher number of QTL detected across environments compared to individual environments can be explained by a higher heritability across several environments instead of only one. At high nitrogen supply two QTL were identified on LG A06 and C04b (Tab. 4.15). At both QTL the 'Alesi-*bzh*' allele improved nitrogen utilization efficiency. Together these QTL explained 30 % of phenotypic variance in nitrogen utilization efficiency at high nitrogen supply. QTL for nitrogen utilization efficiency at low nitrogen (LG A07 and A09) and high nitrogen supply (LG A03, A09 and C07) identified by Miro (2010) were not detected.

Two QTL on LG A06 and A07 were identified for nitrogen use efficiency across low nitrogen environments (Tab. 4.8). The QTL *NUE-A07* explained 9 % of phenotypic variation in nitrogen use efficiency. Miro (2010) identified a QTL for nitrogen use efficiency on LG A07 at low and high nitrogen supply. The explained phenotypic variance for nitrogen use efficiency ranged from 6 % (low nitrogen) to 18 % at high nitrogen supply. In the same study the author reported another QTL for nitrogen use efficiency on chromosome C09 which explained 9 % of variance at low nitrogen supply and 7 % at high nitrogen supply. In the present experiment a QTL for nitrogen use efficiency on LG C09 was detected at low nitrogen supply in Göttingen 2011 only (Tab. 4.5). Other QTL reported

by Miro (2010) on chromosomes A09, A07, C04 and C07 could not be detected. The QTL for nitrogen use efficiency on A06 was mapped to the position of the dwarf gene and was detected at low and high nitrogen supply. At both nitrogen supply levels the dwarf allele from 'Alesi-*bzh*' improved nitrogen use efficiency. At low nitrogen supply the QTL explained 16 % and 42 % of the phenotypic variance in nitrogen use efficiency at high nitrogen supply (Tab. 4.8, Tab. 4.15). Two additional QTL on A03 and A06 were detected at high nitrogen supply accounting for 8 % or 9 % of variance in nitrogen use efficiency, respectively (Tab. 4.15).

In the present experiment different QTL were detected for seed yield and yield related traits (straw yield and harvest index) as well as nitrogen related traits (nitrogen uptake efficiency, nitrogen utilization efficiency and nitrogen use efficiency) among the two nitrogen supply levels. This indicates that different genetic mechanisms (genes or regulatory regions) are involved in the expression of a trait at different nitrogen levels.

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Summary

The cultivation of winter oilseed rape is characterised by high rates of nitrogen fertilisation. This can cause large nitrogen surpluses after seed harvest of oilseed rape. Most of the rapeseed oil is used for the production of biofuels. New regulatory standards in the European Union demand a reduction in greenhouse gas (GHG) emissions from biofuels of 60 % (by 2018) compared to fossil fuels. Energy input to produce mineral nitrogen fertiliser is responsible for a high proportion of GHG emissions in the production chain of rapeseed oil.

The environmental aspects and new legislative regulations illustrate the future challenge of maintaining current seed yields while using less nitrogen fertiliser than today. The development of oilseed rape cultivars with improved nitrogen use efficiency is one contribution to cope with this challenge. The use of semi-dwarf oilseed rape types might be a step towards this direction.

The aim of this study was to analyse the agronomic performance of semi-dwarf and normal oilseed rape hybrids at low and high nitrogen supply. Several nitrogen related traits were analysed with regard to nitrogen use efficiency. The genetic mechanisms controlling these agronomic and nitrogen related traits were analysed by QTL mapping.

A population of 150 oilseed rape hybrids, segregating in 75 semi-dwarf and 75 normal hybrids was developed from a DH population. DH lines were generated from the cross 'Alesi-*bzh*' x 'H30'. 'Alesi-*bzh*' is a dwarf line carrying the *bzh* dwarf allel. The DH population is segregating into dwarf and normal type lines. The hybrids were tested in field experiments in Einbeck and Göttingen at low nitrogen supply (without nitrogen fertilization) in 2010/2011. In 2011/2012 and 2012/2013 108 hybrids were tested at the same locations at low and high nitrogen supply.

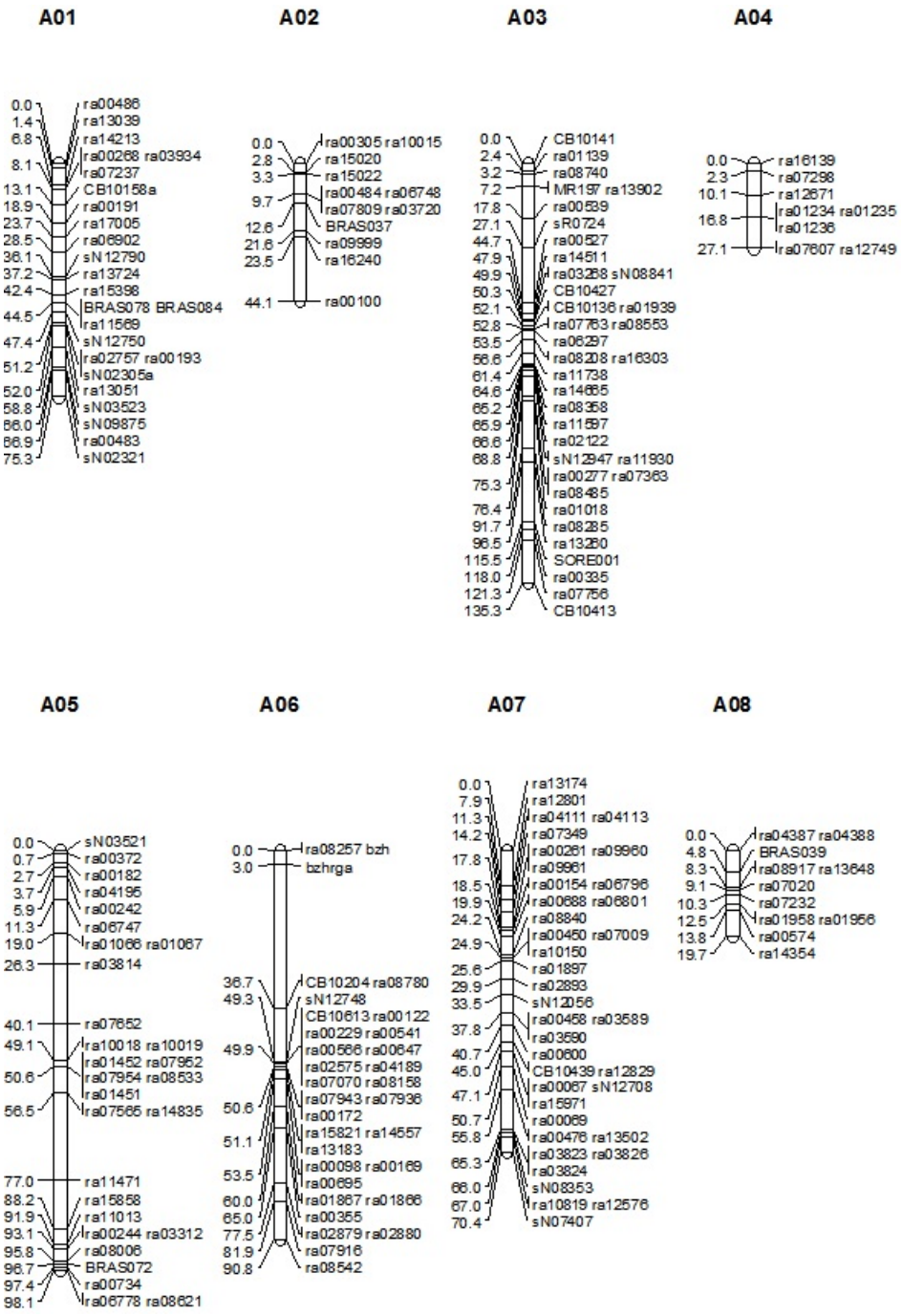
On average, semi-dwarf hybrids produced a significantly higher seed yield (2.4 dt/ha) at low nitrogen supply compared to normal hybrids. At high nitrogen supply both growth types produced similar seed yields with a non-significant difference of 0.2 dt/ha. In general semi-dwarf hybrids formed significantly less straw in all environments than normal hybrids. The combinations of higher or similar seed yields and less straw yield resulted in a higher harvest index of semi-dwarf hybrids at both nitrogen supply levels. Semi-dwarfs had a significantly higher nitrogen utilization efficiency compared to normal hybrids at

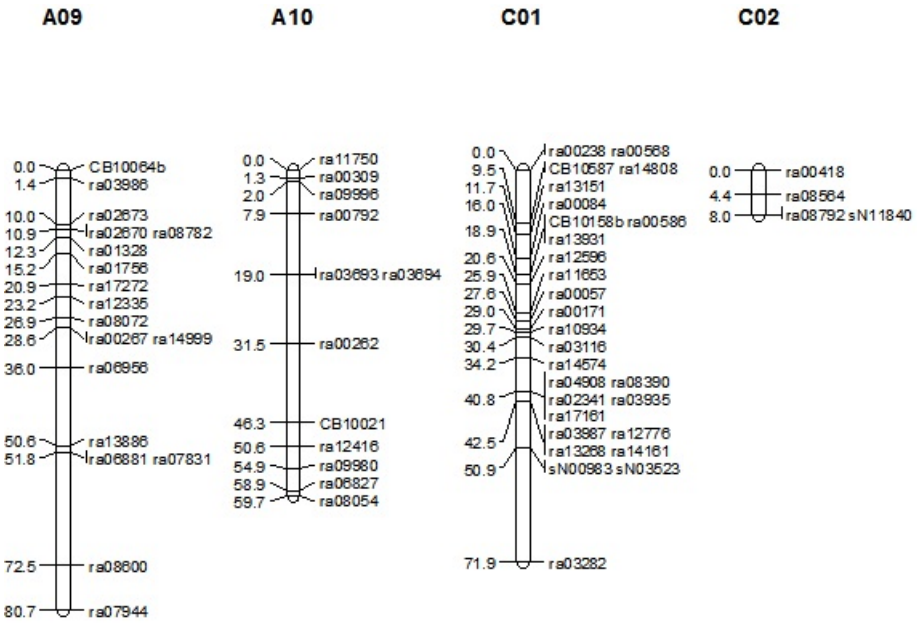
low and high nitrogen supply. That means they produced more kg seed per kg nitrogen taken up. The difference between the growth types was 2.7 and 1.1 kg seeds per kg nitrogen taken up at low and high nitrogen supply, respectively. Therefore, semi-dwarf hybrids revealed a higher nitrogen use efficiency at both nitrogen levels. The variation in nitrogen uptake efficiency contributed more to genetic variation in nitrogen use efficiency than variation in nitrogen utilization efficiency.

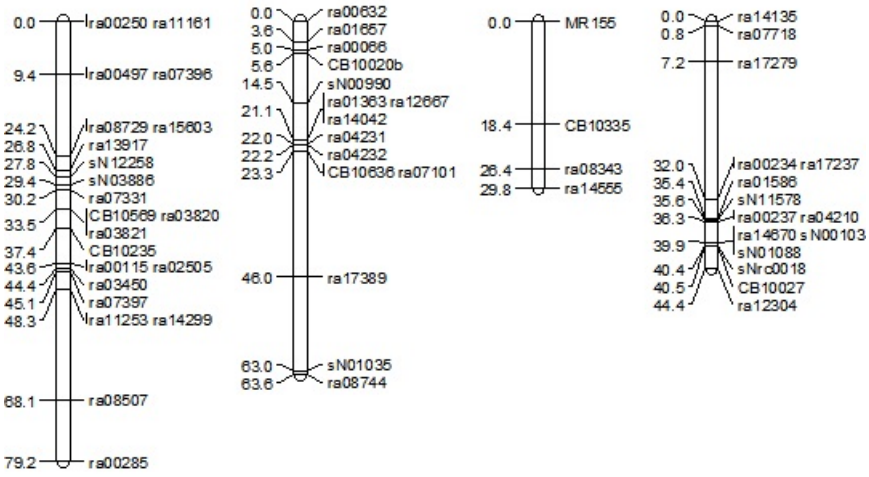
QTL mapping identified a QTL on linkage group (LG) A06 with pleiotropic effects on seed yield, straw yield, harvest index and nitrogen use efficiency at low and high nitrogen supply. Due to the position of the QTL it is most likely the effect of the dwarf gene *Bzh* itself. Another QTL with pleiotropic effects on seed yield, harvest index and nitrogen use efficiency was identified on LG A07 at low nitrogen supply.

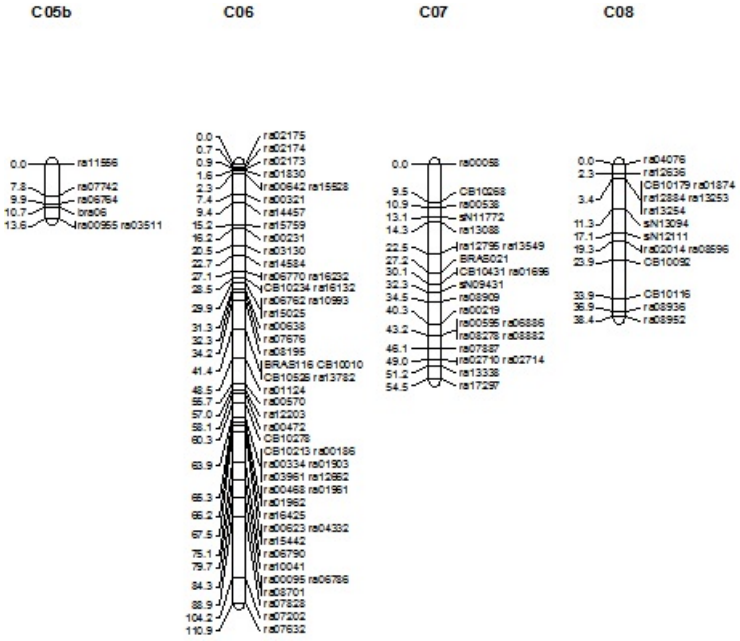
Based on the results of the present study semi-dwarf hybrids produce higher seed yields and show a higher nitrogen use efficiency than normal type hybrids, especially at nitrogen deficient conditions.

Appendix A


A01
0.0 ra00486
1.4 ra13039
6.8 ra14213
8.1 ra00268 ra03934
ra07237
13.1 CB10158a
18.9 ra00191
23.7 ra17005
28.5 ra06902
36.1 sN12790
37.2 ra13724
42.4 ra15398
44.5 BRAS078 BRAS084
ra11569
47.4 sN12750
51.2 ra02757 ra00193
sN02305a
52.0 ra13051
58.8 sN03523
66.0 sN09875
66.9 ra00483
75.3 sN02321
A02
0.0 ra00305 ra10015
2.8 ra15020
3.3 ra15022
ra00484 ra06748
9.7 ra07809 ra03720
12.6 BRAS037
21.6 ra09999
23.5 ra16240
44.1 ra00100
A03
0.0 CB10141
2.4 ra01139
3.2 ra08740
7.2 MR197 ra13902
17.8 ra00539
27.1 sR0724
44.7 ra00527
47.9 ra14511
49.9 ra03268 sN08841
50.3 CB10427
52.1 CB10136 ra01939
52.8 ra07763 ra08553
53.5 ra06297
56.6 ra08208 ra16303
61.4 ra11738
64.6 ra14665
65.2 ra08358
65.9 ra11597
66.6 ra02122
68.8 sN12947 ra11930
75.3 ra00277 ra07363
76.4 ra08485
91.7 ra01018
96.5 ra08285
115.5 ra13260
118.0 SORE001
121.3 ra00335
135.3 ra07756
CB10413
A04
0.0 ra16139
2.3 ra07298
10.1 ra12671
16.8 ra01234 ra01235
ra01236
27.1 ra07607 ra12749
A05
0.0 sN03521
0.7 ra00372
2.7 ra00182
3.7 ra04195
5.9 ra00242
11.3 ra06747
19.0 ra01066 ra01067
26.3 ra03814
40.1 ra07652
49.1 ra10018 ra10019
ra01452 ra07952
50.6 ra07954 ra08533
ra01451
56.5 ra07565 ra14835
77.0 ra11471
88.2 ra15858
91.9 ra11013
93.1 ra00244 ra03312
95.8 ra08006
96.7 BRAS072
97.4 ra00734
98.1 ra06778 ra08621
A06
0.0 ra08257 bzh
3.0 bzhrga
36.7 CB10204 ra08780
49.3 sN12748
CB10613 ra00122
ra00229 ra00541
49.9 ra00566 ra00647
ra02575 ra04189
ra07070 ra08158
50.6 ra07943 ra07936
ra00172
51.1 ra15821 ra14557
ra13183
53.5 ra00098 ra00169
ra00695
60.0 ra01867 ra01866
65.0 ra00355
77.5 ra02879 ra02880
81.9 ra07916
90.8 ra08542
A07
0.0 ra13174
7.9 ra12801
11.3 ra04111 ra04113
14.2 ra07349
17.8 ra00261 ra09960
ra09961
18.5 ra00154 ra06796
19.9 ra00688 ra06801
24.2 ra08840
ra00450 ra07009
24.9 ra10150
25.6 ra01897
29.9 ra02893
33.5 sN12056
ra00458 ra03589
37.8 ra03590
40.7 ra00600
45.0 CB10439 ra12829
47.1 ra00067 sN12708
ra15971
50.7 ra00069
55.8 ra00476 ra13502
65.3 ra03823 ra03826
ra03824
66.0 sN08353
67.0 ra10819 ra12576
70.4 sN07407
A08
0.0 ra04387 ra04388
4.8 BRAS039
8.3 ra08917 ra13648
9.1 ra07020
10.3 ra07232
12.5 ra01958 ra01956
13.8 ra00574
19.7 ra14354


A09
A10
C01
C02
0.0 CB10064b
1.4 ra03986
10.0 ra02673
10.9 ra02670 ra08782
12.3 ra01328
15.2 ra01756
20.9 ra17272
23.2 ra12335
26.9 ra08072
28.6 ra00267 ra14999
36.0 ra06956
50.6 ra13886
51.8 ra06881 ra07831
72.5 ra08600
80.7 ra07944
0.0 ra11750
1.3 ra00309
2.0 ra09996
7.9 ra00792
19.0 ra03693 ra03694
31.5 ra00262
46.3 CB10021
50.6 ra12416
54.9 ra09980
58.9 ra06827
59.7 ra08054
0.0 ra00238 ra00568
9.5 CB10587 ra14808
11.7 ra13151
16.0 ra00084
18.9 CB10158b ra00586
ra13931
20.6 ra12596
25.9 ra11653
27.6 ra00057
29.0 ra00171
29.7 ra10934
30.4 ra03116
34.2 ra14574
40.8 ra04908 ra08390
ra02341 ra03935
ra17161
42.5 ra03987 ra12776
ra13268 ra14161
50.9 sN00983 sN03523
71.9 ra03282
0.0 ra00418
4.4 ra08564
8.0 ra08792 sN11840


C03
C04a
C04b
C05a
0.0 ra00250 ra11161
9.4 ra00497 ra07396
24.2 ra08729 ra15603
26.8 ra13917
27.8 sN12258
29.4 sN03886
30.2 ra07331
33.5 CB10569 ra03820
ra03821
37.4 CB10235
43.6 ra00115 ra02505
44.4 ra03450
45.1 ra07397
48.3 ra11253 ra14299
68.1 ra08507
79.2 ra00285
0.0 ra00632
3.6 ra01657
5.0 ra00066
5.6 CB10020b
14.5 sN00990
ra01363 ra12667
21.1 ra14042
22.0 ra04231
22.2 ra04232
23.3 CB10636 ra07101
46.0 ra17389
63.0 sN01035
63.6 ra08744
0.0 MR155
18.4 CB10335
26.4 ra08343
29.8 ra14555
0.0 ra14135
0.8 ra07718
7.2 ra17279
32.0 ra00234 ra17237
35.4 ra01586
35.6 sN11578
36.3 ra00237 ra04210
ra14670 sN00103
39.9 sN01088
40.4 sNrc0018
40.5 CB10027
44.4 ra12304


C05b
C06
C07
C08
0.0 ra11556
7.8 ra07742
9.9 ra06764
10.7 bra06
13.6 ra00955 ra03511
0.0 ra02175
0.7 ra02174
0.9 ra02173
1.6 ra01830
2.3 ra00642 ra15528
7.4 ra00321
9.4 ra14457
15.2 ra15759
16.2 ra00231
20.5 ra03130
22.7 ra14584
27.1 ra06770 ra16232
28.5 CB10234 ra16132
29.9 ra06762 ra10993
31.3 ra15025
32.3 ra00638
34.2 ra07676
41.4 ra08195
BRAS116 CB10010
CB10526 ra13782
46.5 ra01124
55.7 ra00570
57.0 ra12203
58.1 ra00472
60.3 CB10278
63.9 CB10213 ra00186
ra00334 ra01903
ra03961 ra12662
ra00468 ra01961
65.3 ra01962
66.2 ra16425
67.5 ra00623 ra04332
75.1 ra15442
79.7 ra06790
ra10041
84.3 ra00096 ra06786
88.9 ra08701
104.2 ra07828
110.9 ra07202
ra07632
0.0 ra00058
9.5 CB10268
10.9 ra00538
13.1 sN11772
14.3 ra13088
22.5 ra12795 ra13549
27.2 BRAS021
30.1 CB10431 ra01696
32.3 sN09431
34.5 ra08909
40.3 ra00219
43.2 ra00595 ra06886
ra08278 ra08882
46.1 ra07887
49.0 ra02710 ra02714
51.2 ra13338
54.5 ra17297
0.0 ra04076
2.3 ra12636
3.4 CB10179 ra01874
ra12884 ra13253
ra13254
11.3 sN13094
17.1 sN12111
19.3 ra02014 ra08596
23.9 CB10092
33.9 CB10116
36.9 ra08936
38.4 ra08952

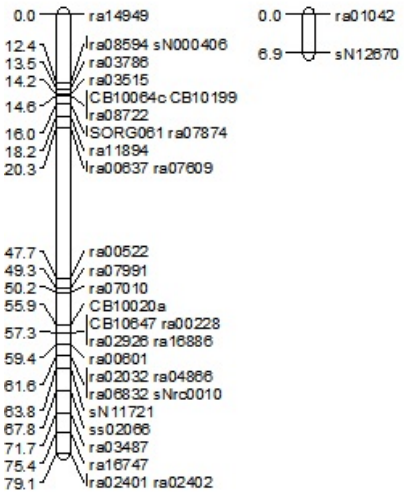

C09
LG22
0.0 ra14949
12.4 ra08594 sN000406
13.5 ra03786
14.2 ra03515
14.6 CB10064c CB10199
ra08722
16.0 SORG061 ra07874
18.2 ra11894
20.3 ra00637 ra07609
47.7 ra00522
49.3 ra07991
50.2 ra07010
55.9 CB10020a
57.3 CB10647 ra00228
ra02926 ra16886
59.4 ra00601
61.6 ra02032 ra04866
ra06832 sNrc0010
63.8 sN11721
67.8 ss02066
71.7 ra03487
75.4 ra16747
79.1 ra02401 ra02402
0.0 ra01042
6.9 sN12670

Table 4.16: Linkage map of 'Alesi-*bzh*' x 'H30' population

No. of marker	Marker name	Position (cM)
LG A01		
1	ra00486	0.0
2	ra13039	1.4
3	ra14213	6.8
4	ra00268	8.1
5	ra03934	8.1
6	ra07237	8.1
7	CB10158b	13.1
8	ra00191	18.9
9	ra17005	23.7
10	ra06902	28.5
11	sN12790	36.1
12	ra13724	37.2
13	ra15398	42.4
14	BRAS078	44.5
15	BRAS084	44.5
16	ra11569	44.5
17	sN12750	47.4
18	ra02757	51.2
19	ra00193	51.2
20	sN02305a	51.2
21	ra13051	52.0
22	sN03523rb	58.8
23	sN09875	66.0
24	ra00483	66.9
25	sN02321	75.3
LG A02		
26	ra00305	0.0
27	ra10015	0.0
28	ra15020	2.8
29	ra15022	3.3
30	ra00484	9.7
31	ra06748	9.7

Continued on next page

Table 4.16 – continued from previous page

No. of marker	Marker name	Position (cM)
32	ra07809	9.7
33	ra03720	9.7
34	BRAS037	12.6
35	ra09999	21.6
36	ra16240	23.5
37	ra00100	44.1
LG A03		
38	CB10141	0.0
39	ra01139	2.3
40	ra08740	3.2
41	MR197	7.2
42	ra13902	7.2
43	ra00539	17.8
44	sr000724	27.1
45	ra00527	44.7
46	ra14511	47.9
47	ra03268	49.9
48	sN08841	49.9
49	CB10427	50.3
50	CB10136	52.1
51	ra01939	52.1
52	ra07763	52.8
53	ra08553	52.8
54	ra06297	53.5
55	ra08208	56.6
56	ra16303	56.6
57	ra11738	61.4
58	ra14665	64.6
59	ra08358	65.2
60	ra11597	65.9
61	ra02122	66.6
62	sN12947	68.8
63	ra11930	68.8
64	ra00277	75.3

Continued on next page

Table 4.16 – continued from previous page

No. of marker	Marker name	Position (cM)
65	ra07363	75.3
66	ra08485	75.3
67	ra01018	76.4
68	ra08285	91.7
69	ra13260	96.5
70	sore0001	115.5
71	ra00335	118.0
72	ra07756	121.3
73	CB10413	135.3
LG A04		
74	ra16139	0.0
75	ra07298	2.3
76	ra12671	10.1
77	ra01234	16.8
78	ra01235	16.8
79	ra01236	16.8
80	ra07607	27.1
81	ra12749	27.1
LG A05		
82	sN03521f	0.0
83	ra00372	0.7
84	ra00182	2.7
85	ra04195	3.7
86	ra00242	5.9
87	ra06747	11.3
88	ra01066	19.0
89	ra01067	19.0
90	ra03814	26.3
91	ra07652	40.1
92	ra10018	49.1
93	ra10019	49.1
94	ra01452	50.6
95	ra07952	50.6
96	ra07954	50.6

Continued on next page

Table 4.16 – continued from previous page

No. of marker	Marker name	Position (cM)
97	ra08533	50.6
98	ra01451	50.6
99	ra07565	56.5
100	ra14835	56.5
101	ra11471	77.0
102	ra15858	88.2
103	ra11013	91.9
104	ra00244	93.1
105	ra03312	93.1
106	ra08006	95.8
107	BRAS72	96.7
108	ra00734	97.4
109	ra06778	98.1
110	ra08621	98.1
LG A06		
111	bzh	0.0
112	bzhrga	3.0
113	CB10204	36.7
114	ra08780	36.7
115	sN12748	49.3
116	CB10613	49.9
117	ra00122	49.9
118	ra00229	49.9
119	ra00541	49.9
120	ra00566	49.9
121	ra00647	49.9
122	ra02575	49.9
123	ra04189	49.9
124	ra07070	49.9
125	ra08158	49.9
126	ra07943	50.6
127	ra07936	50.6
128	ra00172	50.6
129	ra15821	51.1

Continued on next page

Table 4.16 – continued from previous page

No. of marker	Marker name	Position (cM)
130	ra14557	51.1
131	ra13183	51.1
132	ra00098	53.5
133	ra00169	53.5
134	ra00695	53.5
135	ra01867	60.0
136	ra01866	60.0
137	ra00355	65.0
138	ra02879	77.5
139	ra02880	77.5
140	ra07916	81.9
141	ra08542	90.8
LG A07		
142	ra13174	0.0
143	ra12801	7.9
144	ra04111	11.3
145	ra04113	11.3
146	ra07349	14.2
147	ra00261	17.8
148	ra09960	17.8
149	ra09961	17.8
150	ra00154	18.5
151	ra06796	18.5
152	ra00688	19.9
153	ra06801	19.9
154	ra08840	24.2
155	ra00450	24.9
156	ra07009	24.9
157	ra10150	24.9
158	ra01897	25.6
159	ra02893	29.9
160	sN12056	33.5
161	ra00458	37.8
162	ra03589	37.8

Continued on next page

Table 4.16 – continued from previous page

No. of marker	Marker name	Position (cM)
163	ra03590	37.8
164	ra00600	40.7
165	CB10439	45.0
166	ra12829	45.0
167	ra00067	47.1
168	sN12708	47.1
169	ra15971	47.1
170	ra00069	50.7
171	ra00476	55.8
172	ra13502	55.8
173	ra03823	65.3
174	ra03826	65.3
175	ra03824	65.3
176	sN8353	66.0
177	ra10819	67.0
178	ra12576	67.0
179	sN7407	70.4
LG A08		
180	ra04387	0.0
181	ra04388	0.0
182	BRAS039b	4.8
183	ra08917	8.3
184	ra13648	8.3
185	ra07020	9.1
186	ra07232	10.3
187	ra01958	12.5
188	ra01956	12.5
189	ra00574	13.8
190	ra14354	19.7
LG A09		
191	CB10064b	0.0
192	ra03986	1.4
193	ra02673	10.0
194	ra02670	10.9

Continued on next page

Table 4.16 – continued from previous page

No. of marker	Marker name	Position (cM)
195	ra08782	10.9
196	ra01328	12.3
197	ra01756	15.2
198	ra17272	20.9
199	ra12335	23.2
200	ra08072	26.9
201	ra00267	28.6
202	ra14999	28.6
203	ra06956	36.0
204	ra13886	50.6
205	ra06881	51.8
206	ra07831	51.8
207	ra08600	72.5
208	ra07944	80.7
LG A10		
209	ra11750	0.0
210	ra00309	1.3
211	ra09996	2.0
212	ra00792	7.9
213	ra03693	19.0
214	ra03694	19.0
215	ra00262	31.5
216	CB10021	46.3
217	ra12416	50.6
218	ra09980	54.9
219	ra06827	58.9
220	ra08054	59.7
LG C01		
221	ra00238	0.0
222	ra00568	0.0
223	CB10587	9.5
224	ra14808	9.5
225	ra13151	11.7
226	ra00084	16.0

Continued on next page

Table 4.16 – continued from previous page

No. of marker	Marker name	Position (cM)
227	CB10158c	18.9
228	ra00586	18.9
229	ra13931	18.9
230	ra12596	20.6
231	ra11653	25.9
232	ra00057	27.6
233	ra00171	29.0
234	ra10934	29.7
235	ra03116	30.4
236	ra14574	34.2
237	ra04908	40.8
238	ra08390	40.8
239	ra02341	40.8
240	ra03935	40.8
241	ra17161	40.8
242	ra03987	42.5
243	ra12776	42.5
244	ra13268	42.5
245	ra14161	42.5
246	sN00983f	50.9
247	sN03523ra	50.9
248	ra03282	71.9
LG C02		
249	ra00418	0.0
250	ra08564	4.4
251	ra08792	8.0
252	sN11840	8.0
LG C03		
253	ra00250	0.0
254	ra11161	0.0
255	ra00497	9.4
256	ra07396	9.4
257	ra08729	24.2
258	ra15603	24.2

Continued on next page

Table 4.16 – continued from previous page

No. of marker	Marker name	Position (cM)
259	ra13917	26.8
260	sN12258	27.8
261	sN03886i	29.4
262	ra07331	30.2
263	CB10569a	33.5
264	ra03820	33.5
265	ra03821	33.5
266	CB10235	37.4
267	ra00115	43.6
268	ra02505	43.6
269	ra03450	44.4
270	ra07397	45.1
271	ra11253	48.3
272	ra14299	48.3
273	ra08507	68.1
274	ra00285	79.2
LG C04a		
275	MR155	0.0
276	CB10335	18.4
277	ra08343	26.4
278	ra14555	29.8
LG C04b		
279	ra00632s02	0.0
280	ra01657	3.6
281	ra00066	5.0
282	CB10020c	5.6
283	sN00990a	14.5
284	ra01363	21.1
285	ra12667	21.1
286	ra14042	21.1
287	ra04231	22.0
288	ra04232	22.2
289	CB10636	23.3
290	ra07101	23.3

Continued on next page

Table 4.16 – continued from previous page

No. of marker	Marker name	Position (cM)
291	ra17389	46.0
292	sN01035r	63.0
293	ra08744	63.6
LG C05a		
294	ra14135	0.0
295	ra07718	0.8
296	ra17279	7.2
297	ra00234	32.0
298	ra17237	32.0
299	ra01586	35.4
300	sN11578	35.6
301	ra00237	36.3
302	ra04210	36.3
303	ra14670	39.9
304	sN00103	39.9
305	sN01088a	39.9
306	snrc0018	40.4
307	CB10027	40.5
308	ra12304	44.4
LG C05b		
309	ra11556	0.0
310	ra07742	7.8
311	ra06764	9.9
312	BRAS106	10.7
313	ra00955	13.6
314	ra03511	13.6
LG C06		
315	ra02175	0.0
316	ra02174	0.7
317	ra02173	0.9
318	ra01830	1.6
319	ra00642	2.3
320	ra15528	2.3
321	ra00321	7.4

Continued on next page

Table 4.16 – continued from previous page

No. of marker	Marker name	Position (cM)
322	ra14457	9.4
323	ra15759	15.2
324	ra00231	16.2
325	ra03130	20.5
326	ra14584	22.7
327	ra06770	27.1
328	ra16232	27.1
329	CB10234	28.5
330	ra16132	28.5
331	ra06762	29.9
332	ra10993	29.9
333	ra15025	29.9
334	ra00638	31.3
335	ra07676	32.3
336	ra08195	34.2
337	BRAS116	41.4
338	CB10010	41.4
339	CB10526	41.4
340	ra13782	41.4
341	ra01124	48.5
342	ra00570	55.7
343	ra12203	57.0
344	ra00472	58.1
345	CB10278	60.3
346	CB10213	63.9
347	ra00186	63.9
348	ra00334	63.9
349	ra01903	63.9
350	ra03961	63.9
351	ra12662	63.9
352	ra00468	65.3
353	ra01961	65.3
354	ra01962	65.3
355	ra16425	66.2

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Table 4.16 – continued from previous page

No. of marker	Marker name	Position (cM)
356	ra00623	67.5
357	ra04332	67.5
358	ra15442	67.5
359	ra06790	75.1
360	ra10041	79.7
361	ra00095	84.3
362	ra06786	84.3
363	ra08701	84.3
364	ra07828	88.9
365	ra07202	104.2
366	ra07632	110.9
LG C07		
367	ra00058	0.0
368	CB10268	9.5
369	ra00538	10.9
370	sN11772	13.1
371	ra13088	14.3
372	ra12795	22.5
373	ra13549	22.5
374	BRAS121	27.2
375	CB10431	30.1
376	ra01696	30.1
377	sN09431	32.3
378	ra08909	34.5
379	ra00219	40.3
380	ra00595	43.2
381	ra06886	43.2
382	ra08278	43.2
383	ra08882	43.2
384	ra07887	46.1
385	ra02710	49.0
386	ra02714	49.0
387	ra13338	51.2
388	ra17297	54.5

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Table 4.16 – continued from previous page

No. of marker	Marker name	Position (cM)
LG C08		
389	ra04076	0.0
390	ra12636	2.3
391	CB10179	3.4
392	ra01874	3.4
393	ra12884	3.4
394	ra13253	3.4
395	ra13254	3.4
396	sN13094	11.3
397	sN12111	17.1
398	ra02014	19.3
399	ra08596	19.3
400	CB10092	23.9
401	CB10116	33.9
402	ra08936	36.9
403	ra08952	38.4
LG C09		
404	ra14949	0.0
405	ra08594	12.4
406	sN0406	12.4
407	ra03786	13.5
408	ra03515	14.2
409	CB10064c	14.6
410	CB10199a	14.6
411	ra08722	14.6
412	sorg061	16.0
413	ra07874	16.0
414	ra11894	18.2
415	ra00637	20.3
416	ra07609	20.3
417	ra00522	47.7
418	ra07991	49.3
419	ra07010	50.2
420	CB10020b	55.9

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No. of marker	Marker name	Position (cM)
421	CB10647	57.3
422	ra00228	57.3
423	ra02926	57.3
424	ra16886	57.3
425	ra00601	59.4
426	ra02032	61.6
427	ra04866s02	61.6
428	ra06832	61.6
429	snrc0010	61.6
430	sN11721r	63.8
431	ss002066	67.8
432	ra03487	71.7
433	ra16747	75.4
434	ra02401	79.1
435	ra02402	79.1
LG 22		
436	ra01042	0.0
437	sN12670	6.9

Curriculum vitae

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