Comparative analysis of phytohormone translocation, nitrogen metabolism and yield components under nitrate and urea nutrition in oilseed rape

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Summary

Winter oilseed rape is nowadays the most important oilseed crop in Northern Europe. Since the 1980s, the increasing demand for vegetable oil in food and non-food industry led to an elevated world production of more than threefold. Against this background, it is highly problematic that oilseed rape is a crop with a high nitrogen balance surplus caused by a high nitrogen demand during the vegetative growth phase, but a low nitrogen transfer to seeds. As legal regulations and environmental concerns demand a reduction of nitrogen fertilizer use, it is of high importance to increase the nitrogen efficiency of winter oilseed rape.

The work in this thesis aimed to improve nitrogen efficiency by increasing root activity and yield formation in oilseed rape by targeted nitrogen nutrition. Nitrogen was fertilized either in the form of nitrate or urea. Nitrate stimulates cytokinin production in roots. These phytohormones delay plant senescence processes and stimulate shoot branching. Thus, nitrate fertilization was anticipated to prolong nitrogen uptake activity by roots and increase potential sites for pod and seed formation of shoots. Both mechanisms may contribute to a better yield potential. In contrast to nitrate, urea fertilization was supposed to induce earlier plant senescence and lower the yield potential.

Two populations, one consisting of actual oilseed rape breeding lines and the other representing old cultivars, landraces and a resynthesized line, were grown in field trials once or repeatedly over different years and were supplied with the two different forms of nitrogen fertilizer. Xylem sap was collected at different developmental stages, ranging from flower development until pod formation, to measure the translocation of cytokinins and of nitrogen forms as an estimate for root activity. Cytokinin as well as nitrogen export to the shoot were low when no nitrogen was applied but increased with nitrogen fertilization. The fertilized nitrogen form had a strong impact on xylem cytokinins and nitrogen: Consistently over genotypes and years, nitrate application stimulated cytokinin translocation relative to urea supply shortly after fertilization, while this effect disappeared later on or even reversed. Similar results were obtained for nitrogen export to the shoot, so that nitrate appears to represent a highly available nitrogen form stimulating cytokinin translocation over the short-term, while nitrogen availability from the urea fertilizer lasted for longer. However, this differential nitrogen availability did not affect total nitrogen accumulation in the shoot or the nitrogen distribution among above-ground organs.

Initially expected higher branching of nitrate-fertilized plants was only observed in one experimental year, likely because the low stand density due to winter kills promoted shoot branching in response to cytokinins, while in other years a higher stand density might have suppressed this response. Since yield composition is highly plastic in oilseed rape, a higher number of branches and pods was compensated by a lower number of seeds, so that final seed yield was not affected by the fertilized nitrogen form. Bearing in mind that production costs as well as greenhouse gas emissions of urea are lower than from nitrate-based fertilizers, the same agronomic efficacy as nitrate makes urea a valuable nitrogen form for agricultural production of oilseed rape.

In general, yield composition appeared to be less variable when elite lines were assessed, but genotypic variability in final seed yield was similar in both oilseed rape populations. Regardless of genotypes and years, compensation mechanisms among individual yield components had a fundamental impact on final seed yield, which was mainly determined by seed number.

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1 Introduction

Winter oilseed rape (*Brassica napus* L.) is a relatively young species of the *Brassicaceae* family and developed during the first centuries of the Common Era as crossing product of turnip rape (*Brassica rapa* L.) and wild cabbage (*Brassica oleracea* L.). For a long time rapeseed oil was rather used as lamp oil and only in small amounts for human nutrition, since the use of linseed oil was historically more common and negative effects on human health were expected by the long-chained erucic acid (contained to about 50 % in rape oil; Brauer, 2011). Erucic acid can cause fatty degeneration in heart, skeletal muscles and adrenals as shown in rat experiments (Abdellatif and Vles, 1970). At the end of the 1960s and beginning of 1970s, breeding efforts in Canada and Germany resulted in the first erucic acid free ("0") oilseed rape cultivars "Liho" (spring type) and "Lesira" (winter type). Further breeding activities led to the reduction of the glucosinolate content in oilseed rape (first cultivar "Bronowski"), which increased the fodder value of the protein-rich press cake remaining after oil extraction (Brauer, 2011), since glucosinolates lower the palatability and cause thyroid diseases when fed to animals (reviewed by Tripathi and Mishra, 2007). During the 1980s the low glucosinolate trait was crossed into "0" oilseed rape cultivars and since that time the importance of "00" (erucic acid free and low glucosinolate content) rape increased (Brauer, 2011). Today winter rapeseed is the most important oilseed crop in Northern Europe (Rathke *et al.*, 2006). Its oil is used in the food as well as in the non-food sector e.g. as cooking and frying oil, as raw material for margarine and mayonnaise (Schwarz and Erbersdobler, 2011) or for the production of biodiesel (Brauer, 2011), lubricants and hydraulic oil (Harms, 2011). Also in crop rotations oilseed rape plays an important role as it interrupts cycles of pathogenic diseases in cereal crops, as its long taproot improves the soil structure (Christen, 2011) and as it can act as catch crop to prevent nitrogen (N) losses (Rossato *et al.*, 2001).

1.1 Nitrogen in the agricultural production of winter oilseed rape

At the beginning of the 1970s, oilseed rape yielded in average about 20 dt ha^{-1} in Germany (http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor, accessed on 17 Sept. 2015). Today, common yields in central Europe range between 30 and 40 dt ha^{-1} and can reach up to 50 dt ha^{-1} when production site and conditions are favorable. This yield increase is the consequence of breeding success and improved plant management, but relies as well on elevated resource inputs, especially N fertilization (Rathke *et al.*, 2006). The positive influence of N fertilizer application on winter oilseed rape yields has been reported in many studies undertaken in different countries (e.g. Dreccer *et al.*, 2000; Cheema *et al.*, 2001; Zhang *et al.*, 2010; Ulas *et al.*, 2012; Nyikako *et al.*, 2014). Depending on site and production conditions, highest seed yields were reached by applying between 160 to 240 kg N ha^{-1} (Rathke *et al.*, 2005; Sieling and Kage, 2010; Albert, 2011).

N is the mineral nutrient that most often limits the growth of plants (Forde, 2002) and the yield of agricultural crops (Harper, 1987). In plant nutrition, N is classified as a macronutrient accounting for 1 - 5 % of the total plant dry matter as component of proteins, nucleic acids, chlorophyll, enzymes, phytohormones and secondary metabolites (Hawkesford *et al.*, 2012). Thereby, the largest amount of N is needed for establishing and maintaining the photosynthetic apparatus (up to 75 % of the total N content in leaves of C3 plants; Gammelvind *et al.*, 1996). In photosynthesis, the light reactions result in the reduction of the redox carrier $NADP^+$ to NADPH and the formation of the energy carrier ATP (Berg *et al.*, 2002), which are both essential for the production of many different carbon (C) and N assimilates (Lawlor, 2002). Under N deficiency, the amount of structural photosynthetic components,

chlorophyll and ribulose bisphosphate carboxylase (RUBISCO), drops resulting in lower photosynthetic capacity and lower efficiency of the carboxylation reaction (Lam *et al.*, 1996). Since an adequate assimilate supply to developing meristems is needed to maintain their growth (Lawlor, 2002), N deficiency leads to smaller leaf size as well as suppressed shoot formation, branching and tillering in a multitude of plant species (Harper, 1987). Also growth rate and grain filling are highly dependent on adequate assimilate delivery to achieve optimal yields (Lawlor, 2002). Thus, it is not surprising that worldwide 85 - 90 million metric tons of N fertilizers are applied every year to agricultural systems and, since N is a relatively expensive nutrient, major costs in plant production arise from the input of commercial N fertilizers (Masclaux-Daubresse *et al.*, 2010).

Against this background it is highly problematic that about half of the N, which is applied as mineral fertilizer to agricultural systems, is not incorporated into the crop during its growth cycle but either remains in the soil for the following crop or is lost in the form of NH_3, NO, or N_2O emissions to the atmosphere or primarily as nitrate to ground and surface waters (Galloway and Cowling, 2002). The reactive N species emitted to the atmosphere participate in ozone degradation in the stratosphere and ozone formation in the troposphere, favoring global warming and ozone-induced damages in crops, forests and natural ecosystems associated with more intensive pathogen and insect attacks. Humans exposed to high ozone concentrations can develop respiratory and cardiac diseases. Reactive N species also lead to increased precipitation acidity and thus to acidification of soils and surface waters. N leaching from fields to the water body results in eutrophication and hypoxia. On N-saturated soils biodiversity decreases and the abundance of soil organisms, important for the function of distinct ecosystems, is altered (Galloway and Cowling, 2002).

In 1991, the European Union reacted to the described problems with the release of the "Nitrates Directive" (Council Directive 91/676/EEC of 12 Dec. 1991). It aims to protect the European water quality by preventing the pollution of ground and surface waters with nitrate resulting from agricultural sources and by promoting good farming practices (http://ec.europa.eu/environment/water/water-nitrates/index_en.html, accessed on 9 Oct. 2015). A German implementation of the "Nitrates Directive" was released in 1996 with the "Düngeverordnung" (Düngeverordnung, 1996) and in a revised form it stipulates since 2006 for the first time, among other regulations, legal N balance thresholds: Over a three-years period, N balance surpluses (amount of N fertilized minus N offtake by the harvested products) must not exceed 90 kg N ha^{-1} a^{-1} from 2006–2008, declining to 60 kg ha^{-1} a^{-1} starting from 2009–2011 (Sieling and Kage, 2010; Düngeverordnung, 2006). A new draft of the German "Düngeverordnung" foresees a reduction down to 50 kg ha^{-1} a^{-1} from 2018 on (BMEL, 2014). Problematically, only under best crop management conditions oilseed rape production causes N balance surpluses of approximately 60 kg ha^{-1} (Koeslin-Findeklee *et al.*, 2014), but these can also reach values above 100 kg N ha^{-1} and consequently aspired N balance thresholds for three-years crop rotations might be exceeded (Henke *et al.*, 2009).

Further pressure on oilseed rape production is arising from renewable energy regulations. The European "Renewable Energy Directive" (Directive 2009/28/EC of the European Parliament and of the Council of 23 Apr. 2009) was released in 2009 with the aim to increase the share of biofuel in transport energy consumption to at least 10 % by 2020. Until 2012 already 5.1 % were reached, with biodiesel accounting for 75 % of the total biofuel, and oil from rapeseed is with more than 50 % the main contributor to the biodiesel production (Walter *et al.*, 2015). According to the "Renewable Energy Directive" and the German "Biokraftstoff-Nachhaltigkeitsverordnung", biofuels currently must emit 35 % lower greenhouse gasses than their fossil alternatives. These values will increase from 2017 on to 50 % and starting from 2018 to 60 % (Biokraftstoff-Nachhaltigkeitsverordnung, 2009). The present global warming potential of biodiesel from rapeseed is 52 g CO_2 eq. MJ^{-1} and equates thus about 38 % lower

greenhouse gas emission compared to fossil fuel with 83.8 g CO_2 eq. MJ^{-1}. Highest emissions arise thereby from the cultivation process with 29 g CO_2 eq. MJ^{-1} and are mainly caused by greenhouse gas emissions during the production of the N fertilizer as well as by N_2O emissions (the most important greenhouse gas released in agricultural production; Snyder *et al.*, 2009) from production sites after N fertilizer application (UFOP, 2013).

Thus, there is an increasing demand in rapeseed production to reduce the amount of N application and field-remaining N residues after harvest (Koeslin-Findeklee *et al.*, 2014).

1.2 Nitrogen use of winter oilseed rape

Generally, winter oilseed rape is characterized as one of the crops with highest N balance surpluses in European agriculture (Schulte auf'm Erley *et al.*, 2007). Rapeseed couples a high demand for N during the vegetative growth phase (before winter and in spring until flowering) with low N uptake and incomplete N retranslocation from the vegetative organs to the seeds during generative growth (Rathke *et al.*, 2006). Breeding led to considerable improvement of crop cultivars over the last five decades, but the common practice of selecting new cultivars under high N supplies might not have favored N storage capacity, although N transiently stored in vegetative tissues can be mobilized to supply developing sink organs. A large portion of N taken up by the growing rapeseed plant is fixed in falling leaves that shed either during winter or in spring and still contain more than 2 % N of the leaf DW (Rossato *et al.*, 2001). Together with the large amount of N remaining fixed in stems and pod walls on the field (Koeslin-Findeklee and Horst, 2016) only about 50 % of the applied N fertilizer is recovered in the harvested seeds (Rossato *et al.*, 2001). With regard to N fertilizer costs and environmental problems of excessive N application, improving the N use efficiency (NUE) of winter oilseed rape has become of increasing interest in breeding (Nyikako *et al.*, 2014).

Moll *et al.* (1982) defined NUE as the grain production per unit of soil-available N. It depends on two components: The efficiency of N absorption (N uptake efficiency, NupE) and the efficiency of utilizing this absorbed N for grain production (N utilization efficiency, NutE). Thereby, NupE is the amount of N acquired by the plant per unit of soil-available N while NutE is defined as unit grain yield per unit N taken up.

1.2.1 Nitrogen uptake and assimilation

Most plants are able to take up and assimilate N as nitrate, ammonium, urea and amino acids (AAs; Crawford and Glass, 1998). These bioavailable N forms in the soil originate from the degradation of N-containing polymers in organic matter by extracellular enzymes to monomers as AAs, amino sugars or nucleic acids, which can be mineralized by microorganisms to ammonium and further by nitrifying microbes to nitrate, as long as soil N availability is sufficient to meet the N demands for the development of microorganisms executing this N conversion chain (Schimel and Bennett, 2004). Among the soil N forms, nitrate and ammonium are most important for plant N uptake (Hawkesford *et al.*, 2012). Usually, plants exhibit optimal growth when both N forms are supplied, but ammonium uptake is preferred over nitrate when overall N concentrations in the soil solution are low, i.e. approximately in the micromolar range (von Wirén *et al.*, 2000). Interestingly, soil concentrations do not necessarily reflect the uptake ratio of these two N forms: Average ammonium concentrations rarely exceed 50 µM, while nitrate values may range between 0.5 and 10 mM in well-aerated agricultural soils (Miller *et al.*, 2007).

Roots take up nitrate preferentially across the plasma membrane of epidermal and cortical cells (Crawford, 1995), where the uptake is driven by the electrochemical gradient and catalyzed by two transport systems: a high-affinity (HATS) and a low-affinity transport system

(LATS; Forde, 2002). The HATS is a saturable transport system in the plasma membrane with a K_m between 7 and 100 µM and is dominant at external nitrate concentrations up to 200 µM. Above 200 µM nitrate, the non-saturable LATS becomes active in nitrate uptake (von Wirén *et al.*, 1997). Both, HATS and LATS exist in an inducible and a constitutive form (Malagoli *et al.*, 2004). In *Arabidopsis thaliana* and other plant species high-affinity nitrate transporters belong to the NRT2 family, whereas low-affinity nitrate transporters are part of the NRT1 family (von Wirén *et al.*, 1997). However, *AtNRT1.1* encodes for a dual-affinity transporter (Forde, 2002).

Under cold conditions, low pH, accumulation of phenolic-based allelopathic compounds and low oxygen availability in the soil, nitrification ceases while ammonification increases (Britto and Kronzucker, 2002). For the uptake of ammonium plant roots developed a saturable HATS and a non-saturable LATS, too. The HATS is operating at ammonium concentrations below 0.5 mM while LATS is mediating ammonium uptake at external concentrations above 0.5 mM. Transporters of the AMT1 family play the most important role for high-affinity ammonium uptake (Yuan *et al.*, 2007).

Urea is worldwide the major N form supplied as fertilizer to agricultural systems. In soils, urea is hydrolyzed by ureases to ammonium and concurrently nitrified to nitrate by microorganisms (Mérigout *et al.*, 2008). However, urease activity decreases during cold (Moyo *et al.*, 1989) and waterlogging conditions (Pulford and Tabatabai, 1988). Urea uptake from the soil is mediated by a secondary active urea transporter (DUR3) that has a substrate affinity of approximately 4 µM for urea in *Arabidopsis thaliana* and shows saturable kinetics (Kojima *et al.*, 2007). Since ureases, which occur ubiquitously in soil substrates, show a substrate affinity for urea in the millimolar range, the low K_m of DUR3 appears to be an evolutionary adaptation to take up the urea left over from microbial degradation (Kojima *et al.*, 2006). AtDUR3 has orthologs in lower and higher plants (Witte, 2011), including oilseed rape (Kojima *et al.*, 2006). At higher external urea concentrations plasma membrane-localized aquaporins serve as a low-affinity uptake system (Kojima *et al.*, 2006; Witte, 2011).

AAs occur in a range of 1 - 100 µM in agricultural soils and accumulate as products of protein degradation by microbial proteases. Thereby, plants are in strong competition for AA uptake with microbes (Hawkesford *et al.*, 2012). Various families of AA transporters exist in plants: AA permeases (AAP), lysine/histidine-type transporters (LHT), proline/compatible solute transporters (ProT), aromatic-neutral AA transporters (ANT1-like), γ-aminobutyric acid transporters (GAT) and cationic AA transporters (CAT). Among them, LHT1, AAP1, AAP5 and ProT2 are involved in root AA uptake of *Arabidopsis thaliana* (Tegeder, 2012).

Taken together, plants have evolved uptake systems for different N forms and different concentration ranges in the soil. This allows an optimal N provision under any kind of soil N conditions (Kojima *et al.*, 2006). The contribution of N transporters to the NUE in different crop cultivars becomes especially important in terms of NupE under well-fertilized agricultural conditions, where crops usually develop a smaller root/shoot ratio, while under N limitation root systems with enhanced root length densities prevail that are beneficial for N uptake (Garnett *et al.*, 2009). Although winter oilseed rape naturally exhibits a high capacity for N uptake during vegetative growth (Rossato *et al.*, 2001), especially post-flowering N uptake is highly important for N efficiency, at least under N-limited conditions (Ulas *et al.*, 2012).

Most N taken up by plants is assimilated into C skeletons. For this purpose, all inorganic N is first reduced to ammonium, which is then used for the synthesis of glutamine (Gln) and glutamate (Glu). The assimilation of absorbed nitrate starts with a reduction to ammonium (Lam *et al.*, 1996), catalyzed in two steps via cytosolic nitrate reductase and plastidic nitrite reductase (Krapp, 2015). This required demand for reducing equivalents is thought to be the reason for the preferential uptake of ammonium over nitrate (Bloom *et al.*, 1992). The major site of nitrate assimilation differs among plant species, but commonly nitrate reduction takes

place in roots rather at low nitrate supply, while shoot nitrate assimilation becomes increasingly important at higher external nitrate concentrations (Andrews, 1986). Absorbed urea is hydrolyzed by urease (assumed to be cytosolic) to ammonium and carbamate, which is then spontaneously dissociating to ammonium and CO_2 when water is available. Thus, from one urea molecule two molecules of ammonium can be generated (Witte, 2011). Both internally produced and directly absorbed ammonium is assimilated into the amide group of glutamine by glutamine synthetase (GS), and the transfer of this amide group to 2-oxoglutarate is catalyzed by glutamate synthase (GOGAT) forming two glutamate moieties. Glutamine and glutamate are used for the production of further AAs (Masclaux-Daubresse *et al.*, 2006). The chloroplastic GS/GOGAT isoforms GS2 and ferredoxin-GOGAT occur predominantly in leaves and are thought to be involved in primary N assimilation and re-assimilation of photorespiratory ammonia, while cytosolic GS1 and NADH-GOGAT are proposed to function mainly in root N assimilation (Lam *et al.*, 1996). It is generally assumed that assimilation of ammonium (absorbed or produced in the root) takes rather place in roots and that ammonium-N is transported to the shoot mainly in the form of AAs (Finnemann and Schjoerring, 1999). Indeed, in most plants species the major N forms transported in the xylem are amides such as glutamine and asparagine, and acidic AAs such as glutamate and aspartate (Fischer *et al.*, 1998). However, nitrate (Liptay and Arevalo, 2000), ammonium (Finnemann and Schjoerring, 1999) and urea (Liu *et al.*, 2003b) are translocated to the shoot, as well.

Different supplied N forms may thus be likely assimilated in different ways by plants which may in turn influence the delivery of N forms from the root to the shoot. How far the application of different N fertilizer types changes the composition of N forms in the xylem sap of field-grown plants has not been studied in detail.

1.2.2 Nitrogen remobilization

Oilseed rape as monocarpic species (Nishizawa *et al.*, 2010) shows during the course of its reproductive phase a whole-plant dieback (Krupinska, 2007). During the juvenile and the adult phase, plants rapidly increase their size as well as root and shoot mass until adult plants become able to form reproductive organs (Gregory, 2006; Huijser and Schmid, 2011). In rapeseed, newly expanding vegetative organs as stem and leaves are mainly supplied by N from root uptake, although a considerable amount of 10 - 50 % is provided by reallocation of N from older leaves at the bottom of the canopy (Malagoli *et al.*, 2005). The reproductive stage is associated with the transition to senescence, when nutrients are remobilized from vegetative organs to developing flowers and seeds (Krupinska, 2007). Among the vegetative organs of rapeseed, leaves contribute the largest amount of N for the development of reproductive tissues (Malagoli *et al.*, 2005).

Leaf senescence occurs in dependence of plant and leaf age as the last step in leaf development, which is characterized by a decline in photosynthetic activity. Also external factors like shading, temperature or pathogen attack can lead to leaf senescence. Thereby, internal and external factors regulate gene expression patterns of senescence-associated genes (*SAG*s) and other genes of the senescence program (Quirino *et al.*, 2000). Senescence mostly ends up in programmed cell death (Quirino *et al.*, 2000) and proceeds in a highly organized manner (Noh and Amasino, 1999b): Chloroplasts, which contain the largest protein content in leaf cells, are the first leaf organelles targeted to degradation. The nucleus and the mitochondria, which are needed for gene transcription and energy provision, respectively, are kept intact until the last stages of senescence (Quirino *et al.*, 2000). The degradation of chloroplast proteins starts in the chloroplast itself by senescence-induced proteases. Chloroplast components are then guided to the central vacuole, which is kept intact during senescence, via vesicle trafficking for final protein degradation. Senescence-associated vesicles (SAV) exhibit high protease activity and since the *SAG12*-encoded cysteine-protease, which is

uniquely expressed during developmental senescence, was detected in SAVs, these vesicles are thought to play a specific role in chloroplast protein degradation and trafficking to the vacuole during developmental plant senescence. During N starvation-induced leaf senescence autophagosomes take over functions in guiding chloroplast proteins to the vacuole (Masclaux-Daubresse *et al.*, 2010). Cytosolic GS1, NADH-GOGAT and glutamate dehydrogenase (GDH) are involved in re-assimilating N from this protein breakdown into glutamate (Lam *et al.*, 1996). It has been reported that the chloroplast proteins RUBISCO and light-harvesting complex II together with vegetative storage proteins account for the largest amount of organic N which is recycled for remobilization to developing tissues (Fan *et al.*, 2009). In particular AAs (Masclaux-Daubresse *et al.*, 2008) but also vacuolar stored nitrate are preferential forms of N loaded into the phloem (Fan *et al.*, 2009) and partitioned among the competing sinks based on a high phloem unloading capacity of these N forms (Patrick and Offler, 2001).

At the transition from the vegetative to the generative phase, nitrate influx via HATS and LATS decreases drastically in oilseed rape (Malagoli *et al.*, 2004), so that N uptake from the soil during pod development becomes negligible. Consequently, most of the N for grain filling must derive from remobilization (Rossato *et al.*, 2001). Problematically, the sink strength of the grains is not sufficient to ensure an efficient incorporation of the large amount of N taken up during the vegetative stage (Svečnjak and Rengel, 2006). In several studies this resulted in only about 41 %, 31 % and 11 % (Rossato *et al.*, 2001) or 36 %, 34 % and 8 % (Malagoli *et al.*, 2005) of leaf, stem and taproot N, respectively, being reallocated, and finally only 48 % of the N entering the plant was recovered in the pods for seed filling (Rossato *et al.*, 2001).

It has repeatedly been described that N utilization is more important than N uptake for differences in NUE among rapeseed cultivars under high N supplies (Berry *et al.*, 2010; Schulte auf'm Erley *et al.*, 2011, Kessel *et al.*, 2012). Against this view, Yau and Thurling (1987) found N utilization to be more important under limited N conditions. Nevertheless, improving N remobilization is crucial to enhance the N use in oilseed rape (Masclaux-Daubresse *et al.*, 2008) and, according to model calculations, the optimization of leaf N remobilization or reduction of residual N in dead leaves could improve seed yield by approximately 15 % (Avice and Etienne, 2014). In this context, it has been suggested to specifically manipulate the important re-assimilation enzyme GS1 in a developmentally-controlled manner (Hawkesford *et al.*, 2012). Increasing the phloem-loading capacity of re-assimilated AAs and inorganic N (for which peptide and AA transporters or AMT1.1 for ammonium and NRT2.5 for nitrate might play a role) was proposed as well (Masclaux-Daubresse *et al.*, 2008), but N remobilization appears to be limited by an incomplete hydrolysis of leaf proteins rather than by AA transport from mesophyll cells to the phloem (Avice and Etienne, 2014). In any case, efficient N remobilization during leaf senescence for enhancing yield might only be functional when the sink strength in seeds is sufficient (Masclaux-Daubresse *et al.*, 2008).

An interesting result in the context of improving N remobilization was described by Bauer (2014): Nitrate-treated wheat plants appeared to restrain N in the flag leaves while ammonium- and urea-fed plants rather exported N in favor of the grains. Whether N remobilization can be also affected by the N form being fertilized to oilseed rape is, against this, not yet known.

1.3 Yield formation and yield structure in winter oilseed rape

In field-grown rapeseed, final seed yield is determined by the number of plants per area, the number of pods per plant, seeds per pod and the average seed weight. However, several secondary yield parameters, as the pod number per area, the seed number per plant or the

seed yield per pod, can be determined too, and due to a large variation in these individual components oilseed rape exhibits a highly plastic yield structure (Diepenbrock, 2000).

Among the primary yield parameters the number of plants per area has the greatest impact on the formation of yield structure (Diepenbrock, 2000). The plant growth before floral initiation determines the leaf number and subsequently the number of flowers and pod-bearing branches (Habekotté, 1993). However, *Arabidopsis thaliana* plants exhibited an induction of *BRANCHED1* expression (*BRC1*, a negative regulator of shoot branching, see 1.4.4) and thus a decrease in the number of shoot branches in response to higher planting density (Aguilar-Martínez *et al.*, 2007). Several studies in oilseed rape could show that the number of branches per plant decreased with higher planting density, too (e.g. McGregor, 1987; Ozer, 2003). The intraspecific competition among nutrients, light and water reduces not only the number of branches but also the number of pods. Due to poorer crop growth and limited leaf expansion the inflorescence initiation is reduced leading to less flowers and thus less pods per plant (Diepenbrock, 2000). Chay and Thurling (1989) even found a lower pod length at higher planting density in spring rapeseed. Also the pod distribution within the canopy changes: At high planting density most of the fertile pods are formed at the uppermost terminal branch, while at lower density the proportion of pods at the deeper branches increases (Leach *et al.*, 1999).

The number of seeds produced per pod averages 15 - 20 seeds, but can reach up to 30 - 40. Although the ovules are homogenously distributed in the unfertilized pod, differences in seed formation exist in dependence of the cultivar and the environmental conditions (Diepenbrock, 2000). Thereby, assimilate supply during flowering is crucial for seed set (Habekotté, 1993). An important source limitation for seed production appears to be the gap between leaf photosynthesis, which declines from the onset of flowering due to leaf shading by flowers, and pod photosynthesis. The latter starts at earliest approximately two weeks after flowering before compensating efficiently for the drop of lower leaf photosynthesis (Diepenbrock, 2000). Once a dense canopy of pods has developed, earlier formed pods at lower branches are shaded leading to reduced seed development in these pods (Thurling, 1991) and at higher planting density an even lower seed number and seed weight (Diepenbrock, 2000). Typically, the seed number is the parameter most closely related to final seed yield (Berry and Spink, 2006). In contrast to pod and seed number, which are mainly determined during flowering, the seed weight is defined between the end of flowering and pod maturity (Dreccer *et al.*, 2000) and, since it is the last yield component to be formed, it depends rather on the other yield parameters than on environmental factors (Diepenbrock, 2000). The growth rate of the seeds is determined by the assimilate availability during seed filling as well as by the number of seeds competing for assimilates (Habekotté, 1993). Thus, individual seed weight of oilseed rape is typically lower when pod and seed numbers are increasing (Diepenbrock, 2000).

McGregor (1987) as well as Leach *et al.* (1999) reported that area seed yield increased up to a planting density of 40 - 50 and 50 - 60 plants m^{-2}, respectively. However, intraspecific competition at higher planting densities led to reduced branch, pod and seed numbers and thus lower seed yield of individual plants, while the overall area seed yield remained stable in a wide range from about 50 - 150 plants m^{-2} (Leach *et al.*, 1999). After thinning events, such as frost or hail, residual plants are even able to compensate for lost plants (McGregor, 1987), indicating a high plasticity in yield formation also within oilseed rape stands (Diepenbrock, 2000). Despite this deep knowledge on yield formation and the compensatory response among individual yield components in response to N fertilization, little is known to what extent yield components of oilseed rape are affected by different forms of N fertilization.

1.4 Physiological functions of cytokinins and their relation to nitrogen nutrition

Cytokinins (CKs) are plant hormones which play a crucial role in controlling proliferation and differentiation of plant cells, root/shoot balance, transduction of nutritional signals (Sakakibara, 2006), seed dormancy and germination, bud formation and release, stimulation of leaf expansion and responses to abiotic and biotic stress (Spíchal, 2012) as well as in the regulation of plant senescence processes (Jibran *et al.*, 2013).

1.4.1 Cytokinin synthesis

The phytohormone class of CKs naturally comprises adenine derivates with an isoprene-derived or an aromatic side chain at the N^6-terminus (Sakakibara, 2006), whereas CKs with isoprenoid-derived side chains are more commonly found in higher plants (Kamada-Nobusada and Sakakibara, 2009). Among them, N^6-isopentenyl-adenine (IP), *trans*-zeatin (Z), *cis*-zeatin (cZ) and dihydrozeatin are the most abundant (Sakakibara, 2006). CKs are mainly synthesized in active root meristems (Engels *et al.*, 2012) but also in mature floral tissues, in axillary buds, in the fruit abscission zone, in leaf and root phloem companion cells, in the endodermis of the root elongation zone and in the root cap (Hirose *et al.*, 2008). Isopentenyltransferases (IPTs) catalyze the first and most limiting step in the formation of CKs (Schmülling, 2003). Two forms of IPTs, the adenylate IPTs (1) and the tRNA IPTs (2), exist in plants and catalyze the following reactions (Spíchal, 2012):

(1) ADP + DMAPP → N^6-isopentenyladenosine-5´-diphosphate + PP_i
ATP + DMAPP → N^6-isopentenyladenosine-5´-triphosphate + PP_i
(2) tRNA + DMAPP → prenyl-tRNA + PP_i.

In *Arabidopsis thaliana*, seven adenylate IPT and two tRNA IPT isoforms could be identified, which are expressed in a tissue-specific manner (Kamada-Nobusada and Sakakibara, 2009) and are localized in distinct cellular compartments: AtIPT1, AtIPT3, AtIPT5 and AtIPT8 in the plastid, AtIPT2 and AtIPT4 in the cytosol and AtIPT7 in the mitochondria (Spíchal, 2012). To produce active CK forms, a phosphatase reaction (to form the respective monophosphate-CK forms) and subsequent reactions of either a combination of 5´-ribonucleotide phosphohydrolase and adenosine nucleosidase or a phosphoribohydrolase from the "lonely guy" (LOG) family (to cleave the ribose from the purine skeleton) follow the IPT reaction (Spíchal, 2012; an exemplary overview of the Z synthesis including the described steps is shown in Figure 1-1, corresponding reactions in cZ and IP synthesis are only partially presented). Synthesized CKs act locally or in long-distance signaling, the latter requiring a physiologically inactive form for transport (Sakakibara, 2006) via the vascular system (Haberer and Kieber, 2002). The inactivation is caused by modifications at the N^6-located side chain or at the adenine moiety and is specific for distinct CKs (Sakakibara, 2006; modifications described in the following are partially shown in Figure 1-1). Structural changes at the adenine moiety of CKs are the conversion into the respective nucleosides and nucleotides (by 5´-nucleotidase, adenosine nucleosidase, adenine phosphoribosyltransferase and adenosine kinase) or the glycosylation of *N3*, *N7* and *N9*. The N^6-side chain can be *O*-glycosylated (by *O*-glucosyltransferase and *O*-xylosyltransferase) or, in case of an IP side chain, hydroxylated (by the cytochrome P450 monooxygenases CYP735A1 and CYP735A2). While the *N*-glycosylation of *N7* and *N9* is assumed to irreversibly inactivate CKs and leads to degradation via CK oxidases (CKXs), the *N3*-gylcosides as well as the *O*-glycosylation at the N^6-side chain can be converted back to the free CK base by β-glucosidases. *O*-glycosylation even protects CKs against degradation (Spíchal, 2012). Catalyzed by LOG, the nucleosides can be converted back to free CK bases, too.

A further process in regulating the CK levels is an irreversible cleavage of the unsaturated N^6-side chain of some CKs, e.g. Z or IP, by CKXs (Kieber and Schaller, 2014).

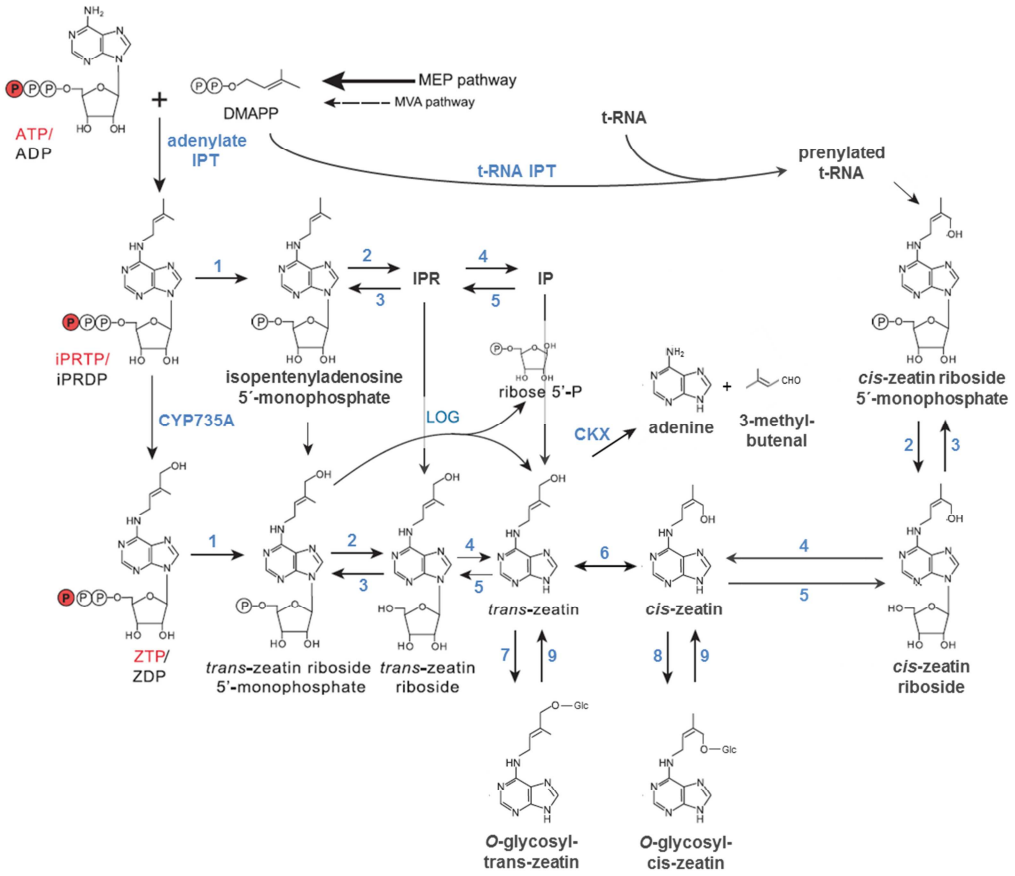


Figure 1-1: Partial model of biosynthetic and metabolic pathways for the adenine-type cytokinins *trans*-zeatin (Z), *cis*-zeatin (cZ) and isopentenyl adenine (IP). Figure reprinted from Kieber and Schaller (2014) and extended after Haberer and Kieber (2002) and Spíchal (2012).
MEP: Methylerythritol 4-phosphate; MVA: Mevalonate; DMAPP: Dimethylallyl pyrophosphate; ATP: Adenosine triphosphate; ADP: Adenosine diphosphate; 1: Phosphatase; 2: 5´-ribonucleotide phosphohydrolase; 3: Adenosine kinase; 4: Purine nucleoside phosphorylase; 5: Adenosine nucleosidase; 6: *cis-trans*-isomerase; 7: *trans*-zeatin-*O*-glycosyltransferase; 8: *cis*-zeatin-*O*-glycosyltransferase; 9: β-glucosidase. Further abbreviations are mentioned in the text.

1.4.2 Role of nitrogen nutrition in cytokinin biosynthesis

The synthesis of CKs is strongly influenced by plant-available N (Sakakibara, 2006). In *Arabidopsis thaliana*, *IPT3* expression is rapidly upregulated in roots and shoots by nitrate supply after N limitation (Takei *et al.*, 2004a) and also glutamine (Gln) application exerts a similar effect (Kamada-Nobusada *et al.*, 2013). In roots, the resulting higher levels of IPT3 products stimulate the expression of *CYP735A2* (Sakakibara *et al.*, 2006), which catalyzes the side-chain hydroxylation of N^6-isopentenyladenosine-di/triphosphate (IPRDP/IPRTP) forming *trans*-zeatin-di/triphosphate (ZRDP/ZRTP, see Figure 1-1; Kieber and Schaller, 2014). CK abundance regulates protein synthesis, macronutrient acquisition and morphological root responses to nitrate supply (Sakakibara *et al.*, 2006). Since *IPT3* is mainly expressed in the phloem companion cells, IPT3-borne CKs might also act in long-distance signaling via the vascular system (Takei *et al.*, 2004a). Plants transport preferentially Z-type CKs via their xylem vessels and IP-type CKs in the phloem (Kudo *et al.*, 2010). Thus, root-synthesized and for allocation inactivated Z is transported as zeatin riboside (ZR) to the shoot, where it mediates, together with CKs produced by shoot IPT3, the nitrate response of the above-ground plant parts (Sakakibara *et al.*, 2006).

Takei *et al.* (2004a) proposed that CKs produced via IPT3 might repress the expression of *IPT5*, while long-term availability of nitrate and ammonium stimulate *IPT5* expression. In *Arabidopsis thaliana*, IPT5 is localized in lateral root primordia and in pericycle cells, suggesting that IPT5-dependent CKs act in local as well as in long-distance signaling. Together with IPT3-borne CKs they mediate plant responses to changing N availabilities in a dual response system (Takei *et al.*, 2004a) and transmit the N status of the root to the shoot (Schmülling *et al.*, 2003). Both IPTs are localized in plastids, where also a few steps of N assimilation take place. These regulatory interactions support the idea of a tight link between N nutrition and CK production (Sakakibara, 2006).

Although the influences of nitrate and ammonium nutrition on CK production have been subject to many studies, literature on the interaction of urea supply with CK synthesis is rare. Mercier *et al.* (1997) showed that urea induced the highest shoot CK and biomass accumulation among the three mentioned N forms in the tank-forming epiphytic bromeliad *Vriesea philippocoburgii*. Contrastingly, in a study of Bauer (2014) hydroponically-grown barley exhibited lowest biomass and shoot N concentration under stabilized urea supply. Furthermore, almost no ZR translocation from root to shoot was observed under urea conditions, while IPR translocation in the xylem was comparable to nitrate or ammonium treatment. However, differences in root *IPT3* expression under the different N-nutritional conditions could not be identified (Bauer, 2014). How xylem CK translocation of field-grown oilseed rape is influenced by differential N nutrition has not been studied so far.

Coinciding with the studies of Mercier *et al.* (1997) and Bauer (2014) CKs appear not only to function in N signaling (Kamínek *et al.*, 2003), but also to play an important role in plant productivity: Together with light and nitrate signals, CKs positively regulate the expression of nitrate reductase genes and thus stimulate the assimilation of nitrate into organic N forms. CKs enhance the sink strength of meristematic and reproductive tissues, which increases the driving force for N uptake, assimilation and allocation within the plant and is therefore beneficial for N economy and plant yield especially under limited N supply (Kamínek *et al.*, 2003).

1.4.3 Cytokinins in the senescence regulation of plants

The progression of plant senescence depends on the interplay of phytohormones with senescence-accelerating function, like ethylene, jasmonic acid (JA), abscisic acid (ABA) and salicylic acid (SA), with those retarding senescence, like auxin, gibberellic acid (GA) and CKs (Jibran *et al.*, 2013). Thereby, the action of phytohormones in senescence regulation is considered as stimulatory rather than as initializing and is integrated into a complex signaling network of developmental and environmental factors regulating the expression of over 100 *SAG* genes, including transcription factors (TFs) of the NAC, WRKY, MYB, C2-H2 zinc-finger, bZIP, and AP2/EREBP families (Besseau *et al.*, 2012). The role of CKs as senescence-delaying phytohormones appears to be universal in plants, and the decrease of CK contents in leaves has been proposed as one key step for the progression of senescence (Jibran *et al.*, 2013). The onset of senescence is accompanied by decreasing CK translocation from the root to the shoot via the xylem and a decreasing capacity of CK synthesis in the leaves (Gan and Amasino, 1996), resulting e.g. in *Arabidopsis thaliana* from a downregulation of *IPT3* expression, altered CK signaling via lower expression of different A-type *AAR* response regulator genes and upregulation of the CK receptor *AHP4*, as well as increased CK degradation due to higher *CKX* expression (Buchanan-Wollaston *et al.*, 2005).

Thereby, the activation of the histidine kinase AHK3 by CKs and the subsequent specific phosphorylation of ARR2 were shown to be critical for retarding leaf senescence (Kim *et al.*, 2006) while the presence of CKs inhibited catabolic enzymes, in particular proteases or lipases (Kamínek *et al.*, 2003). A key player in CK-mediated senescence regulation

appears to be the extracellular cell wall-bound invertase, which hydrolyzes phloem-delivered sucrose to hexose monomers in the apoplast for their subsequent uptake into the sink cells. When CK levels drop at the onset of senescence, invertase activity decreases and consequently sugar supply of the cells declines. This change in source-sink relations marks an important step in senescence regulation (Balibrea Lara *et al.*, 2004). Other targets of altered CK levels are chloroplasts, a primary subject of the senescence program: CKs promote their differentiation and multiplication and activate the expression of *CAB*, which encodes the light-harvesting chlorophyll a/b binding protein, and of *RUBISCO*, while a drop in CK levels provokes chloroplast disintegration as an early ultrastructural modification related to senescence (Gan and Amasino, 1996). As long as *CAB* is expressed and distinct levels of CKs, auxin and the photosynthesis products sucrose, glucose and fructose are present, the expression of *SAG12*, as marker for developmentally-controlled senescence, is inhibited. However, a decline in photosynthesis leads to an upregulation of *SAG12* expression. Although the supply of external CKs can suppress upregulation of *SAG12* (probably via inactivation of a transcriptional activator), this does not lead to a reversion of leaf senescence (Noh and Amasino, 1999a).

On the basis that CKs can delay senescence in plants (Jibran *et al.*, 2013) and that crops displaying delayed leaf senescence can prolong assimilate supply to the roots and thus maintain N uptake after flowering, which is beneficial for yield formation (Schulte auf'm Erley *et al.*, 2007), several approaches have been conducted to enhance seed yield via an altered CK metabolism. Earlier studies showed that the exogenous application of synthetic CKs leads to delayed leaf senescence and higher yield in different plant species, e.g. rice (Ray *et al.*, 1983) and barley (Mishra and Gaur, 1985). Furthermore, kernel and ear abortion of maize could be reduced and the number of flowers of oilseed rape could be enhanced by CK application (Schmülling *et al.*, 2003). The first authors showing that the auto-regulated production of CKs by expressing *IPT* under the *SAG12* promoter could inhibit leaf senescence were Gan and Amasino (1995). Transformed tobacco plants showed a functional stay-green phenotype, in which chlorophyll catabolism was not only impaired (like in cosmetic stay-green plants) but photosynthesis capacity was also maintained, until leaves underwent a sudden death after a significant prolongation of their life-span. As a result biomass and seed yield production substantially increased. In the following years a lot of plant species were transformed with *IPT* under promoters of developmental-senescence controlled genes with similar (e.g. in tomato) or contradictory effects (e.g. wheat and rice; summarized by Gregersen *et al.*, 2013). In oilseed rape, transformation with *SAG-IPT* (Jameson and Song, 2015) or *AtMYB32xs::IPT* (Kant *et al.*, 2015) constructs led to yield increases also under field conditions. Another strategy to enhance endogenous CK levels is the inhibition of their degradation by reducing the expression of *CKX* (Jameson and Song, 2015). Cotton plants with a moderate down-regulation of *GhCKX* showed delayed leaf senescence and increased seed and lint yield (Zhao *et al.*, 2015). Furthermore, Ashikari *et al.* (2005) showed that *OsCKX2*, whose expression is lower in high-yielding lines, proved to be causative for increased grain number in rice and suggested that the resulting higher CK levels in inflorescence meristems stimulated flower number. In wheat, the orthologue of *OsCKX2* was associated with grain size (Jameson and Song, 2015).

As there is a strong interaction between N nutrition and CK synthesis (see 1.4.2) the question arises, whether CK production of crop plants can be influenced by the N form applied to agricultural systems in a sense that nitrate supply delays senescence and increases plant productivity. Singh *et al.* (1992) showed that application of ammonium nitrate increased the CK levels of detached tobacco leaves and was most efficient in retarding leaf senescence compared to other N forms, which were less effective or not able to delay senescence. In a study of Schildhauer *et al.* (2008) barley leaf senescence could be reversed by applying nitrate and ammonium but not by urea treatment. However, the effect of different fertilized N forms on CK production in relation to plant senescence of field-grown crops has not yet been elucidated.

1.4.4 Role of cytokinins in plant architecture

In root and shoot formation, CKs play an opposing role, as they have a positive impact on shoot but a negative impact on root growth and development (Aloni *et al.*, 2006). Regarding root architecture, the formation of lateral roots from pericycle cells is of high importance (Fukaki and Tasaka, 2009). The phytohormone auxin, which acts antagonistically to CKs in many aspects of plant growth (Li *et al.*, 2006), plays a crucial role in the formation of lateral root primordia as well as in the stimulation of their outgrowth (Overvoorde *et al.*, 2010). Originating mainly from shoot organs, auxin is transported from the shoot apical meristem to the root via the vascular tissue (Aloni *et al.*, 2006). At the root tip, auxin is redirected and transported backwards to the shoot (Fukaki and Tasaka, 2009), together with auxin synthesized in the root apex. Auxin pulses, which occur every 15 h in the basal root meristem (transition zone between root meristem and elongation zone), prime the nearby pericycle cells adjacent to the xylem poles via auxin signaling to become regularly spaced. Due to AUX1 (auxin influx carrier)-mediated auxin transport, left-right alternating lateral root founder cells are generated along the primary root axis (De Smet *et al.*, 2007). A distinct auxin gradient, regulated by polar auxin transport (Laplaze *et al.*, 2007), induces a defined program of asymmetric, anticlinal cell divisions and cell differentiations of the founder cells to develop mature lateral root primordia (Overvoorde *et al.*, 2010).

CKs act antagonistically to auxin in the formation of founder cells and mature lateral root primordia by affecting auxin distribution: CKs downregulate *PIN FORMED* (*PIN*) genes, which encode auxin efflux facilitators mediating polar auxin transport. Consequently, the auxin gradient for stimulating exact pericycle cell priming and asymmetric founder cell division is impaired (Laplaze *et al.*, 2007). Furthermore, CKs have a negative effect on cell division during lateral root initiation as they reduce the expression of cyclins important for G2- to M-phase transition, mediated by an AHK4-dependent signaling pathway (Li *et al.*, 2006).

This knowledge was used in an approach undertaken by Werner *et al.* (2010): Tobacco and *Arabidopsis thaliana* plants expressing *CKX*s under the transcriptional control of root-specific promotors, showed significantly lower root CK concentrations associated with increased lateral root number and length. Shoot growth was not affected, but nutrient uptake into the above-ground plant parts was enhanced. These results appear to be interesting in terms of improving nutrient acquisition and thus productivity of crops (Werner *et al.*, 2010) because deeper root systems can be beneficial for N uptake of plants especially under N limitation (Garnett *et al.*, 2009). However, Li *et al.* (2006) showed that lateral root development is stimulated by exogenously applied CKs via induced expression of type-D cyclins mediating the G1- to S-phase transition, and also lateral root elongation was positively affected by CK treatment in this study. Thus, opposite developmental effects may be achieved depending on the way and extent by which CK homeostasis is altered.

How the application of different N forms can manipulate CK homeostasis in barley has been part of the work of Bauer (2014) and was already mentioned in 1.4.2. Thereby, the lowest Z translocation levels from root to shoot found under urea nutrition were associated with the lowest root dry weight production among the N forms urea, nitrate and ammonium. Contrastingly, urea nutrition of green-house cultivated creeping bent grass favored root and shoot biomass accumulation over nitrate (McCrimmon *et al.*, 1993), while tomato roots yielded comparable dry weight under nitrate and urea conditions (Kirkby and Mengel, 1967). To what extent the mentioned N forms influence CK homeostasis and root development in field-grown crops remains rather unclear.

As described for root development, also shoot architecture depends on the interplay of the antagonistically acting phytohormones auxin and CKs. An additional phytohormone class involved in the regulation of shoot axillary bud outgrowth are strigolactones (SLs; Dun *et al.*, 2012). Axillary buds are formed in the axils of leaves from axillary meristems (Janssen *et al.*, 2014): Different genes, such as *LATERAL SUPRESSOR* (*LAS*) or *REGULATOR OF*

AXILLARY MERISTEMS (*RAX*), have been described to be essential for axillary meristem initiation and for the formation of leaf primordia during vegetative plant development or of flower meristems during the reproductive stage. In dependence of developmental and environmental stimuli, initiated axillary meristems grow out or remain arrested as axillary buds (Aguilar-Martínez *et al.*, 2007). It is known for already more than 100 years that the main shoot apex exerts an inhibitory effect on the outgrowth of axillary buds below, a phenomenon called apical dominance (Domagalska and Leyser, 2011). The shoot apex as well as young expanding leaves represent abundant sources of auxin synthesis and via active basipetal transport within the vasculature a polar auxin transport stream evolves (Ongaro and Leyser, 2008). Since auxin cannot move upwards into the axillary buds, two models have been proposed how auxin indirectly inhibits bud outgrowth: the auxin transport canalization-based model and the second messenger hypothesis (Domagalska and Leyser, 2011).

The first model assumes that a bud needs to establish its own auxin export stream to grow out and therefore vascular tissues have to be formed. For the development of vascular strands auxin flows from the source to the sink tissues are gradually canalized into specific cell files of polar auxin transport, since auxin stimulates the expression of *PIN*s, polarizes them in the direction of the auxin flow and inhibits their removal from the membrane in a positive feedback loop (Domagalska and Leyser, 2011). However, if a polar auxin stream from the shoot apex to the root exists in the main stem, auxin produced in the buds cannot be canalized into the direction of the stem vasculature, since the sink strength of the main stem for auxin is too low to establish an auxin flow. Thus, polar auxin transport in the main stem inhibits bud outgrowth. Next to auxin, SLs, synthesized in roots and shoots, act inhibitory on bud outgrowth, as they reduce the accumulation of PIN1 proteins on the basal membrane of xylem parenchyma cells, resulting in a dampening of the polar auxin stream and thus enhancing the competition between the buds for a common auxin sink in the stem (Domagalska and Leyser, 2011).

The other hypothesis for bud outgrowth regulation assumes auxin action via a second messenger, which directly moves into the bud. Possible messengers could be SLs, which inhibit bud outgrowth, and CKs, which have a stimulating action. Apically derived auxin was shown to upregulate the expression of the SL-biosynthesis genes *MORE AXILLARY GROWTH (MAX) 3* and *MAX4* in *Arabidopsis thaliana* as well as homologues in other species resulting in higher SL production (Domagalska and Leyser, 2011), whereas the *MAX4* response could be only observed in roots and hypocotyls (Bainbridge *et al.*, 2005). However, both long-distance signaling via SLs synthesized in roots as well as local SL-signals from the shoot are important for the regulation of shoot branching (Dun *et al.*, 2012). Within the buds, SLs are thought to interact with an α/β-hydroxylase (AtD14), which then binds to an F-box protein (MAX2) activating an SCF-type protein ubiquitin ligase complex. This machinery possibly acts in degrading TFs involved in the regulation of cell cycle, cell division and other pathways necessary for meristem growth (Janssen *et al.*, 2014). An important negative regulator of shoot branching is the bud localized TF BRC1 in *Arabidopsis thaliana* (Aguilar-Martínez *et al.*, 2007) and it was proposed that the SCF^{MAX2} complex degrades a repressor of *BRC1* transcription, finally leading to an inhibition of bud outgrowth via SL signaling (Janssen *et al.*, 2014). In contrast to SLs, CKs play an important role in the activation of bud outgrowth as the supply of CKs can reduce the expression of *BRC1* (Dun *et al.*, 2012), likely via the degradation of a transcriptional activator (Janssen *et al.*, 2014). To maintain the effect of apical dominance, auxin transported basipetally downregulates *IPT* genes at the nodes as well as in the roots (Ongaro and Leyser, 2008) and was also shown to upregulate *CKX2*, resulting in decreased CK levels (Domagalska and Leyser, 2011). Thereby, the expression of *PsIPT2* in the nodal stem correlates strongly with bud release in pea (Shimizu-Sato *et al.*, 2009).

Based on these studies, it may be expected that those yield components which depend on shoot branching, i.e. pod number and seed number, are affected by those fertilizer N forms

which promote CK translocation from roots to shoots. In cereals it was already shown that ammonium nitrate fertilizer can stimulate tillering compared to solely nitrate or ammonium nutrition (Wang and Below, 1995) as well as compared to urea, but influences of the fertilizer N forms on spike formation were not consistent (Bauer, 2014). For oilseed rape, however, such data have not been published so far.

1.5 Aims

As described above, there are several pieces of evidence indicating that a targeted N nutrition can affect yield composition via its interaction with phytohormone biosynthesis or transport. To which extent a variation in N fertilizer forms can be employed in order to improve seed yield formation and N efficiency of field-grown oilseed rape was to be elucidated in this work. A set of more than ten elite breeding cultivars of oilseed rape was grown in two subsequent years in order to describe the influence of two contrasting N fertilizer forms, namely ammonium nitrate and urea, on root activity and N uptake during flower development, flowering and pod formation, on shoot N accumulation and N distribution as well as on yield formation. Thereby, the more nitrate-based nutrition, established by ammonium nitrate supply, was expected to induce CK production in the root and CK export to the shoot to follow two goals:

(i) Delaying plant senescence processes by CKs resulting in a prolonged leaf photosynthetic activity for extended assimilate supply to the roots. This may also prolong root N uptake until flowering or even post-flowering which might then be beneficial for yield formation.
(ii) Increasing shoot branching by CKs to increase the potential sites for pod development and thus for yield potential of a plant.

In contrast to ammonium nitrate, no such CK-based effects were expected to be transmitted by urea, so that urea-fertilized plants might exhibit (i) an earlier senescence, likely associated with earlier N retranslocation from vegetative to generative organs, and (ii) less branching relative to nitrate-fed plants.

Since root development is almost impossible to study directly in field experiments (Hirel *et al.*, 2007) indirect measures were taken for describing root activity and N uptake of the oilseed rape lines. In the present thesis, xylem sap was harvested from field-grown oilseed rape to determine shoot-ward CK translocation rates as marker for root activity. This appeared reasonable since CKs are produced mainly in active root meristems and are transported to the shoot. Likewise, N uptake was evaluated by the export of N forms to the shoot. To what extent the differential N supply influenced CK and N export to the shoot at different developmental stages in the elite lines is presented in 3.1.1 and 3.2.1.

Additionally to xylem CK translocation during generative plant development, the ratio between N accumulation in vegetative organs to N accumulation in pods at the end of pod development was used to estimate senescence progression of these lines under nitrate compared to urea treatment (3.1.2 and 3.2.2). Finally, yield structure and overall seed yield were recorded in order to evaluate if targeted N nutrition can be used to increase branch and pod formation and thus to improve seed production (3.1.3 and 3.2.3). This might be important against the background of legal regulations for reducing N balance surpluses and greenhouse gas emissions in the agricultural production of oilseed rape.

In a further field season, old cultivars, landraces and a resynthesized line were grown to evaluate if such genetic diverse lines carry a higher responsiveness as the elite lines to the described differential N nutrition (3.3). Such a comparison of diverse material and actual elite lines made it also possible to study if distinct types of yield composition, which are more beneficial for overall seed yield, were selected for by breeding (3.1.3, 3.2.3 and 3.3.3).

Reference lines were grown in all three experimental years to estimate the impact of environmental conditions. As they were additionally grown without any N application, the impact of N supply, besides N form, on root activity, shoot N accumulation and final yield formation was recorded to evaluate N-dependent variability in physiological and agronomic traits (3.4).

2 Material and Methods

2.1 Plant material

The winter oilseed rape cultivars (*Brassica napus* L. *subsp. napus*) for this work were supplied by German breeding companies as indicated in Table 2-1. Material with a "PBC" code was provided in frame of the research project "Pre-BreedYield" (sponsored by the German Federal Ministry of Education and Research from September 2011 to May 2015, project ID: 0315964D) and represents a genetic diverse collection of current and old adapted varieties and one resynthesized *B. napus* line from *B. rapa* and *B. oleracea* (PBC021). Other seed material represents actual elite breeding lines.

Table 2-1: Overview of the winter oilseed rape cultivars used in this work and their origin.

Plant material	Producer
PBC001	unknown
PBC002	unknown
PBC007	unknown
PBC009	unknown
PBC011	unknown
PBC013	Norddeutsche Pflanzenzucht Hans-Georg Lembke, Holtsee, Germany
PBC015	Norddeutsche Pflanzenzucht Hans-Georg Lembke, Holtsee, Germany
PBC018	unknown
PBC019	unknown
PBC021	Georg-August-University Göttingen, Germany
PBC022	KWS SAAT, Einbeck, Germany
PBC023	KWS SAAT, Einbeck, Germany
PBC024	KWS SAAT, Einbeck, Germany
PBC027	Deutsche Saatveredelung, Lippstadt, Germany
PBC029	Syngenta Cereals, Hanstedt I, Germany
Alpaga	Norddeutsche Pflanzenzucht Hans-Georg Lembke, Holtsee, Germany
11091433	Syngenta Seeds, Bad Salzuflen, Germany
12091707	Syngenta Seeds, Bad Salzuflen, Germany
BCSNE001	Bayer CropScience Raps, Grundhof, Germany
BCSNE002	Bayer CropScience Raps, Grundhof, Germany
DSV-01	Deutsche Saatveredelung, Lippstadt, Germany
DSV-02	Deutsche Saatveredelung, Lippstadt, Germany
KWS_01	KWS SAAT, Einbeck, Germany
KWS_02	KWS SAAT, Einbeck, Germany
LG00-304E	Limagrain, Peine, Germany
LG02-228D	Limagrain, Peine, Germany
NPZ012	Norddeutsche Pflanzenzucht Hans-Georg Lembke, Holtsee, Germany
NPZ208	Norddeutsche Pflanzenzucht Hans-Georg Lembke, Holtsee, Germany

2.2 Growth conditions

2.2.1 Experimental site

The field experiments were conducted in three subsequent years (2011/2012, 2012/2013 and 2013/2014) on experimental field sites of the IPK Gatersleben (Saxony-Anhalt, Germany). This location is situated 106 m above sea level and is characterized by a long-term mean annual temperature of 9 °C and a precipitation of 486 mm. Loamy clay is the predominant soil type, the soil taxation is rated to 85 points.

2.2.2 Weather conditions

The weather conditions of the experimental years are shown in Figure 2-1. All years were characterized by mild winter temperatures, whereas in 2013/2014 the mean temperatures during the winter never fell below zero. In contrast, the length of the period with low temperatures differed strongly between the experimental years: In 2013/2014 the vegetation period started already in February while in 2012/2013 it did not start before the end of March. The precipitation pattern differed strongly among the experimental years, too: In 2011/2012 and 2013/2014 early spring was affected by drought and the rainfall was increasing until July. Contrastingly, in 2012/2013 early spring precipitation was about twice as much as in the other years, but June and July had about 60 % lower precipitation compared to the same months in 2011/2012 and 2013/2014.

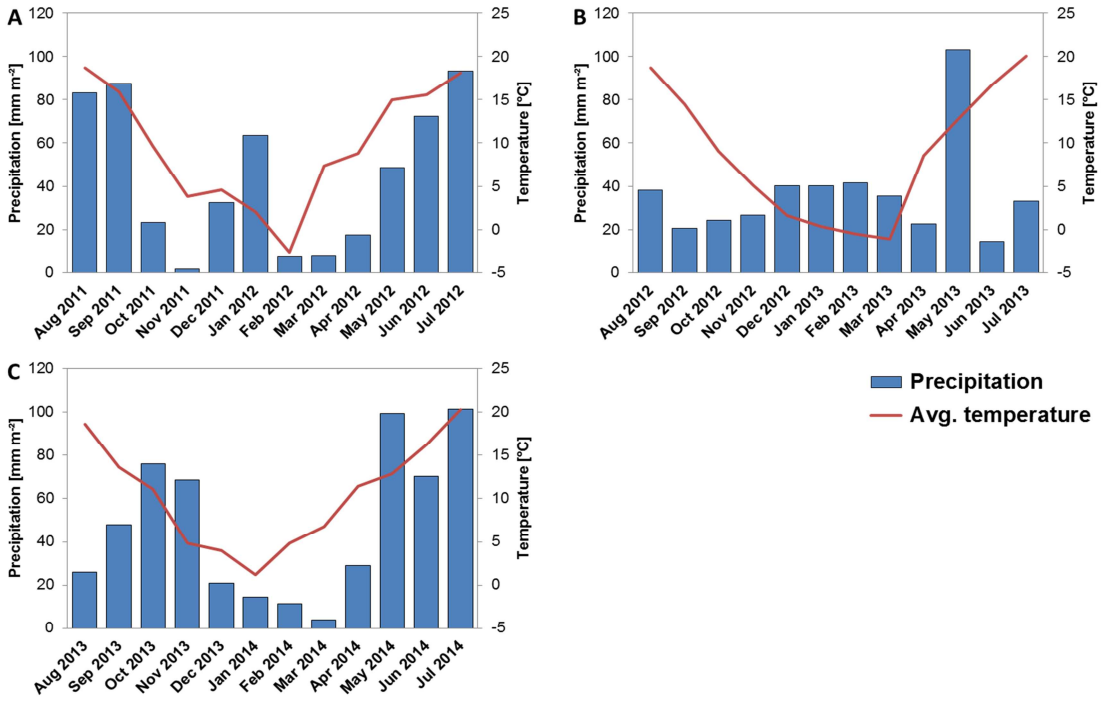


Figure 2-1: Weather conditions during the experimental years 2011/2012 (A), 2012/2013 (B) and 2013/2014 (C) as recorded by the weather station of the IPK Gatersleben.

2.2.3 Seeding of plant material

In every experimental year seeds were sown in late August in field plots with a size of 10 m x 3 m and a density of 30 germinable seeds m^{-2} using a seed drill (Maschinenfabrik Schmotzer, Bad Windsheim, Germany). Winter barley was the preceding crop in every year.

To decrease the risk for fungal and pest infestation of the seedlings, 100 g seeds were coated with a mixture of 1.7 ml Cruiser OSR (Syngenta Agro, Maintal, Germany) - containing 280 g l^{-1} thiamethoxam (insecticide), 33.3 g l^{-1} metalaxyl-M and 8 g l^{-1} fludioxinil (fungicides) - and 0.5 g SEPIRET® PF16 White (Becker Underwood, Colomiers, France) stabilizing Cruiser OSR on the seed surface.

2.2.4 N fertilizer treatments

In this work, three fertilization treatments were applied as indicated in Table 2-2.

Table 2-2: Overview of the applied N fertilizer treatments.

Fertilizer treatment	Fertilization scheme
Control	without any fertilization
Nitrate	20 kg N ha^{-1} as ammonium sulfate in autumn
	40 kg N ha^{-1} as ammonium nitrate in spring (rosette stage)
	60 kg N ha^{-1} as ammonium nitrate in spring (shoot elongation)
Urea	20 kg N ha^{-1} as ammonium sulfate in autumn
	40 kg N ha^{-1} as urea + urease inhibitor in spring (rosette stage)
	60 kg N ha^{-1} as urea + urease inhibitor in spring (shoot elongation)

For the nitrate treatment ammonium nitrate was applied (Yara, Tertre, Belgium). Nitrate from this fertilizer is directly plant available, while the majority of the ammonium will first be fixed by clay minerals before being released over a longer term and partially nitrified to nitrate (Jensen, 2006). Thus, fertilization with ammonium nitrate represented in fact a nitrate-based form of N nutrition. In contrast, in the urea treatment N was applied as urea (SKW Stickstoffwerke Piesteritz, Lutherstadt Wittenberg, Germany) stabilized with the urease inhibitor n-(n-Butyl)-thiophosphoric triamide (NBPT; Agrotain by Koch Agronomic Services, Wichita, USA) as described in the manual (1.13 g NBPT per kg urea fertilizer) to avoid rapid urea degradation and to establish a urea-based N nutrition. Using these two fertilizer N forms allowed a direct comparison between the N treatments nitrate and urea, since the total N amounts applied were identical (see Table 2-6: +N trials). In a separate field a control treatment without any N fertilization was established, which allowed examining the overall N fertilization effect conferred by the two N treatments (see Table 2-6: +N/-N trials).

The amount of applied fertilizer N was estimated according to soil type, average N mobilization from the soil and preceding crop and was kept constant over the experimental years. The fertilization time points were chosen in agreement with common agronomical practice according to the highest N demands during rapeseed development: In autumn N was applied to support optimal plant development and rooting depth before winter. N fertilization in the rosette stage after winter was necessary to provide sufficient N for the regeneration of root, leaf and flower sets since at low early spring temperatures N mobilization from the soil is limited. Fertilization during shoot elongation was meant to stimulate pod formation, to decrease the reduction of branches and to provide sufficient N during the phase of highest N demand (KWS, 2013). The exact BBCH stages for the fertilization time points are given in a synopsis with the sampling of plant material (Figure 2-3).

2.2.5 Determination of N forms in the soil

At the beginning of spring, in every vegetation period 15 samples from 0 - 30 cm and 30 - 60 cm soil depth, respectively, were taken, mixed and analyzed for available mineralized N (N_{min}, Table 2-3) by Agrolab, Oberdorla, Germany.

Table 2-3: N_{min} in different soil depth at the beginning of spring in each experimental year.

Year	N_{min} [kg N ha^{-1}]		
	0-30 cm	30-60 cm	N_{min} total
2012	7	7	14
2013	8	10	18
2014	10	12	22

In spring 2014, soil samples from control, nitrate and urea fertilizer treatment were analyzed for their content of ammonium, nitrate and urea (Table 2-4) by SKW Stickstoffwerke Piesteritz, Cunnersdorf, Germany. For this purpose, nine soil samples per treatment were taken at 0 - 30 cm, 30 - 60 cm and 60 - 90 cm soil depth, mixed and kept on ice until analysis at the same day.

Table 2-4: Concentrations of ammonium, nitrate and urea in the soil before spring N fertilizer application and 7 days after applying N fertilizer at a dose of 40 kg N ha^{-1} and 60 kg N ha^{-1}, respectively, in 2014.

Soil depth [cm]	N treatment	N forms [mg 100 g soil DW^{-1}]		
		NH_4^+	NO_3^-	Urea
Before N fertilization				
0-30	Control	0.35	0.06	0
30-60	Control	0.25	0.05	0
60-90	Control	0.24	0.12	0
0-30	Nitrate	0.36	0.07	0
30-60	Nitrate	0.35	0.07	0
60-90	Nitrate	0.31	0.06	0
0-30	Urea	0.26	0.05	0
30-60	Urea	0.34	0.05	0
60-90	Urea	0.31	0.06	0
7 d after 40 kg N ha^{-1}				
0-30	Control	0.48	0.08	0
30-60	Control	0.51	0.11	0
60-90	Control	0.53	1.17	0
0-30	Nitrate	1.67	1.63	0
30-60	Nitrate	1.23	1.21	0
60-90	Nitrate	2.05	2.50	0
0-30	Urea	0.70	0.12	2.36
30-60	Urea	0.80	0.13	4.01
60-90	Urea	1.00	0.19	7.42
7 d after 60 kg N ha^{-1}				
0-30	Control	0.45	0.20	0
30-60	Control	0.46	0.13	0
60-90	Control	0.43	0.17	0
0-30	Nitrate	2.46	2.70	0
30-60	Nitrate	1.84	0.82	0
60-90	Nitrate	1.44	1.54	0
0-30	Urea	1.62	0.29	2.88
30-60	Urea	0.96	0.17	1.19
60-90	Urea	1.25	0.24	1.43

2.2.6 Crop protection

Crop protection throughout the vegetation periods was undertaken according to local practice whenever needed. An overview is given in Table 2-5.

Table 2-5: Overview of date and amount of the applied compounds for crop protection products during the three experimental years.

Date	Action	Amount	Product	Producer
09/16/2011	Herbizide	2.0 l ha^{-1}	Butisan® Top	BASF, Ludwigshafen am Rhein, Germany
09/16/2011	Insecticide	0.075 l ha^{-1}	Karate® Zeon	Syngenta Agro, Maintal, Germany
10/21/2011	Fungicide	0.8 l ha^{-1}	Folicur®	Bayer CropScience, Langenfeld, Germany
10/21/2011	Herbizide	1.0 l ha^{-1}	Fusilade Max	Syngenta Agro, Maintal, Germany
03/19/2012	Insecticide	0.075 l ha^{-1}	Karate® Zeon	Syngenta Agro, Maintal, Germany
04/12/2012	Insecticide	150 g ha^{-1}	Plenum® 50 WG	Syngenta Agro, Maintal, Germany
04/19/2012	Insecticide	0.3 l ha^{-1}	Biscaya®	Bayer CropScience, Langenfeld, Germany
04/19/2012	Fungicide	0.5 kg ha^{-1}	Cantus® Gold	BASF, Ludwigshafen am Rhein, Germany
09/11/2012	Herbizide	2.0 l ha^{-1}	Butisan® Top	BASF, Ludwigshafen am Rhein, Germany
09/28/2012	Herbizide	1.0 l ha^{-1}	Fusilade® Max	Syngenta Agro, Maintal, Germany
09/28/2012	Fungicide	1.0 l ha^{-1}	Folicur®	Bayer CropScience, Langenfeld, Germany
04/18/2013	Insecticide	0.075 l ha^{-1}	Karate® Zeon	Syngenta Agro, Maintal, Germany
04/29/2013	Insecticide	0.15 l ha^{-1}	Plenum® 50 WG	Syngenta Agro, Maintal, Germany
09/13/2013	Herbizide	2.0 l ha^{-1}	Butisan® Top	BASF, Ludwigshafen am Rhein, Germany
09/13/2013	Herbizide	0.8 l ha^{-1}	Panarex®	BASF, Ludwigshafen am Rhein, Germany
03/20/2014	Insecticide	0.075 l ha^{-1}	Karate® Zeon	Syngenta Agro, Maintal, Germany
04/04/2014	Insecticide	0.3 l ha^{-1}	Biscaya®	Bayer CropScience, Langenfeld, Germany

2.3 Experimental setup

2.3.1 Field trials

Over the three subsequent experimental years field trials with 15 genotypes treated with nitrate or urea (see Table 2-2), were set up to investigate the influence of these fertilizer N forms on xylem CK and N transport, shoot N distribution and yield formation in a broad collection of elite lines and genetic diverse rapeseed genotypes (see Table 2-1). In 2012/2013 and 2013/2014 the reference lines PBC007 and PBC015 were grown in smaller field trials under nitrate, urea and additional control conditions, which allowed to compare the effects of fertilized N form as well as of the fertilized N dose and thus to evaluate the impact of either fertilized N form on N efficiency in different genotypes (as described in 2.7).

The field trials with 15 genotypes under nitrate and urea treatment were named as "+N" trials, the smaller field trials under nitrate, urea and control treatment were named "+N/-N". An overview of the field trials installed in the three experimental years is given in Table 2-6.

Table 2-6: Overview of the field trials in the three experimental years with the plant material grown in each trial. Underlined plant material codes mark reference genotypes grown repeatedly over two (PBC029) or three (PBC007, PBC015) years.

Growing season	Field trial	Plant material
2011/2012	+N	PBC001, PBC002, PBC007, PBC009, PBC011, PBC013, PBC015, PBC018, PBC019, PBC021, PBC022, PBC023 PBC024, PBC027, PBC029
2012/2013	+N	NPZ208, NPZ012, LG02-228D, LG00-304E, KWS_01, KWS_02, DSV-01, DSV-02, 12091707, 11091433, BCSNE001, BCSNE002, Alpaga, PBC007, PBC015
2012/2013	+N/-N	PBC007, PBC015
2013/2014	+N	NPZ208, NPZ012, LG02-228D, LG00-304E, KWS_01, KWS_02, DSV-01, DSV-02, 12091707, 11091433, BCSNE001, BCSNE002, PBC007, PBC015, PBC029
2013/2014	+N/-N	PBC007, PBC015

2.3.2 Field plot design

Every field trial (see Table 2-6) was installed in two replicates which allowed harvesting samples throughout the vegetation period from one trial while keeping the corresponding plots from the other trial intact for the final harvest of seeds (see 2.6.3).

Plots in every field trial were arranged in an extended Latin rectangle (schematically shown in Figure 2-2A). This design allows data correction for soil differences at three levels: rows, columns and quartiles as schematically shown in Figure 2-2B (Bauer, 2014). Every row, column and quartile contained one plot with each genotype under nitrate, urea and in +N/-N trials additionally without N fertilization resulting in four replicates of every genotype x treatment combination in each field trial.

Data could be corrected using the following formula:

$$Value_{plot\ corrected} = value_{plot} - mean_{block} + mean_{whole\ field\ trial}$$.

A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	30	29	28	27	26	25	24	23	22	21	20	19	18	17	16
30	29	28	27	26	25	24	23	22	21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1

B

1	2	3	4	1	2	3	4	1	2	3	4				
3	4	1	2	3	4	1	2	3	4	1	2				
2	1	4	3	2	1	4	3	2	1	4	3				
4	3	2	1	4	3	2	1	4	3	2	1				

Row effects | Column effects | Fourth effects

Figure 2-2: Exemplary field design of a +N trial in a Latin rectangle (A) and the possible blocks to correct data for position effects within this type of field design on the basis of a Latin square (B). The four different grey-scale nuances indicate the four different blocks for correction of row, column or quartile effects.

2.3.3 Harvest und fertilization scheme

Figure 2-3 illustrates the time course of field sampling and N fertilization in the +N field trials (see Table 2-6). The indicated BBCH stages correspond to the standards of phenological plant development of the Federal Biological Agency, Federal Office for Plant Varieties and Chemical Industry (Lancashire *et al.*, 1991). In +N/-N trials xylem sap was additionally taken one week after each fertilization regardless of the BBCH stage.

Samples were always taken from the core of the plot (7 m x 2 m) to minimize border effects between different plots, such as mobility of fertilized N forms to the adjacent plot, misplaced seeds from the neighboring genotype or shadowing by a very high neighboring genotype.

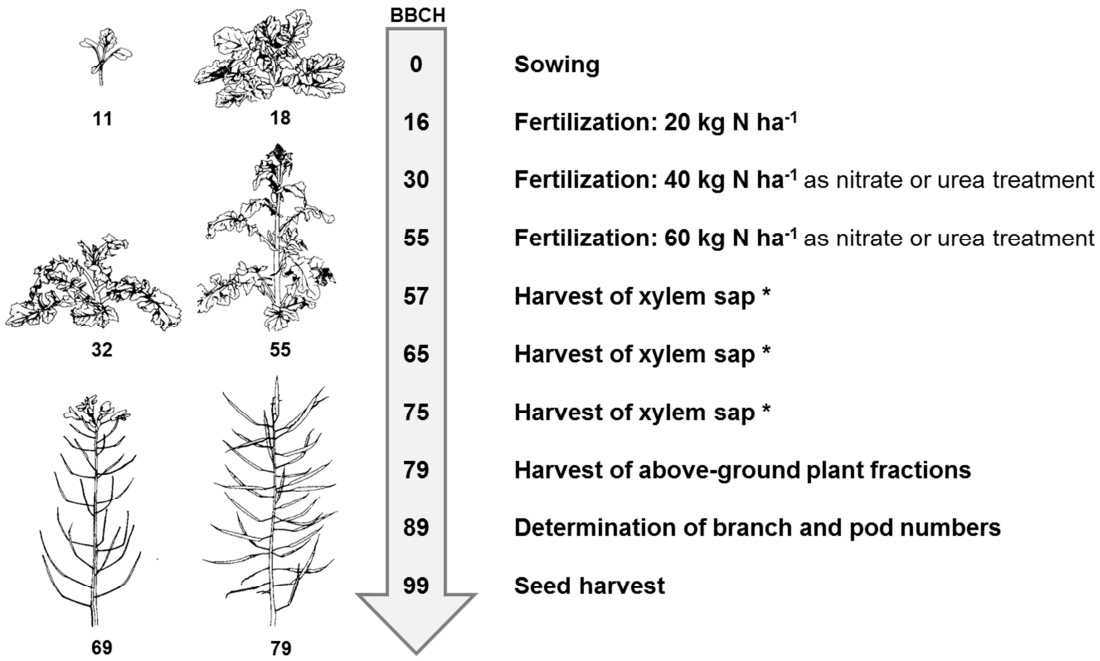


Figure 2-3: Overview of N fertilization and field sampling in the +N field trials (right) and images of exemplary developmental stages (left; © BASF AG, 1990). Xylem sap (*) was collected as indicated in the scheme only in 2012/2013 and 2013/2014, while in 2011/2012 xylem sap was taken at BBCH52, BBCH59 and BBCH69.

2.4 Xylem sap collection and analysis

2.4.1 Harvest of xylem sap from field-grown rapeseed plants

From about 7.30 a.m. until 9.30 a.m. (Central European Time) ten plants per plot were cut 2 cm above the soil surface with a scalpel. Thereby, sampling at BBCH57 started about 1.5 h after sunrise, at BBCH65 about 2.5 h and at BBCH75 about 3 h depending on plant development in the different years. A silicon tube, fitting to the diameter of the stem, was placed on it and closed on top with aluminum foil to avoid degradation of xylem sap components by UV light and to protect the xylem sap from dust during sampling. Remaining leaves were removed from the stem base since the exudation rate decreased when leaves were kept intact. On dry sampling days each examined plant was watered with 1 l tap water to improve water availability to roots for adequate generation of root pressure.

The first 100 µl of exudate were removed from the sampling tube since pretests with cytosolic malate dehydrogenase activity as marker showed cytosolic contamination in up to 100 µl exudate volume. The exact time point of removing contaminated exudate was noted. After 1 - 1.5 h xylem sap of ten plants per plot was pooled in a plastic tube and the exact time point was noted again to determine the exudation rate. The exudate was kept on ice until it was frozen at -80 °C.

Figure 2-4: Harvest of xylem sap from rapeseed. After cutting the stem and removing remaining leaves a silicon tube was mounted on top of the cut stem and closed with aluminum foil. Xylem sap accumulated in the tube.

2.4.2 Cytokinin extraction and determination

The protocol for the extraction and detection of CKs from rapeseed xylem sap was established by Kai Eggert (Molecular Plant Nutrition, IPK Gatersleben, Germany) according to Owen and Abrams (2009) Novak *et al.* (2008) and Kojima *et al.* (2009).

OASIS® HLB columns (Waters Corporation, Milford, USA) were used to clean up and extract CKs from xylem sap. These columns contain a **H**ydrophilic-**L**ipophilic **B**alanced reversed phase sorbent, namely a copolymer of m-divinylbenzol and N-vinylpyrrolidon. The column was connected to a vacuum chamber (Machery-Nagel, Düren, Germany; vacuum pump: KNF Neuberger, Freiburg, Germany) to facilitate the flow of the solvents through the column. 1 ml acetonitrile (VWR International, Leuven, Belgium) and 1 ml methanol (VWR International, Leuven, Belgium) were used to condition the column. Then it was equilibrated with 1 ml H_2O containing 1 % acetic acid (Carl Roth, Karlsruhe, Germany).

In the next step, 500 µl xylem sap sample were acidified with 500 µl H_2O containing 2 % acetic acid (Carl Roth, Karlsruhe, Germany), spiked with internal CK standards for IP, cZ, Z, IPR and cZR (marked with deuterium oxide or heavy N; OlChemIm, Olomouc, Czech Republic), well mixed for 30 sec and centrifuged for 10 min (4 °C, 20800 *g*) before loading it onto the column. Based on extensive pre-tests with xylem sap from field-grown oilseed rape the occurrence of other CK forms in the samples, namely dihydrozeatin, benzyladenine, dihydrozeatinriboside, benzyladenosine, IP-9-glucoside, Z-*O*-glucoside, tZ-9-glucoside and tZ-*O*-glucoside, could be excluded.

The samples were washed with 1 ml H_2O containing 2 % ammonia (Merck, Darmstadt, Germany) and 1 ml H_2O containing 1 % acidic acid (Carl Roth, Karlsruhe, Germany), respectively. To elute the CK from the column 1.7 ml 80 % acetonitrile with 1 % acidic acid (v/v) (acetonitrile: VWR International, Leuven, Belgium; acidic acid: Carl Roth, Karlsruhe, Germany) was used.

The eluted samples were evaporated in a vacuum centrifuge (centrifuge and condensator: Martin Christ Gefriertrocknungsanlagen, Osterode, Germany; pump: Vacuubrand, Wertheim, Germany) and stored at -80 °C until reconstitution in 50 µl mobile phase. Therefore, 10 µl 50 % methanol solution containing 0.5 % formic acid (v/v) (methanol: VWR International, Leuven, Belgium; formic acid: Carl Roth, Karlsruhe, Germany) were added to the samples, well mixed and placed in an ultrasonic bath (Bandelin electronic, Berlin, Germany) for 3 min. 40 µl deionized water was added and the samples were again mixed and sonicated as described before. After 10 min centrifugation (4 °C, 20800 *g*) the supernatant was used for separation and detection of CKs.

CKs were separated by ultra-performance liquid chromatography (UPLC; Acquity system, Waters, Eschborn, Germany) using a BEH C18 column (Acquity UPLC BEH C18, 1.7 µm, 2.1x50 mm, Waters, Eschborn, Germany), heated to 40 °C. LC-MS-grade water with 0.1 % formic acid (v/v) (water: Th. Geyer, Renningen, Germany; formic acid: Carl Roth, Karlsruhe, Germany) was used as mobile phase A. Acetonitrile with 0.1 % formic acid (v/v) (VWR International, Leuven, Belgium; formic acid: Carl Roth, Karlsruhe, Germany) was used as mobile phase B to elute to CKs from the column. The mobile phase gradient is indicated in Table 2-7. One run lasted for 7 min at a flow rate of 0.4 ml/min.

Samples were ionized using the electrospray ionization (ESI) positive ion mode and detected with a triple quadrupole mass spectrometer (MS; Xevo TQ, Waters, Eschborn, Germany). MassLynx Mass Spectrometry software (Waters, Milford, USA) was used to quantify CK amounts based on an external CK standard curve ranging from 2.5 - 1000 nM for IP, cZ, Z, IPR and cZR.

Table 2-7: UPLC gradient for separation of CKs.

Retention time [min]	Eluent A [%]	Eluent B [%]
0	90	10
2.00	85	15
4.70	40	60
5.00	1	99
5.50	1	99
6.00	90	10
7.00	90	10

2.4.3 ACQ-derivatization and determination of amino acids and ammonium

In this work, AAs and ammonium were separated and detected using UPLC coupled with a fluorescence detector. AAs and ammonium were derivatized with the fluorescent agent aminoquinolyl-N-hydroxysuccimidyl carbamate (ACQ). ACQ reacts with the amino group to form stable urea-like structures within the molecule which strongly fluoresce (after Cohen and Michaud, 1993). 3 mg ACQ, prepared at IPK Gatersleben, were dissolved in 1 ml pure acetonitrile (VWR International, Leuven, Belgium). For derivatization, 160 µl 0.2 M boric acid (AppliChem, Darmstadt, Germany), pH 8.8, and 20 µl xylem sap sample were well mixed with 20 µl of prepared ACQ reagent and incubated for 10 min at 55 °C. In parallel, standards with known amounts of 19 proteinogenic AAs and γ-aminobutyric acid (Sigma-Aldrich, Buchs, Switzerland; Trp could not be derivatized, probably due to its structure) as well as ammonium were prepared.

Separation of the ACQ-derivatized AAs and ammonium was performed using UPLC (Acquity H-Class system, Waters, Eschborn, Germany) with a C18 reversed-phase column (ACQ Tag Ultra C18, 1.7 µm, 2.1x100 mm, Waters, Eschborn, Germany). "Eluent A Concentrate" and "Eluent B" for AAs analysis from Waters (Eschborn, Germany) were used to produce different mobile phases. The column was equilibrated with one part of eluent A (pure "Eluent A Concentrate" from Waters, Eschborn, Germany) and nine parts of eluent C (pure "Eluent B" from Waters, Eschborn, Germany). The mobile phase gradient is indicated in Table 2-8 and consisted of four eluents: A (pure "Eluent A concentrate" from Waters, Eschborn, Germany), B (one part "Eluent B" from Waters, Eschborn, Germany, and nine parts LC-MS water from Th. Geyer, Renningen, Germany), C (pure "Eluent B" from Waters, Eschborn, Germany) and D (LC-MS grade water from Th. Geyer, Renningen, Germany). A run lasted 10.2 min at a flow rate of 0.7 ml per min. The column was heated to 50 °C during the run. AAs and ammonium were detected by a fluorescence detector (Acquity UPLC Photodiode Array eλ Detector, Waters, Eschborn, Germany) at an excitation wavelength of 266 nm and emission wavelength of 473 nm and quantified by Empower Pro Software (Waters, USA) using a mixture of standards with different concentrations.

Table 2-8: UPLC gradient for separation of AAs and ammonium.

Retention time [min]	Eluent A [%]	Eluent B [%]	Eluent C [%]	Eluent D [%]
0	10	0	90	0
0.29	9.9	0	90.1	0
5.49	9	80	11	0
7.10	8	15.6	57.9	18.5
7.30	8	15.6	57.9	18.5
7.69	7.8	0	70.9	21.3
7.99	4	0	36.3	59.7
8.59	4	0	36.3	59.7
8.68	10	0	90	0
10.2	10	0	90	0

2.4.4 Determination of nitrate

The quantitative determination of nitrate was based on the nitration of salicylic acid in water-free, acid environment to nitrosalicylic acid. By establishing an alkaline environment, the corresponding base of nitrosalicylic acid is formed, which exhibits a yellowish color and can be determined spectrophotometrically (Wiseman and Jacobson, 1965).

5 µl xylem sap sample was well mixed with 160 µl 1 % salicylic acid (Sigma-Aldrich, Buchs, Switzerland) in sulfuric $acid_{conc.}$ (Laborchemie, Appolda, Germany) and incubated on ice for 20 min. After addition of 1800 µl 3 M sodium hydroxide (Merck, Darmstadt, Germany) absorption was detected spectrophotometrically (Thermo Fischer Scientific, Waltham, USA) at 410 nm and the nitrate concentration in the sample was evaluated based on a standard curve.

2.4.5 Determination of urea

The determination of urea followed the principle described by Yuki *et al.* (1981). Diacetylmonoxime and thiosemicarbazide condense to form a semicarbazon. Urea binds to the product resulting in a cationic, red-colored intermediate, which is stable for a few days before losing its cationic character and therefore its red color. The intermediate can be detected spectrophotometrically.

240 µl xylem sap sample was mixed with a color development reagent containing 4.6 mM diacetylmonoxime (Carl Roth, Karlsruhe, Germany), 1.28 mM thiosemicarbazide (Merck, Darmstadt, Germany), 6.6 % sulfuric acid (Laborchemie, Appolda, Germany), 14.6 µM ferric chloride hexahydrate (Merck, Darmstadt, Germany) and 0.006 % orthophosphoric acid (Laborchemie, Appolda, Germany). The samples were incubated for 15 min at 99 °C. The absorption was determined spectrophotometrically (Thermo Fischer Scientific, Waltham, USA) at 540 nm and the urea concentration in the sample was evaluated based on a standard curve.

2.4.6 Determination of total nitrogen

Total N was determined in samples of 300 µl freeze-dried xylem sap (freeze dryer from Dieter Piatkowski Forschungsgeräte, Munich, Germany) using an elemental analyzer (EA)-MS (EuroEA3000, Hekatech, Wegberg, Germany). Samples were combusted at 1010 °C under concentrated oxygen atmosphere (Dumas principle of Dynamic Flash Combustion). The products NO_x, CO_2, H_2O and SO_2 were reduced by copper; water was detracted in a water trap. Helium (100 ml/min) was used as carrier gas for separation using a gas chromatography (GC)-column heated to 70 °C. C and N were recorded by a thermal conductivity detector. The results were quantified based on 2.5-Bis(5tert-butyl-2-benzo-oxazol-2-yl)thiophen (BBOT) standard with the Callidus software (Hekatech, Wegberg, Germany).

2.5 Analysis of above-ground plant nitrogen allocation

At BBCH79, two plants per plot were cut as close as possible to the soil surface with a secateurs and divided into seven different fractions, pooled from both plants: I) stem, II) branches, pods derived from the III) stem and from the IV) branches, V) youngest leaves derived from the branches, VI) senescent leaves and VII) green leaves derived from the stem. During the sampling, most leaves could be taken from the shoot as they were still attached or could, if necessary, be collected from the ground.

Fresh weight was determined directly on the field and after four days drying at 80 °C dry weight was recorded. The samples were ground (mill from Retsch, Haan, Germany) and C and N contents were determined using EA-MS (EuroEA3000, Hekatech, Wegberg, Germany) as described in 2.4.6.

2.6 Yield parameters

According to Diepenbrock (2000) the seed yield per area is a product of the primary yield components stand density, number of pods per plant, number of seeds per pod and the thousand seed weight.

2.6.1 Stand density

The stand density was determined in early spring by counting the number of plants in a 1 m² frame. Counting was conducted twice per plot and the mean was taken.

2.6.2 Number of branches and pods

At BBCH89 (full maturity) the total number of branches and pods of ten plants per plot was recorded. The mean was calculated as number of branches and pods per plant. The number of pods per branch was calculated by dividing the number of pods per plant by the number of branches per plant.

2.6.3 Seed yield

After BBCH89 seed moisture content was monitored regularly with a seed moisture meter (Pfeuffer, Kitzingen, Germany) until 12 % were reached. Using a combine harvester (Wintersteiger, Ried im Innkreis, Austria) seeds were harvested from the core of each plot (7 m x 1.5 m) and collected in a fabric bag. The seeds were dried on a drying system (Hoopman equipment & engineering, Aalten, The Netherlands), producing an air flow through the harvested material, until 7 % seed moisture content were reached. After cleaning the seeds with a grain sample cleaner (Baumann Saatzuchtbedarf, Waldenburg, Germnany) yield was determined by weighing the seeds.

2.6.4 Thousand seed weight

At 7 % seed moisture content 3 x 1000 seeds were counted (Pfeuffer, Kitzingen, Germany) and the mean calculated as thousand seed weight (TSW).

2.6.5 Number of seeds per pod

The following formula was used to calculate the number of seeds per pod:

$$\text{Seeds pod}^{-1} = \frac{\text{Seed yield m}^{-2}}{\text{Plants m}^{-2}} \div \frac{\text{TSW}}{1000} \div \text{Pods plant}^{-1}.$$

2.6.6 Seed N concentration

Seeds were dried for four days at 80 °C and N content was determined using EA-MS (EuroEA3000, Hekatech, Wegberg, Germany) as described in 2.4.6.

2.7 Agronomic nitrogen-related traits

Data of above-ground N and several yield parameters of the +N/-N trials (see Table 2-6) were used to calculate N uptake efficiency (NupE), N utilization efficiency (NutE), apparent N use, agronomic N efficiency, physiological N efficiency (after Rathke *et al.*, 2006), N harvest index (NHI, after Haas and Friedt, 1990) and N removal:

$$(1)\ \text{NupE} = \frac{\text{Total above-ground N BBCH79 [kg ha}^{-1}\text{]}}{(\text{Total soil N}_{min}\text{ in 0-60 cm depth [kg ha}^{-1}\text{]}^{*} + \text{Total N fertilizer applied in spring [kg ha}^{-1}\text{])}}$$

* see Table 2-3

$$(2)\ \text{NutE} = \frac{\text{Seed yield [kg ha}^{-1}\text{]}}{\text{Total above-ground N BBCH79 [kg ha}^{-1}\text{]}}$$

$$(3)\ \text{N removal} = \text{Seed N [kg kg DW}^{-1}\text{]} \times \text{Seed mass [kg DW ha}^{-2}\text{]}$$

$$(4)\ \text{NHI} = \frac{\text{N removal [kg ha}^{-1}\text{]}}{\text{Total above-ground N BBCH79 [kg ha}^{-1}\text{]}}$$

2.8 Statistical analyses

Statistical analyses were undertaken using the program SigmaPlot 11.0 (Systat Software, Erkrath, Germany). Data were analyzed as proposed in the SigmaPlot 11.0 user manual: Gaussian distribution was checked using the Shapiro-Wilk test at $p>0.05$. If data followed normal distribution, two groups were compared using an unpaired t-test while multiple comparison analysis was done by ANOVA using Tukey´s test as post-hoc test. Correlations of normal distributed data were calculated by Pearson product moment correlation. Nonparametric tests were chosen for data not following Gaussian distribution: Mann-Whitney rank sum test for the comparison of two groups, Kruskal-Wallis one-way ANOVA on ranks and two- or three-way ANOVA on ranks with Tukey´s test for multiple comparisons and Spearman rank order for correlations.

The phenotypic variability of a trait within a rapeseed population was determined using the coefficient of variation (V) allowing the direct comparison of variability for traits with different values by dividing the group specific standard deviation (s) by the group specific mean ($\bar{x}$): $V=\frac{s}{\bar{x}}$ (Kähler, 2007).

3 Results

3.1 Influence of fertilizer nitrogen form on plant nitrogen acquisition and yield formation in a set of oilseed rape elite lines in 2012/2013

A major goal of this work was to describe the genotypic variability of rapeseed elite lines in the translocation of CKs as a measure for root growth and thus potential N uptake activity, in the subsequent shoot N accumulation and distribution and in the yield formation as influenced by the contrasting N fertilizer forms nitrate and urea.

3.1.1 Influence of the applied nitrogen form on xylem nitrogen and cytokinin translocation

Xylem sap was harvested during flower development (BBCH57), during flowering (BBCH65) and during pod filling (BBCH75); these dates corresponded to 2 days, 21 days and 40 days after fertilization with 60 kg N ha^{-1} in the form of nitrate or urea, respectively.

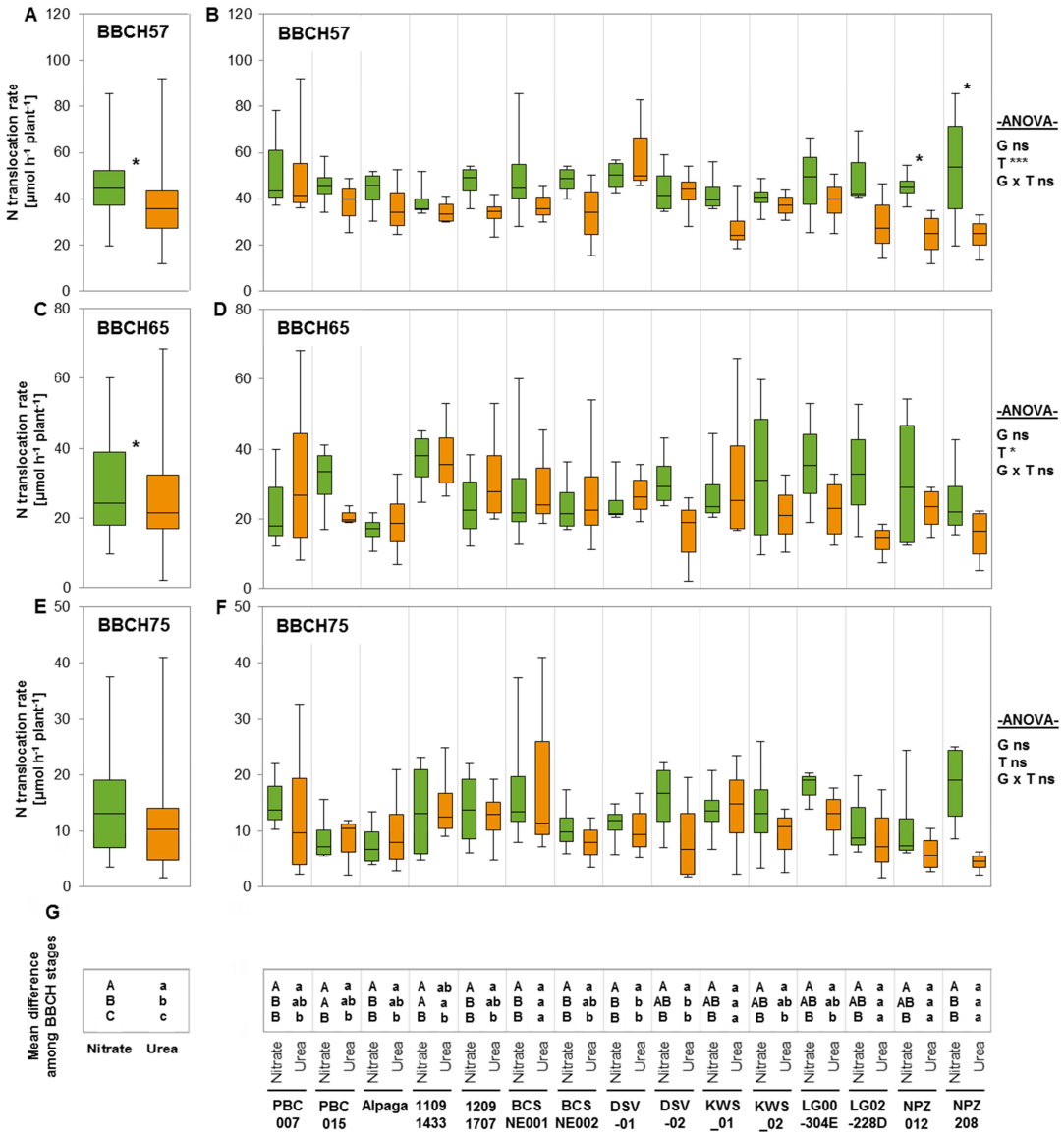


Figure 3-1 (For description see next page.)

Under supply of either fertilizer N form, total N translocation rates were highest during flower development, decreased by about 35 - 40 % at flowering and by 55 % until pod development (Figure 3-1A, C, E, G). The influence of the fertilizer N form on total N translocation became most apparent during flower development with nitrate fertilization leading to 30 % higher N translocation. This difference also held true during flowering with 15 % higher N translocation and in tendency at pod development. The decreasing influence of the N form on total N translocation was most likely related to the decreasing stability of the fertilized N forms in the soil over time.

The genotypic variation in total N transport, as indicated by the coefficient of variation, was 0.28, 0.47 and 0.54 under nitrate and 0.40, 0.57 and 0.74 under urea at BBCH57, BBCH65 and BBCH75, respectively (Figure 3-1B, D, F). Thus, variability in total N transport increased from the first to the last developmental stage, whereby higher variation was consistently found under urea treatment. Shortly after fertilizer application (BBCH57), 13 out of 15 elite lines transported a larger total N amount in the xylem after nitrate treatment compared to urea, even though this difference was significant only in 2 of them. At later developmental stages the fertilizer treatment did not significantly influence N translocation in any of the lines. Interestingly, lines transporting a larger amount of N in the xylem at BBCH65 did that also at BBCH75 under either N treatment (Annex-2).

Since, at least described under N limitation, higher N uptake during reproductive growth is positively correlated with seed yield in oilseed rape (Schulte auf'm Erley *et al.*, 2007), both variants of 11091433, the nitrate variants of LG00-304E and LG02-228D and the urea variant of KWS_01 at BBCH65 as well as both variants of BCSNE001 and the nitrate variants of LG00-304E and NPZ208 at BBCH75 appeared to be interesting candidates for improved seed yield. This was verified by correlations between final seed yield and total N translocation among the elite lines as shown in 3.1.3.

Figure 3-1: Total N translocation in the xylem sap of the elite line collection in the +N field trial 2012/2013 at BBCH57, BBCH65 and BBCH75 as mean over all genotypes (A, C, E) or of single genotypes (B, D, F) and as comparison between the developmental stages over all genotypes (G) or single genotypes (H). All rapeseed genotypes were fertilized in spring with 40 kg N h^{-1} at BBCH30 and 60 kg N h^{-1} at BBCH55 as nitrate or urea. Xylem sap was pooled from ten plants per plot. (A-F) Boxes show median, first and third quartile; whiskers represent minimum and maximum of all data in a group (A, C, E: n=60; B, D, F: n=4). (A, C, E) Significant median differences are indicated by an asterisk according to Mann-Whitney rank sum test at $p<0.05$. (B, D, F) ANOVA on ranks (- -) results *, **, *** indicate significant differences or interactions at $p<0.05$, $p<0.01$, $p<0.001$, respectively; G=genotype, T=N treatment, ns=non-significant. Significant mean difference among nitrate and urea treatment within a genotype is indicated by asterisks according to unpaired t-test at $p<0.05$. There were no significant mean or median differences among the genotypes within nitrate treatment according to Tukey´s test (B, D) or Tukey´s test on ranks (F), respectively, and within urea treatment according to Tukey´s test (F) or Tukey´s test on ranks (B, D), respectively, at $p<0.05$. (G, H) Table shows mean or median differences for xylem N translocation among BBCH stages indicated by different letters in vertical rows of three comparing BBCH57, BBCH65 and BBCH75 (top to bottom). Different upper case letters indicate significant mean (H) or median (G) differences within nitrate treatment, while lower case letters refer to mean (H) or median (G) differences within urea treatment according to Tukey´s test (H) or Tukey´s test on ranks (G) at $p<0.05$; (G) n=60; (H) n=4.

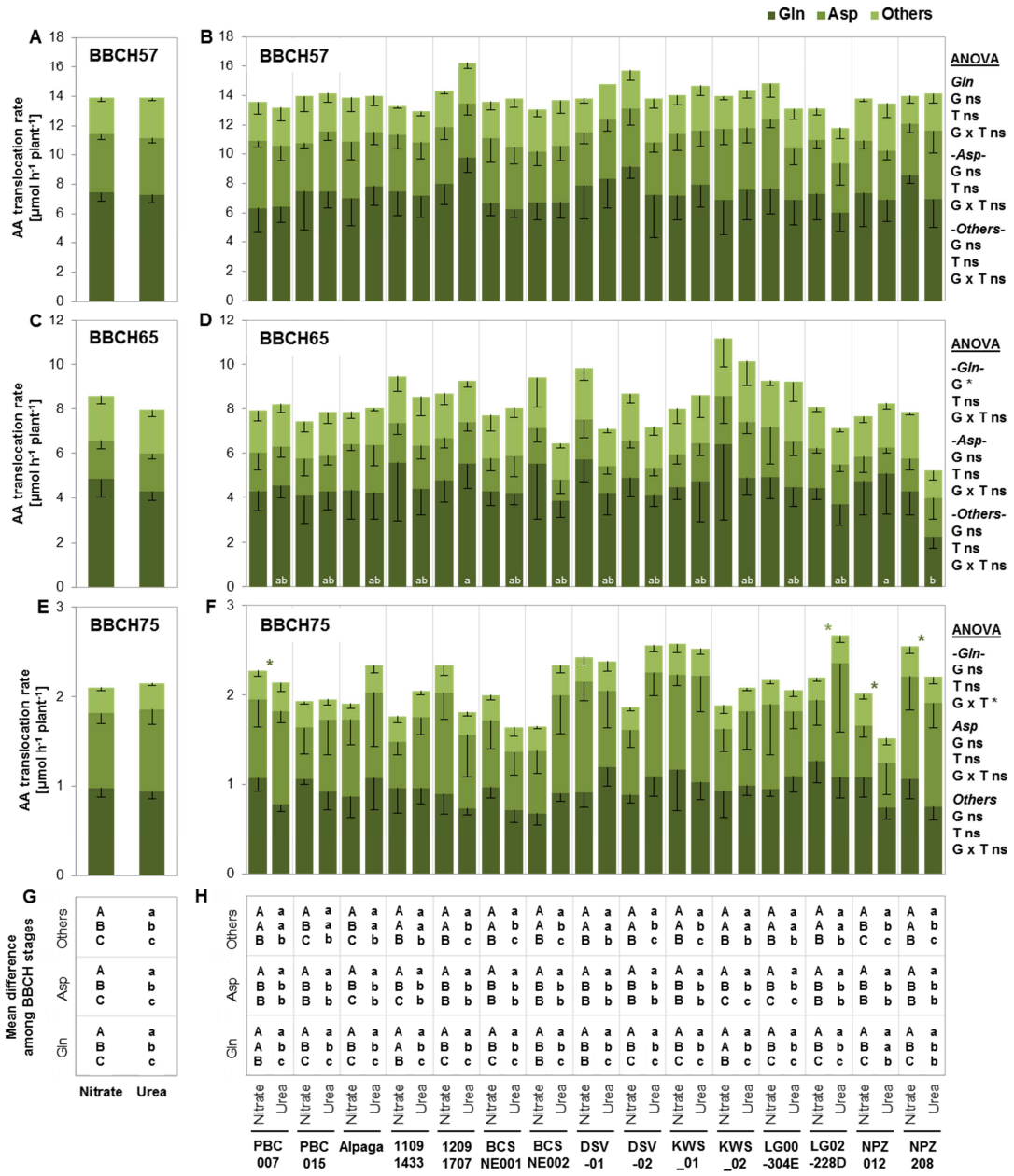


Figure 3-2: Amino acid translocation in the xylem sap of the elite line collection in the +N field trial 2012/2013 at BBCH57, BBCH65 and BBCH75 as mean over all genotypes (A, C, E) or of single genotypes (B, D, F) and as comparison between the developmental stages over all genotypes (G) or single genotypes (H). All rapeseed genotypes were fertilized in spring with 40 kg N h^{-1} at BBCH30 and 60 kg N h^{-1} at BBCH55 as nitrate or urea. Xylem sap was pooled from ten plants per plot. (A-F) Bars show means -SD (A, C, E: n=60; B, D, F: n=4). (A, C, E) There were no significant median differences according to Mann-Whitney rank sum test at p<0.05. (B, D, F) ANOVA or ANOVA on ranks (- -) results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. Significant mean difference among nitrate and urea treatment within a genotype is indicated by asterisks referring to AA color code according to unpaired t-test at p<0.05. Different upper case letters within a bar indicate significant mean or median differences among the genotypes within nitrate treatment according to Tukey´s test (B: ■■, D: ■, F: ■■■) or Tukey´s test on ranks (B: ■, D: ■■), respectively, at p<0.05; different lower case letters indicate significant mean or median differences among the genotypes within urea treatment according to Tukey´s test (B: ■■■, D: ■, F: ■■■) or Tukey´s test on ranks (D: ■), respectively, at p<0.05. (G, H) Table shows mean or median differences for xylem Gln, Asp and residual AAs (Others) translocation among BBCH stages indicated by different letters in vertical rows of three comparing BBCH57, BBCH65 and BBCH75 (top to bottom). Different upper case letters indicate significant mean (H) or median (G) differences within nitrate treatment, while lower case letters refer to mean (H) or median (G) differences within urea treatment according to Tukey´s test (H) or Tukey´s test on ranks (G) at p<0.05; (G) n=60; (H) n=4.

Like observed for total N, largest amounts of AAs were translocated at flower development before they decreased during flowering and further until pod development. At all developmental stages Gln and Asp were the most abundant AAs, whereas until flowering Gln translocation appeared to be preferred over Asp but during pod development both AAs were translocated in similar amounts (Figure 3-2A, C, E, G). These results are contradictory to a study of Balint and Rengel (2011) where proline and alanine were found as most abundantly transported AAs in the xylem after Gln, but in summer types of *Brassica napus*. The fertilizer N form had no significant impact on mean AA translocation rates at any developmental stage.

Within the population of elite lines, genotypic variability in the total translocation rate of AAs was lowest at BBCH57 (0.13 under nitrate, 0.14 under urea) and increased until later plant development (BBCH65: 0.28 under nitrate, 0.24 under urea; BBCH75: 0.22 under nitrate, 0.24 under urea). The fertilizer N form had no influence on the variability in total AA translocation at any of the developmental stages (Figure 3-2B, D, F).

While the fertilizer N form did not significantly influence AA translocation rates in individual lines at earlier developmental stages, 3 lines translocated significantly more Gln under nitrate and 1 line translocated more Asp after urea treatment at BBCH75. However, such impacts of the N treatment did not manifest in the population mean of Gln and Asp translocation rates (Figure 3-2E). Thus, overall mean transport of total or the two most abundant AAs was not influenced by the N fertilizer form at any of the three developmental stages, even though the fertilized N form significantly altered Gln and Asp translocation rates in some of the lines at BBCH75.

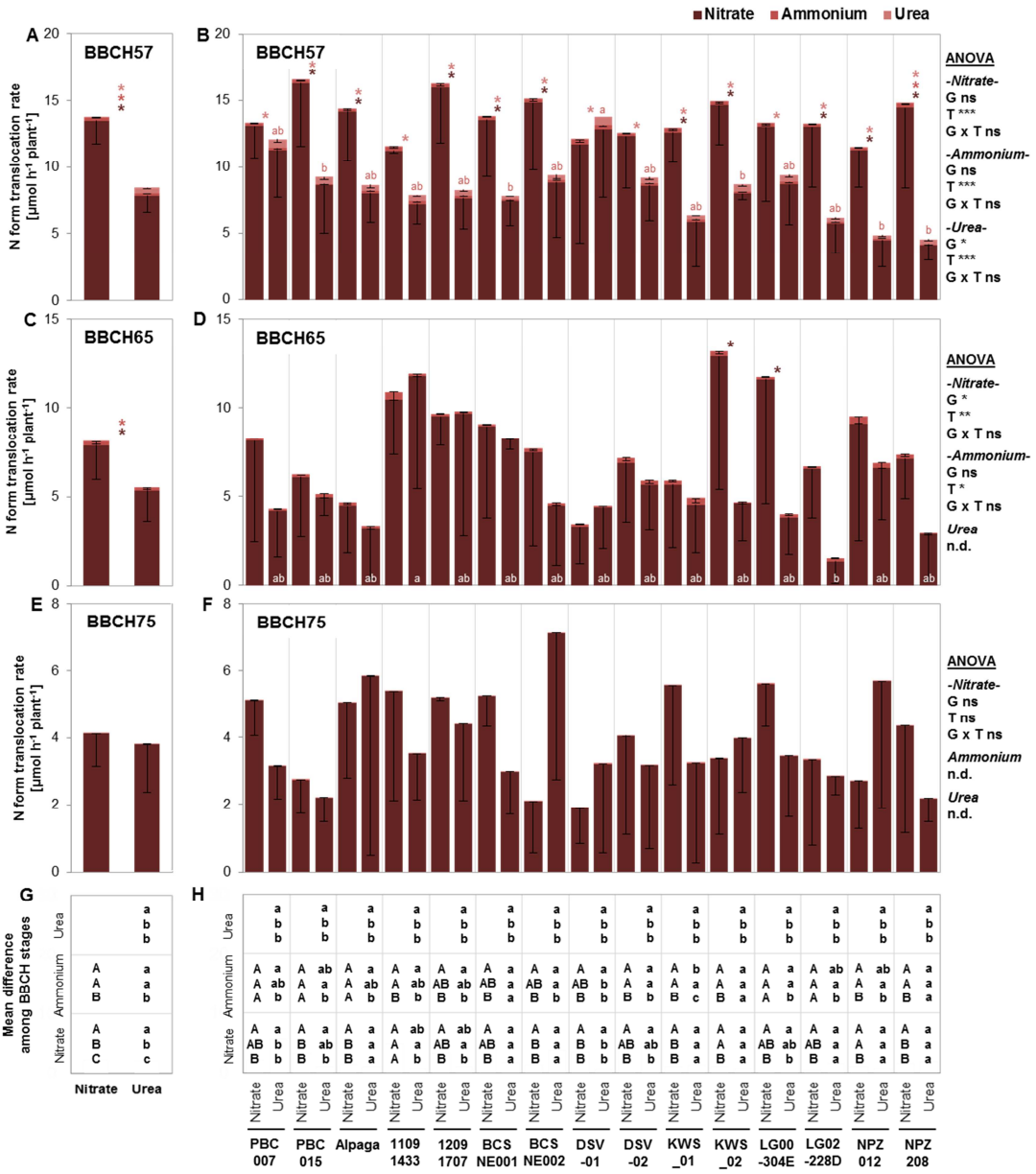


Figure 3-3: Translocation rates of nitrate, ammonium and urea in the xylem sap of the elite line collection in the +N field trial 2012/2013 at BBCH57, BBCH65 and BBCH75 as mean over all genotypes (A, C, E) or of single genotypes (B, D, F) and as comparison between the developmental stages over all genotypes (G) or single genotypes (H). All rapeseed genotypes were fertilized in spring with 40 kg N h^{-1} at BBCH30 and 60 kg N h^{-1} at BBCH55 as nitrate or urea. Xylem sap was pooled from ten plants per plot. (A-F) Bars show means -SD (A, C, E: n=60; B, D, F: n=4). (A, C, E) Significant mean or median differences are indicated by an asterisk referring to the N form color code according to unpaired t-test (A: ■) or Mann-Whitney rank sum test (A: ■■, C: ■■■, E: ■■■), respectively, at p<0.05. (B, D, F) ANOVA or ANOVA on ranks (- -) results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant, n.d.=non-determinable. Significant mean difference among nitrate and urea treatment within a genotype is indicated by asterisks referring to N form color code according to unpaired t-test at p<0.05. Different upper case letters within a bar indicate significant mean or median differences among the genotypes within nitrate treatment according to Tukey´s test (B: ■■, D: ■, F: ■) or Tukey´s test on ranks (B: ■, D: ■■, F: ■■), respectively, at p<0.05; different lower case letters indicate significant mean or median differences among the genotypes within urea treatment according to Tukey´s test (B: ■■■) or Tukey´s test on ranks (D: ■■■, F: ■■■), respectively, at p<0.05. (G, H) Table shows mean or median differences for xylem nitrate, ammonium and urea translocation among BBCH stages indicated by different letters in vertical rows of three comparing BBCH57, BBCH65 and BBCH75 (top to bottom). Different upper case letters indicate significant mean (H) or median (G) differences within nitrate treatment, while lower case letters refer to mean (H) or median (G) differences within urea treatment according to Tukey´s test (H) or Tukey´s test on ranks (G) at p<0.05; (G) n=60; (H) n=4.

As rapeseed plants were fertilized with different N fertilizer forms, it was of interest to track N forms in the xylem and to determine to what extent these N forms are also transported to shoots. Among these N forms, nitrate was by far the most abundant in every genotype and at any developmental stage regardless of the fertilized N form. Mean translocation rates of the population indicated that the translocation pattern of nitrate and ammonium changed significantly over time (Figure 3-3A, C, E, G): Nitrate translocation was highest during flower development and decreased until pod formation, while ammonium translocation remained stable until flowering but decreased afterwards.

Against this, urea transport was rather independent of the developmental stage, but influenced by the fertilizer N treatment as translocation of urea in the xylem sap was only found shortly after urea fertilizer application (BBCH57 - two days after fertilization). The fertilizer N treatment also significantly influenced mean nitrate and ammonium translocation at BBCH57 and BBCH65, when nitrate treatment increased nitrate and ammonium translocation rates compared to urea treatment. Mean translocation rates of ammonium were influenced by nitrate treatment in the same way as those of nitrate. This was most probably due to the application of ammonium nitrate fertilizer for establishing the nitrate treatment.

As expressed by the coefficient of variation, translocation rates of N forms varied among individual genotypes (Figure 3-3B, D, F): At BBCH57, coefficients for nitrate and ammonium translocation rates were 0.30 and 0.44 under nitrate but 0.42 and 0.46 under urea, respectively. At BBCH65, variation in nitrate transport was about 30 % higher, while variability in ammonium transport increased by 40 - 50 %. At BBCH75, variation in nitrate transport decreased only slightly (to 0.55 under nitrate and to 0.70 under urea). Thus, at all developmental stages nitrate translocation was more variable after urea than after nitrate fertilization. Ammonium translocation variations at BBCH75 could not be determined since genotypes did not translocate ammonium. For the same reason variation in urea translocation was only detectable at BBCH57.

An influence of the fertilizer N form on translocation rates of nitrate, ammonium and urea in the xylem sap of the individual genotypes was most prominent at BBCH57. The elevated mean xylem urea transport observed for the whole population was a result of significantly higher urea translocation rates in every of the 15 genotypes shortly after urea fertilization (Figure 3-3B). Interestingly, when longer time passed between fertilizer application and xylem sap harvest, as it was the case at BBCH65 and BBCH75, no urea transport in the xylem sap was found in any genotype, regardless of the fertilized N form. On the other hand, nitrate fertilization led to increased nitrate translocation in 14 genotypes, in 10 of them significantly, as well as to elevated ammonium transport in 13 genotypes, whereas only 1 of them showed significantly higher ammonium transport at BBCH57. This trend held on at BBCH65, while at BBCH75, there was no significant influence of the N fertilizer form on nitrate translocation in any genotype. However, approximately 2/3 of the lines tended to transport more nitrate after nitrate fertilization (Figure 3-3D, F).

Interestingly, at all developmental stages the sum of mean nitrate, ammonium, urea (Figure 3-3) and AA translocation (Figure 3-2) in the xylem sap of the elite line collection in 2012/2013 could only explain about 55 - 60 % of the total N transport (Figure 3-1) indicating a considerable contribution of other N forms such as peptides and proteins (Buhtz *et al.*, 2004; Kehr *et al.*, 2005) or amides (Fischer *et al.*, 1998).

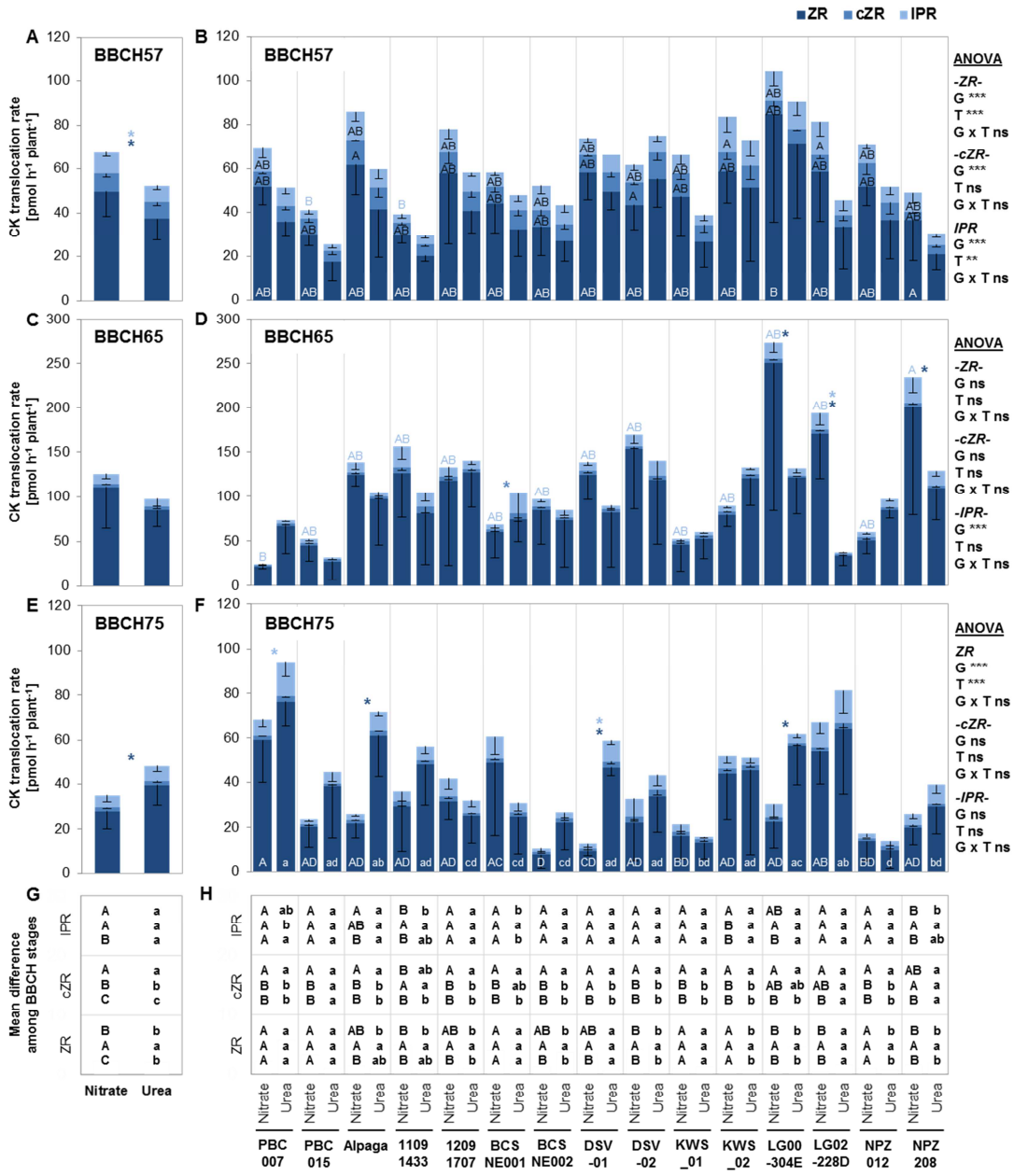


Figure 3-4: CK translocation in the xylem sap of the elite line collection in the +N field trial 2012/2013 at BBCH57, BBCH65 and BBCH75 as mean over all genotypes (A, C, E) or of single genotypes (B, D, F) and as comparison between the developmental stages over all genotypes (G) or single genotypes (H). All rapeseed genotypes were fertilized in spring with 40 kg N h^{-1} at BBCH30 and 60 kg N h^{-1} at BBCH55 as nitrate or urea. Xylem sap was pooled from ten plants per plot. (A-F) Bars show means -SD (A, C, E: n=60; B, D, F: n=4). (A, C, E) Significant median differences are indicated by asterisks referring to the CK color code according to Mann-Whitney rank sum test at p<0.05. (B, D, F) ANOVA or ANOVA on ranks (- -) results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. Significant mean difference among nitrate and urea treatment within a genotype is indicated by asterisks referring to CK color code according to unpaired t-test at p<0.05. Different upper case letters within a bar indicate significant mean or median differences among the genotypes within nitrate treatment according to Tukey´s test (B: ■, F: ■) or Tukey´s test on ranks (B: ■■, D: ■■■, F: ■■), respectively, at p<0.05; different lower case letters indicate significant mean or median differences among the genotypes within urea treatment according to Tukey´s test (B: ■■, D: ■, F: ■) or Tukey´s test on ranks (B: ■, D: ■■, F: ■■), respectively, at p<0.05. (G, H) Table shows mean or median differences for xylem ZR, cZR and IPR translocation among BBCH stages indicated by different letters in vertical rows of three comparing BBCH57, BBCH65 and BBCH75 (top to bottom). Different upper case letters indicate significant mean (H) or median (G) differences within nitrate treatment, while lower case letters refer to mean (H) or median (G) differences within urea treatment according to Tukey´s test (H) or Tukey´s test on ranks (G) at p<0.05; (G) n=60; (H) n=4.

In all samples, ZR, cZR and IPR were detected as the most abundant xylem-transported forms of CKs (Figure 3-4). ZR was the most abundant CK form in every genotype under either N fertilizer treatment and at every sampling time point: ZR accounted for 70 % of mean total CK translocation under nitrate and 75 % under urea at BBCH57, for 90 % (nitrate) and 85 % (urea) at BBCH65 and for about 80 % under both N treatments at BBCH75 (Figure 3-4A, C, E). Translocation rates of cZR and IPR were similar at BBCH57 but IPR translocation was higher than cZR at BBCH65 and BBCH75. This is not in agreement with greenhouse studies on xylem sap analyses in hydroponically and pot-grown oilseed rape as Koeslin-Findeklee *et al.* (2015) reported iP and Singh and Sawhney (1992) found dihydrozeatin as major CK forms. Both authors detected additional non-ribosylated CKs and *O*-glycosylated CK-forms in contrast to this work.

The population mean of ZR and cZR translocation rates indicated a time-dependent pattern under both fertilizer N treatments: From BBCH57 until BBCH65 translocation rates of ZR increased more than twofold, while cZR rates decreased by the same amount under either N treatment, while from BBCH65 until BBCH75 translocation rates of both CK forms decreased regardless of the N form. Thus, mean translocation rates of ZR were highest during flowering and during pod development, but cZR translocation decreased from flower development onwards. In contrast, the translocated amount of IPR remained relatively stable over the three sampling time points (Figure 3-4G).

As hypothesized initially, nitrate fertilization led to a significant increase in mean ZR and IPR translocation but only during flower development when xylem sap was harvested only two days after fertilizer application. Then each CK form was promoted by 20 %. A trend for higher ZR translocation under nitrate was also visible during flowering, while during pod development urea stimulated both ZR and cZR translocation by 25 %. Thus, the fertilizer N form oppositionally affected xylem CK translocation in dependence on the plant developmental stage, which raised the question whether urea itself or whether a longer lasting effect of N availability after urea fertilization caused this increase over the nitrate treatment.

CK translocation rates in the xylem sap of the individual genotypes varied strongly as expressed by the coefficients of variation (Figure 3-4B, D, F). Overall variability in CK translocation was lowest at the end of flower development (ZR, cZR and IPR: 0.43, 0.37 and 0.53 under nitrate and 0.53, 0.45 and 0.51 under urea, respectively) and similar at the other two developmental stages (ZR, cZR and IPR at BBCH65: 0.75, 0.53 and 0.95 under nitrate and 0.54, 0.76 and 1.10 under urea, respectively; ZR, cZR and IPR at BBCH75: 0.73, 0.52 and 0.94 under nitrate and 0.63, 0.57 and 0.83 under urea, respectively). However, IPR translocation rates varied most within the population, especially at BBCH65 and BBCH75.

Nitrate treatment did not promote CK translocation in any genotype at BBCH57, although this was observed for the population mean translocation of ZR and IPR (Figure 3-4A). Nevertheless, 14 or 10 out of the 15 genotypes tended to increase ZR or IPR translocation, respectively. At BBCH65, 3 genotypes showed a significantly and 6 further genotypes in tendency higher ZR translocation after nitrate; also IPR transport was significantly increased in 1 and in tendency increased in 7 genotypes under nitrate. However, this did not result in significantly higher mean translocation rates of ZR or IPR when overlooking the whole population (Figure 3-4C). These proportions changed at BBCH75, when 11 out of 15 lines (3 significant) followed the significantly higher mean ZR translocation and 8 genotypes (2 significant) had higher IPR translocation under urea. At all developmental stages cZR translocation remained rather unaffected by the fertilizer N form.

Since delayed root senescence (measured by higher xylem CK transport in this study) during later plant development could be beneficial for N uptake during generative plant development, the nitrate variants of LG00-304E, LG02-228D and NPZ208 at BBCH65 as well as

both variants of PBC007, LG02-228D and the urea variant of Alpaga at BBCH75 may be promising candidates for an extended lifespan possibly leading to higher seed yield (Figure 3-4). This hypothesis was tested relating CK translocation rates to final seed yield in 3.1.3.

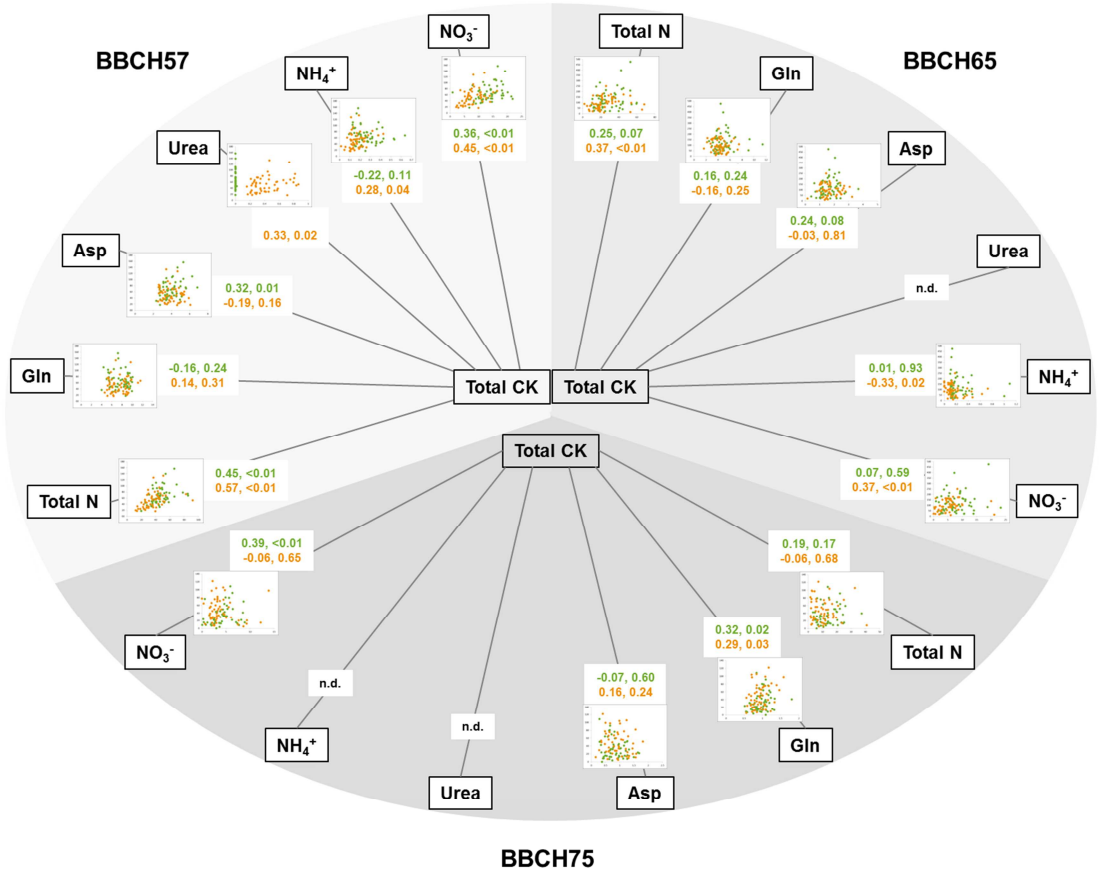


Figure 3-5: Correlations between total CK translocation and different N fractions in the xylem sap of the elite line collection in the +N field trial 2012/2013 at BBCH57, BBCH65 and BBCH75. Components were correlated by Spearman rank order correlation. Values for r and p were calculated separately for nitrate (■) and urea (■) treatment; n=60; n.d.=non-determinable.

To evaluate the relation between N translocation rates, which reflect N uptake (Peuke, 2010), and CK translocation rates, which were meant to serve as root activity markers, these measures were correlated at each of the different developmental stages, and correlation coefficients were calculated separately for nitrate- and urea-fertilized plants (Figure 3-5). Especially during flower development, CK translocation rates were positively associated with the translocation of total N or of nitrate, regardless of the fertilizer treatment. During flowering this correlation became weaker and was rather limited to urea fertilized plants. This may support the notion that urea fertilization provided a longer lasting N provision to roots than nitrate, which is prone to leaching (Arkoun *et al.*, 2012). At pod development, there was only a positive correlation between CK and nitrate translocation rates in nitrate-fertilized plants. Although it is still unclear why this latter correlation was limited to nitrate-fertilized plants, this analysis indicated that CK translocation rates reflect total N translocation rates even closer than nitrate translocation rates, at least during flower development and flowering.

3.1.2 Influence of the applied nitrogen form on shoot nitrogen allocation

As a long-term consequence of N translocation in the xylem, N allocation to the shoot was determined in order to evaluate whether different N forms influenced total shoot N accumulation and N partitioning to different above-ground plant organs. For this purpose, individual above-ground plant organs of the elite lines were harvested separately at the end of pod development for the determination of organ DWs and organ N concentrations.

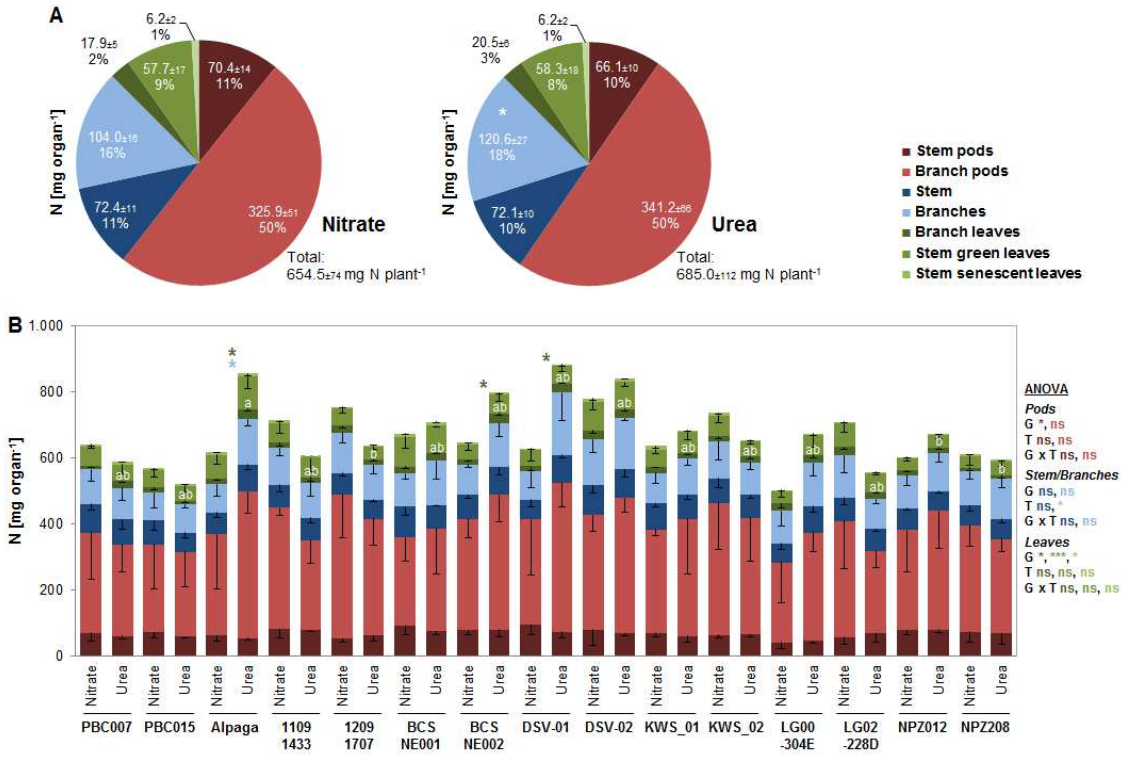


Figure 3-6: Total N pools in the above-ground plant biomass at BBCH79 as mean over all genotypes (A) or in single genotypes (B) in the elite line collection in the +N field trial 2012/2013. All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Two plants per plot were separated into the indicated above-ground plant fractions to determine their N content. (A) Pie charts show N content (means ±SD) of the indicated organs and the corresponding percentage to total plant N (n=60). Asterisks in the right-hand chart indicate significant mean or median differences among nitrate and urea treatment according to unpaired t-test (■■) or Mann-Whitney rank sum test (■■■■■) at p<0.05. (B) Bars show means -SD (n=4). ANOVA or ANOVA on ranks (- -) results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. Significant mean difference among nitrate and urea treatment within a genotype is indicated by asterisks referring to the organ color code according to unpaired t-test at p<0.05. Different upper case letters within a bar indicate significant mean or median differences among the genotypes within nitrate treatment according to Tukey´s test (■■■■■) or Tukey´s test on ranks (■■), respectively, at p<0.05; different lower case letters indicate significant mean or median differences among the genotypes within urea treatment according to Tukey´s test (■■■■) or Tukey´s test on ranks (■■■), respectively, at p<0.05.

The population mean of total shoot N accumulation at the end of pod development was not significantly influenced by the fertilizer N treatment, but tended to be slightly higher after urea fertilization (Figure 3-6A). Also the relative contribution of N pools in the different organ fractions to total above-ground N was similar between the fertilizer N treatments: About 60 % of the total N was allocated to the generative organs, while the rest was remaining in the vegetative plant parts, i.e. about 30 % in stem fractions (stem and branches) and 10 % in leaf fractions. Similar N distribution within the shoot at this stage was also found by Malagoli *et al.* (2005).

Between fertilizer treatments there were only minor differences as urea led to a significantly higher N accumulation in branches and a slightly higher N content in branch leaves, while nitrate-fertilized plants tended to allocate more N to the stem and its pods and green leaves.

Even if significant differences in N allocation existed only in the green leaf fraction of the stem after urea fertilization, trends of variability within the collection were visible (Figure 3-6B): Coefficients of variation of N contents in different organs of nitrate-fertilized plants were lowest for “stem pods” and “branch pods” (0.27 and 0.33), slightly higher for the stem fractions “stem” and “branches” (0.33 and 0.32) and highest for green and senescent leaves (0.46 and 0.62) of the stem. Urea fertilization altered these coefficients of variation in N content only slightly. In general, this genotypic variation in N allocation to different above-ground plant organs was rather based on variation in DWs of the different organs than in their N concentrations, which remained relatively stable among the genotypes (see Annex-3).

The higher N allocation to branches (significant) and branch leaves (trend) described for the whole collection under urea fertilization compared to nitrate (Figure 3-6B) resulted from a higher N allocation to these organs in 7 out of 15 genotypes, partially significant or in trend. N distribution to other plant organs was not significantly influenced by the N treatment in any of the considered genotypes.

To address the question if nitrate fertilization retards plant senescence in oilseed rape, the ratio of N content in generative to N content in vegetative organs was considered: The population mean $N_{generative}/N_{vegetative}$ was slightly higher under nitrate fertilization compared to urea indicating that rather nitrate-fed plants were directed earlier towards senescence and remobilized N thus earlier from vegetative to generative organs. However, only 1/3 of the genotypes were in accordance with this mean, while about half of the rapeseed cultivars did not show any difference in their $N_{generative}/N_{vegetative}$ between the fertilizer treatments (Annex-7).

3.1.3 Influence of the applied nitrogen form on yield formation

According to Diepenbrock (2000) the seed yield per area is a product of the primary yield components stand density, number of pods per plant, number of seeds per pod and the individual seed weight. For a deeper elucidation of the contribution of individual architectural shoot traits to seed yield formation the number of pods per plant was separately analyzed by considering branches per plant and pods per branch.

To illustrate the genetic variability in the individual yield parameters and their dependence on the fertilized N form, individual yield components were separately assessed in all elite lines.

Figure 3-7: Yield parameters of the elite line collection in the +N field trial 2012/2013 as means over all genotypes (A, C, E, G, I) or of the single genotypes (B, D, F, H, J). All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Yield parameters were determined as described in 2.6. Boxes show median, first and third quartile; whiskers represent minimum and maximum of all data in a group (A, C, E, G, I: n=60; B, D, F, H, J: n=4). (A, C, E, G, I) There were no significant differences among the means or medians according to unpaired t-test (C, I) or Mann-Whitney rank sum test (A, E, G), respectively, at $p<0.05$. (B, D, F, H, J) ANOVA results *, **, *** indicate significant differences or interactions at $p<0.05$, $p<0.01$, $p<0.001$, respectively; G=genotype, T=N treatment, ns=non-significant. There were no significant mean difference among nitrate and urea treatment within a genotype according to unpaired t-test at $p<0.05$. Different upper case letters indicate significant mean differences among the genotypes within nitrate treatment according to Tukey´s test at $p<0.05$; different lower case letters indicate significant mean or median differences among the genotypes within urea treatment according to Tukey´s test (B, D, H) or Tukey´s test on ranks (F, J) at $p<0.05$. TSW=Thousand seed weight.

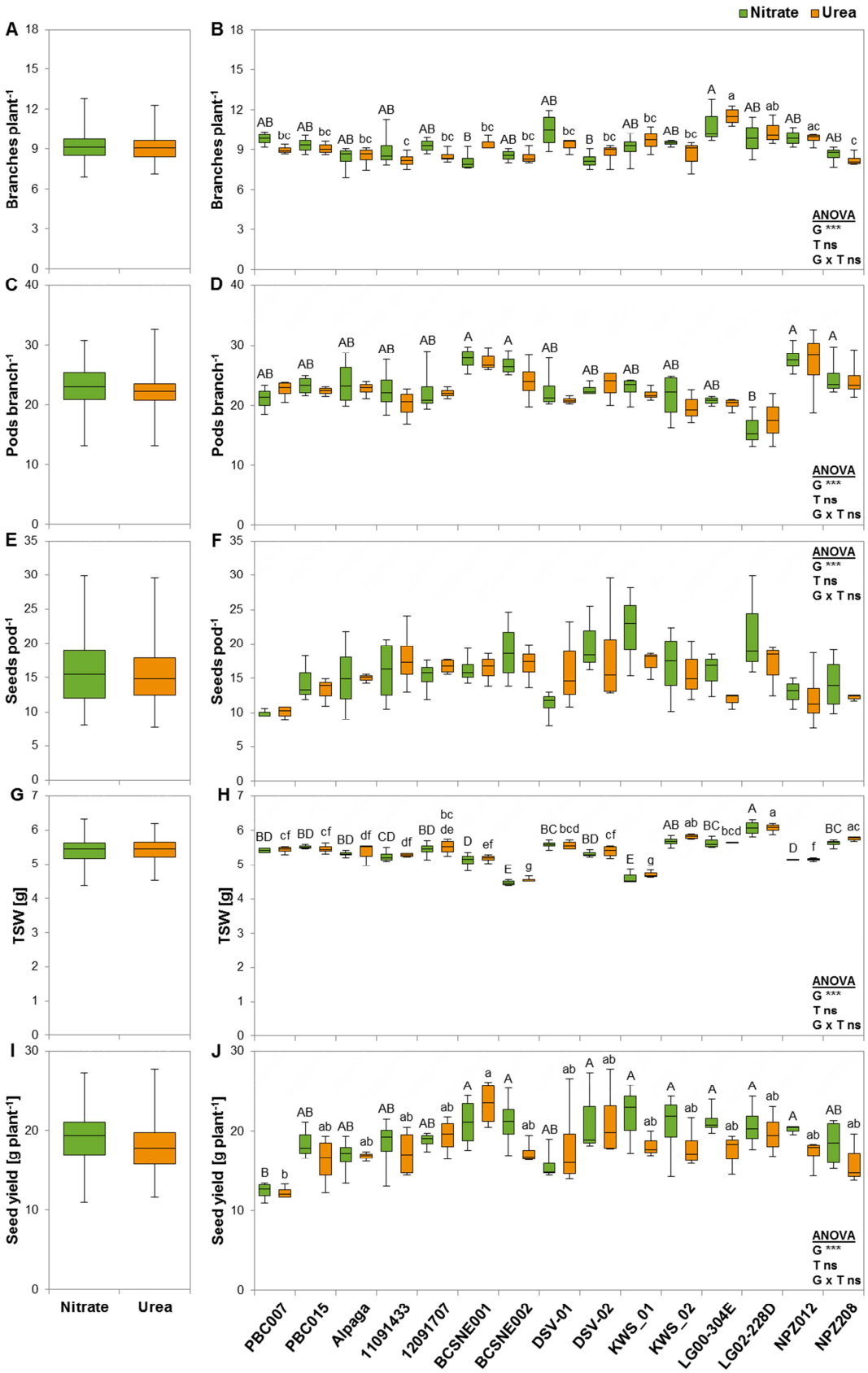


Figure 3-7 (For description see previous page.)

Table 3-1: Stand density and area seed yield of the elite line collection in the +N field trial 2012/2013. All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Yield parameters were determined as described in 2.6. Table shows means ±SD (n=4 for single genotypes, n=60 for all genotypes). ANOVA (Plants m^{-2}) or ANOVA on ranks (Seed yield) results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. Asterisks indicate significant mean difference among nitrate and urea treatment within a single genotype according to unpaired t-test at p<0.05 or among all genotypes according to unpaired t-test (Plants m^{-2}) or Mann-Whitney rank sum test (Seed yield) at p<0.05. Different upper case letters indicate significant mean differences among the genotypes within nitrate treatment according to Tukey´s test at p<0.05; different lower case letters indicate significant mean or median differences among the genotypes within urea treatment according to Tukey´s test (Plants m^{-2}) or Tukey´s test on ranks (Seed yield), respectively, at p<0.05.

Genotype	Plants m^{-2}			Seed yield [g m^{-2}]			
	Nitrate		Urea	Nitrate		Urea	
PBC007	20.0 ±3.0		18.8 ±0.9	246.6 ±19.9	E	229.6 ±7.0	d
PBC015	19.5 ±2.4		18.6 ±1.8	342.3 ±7.8	AD	298.3 ±33.0	bc
Alpaga	21.9 ±3.5		19.2 ±2.6	361.8 ±6.7	A	337.4 ±52.2	ac
11091433	19.1 ±3.3		18.8 ±3.3	341.3 ±20.0	AD	316.3 ±15.9	ac
12091707	18.5 ±0.9		16.5 ±1.5	347.8 ±6.8	AC	317.6 ±25.2	ac
BCSNE001	15.3 ±1.4		14.0 ±2.5	318.3 ±21.9	CD	322.3 ±21.4	ac
BCSNE002	16.5 ±2.0		19.6 ±2.4	344.0 ±18.3	AC	337.6 ±19.9	ac
DSV-01	19.7 ±2.4		16.9 ±3.9	307.5 ±15.8	D	289.5 ±15.6	c
DSV-02	17.0 ±2.7		17.0 ±3.6	355.3 ±7.1	AC	349.1 ±11.0	ab
KWS_01	16.1 ±3.0		17.6 ±1.3	358.0 ±17.7	AB	329.3 ±14.4	ac
KWS_02	17.8 ±4.3		19.0 ±2.1	351.9 ±6.1	AC	337.4 ±16.9	ac
LG00-304E	16.6 ±1.6		18.6 ±2.8	351.5 ±7.5	AC	341.1 ±13.3	ac
LG02-228D	17.5 ±2.9		18.3 ±2.7	355.5 ±12.9	AB	354.7 ±19.3	a
NPZ012	16.1 ±0.4	*	19.6 ±1.8	328.8 ±11.6	AD	335.0 ±13.0	ac
NPZ208	18.0 ±3.3		20.7 ±2.9	324.3 ±8.7	BCD	335.6 ±16.2	ac
All genotypes	18.0 ±2.9		18.2 ±2.7	334.9 ±31.6	*	321.1 ±36.4	
ANOVA	G *, T ns, GxT ns			G ***, T ***, GxT ns			

In general, the fertilizer N form did not affect population means of the individual yield components in the elite lines in 2012/2013 (Figure 3-7A, C, E, G, I). There was only a trend that urea-treated plants formed about 0.5 pods $branch^{-1}$ more and 1 seed pod^{-1} less than nitrate-fertilized plants, which resulted in a final plant seed yield reduction of about 1.5 g $plant^{-1}$. Since the stand density of about 18 plants m^{-2} was comparable in the plots receiving nitrate and urea fertilizer application, the mean final area seed yield (as product of stand density and plant seed yield) was increased by nearly 14 g after nitrate supply (Table 3-1). Thus, a trend for higher pod and seed production under nitrate resulted in a significantly larger seed yield production per area.

Among the lines, the phenotypic coefficients of variation in the formation of the individual yield parameters were highly similar between plants fertilized with either nitrate or urea (Figure 3-7B, D, F, H, J). The variability in seed number produced per pod was highest (0.31 under nitrate, 0.27 under urea), followed by pods per branch (0.16 under nitrate, 0.15 under urea) and branches per plant (0.12 under both N treatments), while the variation in TSW was lowest (0.08 under nitrate, 0.07 under urea). The seed yield per plant (0.19 under nitrate, 0.20 under urea) was twice as variable as the seed yield per square meter (0.09 under nitrate, 0.11 under urea; Table 3-1).

In accordance with the finding that the N fertilizer form had no significant influence on the mean population yield parameters (Figure 3-7A, C, E, G), no single genotype responded significantly in yield parameter formation to fertilizer application (Figure 3-7B, D, F, H). The trend of lower mean plant seed yield (Figure 3-7I) and significantly lower area seed yield (Table 3-1) after urea supply was supported by 11 (Figure 3-7J) or 13 (Table 3-1) of the

15 genotypes, respectively, but in none of them significantly. Thus, it was not possible to influence the formation of yield parameters in any line by applying two contrasting N forms.

However, among the different elite lines diverse patterns in yield formation were observed. More than half of the genotypes (PBC015, Alpaga, 11091433, 12091707, DSV-01, DSV-02, KWS_02 and NPZ208) could be grouped together as they exhibited an average formation of all single yield parameters as well as of final seed yield per plant. NPZ012 showed a similar seed yield as the mentioned genotypes with forming more pods per branch but less seeds per pod and lower TSW. Also BCSNE001 exhibited the yield formation behavior of NPZ012 but with slightly more seeds per pod (in the range of population average) and thus had higher final seed yield. BCSNE002 and KWS_01 showed the lowest TSWs among the population but produced average (under urea) or even above-average (under nitrate) seed yields as result of elevated pod (BCSNE002) and seed (KWS_01) production. LG00-304E formed more branches, while the other yield components were at average under nitrate but seed production was reduced under urea resulting in the trend to form above-average seed yield under nitrate and average yield under urea. Slightly higher branching than the population mean was also exhibited by LG02-228D associated with a decreased pod formation and highest seed weight among the genotypes resulting in average seed yield production. The lowest seed yield in the population was produced by PBC007 as result of low seed formation per pod since the other yield parameters were formed in accordance with the population mean values. Thus, some genotypes had a unique behavior in yield formation, while more than half of the genotypes showed a similar pattern of seed yield formation assuming that there was a relatively high genetic similarity among most of the lines determining a distinct yield formation pattern.

The initial hypothesis that higher root activity during generative plant development might be beneficial for seed yield was verified by correlating seed yield formation among the elite lines with xylem N and CK translocation rates at BBCH65 and BBCH75 (Figure 3-1, Figure 3-4). Against the expectation, no correlations were found between seed yield and CK or N translocation rates at either developmental stage (Annex-21). Thus, higher root activity during flowering appears to have no influence on seed yield formation.

In order to clarify which yield components contribute most to final seed yield in the rapeseed elite lines and how individual yield components relate to each other, a correlation matrix was set up.

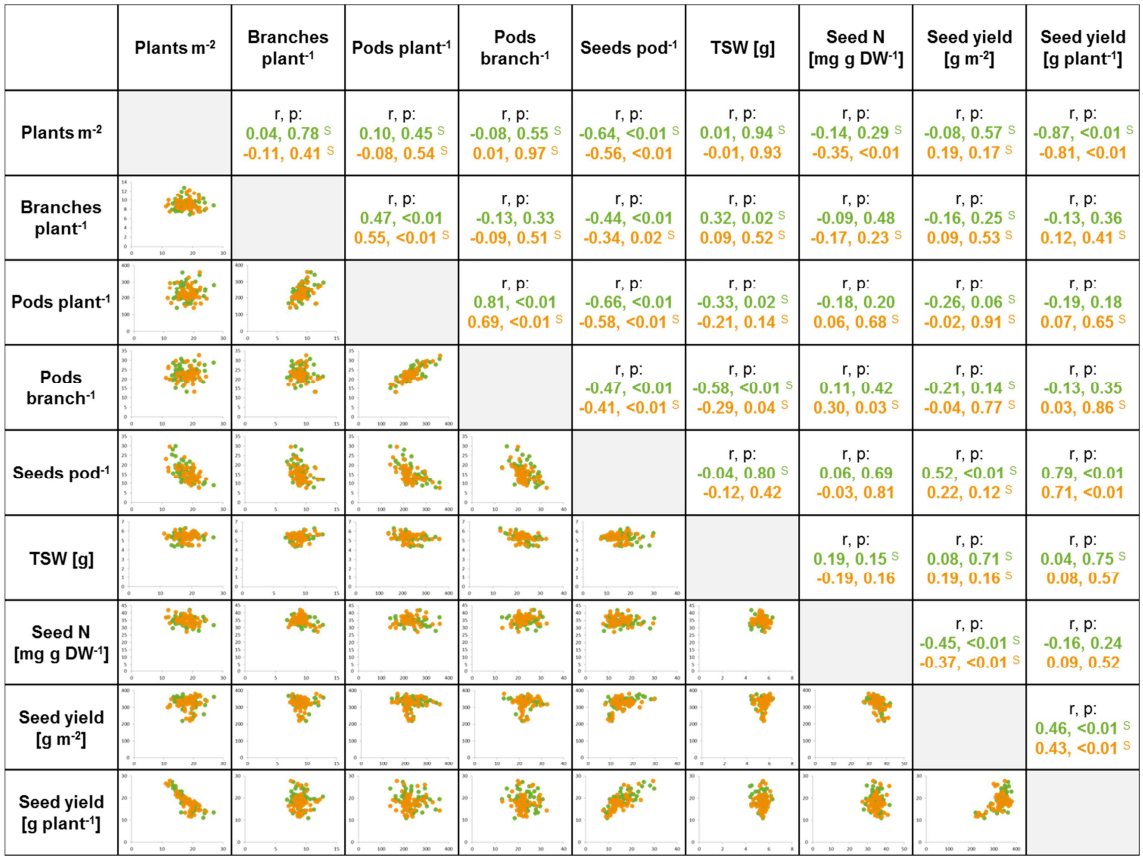


Figure 3-8: Correlations between the yield parameters of the genotypes in the elite line collection in the +N field trial 2012/2013. All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Correlation charts as well as r and p value were calculated separately for nitrate (■) and urea (■) treatment; n=60. ^{S} indicates calculation of r and p value according to Spearman rank order correlation, while unmarked values accord to Pearson product moment correlation. Chart axes correspond to the yield parameter indicated on top or at the left-hand side of the matrix. TSW=Thousand seed weight.

Under nitrate as well as under urea supply, a higher formation of branches per plant was significantly associated with an increased number of pods per plant, and this latter trait was tightly correlated with the number of pods built per branch (Figure 3-8). However, plants with higher shoot branching and increased pod formation produced less seeds per pod. Also the TSW was slightly lower with increasing pod number especially under nitrate supply.

Among the yield parameters, only seed number per pod positively correlated with seed yield per area or per plant, and this observation related mainly to nitrate conditions. Plants producing a higher seed number had in turn lower N concentrations in the seeds indicating sink competition among N.

Overall seed yield per plant was significantly increased when stand density was lower, which resulted mainly from higher seed production per pod than from altered plant architectural traits, i.e. branch and pod formation, at lower stand density.

As most of the correlations between the different yield parameters were highly similar after nitrate or urea supply, the seed yield of the elite lines in 2012/2013 appeared to be rather independent of the fertilizer N treatment.

3.2 Influence of fertilizer nitrogen form on plant nitrogen acquisition and yield formation in a set of oilseed rape elite lines in 2013/2014

To verify robustness of results for plant nitrogen acquisition and yield formation in oilseed rape elite lines under the agriculturally relevant experimental conditions, the same experiment as described in 3.1 was repeated in 2013/2014.

3.2.1 Influence of the applied nitrogen form on xylem nitrogen and cytokinin translocation

As in the previous year, xylem sap was harvested during flower development (BBCH57), during flowering (BBCH65) and during pod filling (BBCH75), but these dates corresponded in 2013/2014 to 11 days, 40 days and 48 days after fertilization with 60 kg N ha^{-1} in the form of nitrate or urea, respectively.

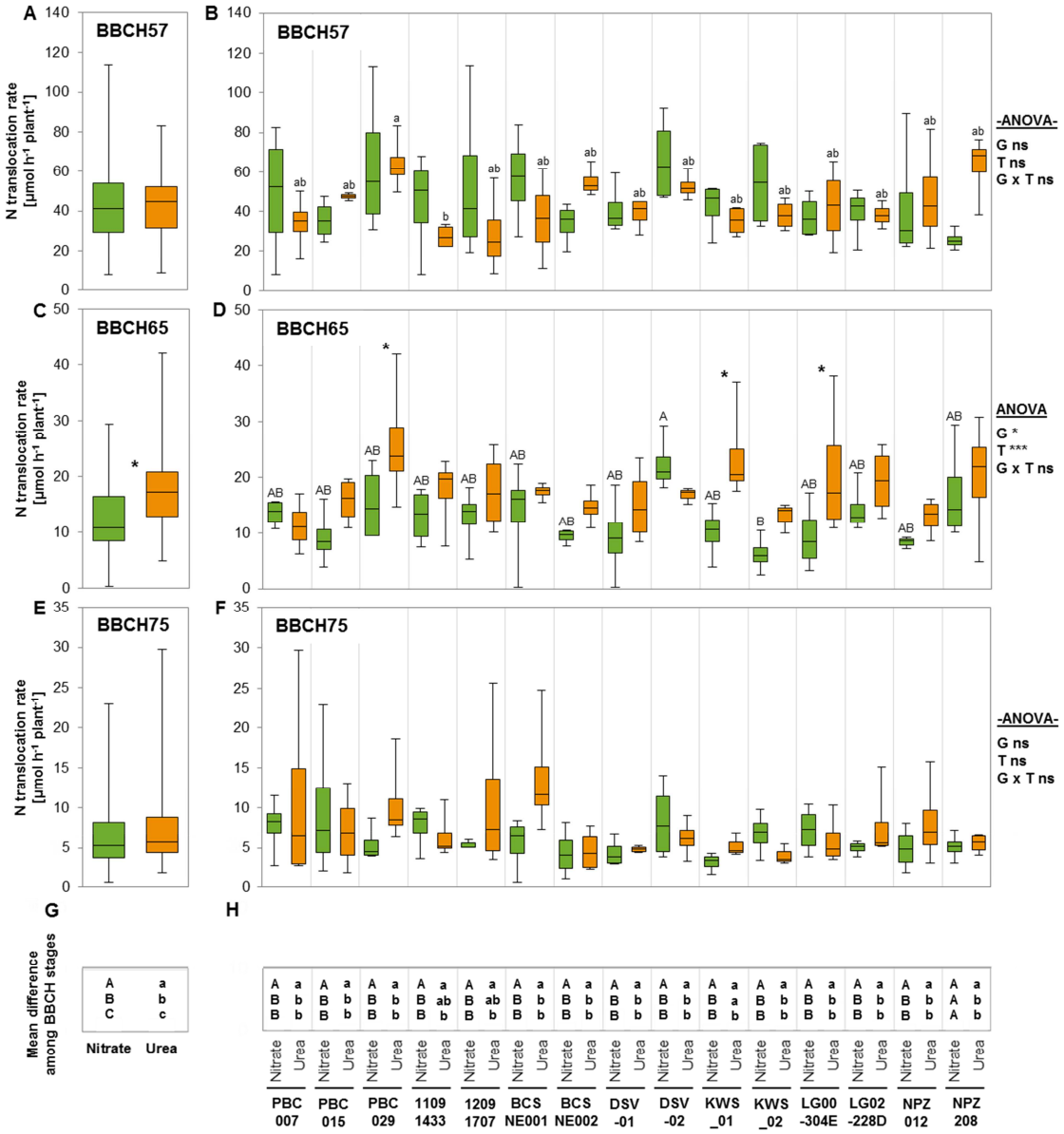


Figure 3-9: Total N translocation in the xylem sap of the elite line collection in the +N field trial 2013/2014 at BBCH57, BBCH65 and BBCH75 as mean over all genotypes (A, C, E) or of single genotypes (B, D, F) and as comparison between the developmental stages over all genotypes (G) or single genotypes (H). All rapeseed genotypes were fertilized in spring with 40 kg N ha^{-1} at BBCH30 and 60 kg N ha^{-1} at BBCH55 as nitrate or urea. Xylem sap was pooled from ten plants per plot. (A-F) Boxes show median, first and third quartile; whiskers represent minimum and maximum of all data in a group (A, C, E: n=60; B, D, F: n=4). (A, C, E) Significant median difference is indicated by an asterisk according to Mann-Whitney rank sum test at $p<0.05$. (B, D, F) ANOVA or ANOVA on ranks (- -) results *, **, *** indicate significant differences or interactions at $p<0.05$, $p<0.01$, $p<0.001$, respectively; G=genotype, T=N treatment, ns=non-significant. Significant mean difference among nitrate and urea treatment within a genotype is indicated by asterisks according to unpaired t-test at $p<0.05$. Different upper case letters within a bar indicate significant mean or median differences among the genotypes within nitrate treatment according to Tukey´s test (B, D) or Tukey´s test on ranks (F) respectively, at $p<0.05$; different lower case letters indicate significant mean or median differences among the genotypes within urea treatment according to Tukey´s test (B, D) or Tukey´s test on ranks (F), respectively, at $p<0.05$. (G, H) Table shows mean or median differences for xylem N translocation among BBCH stages indicated by different letters in vertical rows of three comparing BBCH57, BBCH65 and BBCH75 (top to bottom). Different upper case letters indicate significant mean (H) or median (G) differences within nitrate treatment, while lower case letters refer to mean (H) or median (G) differences within urea treatment according to Tukey´s test (H) or Tukey´s test on ranks (G) at $p<0.05$; (G) n=60; (H) n=4.

Under the two fertilizer N treatments nitrate and urea, the population mean of total N translocation rates in the xylem were highest during flower development and decreased steadily until pod development by 65 - 75 % from BBCH57 to BBCH65 and by 45 - 55 % from BBCH65 to BBCH75 (Figure 3-9A, C, E, G). This was in accordance with the results of the previous experiment, although overall N translocation rates were lower in 2013/2014 assuming seasonal effects.

Against this, the N form influence on xylem N translocation was opposed: In 2012/2013, nitrate promoted N translocation during flower development and during flowering (Figure 3-1) but in 2013/2014 N translocation from root to shoot was only affected during flowering and there urea enhanced N translocation from root to shoot by about 30 % indicating longer availability of N from urea fertilizer in the soil.

Variability in total N transport among the genotypes within the population, as expressed by the coefficient of variation, were 0.51, 0.51 and 0.59 under nitrate and 0.38, 0.42 and 0.85 under urea at BBCH57, BBCH65 and BBCH75, respectively (Figure 3-9B, D, F). Thus, variation in xylem N transport was relatively stable over the considered developmental stages under nitrate but increased from the first to the last stage under urea, as found already in the 2012/2013 experiment. The N form effect on mean total N translocation at BBCH65 was reflected by 13 out of 15 genotypes, in 3 of them even significantly.

Interesting candidates for potentially increased yield formation due to higher root-to-shoot translocation of N at later developmental stages are the urea variants of PBC029, KWS_01, LG00-228D and the nitrate variant of DSV-02 at BBCH65 and at BBCH75 the urea variants of PBC007, PBC029 and BCSNE001 and the nitrate variants of PBC015 and DSV-02. Therefore, xylem total N translocation rates within the population were correlated with final seed yield production which is shown in 3.2.3.

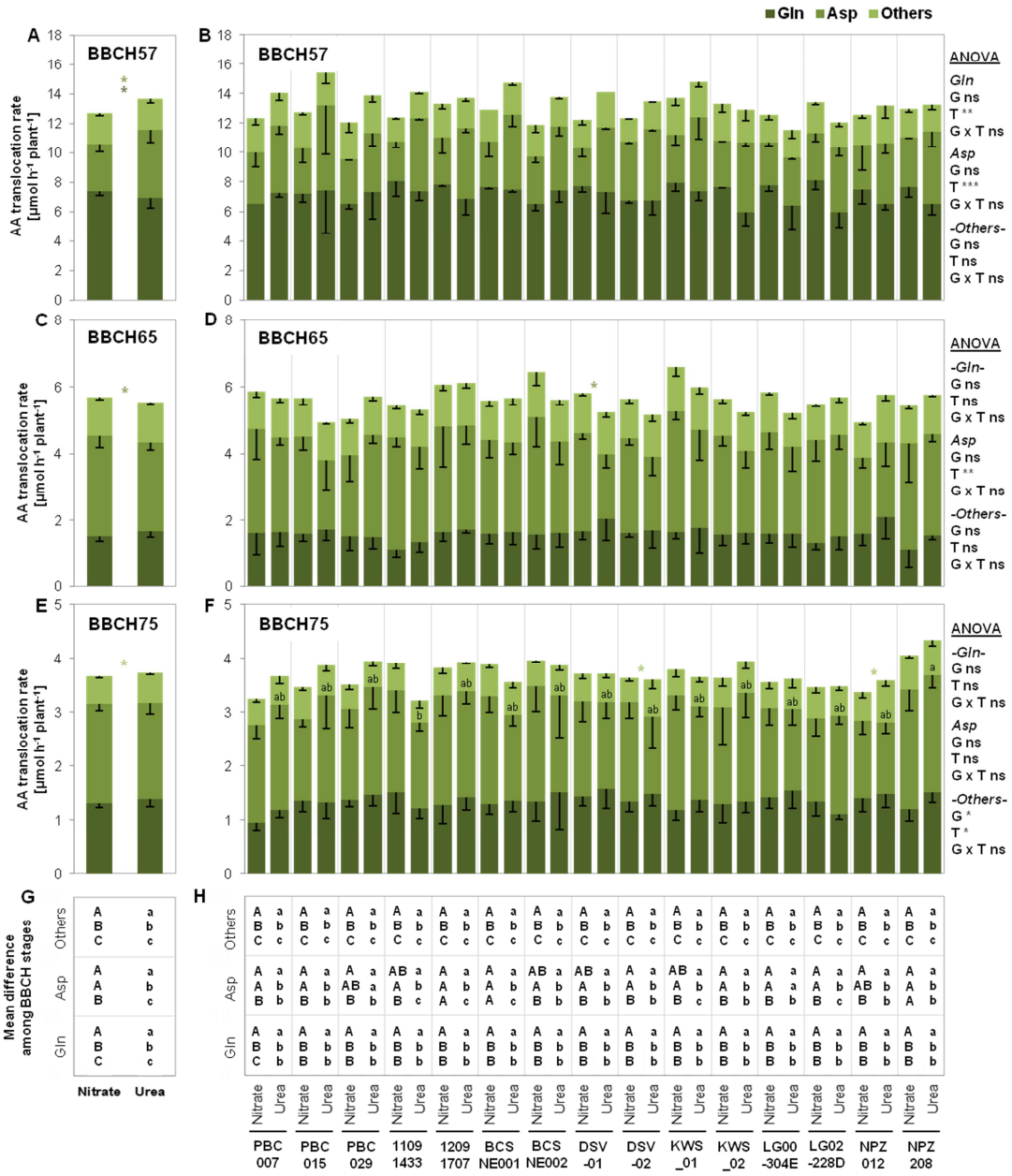


Figure 3-10: Amino acid translocation in the xylem sap of the elite line collection in the +N field trial 2013/2014 at BBCH57, BBCH65 and BBCH75 as mean over all genotypes (A, C, E) or of single genotypes (B, D, F) and as comparison between the developmental stages over all genotypes (G) or single genotypes (H). All rapeseed genotypes were fertilized in spring with 40 kg N ha^{-1} at BBCH30 and 60 kg N ha^{-1} at BBCH55 as nitrate or urea. Xylem sap was pooled from ten plants per plot. (A-F) Bars show means -SD (A, C, E: n=60; B, D, F: n=4). (A, C, E) Significant median difference in xylem translocation of a distinct N form is indicated by an asterisk referring to the AA color code according to Mann-Whitney rank sum test at p<0.05. (B, D, F) ANOVA or ANOVA on ranks (- -) results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. Significant mean difference among nitrate and urea treatment within a genotype is indicated by asterisks referring to AA color code according to unpaired t-test at p<0.05. Different upper case letters within a bar indicate significant mean or median differences among the genotypes within nitrate treatment according to Tukey´s test (D: ■■, F: ■■■) or Tukey´s test on ranks (B: ■■■, D: ■), respectively, at p<0.05; different lower case letters indicate significant mean or median differences among the genotypes within urea treatment according to Tukey´s test (D: ■■, F: ■■) or Tukey´s test on ranks (B: ■■■, D: ■, F: ■), respectively, at p<0.05. (G, H) Table shows mean or median differences for xylem Gln, Asp and residual AAs (Others) translocation among BBCH stages indicated by different letters in vertical rows of three comparing BBCH57, BBCH65 and BBCH75 (top to bottom). Different upper case letters indicate significant mean (H) or median (G) differences within nitrate treatment, while lower case letters refer to mean (H) or median (G) differences within urea treatment according to Tukey´s test (H) or Tukey´s test on ranks (G) at p<0.05; (G) n=60; (H) n=4.

Like total N translocation also mean population AA translocation was highest during flower development and decreased until pod formation (Figure 3-10A, C, E, G). Gln and Asp were the most abundant AAs in the xylem sap at the considered developmental stages. However, their proportions changed with progressing plant development as at BBCH57 Gln accounted for 50 - 60 % of total xylem AAs, while at BBCH65 and BBCH75 Asp made out 40 - 50 %. This follows in principle the observations made in the same population in 2012/2013.

Until flowering, application of nitrate promoted translocation of the respective most abundant AAs, Gln at BBCH57 and Asp at BBCH65, each by 10 % and during pod development the translocation of residual AAs by 15 %. Against this, Asp translocation was 30 % elevated at BBCH57 after urea fertilization. Since in the previous year no impact of the fertilizer N form on AA translocation was observed, the responsiveness of the same oilseed rape lines for different N forms may have depended on the environmental conditions.

Variations in translocation rates of AAs within the elite line population were similar at all three developmental stages with coefficients of variation of between 0.11 and 0.14 at each N condition and developmental stage, indicating only minor influence of both factors on phenotypic variability in AA translocation (Figure 3-10B, D, F). A similar situation was found in the previous year, although variability in AA translocation among the elite lines was higher from flowering onwards.

Although population mean Gln and Asp translocation was influenced by the fertilizer N treatment at BBCH57 (Figure 3-10A), no single genotype reflected this significantly but 10 out of 15 genotypes had in trend higher Gln translocation under nitrate and 14 of 15 displayed higher Asp levels under urea in accordance with the whole population means (Figure 3-10B). At BBCH65, mean Asp transport was promoted by nitrate treatment (Figure 3-10C) reflected by 1 of the 15 single genotypes significantly and by further 9 genotypes in trend (Figure 3-10D). At BBCH75, 6 of the 15 genotypes, 2 of them significantly, showed increased translocation rates of the sum of residual AAs in the xylem under urea treatment (Figure 3-10F), which manifested in a higher population mean translocation of residual AAs (Figure 3-10E).

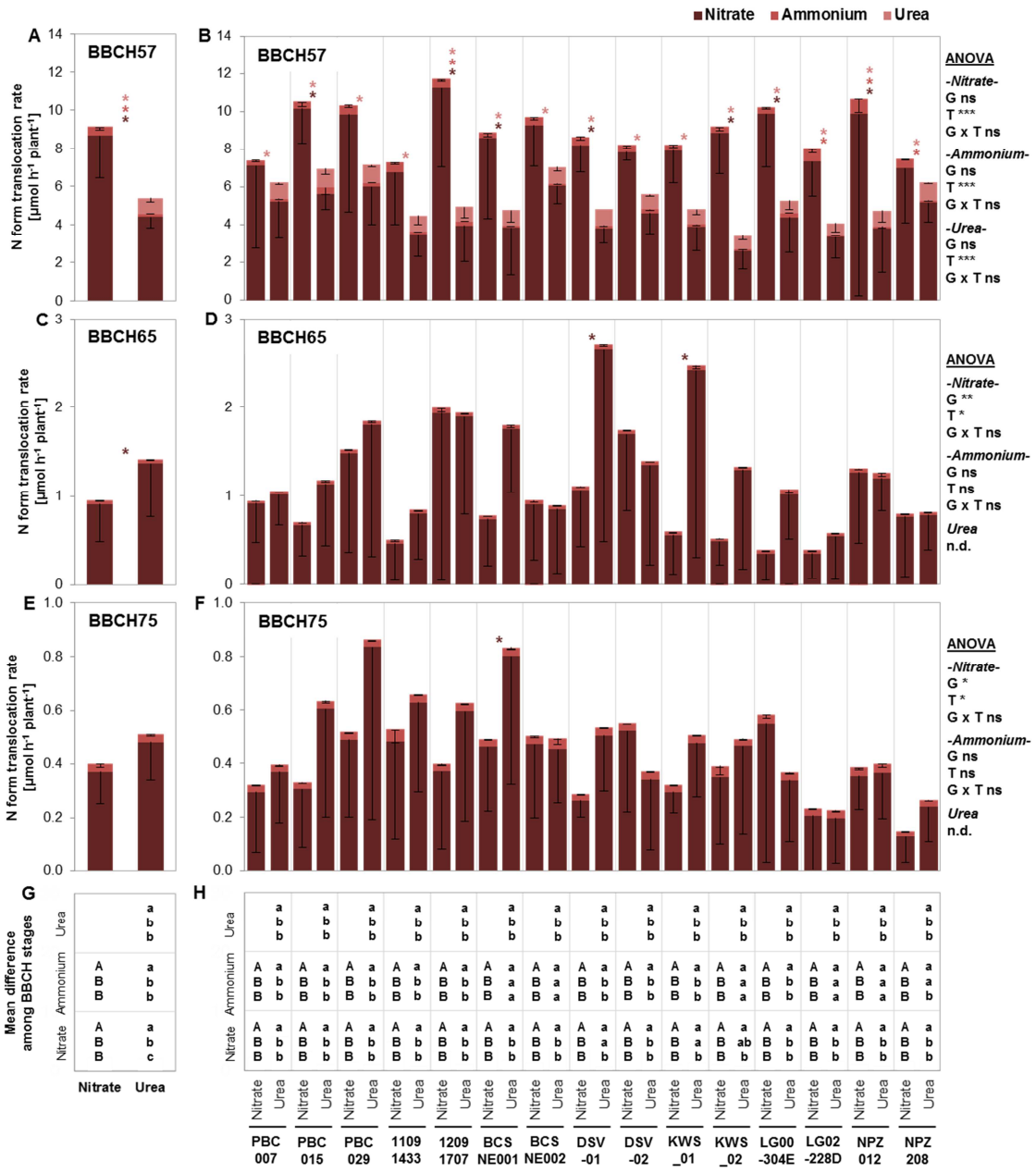


Figure 3-11: Translocation rates of nitrate, ammonium and urea in the xylem sap of the elite line collection in the +N field trial 2013/2014 at BBCH57, BBCH65 and BBCH75 as mean over all genotypes (A, C, E) or of single genotypes (B, D, F) and as comparison between the developmental stages over all genotypes (G) or single genotypes (H). All rapeseed genotypes were fertilized in spring with 40 kg N ha^{-1} at BBCH30 and 60 kg N ha^{-1} at BBCH55 as nitrate or urea. Xylem sap was pooled from ten plants per plot. (A-F) Bars show means -SD (A, C, E: n=60; B, D, F: n=4). (A, C, E) Significant median difference in xylem translocation of a distinct N form is indicated by an asterisk referring to the N form color code according to Mann-Whitney rank sum test at p<0.05. (B, D, F) ANOVA on ranks (- -) results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant, n.d.=non-determinable. Significant mean difference among nitrate and urea treatment within a genotype is indicated by asterisks referring to N form color code according to unpaired t-test at p<0.05. There were no significant median differences among the genotypes within nitrate treatment according to Tukey´s test on ranks and no significant mean or median differences within urea treatment according to Tukey´s test (B: ■■, D: ■) or Tukey´s test on ranks (B: ■, D: ■■, F: ■■■), respectively, at p<0.05. (G, H) Table shows mean or median differences for xylem nitrate, ammonium and urea translocation among BBCH stages indicated by different letters in vertical rows of three comparing BBCH57, BBCH65 and BBCH75 (top to bottom). Different upper case letters indicate significant mean (H) or median (G) differences within nitrate treatment, while lower case letters refer to mean (H) or median (G) differences within urea treatment according to Tukey´s test (H) or Tukey´s test on ranks (G) at p<0.05; (G) n=60; (H) n=4.

Nitrate and ammonium were translocated from root to shoot in every genotype and at any developmental stage regardless of the fertilized N form, but nitrate to much higher extent (Figure 3-11A, C, E). Translocation rates of both N forms in the xylem decreased with progressing plant development, whereas ammonium with -95 % (under both N treatments) decreased even sharper than nitrate with -90 % (under nitrate treatment) or -70 % (under urea treatment) from BBCH57 to BBCH65, while from BBCH65 to BBCH75 translocation of both N forms decreased by 60 % regardless of the N treatment. Thus, translocation of nitrate and ammonium followed the same trend as total N (Figure 3-9) and AA translocation (Figure 3-10), but only about half of total N translocation rates at each developmental stage and N treatment could be explained by the sum of nitrate, ammonium, urea and AAs. This was already observed in the elite lines in 2012/2013, even to a similar extent.

As in the previous year, urea was solely translocated in the xylem shortly after urea application (at BBCH57 - 11 days after fertilization), then reaching significantly higher levels than the nitrate variant. Against this, mean nitrate and ammonium translocation rates were significantly increased after nitrate treatment at BBCH57, each by 50 %, most likely due to application of ammonium nitrate fertilizer as nitrate treatment. However, at BBCH65 nitrate translocation was about 35 % higher after urea treatment and this trend was also visible at BBCH75.

Nitrate translocation rates in the xylem sap varied least among the individual lines at BBCH57 with variation coefficients of 0.42 under nitrate treatment and 0.38 under urea; until BBCH65 genotypic variability in nitrate translocation almost doubled and until BBCH75 variation decreased to 0.7 regardless of the applied N treatment. Genotypic variation in ammonium translocation was more influenced by the N treatment as variation coefficients under nitrate were relatively stable over time at 0.4 - 0.5, while urea treatment led to decreasing variability in ammonium translocation from flower development until flowering with 0.69 at BBCH57, 0.47 at BBCH65 and 0.25 at BBCH75. Urea translocation variation within the population could only be determined at BBCH57 under urea treatment and showed a variation coefficient of 0.42, similar to nitrate translocation at BBCH57 (Figure 3-11B, D, F).

The applied N form most prominently influenced translocation rates of the three N forms in the xylem sap of the individual genotypes at BBCH57, shortly after fertilizing with ammonium nitrate or urea. Thereby, urea treatment significantly promoted urea translocation in each of the 15 genotypes and nitrate treatment had the same effect on nitrate translocation, in 7 of the 15 lines significantly. Ammonium translocation was enhanced in 13 out of 15 genotypes, in 4 of them significantly, under nitrate treatment (Figure 3-11B). At BBCH65, the applied N form had an opposite influence on nitrate translocation in the xylem as 2/3 of the genotypes showed elevated nitrate translocation rates under urea conditions, 2 of them significantly (Figure 3-11D). Also at BBCH75, 11 of 15 genotypes (1 significant) had increased nitrate translocation under urea treatment, but this was not manifested in higher population mean nitrate translocation under urea fertilizer conditions (Figure 3-11E, Figure 3-11F).

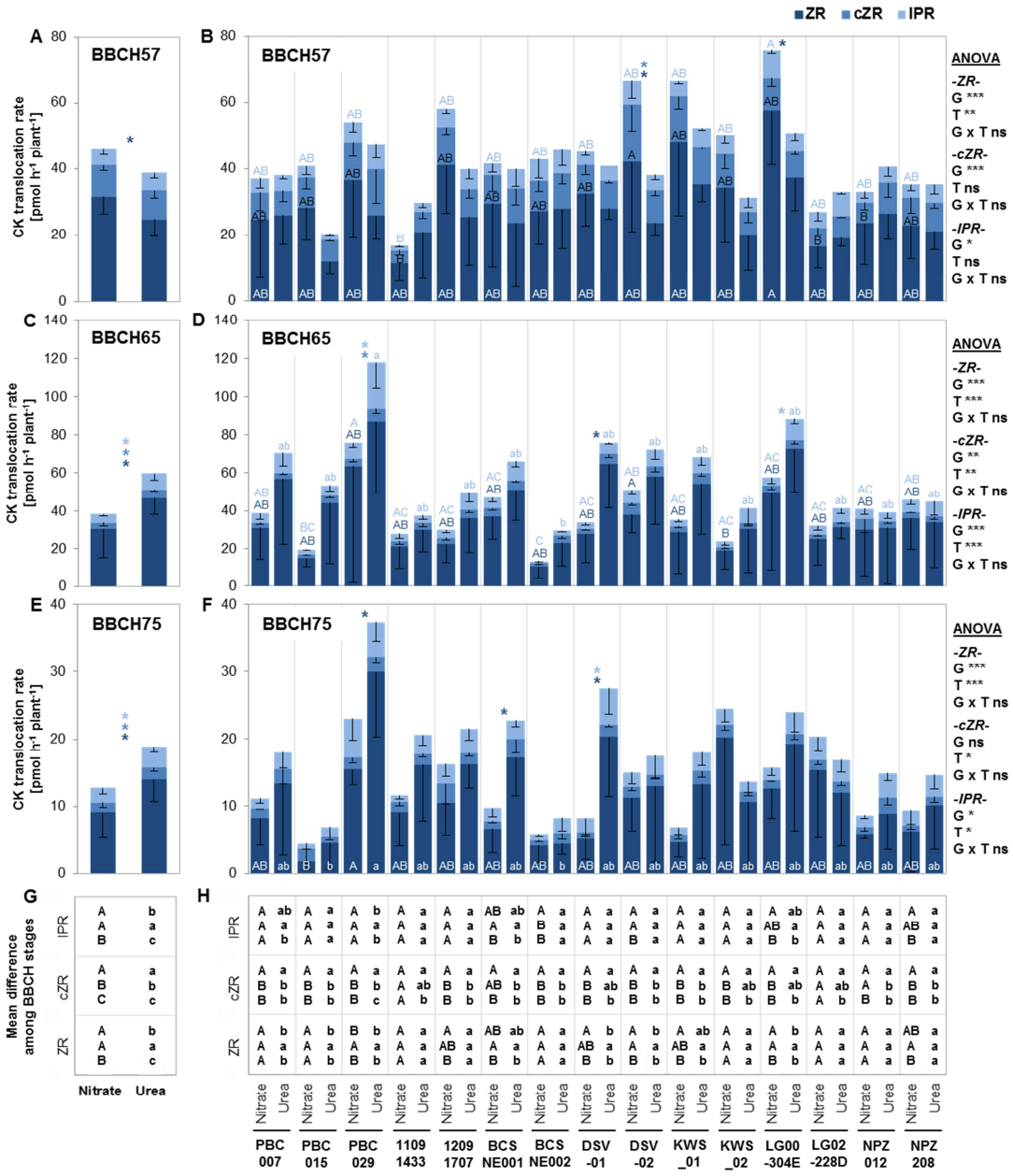


Figure 3-12: CK translocation in the xylem sap of the elite line collection in the +N field trial 2013/2014 at BBCH57, BBCH65 and BBCH75 as mean over all genotypes (A, C, E) or of single genotypes (B, D, F) and as comparison between the developmental stages over all genotypes (G) or single genotypes (H). All rapeseed genotypes were fertilized in spring with 40 kg N ha^{-1} at BBCH30 and 60 kg N ha^{-1} at BBCH55 as nitrate or urea. Xylem sap was pooled from ten plants per plot. (A-F) Bars show means -SD (A, C, E: n=60; B, D, F: n=4). (A, C, E) Significant median difference is indicated by an asterisk referring to the CK color code according to Mann-Whitney rank sum test at $p<0.05$. (B, D, F) ANOVA on ranks (- -) results *, **, *** indicate significant differences or interactions at $p<0.05$, $p<0.01$, $p<0.001$, respectively; G=genotype, T=N treatment, ns=non-significant. Significant mean difference among nitrate and urea treatment within a genotype is indicated by asterisks referring to CK color code according to unpaired t-test at $p<0.05$. Different upper case letters within a bar indicate significant mean or median differences among the genotypes within nitrate treatment according to Tukey´s test (B: ■, D: ■■) or Tukey´s test on ranks (B: ■■, D: ■, F: ■■■), respectively, at $p<0.05$; different lower case letters indicate significant mean or median differences among the genotypes within urea treatment according to Tukey´s test (F: ■) or Tukey´s test on ranks (B: ■■■, D: ■■■, F: ■■), respectively, at $p<0.05$. (G, H) Table shows mean or median differences for xylem ZR, cZR and IPR translocation among BBCH stages indicated by different letters in vertical rows of three comparing BBCH57, BBCH65 and BBCH75 (top to bottom). Different upper case letters indicate significant mean (H) or median (G) differences within nitrate treatment, while lower case letters refer to mean (H) or median (G) differences within urea treatment according to Tukey´s test (H) or Tukey´s test on ranks (G) at $p<0.05$; (G) n=60; (H) n=4.

Regardless of the N form and developmental stage, ZR was the most abundant CK form in the xylem by accounting for 65 - 75 % of total CK translocation rates among the elite line population at each developmental stage, followed by cZR at BBCH57 and IPR each at BBCH65 and BBCH75 (Figure 3-12A, C, E). This is in accordance with the results of the previous year.

Mean population translocation rates of cZR decreased regardless of the fertilizer N treatment steadily during the course of plant development, i.e. by 60 - 70 % from BBCH57 to BBCH65 and further by 25 - 30 % until BBCH75, which repeated the findings of 2012/2013. ZR and IPR translocation varied as well during plant development but in different patterns depending on the applied N treatment: While under nitrate translocation of both ZR and IPR was stable from BBCH57 to BBCH65 and decreased afterwards by 70 % and 40 %, respectively, translocation rates of ZR and IPR under urea increased both by about 40 % from BBCH57 to BBCH65 and decreased until BBCH75 by 70 % and 60 %, respectively (Figure 3-12G). These results were partially shown also in 2012/2013, i.e. for IPR under nitrate and for ZR under urea treatment, indicating seasonal variation in the response of xylem CK translocation to contrasting N fertilizer forms.

The N treatment significantly influenced population mean translocation rates of ZR at all developmental stages, but while as initially expected shortly after fertilization (at BBCH57 - 11 days after fertilizer application) nitrate promoted ZR translocation by about 20 %, later after fertilizer application urea stimulated xylem ZR transport by 30 % (BBCH65) and 40 % (BBCH75). Also IPR and cZR translocation from root to shoot was enhanced under urea treatment at BBCH65 and BBCH75. Similar results were obtained in the previous year shortly after fertilizer application and at the latest developmental stage assuming a robust effect of N forms on xylem CK translocation under different environmental conditions.

The variation in xylem CK translocation rates among the individual genotypes was mostly higher under nitrate conditions and appeared in general to increase during plant development (ZR, cZR and IPR at BBCH57: 0.55, 0.51 and 0.61 under nitrate and 0.41, 0.43 and 0.57 under urea, respectively; ZR, cZR and IPR at BBCH65: 0.79, 0.69 and 0.57 under nitrate and 0.59, 0.43 and 0.80 under urea, respectively; ZR, cZR and IPR at BBCH75: 0.79, 0.74 and 0.88 under nitrate and 0.69, 0.56 and 0.72 under urea, respectively; Figure 3-12B, D, F). Genotypic variability in xylem CK translocation among the elite lines appeared to be rather unaffected by seasonal conditions as it was similar in both years.

The significant effects of nitrate treatment on mean population ZR translocation at BBCH57 was reflected by 2/3 (2 of them significantly) of the single genotypes (Figure 3-12B). Even more pronounced was the impact of urea on ZR translocation rates at later plant development as 13 out of 15 genotypes showed higher ZR levels at both stages, with 1 genotype at BBCH65 and 3 genotypes at BBCH75 showing significant increases. Also IPR and cZR translocation were enhanced by urea in 13 genotypes at both developmental stages BBCH65 and BBCH75, whereas this effect was significant for cZR in 1 genotype at BBCH65 and for IPR in 2 genotypes at BBCH65 and 1 genotype at BBCH75. Interestingly, trends for repeated influence of the applied N form on total CK transport in 2012/2013 and 2013/2014 were observed in 7 of the 15 individual lines at BBCH57 (increased CK transport after nitrate treatment) and in 6 and 8 genotypes at BBCH65 and BBCH75, respectively, with higher CK transport under urea.

As candidates for improved seed yield due to delayed root senescence at later developmental stages the urea variants of PBC007, BCSNE001, DSV-01, LG00-304E and both variants of PBC029 appeared to be highly interesting, since all of these variants showed elevated total CK translocation (as proposed measure for delayed root senescence in this study) at BBCH65 as well as at BBCH75. This was tested by correlations between CK translocation rates and final seed yield in 3.2.3.

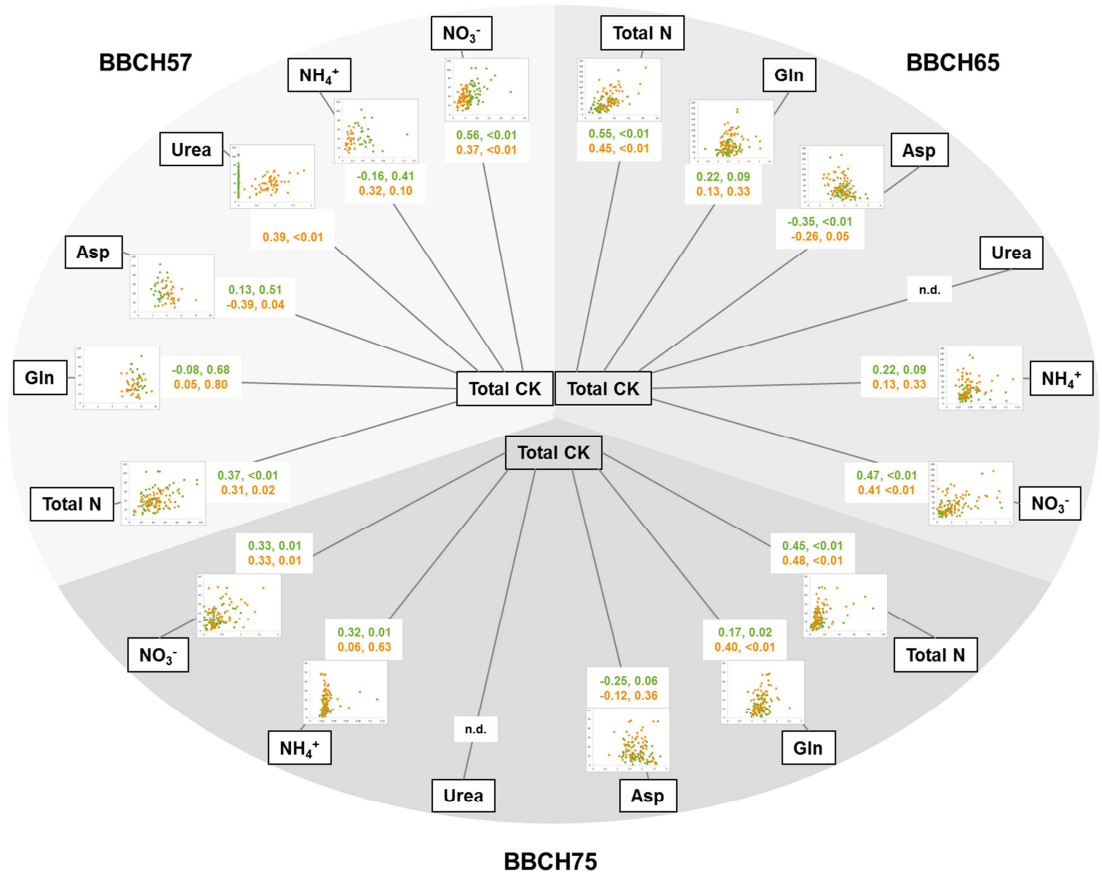


Figure 3-13: Correlations between total CK translocation and different N fractions in the xylem sap of the elite line collection in the +N field trial 2013/2014 at BBCH57, BBCH65 and BBCH75. Components were correlated by rank order correlation. Values for r and p were calculated separately for nitrate (■) and urea (■) treatment; n=60.

To evaluate robustness of the relations between N and CK translocation rates found in 2012/2013 (Figure 3-5), a correlation study between N compounds and CKs in the xylem sap of the elite lines was undertaken again in 2013/2014. Indeed, significant positive associations between CKs and total N as well as nitrate translocation during flower development could be repeatedly observed, regardless of the applied N treatment (Figure 3-13). Against the results of 2012/2013, same correlations were present also at BBCH65 and BBCH75 under either N treatment. This indicates that CK translocation can serve as relative robust indirect marker for total N as well as nitrate translocation in the xylem sap at earlier plant development, while at later phases relations between xylem CK and N translocation are more prone to seasonal variation.

3.2.2 Influence of the applied nitrogen form on above-ground nitrogen allocation

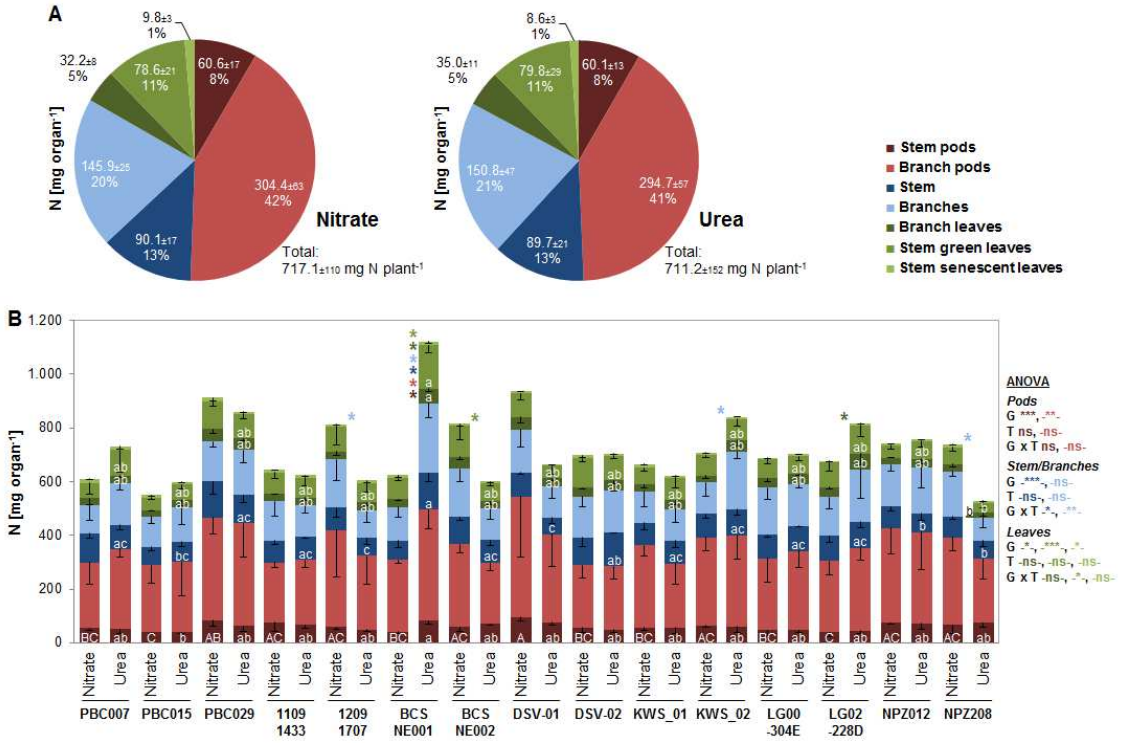


Figure 3-14: Total N pools in the above-ground plant biomass at BBCH79 in single genotypes (A) or as mean over all genotypes (B) in the elite line collection in the +N field trial 2013/2014. All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Two plants per plot were seperated into the indicated above-ground plant fractions to determine their N content. (A) Pie charts show N content (means ±SD) of the indicated organs and the corresponding percentage to total plant N (n=60). There were no significant median differences in N allocation to the indicated organs among nitrate and urea treatment according to Mann-Whitney rank sum test at p<0.05. (B) Bars show means -SD (n=4). ANOVA or ANOVA on ranks (- -) results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. Significant mean difference among nitrate and urea treatment within a genotype is indicated by asterisks referring to the organ color code according to unpaired t-test at p<0.05. Different upper case letters within a bar indicate significant mean or median differences among the genotypes within nitrate treatment according to Tukey´s test (■■) or Tukey´s test on ranks (■■■■), respectively, at p<0.05; different lower case letters indicate significant mean or median differences among the genotypes within urea treatment according to Tukey´s test (■■■) or Tukey´s test on ranks (■■■■), respectively, at p<0.05.

Mean N accumulation and N distribution in the shoot at the end of pod development among the elite line population was, as already seen in 2012/2013, not affected by the applied fertilizer N treatment (Figure 3-14A). However, in contrast to the previous experimental year when at BBCH79 about 60 % of total above-ground N was allocated to pods (Figure 3-6A), N accumulation in the pod fraction in 2013/2014 was only about 50 %, while especially relative N contents in branches and in the green leaf fractions were higher in 2013/2014. This raised the question whether plant material may have been harvested at an earlier plant developmental stage in 2013/2014, as N retranslocation had apparently not proceeded to the same extent as in 2012/2013 (Annex-7).

Overall above-ground N accumulation was about 50 - 60 mg plant^{-1} higher than in the previous year, but in that year stand density was higher suggesting better N uptake abilities in stands with less intraspecific competition among N (Table 3-1, Table 3-2). However, also literature values for shoot N accumulation, mainly determined at seed harvest, vary strongly depending on experimental conditions, e.g. 360 mg plant^{-1} (Svečnjak and Rengel, 2006), around 1300 mg plant^{-1} (Malagoli *et al.*, 2005) or even about 2200 mg plant^{-1} (Zhang *et al.*, 2012).

Phenotypic variability in N allocation to different above-ground organs within the collection was comparable to the previous experimental year 2012/2013 (Figure 3-6B): Coefficients of variation were 0.35 for both pod fractions, 0.28 and 0.35 for the stem fractions “stem” and “branches”, 0.43 for both green leaf fractions and 0.60 for “stem senescent leaves” under nitrate. Similar population variability in N content was found for the pod fractions under urea with 0.31 for both “stem pods” and “branch pods”, for the stem with 0.30 and for the green leaf fractions with 0.49 for both, while variation in N content of the branches was about 10 % higher under urea. An about 10 % lower variation was found for N allocation to the “stem senescent leaves”. Variability in total plant N was 0.27 under nitrate and 0.30 under urea and thus similar between the two fertilizer N treatments and comparable to the results of 2012/2013. As in the previous experimental year, differences in N allocation to plant organs were rather subject to dry weight variation among the organs than to their N concentration, which remained relatively stable among the genotypes (Annex-6).

Regarding N allocation to different above-ground fractions in the individual genotypes (Figure 3-14B), significant differences among the lines existed rather under urea (in 4 of the 7 fractions) than under nitrate treatment (in 1 fraction). Especially urea treated BCSNE001 showed the highest N accumulation in all plant organ fractions assuming that plants harvested from this genotype were accidently exceptional big exemplars as no such strong impact was found in 2012/2013. The population mean trend for higher N allocation to branch pods under urea was reflected by 7 of the 15 genotypes (significant in 1) but against this, 2 genotypes showed even significantly lower N in the branch pods under urea. Although the population means for N allocation to both of the green leaf fractions were not significantly different between the N fertilizer treatments, 1 genotype of the collection showed significantly higher N in branch leaves and main shoot green leaves.

As in the previous experimental year, $N_{generative}/N_{vegetative}$ at BBCH79 was considered to evaluate if nitrate fertilization directs oilseed rape with delay into N retranslocation. $N_{generative}/N_{vegetative}$ was neither significantly influenced by the N treatment regarding the population mean nor any individual genotype, although there was a slight tendency for rather earlier N retranslocation to pods under nitrate within the whole population (Annex-7). This trend was not in agreement with the initial expectation, but appeared to be valid under different seasonal influences as the same phenomenon was observed also in 2012/2013.

Figure 3-15: Yield parameters of the elite line collection in the +N field trial 2013/2014 as means over all genotypes (A, C, E, G, I) or of the single genotypes (B, D, F, H, J). All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Yield parameters were determined as described in 2.6. Boxes show median, first and third quartile; whiskers represent minimum and maximum of all data in a group (A, C, E, G, I: n=60; B, D, F, H, J: n=4). (A, C, E, G, I) There were no significant differences among the means or medians according to unpaired t-test (A, G) or Mann-Whitney rank sum test (C, E, I), respectively, at $p<0.05$. (B, D, F, H, J) ANOVA (B, D) or ANOVA on ranks (F, H, I) results *, **, *** indicate significant differences or interactions at $p<0.05$, $p<0.01$, $p<0.001$, respectively; G=genotype, T=N treatment, ns=non-significant. Asterisks indicate significant mean difference among nitrate and urea treatment within a genotype according to unpaired t-test at $p<0.05$. Different upper case letters indicate significant mean or median differences among the genotypes within nitrate treatment according to Tukey´s test (B, F, J) or Tukey´s test on ranks (D, H), respectively, at $p<0.05$; different lower case letters indicate significant mean or median differences among the genotypes within urea treatment according to Tukey´s test (B, D) or Tukey´s test on ranks (F, H, J) at $p<0.05$. TSW=Thousand seed weight.

3.2.3 Influence of the applied nitrogen form on yield formation

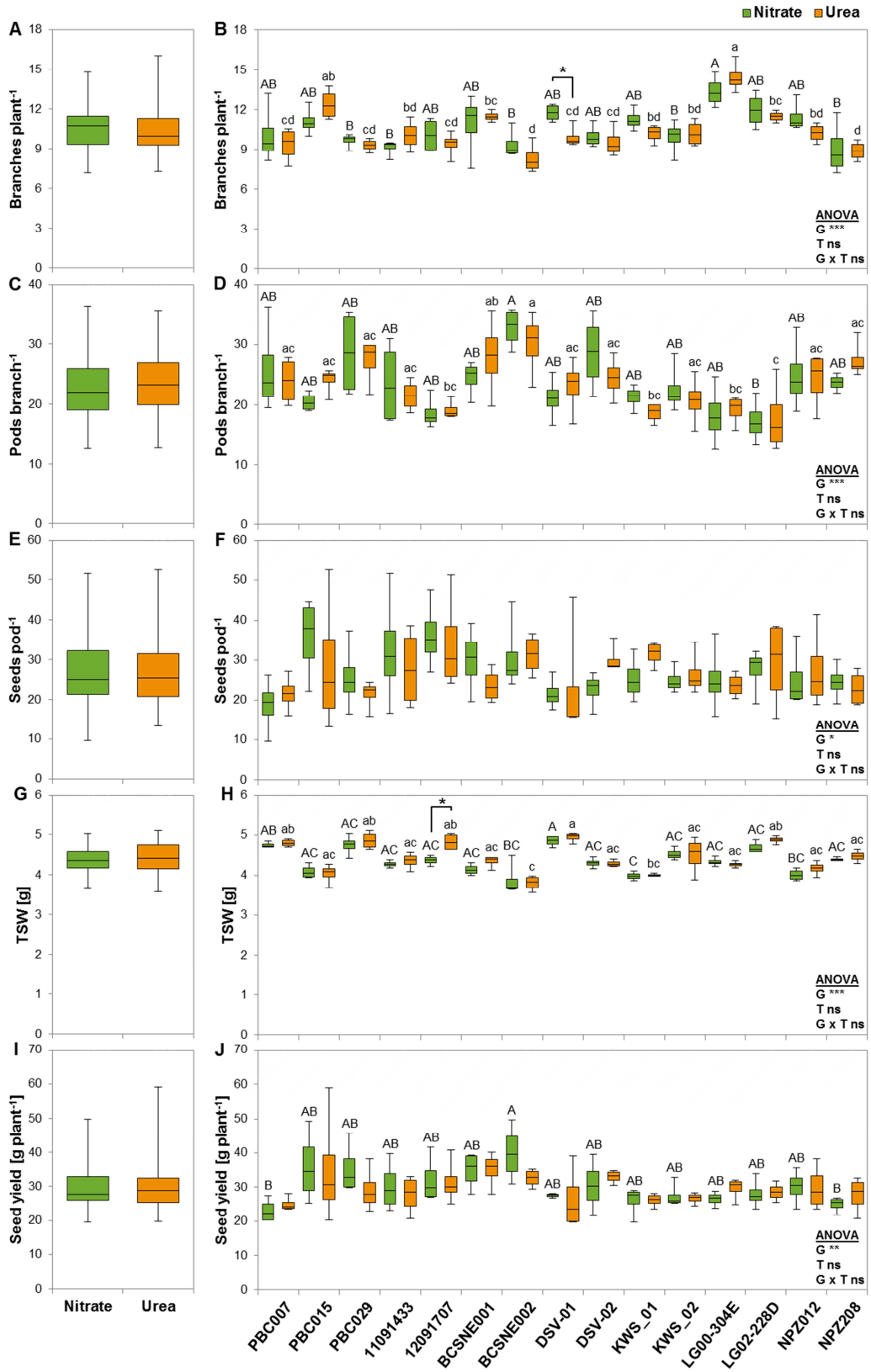


Figure 3-15 (For description see previous page.)

Table 3-2: Stand density and area seed yield of the elite line collection in the +N field trial 2013/2014. All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Yield parameters were determined as described in 2.6. Table shows means ±SD (n=4 for single genotypes, n=60 for all genotypes). ANOVA (Plants m^{-2}) or ANOVA on ranks (Seed yield) results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. Asterisks indicate significant mean difference among nitrate and urea treatment within a single genotype according to unpaired t-test at p<0.05 or among all genotypes according to unpaired t-test (Plants m^{-2}) or Mann-Whitney rank sum test (Seed yield) at p<0.05. Different upper case letters indicate significant mean differences among the genotypes within nitrate treatment according to Tukey´s test at p<0.05; different lower case letters indicate significant mean or median differences among the genotypes within urea treatment according to Tukey´s test (Plants m^{-2}) or Tukey´s test on ranks (Seed yield), respectively, at p<0.05.

Genotype	Plants m^{-2}			Seed yield [g m^{-2}]	
	Nitrate		Urea	Nitrate	Urea
PBC007	14.2 ±1.6	AB	13.0 ±1.3	323.6 ±17.2	324.8 ±27.0
PBC015	12.1 ±3.3	AB	12.8 ±4.8	410.9 ±43.0	392.3 ±48.0
PBC029	11.7 ±1.9	AB	12.8 ±1.8	404.0 ±51.5	366.3 ±28.0
11091433	13.6 ±2.7	AB	14.8 ±2.6	398.6 ±35.9	400.9 ±21.8
12091707	12.1 ±2.4	AB	13.5 ±2.1	386.9 ±86.6	418.0 ±47.1
BCSNE001	11.6 ±2.2	AB	11.2 ±1.4	396.1 ±33.3	388.6 ±28.2
BCSNE002	9.1 ±1.7	B *	11.8 ±1.0	355.4 ±26.1	384.5 ±34.9
DSV-01	14.0 ±0.3	AB	15.0 ±4.5	384.4 ±14.6	368.3 ±5.6
DSV-02	12.7 ±3.1	AB	11.2 ±0.7	371.2 ±20.0	368.7 ±32.9
KWS_01	15.8 ±2.7	A	15.5 ±1.3	402.1 ±38.3	404.5 ±45.2
KWS_02	14.6 ±1.6	AB	14.9 ±1.1	397.7 ±11.2	396.8 ±21.1
LG00-304E	14.8 ±0.6	A	12.4 ±1.4	391.0 ±24.5	362.9 ±14.7
LG02-228D	14.8 ±2.3	A	14.2 ±2.0	407.0 ±10.5	400.6 ±20.2
NPZ012	13.4 ±2.7	AB	12.4 ±2.4	393.7 ±46.3	358.4 ±23.5
NPZ208	15.0 ±1.7	A	14.2 ±1.1	371.3 ±18.9	390.0 ±53.6
All genotypes	13.3 ±2.6		13.3 ±2.4	386.3 ±39.5	381.7 ±36.7
ANOVA	G ***, T ns, GxT ns			G **, T ns, GxT ns	

Like in the previous experimental year, means of the individual yield components within the oilseed rape elite line population were not influenced by the applied N form (Figure 3-15A, C, E, G). Thus, final mean seed yield per plant was comparable between the fertilizer N treatments, too, and the same was observed for area seed yield as also equal mean stand densities could establish after winter in both N treatments (Figure 3-15I, Table 3-2). Since in total about 6 plants less established per square meter compared to the previous year, plants in 2013/2014 could develop 3 branches $plant^{-1}$ and 10 seeds pod^{-1} more which went on cost of the TSW but led to higher seed yield per plant (+10 g) and per square meter (+60 g; Figure 3-7, Figure 3-15, Table 3-1, Table 3-2). This indicates that the yield formation of oilseed rape depends highly on stand establishment and seasonal conditions.

Also the phenotypic variation in yield and yield parameter formation among the elite line collection was comparable between the fertilizer N treatments for the single traits, but varied between the different yield parameters: The trait “seeds per pod” was most variable with variation coefficients of 0.31 and 0.32 under nitrate and urea, respectively, followed by the pods per branch with 0.25 and 0.22, the stand density with 0.19 and 0.18, the branches per plant with 0.16 and 0.17 and the TSW with 0.08 and 0.09. Variability in plant seed yield formation was 0.23 under nitrate and 0.22 under urea and thus two times higher than in seed yield formation per square meter with 0.10 under both N treatments (Figure 3-15, B, D, F, H, J, Table 3-2). This reflected highly the genotypic variation in individual yield components of the previous year.

Although DSV-01 showed a significantly higher branching under nitrate and 12091707 had a higher TSW under urea, mean population yield parameter formation was even not altered in trend by the applied N treatment.

However, various patterns of yield formation existed among the lines, regardless of the fertilizer N treatment: The individual yield parameters and the final seed yield of the six genotypes 11091433, BCSNE001, DSV-02, KWS_02, NPZ012 and NPZ208 were close to the population means, while the other genotypes had a rather unique behavior of yield formation. DSV-01, KWS_01, LG00-304E and LG02-228D produced nearly identical seed yield but with different formation patterns: DSV-01 and KWS_01 formed rather average numbers of branches and pods, but slightly more seeds of KWS_01 were compensated for by lower TSW compared to DSV-01. LG00-304E showed an elevated branching behavior and decreased pod formation associated with decreased average seed production and TSW, while LG02-228D showed only slightly higher branching, pod and seed formation (comparable to LG00-304E) but elevated TSW. PBC029 and 12091707 produced average plant seed yields with average formation of branches, but while PBC029 had slightly above-average pod formation and average seed production associated with above-average TSW, 12091707 had decreased pod numbers per branch filled with increased numbers of seeds with average TSW under nitrate or above-average TSW under urea (thereby potentially compensating for slightly lower branching and seed production under urea compared to nitrate). The nitrate variant of BCSNE002 produced the highest seed yield in the population by low branching associated with highest pod formation per branch, average seed filling but very low TSW, while the urea variant tended to produce even less branches and pods than the nitrate variant and resulted in slightly above-average seed yield. The reference genotype PBC007 exhibited lowest yield production in the population with branch and pod numbers in the range of the population mean, but decreased seed production associated with higher TSW. Against this, PBC015 showed the highest seed yield in the population which was based under nitrate on average branching, pod formation below average but high seed production with below-average TSW, while the urea variant exhibited elevated branching with average pod and seed production and a seed weight comparable to the nitrate variant. Unlike the 2012/2013 experiment, only six of the elite lines could be grouped together in 2013/2014 based on their similarity in yield formation.

Interestingly, yield formation behavior of 11091433, DSV-02, KWS_01, LG00-304E, LG02-228D and NPZ208 relative to population mean yield formation was consistent over both experimental years, indicating that adaptation of yield formation to different seasonal conditions is to some degree genetically determined.

Final seed yield of the elite lines was correlated with xylem N and CK translocation rates at BBCH65 and BBCH75 in order to test if higher root activity during later plant developmental stages might be beneficial for seed yield. Like in 2012/2013, no correlation of the parameters could be observed in 2013/2014 (Annex-22). This indicates that under sufficient N supply seed yield formation might not profit from N uptake during generative plant development as it was described for N-deficient conditions by Schulte auf'm Erley *et al.* (2007).

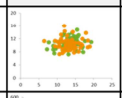
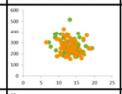
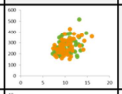
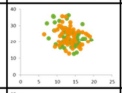
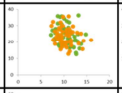
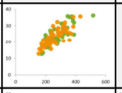
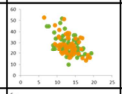
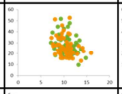
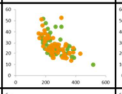
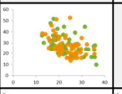
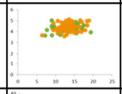
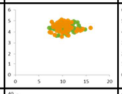
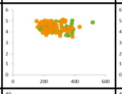
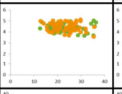
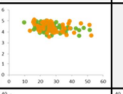
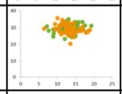
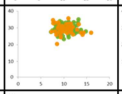
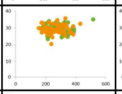
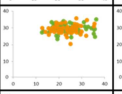
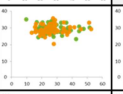
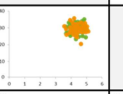
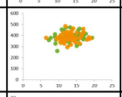
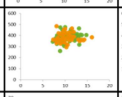
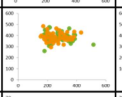
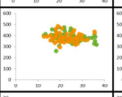
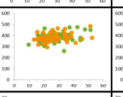
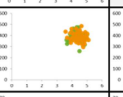
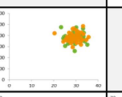
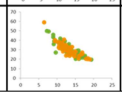
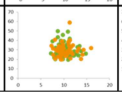
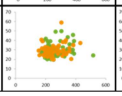
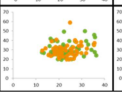
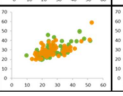
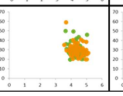
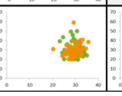
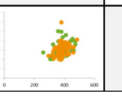
	Plants m^{-2}	Branches plant^{-1}	Pods plant^{-1}	Pods branch^{-1}	Seeds pod^{-1}	TSW [g]	Seed N [mg g DW^{-1}]	Seed yield [g m^{-2}]	Seed yield [g plant^{-1}]
Plants m^{-2}		r, p: 0.19, 0.14 -0.01, 0.97 ^{S}	r, p: -0.26, 0.05 ^{S} -0.17, 0.18	r, p: -0.31, 0.02 ^{S} -0.20, 0.12	r, p: -0.46, <0.01 ^{S} -0.43, <0.01 ^{S}	r, p: 0.18, 0.17 0.27, 0.04	r, p: 0.21, 0.11 -0.30, 0.02	r, p: 0.16, 0.24 0.13, 0.31	r, p: -0.86, <0.01 ^{S} -0.84, <0.01 ^{S}
Branches plant^{-1}			r, p: 0.30, 0.02 ^{S} 0.42, <0.01 ^{S}	r, p: -0.26, 0.05 ^{S} -0.91, 0.14 ^{S}	r, p: -0.30, 0.02 ^{S} -0.28, 0.03 ^{S}	r, p: -0.01, 0.98 -0.07, 0.62 ^{S}	r, p: 0.06, 0.66 -0.06, 0.66 ^{S}	r, p: 0.26, 0.05 0.10, 0.45 ^{S}	r, p: -0.03, 0.82 ^{S} 0.03, 0.83 ^{S}
Pods plant^{-1}				r, p: 0.81, <0.01 ^{S} 0.79, <0.01	r, p: -0.59, <0.01 ^{S} -0.63, <0.01 ^{S}	r, p: -0.17, 0.19 ^{S} -0.19, 0.16	r, p: 0.01, 0.99 ^{S} 0.09, 0.49	r, p: -0.10, 0.47 ^{S} -0.01, 0.96	r, p: 0.05, 0.18 ^{S} 0.24, 0.65 ^{S}
Pods branch^{-1}					r, p: -0.45, <0.01 ^{S} -0.47, <0.01 ^{S}	r, p: -0.10, 0.49 ^{S} -0.16, 0.21	r, p: -0.05, 0.73 ^{S} 0.15, 0.25	r, p: -0.31, 0.02 ^{S} -0.02, 0.88	r, p: 0.21, 0.11 ^{S} 0.17, 0.19 ^{S}
Seeds pod^{-1}						r, p: -0.29, 0.25 ^{S} -0.29, 0.26 ^{S}	r, p: -0.09, 0.47 ^{S} 0.21, 0.10 ^{S}	r, p: 0.24, 0.07 ^{S} 0.26, 0.05 ^{S}	r, p: 0.55, <0.01 ^{S} 0.57, <0.01 ^{S}
TSW [g]							r, p: 0.01, 0.93 -0.01, 0.92	r, p: -0.09, 0.50 -0.10, 0.45	r, p: -0.27, 0.03 ^{S} -0.29, 0.03 ^{S}
Seed N [mg g DW^{-1}]								r, p: 0.01, 0.94 0.06, 0.65	r, p: -0.18, 0.17 ^{S} 0.33, 0.01 ^{S}
Seed yield [g m^{-2}]									r, p: 0.28, 0.03 ^{S} 0.39, <0.01 ^{S}
Seed yield [g plant^{-1}]									

Figure 3-16: Correlations between the yield parameters of the genotypes in the elite line collection in the +N field trial 2013/2014. All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Correlation charts as well as r and p value were calculated separately for nitrate (■) and urea (■) treatment; n=60. ^{S} indicates calculation of r and p value according to Spearman rank order correlation, while unmarked values accord to Pearson product moment correlation. Chart axes correspond to the yield parameter indicated on top or at the left-hand side of the matrix. TSW=Thousand seed weight.

Relations among the individual yield parameters of the elite line population in 2013/2014 were highly similar to the previous year: Regardless of the fertilizer N treatment, higher branching of a line led to higher pod formation per plant but this appeared to be on cost of seed formation per pod (Figure 3-16).

However, in both years the trait “seeds per pod” was the most positively associated with final plant seed yield but both parameters decreased with increasing stand density, assuming intraspecific competition for nutrients (Diepenbrock, 2000) and light (Thurling, 1991). The number of seeds produced per pod was also moderately positively associated with area seed yield and that in both experimental years, indicating that the seed production per pod is the most important trait among the considered yield parameters for the determination of final plant and area seed yield, regardless of seasonal variations.

3.3 Influence of fertilizer nitrogen form on xylem cytokinin translocation, shoot nitrogen distribution and yield formation in a set of genetic diverse oilseed rape lines in 2011/2012

Since in both experimental years 2012/2013 and 2013/2014 a similar effect of nitrate stimulating xylem CK translocation shortly after fertilizer application was observed in the elite lines (3.1.1, 3.2.1), the question arose if this reaction holds true also for other genotypes. Thus, CK translocation rates in xylem sap samples obtained from a field trial with genetic diverse oilseed rape lines in 2011/2012 were evaluated considering this aspect. Since the material from this field trial additionally allowed to study the influence of the different N forms nitrate and urea on shoot N accumulation and yield formation, emphasis was put on the question if within a collection of genetically more diverse lines the responsiveness for the contrasting N fertilizer forms is higher than in the elite lines where nearly no impact of nitrate and urea on shoot N distribution and yield formation could be identified (3.1.2, 3.1.3; 3.2.2, 3.2.3).

3.3.1 Influence of the applied nitrogen form on xylem cytokinin translocation

In the genetic diverse collection grown in the +N field trial in 2011/2012, xylem sap harvests were undertaken earlier than in the elite population, i.e. during early and late flower development (at BBCH52 and BBCH59) and during the end of flowering (BBCH69). Thereby, xylem sap was harvested at BBCH52, i.e. 27 days after fertilization with 40 kg N ha^{-1}, while harvests at BBCH59 and BBCH69 were undertaken 7 days and 42 days after fertilization with 60 kg N ha^{-1}.

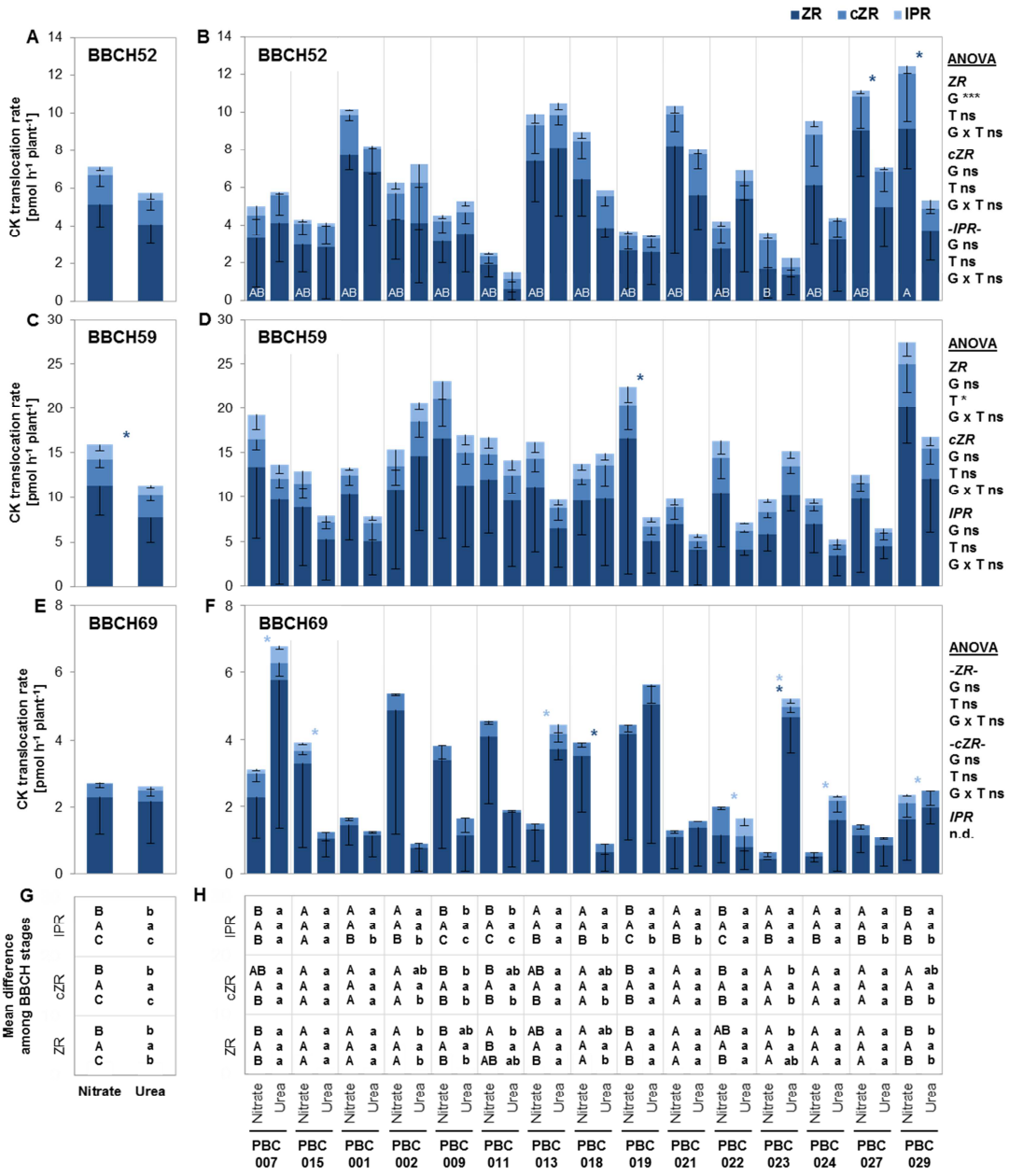


Figure 3-17: CK translocation in the xylem sap of the genetic diverse collection in the +N field trial 2011/2012 at BBCH52, BBCH59 and BBCH69 as mean over all genotypes (A, C, E) or of single genotypes (B, D, F) and as comparison between the developmental stages over all genotypes (G) or single genotypes (H). All rapeseed genotypes were fertilized in spring with 40 kg N ha^{-1} at BBCH30 and 60 kg N ha^{-1} at BBCH55 as nitrate or urea. Xylem sap was pooled from ten plants per plot. (A-F) Bars show means -SD (A, C, E: n=45; B, D, F: n=3). (A, C, E) Significant median differences are indicated by an asterisk referring to the CK color code according to Mann-Whitney rank sum test at $p<0.05$. (B, D, F) ANOVA or ANOVA on ranks (- -) results *, **, *** indicate significant differences or interactions at $p<0.05$, $p<0.01$, $p<0.001$, respectively; G=genotype, T=N treatment, ns=non-significant, n.d.=non-determinable. Significant mean difference among nitrate and urea treatment within a genotype is indicated by asterisks referring to CK color code according to unpaired t-test at $p<0.05$. Different upper case letters within a bar indicate significant mean or median differences among the genotypes within nitrate treatment according to Tukey´s test (B: ■■■, D: ■■■, F: ■) or Tukey´s test on ranks (F: ■■), respectively, at $p<0.05$; different lower case letters indicate significant mean or median differences among the genotypes within urea treatment according to Tukey´s test (B: ■■, D: ■■■, F: ■) or Tukey´s test on ranks (B: ■, F: ■■), respectively, at $p<0.05$. (G, H) Table shows mean or median differences for xylem ZR, cZR and IPR translocation among BBCH stages indicated by different letters in vertical rows of three comparing BBCH52, BBCH59 and BBCH69 (top to bottom). Different upper case letters indicate significant mean (H) or median (G) differences within nitrate treatment, while lower case letters refer to mean (H) or median (G) differences within urea treatment according to Tukey´s test (H) or Tukey´s test on ranks (G) at $p<0.05$; (G) n=45; (H) n=3.

As already observed in the elite lines, ZR was the most abundant CK form in every genotype of the diversity collection at each N fertilizer treatment and developmental stage, followed by cZR and IPR, whereas the latter was not detected in all samples at BBCH69. Regardless of the fertilizer N treatment, ZR accounted for 2/3 (BBCH52) or up to 75 % (BBCH59 and BBCH69) of the total population CK translocation, while cZR made out about 20 % (BBCH59 and BBCH69) to 30 % (BBCH52) and IPR the residual 5 - 10 % (Figure 3-17A, C, E).

Population mean CK translocation rates varied during the progress of plant development and appeared to be dependent on the applied N fertilizer form (Figure 3-17G): All three CK forms increased from BBCH52 to BBCH59, thereby both ZR and IPR by 2 (urea)- to 2.5 (nitrate)-fold and cZR by about 0.5-fold under either N treatment, and decreased until BBCH69 by 4 (urea)- to 6 (nitrate)-fold for ZR, 6-fold for cZR under both N treatments and 3 (urea)- to 20 (nitrate)-fold for IPR.

Mean ZR translocation was significantly promoted by 30 % seven days after nitrate application at BBCH59, and IPR translocation rates increased in trend at this developmental stage compared to urea. Since xylem CK translocation rates were also enhanced shortly after nitrate application in both elite line experiments, this effect appeared to be quite robust for any kind of seasonal condition or for any considered genotype. A trend for higher ZR translocation in the xylem was also observed at BBCH52 after nitrate treatment, while at BBCH69 urea tended to enhance IPR translocation. The latter was also the case in the elite line population of 2013/2014.

Expressed by the coefficient of variation, CK translocation rates in the xylem sap of the individual genotypes were subject to genotypic variability, and this to largest extent at the end of flowering with 0.66 and 0.87 for cZR and ZR translocation, respectively, under nitrate and 0.89 and 1.10 under urea treatment, while variation in IPR ranged from 0 - 0.25 pmol h^{-1} $plant^{-1}$ under nitrate and 0 - 0.5 pmol h^{-1} $plant^{-1}$ under urea. The variation in translocation rates of individual CKs at earlier plant development were comparable under each N treatment and developmental stage with values between 0.65 and 0.75 (Figure 3-17B, D, F).

An influence of the fertilized N form on CK translocation rates of individual genotypes was hardly observed at each stage of flower development. Although nitrate significantly promoted population mean ZR translocation at BBCH59 (Figure 3-17C), only 1 line (PBC019) reflected this effect significantly. Nevertheless, further 11 genotypes followed this trend. Translocation rates of other CK forms in individual genotypes followed the pattern observed for ZR translocation, but were not significantly influenced by the fertilizer N treatment at BBCH59. At BBCH52, mean population ZR translocation tended to increase under nitrate which was even significant in 2 cultivars (PBC027 and PBC029) and in trend in 7 further cultivars. Among the cultivars still translocating IPR at BBCH69, 5 had increased levels after urea and 2 after nitrate fertilization leading to a general trend of higher IPR translocation after urea treatment over the whole population.

3.3.2 Influence of the applied nitrogen form on shoot nitrogen allocation

The total above-ground N acquisition and allocation to the shoots of the genetic diverse rapeseed cultivars was determined like in the elite line collection at BBCH79.

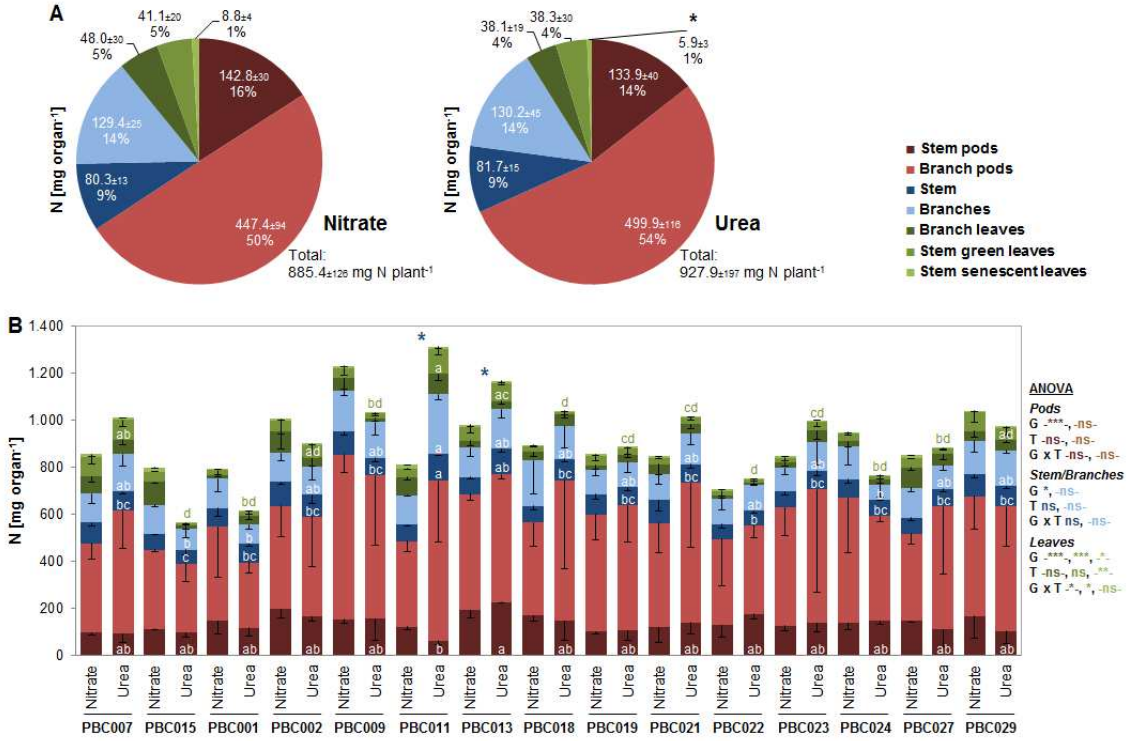


Figure 3-18: Total N pools in the above-ground plant biomass at BBCH79 in single genotypes (A) or as mean over all genotypes (B) in the genetic diverse collection in the +N field trial 2011/2012. All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Two plants per plot were separated into the indicated above-ground plant fractions to determine their N content. (A) Pie charts show N content (means ±SD) of the indicated organs and the corresponding percentage to total plant N (n=45). Asterisks in the right-hand chart indicate significant mean or median differences among nitrate and urea treatment according to unpaired t-test (■) or Mann-Whitney rank sum test (■■■■■) at p<0.05. (B) Bars show means -SD (n=3). ANOVA or ANOVA on ranks (- -) results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. Significant mean difference among nitrate and urea treatment within a genotype is indicated by asterisks referring to the organ color code according to unpaired t-test at p<0.05. Different upper case letters within a bar indicate significant mean or median differences among the genotypes within nitrate treatment according to Tukey´s test (■■■■■) or Tukey´s test on ranks (■■), respectively, at p<0.05; different lower case letters indicate significant mean or median differences among the genotypes within urea treatment according to Tukey´s test (■■■■■) or Tukey´s test on ranks (■■), respectively, at p<0.05.

Although the mean total above-ground N content of the genetic diverse population plants was slightly higher after urea fertilization compared to nitrate, the relative N contribution of the different organs was similar under both treatments as about 2/3 of the total above-ground N was allocated to the generative organs, while at BBCH79 about 1/3 remained in the vegetative plant parts (Figure 3-18A). Interestingly, N allocation to stem and branches were identical under both treatments and contributed with 9 % and 14 % to total above-ground N. N allocation to the leaves was slightly higher under nitrate as N contents in both stem leaf fractions accounted each for 5 % of the total N acquisition while under urea their contribution was only 4 %, and N allocation to senescent leaves was even significantly higher under nitrate. Against this, N distribution to generative organs was slightly increased in urea treated plants: The sum of N allocated to both pod fractions accounted for 68 % under urea but only for 66 % under nitrate. In the genetic diverse collection this suggests a trend for delayed N retranslocation especially from leaves to pods by nitrate fertilization which was also manifested in a lower $N_{generative}/N_{vegetative}$ ratio under nitrate (Annex-12). However, this trend

was opposite to the results observed for the elite lines in 2012/2013 as well as in 2013/2014, when nitrate treated plants tended to translocate N earlier to pods (Figure 3-6, Figure 3-14).

There were huge differences between some of the genotypes in total N and N distribution among the organ fractions (Figure 3-18B). Variability within the population as expressed by the coefficient of variation was highest for the leaf fractions and then more variable under urea (0.79 and 0.66 for branch leaves, 0.67 and 0.89 for stem green leaves and 0.63 and 0.75 for stem senescent leaves under nitrate and urea, respectively). Genotypic variation in N allocation to the residual organ fractions was much lower with 0.26 and 0.39 for stems and branches, respectively, and 0.31 for both pod fractions under nitrate. Urea increased this variation in average by about 0.1. Thus, variability in shoot N distribution among the genetic diverse oilseed rape genotypes was comparable to the variations found in the elite lines. Similar to the elite lines, differences in shoot N allocation among the genotypes were rather a product of variation in organ DW than in N concentration of the organs, since N concentrations of most above-ground plant fractions remained relatively stable among the genotypes (except of branch leaves and main shoot pods), while the DWs of the organs differed to a larger extent (see Annex-9).

The fertilizer N form had no consistent effect on above-ground N allocation in the individual genotypes, although the N content in the branches was significantly higher in urea treated PBC011 and PBC013. In accordance with the population mean trend for higher N allocation to leaves but lower N allocation to pods under nitrate treatment compared to urea at the end of pod development (Figure 3-18A), 2/3 of the genotypes in the population showed a decreased $N_{generative}/N_{vegetative}$ ratio under nitrate (Annex-12).

3.3.3 Influence of the applied nitrogen form on yield formation

All yield parameters recorded in the elite line population were also accessed in the genetic diverse oilseed rape population.

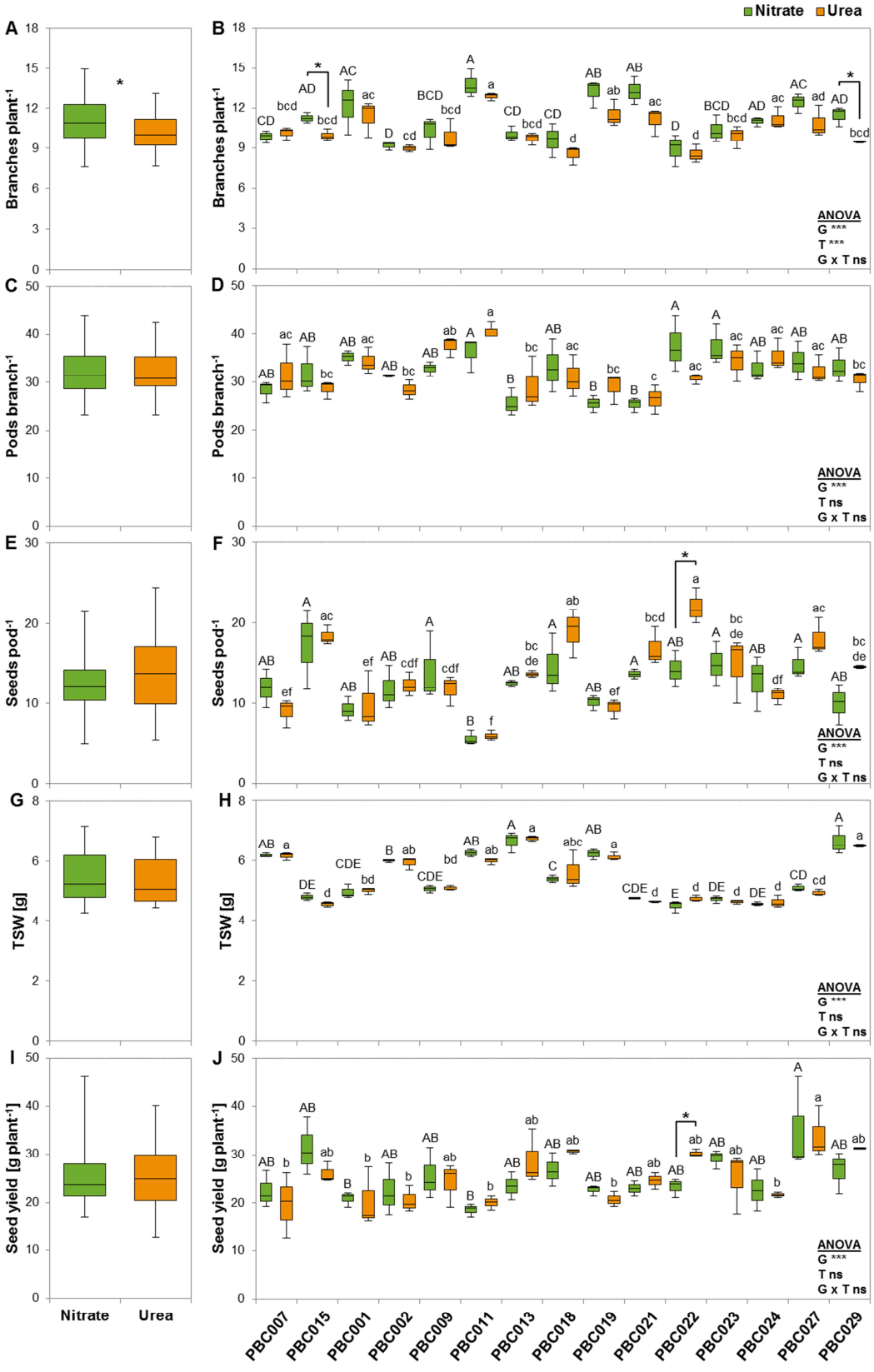


Figure 3-19 (For description see next page.)

Table 3-3: Stand density and area seed yield of the genetic diverse collection in the +N field trial 2011/2012. All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Yield parameters were determined as described in 2.6. Table shows means ±SD (n=3 for single genotypes, n=45 for all genotypes). ANOVA results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. Asterisks indicate significant mean difference among nitrate and urea treatment within a single genotype or among all genotypes according to unpaired t-test at p<0.05. Different upper case letters indicate significant mean differences among the genotypes within nitrate treatment according to Tukey´s test at p<0.05; different lower case letters indicate significant mean differences among the genotypes within urea treatment according to Tukey´s test at p<0.05.

Genotype	Plants m^{-2}				Seed yield [g m^{-2}]			
	Nitrate		Urea		Nitrate		Urea	
PBC007	11.5 ±1.9	AD	14.4 ±4.7	ab	252.9 ±4.3	E	263.7 ±27.1	ef
PBC015	10.9 ±1.2	BCD	13.2 ±1.9	ab	336.1 ±25.4	AB	340.2 ±23.9	ad
PBC001	12.5 ±0.5	AD	12.0 ±2.4	ab	260.0 ±9.9	DE	249.3 ±22.0	ef
PBC002	13.0 ±2.2	AC	14.2 ±1.2	ab	283.8 ±18.5	CDE	289.3 ±14.4	bcde
PBC009	10.9 ±2.6	BCD	11.3 ±1.8	ab	269.2 ±12.8	DE	269.4 ±20.7	ef
PBC011	10.7 ±0.2	BCD	10.3 ±0.8	ab	198.8 ±16.4	F	205.7 ±0.59	f
PBC013	14.0 ±1.4	AC	11.0 ±1.7	ab	325.5 ±13.0	AC	310.7 ±28.4	ae
PBC018	11.5 ±1.7	AD	10.0 ±1.1	ab	305.3 ±12.8	BCD	305.9 ±36.4	ae
PBC019	11.4 ±0.5	BCD	12.7 ±1.3	ab	257.7 ±2.3	E	260.9 ±7.8	ef
PBC021	12.3 ±1.0	AD	11.4 ±0.6	ab	268.8 ±19.9	DE	279.2 ±24.9	cde
PBC022	14.3 ±0.8	AB *	11.3 ±1.0	ab	332.3 ±12.5	AB	343.4 ±20.7	ac
PBC023	11.7 ±1.4	AD	14.8 ±4.3	ab	338.8 ±16.6	AB	352.4 ±3.0	ab
PBC024	16.0 ±3.5	A	16.5 ±0.8	a	352.1 ±9.8	A	356.1 ±8.3	a
PBC027	8.0 ±1.6	D	8.3 ±1.8	b	270.6 ±17.6	DE	276.7 ±25.2	de
PBC029	9.3 ±1.0	CD	8.6 ±1.0	b	246.1 ±22.2	E	282.2 ±33.7	bcde
All genotypes	11.9 ±2.4		12.0 ±2.8		286.9 ±44.6		292.6 ±45.7	
ANOVA	G ***, T ns, GxT ns				G ***, T ns, GxT ns			

Among the mean yield parameters in the population (Figure 3-19A, C, E, G, I) the number of branches per plant was significantly influenced by the fertilizer N treatment, as nitrate led to the formation of about one additional branch per plant compared to urea. Against this, urea fertilized plants tended to have increased plant height (see Annex-10). The number of pods per branch was not influenced by the N treatment and thus the total number of pods per plant was increased by the number of pods produced at one branch (about 30) in nitrate fertilized plants (see Annex-11). This higher pod production per plant resulted in the trend to form about 1.5 seeds pod^{-1} less under nitrate. However, the TSW was not affected by the applied N form. Since the stand density after winter (Table 3-3) as well as the seed yield per plant was similar in both N treatment groups, the final seed yield per square meter was not differing between the N treatments, too. Thus, although lines in the genetic diverse collection appeared to modify plant architectural traits in response to the applied N form, compensatory mechanisms in yield formation impeded their translation into enhanced seed yield.

Figure 3-19: Yield parameters of the genetic diverse collection in the +N field trial 2011/2012 as means over all genotypes (A, C, E, G, I) or of the single genotypes (B, D, F, H, J). All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Yield parameters were determined as described in 2.6. Boxes show median, first and third quartile; whiskers represent minimum and maximum of all data in a group (A, C, E, G, I: n=45; B, D, F, H, J: n=3). (A, C, E, G, I) Asterisks indicate significant differences among the means or medians according to unpaired t-test (A, C, I) or Mann-Whitney rank sum test (E, G), respectively, at p<0.05. (B, D, F, H, J) ANOVA results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. Asterisks indicate significant mean difference among nitrate and urea treatment within a genotype according to unpaired t-test at p<0.05. Different upper case letters indicate significant mean differences among the genotypes within nitrate treatment according to Tukey´s test at p<0.05; different lower case letters indicate significant mean differences among the genotypes within urea treatment according to Tukey´s test at p<0.05. TSW=Thousand seed weight.

Phenotypic variation in yield parameters among the lines was only little influenced by the N treatment and laid at 0.15 for the number of branches per plant, for the pods per branch, for the TSW and for the area seed yield, while variation in plant seed yield was higher (0.22). The number of seeds per pod was most variable among the lines with coefficients of 0.29 and 0.35 under nitrate and urea, respectively (Figure 3-19B, D, F, H, J, Table 3-3). Although in the genetic diverse oilseed rape collection a higher genotypic variability in the formation of individual yield components and in seed yield than in the elite lines was expected, only TSW appeared to be considerably more variable in genetic diverse lines with about twofold higher variation coefficients.

The already mentioned finding that most of the mean yield parameters and the seed yield were not influenced by the fertilizer N treatment was supported by the fact that the N form influence on yield and yield parameters of the individual genotypes was minor, too. However, the significant stimulating effect of nitrate on shoot branching observed for the population mean (Figure 3-19A) was based on two genotypes (PBC015 and PBC029), which differed significantly and 12 further genotypes which differed in tendency. Interestingly, PBC022 was the only genotype showing a significantly different behavior in yield formation under nitrate compared to urea as the urea variant produced a higher number of seeds per pod (Figure 3-19F) and thus a higher plant seed yield (Figure 3-19J). This was associated with a significantly lower number of plants per square meter in the urea plots so that the observed differences in yield formation of PBC022 appeared to be rather compensatory mechanisms for varying stand density than responses to the contrasting N fertilizer forms (Table 3-3).

Since it was of interest to identify distinct patterns of yield structure which might be potentially beneficial for final seed yield, yield formation was compared between the genotypes of the genetic diverse collection. In contrast to the elite lines, where up to 1/2 of the genotypes showed similar yield structure, most of the genetic diverse lines had a unique pattern of yield formation. However, several small groups existed: Compared to the population mean values PBC007 as well as PBC002 had rather low branch, pod and seed formation associated with elevated TSW resulting in plant seed yield below average. The yield formation in PBC013 was similar to that in PBC007 and PBC002, but seed number and seed weight were slightly higher resulting in a rather average seed yield. PBC001 and PBC011 formed also low plant seed yield, but based on a slightly different interaction among yield parameters: Both genotypes produced branches and pods above average and seeds below average, and although TSW was above average in PBC011 seed yield was the lowest in the population. PBC001 showed lower TSW than the population average and exhibited finally the second lowest seed yield. PBC015 and PBC027 built a rather high number of branches, average pod numbers and especially high numbers of seeds per pod with TSWs below average, resulting in highest seed yields among the population. Above-average seed yields were also found for PBC018 and PBC029 but with differing formation pattern: Both variants of PBC018 and the urea variant of PBC029 had branch numbers below average, average pod formation and seed production above average, but TSW was at average in PBC018 while PBC029 showed the second highest TSW in the population. Compared to the urea variant, nitrate-supplied PBC029 had a significantly higher branching and lower seed production, while the other parameters and the final seed yield were not changed. PBC009, PBC023 and PBC024 showed rather average branching and formation of pods and seeds with a TSW below average resulting in average seed yield production. Relatively similar yields were formed by PBC019 and PBC021 with another behavior: Both genotypes had branches above average with the trend for higher branch formation under nitrate. Against this, pod formation was below average and even lower under nitrate compared to urea, but while PBC019 produced less seeds with higher TSW, PBC021 behaved rather in the opposite way. Thus, most of the lines showed different yield

formation behavior, indicating a high diversity in yield formation among the genetic diverse oilseed rape collection.

Depending on yield formation of the individual genotypes general yield levels as well as yield structure of the populations in the three experimental years varied (Figure 3-7, Figure 3-15, Figure 3-19): Mean population branching was lowest in the elite line population 2012/2013 and comparable between the genetic diverse collection in 2011/2012 and the elite lines in 2013/2014, while pod production was highest in the 2011/2012 and comparable between the two following years. Seed formation per pod was strongly enhanced in 2013/2014 compared to the years before, but coincided with a lower mean population TSW. Total plant seed yield was highest in the elite line collection 2013/2014, followed by the diverse collection 2011/2012 with about 15 % lower yield and the elite lines 2012/2013 with about 35 % lower plant seed yield production. Since thus even yield patterns in the same population (elite lines in 2012/2013 and 2013/2014) appeared to be subject to seasonal influences, the reference lines PBC007 and PBC015 were used to evaluate the impact of environmental conditions for genotypic yield formation: Differences in yield levels of both lines followed a similar seasonal pattern as the population means (15 % lower yield in 2011/2012 and 50 % lower in 2012/2013 compared to 2013/2014), but it is also noteworthy that in the first and in the third year yields of PBC007 and PBC015 laid below and above the population average, respectively, while in the second year PBC015 produced rather average yield and PBC007 laid far below average. Interestingly, a superior seed yield formation per plant of about 30 % of PBC015 over PBC007 was observed in all three experimental years. However, their yield formation differed among the years: While in 2011/2012 and 2013/2014 a low stand density tended to lead to a higher branch formation in PBC015 (especially under nitrate in 2011/2012 and under urea in 2013/2014) than in PBC007, this effect was not visible at higher stand densities in 2012/2013. The pod number per branch was similar between both genotypes in all years, but seed production per pod in PBC015 was higher than in PBC007. Higher seed production of PBC015 appeared to be associated with lower seed weight in 2011/2012 and 2013/2014, while TSW formed in 2012/2013 was identical in the two reference genotypes. Thus, higher seed yield production of PBC015 appeared to be genetically determined, while the formation of individual yield components contributing to seed yield were rather subject to seasonal variation.

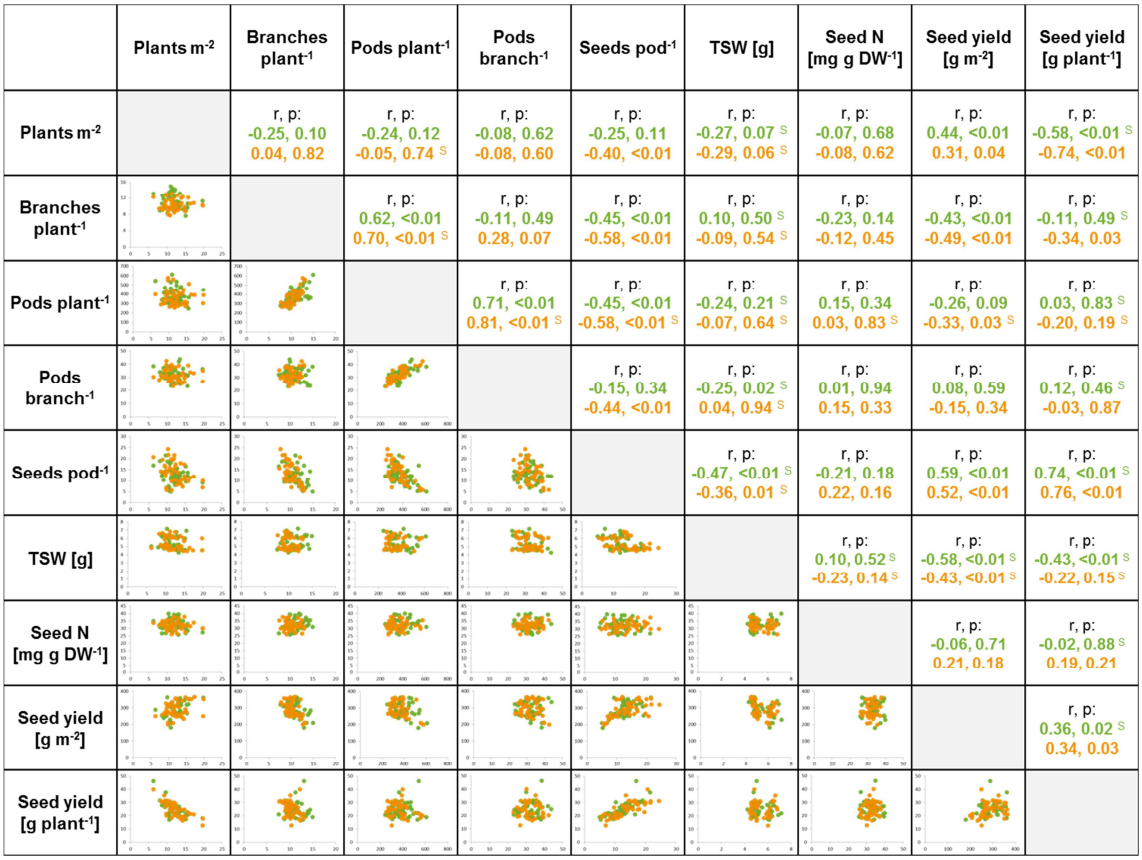


Figure 3-20: Correlations between the yield parameters of the genotypes in the genetic diverse collection in the +N field trial 2011/2012. All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Correlation charts as well as r and p value were calculated separately for nitrate (■) and urea (■) treatment; n=45. ^{S} indicates calculation of r and p value according to Spearman rank order correlation, while unmarked values accord to Pearson product moment correlation. Chart axes correspond to the yield parameter indicated on top or at the left-hand side of the matrix. TSW=Thousand seed weight.

Relations among the individual yield components of the genetic diverse oilseed rape population showed that under both N treatments the plant architectural traits number of branches and pods per plant were highly positively related, indicating that genotypes with higher shoot branching are also able to produce more pods (Figure 3-20).

However, higher numbers of branches and pods were associated with a lower seed formation per pod. Only under urea treatment plants produced less seeds per pod at higher stand densities. Regardless of the N form, higher seed formation per pod was associated with lower seed weight, while other yield parameters showed no relation to TSW.

The final plant as well as area seed yield in the genetic diverse collection of 2011/2012 was most depended on the number of seeds produced per pod. In accordance with this result, TSW was negatively correlated with plant and area seed yield formation. While the plant seed yield was significantly decreasing with increasing stand density, area seed yield production benefited from a higher number of plants per square meter. Against this, an increased branch and pod formation, which was not related to plant seed yield formation, appeared to be disadvantageous for area seed yield. However, the recorded seed yield per plant and per area was significantly positively correlated under both N conditions.

Thus, relationships between the recorded yield parameters were quite stable in all experimental years (2011/2012-2013/2014), suggesting that core mechanisms of compensatory responses in yield formation might be valid regardless of genotype, N form influence or environmental conditions (Figure 3-8, Figure 3-16, Figure 3-20). Thereby, final seed yield appeared to benefit most robustly from the number of seeds formed per pod.

3.4 Influence of nitrogen fertilization and form of fertilized nitrogen on plant nitrogen acquisition and yield formation in the oilseed rape cultivars PBC007 and PBC015

In parallel to investigations on the influence of different N fertilizer forms, +N/-N field trials were installed using two different N levels (see 2.2.4. and 2.3.1) with the aim to characterize the effect of N fertilization per se on the root-to-shoot translocation of N and CK forms, above-ground N allocation, yield formation and finally N efficiency of oilseed rape. Therefore, the two reference genotypes PBC007 and PBC015 were chosen from the genetic diverse collection (see 2.1) based on their contrasting seed yield in 2011/2012 (Figure 3-19) in combination with contrasting N concentrations in the youngest leaves of the stem (see Annex-13): PBC015 reached a higher yield, regardless of the fertilized N form, which was associated with lower leaf N concentration. Therefore, PBC015 was assumed to utilize fertilized N more efficiently for retranslocation from leaves to developing seeds and thus to show a higher N efficiency than PBC007.

3.4.1 Influence of nitrogen fertilization and fertilized nitrogen form on xylem nitrogen and cytokinin translocation

In order to characterize the effect of N fertilization on the root-to-shoot translocation of N, N forms and CKs, the two genotypes PBC007 and PBC015 were grown in two subsequent years in field trials applying either no N fertilizer or an equal amount of N in the form of nitrate or urea.

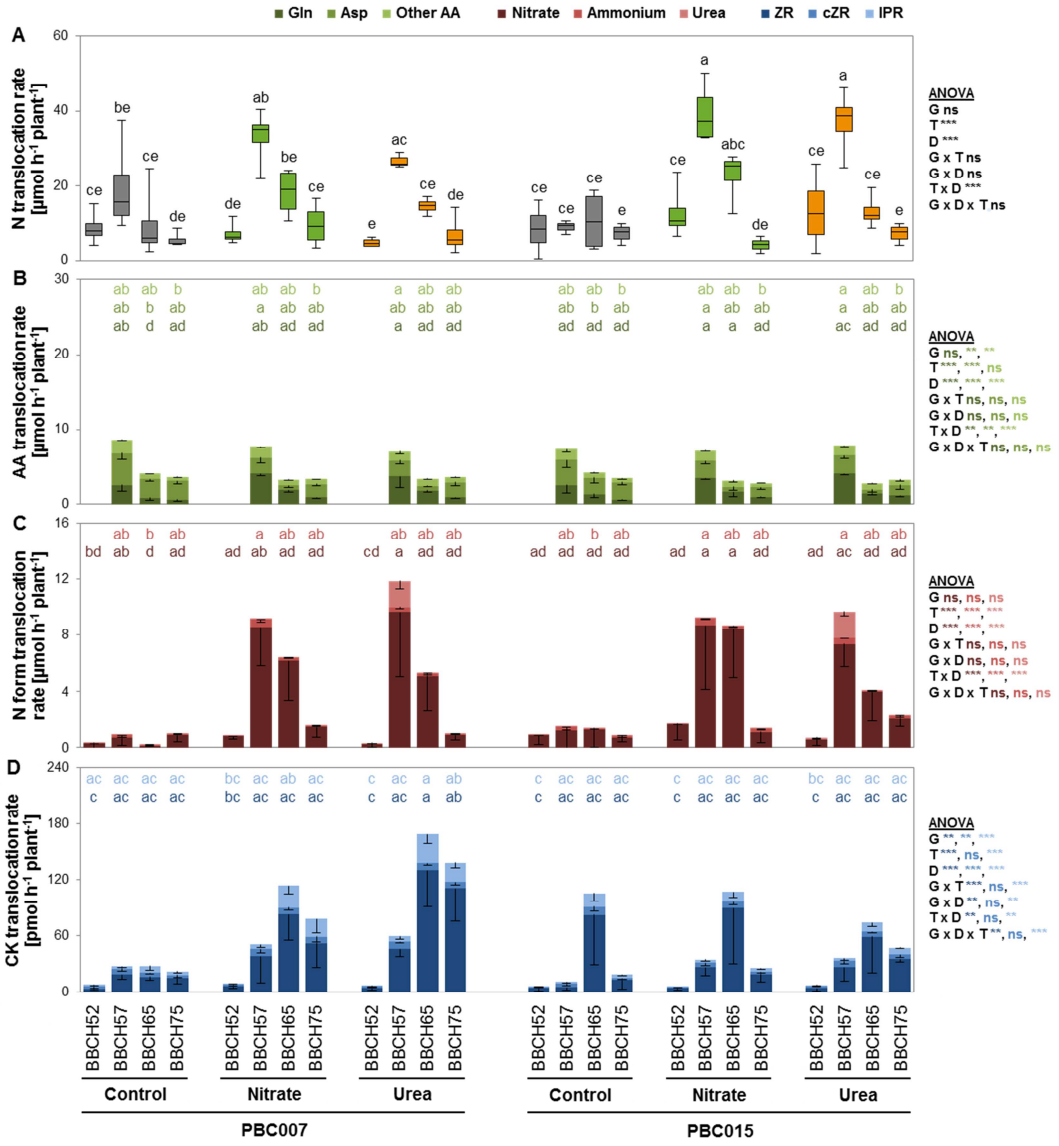


Figure 3-21: Translocation of total N (A), AAs (B), N forms (C) and CKs (D) in the xylem sap of the reference genotypes PBC007 and PBC015 in the +N/-N field trials 2012/2013 at BBCH52, BBCH57, BBCH65 and BBCH75. Both rapeseed genotypes were fertilized in spring with 40 kg N ha^{-1} at BBCH30 and 60 kg N ha^{-1} at BBCH55 as nitrate or urea, while control treatment received no additional N fertilization. Xylem sap from ten plants per plot was pooled. (A-C) Bars show means -SD; n=4. (D) Boxes show median, first and third quartile; whiskers represent minimum and maximum of all data in a group; n=4. (A-D) ANOVA (C: ■, D) or ANOVA on ranks (A: ■■■, B: ■■■, C: ■■) results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively, and refer to the compound color code; G=genotype, T=N treatment, D=developmental stage, ns=non-significant. Different letters indicate significant differences among the means or medians according to Tukey´s test (C: ■, D) or Tukey´s test on ranks (A: ■■■, B: ■■■, C: ■■) at p<0.05. (B, C) Ammonium and AAs in the xylem sap could not be determined.

In the 2012/2013 trial, non-fertilized plants of both genotypes showed a rather constant and low rate of total N translocation in the xylem sap, while in fertilized plants total N translocation rates in both genotypes increased up to fourfold between BBCH52 and BBCH57 before decreasing at BBCH65 and reaching comparably low levels as at BBCH52 at BBCH75 (Figure 3-21A). There were no significant differences in total N translocation rates between N fertilizer forms and the two genotypes.

The peak in total N translocation rates at BBCH57 was reflected by a peak in xylem translocation rates of AAs (Figure 3-21B). While AA translocation rates could not be determined at BBCH52 due to too low sample volumes recovered, they decreased from

BBCH57 to later developmental stages by half regardless of genotype or N treatment. Remarkably, this pattern of AA translocation rates was independent of N fertilization, suggesting that residual soil N was sufficient to increase AA translocation rates at BBCH57. Nevertheless, N fertilization led to a significant change in AA composition as at BBCH57 and BBCH65 non-fertilized plants transported more Asp, while N-treated plants showed a higher translocation of Gln. Similar to the translocation of total N also translocation of AA remained independent of treatment and genotype.

Among the determined N forms in the xylem sap, nitrate was by far the most abundant N form (Figure 3-21C). Relative to nitrate, ammonium translocation mounted up to max. 10 % in fertilized and up to 25 % in non-fertilized plants, while urea was only found in the xylem sap after urea fertilization, then reaching up to 30 % of the translocation rate of nitrate. However, urea translocation was only seen shortly after urea application (at BBCH52 and BBCH57). Xylem translocation rates of these three N forms peaked at BBCH57, which was only seven days after application of 60 kg N ha^{-1} at BBCH55 (see Annex-14). This peak must have been an immediate consequence of the N fertilization as non-fertilized plants showed no such peak. In particular after this second spring N fertilization, the amount of N contained in the three N forms nitrate, ammonium and urea in the xylem sap was similar as to that of AAs. Although translocation rates of these three N forms together also decreased at later developmental stages, they showed a more differentiated seasonal pattern and thus appeared to contribute more to the fluctuations observed in total N translocation rates than in translocation rates of AAs. Calculating the sum of ammonium, nitrate, urea and AA (Figure 3-21B, Figure 3-21C), the total N translocation in the xylem sap could be fully explained by these N compounds in fertilized plants at BBCH75 and in all samples of non-fertilized plants, while at earlier developmental stages total N translocation in fertilized plants was higher.

In all samples, ZR, cZR and IPR were detected as the most abundant xylem-transported forms of CKs (Figure 3-21D). In each sample ZR was the major form followed by either cZR during flower development or by IPR at later developmental stages.

Total CK translocation rates did not follow the same time-dependent pattern as those of total N or any of the N forms. Instead, CK translocation peaked later at BBCH65 and thus was most likely not directly related to the N fertilizer treatment or to the amount of N being translocated to shoots. In contrast to the translocation of total N or N forms, translocation of CKs was different between the two cultivars: PBC007 showed mostly a higher CK translocation than PBC015, which was on top enhanced under N fertilization. In PBC015, CK translocation at BBCH65 showed a similar peak in non-fertilized and fertilized plants, indicating genotypic differences in CK translocation behavior between the two lines, which were independent of their N translocation and thus presumably also independent of overall N uptake.

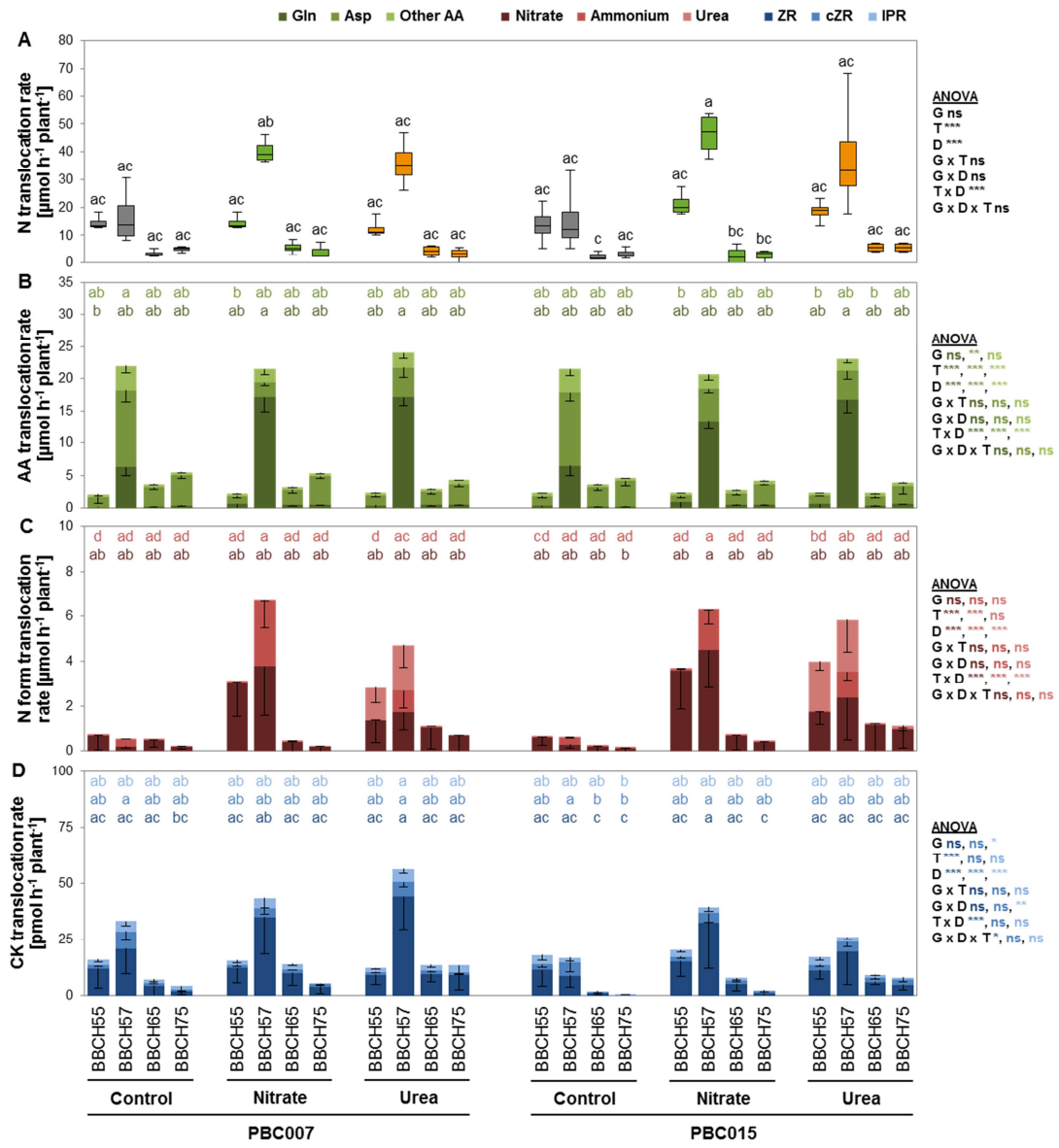


Figure 3-22: Translocation of total N (A), AAs (B), N forms (C) and CKs (D) in the xylem sap of the reference genotypes PBC007 and PBC015 in the +N/-N field trials 2013/2014 at BBCH55, BBCH57, BBCH65 and BBCH75. Both rapeseed genotypes were fertilized in spring with 40 kg N ha^{-1} at BBCH30 and 60 kg N ha^{-1} at BBCH55 as nitrate or urea, while control treatment received no additional N fertilization. Xylem sap from ten plants per plot was pooled. (A-C) Bars show means -SD; n=4. (D) Boxes show median, first and third quartile; whiskers represent minimum and maximum of all data in a group; n=4. (A-D) ANOVA (D) or ANOVA on ranks (A, B, C) results *, **, *** indicate significant differences or interactions at $p<0.05$, $p<0.01$, $p<0.001$, respectively, and refer to the compound color code; G=genotype, T=N treatment, D=developmental stage, ns=non-significant. Different letters indicate significant differences among the means or medians according to Tukey´s test (D) or Tukey´s test on ranks (A, B, C) at $p<0.05$.

To verify the influence of seasonal variations in the response of N and CK translocation to N fertilization, the same experiment was repeated in 2013/2014 (Figure 3-22). Again, total N translocation rates peaked at BBCH57 only in N-fertilized plants of both genotypes before dropping a bit sharper than in 2012/2013 at later developmental stages (Figure 3-22A). This peak was not caused by changes in the translocation of AAs, which also peaked at BBCH57 but irrespective of N fertilization (Figure 3-22B), confirming that in non-fertilized plants AA translocation was covered by uptake of residual soil N. Changes in the pattern of total N translocation were closely reflected by those in the translocation of nitrate, ammonium and urea (Figure 3-22C), which together accounted only for approximately 1/3 of the N being

translocated in the form of AAs. Remarkably, at BBCH57 - seven days after the application of 60 kg N ha^{-1} (see Annex-14) - ammonium translocation was just slightly lower than that of nitrate in nitrate-fertilized plants, while urea and ammonium translocation together surpassed that of nitrate after urea fertilization. Compared to 2012/2013, this more pronounced response to the fertilizer treatment in translocated N forms might have resulted from lower precipitation between fertilization and xylem sap sampling (14.6 mm in 2013 and only 2.6 mm in 2014), which probably reduced the conversion of the applied N forms and thus kept ammonium, nitrate (both from ammonium nitrate fertilizer) and urea more prominent in the soil for plant uptake.

As in the previous year, CK translocation rates in 2013/2014 were considerably higher in PBC007 than in PBC015. In both cultivars, however, they peaked at BBCH57. Over time and plant development, CK translocation rates followed most closely those of total N, as even subtle increases in total N translocation observed in non-fertilized plants were reflected in similarly subtle changes in CK translocation. As total N translocation correlates with total N uptake (Peuke, 2010), this coincidence together with positive relations between N and CK translocation observed in the elite line collection (Figure 3-5, Figure 3-13) suggested that xylem CK translocation may also be seen as an indirect measure for root activity.

3.4.2 Influence of nitrogen fertilization and fertilized nitrogen form on shoot nitrogen allocation

To verify an impact of different N treatments on total shoot N accumulation and N allocation to different plant organs, individual above-ground plant organs were harvested separately at the end of pod development for the determination of organ DWs and organ N concentrations.

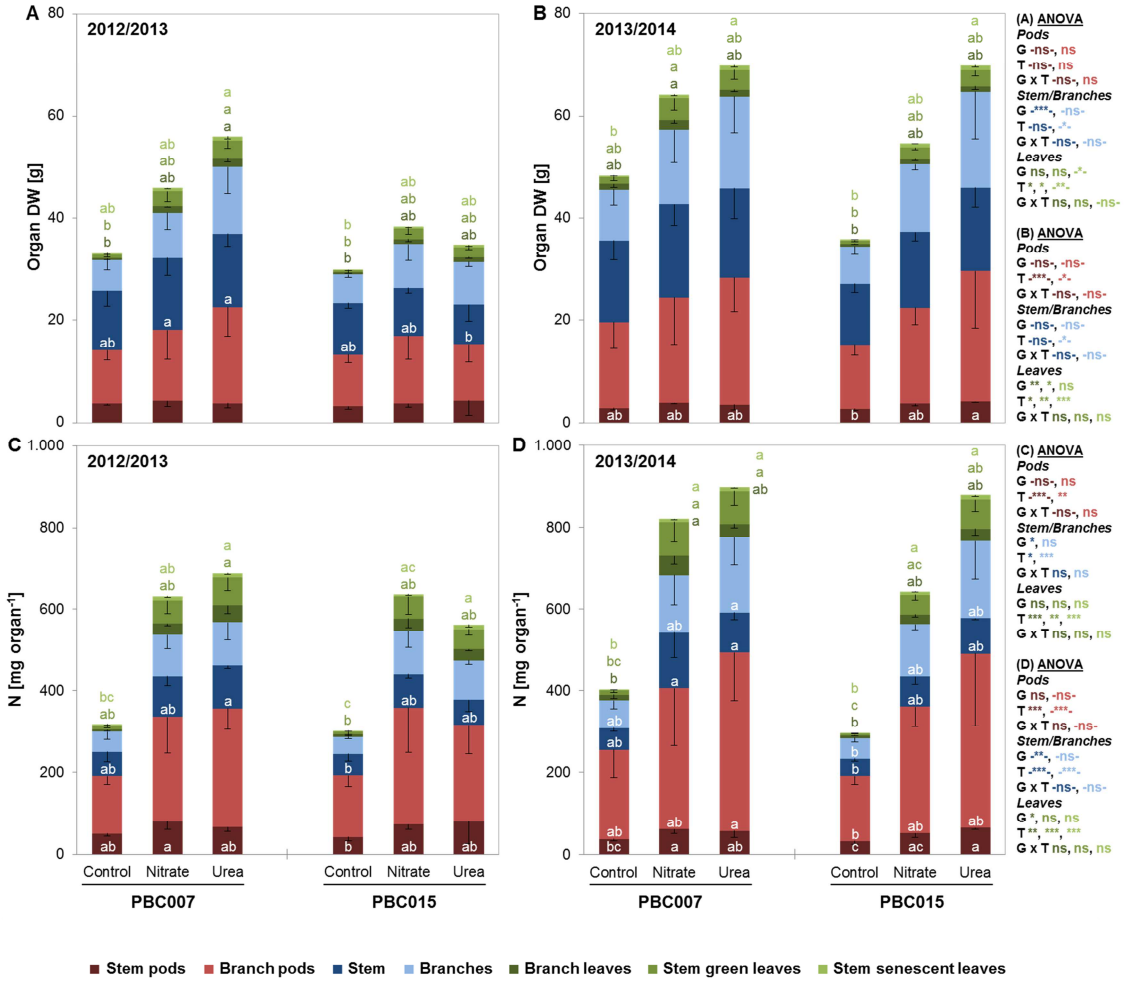


Figure 3-23: Organ dry weight (A, B) and total N pools in the above-ground plant biomass (C, D) at BBCH79 in the reference genotypes PBC007 and PBC015 in the +N/-N field trials 2012/2013 and 2013/2014. Both rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea while control treatment received no additional N fertilization. Two plants per plot were separated into the indicated above-ground plant fractions to determine their N content. (C, D) Values were calculated from the organ DW (A, B) and the N concentration of these organs (Annex-16). (A-D) Bars show means ±SD; n=4. ANOVA or ANOVA on ranks (- -) results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively, and refer to the organ color code; G=genotype, T=N treatment, ns=non-significant. Different letters indicate significant mean or median differences according to Tukey´s test (A: ■■■; B: ■■■; C: ■■■; D: ■■■■) or Tukey´s test on ranks (A: ■■■■; B: ■■■■; C: ■■■■; D: ■■■), respectively, at p<0.05.

Table 3-4: Relative increase in N allocation to above-ground plant organs in the reference lines PBC007 and PBC015 after nitrogen fertilization in the +N/-N field trials 2012/2013 and 2013/2014. Both rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea while control treatment received no additional N fertilization. Means of control treatment were set to 100 % and were used as basis to calculate the relative increase in N allocation to an organ after nitrate or urea treatment compared to the control; n=4.

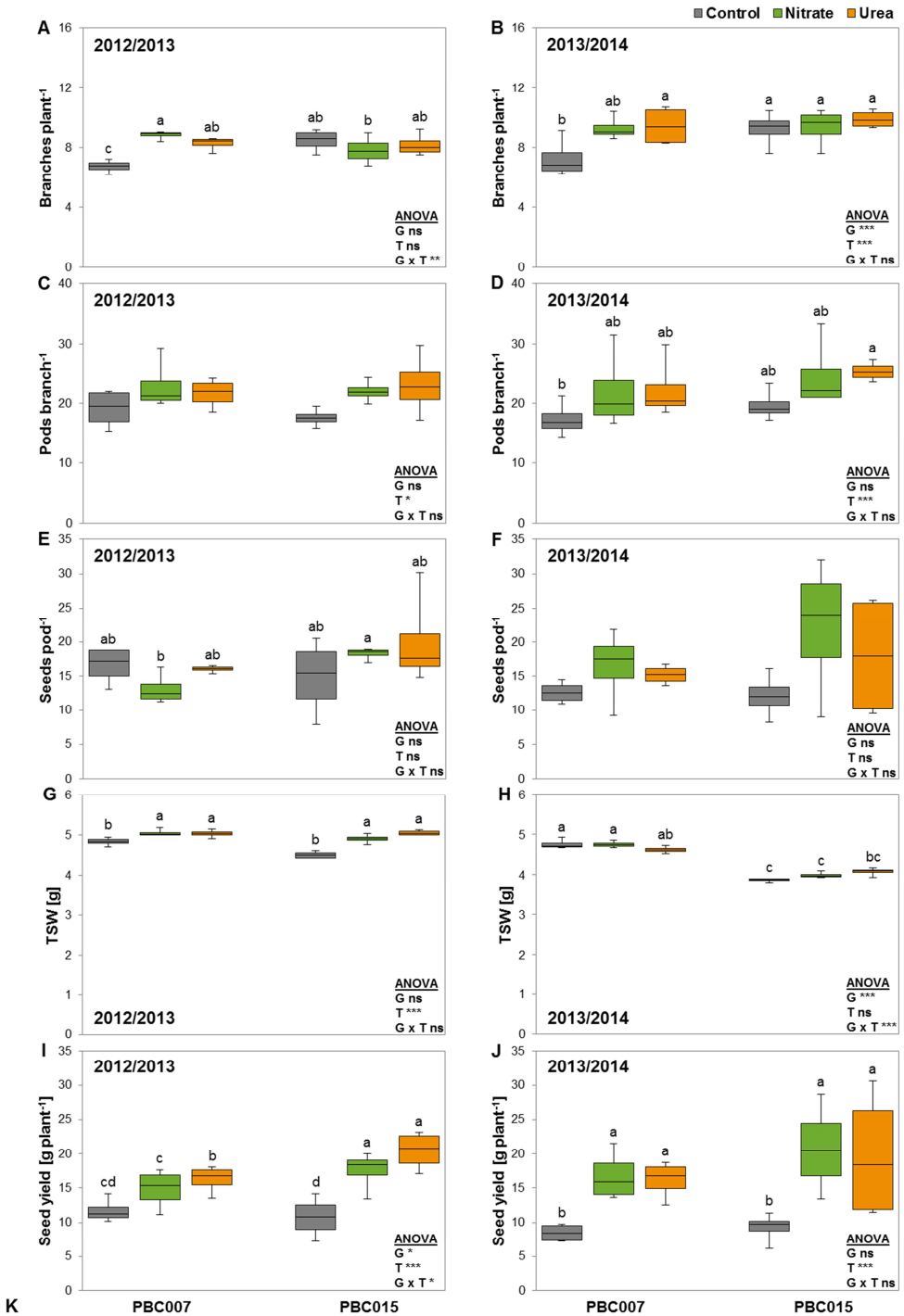
Plant organ	Relative changes in N allocation to indicated organ after N treatment compared to control [%]							
	2012/2013				2013/2014			
	PBC007		PBC015		PBC007		PBC015	
	Nitrate	Urea	Nitrate	Urea	Nitrate	Urea	Nitrate	Urea
Stem pods	66.1	74.4	53.1	15.1	156.7	104.0	73.0	107.2
Branch pods	107.7	118.8	155.8	131.6	109.8	204.2	157.2	281.3
Stem	60.2	31.8	72.0	88.2	65.1	63.6	59.0	99.1
Branches	81.3	106.3	90.0	57.4	57.6	114.2	94.6	167.6
Branch leaves	434.9	699.3	413.5	358.5	228.7	133.1	244.5	322.5
Stem green leaves	419.8	523.4	721.4	638.8	676.1	705.3	1089.1	1482.5
Stem senescent leaves	249.3	291.9	197.5	545.7	367.9	527.2	497.9	786.0

In 2012/2013, N supply increased N accumulation in most plant fractions irrespective of the fertilized N form (Figure 3-23). In case of the stem and the stem pods this was mainly due to higher N concentrations (see Annex-16) as dry weights were rather similar, while increased N accumulation in the other organs resulted from a combination of larger N concentration and larger organ dry weight. However, overall N pools increased in the different organs not to the same extent. N pools in green leaves at the stem or at branches profited most from N fertilization, followed by the N pools of senescent leaves (Table 3-4). In total, the overall N allocation to the shoot was approximately twofold higher under N fertilization. Although PBC015 produced lower biomass in most above-ground plant organs, total N allocation to the shoot and the individual shoot organs was quite similar to PBC007 due to higher N concentrations in PBC015 organs.

In 2013/2014, N fertilization promoted increases in shoot dry weight and N pools in both genotypes to a similar extent. Although all shoot fractions responded to N supply with an increase in biomass and N accumulation, leaves (and this time in particular green leaves from the stem) accumulated most of the fertilized N. In both lines, urea fertilization tended to increase N pools in branches, branch pods and in the stem leaf fractions even more than nitrate fertilization. Comparing the relative changes in N allocation to the different above-ground plant fractions in fertilized and non-fertilized plants of both genotypes emphasized that N fertilization increased N accumulation mostly in leaf fractions: In both years, N accumulated in the green leaf fraction of the stem was highest compared to control, followed by the green leaves derived from branches in 2012/2013 or the senescent leaves from stems in 2013/2014.

3.4.3 Influence of nitrogen fertilization and fertilized nitrogen form on yield formation

To analyze the impact of N fertilization with respect to the fertilized N form on the formation of individual yield parameters and potential compensatory mechanisms in yield formation stand density, number of branches per plant, number of pods per branch, number of seeds per pod and the individual seed weight were determined. Finally seed yield was recorded.



	2012/2013						2013/2014					
	PBC007			PBC015			PBC007			PBC015		
	C	N	U	C	N	U	C	N	U	C	N	U
Plants m^{-2}	18.9 ±1.1	20.4 ±2.0	20.2 ±0.7	18.1 ±2.6	17.8 ±2.4	19.1 ±3.9	18.3 ±3.8	17.0 ±4.3	17.3 ±2.1	16.8 ±3.5	14.5 ±4.2	16.0 ±5.9
Seed yield [g m^{-2}]	224.6 ±19.8 c	294.8 ±51.2 b	304.4 ±65.5 b	215.6 ±67.4 c	352.2 ±17.5 a	395.5 ±58.1 a	151.6 ±19.4 b	273.1 ±26.8 a	276.5 ±28.7 a	150.0 ±14.7 b	283.7 ±50.7 a	274.4 ±31.5 a

Figure 3-24 (For description see next page.)

Table 3-5: Relative changes in yield parameters of the reference lines PBC007 and PBC015 in response to nitrogen fertilization in the +N/-N field trials 2012/2013 and 2013/2014. Both rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea while control treatment received no additional N fertilization. Means of control treatment were set to 100 % and were used as basis to calculate the relative increase (■) or decrease (■) in a yield parameter after nitrate or urea treatment compared to the control; n=4.

Yield parameter	Relative changes in yield parameters after N treatment compared to control [%]							
	2012/2013				2013/2014			
	PBC007		PBC015		PBC007		PBC015	
	Nitrate	Urea	Nitrate	Urea	Nitrate	Urea	Nitrate	Urea
Branches $plant^{-1}$	31.4	22.9	7.9	3.5	27.8	30.2	1.4	7.0
Pods $branch^{-1}$	20.4	13.5	24.8	31.5	26.9	28.7	25.3	28.8
Seeds pod^{-1}	21.0	3.3	23.1	35.3	31.2	20.7	84.0	48.2
TSW	4.5	4.1	8.9	12.2	0.0	2.8	3.2	5.4
Plant seed yield	26.9	38.9	63.0	90.0	97.4	91.2	123.9	112.8
Area seed yield	31.2	35.5	63.4	83.4	80.2	82.4	89.1	82.9

In both experimental years, PBC007 had a considerably lower number of branches, i.e. 6 - 7 branches $plant^{-1}$, when N fertilization was omitted, while it formed approximately 2 branches $plant^{-1}$ more when either nitrate or urea was supplied. Interestingly, this was not the case in PBC015 which formed 8 - 9 branches $plant^{-1}$ irrespective of the level and form of N fertilization (Figure 3-24A, B). Comparing the overall branch number over both experimental years, plants in 2013/2014 consistently formed about 1 branch $plant^{-1}$ more in each genotype and treatment than in 2012/2013, as plants showed a better winter survival and thus a higher stand density than in the second year (Figure 3-24K). This difference in branch number plant per plant between non-fertilized PBC007 and PBC015 raised the question whether these two lines respond differently in yield composition to low N availability.

The effect of N fertilization on the formation of pods per branch was comparable between the genotypes as both formed in each year about 20 - 30 % less pods when N fertilization was omitted (Figure 3-24C, D, Table 3-5). The overall pod formation in the two lines was similar in both years.

Yield components formed during further progression of generative growth responded to N fertilization in a more variable manner. The seed number per pod of the two lines did not respond to N fertilization in 2012/2013, while in 2013/2014, seed production in both genotypes tended to increase after N application by about 25 % in PBC007 and, in spite of the high variation within the data groups for nitrate and urea, by 50 - 80 % in PBC015 (Figure 3-24E, F, Table 3-5). The larger seed number in PBC015 may have been associated with a lower stand density, because of less intraspecific competition for nutrients (Diepenbrock, 2000) and lower shading of pods by organs of other plants leading to increased photosynthetic capacity of pod walls and thus improved assimilate supply for seed development (Thurling, 1991).

Among all yield components, TSW showed the least variability within a treatment. In 2012/2013 there was a significant response of TSW to N fertilization in both genotypes, which, however, disappeared in 2013/2014 when overall TSW was lower (Figure 3-24G, H).

Figure 3-24: Yield parameters of the reference genotypes PBC007 and PBC015 in the +N/-N field trials 2012/2013 (A, C, E, G, I, K) and 2013/2014 (B, D, F, H, J, K). Both rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea while control treatment received no additional N fertilization. Yield parameters were determined as described in 2.6. (A-J) Boxes show median, first and third quartile; whiskers represent minimum and maximum of all data in a group; n=4. ANOVA results *, **, *** indicate significant differences or interactions at $p<0.05$, $p<0.01$, $p<0.001$, respectively; G=genotype, T=N treatment, ns=non-significant. (K) Table shows means ±SD; n=4. C=Control, N=Nitrate, U=Urea. (A-K) Different letters indicate significant differences among the means according to Tukey´s test at $p<0.05$. TSW=Thousand seed weight.

Thus, in both experimental years branches per plant and in tendency also pods per branch responded to N fertilization in a genotype-dependent manner, whereas in particular the formation of seeds was more strongly influenced by the environmental conditions of an individual year.

In each genotype and year seed yield per plant (Figure 3-24I, J) was lower when plants were not fertilized (Table 3-5). However, plant seed yield under N-deficient conditions appeared to be slightly higher in 2012/2013, while this was not the case under fertilized conditions when seed yield was more similar. This indicates a minor influence of the seasonal conditions and of the stand density on the genetically determined total seed yield production per plant when sufficient N was available. However, formation of these yields was variable as higher seed production per pod at lower stand density in 2013/2014 appeared to be compensated for by lower TSW. Also under N deficiency such compensatory effects appeared as PBC007 plants had a significantly lower number of branches per plant but higher TSW and thus achieved the same seed yield per plant as PBC015. This indicated that rapeseed possesses a wide range of compensatory responses in yield composition as also shown for the genetic diverse and the elite lines in the +N field trials (3.1-3.3).

3.4.4 Influence of nitrogen fertilization and fertilized nitrogen form on nitrogen-related agronomic traits

The agronomic interest of measuring N pools in plant fractions and their influence on yield composition lies in the determination of N efficiency parameters. As the overall N use efficiency in plants is a direct product of the two components N uptake efficiency (NupE), i.e. the total N uptake per unit N supply, and N utilization efficiency (NutE), i.e. the seed yield per total N uptake (Nyikako *et al.*, 2014), these two measures were calculated first.

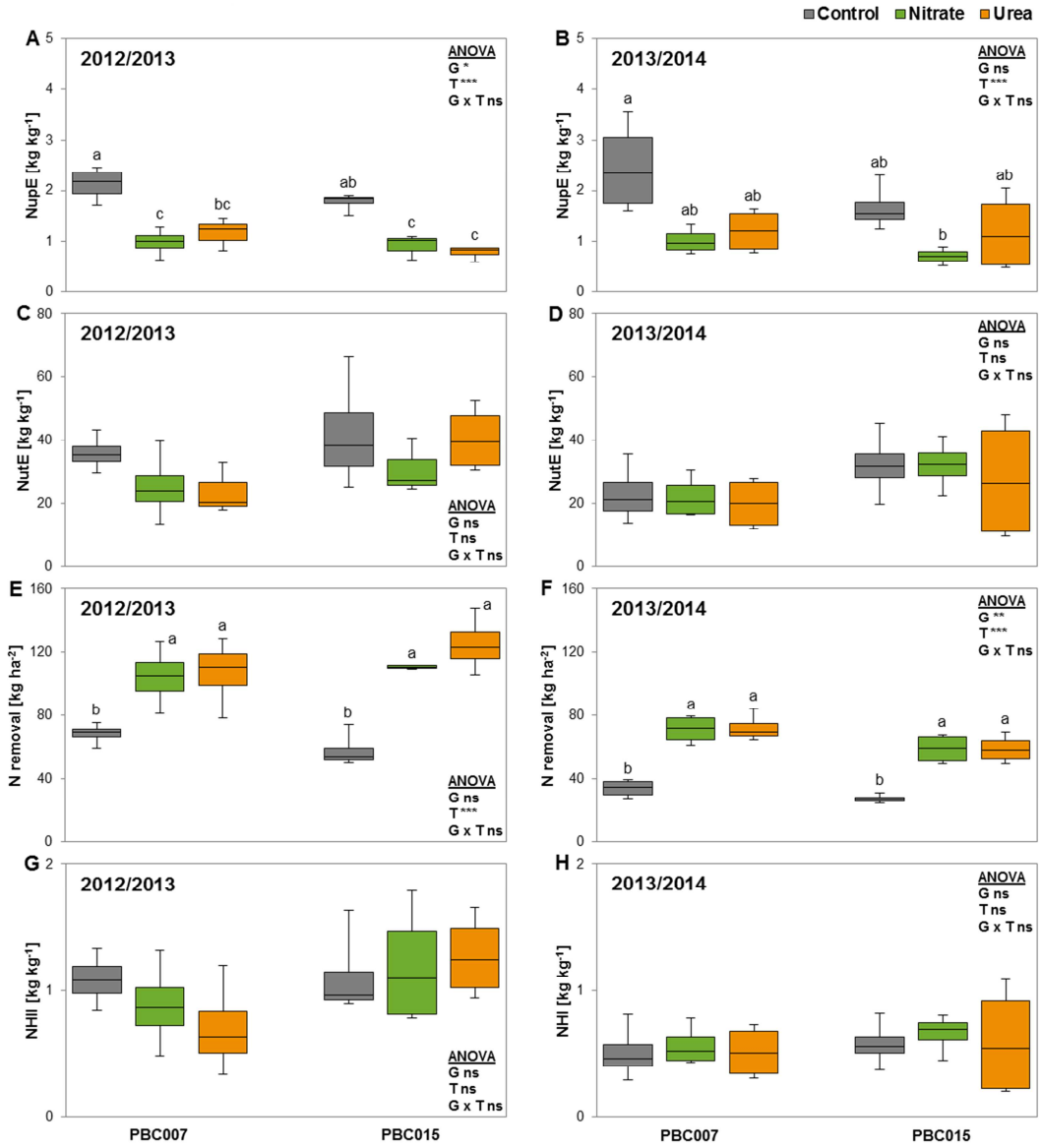


Figure 3-25: Agronomic N-related traits of the reference genotypes PBC007 and PBC015 in the +N/-N field trials 2012/2013 (A, C, E, G) and 2013/2014 (B, D, F, H). Both rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea while control treatment received no additional N fertilization. Traits were calculates as described in 2.7. Boxes show median, first and third quartile; whiskers represent minimum and maximum of all data in a group; n=4. ANOVA (A, C, E, G, F) or ANOVA on ranks (B, D, H) results *, **, *** indicate significant differences or interactions at $p<0.05$, $p<0.01$, $p<0.001$, respectively; G=genotype, T=N treatment, ns=non-significant. Different letters indicate significant differences among the means or medians according to Tukey´s test (A, C, E, G, F) or Tukey´s test on ranks (B, D, H), respectively, at $p<0.05$. NupE=N uptake efficiency, NutE=N utilization efficiency, NHI=N harvest index.

In both experimental years the influence of N treatments on NupE was nearly identical (Figure 3-25A, B): Non-fertilized plants of both genotypes consistently took up about

2 kg N per kg of available N, while in fertilized plants this ratio was about half, regardless of the fertilized N form. NutE did not significantly differ among N treatments in both lines in each year (Figure 3-25C, D), but PBC015 had a trend for higher NutE of about 10 kg seed yield per kg of N taken up in the fertilized variants in both years and in all N treatments in 2013/2014.

Since only NupE but not NutE significantly increased in non-fertilized plants, N efficiency (as the product of NupE and NutE) was consistently higher under low N supply in both genotypes over both experimental years and reached 75 kg (in 2012/2013) and 55 kg (in 2013/2014) seed yield per kg applied N in both lines. With regard to the fertilized N form, the urea-variant of PBC015 yielded consistently 30 kg seeds per kg available N compared to 22 kg of the nitrate-variant, while N efficiency of PBC007 appeared not to respond to the fertilizer N form and laid for both at 22 kg seeds per kg available N in every years.

Total N removal from the field significantly increased with N fertilization in each genotype and year (Figure 3-25E, F), while the fertilized N form tended to have only a marginal influence. In PBC007, N fertilization led to higher N removal of approximately 40 kg N ha^{-1} in both years, although N removal by PBC007 in 2013/2014 was much lower than in the first year. In principle, a highly similar observation was made for PBC015.

With a N recovery of about 0.5 kg N per kg of N taken up by the plants, NHI was relatively stable across genotypes and N treatments in 2013/2014, while in 2012/2013 NHI was about twofold higher (Figure 3-25G, H). Thus, despite an approximately twofold higher but - even though not significant - N removal in fertilized plants, NHI turned out to be almost non-responsive to N fertilization, as already described by Hocking *et al.* (1997) or Schulte auf'm Erley *et al.* (2011), but mostly influenced by seasonal differences from year to year. This clearly indicated that NHI is neither subject to genotypic variation nor to N management, which makes this parameter difficult to be used for the improvement of N fertilizer use efficiency in this study.

4 Discussion

4.1 Root-to-shoot translocation of cytokinins and nitrogen in oilseed rape in dependence of the amount and form of applied nitrogen fertilizer

4.1.1 Xylem translocation of *trans*-zeatin riboside appears to reflect root activity in the course of oilseed rape development

When ZR translocation rates in the xylem sap of different oilseed rape cultivars were determined at different developmental stages in different years they showed a developmental pattern with increasing rates until flowering and a decrease afterwards (Figure 3-4, Figure 3-12, Figure 3-17). Among the different CK forms, the relative contribution of ZR to total xylem CK translocation remained stable at 65 - 75 % in all years and at all developmental stages. IPR translocation followed in principle the same developmental pattern as ZR, but was considerably less abundant in the xylem sap. This coincides with the general finding of ZR being the major xylem CK transport form, probably because the monooxygenase CYP735A required for Z-type CK synthesis is predominantly expressed in roots; at least this was found in *Arabidopsis thaliana* (Takei *et al.*, 2004b). In contrast, IP-type CKs are most abundantly found in the phloem (Kudo *et al.*, 2010). Based on cell-culture activity assays Z- and IP-type CKs are regarded as the most active natural CK forms (Schäfer *et al.*, 2015). During flower development, which is accompanied by shoot elongation in oilseed rape, shoot as well as root growth are strong (Deligios, 2009). High ZR translocation into the shoot in this phase appears to be important to meet the requirement of shoot apical and floral meristems for CKs mediating cell division. Therefore, xylem-translocated ZR is converted into the biologically active Z via a LOG-type phosphoribohydrolase, which is specifically expressed in the shoot apical meristem (Kyozuka, 2007). However, as CKs are mainly synthesized in active root meristems (Engels *et al.*, 2012) the increasing xylem ZR levels may also indicate the growth rate or even more the physiological activity of the root system, which appeared to increase during flower development until mid of flowering (Figure 3-4, Figure 3-12, Figure 3-17). Since N is needed as macronutrient for plant growth and development (Hawkesford *et al.*, 2012), the simultaneously decreasing root-to-shoot translocation rates of N may reflect an enhanced requirement of the growing root system for N (Figure 3-1, Figure 3-2, Figure 3-3, Figure 3-9, Figure 3-10, Figure 3-11). Although the relative amount of N to CKs in the xylem sap decreased from BBCH57 to BBCH65, xylem CK translocation rates were positively associated with total N and partially with nitrate in both experimental years (Figure 3-5, Figure 3-13). This suggests that plants exporting a larger amount of cell division-stimulating CKs into the shoot deliver also larger amounts of N at that individual stage to meet the demand of the growing shoot for N. This relation held true only until flowering.

After flowering overall CK as well as N translocation rates decreased in the xylem sap (Figure 3-1, Figure 3-2, Figure 3-3, Figure 3-4, Figure 3-9, Figure 3-10, Figure 3-11, Figure 3-12, Figure 3-17). This may reflect ceasing CK production and N uptake by roots resulting from decreasing root activity from the end of flowering onwards (Gregory, 2006). However, especially at this later plant developmental stage total N transport from root-to-shoot was not consistently positively associated with CK levels (Figure 3-5, Figure 3-13). This suggests that, at least in 2012/2013, N retranslocation from the root to the shoot might have contributed to xylem N translocation as well. However, in previous studies the taproot has been regarded only as a minor source of endogenous N for reallocation during pod filling compared to leaves and stems (Rossato *et al.*, 2001; Malagoli *et al.*, 2005).

Although the mean IPR translocation in the xylem sap mostly decreased at the end of flowering, the relative contribution of IPR to total xylem CKs increased from about 10 - 15 % between flower development and flowering (monitored in all experimental years) to 25 % at pod formation (Figure 3-4, Figure 3-12, Figure 3-17). These relatively stable results suggest an environmentally-independent process in IPR/total CK translocation ratios when plants come to maturity. One possibility is that the conversion of IP-type nucleotides for the synthesis of ZR-type CKs, which in *Arabidopsis thaliana* is catalyzed by the predominantly root-expressed CYP735A monooxygenases (Takei *et al.*, 2004b; see also Figure 1-1), was decreasing with progressing plant development resulting in higher xylem translocation of IPR. This increasing delivery of IPR to the shoot may be involved in pod development as ripening fruits of grapevine, tomato and strawberry also contained a large amount of IP-type CKs. However, in that study synthesis and activation of IP-type CKs appeared to take place directly in the grape fruits rather than being delivered via the vascular system (Böttcher *et al.*, 2015). So, if there is an involvement of xylem-translocated IPR in pod ripening, it could be rather in long-distance signaling.

In contrast to ZR and IPR, cZR translocation rates mostly decreased steadily from flower development until pod formation (Figure 3-4, Figure 3-12, Figure 3-17). In *Arabidopsis thaliana*, cZ-type CKs were described to account for 70 % of total CKs in seeds, but their levels are lower in vegetative plant tissues where Z-type CKs become dominant and may represent over 90 % of the CK forms. During early plant senescence, however, cZ levels increase to about 20 % of total CKs (Gajdošová *et al.*, 2011). Similar relative amounts of cZR were also observed in this work to be translocated from root to shoot in the xylem sap shortly before flowering, with cZR accounting for 15 % (2012/2013) to 30 % (2011/2012 and 2013/2014) of total CK translocation at BBCH57 or BBCH59. In 2011/2012, when first xylem sap was taken already at BBCH52, mean population cZR translocation accounted even for 40 % at the beginning of flower development. As oilseed rape also accumulates high levels of cZR in shoot apices during vernalization (Tarkowská *et al.*, 2012) the abundance of cZ-type CKs could play a role in flowering control.

Schäfer *et al.* (2015) proposed a shift in the cZ(R)/Z(R) ratio towards the *cis*-isomers during plant senescence. This may be related to their weaker action on cell division. In the present study, against this, cZ(R)/Z(R) ratios were decreasing from flower development to flowering in every year, but to different extents, and remained stable until pod formation (Figure 3-4, Figure 3-12, Figure 3-17). However, since cZ-type CKs appear to be also important in mediating responses to growth-limiting conditions resulting from various abiotic and biotic stresses (Schäfer *et al.*, 2015), development-dependent cZR abundance in the xylem could have been influenced by unfavorable environmental conditions, which may have occurred manifold during the field experiments, even though biotic stresses were mitigated by chemical pest and weed management (Table 2-5).

4.1.2 Nitrogen fertilization stimulates the translocation of nitrogen compounds to the shoot especially before flowering

At relatively early stages of plant development in spring, BBCH52 and BBCH55, the genotypes PBC007 and PBC015 showed no differences in total N transport to the shoot between non-fertilized and fertilized variants, the latter receiving a N dose of 40 kg N ha^{-1} one week before xylem sap was harvested. Translocation rates of N compounds were even identical in both cultivars and both experimental years (Figure 3-21, Figure 3-22). Thus, both cultivars had apparently similar seasonally-independent N demands during early flower development, and these demands might have been fully met by the N provision from the soil at that time of spring, when about 20 kg N ha^{-1} were available as N_{min} before the N treatment (Table 2-3). Malagoli *et al.* (2005) showed that the N accumulation by the winter oilseed rape cultivar

Capitol increased from the later phase of stem extension to the stage of visible flower buds by approximately 50 mg per plant, which corresponds to an uptake of 7.5 - 10 kg N ha^{-1} at stand densities of 15 - 20 plants m^{-2} as observed in this work (Figure 3-24). Also the similar CK export to the shoot supposed that non-fertilized plants were not negatively affected in their root growth in early spring.

Until BBCH57 both genotypes increased their root-to-shoot N translocation by about fourfold when additional 60 kg N ha^{-1} were applied, while in non-fertilized plants N translocation in the xylem sap remained at the level of the previous developmental stage, and this was again a season-independent observation (Figure 3-21, Figure 3-22). Thus, plants relying solely on soil N resources appeared to be limited in N availability at the end of flower development and invested probably considerable proportions of remaining N into root growth to reach soil N reservoirs rather than exporting N to the shoot (Hawkesford *et al.*, 2012). Accordingly, the substantially lower translocation of nitrate to the shoot observed in non-fertilized plants in both experimental years might have been a result of higher root nitrate assimilation under N deficiency (Andrews, 1986). Since roots have a larger need for C skeletons under these conditions (Marschner *et al.*, 1996), it appears logic that N-limited oilseed rape plants cycled 50 % of their AAs in form of Asp to the shoot, which contains one C atom less than Gln (Schlatter and Langer, 2008) - the most abundant AA in the xylem sap of fertilized variants. Moreover, due to the fact that Asp contains also one amino group less than Gln (Schlatter and Langer, 2008), AAs might act as mobile messengers of the root N status (Barneix and Causin, 1996). The information of a lower N availability for non-fertilized plants appeared to be transmitted to the shoot also by lower xylem ZR translocation rates (Figure 3-21, Figure 3-22). Under such conditions above-ground CK synthesis must increase to ensure sufficient CK supply to the growing shoot organs (Aloni *et al.*, 2006). Unlike ZR translocation, however, cZR export to the shoot was increased in non-fertilized plants, at least in 2013/2014, probably to reduce cell division activity in shoot meristems (Schäfer *et al.*, 2015) and to transmit the information of lower cell division activity in root meristems to the shoot. Furthermore, since impaired cZ biosynthesis in roots may lead to reduced root meristem size and misplaced protoxylem formation (Schäfer *et al.*, 2015), certain amounts of cZ-type CKs appear to be important for enabling root growth under N limitation.

Usually, N-deficient plants are more inhibited in shoot than in root growth and thus increase their root/shoot ratio (Gruber *et al.*, 2013). To what extent, however, root growth was affected by lacking N fertilization in this work can only be indirectly concluded from the originally contemplated marker “xylem CK translocation”, since the production of these phytohormones may have been too closely related to the signaling of different N nutritional conditions. Hence, it is critical to employ them rather as suitable marker for meristem activity, especially under varied N supplies.

Xylem translocation of N until flowering (BBCH65) decreased in the fertilized variants of both genotypes in both years, likely indicating a declining root N uptake activity (Figure 3-21, Figure 3-22). Indeed, nitrate influx via HATS and LATS drastically decreases at the transition from vegetative to generative plant development in oilseed rape (Malagoli *et al.*, 2004) until N uptake becomes insignificant during pod filling (Rossato *et al.*, 2001). However, xylem N translocation dropped sharper in 2013/2014 so that fertilized plants exported almost the same small amounts of total N, AAs and nitrate to the shoot as non-fertilized plants, while in 2012/2013 N delivery to the shoot was still two- to threefold higher when N was supplied. This could have been a result of the exceptional long cold period at the beginning of the year 2013, when temperatures were close to the freezing point until the end of March. Such growth conditions may have forced plants to pass through their development from April onwards much faster in 2013 than in 2014 and thus to start flowering although stem elongation was not yet completed (Figure 2-1). On the other hand, as several studies describe an increase in root growth (Kappen *et al.*, 2000; Kamh *et al.*, 2005) or nitrate uptake (Rossato *et al.*, 2001)

between flowering and pod filling, the question arises if in 2013/2014 the root-to-shoot translocation of N at BBCH65 was limited. Considering that in total 100 kg N ha^{-1} were added on top of the soil reservoir of 20 kg N_{min} ha^{-1} during spring but total accumulated above-ground N, as determined at BBCH79, was between 130 and 150 kg N ha^{-1} (calculated from Figure 3-23D and Figure 3-24K) and that almost all AAs translocated to the shoot at BBCH65 were in form of the putatively N-deficiency signaling Asp, it might be possible that also the fertilized variants became N limited during generative plant development in the field season 2013/2014. However, data on N accumulation in the above-ground biomass before spring, which also contributes to the shoot N pool, were not collected. Furthermore, the data shown for all xylem-translocated compounds represent snapshots and could have been influenced by short-term environmental conditions or stresses during their harvest.

At pod development (BBCH75) differences in N transport to the shoot between N-deficient and N-supplemented plants disappeared in both experimental years, most likely indicating a developmental influence by root senescence and thus a ceasing root activity under which N could not be further taken up.

4.1.3 The type of applied nitrogen fertilizer affects the composition of nitrogen forms in the xylem sap

A very pronounced effect, which could be observed in every genotype and year, was the transport of urea to the shoot whenever urea fertilizer was applied shortly before (Figure 3-3, Figure 3-11: BBCH57; Figure 3-21, Figure 3-22: BBCH52/BBCH55 and BBCH57). Most likely there was a short-term excess of urea over nitrate and ammonium in the soil stimulating the uptake of urea into the plant (Mérigout *et al.*, 2008). Indeed, as determined in 2014, seven days after the respective two spring fertilization events, urea was considerably more abundant in the soil than the other N forms (Table 2-4). Also Bauer (2014) found similar situations up to ten days after the application of urea fertilizer to field-grown wheat. Since in *Arabidopsis thaliana* the saturable, high-affinity urea transporter DUR3 is most likely negatively feedback-regulated by prolonged exposure to high concentrations of urea (Pinton *et al.*, 2016), urea uptake in the present study might have been rather conducted by aquaporins, which are known to mediate urea uptake in the low-affinity range (Liu *et al.*, 2003a). Most of the urea is thought to be assimilated already in roots (Pilbeam, 2015), which appears to accord with slightly higher total AA translocation rates under urea treatment in 2013/2014 (Figure 3-10). However, some urea is also translocated to the shoot (Pilbeam, 2015). Interestingly, xylem translocation rates of urea were similar in all considered genotypes at a distinct sampling time point. This may indicate a strong dependence of urea translocation on the mass flow of water to the shoot (Wilson and Walker, 1988; Liu *et al.*, 2003a). Indeed, higher xylem urea translocation appeared to be associated with higher xylem exudation rates (Annex-1, Annex-4, Annex-15).

At the same time as urea-fed plants translocated detectable amounts of urea to the shoot, nitrate and ammonium translocation decreased by about half compared to nitrate-treated plants, likely resulting from a lower availability of these two N forms in the soil shortly after urea application. In addition, transcriptional downregulation of the nitrate transporters *BnNRT1.1* and *BnNRT2.1* by urea may have contributed to lower nitrate translocation (Arkoun *et al.*, 2012). Moreover, at least in 2013/2014, urea-treated plants exported significantly larger amounts of their AAs in form of Asp, while nitrate-fertilized plants translocated larger quantities of Gln carrying one amino group more (Schlatter and Langer, 2008). This led, significantly or at least in trend, to an overall lower root-to-shoot translocation of total N relative to nitrate-fertilized plants (Figure 3-1, Figure 3-9, Figure 3-21, Figure 3-22). In the elite lines grown in 2012/2013 this lower N translocation held yet true until BBCH65 - 21 days after applying 60 kg N ha^{-1} - but without showing any translocation of urea anymore.

Against this, plants at BBCH65 in 2013/2014 even exported significantly more nitrate and consequently more total N to the shoot after urea application. However, in that year the fertilization event was already 40 days ago.

When solely urea is applied, i.e. without urease inhibitor, it is rapidly hydrolyzed by soil ureases so that under warm and humid conditions urea-N losses in the form of ammonia gas from the soil surface can reach up to 47 %. For this reason, urea fertilizers are often stabilized with urease inhibitors to slow down the hydrolysis of urea and allow longer-lasting availability of urea in the soil (Watson, 2005). NBPT, which was used in this work, is a structural analogue of urea with a stability of 10 - 14 days (Watson, 2005). It was therefore not surprising that shortly after fertilizer application urea was found to be present in soil samples and plants. Since the uptake capacity for urea is generally lower than that for nitrate (Tan *et al.*, 2000; Arkoun *et al.*, 2012), total N transport to the shoot was lower under urea supply. Furthermore, it was proposed that increased Gln and Asn levels in plants fed exclusively with urea inhibit N assimilation and lower N uptake (Witte, 2011). In the present study, at least in the xylem sap no higher amide levels were found after urea application. It appeared rather that plants in 2013/2014 at BBCH57 already shifted xylem AA levels towards Asp as indication of a temporary mild N deficiency likely resulting from 11 days of urea availability (Figure 3-10). Such symptoms were also observed by Arkoun *et al.* (2012) after ten days of rapeseed cultivation in hydroponics using urea as sole N form, and might have not been seen in this work in 2012/2013, since urea application was only two days before xylem sap harvest at BBCH57 (Figure 3-2, Figure 3-10). As result of the lower uptake capacity for urea, fertilizer-N might have been depleted at a relatively slower rate when urea was supplied compared to ammonium nitrate. However, since after a longer period between fertilizer treatment and xylem sap harvest NBPT will be degraded, applied urea becomes more and more hydrolyzed, forming first ammonium and further nitrate via nitrification (Mérigout *et al.*, 2008), which become then more important forms of N uptake for the urea-fertilized variants. It thus appears that urea application led to a delayed formation of nitrate-N in the soil, increasing N provision to plants for a longer time after N fertilization took place. This appeared to be reflected also in the profile of xylem-translocated AAs, as urea-fed plants had lower Asp and in trend higher Gln levels at BBCH65 in 2013/2014. Another reason for higher N translocation to the shoot 40 days after urea application compared to nitrate could have been that soil-N levels under nitrate decreased faster, since nitrate-N is very prone to leaching into deeper soil layers (Thorup-Kristensen, 2001) and ammonium-N can be lost as ammonia gas to the atmosphere (Arkoun *et al.*, 2012). This might have been partially the case, since total above-ground N accumulation, as measured in every experimental year at BBCH79, was mostly slightly higher in the urea variants (Figure 3-6, Figure 3-14, Figure 3-18, Figure 3-23).

At BBCH75, differences in N translocation to the shoot between the two N variants were not significant any longer, likely indicating that the abundance of plant-available N forms in the soil reached similar levels. Moreover, during pod development in oilseed rape overall N export to the shoot may be rather determined by N retranslocation from the root (Malagoli *et al.*, 2005) than by de-novo uptake of N from the soil (Malagoli *et al.*, 2004).

4.1.4 Nitrate fertilization has a stimulating short-term effect on xylem cytokinin transport

Shortly after the application of 60 kg N ha^{-1} in the form of nitrate, xylem translocation rates of ZR were higher than in urea-fertilized plants (Figure 3-4, Figure 3-12, Figure 3-17). In contrast, root-to-shoot translocation of cZR and mostly also of IPR remained unaffected, probably because the abundance of tRNA-IPTs, serving as precursors for cZ-type CK synthesis, is generally not affected by the plant nutrient status (Schäfer *et al.*, 2015) and IP-type CK synthesis in response to nitrate mainly takes place in shoots (Sakakibara *et al.*, 2006). This phenomenon was observed in every experimental year when regarding population means, and

at least in trend when regarding individual genotypes (Figure 3-4, Figure 3-12: BBCH57; Figure 3-17: BBCH59; Figure 3-21, Figure 3-22: BBCH52/BBCH55 and BBCH57). This accords with the common view that nitrate and ammonium stimulate Z synthesis (Takei *et al.*, 2004a; see also 1.4.2), while urea appeared to exert no such pronounced effect. It is thus most likely that the higher uptake capacity for nitrate as N source (Arkoun *et al.*, 2012), as seen by the higher xylem N translocation shortly after nitrate application (Figure 3-1, Figure 3-9, Figure 3-21, Figure 3-22), is transmitted via a CK signal to the shoot to mediate above-ground N responses (Sakakibara *et al.*, 2006) and to adjust cell-division activity to N supply (Böttcher *et al.*, 2015). A certain role might also play the xylem exudation rate, which was mostly a bit higher in the nitrate-fed variants (Annex-1, Annex-4, Annex-8, Annex-15). Possibly, nitrate itself stimulated the transfer of water within the root (Sakakibara *et al.*, 2006) by controlling water channel activity (Hoarau *et al.*, 1996). Furthermore, nitrate-induced CKs promote stomata opening (Sakakibara *et al.*, 2006) which might have resulted in a higher water flow through the xylem. However, such differences in xylem exudation rates were only observed in tendency but were not significant.

Whether de-novo synthesis of Z in response to nitrate availability was the origin of higher ZR levels in the xylem shortly after nitrate application cannot be answered using the investigated parameters in this work. Bauer (2014) described that the expression of *IPT3* in barley roots was not differentially influenced by the N forms nitrate and urea. However, as the author discussed, grasses could use another isoform than *IPT3* being responsive to different N forms or could generally use other proteins for N-mediated CK synthesis. Since in the experiment of Bauer (2014) plants were already cultivated for 40 days on the respective N-forms, Z synthesis via IPT5 might have played also a role as this enzyme is known to be stimulated by longer-lasting nitrate and ammonium availability (Takei *et al.*, 2004a).

A close relation between N availability and CKs (Sakakibara, 2006) was seen even longer time after fertilizer application: In 2012/2013, higher total N translocation to the shoot 21 days after nitrate application (BBCH65) was associated with, at least in tendency, higher xylem ZR translocation rates, whereas in 2013/2014, when urea led to higher N export to the shoot at BBCH65, all CK forms were more abundant in urea-treated plants (Figure 3-1, Figure 3-4, Figure 3-9, Figure 3-12). At BBCH75, plants in both experimental years had still higher translocation rates especially of ZR although differences in N export to the shoot became already insignificant. This could indicate that plants still sensed a higher N availability for a longer time after fertilization of urea compared to nitrate (as discussed in 4.1.3), although root N uptake may have already declined in response to plant senescence (Malagoli *et al.*, 2004). However, the mostly positive correlations between xylem nitrate or total N translocation and CK abundance at individual developmental stages support the notion that oilseed rape transmitted information on the actual N availability via a CK signal from root to shoot (Figure 3-5, Figure 3-13). This matches the common view of CKs being signaling molecules for the plant N status as already described for other plant species, such as *Arabidopsis thaliana*, maize, barley or wheat (Kamada-Nobusada *et al.*, 2013).

4.2 Influence of nitrogen fertilization on nitrogen allocation to the shoot

4.2.1 Nitrogen fertilization enhances shoot nitrogen accumulation and stimulates nitrogen allocation especially to leaves

Consistently over both experimental years 2012/2013 and 2013/2014, the reference genotypes PBC007 and PBC015 accumulated considerably higher levels of above-ground N when N fertilizer was applied (Figure 3-23). This is not surprising, since in the non-fertilized variants N availability in the soil appeared to be limiting from late flower development onwards (Figure 3-21, Figure 3-22; see also 4.1.2). It is likely that a large part of the stem and partially also of branches was built before that stage, which could explain why N accumulation in these vegetative shoot organs was least affected by differential N fertilization (Table 3-4). In contrast, N accumulation in leaf fractions was much stronger affected due to significantly higher organ dry weights coupled with higher organ N concentrations. Under well-fertilized conditions, oilseed rape mainly covers the N demand of newly expanding vegetative organs directly via root N uptake (Malagoli *et al.*, 2005), allowing to develop a large leaf area index (Rathke *et al.*, 2006). Against this, limited soil N reserves force plants to earlier recycle N from older leaves to support the development of younger leaves, so that under these conditions sequential leaf senescence proceeds faster (Avice and Etienne, 2014) and the photosynthetically active period becomes shorter (Rathke *et al.*, 2006). Since also recycled N is not sufficient to meet the N demand of developing leaves, leaves remain smaller than under N-sufficient conditions (Rathke *et al.*, 2006). This may explain the strong increase in biomass of the leaf fractions when N was supplied (Figure 3-23). Especially in 2012/2013, N concentrations of the leaves appeared to be less affected by N supply than leaf biomass. This fits to the fact that leaf photosynthetic capacity is known to be only slightly increased under adequate N supply, while higher productivity mainly results from larger leaf area and prolonged photosynthetic activity (Rathke *et al.*, 2006). Since adequate assimilate supply is needed to ensure optimal meristem growth (Harper, 1987), N-fed plants were likely able to produce more pods and, most likely, also to increase assimilate production in pod walls to supply growing seeds under N-sufficient conditions (Gammelvind *et al.*, 1996). This may have resulted in a larger N pool size of the pod fractions and was probably more pronounced in 2013/2014, since lower stand density enabled better N availability for the individual plants (Figure 3-23, Figure 3-24).

As already observed by Svečnjak and Rengel (2006) the distribution of N among shoot organs might have been influenced by the applied N level rather than by the genotype: Under N-deficient conditions, above-ground N pools appeared to be reduced to similar sizes regardless of the genotype or experimental year (probably also because stand densities in control plots were similar). Genotypic differences in N pool sizes could only be observed under N-sufficient conditions so that then individual genotypic N uptake, N use, responsiveness to different N forms and also environmental impacts appeared to be important as well (Figure 3-6, Figure 3-14, Figure 3-18, Figure 3-23).

4.2.2 The applied nitrogen form does not significantly influence the relative distribution of nitrogen among shoot organs

The applied N fertilizer form had no substantial impact on the overall accumulation of N in the shoot when oilseed rape plants were examined close to maturity (Figure 3-6, Figure 3-14, Figure 3-18). However, in the first two experimental years there was a trend for higher N accumulation when urea was supplied. This is in accordance with a study of Arkoun *et al.* (2012) in which stabilized urea led to the highest plant N accumulation compared to non-stabilized urea, ammonium sulfate or ammonium nitrate. Likely, N from urea fertilizer was

available for a longer period (see 4.1.3) leading to higher plant N accumulation. Furthermore, Zanin *et al.* (2015) found that root growth of maize was stimulated by the presence of urea, so that plants treated with urea could likely explore more soil N reserves. Pinton *et al.* (2016) suggested that a simultaneous availability of nitrate with urea increases the efficiency of total N assimilation. Under these nutritional conditions, urea-originating ammonium is assimilated by a putative cytosolic pathway, including GS1 and an Asn synthase, while the assimilation of nitrate-originating ammonium is mediated by GS/GOGAT (Pinton *et al.*, 2016). Such situation might have been the case for several days after applying urea fertilizer in addition to soil nitrate reserves, but not after applying nitrate as fertilizer since, at least in 2013/2014 but likely also in the other years, no soil urea could be detected (Table 2-4).

In contrast to the first and second experimental year, such trend for higher N accumulation in urea-treated plants was only seen in about half of the genotypes and consequently not in the whole population mean of the third year. In that year, plants for the determination of above-ground N accumulation might have been accidentally harvested too early from the field as indicated by still about 15 % higher N allocation to vegetative organs - and thus less progression of N retranslocation to generative plant parts. Also N retranslocation from the root N pool to the shoot (Malagoli *et al.*, 2005) might have been less advanced under these conditions. Thus, a comparison of total shoot N accumulation among the years is difficult and one cannot say if at later progression of N retranslocation to the shoot a trend for higher shoot N accumulation under urea would have been manifested in the third year, as well. On the other hand, Fismes *et al.* (2000) detected also contrasting trends for above-ground N accumulation at maturity after nitrate and urea application in different experimental years, so that a trend for higher N accumulation under urea may not be valid under every environmental condition.

Indications for the initial idea that nitrate fertilization might have the potential to retard plant senescence relative to urea (see 1.5), could only be seen in the genetic diverse collection in 2011/2012: About 2/3 of the genotypes responded to nitrate with a later start of flowering and an extension of the flowering period by about one day (Annex-17). Since the second spring fertilization was given only one week before the end of flower development, the differentially fertilized N forms might have been still very prominent in the soil (as discussed in 4.1.3) at the transition phase to flowering. Thus, nitrate-induced ZR export to the shoot (in 2011/2012 measured at BBCH59, Figure 3-17; see also 4.1.4) might have led to delayed shoot senescence (Jibran *et al.*, 2013), resulting in a delay of flowering. Furthermore, flowering is naturally accompanied by the onset of leaf senescence (Hocking *et al.*, 1997), and interestingly, the trend for delayed N retranslocation from vegetative organs to pods after nitrate application, as monitored in the genetic diverse collection, was mainly resulting from higher percentages of N in the leaf fractions (Figure 3-18, Annex-12). This might indicate that also leaf senescence was retarded by nitrate, which stimulated Z-type CKs slowing down N recycling to generative organs.

In the two following years, flowering behavior of the elite line collection was not influenced by the given N forms (data not shown). The fertilizer application about two weeks before onset of flowering could have been too early to influence flowering. Also the trend of delayed plant senescence as monitored by lower N remobilization to pods under nitrate did not repeat: Although the bulk of the diverse genotypes tended to respond to nitrate fertilization with symptoms of delayed senescence, only two (2012/2013) or six (2013/2014) of the elite lines behaved in a similar way, while five (2012/2013) or seven (2013/2014) of these lines tended to show earlier N remobilization under nitrate (Annex-7). It appeared that the trend for earlier N retranslocation by nitrate-fed variants was associated with lower CK export to the shoot during pod formation (BBCH75). This might have resulted from lower N levels in the soil later after nitrate application (see 4.1.3), so that shoots of nitrate-fertilized plants did not receive that much senescence-retarding CKs as the urea variants after flowering (Figure 3-4, Figure 3-12). It

is unclear, if the applied N treatment exerted reverse effects on xylem CK levels during pod formation in the genetic diverse collection in 2011/2012, which could explain the observed trend for delayed plant senescence under nitrate. In that year, CK translocation rates were not determined during pod formation, and the data from BBCH69 did not show any influence of the N form on CK levels in the xylem sap (Figure 3-17). However, it has to be considered that overall N retranslocation appeared to have progressed faster in 2011/2012 than in the following years, as the senescent leaf mass was substantially higher and their N concentration was lower than in 2012/2013 and 2013/2014 (Annex-3, Annex-6, Annex-9). This may also have been caused by unintended shifts of the sampling time points.

By regarding the reference genotypes PBC007 and PBC015 in the three subsequent years it becomes evident that differences in sampling, fertilizer management or general environmental conditions might have had an important influence on the genotypic behavior: Both genotypes showed lower $N_{generative}/N_{vegetative}$ ratios under nitrate in 2011/2012, no differences in 2012/2013 and at least PBC015 a slightly higher ratio under nitrate in 2013/2014. Thus, nitrate application may have the intended potential to delay plant senescence for a prolonged assimilate supply to roots and thus N uptake after flowering in oilseed rape (Schulte auf'm Erley *et al.*, 2007), but probably this can only be detected when leaf senescence has proceeded to a certain extent. Also the data of 2011/2012 showed only a trend for nitrate delaying N remobilization, most likely because the abundance of applied N forms decreased in the soil over time (see 4.1.3). Repeated application of the N forms could be a solution to prolong the availability of a certain N form and thus their physiological action on plant development. In tobacco, leaf senescence could be retarded by nitrate, compared to urea, only when N was given several times (Singh *et al.*, 1992). On the other hand, also the timing of N application is critical for plant development. It could be shown in wheat that late fertilization with nitrate led to higher CK accumulation in the flag leaf compared to urea, and that this effect was more pronounced the later N was applied. However, at the end of the vegetation period this resulted in lower grain protein because of retarded N recycling from leaves to grains (Bauer, 2014). Thus, the question arises whether a late urea application could be beneficial for oilseed rape to increase the amount of retranslocated N from vegetative organs, among which especially leaves are subject to poor N remobilization efficiency (Hocking *et al.*, 1997; also visible in this work by considerably higher N concentrations in senescing leaves than in stems and branches, Annex-9). However, common fertilizer management practice in oilseed rape production does not foresee such a late application, since N uptake from the soil decreases during generative growth (Sieling and Kage, 2010).

4.3 Yield structure in dependence of nitrogen supply and genotypic variation

4.3.1 Nitrogen supply led to enhanced seed yield in both reference lines but their responsiveness to the applied fertilizer differed

In accordance with many studies (e.g. Dreccer *et al.*, 2000; Cheema *et al.*, 2001; Zhang *et al.*, 2010; Ulas *et al.*, 2012; Nyikako *et al.*, 2014) the application of N fertilizer resulted in considerably higher seed yields in the genotypes PBC007 and PBC015 in both experimental years (Figure 3-24). Yet, both lines responded differently to the given N dose regarding their yield structure. While N application to PBC007 stimulated shoot branching, PBC015 was able to build a similar number of branches as N-fed PBC007 under both N levels, and this was the case in both experimental years to a similar extent. N limitation usually causes a reduction in shoot branching (Harper, 1987; Domagalska and Leyser, 2011), which might have been somehow impaired PBC015 in its branching response to N availability. The outgrowth of axillary buds in response to the nutrient status is thought to be mediated by systemic signaling involving the hormones auxin, SLs and CKs (de Jong *et al.*, 2014; see also 1.4.4). Since CK translocation in the xylem sap of PBC015 was affected by the given N level in a similar manner as in PBC007 and nitrate-treated PBC015 was even one of the genotypes in 2011/2012 exporting a significantly larger amount of CKs to the shoot than after urea supply, an impaired responsiveness of CK signaling to N availability is unlikely the cause for poor branching (Figure 3-17, Figure 3-21, Figure 3-22).

In contrast to branching, pod formation at the branches was stimulated by N application in both lines to a similar extent. N omission limited biomass and N accumulation of leaves, which most likely reduced the photosynthesis capacity of N-deficient plants, resulting in lower assimilate supply to flowers and consequently lower pod numbers (Rathke *et al.*, 2006; Figure 3-23, Annex-16). Under N limitation plants likely delivered a large amount of the synthesized carbohydrates to the roots to establish a higher root/shoot ratio (Gruber *et al.*, 2013). Consequently, carbohydrate supply to developing pods and seeds was lower (Kamh *et al.*, 2005), which then resulted either in a lower production of seeds per pod (in 2013/2014) or in lower TSW (in 2012/2013). Although the numbers of pods per branch and seeds per pod were comparable in both genotypes and years when no N fertilizer was supplied, TSW of PBC015 was consistently lower than that of PBC007 indicating that this yield parameter compensated for higher branching in PBC015, so that both lines reached a similar final seed yield under -N.

Seed yield of PBC007 and PBC015 was limited to a similar extent by N deficiency in both experimental years (Figure 3-24). Most likely, this was caused by similar intraspecific competition for N since soil N_{min} reserves after winter as well as stand densities in the -N plots were comparable in both years (Table 2-3, Figure 3-24). Furthermore, similar restrictions in total shoot N accumulation in both genotypes and years appear to point to comparable N availabilities for individual plants in the -N plots (Figure 3-23). It was thus possible to directly compare N-efficiency parameters of N-deficient PBC007 and PBC015 in both experimental years: In the established low-N environments, both genotypes had a similar ability to take up available N and convert it into seed yield (Figure 3-25). The calculated NupE of about 2 kg absorbed N per kg of soil-available N appears high, as this means that plants took up two times more N than N being available at the beginning of spring (Table 2-3). However, N_{min} was only measured up to 60 cm soil depth, but roots of oilseed rape can reach much deeper soil layers, i.e. in less compacted soils up to 180 cm to explore further soil N reserves (Diepenbrock, 2011). In addition, N mineralization by microbes is an ongoing process in the soil (Schimel and Bennett, 2004), so that the N_{min} pools measured at the beginning of spring underrepresent the total N delivery from the soil organic matter during the whole plant growth period. To achieve more accurate NupE values in future, the experimental design could

be improved, e.g. by determining N_{min} more often and also in deeper soil layers during the vegetation period, by measuring potential volatile N losses via leaves which might underestimate NupE (Xu *et al.*, 2012) and by considering root N accumulation.

As for NupE, also NutE did not vary significantly between the two genotypes under -N conditions, so that overall N efficiency was also similar (Figure 3-25). Thus, it appears that the original selection of the reference genotypes PBC007 and PBC015 based on putatively contrasting NutE, which was apparent solely under N-sufficient conditions (see 3.4), did not manifest when comparing agronomic N traits under N deficiency. However, differences in seed yield existed between the two genotypes in most of the conducted experimental trials that included N fertilization. This strongly suggests that PBC007 and PBC015 exhibit a contrasting responsiveness to N application. PBC015 might therefore be regarded as a so-called "N responder line" (Nyikako *et al.*, 2014), which means that it shows a higher efficacy in converting fertilized N into seed yield (Sattelmacher *et al.*, 1994; Figure 3-7, Figure 3-15, Figure 3-19, Figure 3-24).

According to the general observation in oilseed rape that the N recovery rate decreases along with increasing fertilizer N levels (Rathke *et al.*, 2006), NupE was lower in N-fertilized plants, regardless of genotype and year (Figure 3-25). N-fertilized plants had a NupE of about 1 kg absorbed N per kg of available N, which corresponds to values measured under N deficiency by Koeslin-Findeklee and Horst (2016). This suggests that the applied amount of 100 kg N ha^{-1} in spring was used up completely by the plants so that they probably went into a mild N deficiency (as also already implied in 4.1.2) and did not reach their full yield potential. However, as resource inputs of currently 160 - 240 kg N ha^{-1} in oilseed rape production (Rathke *et al.*, 2005; Sieling and Kage, 2010; Albert, 2011) have to be reduced due to different governmental regulations (see 1.1), selecting for genotypes that respond to moderate N application appears now as an urgent breeding goal (Hirel *et al.*, 2007). Since breeders have selected for high yielding genotypes mainly under high N inputs during the last decades, NUE of modern cultivars may have suffered compared to former varieties, which underwent less favorable selection conditions. Thus, older oilseed rape cultivars could be a valuable source for breeding programs under moderate N supply (Stahl *et al.*, 2015).
In the present study, PBC015, which was approved in 1991 (http://www.proplanta.de/Pflanzenbauberater/Sorten/Wotan--Winterraps-Hauptfruchtanbau_sks_531RAW1.html, accessed on 30 May 2016), was able to use applied N fertilizer very efficiently. This is supported by seed yields above the population averages of the genetic diverse collection and even of the actual breeding elite lines (Figure 3-7, Figure 3-15, Figure 3-19, Figure 3-24). PBC015 tended also to respond to the applied N form, as NUE under urea was slightly higher than under nitrate, probably due to a longer-lasting N availability of the urea fertilizer (see 4.1.3; Figure 3-25). Interestingly, yields of this genotype laid consistently between 30 and 40 dt ha^{-1}, which are the present mean seed yields in central European oilseed rape production (Rathke *et al.*, 2006), so that PBC015 might be an interesting breeding candidate for the development of lines with maximum seed yield at moderate N supply.

In contrast to N uptake, which is strongly influenced by environmental conditions, N utilization is more determined by the genotype (Nyikako *et al.*, 2014) and mainly causes differences in NUE among rapeseed cultivars when N is applied (Berry *et al.*, 2010; Schulte auf'm Erley *et al.*, 2011, Kessel *et al.*, 2012). In agreement with these observations, fertilized PBC015 tended to have a higher NutE than PBC007 while NupE did not vary (Figure 3-25). Just recently it has been shown that the compartmentalization of absorbed nitrate in root cells has a high impact on N efficiency in oilseed rape: A cultivar with reduced activity of vacuolar proton pumps accumulated more nitrate in the cytosol, which stimulated the export of nitrate to the shoot via induction of *NRT1.5*. In the shoot, energy costs for nitrate assimilation are lower than in roots, resulting in a higher N-efficiency of this cultivar compared

to another cultivar, which translocated less amounts of nitrate to the shoot but transferred and stored more nitrate in root vacuoles (Han *et al.*, 2016). As in the present study both genotypes PBC007 and PBC015 did not consistently differ in their above-ground N accumulation such mechanism might be excluded (Figure 3-6, Figure 3-14, Figure 3-18, Figure 3-23). Instead, genotypic differences in NutE may be based on differential N remobilization from vegetative organs for pod and seed filling, e.g. by re-assimilation of N from protein-breakdown during leaf senescence (Hawkesford *et al.*, 2012) or by improved phloem-loading of re-assimilated AAs and inorganic N components (Masclaux-Daubresse *et al.*, 2008). Nevertheless, such differences between the two genotypes were not yet visible at BBCH79.

4.3.2 Nitrate is able to stimulate shoot branching in genetically diverse oilseed rape cultivars, while overall yield structure is little influenced by the applied nitrogen form

One aim of the present work was to enhance seed yield of field-grown oilseed rape by stimulating shoot branching and subsequent pod and seed formation by exploiting nitrate-induced root CK production and export to the shoot (see 1.4.4 and 1.5). Although the expected higher export of mainly ZR was observed in every experimental year shortly after nitrate application (see 4.1.4), an influence of nitrate on shoot branching was only visible in 2011/2012 (Figure 3-7, Figure 3-15, Figure 3-19). Under nitrate fertilization, about 2/3 of the genotypes of the collection, significantly or in tendency, formed a higher number of branches per plant resulting in more pods per plant (Annex-11). In contrast, at similar total shoot N accumulation urea-treated plants appeared to invest more N to form a higher stem (Annex-10). It is important to note that such responses to contrasting N nutrition in plant architecture appeared just in the genetic diverse collection of oilseed rape genotypes but not in the elite line collection. The diverse genepool may thus carry the relevant genetic information for mediating the response to different N forms, whereas this genetic information got lost during breeding. On the other hand, the reference line PBC015 was able to produce significantly more branches in response to nitrate only in 2011/2012 but not in the other years. This observation makes an influence of the environmental conditions on shoot architecture and yield composition under different fertilizer N forms more likely. One decisive environmental factor might have been the stand density. 2011/2012 was the year with the largest spacing between the plants (Figure 3-7, Table 3-1, Table 3-2, Table 3-3). Maybe, nitrate-induced CK delivery from the root reduced the expression of the negative regulator of shoot branching *BRC1* in axillary buds, allowing their outgrowth (Janssen *et al.*, 2014; see 1.4.4) and further development (Dun *et al.*, 2012). Additionally, Bainbridge *et al.* (2005) reported that a local CK treatment to *Arabidopsis thaliana* roots prevented the auxin-induced upregulation of the SL-biosynthesis gene *MAX4*. A similar mechanism could have been relevant for the present study as nitrate stimulated root CK synthesis, here ZR, shortly after fertilizer application (Figure 3-4, Figure 3-12, Figure 3-17, Figure 3-21, Figure 3-22).

In the following two experimental years, stand density was on average only 1 (2013/2014) or up to 6 (2012/2013) plants m^{-2} higher, but this could have been enough to induce a spacing-depended induction of *BRC1* expression like it was shown in *Arabidopsis thaliana* at increasing planting densities (Aguilar-Martínez *et al.*, 2007). In general, stand densities of more than 9 plants m^{-2} lead to intraspecific competition (Diepenbrock, 2000). Such a regulatory mechanism could have overlain a potential action of CK signaling on bud outgrowth. Thus, it is not possible to exclude that the elite lines are able to produce more branches per plant in response to nitrate whenever stand densities are sufficiently low. At least in 2013/2014, there was a tendency for higher branch formation under nitrate. Anyway, as common stand densities in oilseed rape production lie between 25 and 50 plants m^{-2} (Vosshenrich and Dölger, 2011), planting densities without intraspecific competition are not relevant for

agricultural practice. However, if a stand was reduced, e.g. by strong winter kills, farmers could use a nitrate-based spring fertilization to enhance branching to gain a higher potential for pod and seed formation (Orlovius, 2003), supposing that low stand densities really allow a branching response to nitrate-induced CKs. As seen in the present study, higher branch and pod production appeared to be compensated for by less pod filling and were thus not beneficial for overall seed production. On the other hand, plants in this study received only moderate N supply and may not have evolved their full yield potential (see 4.1.2 and 4.3.1). Under high N supply, the anticipated effects of nitrate fertilization on branching, pod and seed production may have been more pronounced.

As observed for shoot architecture, also final seed yield was not consistently influenced by the two contrasting N forms (Figure 3-7, Table 3-1, Figure 3-15, Table 3-2, Figure 3-19, Table 3-3). Only in 2012/2013 area seed yield was significantly higher in nitrate-treated plants. In that year, an exceptional long cold period at the beginning of the year likely forced a faster plant development in spring, so that the time period between N fertilization and seed development was shorter than in the other years (Figure 2-1). Thus, nitrate-stimulated higher N delivery to the shoot tended to last until post-flowering development , then potentially improving pod wall photosynthesis and seed development (Diepenbrock, 2000; Figure 3-1: BBCH75). In contrast to N, a higher post-flowering export of CKs to the shoot was apparently rather disadvantageous for seed yield, as in that year nitrate-fertilized plants translocated significantly less ZR than the urea variants (Figure 3-4). It has been shown in several plant species that higher CK levels in developing seeds, as achieved via upregulation of *IPT*s, downregulation of *CKX*s or by external CK application, stimulated cell division and sink strength in seeds, which had a positive influence on final seed yield (Song *et al.*, 2015). In the present study, however, CK levels were determined in the xylem sap and not directly in seeds. In correlation studies among all individual genotypes it appeared that, at least at the considered developmental stages, genotype-specific higher CK delivery from the root exerts either no effect on seed yield or partially tends to influence seed yield even negatively (Annex-21, Annex-22, Annex-23). In contrast, lines naturally showing higher xylem CK levels at later plant development often accumulated more N in vegetative organs during pod development. Thus, CKs exported from the root to shoot may retard plant senescence to some extent (Jibran *et al.*, 2013) but this appeared to be disadvantageous for seed yield. This is in accordance with the study of Bauer (2014) where CKs stimulated also N retention in flag on the cost of N retranslocation to grains. Thus, choosing cultivars with naturally higher CK delivery to the shoot during pod and seed development or even stimulating higher xylem CK levels by targeted N fertilization during that developmental phase appears to be an inappropriate way to enhance seed yield.

Nonetheless, as oilseed rape usually receives N fertilization before the phase of yield formation (Sieling and Kage, 2010), an effect of targeted N application on CK levels may get lost until seed development (Kamínek *et al.*, 2003). Accordingly, also other studies on oilseed rape show that the application of ammonium nitrate and urea as contrasting N fertilizer forms had no impact on total seed yield in oilseed rape as well (Fismes *et al.*, 2000; Arkoun *et al.*, 2012). Consequently, the choice of nitrate versus urea may be more suitable to regulate branching, supposing that stand densities allow a nitrate-promoted increase. This is in contrast to cereal crops, where N fertilizer is applied more frequently, providing a more continuous and longer-lasting effect of the chosen N form. In oilseed rape, the choice of the fertilizer N form may be better made on economic criteria. Urea is less costly than ammonium nitrate-based fertilizers (Pinton *et al.*, 2016). Furthermore, as the fabrication of urea fertilizer does not require nitric acid production, no N_2O emissions occur, which lowers greenhouse gas emission compared to ammonium nitrate fabrication by about 2/3 (Snyder *et al.*, 2009) and appears thus to be interesting regarding regulations in the frame of the Renewable Energy Directive (see 1.1). However, when urea is applied to agricultural sites without urease inhibitor

its fast degradation may cause gaseous losses of up to 50 % of urea-N (Watson, 2005) and additional losses of almost all urea-C as CO_2 (Snyder *et al.*, 2009). Total volatile N losses are then often higher than those from ammonium nitrate-fertilized sites (Snyder *et al.*, 2009), but the addition of an urease inhibitor can decrease these volatile N emissions by 50 - 70 % (SKW Piesteritz, 2014; Arkoun *et al.*, 2012). Of course, the emission of greenhouse gases from agricultural sites is also strongly influenced by soil and environmental conditions as well as by crop management practices (Snyder *et al.*, 2009).

4.3.3 Yield structure is highly plastic

In the present study, yield formation in genetic diverse as well as elite breeding lines was monitored over one, two or three subsequent years (Figure 3-7, Figure 3-15, Figure 3-19). Thereby, it was expected that in the genetic diverse population grown in the first year a higher variation in single yield parameters could be observed than in the elite breeding lines. Since seed material of the latter might have undergone stronger selection for yield and quality during breeding programs, genetic variation was likely reduced (Stahl *et al.*, 2015). In fact, TSW was the only yield parameter, which was considerably more variable in the genetic diverse collection (3.1.3, 3.2.3, 3.3.3). As TSW is the yield parameter with the weakest dependency on environmental conditions (Diepenbrock, 2000), it may have often been tackled during breeding. Thus, genetically determined differences in TSW (Koeslin-Findeklee *et al.*, 2014) might have been reduced by selective breeding, potentially leading to lower effects of unfavorable TSW on yield formation. Interestingly, a negative impact of TSW on seed yield was found to be more pronounced in the genetic diverse collection than in the elite lines (Figure 3-8, Figure 3-16, Figure 3-20).

Although the variation in individual yield parameters was not that much pronounced in the two populations, the overall yield structure was apparently more similar among the elite lines than in the genetic diverse lines: More than half (2012/2013) or at least 1/3 (2013/2014) of the elite lines showed a similar pattern of yield parameters, while almost every line in the genetic diverse collection exhibited a rather unique yield structure (2011/2012; Figure 3-7, Figure 3-15, Figure 3-19). However, yield formation of oilseed rape genotypes also strongly depends on the number of plants established per square meter (Diepenbrock, 2000; see also 1.3) and usually the genetic potential for yield parameter formation is better visible at low planting densities (Thurling, 1991). The yield structure of the reference genotype PBC015 exemplarily appears to accord quite well with the conclusion of Thurling (1991): In 2011/2012 and 2013/2014, when stand densities were low (11 - 13 plants m^{-2}), yield structure was characterized by above-average branch and seed formation, below-average TSW and a final seed yield above the respective population averages (Table 3-1, Table 3-3). Contrastingly, at higher stand density in 2012/2013 (about 19 plants m^{-2}) PBC015´s characteristic yield structure was lost and met then exactly the population average, so that its superior yield formation disappeared (Table 3-2). Thus, PBC015 achieved a higher yield potential at low stand density. A similar phenomenon may apply to other lines in 2012/2013, which could also explain why especially in that year so many of the elite genotypes showed a comparable overall yield structure and why the variability in the formation of the individual yield parameters was slightly lower (3.1.3, 3.2.3). Among all yield parameters, the reduction of stand density appeared to have the strongest impact on the genotypes´ ability to produce pods. This was likely due to the additional production of pods at lower branches, favored by a less dense canopy (Diepenbrock, 2000). Berry and Spink (2006) reported that incoming radiation will not be fully trapped, if fertile pods fall short of number of 6000 - 8000 m^{-2}. However, in all experimental years average pod number per square meter consistently reached about 4000 (calculated from "pods $branch^{-1}$", "$branches\ plant^{-1}$" and "$plants\ m^{-2}$" formed in the individual years). Thus, the suboptimal N

supply in the present trials (as discussed in 4.1.2) might have limited the genotypic potential for pod formation as well.

The question, if a distinct yield structure favors higher yield formation, will be difficult to answer from the recorded data, as several types of yield composition appeared beneficial. PBC015 and PBC027, for example, achieved the highest yields among the genetic diverse collection in 2011/2012 with a rather similar structure of above-average branching, average pod numbers and considerably higher seed numbers coupled with very low TSW. In contrast to PBC015 and PBC027, both variants of PBC018 and the urea variant of PBC029 formed high yields with branch numbers below average, average pod formation and above-average seed production, while TSW was at average in PBC018 and high in PBC029 (Figure 3-19). In the elite lines, a higher pod number appeared to be beneficial for total seed yield rather than seed numbers per pod (e.g. BCSNE001 2012/2013 or BCSNE002 in 2013/2014; Figure 3-7, Figure 3-15). Thus, there is no ideotype for high seed yield formation to be deduced from these experiments. Especially by comparing yield structure of the individual lines over two or three years, it becomes obvious that most genotypes adjusted yield parameters to the given environmental conditions when a certain parameter was formed. Of course, these environmental conditions include not only the mentioned stand density and nutrient availability but also crop management, soil and weather conditions, making long-term testing of a genotype´s yield potential over different years and locations necessary (Weymann *et al.*, 2015).

4.3.4 Seed number is the most important trait influencing seed yield

Among all recorded yield parameters, the trait seed number was most closely related to seed yield in every experimental year, which is in accordance with previous observations (Berry and Spink, 2006; Figure 3-8, Figure 3-16, Figure 3-20). Thereby, the correlation coefficients were higher when relating seed number per pod to seed yield per plant rather than to area seed yield. This appears plausible as the seed forming ability and thus final seed yield of individual plants strongly depends on competition for nutrients, light and water within a stand, while on areal scale seeds per pod are formed in trade-off to the given stand density (Diepenbrock, 2000).

Individual oilseed rape plants adjust the formation of fertile branches to the given stand density (Diepenbrock, 2000). This was also seen in the present work regarding the higher mean branch numbers of plants grown in 2011/2012 and 2013/2014 at lower stand densities compared to lower branching in 2013/2014 in more dense stands (Figure 3-8, Table 3-1, Figure 3-16, Table 3-2, Figure 3-20, Table 3-3). However, even at similar stand densities there were large genotypic differences in branch numbers, so that no correlation occured between these two yield parameters (Figure 3-8, Figure 3-16, Figure 3-20). A higher number of branches increased the potential of individual plants of a genotype to produce pods, as seen by positive correlations between branching and pod formation per plant. In contrast to pod number per plant, pod formation per branch was not necessarily related to the total number of branches, since differences in the location among branches determined their pod-forming abilities. Especially at dense canopies, lower branches bear less pods (Diepenbrock, 2000). N deficiency can also limit pod formation, as a lower leaf area index evolves, resulting in reduced assimilate delivery to buds and flowers and thus a weaker initiation of pods (Rathke *et al.*, 2006). Sufficient N supply during flowering is also crucial for seed set within the pods (Habekotté, 1993), considering that the final number of pods and seeds of oilseed rape is determined 1 - 2 weeks after flowering (Sieling and Kage, 2010). Interestingly, pod formation per square meter appeared to be restricted to a maximum of 4000 (as mentioned in 4.3.3) in the present study. Since plants only received a moderate N supply (as discussed in 4.1.2 and 4.3.1), the amount of absorbed N to be retranslocated for pod and seed growth (Rathke *et al.*, 2006),

might have been not enough to ensure the development of an optimal number of 6000 - 8000 pods m^{-2} (Berry and Spink, 2006). Genotypic differences in pod formation per square meter were seen in every year, whereby the formation of a higher number of pods resulted in a lower seed number per pod (Figure 3-8, Figure 3-16, Figure 3-20). This compensation among yield components is well known (Thurling, 1991) and might have resulted from the competition of pods, whose numbers vary among genotypes, for absorbed N for pod wall photosynthesis and seed filling (Habekotté, 1993; Diepenbrock, 2000; Figure 3-6, Figure 3-14, Figure 3-18).

Seed yield formation of individual plants in a stand depends to a large extent on the competition among plants (Diepenbrock, 2000). This became evident in the present study, as plants in 2012/2013 grew in highest stand densities and showed the lowest mean plant seed yield (Figure 3-8, Table 3-1, Figure 3-16, Table 3-2, Figure 3-20, Table 3-3). It has been reported that area seed yield of a stand increases up to a number of 50 plants m^{-2} (McGregor, 1987; Leach *et al.*, 1999). This could not be observed in the present study, as the elite lines showed a higher plant and area seed yield in the year with the lower stand density, namely 2013/2014, although in each year stand densities were even below 20 plants m^{-2}. Weather conditions were likely more beneficial for yield formation in 2013/2014, since plants probably developed continuously during spring. In 2012/2013, the exceptionally long cold period at the beginning of the year may have contrariwise forced plants to develop faster in spring (Figure 2-1). Another possibility is that a higher seed number per individual plant, as it was the case in 2013/2014, might have caused higher sink strength in these plants, likely resulting in higher area seed yield. However, Weymann *et al.* (2015) suggested that at high seed sink strength source limitation sets in so that TSW is no longer related to seed number per area, while at low seed sink strength TSW is negatively related to seed number. Exactly this was the case in both experimental years and to an even larger extent in 2013/2014, rendering higher sink strength in that year rather unlikely (Annex-18, Annex-19).

In most cases, TSW appears to be negatively associated with the seed number (Diepenbrock, 2000). Following the suggestion of Weymann *et al.* (2015) this might be due to oilseed rape being limited in sink strength rather than in source capacity (Rossato *et al.*, 2001). At least in 2011/2012, when the largest differences in TSW existed among lines, there tended to be a negative relation between TSW and N removal by seeds (Annex-20). In contrast, seed number was consistently positively associated with N removal from the field (Annex-18, Annex-19, Annex-20). This indicates that the seed number has a high impact on the N sink size of a plant and thereby plays a crucial role for final seed yield (Berry and Spink, 2006). Even though seed formation depends to a certain extent on environmental conditions (Diepenbrock, 2000), oilseed rape lines with a higher genetic potential for seed formation, e.g. by breeding for longer pods, might even under unfavorable environmental conditions have a higher potential for yield formation (Thurling, 1991).

N removal from the field appeared to increase when N fertilizer was applied to PBC007 and PBC015, most likely because the overall N availability for seed formation was higher (Figure 3-23, Figure 3-24, Figure 3-25). However, as the NHI did not vary among the N treatments, N fertilization must have led to higher N residues remaining on the field, as well. Improving NHI is important in terms of increasing the N efficiency of oilseed rape (Schulte auf'm Erley *et al.*, 2011), but it is problematic that higher N accumulation in the seed fraction is only achieved when plants show also higher N contents in vegetative plant parts (Svečnjak and Rengel, 2006).

Although a higher seed protein content increases the value of the press-cake, remaining after oil extraction from seeds for animal feeding (Koeslin-Findeklee, 2014), it is problematic that usually a negative correlation exists between seed protein and oil content (Orlovius, 2003) Consequently, breeding for lower seed protein content resulted in higher oil yield but favored

concomitantly low NHIs of oilseed rape (Masclaux-Daubresse *et al.*, 2008). To overcome this problem breeding for cultivars with shorter straw has been suggested (Nyikako *et al.*, 2014), while maintaining seed yield and oil content is crucial (Masclaux-Daubresse *et al.*, 2008). Also in the present study, it appeared that genotypes with a lower shoot N content had a higher NHI (Annex-18, Annex-19, Annex-20). This could, however, not be influenced by the fertilizer N form as nitrate and urea had no impact on N retranslocation from vegetative organs to seeds (see 4.2.2). Thus, an improvement of the N efficiency in oilseed rape by choosing a distinct N fertilizer form appears not implementable, also because the fertilizer management does not foresee a late N application (Sieling and Kage, 2010).

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Annex

Annex-1: Xylem exudation rates in the elite line collection in the +N field trial 2012/2013 at BBCH57, BBCH65 and BBCH75. All rapeseed genotypes were fertilized in spring with 40 kg N ha^{-1} at BBCH30 and 60 kg N ha^{-1} at BBCH55 as nitrate or urea. Xylem sap was pooled from ten plants per plot. Table shows means ±SD (n=4 for single genotypes, n=60 for all genotypes). ANOVA (BBCH57) or ANOVA on ranks (BBCH65, BBCH75) results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. There were no significant mean or median differences among nitrate and urea treatment within any single genotype according to unpaired t-test at p<0.05 or among all genotypes according to unpaired t-test (BBCH57) or Mann-Whitney rank sum test (BBCH65, BBCH75), respectively, at p<0.05. There were no significant mean or median differences among the genotypes within nitrate treatment according to Tukey´s test (BBCH75) or Tukey´s test on ranks (BBCH57, BBCH65), respectively at p<0.05; there were no significant mean or median differences among the genotypes within urea treatment according to Tukey´s test (BBCH57) or Tukey´s test on ranks (BBCH65, BBCH75), respectively, at p<0.05.

Genotype	**Xylem exudation rate [ml $h^{-1}plant^{-1}$]**					
	BBCH57		**BBCH65**		**BBCH75**	
	Nitrate	**Urea**	**Nitrate**	**Urea**	**Nitrate**	**Urea**
PBC007	1.27 ±0.46	1.23 ±0.29	1.14 ±0.70	1.01 ±0.88	0.66 ±0.32	0.65 ±0.24
PBC015	1.31 ±0.55	1.21 ±0.35	1.11 ±0.70	1.09 ±0.68	0.40 ±0.08	0.56 ±0.29
Alpaga	1.19 ±0.46	1.07 ±0.27	0.84 ±0.60	0.92 ±0.69	0.65 ±0.11	0.57 ±0.07
11091433	0.89 ±0.43	0.87 ±0.36	1.64 ±0.75	2.18 ±0.55	0.77 ±0.35	0.72 ±0.15
12091707	1.22 ±0.25	0.96 ±0.22	1.00 ±0.72	1.15 ±0.67	0.68 ±0.23	0.52 ±0.18
BCSNE001	0.91 ±0.53	0.75 ±0.31	1.34 ±1.00	1.56 ±1.20	0.74 ±0.28	0.78 ±0.32
BCSNE002	1.10 ±0.42	1.05 ±0.56	0.96 ±0.49	0.93 ±0.79	0.32 ±0.16	0.71 ±0.26
DSV-01	1.03 ±0.33	1.33 ±0.20	0.88 ±0.68	0.95 ±0.63	0.37 ±0.12	0.77 ±0.25
DSV-02	1.16 ±0.39	1.21 ±0.67	1.18 ±0.71	0.86 ±0.96	0.87 ±0.45	0.71 ±0.14
KWS_01	1.04 ±0.28	0.73 ±0.33	0.86 ±0.52	1.02 ±0.71	0.52 ±0.12	0.50 ±0.29
KWS_02	1.05 ±0.29	0.78 ±0.26	1.45 ±0.40	0.91 ±0.27	0.62 ±0.15	0.87 ±0.37
LG00-304E	0.96 ±0.10	0.88 ±0.37	1.68 ±0.87	0.94 ±0.64	0.61 ±0.23	0.58 ±0.26
LG02-228D	1.06 ±0.31	0.90 ±0.49	1.38 ±0.78	0.62 ±0.67	0.53 ±0.21	0.85 ±0.80
NPZ012	0.83 ±0.33	0.55 ±0.26	1.08 ±0.54	0.84 ±0.45	0.39 ±0.23	0.71 ±0.59
NPZ208	0.89 ±0.47	0.60 ±0.22	1.24 ±0.50	1.19 ±0.98	0.52 ±0.21	0.44 ±0.14
All genotypes	1.06 ±0.37	0.94 ±0.39	1.17 ±0.67	1.08 ±0.74	0.58 ±0.26	0.65 ±0.31
ANOVA	G ns, T ns, GxT ns		G ns, T ns, GxT ns		G ns, T ns, GxT ns	

Annex-2: Correlations between translocation of xylem sap components in the elite line collection in the +N field trial 2012/2013 at BBCH57, BBCH65 and BBCH75. Components A and B were correlated by Pearson product moment (Test: P) or Spearman rank order correlation (Test: S). Values for r and p were calculated separately for nitrate (■) and urea (■) treatment; n=60; n.d.=not determined.

Component A	Component B	Nitrate			Urea		
		p	r	Test	p	r	Test
Total N [µmol h^{-1} $plant^{-1}$]							
N BBCH57	N BBCH65	0.13	0.34	S	-0.13	0.35	S
N BBCH57	N BBCH75	0.08	0.56	S	0.15	0.27	S
N BBCH65	N BBCH75	0.51	<0.01	S	0.62	<0.01	S
Amino acids [µmol h^{-1} $plant^{-1}$]							
Other AA BBCH57	Asp BBCH57	-0.32	0.01	S	-0.03	0.82	S
Other AA BBCH57	Gln BBCH57	0.02	0.91	S	0.07	0.57	S
Asp BBCH57	Gln BBCH57	-0.26	0.04	S	-0.19	0.15	S
Other AA BBCH65	Asp BBCH65	0.37	<0.01	S	0.40	<0.01	S
Other AA BBCH65	Gln BBCH65	0.64	<0.01	S	0.58	<0.01	S
Asp BBCH65	Gln BBCH65	0.42	<0.01	S	0.26	0.04	P
Other AA BBCH75	Asp BBCH75	0.32	0.02	P	0.42	<0.01	S
Other AA BBCH75	Gln BBCH75	0.50	<0.01	S	0.33	0.01	S
Asp BBCH75	Gln BBCH75	0.02	0.86	S	0.10	0.44	P
Other AA BBCH57	Other AA BBCH65	0.05	0.70	S	0.30	0.02	S
Other AA BBCH57	Other AA BBCH75	0.04	0.75	S	0.27	0.03	S
Other AA BBCH65	Other AA BBCH75	0.10	0.46	S	-0.02	0.89	S
Asp BBCH57	Asp BBCH65	0.01	0.96	S	0.03	0.84	S
Asp BBCH57	Asp BBCH75	0.09	0.51	S	-0.16	0.22	S
Asp BBCH65	Asp BBCH75	-0.11	0.41	S	0.00	0.97	S
Gln BBCH57	Gln BBCH65	0.18	0.18	S	0.09	0.51	S
Gln BBCH57	Gln BBCH75	-0.02	0.87	S	-0.02	0.87	S
Gln BBCH65	Gln BBCH75	-0.12	0.38	S	0.13	0.34	S
N forms [µmol h^{-1} $plant^{-1}$]							
Urea BBCH57	NH_4^+ BBCH57	n.d.			0.16	0.26	S
Urea BBCH57	NO_3^- BBCH57	n.d.			0.55	<0.01	S
NH_4^+ BBCH57	NO_3^- BBCH57	0.24	0.08	S	0.40	<0.01	S
NH_4^+ BBCH65	NO_3^- BBCH65	0.19	0.15	S	-0.04	0.76	S
NH_4^+ BBCH57	NH_4^+ BBCH65	0.51	<0.01	S	-0.02	0.87	S
NO_3^- BBCH57	NO_3^- BBCH65	-0.21	0.12	S	-0.13	0.36	S
NO_3^- BBCH57	NO_3^- BBCH75	-0.13	0.36	S	0.03	0.81	S
NO_3^- BBCH65	NO_3^- BBCH75	0.23	0.09	S	-0.06	0.68	S
CKs [pmol h^{-1} $plant^{-1}$]							
IPR BBCH57	ZR BBCH57	0.79	<0.01	S	0.78	<0.01	S
cZR BBCH57	ZR BBCH57	0.54	<0.01	S	0.78	<0.01	S
IPR BBCH65	cZR BBCH65	0.65	<0.01	S	0.51	<0.01	S
IPR BBCH65	ZR BBCH65	0.85	<0.01	S	0.65	<0.01	S
cZR BBCH65	ZR BBCH65	0.58	<0.01	S	0.67	<0.01	S
IPR BBCH75	cZR BBCH75	0.74	<0.01	S	0.53	<0.01	S
IPR BBCH75	ZR BBCH75	0.82	<0.01	S	0.81	<0.01	S

Annex-2 continued on page 112. ▸

Component A	Component B	Nitrate			Urea		
		p	r	Test	p	r	Test
cZR BBCH75	ZR BBCH75	0.63	<0.01	S	0.49	<0.01	S
IPR BBCH57	IPR BBCH65	-0.01	0.92	S	-0.08	0.56	S
IPR BBCH57	IPR BBCH75	0.18	0.20	S	-0.07	0.64	S
IPR BBCH65	IPR BBCH75	0.09	0.51	S	-0.12	0.40	S
cZR BBCH57	cZR BBCH65	-0.26	0.05	S	0.01	0.92	S
cZR BBCH57	cZR BBCH75	0.05	0.73	S	-0.06	0.68	S
cZR BBCH65	cZR BBCH75	-0.21	0.13	S	0.07	0.60	S
ZR BBCH57	ZR BBCH65	0.04	0.79	S	0.19	0.16	S
ZR BBCH57	ZR BBCH75	0.13	0.36	S	0.03	0.83	S
ZR BBCH65	ZR BBCH75	-0.01	0.94	S	-0.03	0.84	S

Annex-3: Dry weight and N concentration of different above-ground plant fractions in the elite line collection in the +N field trial 2012/2013. All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Two plants per plot were separated into the indicated above-ground plant fractions. Table shows means ±SD (n=4 for single genotypes, n=60 for all genotypes). ANOVA or ANOVA on ranks (■) results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. Significant mean difference among nitrate and urea treatment within a genotype is indicated by asterisks according to unpaired t-test at p<0.05; there were no significant mean or median differences between nitrate and urea treatment among all genotypes according to unpaired t-test or Mann-Whitney rank sum test (■), respectively, at p<0.05. Different upper case letters indicate significant mean or median differences among the genotypes within nitrate treatment according to Tukey´s test or Tukey´s test on ranks (■), respectively, at p<0.05; different lower case letters indicate significant mean or median differences among the genotypes within urea treatment according to Tukey´s test or Tukey´s test on ranks (■), respectively, at p<0.05.

Genotype	Organ DW [g]			N concentration [mg g DW^{-1}]	
	Nitrate	Urea		Nitrate	Urea
Main shoot pods					
PBC007	3.7 ±1.0	3.4 ±1.6		18.9 ±0.5	18.6 ±3.4
PBC015	4.0 ±0.3	3.1 ±0.4		17.8 ±2.2	18.9 ±2.0
Alpaga	3.5 ±1.0	2.9 ±0.5		18.1 ±2.5	18.8 ±2.5
11091433	4.8 ±1.1	4.4 ±1.4		16.7 ±1.0	18.3 ±3.4
12091707	3.1 ±0.7	3.4 ±0.8		17.0 ±1.8	17.7 ±3.3
BCSNE001	4.9 ±1.2	3.5 ±0.7		18.5 ±1.7	21.2 ±5.3
BCSNE002	4.6 ±1.0	4.8 ±1.8		16.9 ±1.7	17.4 ±3.3
DSV-01	5.4 ±1.3	3.8 ±0.4		17.4 ±1.7	20.6 ±1.8
DSV-02	4.1 ±2.1	3.9 ±0.5		18.9 ±1.9	17.4 ±1.6
KWS_01	3.8 ±0.6	3.5 ±1.1		17.7 ±2.0	17.2 ±1.4
KWS_02	3.4 ±1.1	3.8 ±0.4		19.4 ±2.3	17.2 ±1.8
LG00-304E	2.8 ±1.3	3.0 ±0.8		15.1 ±2.4	15.1 ±1.4
LG02-228D	3.5 ±1.4	3.7 ±0.9		16.8 ±1.5	18.6 ±0.3
NPZ012	4.0 ±0.8	4.3 ±0.4		19.5 ±2.4	18.4 ±2.0
NPZ208	4.1 ±1.0	4.0 ±1.5		17.5 ±2.5	16.8 ±2.2
All genotypes	4.0 ±1.2	3.7 ±1.0		17.8 ±2.0	18.1 ±2.7
ANOVA	G *, T ns, GxT ns			G ns, T ns, GxT ns	
Branch pods					
PBC007	14.8 ±4.5	14.1 ±4.5		20.4 ±2.2	20.2 ±3.5
PBC015	14.4 ±6.0	14.0 ±6.0		18.3 ±1.9	18.5 ±1.4
Alpaga	16.9 ±6.6	22.0 ±3.0		17.9 ±1.8	20.1 ±1.4
11091433	20.7 ±3.2	17.4 ±6.7		17.8 ±1.2	16.1 ±2.3
12091707	23.5 ±5.8	19.6 ±6.1		18.5 ±0.8	17.9 ±2.4
BCSNE001	14.6 ±4.3	16.1 ±7.6		18.7 ±1.0	20.3 ±3.1
BCSNE002	19.7 ±5.9	23.6 ±5.2		17.3 ±1.7	17.5 ±3.0
DSV-01	17.8 ±6.4	19.9 ±9.7		17.7 ±1.4	18.5 ±8.4
DSV-02	17.9 ±1.6	21.8 ±2.6		19.4 ±1.4	18.8 ±1.6
KWS_01	16.5 ±3.6	19.2 ±8.1		18.8 ±2.5	18.7 ±1.1
KWS_02	19.5 ±6.4	19.6 ±7.0		20.7 ±2.7	17.8 ±2.6
LG00-304E	14.5 ±5.0	19.9 ±3.8		16.2 ±3.0	16.6 ±1.5
LG02-228D	21.5 ±8.3	13.5 ±2.4		16.3 ±1.0	18.6 ±2.1
NPZ012	17.0 ±8.1	19.7 ±6.5		18.1 ±0.9	17.9 ±2.4
NPZ208	19.5 ±2.1	16.6 ±2.4		16.5 ±1.2	17.2 ±1.9
All genotypes	18.0 ±5.5	18.6 ±5.8		18.2 ±2.0	18.3 ±2.8
ANOVA	G ns, T ns, GxT ns			G ns, T ns, GxT ns	
Main shoot					
PBC007	11.1 ±1.6	11.3 ±3.2	ab	7.9 ±1.1	7.0 ±1.8
PBC015	10.8 ±2.1	9.6 ±1.6	ab	6.5 ±1.1	6.1 ±0.1
Alpaga	11.6 ±2.1	12.6 ±0.8	ab	5.8 ±0.6	6.5 ±0.9
11091433	10.2 ±1.6	11.0 ±2.1	ab	6.8 ±1.3	6.3 ±1.8
12091707	9.1 ±1.6	8.8 ±1.9	ab	7.1 ±1.1	6.8 ±2.6
BCSNE001	10.8 ±2.2	11.6 ±3.4	ab	8.6 ±1.6	6.4 ±1.8

Annex-3 continued on page 114. ▸

Genotype	Organ DW [g]			N concentration [mg g DW^{-1}]		
	Nitrate	Urea		Nitrate		Urea
BCSNE002	9.0 ±2.0	11.2 ±4.5	ab	8.2 ±1.6		8.0 ±3.1
DSV-01	8.7 ±1.3	8.7 ±2.4	ab	6.9 ±1.2	*	10.6 ±1.8
DSV-02	12.0 ±1.4	14.2 ±1.6	a	7.5 ±1.6		6.2 ±1.5
KWS_01	12.0 ±1.8	12.5 ±1.7	ab	6.7 ±1.6		5.8 ±0.9
KWS_02	11.1 ±2.5	11.2 ±2.2	ab	6.8 ±2.5		6.1 ±0.2
LG00-304E	9.7 ±2.5	12.2 ±2.7	ab	6.1 ±1.4		6.8 ±1.7
LG02-228D	12.0 ±3.5	10.7 ±0.6	ab	5.9 ±0.4		6.2 ±0.8
NPZ012	7.6 ±1.6	7.9 ±0.9	b	8.8 ±1.5		7.6 ±1.8
NPZ208	9.3 ±1.8	9.3 ±0.5	ab	6.7 ±2.4		6.8 ±1.6
All genotypes	10.3 ±2.2	10.9 ±2.6		7.1 ±1.6		6.9 ±1.8
ANOVA	G ***, T ns, GxT ns			G *, T ns, GxT ns		
Branches						
PBC007	8.9 ±2.3	8.3 ±2.6		11.6 ±0.7		11.6 ±2.3
PBC015	7.7 ±3.2	7.8 ±2.4		10.7 ±1.3		10.9 ±0.8
Alpaga	9.5 ±3.6	14.0 ±3.2		9.0 ±0.8		10.0 ±1.5
11091433	10.2 ±2.1	9.6 ±3.8		11.1 ±1.2		10.9 ±1.2
12091707	11.8 ±2.9	9.0 ±3.0		10.6 ±1.3		11.3 ±2.9
BCSNE001	9.0 ±2.7	12.2 ±5.7		11.6 ±2.1		11.7 ±2.1
BCSNE002	8.6 ±2.0	11.6 ±2.8		10.6 ±1.2		11.6 ±2.2
DSV-01	8.1 ±3.2	11.4 ±6.9		10.5 ±1.6		13.2 ±2.0
DSV-02	11.9 ±1.2	14.8 ±1.7		11.5 ±1.7		10.6 ±1.6
KWS_01	9.0 ±2.9	10.9 ±2.6		9.7 ±2.0		10.2 ±1.4
KWS_02	10.7 ±4.4	9.7 ±3.3		10.5 ±1.3		10.1 ±0.6
LG00-304E	9.5 ±3.4	12.1 ±2.5		10.2 ±1.7		10.7 ±1.0
LG02-228D	12.7 ±5.2	8.6 ±1.0		10.0 ±0.7		10.6 ±0.9
NPZ012	8.8 ±4.0	10.3 ±4.1		11.8 ±1.5		10.8 ±1.8
NPZ208	10.5 ±1.9	11.6 ±1.1		10.0 ±1.9		10.5 ±2.0
All genotypes	9.8 ±3.0	10.9 ±3.5		10.6 ±1.5		10.9 ±1.7
ANOVA	G ns, T ns, GxT ns			G ns, T ns, GxT ns		
Branch leaves						
PBC007	0.5 ±0.2	0.7 ±0.2		23.2 ±6.1		27.1 ±3.0
PBC015	0.7 ±0.3	0.5 ±0.3		24.6 ±5.8		26.8 ±3.5
Alpaga	0.8 ±0.1	1.4 ±0.5		22.2 ±5.5		22.0 ±4.8
11091433	0.7 ±0.4	0.7 ±0.4		24.5 ±1.9		23.6 ±1.9
12091707	0.9 ±0.4	0.8 ±0.4		24.5 ±2.5		22.5 ±5.3
BCSNE001	0.9 ±0.3	1.2 ±0.2		24.8 ±1.7		20.1 ±1.8
BCSNE002	0.7 ±0.2	1.0 ±0.3		23.4 ±3.6		28.5 ±6.5
DSV-01	0.7 ±0.6	1.8 ±1.0		23.5 ±2.6		25.5 ±3.0
DSV-02	1.1 ±0.5	1.2 ±0.5		25.9 ±4.1		24.6 ±1.5
KWS_01	0.7 ±0.1	0.7 ±0.2		27.7 ±4.8		23.9 ±5.4
KWS_02	0.6 ±0.2	0.7 ±0.2		24.2 ±4.4		24.6 ±1.1
LG00-304E	1.1 ±0.5	1.1 ±0.4		18.6 ±3.9		21.1 ±2.2
LG02-228D	1.3 ±0.5	0.9 ±0.1		21.8 ±2.5		22.0 ±5.0
NPZ012	0.6 ±0.1	0.7 ±0.3		25.6 ±1.4		23.8 ±3.1
NPZ208	0.4 ±0.3	0.5 ±0.3		23.4 ±2.1		21.6 ±5.1
All genotypes	0.8 ±0.4	0.9 ±0.5		24.0 ±3.8		23.8 ±4.1
ANOVA	G ***, T ns, GxT ns			G ns, T ns, GxT ns		
Main shoot green leaves						
PBC007	2.9 ±0.9	2.2 ±0.8		21.5 ±4.0		22.1 ±3.3
PBC015	2.4 ±0.8	2.0 ±1.0		22.0 ±3.5		22.6 ±1.4
Alpaga	3.3 ±1.1	4.3 ±1.0		21.5 ±3.0		22.7 ±3.6
11091433	2.6 ±1.0	2.8 ±1.7		22.2 ±4.6		21.4 ±1.8
12091707	2.3 ±0.7	1.9 ±0.6		22.6 ±2.1		21.6 ±3.7
BCSNE001	3.8 ±1.5	3.7 ±0.2		23.8 ±1.0		22.5 ±3.5

Annex-3 continued on page 115. ▸

Genotype	Organ DW [g]		N concentration [mg g DW^{-1}]	
	Nitrate	Urea	Nitrate	Urea
BCSNE002	2.1 ±0.8	2.4 ±1.1	22.2 ±3.6	25.4 ±7.0
DSV-01	2.3 ±1.6	3.5 ±2.6	21.6 ±1.1	23.4 ±2.5
DSV-02	3.7 ±1.4	3.4 ±0.4	24.1 ±2.7	23.8 ±2.4
KWS_01	2.3 ±0.8	3.1 ±1.0	23.1 ±3.7	21.4 ±3.9
KWS_02	3.0 ±0.6	2.2 ±0.3	21.0 ±3.5	20.9 ±0.9
LG00-304E	2.1 ±0.8	2.9 ±1.5	17.6 ±2.4	20.9 ±1.4
LG02-228D	3.3 ±1.4	2.8 ±0.3	21.5 ±1.5	21.0 ±2.6
NPZ012	1.6 ±0.3	1.7 ±1.0	23.0 ±3.2	22.1 ±2.9
NPZ208	1.5 ±0.8	1.9 ±0.4	22.7 ±3.7	21.7 ±4.2
All genotypes	2.6 ±1.1	2.7 ±1.2	22.1 ±3.0	22.2 ±3.2
ANOVA	G ***, T ns, GxT ns		G ns, T ns, GxT ns	
Main shoot senescent leaves				
PBC007	0.5 ±0.3	0.6 ±0.2	13.9 ±0.5	14.5 ±4.1
PBC015	0.3 ±0.2	0.3 ±0.0	16.4 ±1.9	15.3 ±1.7
Alpaga	0.5 ±0.1	0.6 ±0.4	14.6 ±1.9	16.4 ±1.4
11091433	0.5 ±0.3	0.5 ±0.3	15.7 ±2.8	13.2 ±1.1
12091707	0.4 ±0.4	0.3 ±0.1	12.7 ±1.8	13.3 ±3.0
BCSNE001	0.6 ±0.2	0.6 ±0.2	16.0 ±4.1	18.7 ±10.2
BCSNE002	0.4 ±0.1	0.4 ±0.3	14.3 ±1.5	13.6 ±2.2
DSV-01	0.3 ±0.2	0.3 ±0.3	14.4 ±2.4	14.8 ±0.8
DSV-02	0.5 ±0.4	0.6 ±0.1	16.3 ±2.5	16.7 ±1.2
KWS_01	0.6 ±0.2	0.4 ±0.3	18.0 ±5.5	13.6 ±2.9
KWS_02	0.6 ±0.2	0.4 ±0.1	12.8 ±2.7	12.9 ±1.3
LG00-304E	0.2 ±0.1	0.3 ±0.2	12.9 ±2.5	12.6 ±2.1
LG02-228D	0.4 ±0.2	0.4 ±0.3	12.0 ±1.8	14.7 ±1.0
NPZ012	0.2 ±0.1	0.2 ±0.1	16.8 ±2.4	16.7 ±3.6
NPZ208	0.3 ±0.1	0.5 ±0.2	14.7 ±1.4	13.5 ±3.2
All genotypes	0.4 ±0.2	0.4 ±0.2	14.9 ±2.9	14.7 ±3.3
ANOVA	G *, T ns, GxT ns		G ns, T ns, GxT ns	

Annex-4: Xylem exudation rates in the elite line collection in the +N field trial 2013/2014 at BBCH57, BBCH65 and BBCH75. All rapeseed genotypes were fertilized in spring with 40 kg N ha^{-1} at BBCH30 and 60 kg N ha^{-1} at BBCH55 as nitrate or urea. Xylem sap was pooled from ten plants per plot. Table shows means ±SD (n=4 for single genotypes, n=60 for all genotypes). ANOVA on ranks results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. There were no significant mean or median differences among nitrate and urea treatment within any single genotype according to unpaired t-test at p<0.05 or among all genotypes according to Mann-Whitney rank sum test, respectively, at p<0.05. There were no significant mean or median differences among the genotypes within nitrate treatment according to Tukey´s test (BBCH57) or Tukey´s test on ranks (BBCH65, BBCH75), respectively at p<0.05; there were no significant mean or median differences among the genotypes within urea treatment according to Tukey´s test (BBCH65) or Tukey´s test on ranks (BBCH57, BBCH75), respectively, at p<0.05.

Genotype	**Xylem exudation rate [ml $h^{-1}plant^{-1}$]**					
	BBCH57		**BBCH65**		**BBCH75**	
	Nitrate	**Urea**	**Nitrate**	**Urea**	**Nitrate**	**Urea**
PBC007	1.12 ±0.60	1.14 ±0.26	0.83 ±0.24	0.98 ±0.13	0.65 ±0.14	0.88 ±0.79
PBC015	1.46 ±0.34	1.32 ±0.14	0.71 ±0.17	1.30 ±0.40	0.89 ±0.79	0.61 ±0.26
PBC029	1.48 ±0.44	1.46 ±0.45	1.03 ±0.31	1.59 ±0.15	0.61 ±0.24	1.00 ±0.31
11091433	0.99 ±0.37	0.96 ±0.29	0.89 ±0.32	0.93 ±0.36	0.65 ±0.10	0.62 ±0.14
12091707	1.14 ±0.09	1.08 ±0.37	0.94 ±0.15	1.10 ±0.44	1.16 ±0.95	0.66 ±0.18
BCSNE001	1.19 ±0.33	1.05 ±0.57	1.15 ±0.32	1.16 ±0.32	0.61 ±0.12	1.18 ±0.52
BCSNE002	1.38 ±0.53	1.34 ±0.15	0.58 ±0.11	0.91 ±0.16	0.40 ±0.17	0.49 ±0.18
DSV-01	1.27 ±0.29	1.03 ±0.16	0.87 ±0.27	1.29 ±0.26	0.37 ±0.13	0.58 ±0.23
DSV-02	1.55 ±0.52	1.07 ±0.16	1.27 ±0.32	1.07 ±0.27	0.82 ±0.26	0.57 ±0.14
KWS_01	1.09 ±0.29	0.93 ±0.08	0.88 ±0.20	1.31 ±0.43	0.39 ±0.15	0.64 ±0.25
KWS_02	1.34 ±0.56	0.89 ±0.19	0.57 ±0.15	1.04 ±0.35	0.61 ±0.19	0.38 ±0.27
LG00-304E	1.50 ±0.31	1.12 ±0.15	0.68 ±0.42	1.19 ±0.59	0.57 ±0.14	0.60 ±0.18
LG02-228D	1.14 ±0.39	0.79 ±0.53	0.88 ±0.35	1.01 ±0.24	0.55 ±0.16	0.69 ±0.43
NPZ012	1.02 ±0.47	1.14 ±0.53	1.44 ±0.96	0.82 ±0.10	0.43 ±0.18	0.67 ±0.24
NPZ208	1.02 ±0.21	1.37 ±0.24	0.93 ±0.22	1.01 ±0.50	0.47 ±0.23	0.51 ±0.14
All genotypes	1.25 ±0.60	1.11 ±0.34	0.91 ±0.55	1.11 ±0.35	0.61 ±0.39	0.67 ±0.35
ANOVA	G ns, T ns, GxT ns		G ns, T ns, GxT ns		G ns, T ns, GxT ns	

Annex-5: Correlations between translocation of xylem sap components in the elite line collection in the +N field trial 2013/2014 at BBCH57, BBCH65 and BBCH75. Components A and B were correlated by Pearson product moment (Test: P) or Spearman rank order correlation (Test: S). Values for r and p were calculated separately for nitrate (■) and urea (■) treatment; n=60.

Parameter A	Parameter B	Nitrate			Urea		
		p	r	Test	p	r	Test
CKs [pmol h^{-1} $plant^{-1}$]							
IPR BBCH57	cZR BBCH57	0.75	<0.01	S	0.58	<0.01	S
IPR BBCH57	ZR BBCH57	0.73	<0.01	S	0.67	<0.01	S
cZR BBCH57	ZR BBCH57	0.83	<0.01	S	0.72	<0.01	S
IPR BBCH65	cZR BBCH65	0.71	<0.01	S	0.57	<0.01	S
IPR BBCH65	ZR BBCH65	0.75	<0.01	S	0.79	<0.01	S
cZR BBCH65	ZR BBCH65	0.83	<0.01	S	0.73	<0.01	S
IPR BBCH75	cZR BBCH75	0.63	<0.01	S	0.63	<0.01	S
IPR BBCH75	ZR BBCH75	0.80	<0.01	S	0.75	<0.01	S
cZR BBCH75	ZR BBCH75	0.63	<0.01	S	0.49	<0.01	S
IPR BBCH57	IPR BBCH65	-0.01	0.97	S	0.34	0.01	S
IPR BBCH57	IPR BBCH75	0.22	0.09	S	0.18	0.18	S
IPR BBCH65	IPR BBCH75	0.36	0.01	S	0.13	0.34	S
cZR BBCH57	cZR BBCH65	0.07	0.59	S	0.25	0.06	S
cZR BBCH57	cZR BBCH75	0.12	0.37	S	0.04	0.75	S
cZR BBCH65	cZR BBCH75	0.07	0.61	S	-0.05	0.72	S
ZR BBCH57	ZR BBCH65	0.05	0.69	S	0.26	0.05	S
ZR BBCH57	ZR BBCH75	0.12	0.38	S	0.18	0.18	S
ZR BBCH65	ZR BBCH75	0.38	<0.01	S	0.30	0.02	S
N forms [µmol h^{-1} $plant^{-1}$]							
Urea BBCH57	NH_4^+ BBCH57	n.d.			0.07	0.73	S
Urea BBCH57	NO_3^- BBCH57	n.d.			0.50	<0.01	P
NH_4^+ BBCH57	NO_3^- BBCH57	0.26	0.17	S	0.46	0.02	S
NH_4^+ BBCH65	NO_3^- BBCH65	0.43	<0.01	S	0.58	<0.01	S
NH_4^+ BBCH75	NO_3^- BBCH75	0.32	0.01	S	0.15	0.26	S
NH_4^+ BBCH57	NH_4^+ BBCH65	-0.33	0.07	S	0.30	0.13	S
NH_4^+ BBCH57	NH_4^+ BBCH75	-0.10	0.61	S	-0.19	0.33	S
NH_4^+ BBCH65	NH_4^+ BBCH75	0.32	0.01	S	-0.03	0.81	S
NO_3^- BBCH57	NO_3^- BBCH65	-0.13	0.35	S	-0.06	0.67	S
NO_3^- BBCH57	NO_3^- BBCH75	0.08	0.55	S	-0.07	0.62	S
NO_3^- BBCH65	NO_3^- BBCH75	0.19	0.15	S	0.23	0.08	S
Amino acids [µmol h^{-1} $plant^{-1}$]							
Other AA BBCH57	Asp BBCH57	0.38	0.04	P	0.18	0.34	S
Other AA BBCH57	Gln BBCH57	-0.04	0.84	P	0.28	0.14	P
Asp BBCH57	Gln BBCH57	-0.30	0.11	P	0.13	0.49	S
Other AA BBCH65	Asp BBCH65	0.33	0.01	S	0.01	0.99	S
Other AA BBCH65	Gln BBCH65	0.25	0.06	S	0.35	<0.01	S
Asp BBCH65	Gln BBCH65	-0.16	0.24	P	-0.30	0.02	S
Other AA BBCH75	Asp BBCH75	0.01	0.97	P	-0.03	0.81	S
Other AA BBCH75	Gln BBCH75	0.20	0.13	P	0.39	<0.01	S

Annex-5 continued on page 118. ▸

Parameter A	Parameter B	Nitrate			Urea		
		p	r	Test	p	r	Test
Asp BBCH75	Gln BBCH75	-0.27	0.04	P	-0.28	0.03	S
Other AA BBCH57	Other AA BBCH65	-0.03	0.86	S	-0.15	0.42	S
Other AA BBCH57	Other AA BBCH75	0.13	0.50	S	0.37	0.04	S
Other AA BBCH65	Other AA BBCH75	-0.10	0.45	S	0.18	0.18	S
Asp BBCH57	Asp BBCH65	-0.11	0.55	S	-0.36	0.05	S
Asp BBCH57	Asp BBCH75	0.18	0.33	S	0.13	0.48	S
Asp BBCH65	Asp BBCH75	0.16	0.22	S	0.15	0.27	S
Gln BBCH57	Gln BBCH65	0.17	0.38	S	-0.34	0.07	S
Gln BBCH57	Gln BBCH75	0.26	0.17	S	0.17	0.38	S
Gln BBCH65	Gln BBCH75	0.20	0.13	S	0.09	0.49	S
Total N [µmol h^{-1} $plant^{-1}$]							
N BBCH57	N BBCH65	0.14	0.29	S	0.05	0.69	S
N BBCH57	N BBCH75	-0.01	0.98	S	-0.04	0.75	S
N BBCH65	N BBCH75	0.12	0.36	S	0.20	0.13	S

Annex-6: Dry weight and N concentration of different above-ground plant fractions in the elite line collection in the +N field trial 2013/2014. All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Two plants per plot were cut into the indicated above-ground plant fractions to determine their DW and N concentration. Table shows means ±SD (n=4 for single genotypes, n=60 for all genotypes). ANOVA or ANOVA on ranks (■) results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. Significant mean difference among nitrate and urea treatment within a genotype is indicated by asterisks according to unpaired t-test at p<0.05; there were no significant mean or median differences between nitrate and urea treatment among all genotypes according to unpaired t-test or Mann-Whitney rank sum test (■), respectively, at p<0.05. Different upper case letters indicate significant mean or median differences among the genotypes within nitrate treatment according to Tukey´s test or Tukey´s test on ranks (■), respectively, at p<0.05; different lower case letters indicate significant mean or median differences among the genotypes within urea treatment according to Tukey´s test or Tukey´s test on ranks (■), respectively, at p<0.05.

Genotype	Organ DW [g]				N concentration [mg g DW^{-1}]			
	Nitrate		Urea		Nitrate		Urea	
Main shoot pods								
PBC007	3.3 ±0.5	AB	2.9 ±0.7	ab	16.9 ±1.5		17.1 ±2.1	
PBC015	2.3 ±0.4	B	2.3 ±0.4	b	17.0 ±0.9		16.7 ±2.3	
PBC029	4.4 ±0.8	AB	3.7 ±1.1	ab	18.7 ±0.8		16.9 ±1.1	
11091433	4.0 ±0.7	AB	3.6 ±0.3	ab	18.3 ±3.9		18.1 ±2.6	
12091707	3.7 ±0.5	AB	2.8 ±0.1	ab	15.8 ±2.1		17.9 ±1.6	
BCSNE001	2.6 ±1.1	AB *	4.4 ±0.5	a	19.6 ±2.6		18.9 ±2.8	
BCSNE002	3.1 ±1.0	AB	4.0 ±0.5	ab	18.6 ±1.1		17.5 ±0.7	
DSV-01	5.6 ±1.0	A *	4.3 ±0.9	a	17.4 ±2.4		17.7 ±1.6	
DSV-02	2.9 ±0.8	AB	2.7 ±0.9	ab	19.3 ±2.6		18.3 ±0.7	
KWS_01	3.5 ±0.9	AB	3.0 ±0.7	ab	16.3 ±1.3		18.5 ±2.2	
KWS_02	3.4 ±0.3	AB	3.4 ±1.4	ab	19.3 ±2.7		17.4 ±1.4	
LG00-304E	2.8 ±1.0	AB	2.9 ±0.6	ab	16.8 ±0.9		16.2 ±0.8	
LG02-228D	2.3 ±0.6	B	2.7 ±0.5	ab	16.6 ±1.9		16.6 ±1.9	
NPZ012	4.4 ±0.6	AB	4.1 ±0.5	ab	16.7 ±1.1		17.4 ±4.0	
NPZ208	3.9 ±1.3	AB	4.3 ±1.1	a	17.3 ±0.5		17.6 ±1.9	
All genotypes	3.5 ±1.1		3.4 ±0.9		17.6 ±2.1		17.5 ±1.9	
ANOVA	G ***, T ns, GxT ns				G ns, T ns, GxT ns			
Branch pods								
PBC007	12.8 ±3.0		16.1 ±2.3		18.9 ±1.4		18.7 ±1.9	
PBC015	13.6 ±3.7		13.9 ±6.3		18.5 ±1.5		19.2 ±2.9	
PBC029	24.2 ±10.4		22.2 ±8.0		19.6 ±2.2		16.9 ±1.9	
11091433	11.3 ±0.7		13.3 ±3.3		19.8 ±1.5		18.5 ±2.1	
12091707	21.2 ±6.4		14.4 ±3.6		16.7 ±2.5		18.6 ±2.7	
BCSNE001	14.6 ±4.6		20.5 ±2.4		18.4 ±2.0		20.2 ±2.1	
BCSNE002	16.9 ±10.0		12.0 ±2.3		19.6 ±5.8		19.2 ±1.9	
DSV-01	22.4 ±8.8		18.5 ±6.9		19.8 ±2.9		17.9 ±1.1	
DSV-02	11.4 ±2.0		11.9 ±2.2		20.6 ±1.7		19.9 ±1.0	
KWS_01	16.6 ±3.1		11.0 ±3.2		18.4 ±3.8		21.7 ±0.8	
KWS_02	16.4 ±4.3		17.7 ±2.0		20.0 ±2.3		19.3 ±4.1	
LG00-304E	14.4 ±3.6		15.4 ±2.7		18.7 ±2.2		19.2 ±2.0	
LG02-228D	14.2 ±1.9		16.8 ±1.6		18.7 ±1.4		18.3 ±2.9	
NPZ012	20.2 ±5.6		17.7 ±8.0		17.7 ±1.5		20.0 ±2.5	
NPZ208	17.9 ±4.1		12.7 ±4.0		18.3 ±1.0		18.7 ±2.2	
All genotypes	16.5 ±6.2		15.6 ±5.1		18.9 ±2.4		19.1 ±2.3	
ANOVA	G ***, T ns, GxT ns				G ns, T ns, GxT ns			
Main shoot								
PBC007	18.2 ±3.3		17.5 ±2.0	ac	6.0 ±0.4		5.1 ±0.6	
PBC015	13.4 ±2.3		13.1 ±3.6	ad	5.2 ±1.3		6.0 ±1.8	
PBC029	18.6 ±5.4		18.8 ±2.8	ab	7.3 ±0.3	*	5.5 ±0.8	
11091433	14.5 ±3.0		15.8 ±1.1	ad	5.8 ±1.4		5.5 ±0.7	
12091707	13.9 ±3.7		10.3 ±2.0	cd	5.8 ±1.0		6.4 ±1.3	

Annex-6 continued on page 120. ▸

Genotype	Organ DW [g]				N concentration [mg g DW^{-1}]			
	Nitrate		Urea		Nitrate		Urea	
BCSNE001	13.7 ±3.9		20.9 ±6.2	a	5.2 ±0.6		6.7 ±1.1	
BCSNE002	14.4 ±3.4		14.1 ±2.6	ad	7.2 ±0.8		6.0 ±0.4	
DSV-01	14.3 ±3.6		12.5 ±2.7	bd	6.5 ±1.8		5.0 ±1.1	
DSV-02	17.1 ±3.3		19.2 ±2.2	ab	6.1 ±1.7		6.2 ±1.2	
KWS_01	15.2 ±2.3		15.5 ±4.8	ad	5.4 ±0.8		5.7 ±1.3	
KWS_02	14.5 ±3.5		17.7 ±2.7	ac	6.2 ±1.1		5.5 ±0.8	
LG00-304E	15.5 ±1.9		16.3 ±2.2	ad	5.9 ±0.4		5.8 ±0.5	
LG02-228D	15.2 ±1.9		17.4 ±2.8	ad	6.1 ±1.4		5.6 ±0.8	
NPZ012	14.6 ±4.4	*	9.5 ±3.5	d	5.6 ±0.8	*	8.0 ±2.6	
NPZ208	14.2 ±3.8		10.1 ±2.6	cd	5.8 ±1.5		6.8 ±1.3	
All genotypes	15.1 ±3.4		15.2 ±4.4		6.0 ±1.2		6.0 ±1.3	
ANOVA	G ***, T ns, GxT ns				G ns, T ns, GxT ns			
Branches								
PBC007	10.1 ±4.4		16.5 ±1.8		10.5 ±0.4		9.6 ±0.9	
PBC015	10.9 ±3.8		10.7 ±5.4		10.5 ±0.9		12.2 ±2.0	
PBC029	19.4 ±10.7		19.5 ±4.5		10.4 ±1.4		8.6 ±0.9	
11091433	12.3 ±3.5		11.0 ±3.0		11.7 ±2.4		10.9 ±1.3	
12091707	16.7 ±7.2		9.8 ±3.3		10.6 ±1.0		10.4 ±1.0	
BCSNE001	12.6 ±4.5	*	21.4 ±5.4		9.4 ±1.7	*	12.1 ±2.1	
BCSNE002	17.9 ±7.9		11.6 ±2.8		10.1 ±1.7		10.0 ±1.0	
DSV-01	15.1 ±6.1		11.8 ±5.2		10.6 ±0.3		9.3 ±1.6	
DSV-02	13.4 ±3.5		14.7 ±2.7		11.3 ±0.8		10.5 ±1.8	
KWS_01	11.7 ±5.5		10.8 ±4.7		10.2 ±1.6		11.1 ±0.9	
KWS_02	13.4 ±3.4		19.1 ±3.6		8.8 ±2.6	*	11.2 ±0.9	
LG00-304E	15.5 ±1.0		14.4 ±2.4		11.4 ±2.9		10.7 ±0.1	
LG02-228D	13.6 ±3.2	*	22.1 ±13.2		10.7 ±0.6		9.0 ±0.8	
NPZ012	16.4 ±3.8		16.5 ±7.7		9.8 ±1.3		11.0 ±2.3	
NPZ208	15.3 ±5.7		8.7 ±4.5		11.4 ±2.0		10.3 ±1.3	
All genotypes	14.3 ±5.4		14.6 ±6.4		10.5 ±1.6		10.5 ±1.6	
ANOVA	G ns, T ns, GxT ns				G ns, T ns, GxT ns			
Branch leaves								
PBC007	0.9 ±0.6		1.1 ±0.2		25.3 ±5.4		25.7 ±2.3	ac
PBC015	0.8 ±0.2		1.0 ±0.6		29.5 ±2.7		31.2 ±1.7	a
PBC029	1.9 ±0.8		1.8 ±0.5		24.7 ±1.2		23.2 ±1.6	bc
11091433	1.0 ±0.1		1.0 ±0.3		26.8 ±2.8		27.4 ±3.2	ac
12091707	1.0 ±0.4		1.0 ±0.4		28.4 ±3.5		28.2 ±3.6	ac
BCSNE001	1.2 ±0.3		1.8 ±0.4		25.6 ±3.1		30.2 ±3.1	ab
BCSNE002	1.5 ±0.7		1.0 ±0.4		29.9 ±4.2		28.2 ±3.1	ac
DSV-01	1.7 ±0.7		1.2 ±0.4		28.0 ±1.6		24.7 ±2.9	ac
DSV-02	1.0 ±0.4		1.3 ±0.3		32.1 ±2.0		28.6 ±1.4	ac
KWS_01	1.0 ±0.2		1.1 ±0.5		25.8 ±8.5		28.5 ±3.7	ac
KWS_02	1.0 ±0.1		1.6 ±0.6		25.7 ±4.1		27.1 ±1.5	ac
LG00-304E	1.5 ±0.4		1.5 ±0.1		23.4 ±4.5		25.4 ±2.7	ac
LG02-228D	1.4 ±0.1	*	2.7 ±1.1		27.2 ±5.8		23.1 ±2.6	c
NPZ012	0.9 ±0.3		1.0 ±0.5		28.0 ±2.1		30.6 ±3.5	a
NPZ208	1.1 ±0.6		0.7 ±0.4		26.0 ±2.5		26.0 ±3.5	ac
All genotypes	1.2 ±0.5		1.3 ±0.8		27.1 ±4.2		27.2 ±3.4	
ANOVA	G ***, T ns, GxT ns				G **, T ns, GxT ns			
Main shoot green leaves								
PBC007	2.8 ±1.0	AB	3.8 ±1.9	ab	20.1 ±5.2		23.0 ±5.0	ab
PBC015	1.9 ±0.2	B	2.3 ±0.8	ab	24.3 ±2.3		25.5 ±2.1	ab
PBC029	6.5 ±3.0	A	5.0 ±1.3	a	19.9 ±2.8		18.6 ±1.0	b
11091433	3.9 ±1.2	AB	3.0 ±0.7	ab	20.6 ±1.4		24.4 ±2.5	ab
12091707	3.7 ±2.2	AB	2.9 ±1.0	ab	24.2 ±2.7		25.0 ±2.6	ab

Annex-6 continued on page 121. ▸

Genotype	Organ DW [g]				N concentration [mg g DW^{-1}]			
	Nitrate		Urea		Nitrate		Urea	
BCSNE001	3.5 ±1.0	AB *	6.3 ±1.5	a	22.2 ±2.8		25.9 ±2.8	ab
BCSNE002	4.6 ±1.7	AB	2.9 ±0.7	ab	24.8 ±3.3		23.7 ±2.8	ab
DSV-01	3.7 ±1.5	AB	2.2 ±1.3	ab	24.4 ±4.6		21.6 ±3.5	ab
DSV-02	4.2 ±0.7	AB	3.9 ±1.0	ab	26.2 ±1.5		23.7 ±1.4	ab
KWS_01	2.5 ±0.8	AB	3.6 ±1.2	ab	25.1 ±2.9		23.2 ±2.8	ab
KWS_02	3.3 ±1.3	AB	3.6 ±0.8	ab	22.9 ±3.8		21.2 ±3.4	ab
LG00-304E	3.2 ±0.4	AB	3.4 ±0.9	ab	21.6 ±2.0		20.1 ±4.1	b
LG02-228D	3.9 ±1.3	AB	4.8 ±2.4	ab	22.8 ±3.2		22.0 ±2.6	ab
NPZ012	2.1 ±0.4	AB	2.5 ±0.8	ab	22.0 ±5.3	*	27.6 ±2.6	a
NPZ208	2.6 ±1.1	AB	1.6 ±0.7	b	23.1 ±2.0		22.3 ±1.8	ab
All genotypes	3.5 ±1.6		3.5 ±1.6		22.9 ±3.4		23.2 ±3.4	
ANOVA	G ***, T ns, GxT ns				G **, T ns, GxT ns			
Main shoot senescent leaves								
PBC007	1.4 ±0.8		1.4 ±0.7	ab	20.1 ±5.2		23.0 ±5.0	ab
PBC015	0.9 ±0.6		0.6 ±0.2	ab	24.3 ±2.3		25.5 ±2.1	a
PBC029	1.5 ±0.9	*	0.9 ±0.2	ab	19.9 ±2.8		18.6 ±1.0	b
11091433	0.9 ±0.4		0.8 ±0.2	ab	20.6 ±1.4		24.4 ±2.5	ab
12091707	0.7 ±0.3		0.8 ±0.4	ab	24.2 ±2.7		25.0 ±2.6	ab
BCSNE001	1.0 ±0.1		1.1 ±0.2	a	22.2 ±2.8		25.9 ±2.8	ab
BCSNE002	0.7 ±0.5		0.7 ±0.4	ab	24.8 ±3.3		23.7 ±2.8	ab
DSV-01	0.6 ±0.2		0.3 ±0.1	b	24.4 ±4.6		21.6 ±3.5	ab
DSV-02	1.1 ±0.6		0.9 ±0.4	ab	26.2 ±1.5		23.7 ±1.4	ab
KWS_01	1.2 ±0.7		1.0 ±0.6	ab	25.1 ±2.9		23.2 ±2.8	ab
KWS_02	0.9 ±0.3		0.9 ±0.1	ab	22.9 ±3.8		21.2 ±3.4	ab
LG00-304E	0.7 ±0.2		0.8 ±0.1	ab	21.6 ±2.0		20.1 ±4.1	ab
LG02-228D	0.6 ±0.3		0.8 ±0.1	ab	22.8 ±3.2		22.0 ±2.6	ab
NPZ012	0.6 ±0.2		0.4 ±0.1	ab	22.0 ±5.3		27.6 ±2.6	a
NPZ208	1.1 ±0.3		0.8 ±0.4	ab	23.1 ±2.0		22.3 ±1.8	ab
All genotypes	0.9 ±0.5		0.8 ±0.5		22.9 ±3.4		23.2 ±3.4	
ANOVA	G ***, T ns, GxT ns				G ns, T ns, GxT ns			

Annex-7: Ratio of N pool size in generative organs to N pool size in vegetative organs ($N_{generative}/N_{vegetative}$) at BBCH79 in the genotypes of the elite line collection in the +N field trials in 2012/2013 and 2013/2014. All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Two plants per plot were separated into the indicated above-ground plant fractions. Table shows means ±SD (n=4 for single genotypes, n=60 for all genotypes). ANOVA on ranks results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. There were no significant mean differences among nitrate and urea treatment within any genotype according to unpaired t-test at p<0.05 or among all genotypes according to Mann-Whitney rank sum test at p<0.05. Different upper case letters indicate significant mean or median differences among the genotypes within nitrate treatment, while different lower case letters indicate significant mean or median differences among the genotypes within urea treatment according to Tukey´s test (2012/2013: urea, 2013/2014: nitrate) or Tukey´s test on ranks (2012/2013: nitrate, 2013/2014: urea), respectively, at p<0.05.

Genotype	$N_{generative}/N_{vegetative}$			
	2012/2013		**2013/2014**	
	Nitrate	**Urea**	**Nitrate**	**Urea**
PBC007	1.4 ±0.2	1.4 ±0.3 ab	1.0 ±0.1 AC	1.0 ±0.3
PBC015	1.5 ±0.1	1.5 ±0.3 ab	1.1 ±0.1 AC	1.0 ±0.2
Alpaga	1.5 ±0.3	1.4 ±0.1 ab		
PBC029			1.1 ±0.1 AC	1.0 ±0.2
11091433	1.8 ±0.8	1.4 ±0.3 ab	0.9 ±0.2 AC	1.0 ±0.1
12091707	1.8 ±0.1	1.9 ±0.2 a	1.1 ±0.2 AC	1.2 ±0.3
BCSNE001	1.2 ±0.2	1.2 ±0.2 b	1.0 ±0.2 AC	0.8 ±0.2
BCSNE002	1.8 ±0.1	1.6 ±0.2 ab	0.8 ±0.3 AC	1.0 ±0.2
DSV-01	2.0 ±0.5	1.6 ±0.4 ab	1.4 ±0.1 A	1.7 ±0.4
DSV-02	1.2 ±0.1	1.3 ±0.1 ab	0.7 ±0.2 C	0.6 ±0.1
KWS_01	1.5 ±0.2	1.5 ±0.4 ab	1.2 ±0.2 AB	0.9 ±0.2
KWS_02	1.7 ±0.3	1.7 ±0.4 ab	1.3 ±0.1 AC	0.9 ±0.3
LG00-304E	1.3 ±0.1	1.3 ±0.2 ab	0.7 ±0.1 C	1.0 ±0.2
LG02-228D	1.3 ±0.1	1.3 ±0.1 ab	0.8 ±0.1 BC	0.8 ±0.2
NPZ012	1.7 ±0.4	1.9 ±0.3 a	1.4 ±0.2 A	1.2 ±0.2
NPZ208	1.9 ±0.3	1.5 ±0.2 ab	1.4 ±0.1 A	1.5 ±0.4
All genotypes	1.6 ±0.3	1.5 ±0.3 ab	1.1 ±0.3 AC	1.0 ±0.3
ANOVA	G ***, T ns, GxT ns		G ***, T ns, GxT ns	

Annex-8: **Xylem exudation rates in the genetic diverse collection in the +N field trial 2011/2012 at BBCH52, BBCH59 and BBCH69.** All rapeseed genotypes were fertilized in spring with 40 kg N ha^{-1} at BBCH30 and 60 kg N ha^{-1} at BBCH55 as nitrate or urea. Xylem sap was pooled from ten plants per plot. Table shows means ±SD (n=3 for single genotypes, n=45 for all genotypes). ANOVA (BBCH69) or ANOVA on ranks (BBCH52, BBCH59) results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. There were no significant mean or median differences among nitrate and urea treatment within any single genotype according to unpaired t-test at p<0.05 or among all genotypes according to unpaired t-test (BBCH69) or Mann-Whitney rank sum test (BBCH52, BBCH59), respectively, at p<0.05. There were no significant mean differences among the genotypes within nitrate treatment according to Tukey´s test at p<0.05; there were no significant mean or median differences among the genotypes within urea treatment according to Tukey´s test (BBCH59, BBCH69) or Tukey´s test on ranks (BBCH52), respectively, at p<0.05.

Genotype	**Xylem exudation rate [ml $h^{-1}plant^{-1}$]**					
	BBCH52		**BBCH59**		**BBCH69**	
	Nitrate	**Urea**	**Nitrate**	**Urea**	**Nitrate**	**Urea**
PBC007	1.10 ±0.46	0.66 ±0.39	1.24 ±0.68	0.89 ±0.43	0.93 ±0.57	0.75 ±0.45
PBC015	1.06 ±0.37	0.78 ±0.26	0.74 ±0.18	0.91 ±0.27	0.82 ±0.25	0.87 ±0.37
PBC001	1.14 ±0.34	0.91 ±0.22	1.79 ±0.75	1.88 ±0.68	0.35 ±0.22	0.48 ±0.11
PBC002	0.93 ±0.32	1.01 ±0.30	0.55 ±0.08	0.70 ±0.64	0.39 ±0.14	0.56 ±0.08
PBC009	1.12 ±0.37	0.76 ±0.41	0.92 ±0.60	2.00 ±0.91	0.58 ±0.16	0.85 ±0.22
PBC011	1.23 ±0.15	0.95 ±0.71	1.87 ±1.36	1.19 ±0.78	0.43 ±0.16	0.72 ±0.34
PBC013	1.29 ±0.18	1.09 ±0.39	1.44 ±0.97	1.26 ±0.60	0.56 ±0.21	0.43 ±0.35
PBC018	1.02 ±0.37	1.08 ±0.39	0.56 ±0.28	0.77 ±0.21	0.69 ±0.08	0.42 ±0.09
PBC019	1.26 ±0.42	1.14 ±0.81	1.31 ±0.91	1.35 ±0.73	0.72 ±0.17	0.41 ±0.10
PBC021	0.96 ±0.10	1.06 ±0.74	1.68 ±0.87	1.01 ±0.91	0.61 ±0.23	0.64 ±0.07
PBC022	0.83 ±0.50	0.72 ±0.32	1.33 ±0.55	1.20 ±0.59	0.83 ±0.39	0.48 ±0.17
PBC023	1.19 ±0.23	1.36 ±0.25	1.08 ±0.50	0.78 ±0.68	0.62 ±0.18	0.55 ±0.11
PBC024	0.92 ±0.18	1.37 ±0.26	1.41 ±0.47	0.59 ±0.11	0.56 ±0.11	0.90 ±0.18
PBC027	1.21 ±0.69	0.88 ±0.22	1.17 ±0.97	0.46 ±0.15	0.55 ±0.21	0.38 ±0.17
PBC029	1.17 ±0.40	1.13 ±0.15	1.02 ±0.79	1.01 ±0.59	0.39 ±0.14	0.60 ±0.18
All genotypes	1.09 ±0.32	1.00 ±0.43	1.20 ±0.71	1.10 ±0.71	0.60 ±0.26	0.60 ±0.23
ANOVA	G ns, T ns, GxT ns		G ns, T ns, GxT ns		G ns, T ns, GxT ns	

Annex-9: Dry weight and N concentration of different above-ground plant fractions in the genetic diverse collection in the +N field trial 2011/2012. All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Two plants per plot were separated cut into the indicated above-ground plant. Table shows means ±SD (n=3 for single genotypes, n=45 for all genotypes). ANOVA or ANOVA on ranks (■) results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. There were no significant mean or median differences among nitrate and urea treatment within any single genotype according to unpaired t-test at p<0.05 or among all genotypes according to unpaired t-test or Mann-Whitney rank sum test (■), respectively, at p<0.05. Different upper case letters indicate significant mean or median differences among the genotypes within nitrate treatment according to Tukey´s test or Tukey´s test on ranks (■), respectively, at p<0.05; different lower case letters indicate significant mean or median differences among the genotypes within urea treatment according to Tukey´s test or Tukey´s test on ranks (■), respectively, at p<0.05.

Genotype	Organ DW [g]				N concentration [mg g DW^{-1}]		
	Nitrate		Urea		Nitrate	Urea	
Main shoot pods							
PBC007	5.6 ±0.8	AB	5.4 ±2.0		17.7 ±0.7	17.0 ±1.8	ab
PBC015	7.9 ±0.8	AB	6.3 ±1.0		16.1 ±0.8	15.7 ±1.4	b
PBC001	7.9 ±2.9	AB	7.1 ±2.1		18.6 ±0.6	16.8 ±0.2	ab
PBC002	10.3 ±2.0	A	9.9 ±1.1		19.4 ±2.7	16.9 ±0.3	ab
PBC009	9.1 ±1.8	AB	8.3 ±4.8		17.5 ±1.8	19.0 ±1.8	ab
PBC011	3.6 ±0.5	B	3.8 ±1.3		17.3 ±1.9	17.7 ±1.6	ab
PBC013	9.8 ±0.4	A	11.1 ±0.3		19.7 ±3.2	20.6 ±0.8	a
PBC018	9.8 ±0.8	A	8.1 ±3.5		17.4 ±1.4	17.9 ±3.1	ab
PBC019	5.9 ±0.8	AB	6.1 ±1.8		17.4 ±0.5	17.3 ±1.7	ab
PBC021	6.4 ±3.3	AB	7.4 ±2.4		18.8 ±2.0	18.9 ±1.4	ab
PBC022	7.5 ±2.5	AB	9.4 ±1.3		17.1 ±1.3	18.9 ±0.3	ab
PBC023	7.1 ±1.9	AB	7.6 ±1.8		18.3 ±2.6	18.2 ±1.1	ab
PBC024	8.6 ±1.6	AB	8.8 ±0.5		16.2 ±0.2	16.9 ±1.1	ab
PBC027	8.8 ±0.9	AB	5.7 ±3.6		20.7 ±0.9	20.0 ±1.2	ab
PBC029	8.1 ±3.4	AB	6.0 ±3.5		20.2 ±2.7	18.1 ±2.4	ab
All genotypes	7.8 ±2.4		7.4 ±2.7		18.2 ±2.0	18.0 ±1.8	
ANOVA	G ***, T ns, GxT ns				G ***, T ns, GxT ns		
Branch pods							
PBC007	20.4 ±2.8		27.5 ±8.8		18.5 ±1.8	19.1 ±0.6	
PBC015	32.8 ±7.5		17.4 ±5.0		17.3 ±0.4	16.9 ±0.6	
PBC001	21.9 ±12.1		15.6 ±3.3		18.3 ±0.6	17.8 ±1.0	
PBC002	23.8 ±6.2		22.8 ±9.6		18.4 ±2.5	18.2 ±2.5	
PBC009	41.4 ±8.8		32.1 ±14.5		17.1 ±1.6	18.8 ±2.4	
PBC011	21.5 ±4.1		35.6 ±17.5		18.8 ±2.2	19.7 ±2.1	
PBC013	22.6 ±4.4		29.3 ±2.9		19.7 ±0.4	18.6 ±1.3	
PBC018	22.9 ±4.1		35.2 ±22.1		17.1 ±2.0	17.0 ±0.1	
PBC019	27.8 ±4.1		27.7 ±4.6		17.7 ±1.4	18.9 ±2.9	
PBC021	23.6 ±8.4		31.7 ±14.7		19.1 ±3.5	18.6 ±1.5	
PBC022	21.6 ±11.2		20.9 ±5.0		16.7 ±0.6	18.3 ±2.0	
PBC023	29.4 ±5.4		32.2 ±23.0		17.0 ±1.9	17.4 ±1.4	
PBC024	31.3 ±14.5		26.4 ±1.8		17.2 ±0.7	16.9 ±0.7	
PBC027	38.8 ±15.9		27.4 ±13.0		19.1 ±2.2	18.6 ±1.3	
PBC029	24.6 ±6.8		28.4 ±11.8		20.7 ±0.4	19.0 ±1.7	
All genotypes	27.0 ±9.6		27.3 ±11.8		18.1 ±1.8	18.3 ±1.6	
ANOVA	G ns, T ns, GxT ns				G ns, T ns, GxT ns		
Main shoot							
PBC007	19.7 ±2.2		18.4 ±0.3	ab	4.5 ±0.3	4.4 ±0.4	
PBC015	19.2 ±3.2		13.9 ±3.6	b	4.4 ±0.2	4.1 ±0.5	
PBC001	16.7 ±0.4		15.4 ±3.2	b	4.4 ±0.8	5.2 ±0.6	
PBC002	18.4 ±4.6		19.4 ±4.7	ab	5.8 ±1.6	4.7 ±0.7	
PBC009	20.8 ±2.3		18.1 ±3.5	ab	4.7 ±0.2	4.1 ±0.3	
PBC011	19.1 ±2.0		15.4 ±1.6	a	4.8 ±0.5	4.5 ±0.2	

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PBC013	18.3 ±1.4	22.3 ±1.3	ab	3.9 ±0.7		4.9 ±0.4	
PBC018	15.3 ±3.1	15.9 ±5.1	ab	4.5 ±0.3		5.8 ±1.1	
PBC019	17.9 ±0.3	16.2 ±0.6	ab	4.8 ±1.1		4.8 ±1.1	
PBC021	15.6 ±2.8	17.3 ±3.8	ab	6.4 ±3.2		4.7 ±0.6	
PBC022	16.3 ±4.8	14.8 ±2.6	b	4.0 ±1.0		4.2 ±0.4	
PBC023	16.8 ±1.7	15.8 ±4.1	ab	4.1 ±0.5		5.0 ±0.5	
PBC024	16.9 ±4.5	15.4 ±3.3	b	4.9 ±0.6		4.6 ±0.7	
PBC027	21.6 ±4.9	17.9 ±1.6	ab	4.6 ±1.0		4.1 ±0.2	
PBC029	18.1 ±0.2	18.5 ±4.3	ab	5.1 ±0.7		4.8 ±1.3	
All genotypes	18.1 ±3.2	17.6 ±4.0		4.7 ±1.1		4.7 ±0.7	
ANOVA	G **, T ns, GxT ns			G ns, T ns, GxT ns			
Branches							
PBC007	16.8 ±5.2	20.3 ±7.5	ab	7.2 ±0.4		8.0 ±1.2	
PBC015	20.9 ±6.8	10.3 ±3.9	b	8.1 ±0.4		8.5 ±1.0	
PBC001	14.1 ±6.2	9.4 ±2.3	b	8.6 ±0.3		8.8 ±0.8	
PBC002	13.9 ±3.3	14.5 ±5.6	ab	8.7 ±0.4		8.2 ±1.3	
PBC009	23.1 ±4.7	16.5 ±5.0	ab	7.7 ±0.7		9.0 ±0.9	
PBC011	20.5 ±3.8	34.7 ±9.4	a	8.0 ±0.4		7.5 ±1.2	
PBC013	15.6 ±3.7	19.8 ±4.0	ab	7.9 ±0.8		8.4 ±0.4	
PBC018	23.2 ±18.2	19.3 ±10.0	ab	8.6 ±0.5		6.8 ±2.3	
PBC019	14.3 ±3.1	13.4 ±3.8	b	7.2 ±0.3		7.8 ±1.3	
PBC021	15.3 ±3.0	16.1 ±6.4	b	7.0 ±1.6		8.2 ±0.1	
PBC022	15.7 ±6.4	10.9 ±1.5	b	7.2 ±2.6		10.2 ±1.2	
PBC023	13.4 ±4.2	14.6 ±11.8	b	7.5 ±0.2		8.6 ±0.3	
PBC024	15.4 ±9.2	11.4 ±0.9	b	9.2 ±8.0		8.8 ±0.6	
PBC027	34.5 ±12.9	13.1 ±2.6	b	8.1 ±1.0		7.7 ±1.0	
PBC029	16.3 ±4.7	19.6 ±6.3	ab	8.6 ±0.9		8.2 ±1.8	
All genotypes	18.2 ±8.3	16.4 ±8.0		8.0 ±1.1		8.3 ±1.2	
ANOVA	G *, T ns, GxT ns			G ns, T ns, GxT ns			
Branch leaves							
PBC007	1.1 ±0.8	1.1 ±0.3		21.9 ±3.9	AB	22.0 ±1.7	ac
PBC015	0.4 ±0.2	0.4 ±0.3		25.8 ±2.2	A	25.4 ±2.7	ab
PBC001	0.4 ±0.3	0.1 ±0.0		23.8 ±2.8	AB	19.9 ±3.0	bc
PBC002	0.4 ±0.2	0.5 ±0.3		24.5 ±3.8	AB	22.6 ±3.3	ac
PBC009	1.0 ±0.4	0.4 ±0.3		22.5 ±4.3	AB	23.1 ±2.3	ac
PBC011	1.1 ±0.6	1.1 ±0.8		20.2 ±2.8	AB	19.6 ±1.5	bc
PBC013	0.8 ±0.6	1.3 ±0.5		19.4 ±1.6	AB	19.2 ±2.5	bc
PBC018	0.7 ±0.5	0.4 ±0.3		26.2 ±1.8	A	22.4 ±1.7	ac
PBC019	0.7 ±0.5	0.8 ±0.7		21.0 ±1.5	AB	19.6 ±0.6	bc
PBC021	1.0 ±0.4	0.3 ±0.1		23.7 ±2.4	AB	23.8 ±2.0	ac
PBC022	0.7 ±0.5	0.3 ±0.1		15.9 ±6.1	B	26.9 ±1.6	a
PBC023	0.6 ±0.3	0.8 ±0.1		25.1 ±2.3	AB	25.2 ±2.1	ac
PBC024	0.3 ±0.1	0.4 ±0.3		26.7 ±0.9	A	24.3 ±2.9	ac
PBC027	2.1 ±1.6	0.6 ±0.4		22.6 ±2.6	AB	21.2 ±1.5	ac
PBC029	0.4 ±0.3	0.6 ±0.5		25.5 ±6.0	A	18.6 ±2.6	c
All genotypes	0.8 ±0.7	0.6 ±0.5		22.9 ±4.1		22.3 ±3.2	
ANOVA	G *, T ns, GxT ns			G *, T ns, GxT ns			
Main shoot green leaves							
PBC007	3.9 ±1.6	4.3 ±1.0	ab	21.5 ±1.2	AB	19.8 ±2.2	
PBC015	1.8 ±0.9	0.4 ±0.3	c	23.6 ±1.1	A	21.0 ±1.8	
PBC001	0.9 ±0.8	1.1 ±0.6	c	23.1 ±1.9	A	16.9 ±2.0	
PBC002	1.8 ±1.1	2.5 ±1.9	bc	23.6 ±3.7	A	20.5 ±3.1	
PBC009	1.9 ±1.1	0.9 ±0.6	c	22.0 ±3.9	AB	22.0 ±3.9	
PBC011	3.9 ±1.2	5.8 ±1.7	a	19.9 ±1.6	AB	18.8 ±2.0	
PBC013	3.1 ±1.5	4.3 ±0.5	ab	18.5 ±3.2	AB	17.7 ±2.8	
PBC018	1.1 ±0.2	0.6 ±0.2	c	21.8 ±2.0	AB	19.9 ±1.9	

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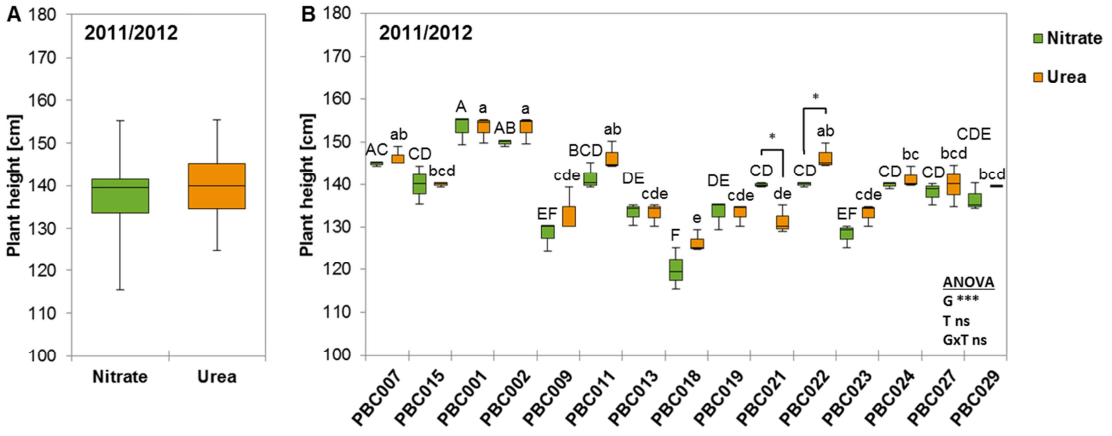
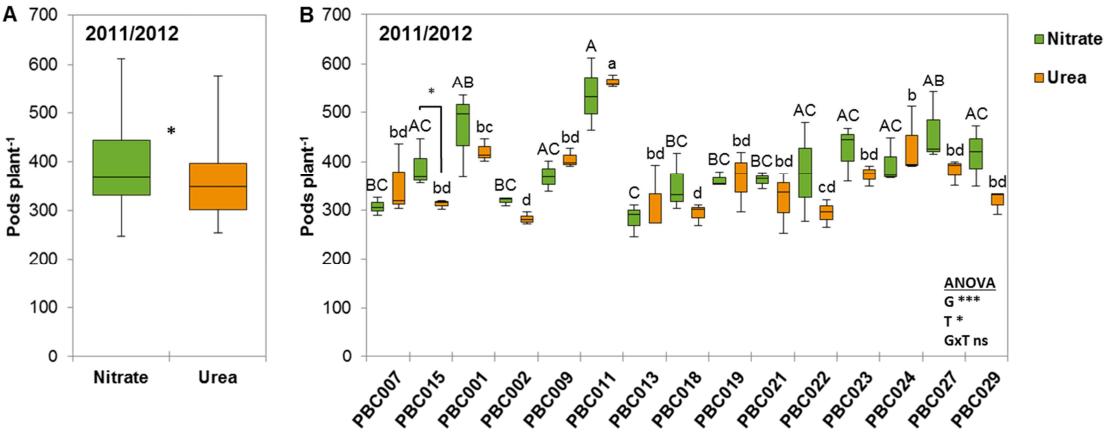
PBC019	2.2 ±1.0	1.6 ±1.5	bc	17.3 ±1.1	AB	17.6 ±2.8
PBC021	1.4 ±0.3	1.4 ±0.6	bc	18.6 ±3.0	AB	20.7 ±2.7
PBC022	1.6 ±1.5	0.4 ±0.3	c	13.3 ±4.8	B	24.0 ±3.0
PBC023	0.8 ±0.5	1.6 ±0.8	bc	23.9 ±2.3	A	21.7 ±1.7
PBC024	1.4 ±0.3	0.8 ±0.5	c	24.3 ±3.0	A	22.9 ±3.2
PBC027	2.4 ±1.4	1.1 ±0.3	c	21.1 ±1.6	AB	20.5 ±2.1
PBC029	4.2 ±3.5	3.1 ±1.6	ac	22.5 ±6.6	AB	17.0 ±1.9
All genotypes	2.2 ±1.6	2.0 ±1.8		21.0 ±3.9		20.1 ±3.0
ANOVA	G ***, T ns, GxT ns			G ns, T ns, GxT ns		
Main shoot senescent leaves						
PBC007	3.3 ±0.5	2.8 ±0.9	ab	8.5 ±1.7		7.8 ±0.8
PBC015	1.0 ±0.4	0.6 ±0.5	b	11.4 ±1.2		10.6 ±0.7
PBC001	0.6 ±0.3	1.9 ±0.8	ab	11.2 ±3.5		9.8 ±2.0
PBC002	3.6 ±2.9	1.6 ±1.4	b	13.0 ±5.3		10.8 ±2.7
PBC009	2.4 ±2.0	0.8 ±0.5	b	10.0 ±1.7		10.5 ±1.9
PBC011	1.9 ±0.3	4.4 ±1.6	a	9.7 ±0.6		11.0 ±1.8
PBC013	1.5 ±0.9	1.8 ±0.5	b	11.4 ±2.0		8.2 ±1.3
PBC018	1.3 ±0.7	2.1 ±0.6	ab	9.4 ±0.5		10.3 ±2.5
PBC019	0.9 ±0.8	1.6 ±0.5	b	10.8 ±0.8		9.9 ±1.2
PBC021	1.8 ±1.1	1.6 ±0.6	b	8.9 ±1.0		10.2 ±0.2
PBC022	0.8 ±0.4	0.4 ±0.3	b	13.4 ±7.9		12.3 ±3.0
PBC023	0.9 ±0.6	1.8 ±1.3	ab	12.6 ±1.0		9.9 ±1.8
PBC024	0.8 ±0.5	0.8 ±0.5	b	11.4 ±1.0		12.0 ±2.0
PBC027	2.9 ±1.3	2.3 ±1.0	ab	10.0 ±1.9		10.3 ±2.3
PBC029	1.8 ±1.5	2.3 ±0.7	ab	12.2 ±3.0		9.9 ±1.4
All genotypes	1.7 ±1.4	1.8 ±1.2		10.9 ±2.8		10.2 ±1.9
ANOVA	G ***, T ns, GxT ns			G ns, T ns, GxT ns		



Annex-10: Plant height in the genetic diverse collection in the +N field trial 2011/2012 as mean over all genotypes (A) or of the single genotypes (B). All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Plant height was determined at the day of seed harvest as mean of the main shoot length from ten plants per plot. Boxes show median, first and third quartile; whiskers represent minimum and maximum of all data in a group (A: n=45; B: n=3). (A) There was no significant difference among the means according to unpaired t-test at p<0.05. (B) ANOVA results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. Asterisks indicate significant mean difference among nitrate and urea treatment within a genotype according to unpaired t-test at p<0.05. Different upper case letters indicate significant mean differences among the genotypes within nitrate treatment according to Tukey´s test at p<0.05; different lower case letters indicate significant mean differences among the genotypes within urea treatment according to Tukey´s test at p<0.05.

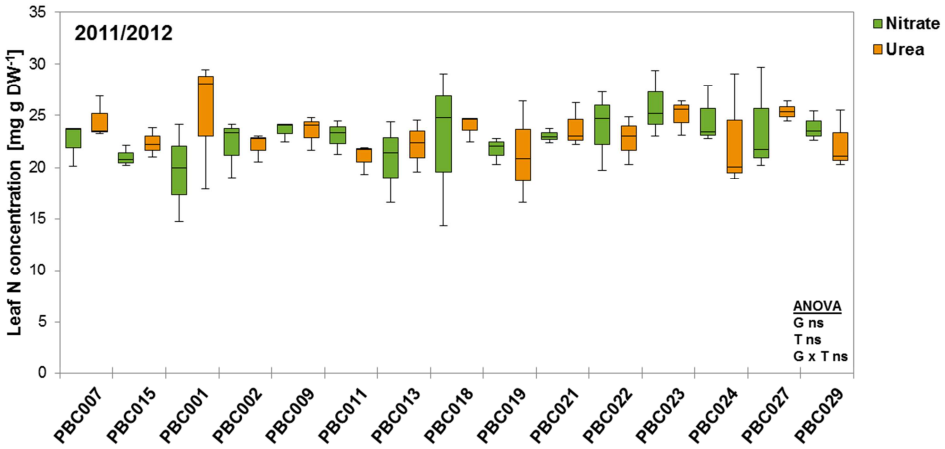


Annex-11: Pod number per plant in the genetic diverse collection in the +N field trial 2011/2012 as mean over all genotypes (A) or of the single genotypes (B). All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Pod number from 10 plants per plot was determined. Boxes show median, first and third quartile; whiskers represent minimum and maximum of all data in a group (A: n=45; B: n=3). (A) Asterisk indicates differences among the means according to unpaired t-test at p=0.07. (B) ANOVA results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. Asterisks indicate significant mean difference among nitrate and urea treatment within a genotype according to unpaired t-test at p<0.05. Different upper case letters indicate significant mean differences among the genotypes within nitrate treatment according to Tukey´s test at p<0.05; different lower case letters indicate significant median differences among the genotypes within urea treatment according to Tukey´s test on ranks at p<0.05.

Annex-12: Ratio of N pool size in generative organs to N pool size in vegetative organs ($N_{generative}/N_{vegetative}$) at BBCH79 in the genotypes of the genetic diverse collection in the +N field trial 2011/2012. All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Two plants per plot were separated into the indicated above ground plant fractions to determine their N content. Table shows means ±SD (n=3 for single genotypes, n=45 for all genotypes). ANOVA on ranks results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. There were no significant mean differences among nitrate and urea treatment within any genotype according to unpaired t-test at p<0.05 or among all genotypes according to Mann-Withney rank sum test at p<0.05. There were no significant mean differences among the genotypes under nitrate as well as under urea treatment according to Tukey´s test on ranks at p<0.05.

Genotype	$N_{generative}/N_{vegetative}$	
	Nitrate	Urea
PBC007	1.3 ±0.1	1.6 ±0.3
PBC015	1.3 ±0.1	2.3 ±0.6
PBC001	2.2 ±0.1	1.8 ±0.2
PBC002	1.7 ±0.4	1.9 ±0.4
PBC009	2.3 ±0.5	2.9 ±1.2
PBC011	1.5 ±0.1	1.3 ±0.4
PBC013	2.3 ±0.8	2.0 ±0.4
PBC018	1.7 ±0.6	2.5 ±0.7
PBC019	2.3 ±0.4	2.6 ±0.6
PBC021	2.0 ±0.4	2.6 ±0.7
PBC022	2.3 ±1.4	2.8 ±0.4
PBC023	2.9 ±0.5	2.4 ±0.3
PBC024	2.4 ±0.2	3.4 ±2.3
PBC027	1.5 ±0.1	2.5 ±0.9
PBC029	1.9 ±0.7	1.9 ±0.8
All genotypes	2.0 ±0.6	2.3 ±0.9

ANOVA
G ***
T ns
GxT ns



Annex-13: N concentration in the youngest main shoot leaf at BBCH69 of the in the genetic diverse collection in the +N field trial 2011/2012. All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Leaf material was grinded to determine N concentration. Boxes show median, first and third quartile; whiskers represent minimum and maximum of all data in a group; n=3. There were no significant differences among the means according to Tukey´s test at p<0.05. ANOVA results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant.

Annex-14: Duration between fertilizer N application events and xylem sap harvests in the +N/-N field trials in 2012/2013 and 2013/2014.

Fertilization events	Plant BBCH stages at xylem sap harvest		Time span between last fertilization event and xylem sap harvest	
	2012/2013	2013/2014	2012/2013	2013/2014
40 kg N ha^{-1} (at BBCH30)				
	52	55	7 d	7 d
60 kg N ha^{-1} (at BBCH55)				
	57	57	7 d	7 d
	65	65	36 d	42 d
	75	75	42 d	48 d

Annex-15: Xylem exudation rates of the reference genotypes PBC007 and PBC015 at different developmental stages in the +N/-N field trials 2012/2013 and 2013/2014. All rapeseed genotypes were fertilized in spring with 40 kg N ha^{-1} at BBCH30 and 60 kg N ha^{-1} at BBCH55 as nitrate or urea while control treatment received no additional N fertilization. Xylem sap was pooled from ten plants per plot. Table shows means ±SD; n=4. Comparing exudation rates of genotypes and N treatment within one year and one BBCH stage, there were no significant mean or median differences according to Tukey´s test or Tukey´s test on ranks (■), respectively, as well as no significant genotype, treatment or genotype x treatment interaction effects according to ANOVA or ANOVA on ranks (■) at $p<0.05$.
* In 2012/2013 first harvest equates BBCH52, in 2013/2014 BBCH55.

Fertilizer treatment	Xylem exudation rate [ml h^{-1} $plant^{-1}$]			
	2012/2013		2013/2014	
	PBC007	PBC015	PBC007	PBC015
*BBCH52/55**				
Control	0.44 ±0.24	0.48 ±0.29	0.54 ±0.21	0.46 ±0.18
Nitrate	0.46 ±0.16	0.66 ±0.30	0.52 ±0.13	0.60 ±0.08
Urea	0.28 ±0.06	0.65 ±0.38	0.49 ±0.10	0.57 ±0.05
BBCH57				
Control	1.18 ±0.71	1.29 ±1.09	0.80 ±0.25	0.70 ±0.55
Nitrate	1.49 ±0.37	1.73 ±0.68	1.06 ±0.53	0.90 ±0.21
Urea	1.56 ±0.94	1.56 ±0.30	0.95 ±0.34	0.80 ±0.40
BBCH65				
Control	1.58 ±0.33	2.36 ±1.52	0.93 ±0.08	0.68 ±0.39
Nitrate	2.49 ±1.07	2.72 ±1.07	0.79 ±0.15	0.75 ±0.24
Urea	2.51 ±0.50	2.03 ±0.67	0.92 ±0.23	0.94 ±0.24
BBCH75				
Control	1.11 ±0.60	0.84 ±0.30	1.38 ±1.34	0.57 ±0.10
Nitrate	1.36 ±1.20	0.86 ±0.75	0.61 ±0.41	0.57 ±0.28
Urea	0.86 ±0.44	3.19 ±3.80	1.14 ±0.96	1.63 ±1.02

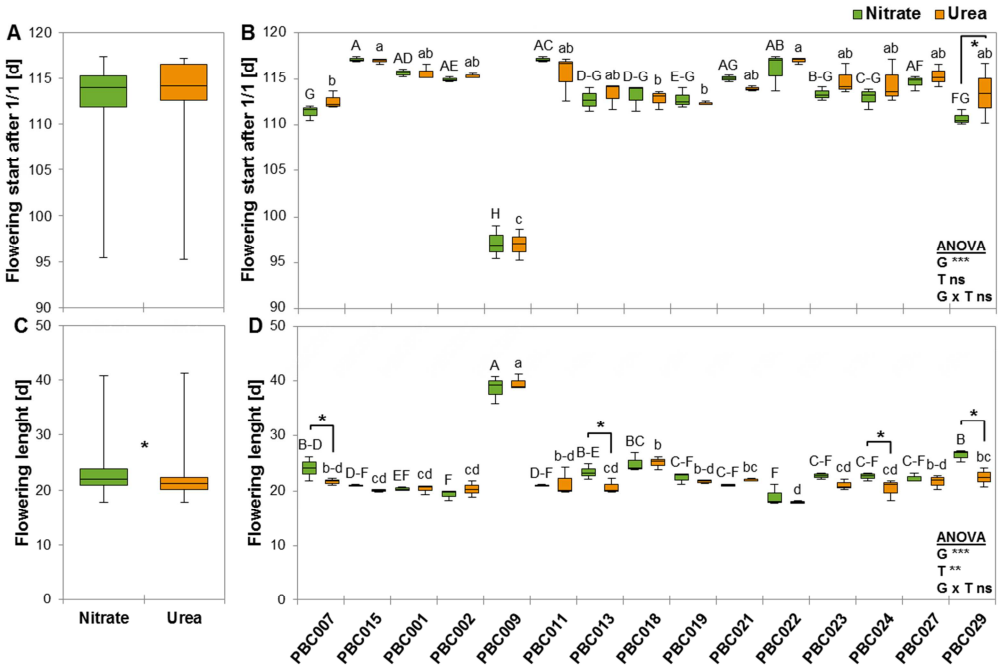
	N concentration [mg g DW^{-1}]											
	2012/2013						2013/2014					
	PBC007			PBC015			PBC007			PBC015		
	C	N	U	C	N	U	C	N	U	C	N	U
Stem pods	13.5 ±0.6 b	19.1 ±1.1 a	19.3 ±2.5 a	13.5 ±1.6 b	19.9 ±0.5 a	18.9 ±1.5 a	13.0 ±0.6 bc	15.7 ±1.9 ab	16.3 ±0.7 a	11.8 ±1.1 c	14.1 ±2.7 ac	15.7 ±0.5 ab
Branch pods	13.7 ±1.2 - -	19.1 ±2.4 - -	21.7 ±3.7 - -	14.9 ±1.4 - -	16.3 ±10.8 - -	21.7 ±2.6 - -	13.0 ±0.9 b	17.0 ±0.8 a	17.6 ±0.9 a	12.8 ±0.5 b	16.6 ±0.5 a	16.8 ±1.2 a
Stem	5.3 ±2.3 - -	7.2 ±1.4 - -	9.8 ±4.8 - -	5.4 ±2.3 - -	8.8 ±1.2 - -	7.8 ±1.8 - -	3.4 ±0.3 -b-	7.2 ±1.8 -a-	5.9 ±1.1 -ab-	3.6 ±0.5 -ab-	4.9 ±1.2 -ab-	5.5 ±1.1 -ab-
Branches	7.9 ±1.4 b	11.5 ±2.1 a	11.7 ±1.7 a	7.5 ±0.5 b	12.3 ±0.8 a	11.7 ±1.1 a	6.8 ±0.8 b	9.5 ±1.1 a	10.4 ±0.2 a	6.8 ±0.3 b	9.6 ±1.1 a	10.0 ±1.0 a
Branch leaves	15.6 ±3.1 b	22.4 ±3.4 a	25.9 ±1.9 a	15.8 ±3.4 b	29.1 ±2.0 a	28.2 ±2.2 a	12.9 ±3.2 b	24.5 ±3.6 a	24.6 ±0.9 a	13.7 ±1.7 b	25.5 ±1.6 a	28.0 ±0.8 a
Stem green leaves	16.6 ±3.8 c	20.8 ±3.1 bc	22.9 ±1.3 ab	16.7 ±1.4 c	27.3 ±2.9 a	24.7 ±2.4 ab	8.2 ±2.5 b	19.8 ±1.9 a	21.0 ±1.5 a	6.9 ±0.8 b	20.9 ±2.8 a	22.2 ±2.9 a
Stem senescent leaves	11.5 ±4.2 c	15.9 ±2.2 bc	14.8 ±1.4 bc	13.3 ±4.6 bc	19.7 ±4.5 ab	23.6 ±2.0 a	5.2 ±0.2 b	9.6 ±1.1 a	10.6 ±1.4 a	6.6 ±0.6 b	13.3 ±1.3 a	11.5 ±2.2 a

(2012/2013) ANOVA
Pods
G ns, -ns-
T ***, -*-
G x T ns, -ns-
Shoot
G -ns-, ns
T -*-, ***
G x T -ns-, ns
Leaves
G *, *, -**-
T ***, ***, -**-
G x T ns, ns, -ns-

(2013/2014) ANOVA
Pods
G -ns-, ns
T -***-, ***
G x T -ns-, ns
Shoot
G ns, ns
T ***, ***
G x T ns, ns
Leaves
G ns, ns, ns
T ***, ***, ***
G x T ns, ns, ns

■ Stem pods ■ Branch pods ■ Stem ■ Branches ■ Branch leaves ■ Stem green leaves ■ Stem senescent leaves

Annex-16: N concentration in different plant organs at BBCH79 in the reference genotypes PBC007 and PBC015 in the +N/-N field trials 2012/2013 and 2013/2014. All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea while control treatment received no additional N fertilization. Two plants per plot were separated into the indicated above-ground plant fractions to determine their N content. Table shows means ±SD; n=4. ANOVA or ANOVA on ranks (- -) results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively, and refer to the organ color code indicated below the table; G=genotype, T=N treatment, ns=non-significant. Different letters indicate significant mean or median differences in N concentration of the indicated organs among both genotypes under three treatments (C=Control, N=Nitrate, U=Urea) within one experimental year according to Tukey´s test or Tukey´s test on ranks (- -), respectively, at p<0.05.



Annex-17: Flowering start after 1/1/2012 and the length of flowering in the genetic diverse collection in the +N field trial 2011/2012 as mean over all genotypes (A, C) or of the single genotypes (B, D). All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Flowering start was rated when at about 10 % of the plants of a plot first petals were visible. Flowering end was rated when on not more than about 10 % of the plants of a plot last petals were visible. Boxes show median, first and third quartile; whiskers represent minimum and maximum of all data in a group (A, C: n=90; B, D: n=6). (A, C) Asterisks indicate differences among the medians according to Mann-Whitney rank sum test at p<0.05. (B, D) ANOVA results *, **, *** indicate significant differences or interactions at p<0.05, p<0.01, p<0.001, respectively; G=genotype, T=N treatment, ns=non-significant. Asterisks indicate significant mean difference among nitrate and urea treatment within a genotype according to unpaired t-test at p<0.05. Different upper case letters indicate significant mean differences among the genotypes within nitrate treatment according to Tukey´s test at p<0.05; different lower case letters indicate significant mean differences among the genotypes within urea treatment according to Tukey´s test at p<0.05.

	Plants m^{-2}	Branches m^{-2}	Pods m^{-2}	Seeds m^{-2}	TSW [g]	Seed yield [g m^{-2}]	Seed yield [g $plant^{-1}$]	Seed N [g m^{-2}] (N removal)	NHI [g g^{-1}]	Total shoot N [g $plant^{-2}$]	Total shoot N [g m^{-2}]
Plants m^{-2}		**0.76** <0,01	**0.55** 0.03	**-0.38** 0.17	**0.34** 0.21	-0.11 0.69	**-0.79** <0,01	**-0.27** 0.34	**-0.69** <0,01	**-0.05** 0.86	**0.64** 0.01
Branches m^{-2}	0.59 0.02		**0.54** 0.04	**-0.58** 0.02	**0.53** 0.04	-0.16 0.57	**-0.73** <0,01	**-0.39** 0.15	**-0.41** 0.13	**-0.30** 0.28	**0.30** 0.28
Pods m^{-2}	**0.46** 0.08	0.66 0.01		**-0.23** 0.42	**-0.09** 0.75	-0.48 0.07	**-0.62** 0.01	**-0.38** 0.16	**-0.17** 0.54	**-0.46** 0.09	**0.03** 0.93
Seeds m^{-2}	**0.03** 0.90	0.13 0.64	**0.25** 0.37		**-0.71** <0,01	0.62 0.01	**0.70** <0,01	**0.48** 0.07	**0.39** 0.15	**0.07** 0.80	**-0.20** 0.47
TSW [g]	**0.08** 0.79	0.18 0.51	**-0.28** 0.31	**-0.62** 0.01		0.06 0.82	**-0.26** 0.34	**0.06** 0.82	**-0.19** 0.49	**0.04** 0.89	**0.27** 0.34
Seed yield [g m^{-2}]	0.21 0.44	**0.30** 0.28	0.12 0.67	0.58 0.02	0.15 0.58		0.52 0.05	0.49 0.06	0.17 0.54	0.19 0.48	0.18 0.52
Seed yield [g $plant^{-1}$]	**-0.73** <0,01	-0.44 0.09	**-0.31** 0.27	**0.46** 0.08	**-0.02** 0.95	0.33 0.22		**0.67** 0.01	**0.70** <0,01	**0.12** 0.66	**-0.44** 0.10
Seed N [g m^{-2}] (N removal)	**-0.17** 0.54	-0.25 0.35	**0.05** 0.85	**0.67** 0.01	**-0.12** 0.66	0.50 0.06	**0.72** <0,01		**0.38** 0.16	**0.31** 0.26	**0.05** 0.86
NHI [g g^{-1}]	**-0.54** 0.04	-0.19 0.50	**-0.27** 0.32	**-0.08** 0.78	**0.27** 0.33	-0.12 0.66	**0.57** 0.03	**0.34** 0.21		**-0.55** 0.03	**-0.90** <0,01
Total shoot N [g $plant^{-2}$]	**-0.18** 0.53	-0.34 0.20	**0.03** 0.91	**0.52** 0.05	**-0.44** 0.11	0.32 0.24	**0.34** 0.22	**0.42** 0.12	**-0.52** 0.05		**0.74** <0,01
Total shoot N [g m^{-2}]	**0.41** 0.13	0.09 0.75	**0.29** 0.30	**0.51** 0.05	**-0.37** 0.17	0.44 0.10	**-0.11** 0.69	**0.28** 0.32	**-0.80** <0,01	**0.83** <0,01	

Annex-18: Correlations between agronomic relevant N traits and yield parameters of the genotypes in the elite line collection in the +N field trial 2012/2013. All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Yield parameters and N traits were obtained as described in 2.6 and 2.7. r values are given in bold, while normal letters represent p values. r and p values were calculated separately for nitrate (▫) and urea (▫) treatment according to Pearson product moment correlation (■) or Spearman rank order correlation (■); n=15. TSW=Thousand seed weight, NHI=Nitrogen harvest index.

	Plants m^{-2}	Branches m^{-2}	Pods m^{-2}	Seeds m^{-2}	TSW [g]	Seed yield [g m^{-2}]	Seed yield [g plant^{-1}]	Seed N [g m^{-2}] (N removal)	NHI [g g^{-1}]	Total shoot N [g plant^{-2}]	Total shoot N [g m^{-2}]
Plants m^{-2}		**0.82** <0,01	**0.51** 0.05	**-0.16** 0.58	**0.33** 0.23	0.08 0.76	**-0.92** <0,01	**0.36** 0.19	**-0.46** 0.08	**-0.27** 0.33	**0.56** 0.03
Branches m^{-2}	**0.52** 0.05		**0.48** 0.07	**0.02** 0.95	**0.22** 0.43	0.15 0.58	**-0.70** <0,01	**0.38** 0.16	**-0.32** 0.25	**-0.22** 0.43	**0.47** 0.08
Pods m^{-2}	**0.02** 0.95	**0.31** 0.26		**-0.23** 0.40	**0.12** 0.68	-0.29 0.28	**-0.59** 0.02	**0.05** 0.86	**-0.36** 0.19	**-0.08** 0.76	**0.32** 0.24
Seeds m^{-2}	**0.00** 1.00	**0.06** 0.82	**0.05** 0.86		**-0.81** <0,01	0.49 0.06	**0.46** 0.0879	**0.45** 0.10	**0.58** 0.02	**-0.27** 0.33	**-0.35** 0.20
TSW [g]	**0.31** 0.27	**0.07** 0.80	**-0.15** 0.59	**-0.84** <0,01		-0.11 0.68	**-0.44** 0.10	**-0.01** 0.98	**-0.58** 0.02	**0.36** 0.19	**0.58** 0.02
Seed yield [g m^{-2}]	**0.38** 0.16	**0.24** 0.38	**-0.11** 0.69	**0.66** 0.01	**-0.15** 0.61		0.24 0.38	0.61 0.01	0.21 0.45	-0.25 0.37	0.10 0.71
Seed yield [g plant^{-1}]	**-0.75** <0,01	**-0.26** 0.34	**0.14** 0.63	**0.43** 0.11	**-0.43** 0.11	**0.23** 0.40		**-0.13** 0.64	**0.57** 0.03	**0.20** 0.48	**-0.56** 0.03
Seed N [g m^{-2}] (N removal)	**0.11** 0.71	**0.01** 0.96	**-0.27** 0.33	**0.50** 0.06	**-0.09** 0.75	**0.76** <0,01	**0.35** 0.20		**0.08** 0.78	**0.02** 0.96	**0.31** 0.26
NHI [g g^{-1}]	**-0.31** 0.27	**-0.41** 0.13	**-0.30** 0.28	**0.48** 0.07	**-0.48** 0.07	**0.21** 0.44	**0.47** 0.08	**0.12** 0.68		**-0.64** 0.01	**-0.90** <0,01
Total shoot N [g plant^{-2}]	0.01 0.97	0.22 0.42	0.11 0.70	-0.39 0.15	0.38 0.16	-0.25 0.37	-0.16 0.57	0.10 0.71	-0.92 <0,01		**0.64** <0,01
Total shoot N [g m^{-2}]	**0.26** 0.35	**0.33** 0.23	**0.19** 0.50	**-0.28** 0.31	**0.42** 0.12	**0.06** 0.84	**-0.26** 0.35	**0.24** 0.38	**-0.92** <0,01	0.89 <0,01	

Annex-19: Correlations between agronomic relevant N traits and yield parameters of the genotypes in the elite line collection in the +N field trial 2013/2014. All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Yield parameters and N traits were obtained as described in 2.6 and 2.7. r values are given in bold, while normal letters represent p values. r and p values were calculated separately for nitrate (▫) and urea (▫) treatment according to Pearson product moment correlation (■) or Spearman rank order correlation (■); n=15. TSW=Thousand seed weight, NHI=Nitrogen harvest index.

	Plants m^{-2}	Branches m^{-2}	Pods m^{-2}	Seeds m^{-2}	TSW [g]	Seed yield [g m^{-2}]	Seed yield [g plant^{-1}]	Seed N [g m^{-2}] (N removal)	NHI [g g^{-1}]	Total shoot N [g plant^{-2}]	Total shoot N [g m^{-2}]
Plants m^{-2}		**0.65** 0.01	**0.57** 0.03	**0.49** 0.06	**-0.26** 0.36	**0.57** 0.03	**-0.55** 0.03	**0.48** 0.07	**-0.30** 0.27	-0.21 0.45	**0.74** <0,01
Branches m^{-2}	**0.87** <0,01		**0.69** <0,01	**0.17** 0.54	**-0.19** 0.51	**0.08** 0.78	**-0.62** 0.01	**0.11** 0.69	**-0.30** 0.28	-0.44 0.10	**0.38** 0.16
Pods m^{-2}	**0.73** <0,01	**0.91** <0,01		0.40 0.14	-0.46 0.09	0.17 0.54	-0.42 0.11	0.21 0.43	-0.15 0.59	-0.46 0.08	0.22 0.42
Seeds m^{-2}	**0.43** 0.11	**0.23** 0.40	**0.17** 0.55		**-0.86** <0,01	**0.89** <0,01	**0.34** 0.21	**0.79** <0,01	**0.41** 0.13	-0.20 0.47	**0.29** 0.30
TSW [g]	**-0.29** 0.30	**-0.27** 0.34	**-0.26** 0.35	**-0.83** <0,01		-0.54 0.04	-0.16 0.55	-0.52 0.04	-0.30 0.28	0.29 0.28	-0.30 0.28
Seed yield [g m^{-2}]	**0.41** 0.13	**0.09** 0.75	**-0.01** 0.96	**0.87** <0,01	**-0.46** 0.09		**0.35** 0.20	**0.90** <0,01	**0.40** 0.15	-0.16 0.57	**0.37** 0.18
Seed yield [g plant^{-1}]	**-0.69** <0,01	**-0.81** <0,01	**-0.75** <0,01	**0.23** 0.41	**-0.06** 0.84	**0.35** 0.20		**0.33** 0.23	**0.72** <0,01	0.07 0.80	**-0.41** 0.13
Seed N [g m^{-2}] (N removal)	**0.31** 0.26	**-0.01** 0.99	**-0.08** 0.77	**0.90** <0,01	**-0.56** 0.03	**0.95** <0,01	**0.41** 0.13		**0.56** 0.03	-0.22 0.42	**0.22** 0.43
NHI [g g^{-1}]	**-0.31** 0.26	**-0.44** 0.10	**-0.48** 0.07	**0.47** 0.08	**-0.35** 0.20	**0.45** 0.09	**0.66** 0.01	**0.57** 0.03		-0.50 0.05	**-0.66** 0.01
Total shoot N [g plant^{-2}]	**-0.20** 0.47	**-0.09** 0.74	**0.05** 0.86	**-0.47** 0.07	**0.42** 0.12	**-0.40** 0.14	**-0.12** 0.68	**-0.42** 0.12	**-0.75** <0,01		0.35 0.19
Total shoot N [g m^{-2}]	**0.55** 0.03	**0.53** 0.04	**0.54** 0.04	**-0.10** 0.72	**0.18** 0.53	**-0.03** 0.91	**-0.58** 0.02	**-0.14** 0.62	**-0.87** <0,01	**0.70** <0,01	

Annex-20: Correlations between agronomic relevant N traits and yield parameters of the genotypes in the genetic diverse collection in the +N field trial 2011/2012. All rapeseed genotypes were fertilized with a total of 100 kg N ha^{-1} in spring as nitrate or urea. Yield parameters and N traits were obtained as described in 2.6 and 2.7. r values are given in bold, while normal letters represent p values. r and p values were calculated separately for nitrate (▫) and urea (▫) treatment according to Pearson product moment correlation (■) or Spearman rank order correlation (■); n=15. TSW=Thousand seed weight, NHI=Nitrogen harvest index.

Annex-21: Correlations between total translocation rates of CKs and N in the xylem at different developmental stages and N accumulation in different shoot organs at BBCH79 as well as final plant seed yield of the genotypes in the elite line collection in the +N field trial 2012/2013. All rapeseed genotypes were fertilized in spring with 40 kg N ha^{-1} at BBCH30 and 60 kg N ha^{-1} at BBCH55 as nitrate or urea. At the indicated BBCH stages, xylem sap was pooled from ten plants per plot to determine CK and N translocation rates. At BBCH79, two plants per plot were separated into the indicated above-ground plant fractions to determine their N content (unit: mg N $organ^{-1}$). Plant and area seed yield were determined as described in 2.6 (units: g $plant^{-1}$, g m^{-2}). Correlations were calculated separately for nitrate and urea treatment; n=15. r and p values were calculated according to Pearson product moment correlation (■) or Spearman rank order correlation (■).

	Total xylem CK translocation [pmol h^{-1} $plant^{-1}$]						Total xylem N translocation [µmol h^{-1} $plant^{-1}$]					
	BBCH57		BBCH65		BBCH75		BBCH57		BBCH65		BBCH75	
	r	p	r	p	r	p	r	p	r	p	r	p
Nitrate												
Stem senescent leaves	-0.25	0.37	-0.48	0.07	0.20	0.47	-0.01	0.97	0.29	0.29	0.39	0.15
Stem green leaves	-0.03	0.90	-0.17	0.55	0.54	0.04	0.22	0.42	-0.08	0.76	0.43	0.11
Branch leaves	0.28	0.31	0.36	0.19	0.22	0.44	-0.06	0.84	-0.29	0.30	0.21	0.46
Branches	0.07	0.80	0.30	0.28	0.52	0.05	-0.13	0.66	-0.23	0.42	-0.05	0.85
Stem	-0.28	0.32	-0.54	0.04	0.48	0.07	0.19	0.51	0.16	0.56	0.43	0.11
Veg. organs total	-0.04	0.89	-0.08	0.79	0.61	0.02	0.09	0.76	-0.10	0.71	0.33	0.24
Branch pods	-0.02	0.95	0.02	0.95	0.16	0.56	-0.13	0.64	0.13	0.64	-0.13	0.65
Stem pods	-0.64	0.01	-0.41	0.13	-0.20	0.48	0.28	0.31	0.19	0.50	0.11	0.71
Pods total	-0.23	0.41	-0.09	0.75	0.07	0.80	-0.10	0.74	0.11	0.69	-0.17	0.54
Plant seed yield	0.05	0.85	0.12	0.67	-0.09	0.75	-0.21	0.45	-0.09	0.74	<0.01	0.99
Area seed yield	0.02	0.89	0.24	0.09	-0.23	0.10	-0.25	0.07	-0.18	0.20	-0.11	0.42
Urea												
Stem senescent leaves	0.04	0.89	0.16	0.57	0.27	0.33	0.21	0.45	-0.06	0.82	0.40	0.14
Stem green leaves	0.21	0.46	0.07	0.81	0.19	0.50	0.16	0.58	-0.07	0.81	0.41	0.13
Branch leaves	0.57	0.03	0.16	0.58	0.21	0.45	0.49	0.06	-0.07	0.81	0.16	0.58
Branches	0.45	0.09	0.38	0.17	-0.10	0.72	0.47	0.08	-0.08	0.79	0.01	0.97
Stem	0.52	0.05	0.16	0.56	0.29	0.29	0.58	0.02	0.01	0.99	0.15	0.60
Veg. organs total	0.44	0.10	0.32	0.25	0.07	0.80	0.29	0.29	-0.11	0.70	0.23	0.40
Branch pods	0.52	0.05	0.37	0.17	-0.23	0.42	0.30	0.28	-0.03	0.92	-0.13	0.64
Stem pods	-0.39	0.16	-0.04	0.88	-0.40	0.14	-0.15	0.59	0.15	0.61	-0.11	0.70
Pods total	0.38	0.16	0.27	0.33	-0.21	0.45	0.40	0.14	0.05	0.85	-0.12	0.67
Plant seed yield	0.21	0.50	0.24	0.40	-0.39	0.15	-0.15	0.60	-0.13	0.65	0.33	0.23
Area seed yield	-0.02	0.87	0.18	0.21	-0.08	0.57	-0.37	0.01	-0.09	0.51	-0.12	0.43

Annex-22: Correlations between total translocation rates of CKs and N in the xylem at different developmental stages and N accumulation in different shoot organs at BBCH79 as well as final plant seed yield of the genotypes in the elite line collection in the +N field trial 2013/2014. All rapeseed genotypes were fertilized in spring with 40 kg N ha^{-1} at BBCH30 and 60 kg N ha^{-1} at BBCH55 as nitrate or urea. At the indicated BBCH stages, xylem sap was pooled from ten plants per plot to determine CK and N translocation rates. At BBCH79, two plants per plot were separated into the indicated above-ground plant fractions to determine their N content (unit: mg N $organ^{-1}$). Plant and area seed yield were determined as described in 2.6 (units: g $plant^{-1}$, g m^{-2}). Correlations were calculated separately for nitrate and urea treatment; n=15. r and p values were calculated according to Pearson product moment correlation (■) or Spearman rank order correlation (■).

	Total xylem CK translocation [pmol h^{-1} $plant^{-1}$]						Total xylem N translocation [µmol h^{-1} $plant^{-1}$]					
	BBCH57		BBCH65		BBCH75		BBCH57		BBCH65		BBCH75	
	r	p	r	p	r	p	r	p	r	p	r	p
Nitrate												
Stem senescent leaves	0.10	0.72	0.38	0.16	-0.03	0.93	0.44	0.10	0.47	0.08	0.13	0.64
Stem green leaves	0.31	0.25	-0.01	0.97	0.36	0.18	0.32	0.24	0.40	0.14	-0.07	0.80
Branch leaves	0.14	0.63	0.23	0.41	0.18	0.52	0.06	0.83	0.13	0.64	-0.34	0.20
Branches	0.16	0.58	0.13	0.64	0.03	0.92	-0.22	0.44	0.09	0.75	-0.19	0.48
Stem	0.21	0.46	0.49	0.06	0.49	0.06	0.48	0.07	0.25	0.36	-0.06	0.81
Veg. organs total	0.19	0.50	0.26	0.36	0.32	0.25	0.26	0.36	0.34	0.22	-0.08	0.76
Branch pods	0.14	0.62	0.13	0.65	0.09	0.75	-0.01	0.98	-0.23	0.41	-0.42	0.12
Stem pods	-0.12	0.67	0.19	0.51	0.08	0.78	0.11	0.69	-0.06	0.83	-0.31	0.26
Pods total	0.08	0.78	0.17	0.54	0.10	0.72	0.05	0.87	-0.18	0.52	-0.51	0.05
Plant seed yield	-0.05	0.86	-0.16	0.57	-0.15	0.59	0.17	0.56	-0.001	1.00	0.16	0.57
Area seed yield	0.12	0.38	0.10	0.43	0.18	0.16	0.11	0.39	-0.06	0.68	0.17	0.20
Urea												
Stem senescent leaves	-0.14	0.61	-0.04	0.89	-0.13	0.64	-0.39	0.16	0.20	0.47	0.58	0.02
Stem green leaves	0.04	0.88	0.20	0.47	0.25	0.38	-0.28	0.32	0.09	0.74	0.66	0.01
Branch leaves	-0.001	1.00	0.20	0.48	0.25	0.36	-0.17	0.55	-0.17	0.55	0.27	0.34
Branches	-0.09	0.75	0.14	0.63	0.15	0.60	-0.13	0.64	-0.12	0.66	0.28	0.32
Stem	0.05	0.87	0.32	0.24	0.21	0.45	0.05	0.85	0.18	0.53	0.29	0.30
Veg. organs total	-0.02	0.95	0.17	0.55	0.22	0.43	-0.22	0.42	0.04	0.90	0.47	0.08
Branch pods	0.06	0.82	0.41	0.13	0.54	0.04	-0.03	0.91	0.02	0.95	0.34	0.22
Stem pods	0.17	0.55	-0.12	0.68	0.20	0.48	0.16	0.57	-0.09	0.75	-0.01	0.96
Pods total	0.08	0.76	0.34	0.22	0.55	0.03	0.02	0.93	0.00	0.99	0.29	0.29
Plant seed yield	-0.25	0.38	-0.08	0.78	-0.24	0.38	-0.18	0.53	0.06	0.82	0.11	0.69
Area seed yield	-0.06	0.66	-0.21	0.10	-0.16	0.21	-0.11	0.38	0.32	0.01	0.08	0.53

Annex-23: Correlations between total translocation rates of CKs in the xylem at different developmental stages and N accumulation in different shoot organs at BBCH79 as well as final plant seed yield of the genotypes in the genetic diverse collection in the +N field trial 2011/2012. All rapeseed genotypes were fertilized in spring with 40 kg N ha^{-1} at BBCH30 and 60 kg N ha^{-1} at BBCH55 as nitrate or urea. At the indicated BBCH stages, xylem sap was pooled from ten plants per plot to determine CK translocation rates. At BBCH79, two plants per plot were separated into the indicated above-ground plant fractions to determine their N content (unit: mg N $organ^{-1}$). Plant and area seed yield were determined as described in 2.6 (units: g $plant^{-1}$, g m^{-2}). Correlations were calculated separately for nitrate and urea treatment; n=15. r and p values were calculated according to Pearson product moment correlation (■) or Spearman rank order correlation (■).

	Total xylem CK translocation [pmol h^{-1} $plant^{-1}$]					
	BBCH52		BBCH59		BBCH69	
	r	p	r	p	r	p
Nitrate						
Stem senescent leaves	-0.31	0.26	-0.07	0.81	0.27	0.34
Stem green leaves	0.23	0.41	0.60	0.02	0.37	0.17
Branch leaves	-0.17	0.54	0.01	0.99	0.51	0.05
Branches	0.48	0.07	0.16	0.55	0.06	0.83
Stem	0.16	0.56	0.39	0.15	0.27	0.32
Veg. organs total	0.11	0.70	0.43	0.11	0.52	0.04
Branch pods	0.22	0.43	0.11	0.69	-0.28	0.30
Stem pods	0.50	0.06	0.08	0.77	0.06	0.84
Pods total	0.20	0.48	0.32	0.25	-0.08	0.78
Plant seed yield	0.19	0.49	-0.16	0.57	-0.22	0.44
Area seed yield	0.06	0.72	-0.23	0.13	-0.31	0.06
Urea						
Stem senescent leaves	-0.31	0.26	0.31	0.26	0.61	0.02
Stem green leaves	0.06	0.82	0.36	0.19	0.48	0.07
Branch leaves	-0.28	0.31	0.34	0.21	0.05	0.84
Branches	-0.21	0.45	0.49	0.06	0.34	0.20
Stem	0.09	0.74	0.45	0.10	0.10	0.71
Veg. organs total	-0.17	0.54	0.48	0.07	0.27	0.32
Branch pods	-0.29	0.30	0.34	0.21	0.23	0.40
Stem pods	0.64	0.01	-0.01	0.99	-0.23	0.40
Pods total	-0.04	0.90	0.36	0.19	0.16	0.55
Plant seed yield	0.30	0.28	-0.12	0.68	-0.30	0.26
Area seed yield	-0.07	0.67	-0.15	0.35	-0.04	0.83

Abbreviations

AA(s)	Amino acid(s)
AAP	amino acid permeases
AAR	*Arabidopsis thaliana* A-type response regulator
ABA	Abscisic acid
ACQ	Aminoquinolyl-N-hydroxysuccimidyl carbamate
ADP	Adenosine diphosphate
AHK	*Arabidopsis thaliana* histidine kinase
AHP	*Arabidopsis thaliana* histidine-containing phosphotransfer protein
AMT	Ammonium transporter
ANT	Aromatic-neutral amino acid transporters
AP2/EREBP	APETALA2/Ethylene-responsive element binding protein
Asp	Aspartate
ATP	Adenosine triphosphate
AUX	Auxin
BBCH	Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie
BBOT	2.5-Bis(5tert-butyl-2-benzo-oxazol-2-yl)thiophen
BMEL	Bundesministerium für Ernährung und Landwirtschaft
BRC	Branched
bZIP	Basic region/leucine zipper motif
CAB	Chlorophyll a/b binding protein
CAT	Cationic amino acid transporters
CDK(s)	Cyclin-dependent kinase(s)
CHASE	Cyclases/histidine kinases associated sensory extracellular
CHK	CHASE-containing histidine kinase
CK(s)	Cytokinin(s)
CKX(s)	Cytokinin oxidase(s)
CRF(s)	Cytokinin responsive factor(s)
CYC(s)	Cyclin(s)
CYP	Cytochrome P450
cZ	*cis*-zeatin
cZR	*cis*-zeatin riboside
DMAPP	Dimethylallyl pyrophosphate
DUR	Degradation of urea
DW	Dry weight
EA	Elemental analyzer
ENT	Equilibrative nucleoside transporter
ER	Endoplasmatic reticulum
ESI	Electrospray ionization
et al.	*et alii*
FAOSTAT	Food and agriculture organization corporate statistical database
GA	Gibberellic acid
GAT	γ-aminobutyric acid transporters
GC	Gas chromatography
GDH	Glutamate dehydrogenase
Gln	Glutamine
GOGAT	Glutamate synthase
GS	Glutamine synthetase
HATS	High-affinity transport system
ICP	Inductively-coupled plasma

IP	Isopentenyl adenine
IPR	Isopentenyl adenosine
IPRDP	N^6-isopentenyladenosine-diphosphate
IPRTP	N^6-isopentenyladenosine-triphosphate
IPT(s)	Isopentenyl transferase(s)
JA	Jasmonic acid
KRP	Kip-related protein
LAS	Lateral suppressor
LATS	Low-affinity transport system
LAX	Like AUX1
LC	Liquid chromatography
LHT	Lysine/histidine-type transporters
LOG	Lonely guy
MAX	More axillary growth
MEP	Methylerythritol 4-phosphate
MS	Mass spectrometry
MVA	Mevalonate
MYB	Myeloblastosis
N	Nitrogen
NAC	NAM, ATAF, CUC
NHI	Nitrogen harvest index
N_{min}	Mineralized nitrogen
NRT	Nitrate transporter
NUE	Nitrogen use efficiency
NupE	Nitrogen uptake efficiency
NutE	Nitrogen utilization efficiency
PBC	Pre-breeding collection
PIN	Pin-formed
ProT	Proline/compatible solute transporters
PUP	Phosphorus uptake
RAX	Regulator of axillary meristems
RUBISCO	Ribulose 1,5-bisphosphate carboxylase
SA	Salicylic acid
SAG(s)	Senescence-associated gene(s)
SAV	Senescence-associated vesicle
SCF	Skp, Cullin, F-box
SD	Standard deviation
SL(s)	Strigolactones(s)
TF(s)	Transcription factor(s)
UFOP	Union zur Förderung von Öl- und Proteinpflanzen e.V.
UPLC	Ultra-performance liquid chromatography
WRKY	Transcription factor with highly conserved WRKY amino acid sequence
Z	*trans*-zeatin
ZR	*trans*-zeatin riboside
ZRDP	*trans*-zeatin-diphosphate
ZRTP	*trans*-zeatin-triphosphate

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10th Plant Science Student Conference, June 2nd - 4th 2014, Gatersleben, Germany

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Gatersleben, March 8th 2017

Affirmation

I hereby declare that this thesis was written by me without having used other than the mentioned references and resources. All phrases taken literally or analogously from published or unpublished sources were indicated. This thesis has neither been previously submitted in its original or in similar form for the purpose of academic examination.

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Signature Place, Date

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