Genetic improvement of nitrogen use efficiency in European winter oilseed rape

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Introduction

Rapeseed, (*Brassica napus* L.) is of major economic importance, mainly grown for its oil-rich seeds. It is the third most important oil crop in the world, behind soybean and palm oil. Rapeseed production requires relative high mineral nitrogen (N) inputs which can cause severe environmental damage. For this reason enhanced nitrogen use efficiency (NUE) has become a major aim in rapeseed production in order to ensure a sustainable agricultural production, particularly in association with the reduced release of nitrogen-derived greenhouse gases from soils and nitrate contamination of waterways. The use of genetic variation to breed more efficient varieties is seen a promising option to improve agricultural sustainability, but first requires detection of suitable variants by appropriate phenotyping procedures.

Material and Methods

In a two-year field study we assessed the genetic improvement of NUE in 30 elite open pollinated and hybrid varieties, spanning 25 years of breeding progress, at six locations across Germany. Two distinct nitrogen fertilization levels were applied (120 and 220 kg N/ha respectively, both included N_{min}). Furthermore, full control of weeds, pest (insects), and diseases (fungi) and straw stiffness were conducted at each side on a local appropriate level. Thus, only N was varied and all other factors were kept on an intensive level in both treatments. At flowering (developmental stage BBCH 67-69), stems, leaves, flowers and pods of plants were harvested separately to determine the respective N concentration in the particular plant segments. Later, at seed maturity, the N concentration of seeds and plant residues was analyzed alongside the primary yield components.

In a second study 30 diverse winter oilseed rape accessions (see Stahl et al., 2016) from the ERANET-ASSYST diversity panel (Westermeier et al., 2009; Bus et al., 2011) were studied in Mitscherlich pots under contrasting nitrogen fertilisation levels. Again, at flowering the genetic material was partitioned into different tissue samples, to gather detailed information on the macro-physiological N responses.

A third experiment aimed to provide a proof-of-concept that enables plant cultivation over the entire lifecycle under semi-controlled environmental conditions with minimal constriction of roots. Therefore, a controlled plant growth platform comprising 144 transportable household refuse containers with a volume of 120 L and a quadratic planting area of 0.16 m² was established (Figure 1). Bins were filled to a depth of 90 cm with a dried soil mixture. Exact control of water supply was given by weighing the containers. In spring the containers with the low nitrogen (LN) treatment received 0.64 g N each, while the containers with the high nitrogen (HN) treatment received two times 1.6 g N (Hohmann et al., 2016). Immediately after the harvest of aboveground plant material, each container was sprayed with water in order to initiate the outflow of the soil. Subsequently the length of the longest root per container was measured, and roots were then dried for 72 h at 70°C until constancy of weight before determination of root dry biomass per container (Stahl, 2016).

Figure 1: Process of root phenotyping. Images depict in direction of arrows how roots were washed out of the soil after harvest of aboveground biomass (Stahl, 2016).

Results:

Field study:

The data confirm under both nitrogen fertilization levels (NFL) a breeding driven enhanced NUE. We observed a highly significant genetic-driven increase in the seed oil concentration and seed yield *per se* and, thus, increased NUE at both NFL. On average, seed yield from modern open pollinated varieties and modern hybrids was higher than from old open pollinated varieties and old hybrids.

Mitscherlich pot experiment:

At LN, modern varieties showed a stronger correlation of leaf N concentration to oil and seed yield, and therefore also to NutE and NUE. This correlation was not found for older varieties. Interestingly, at HN the reverse situation was observed. Hence, modern varieties appear better at converting increased leaf N concentration into yield, making them more N-efficient than older varieties (Stahl et al., 2016). In modern cultivars, early flowering at LN tends to be associated with increased overall NUE, whereas at HN the older varieties showed a similar but weaker, non-significant association (Figure 2). Furthermore, in modern varieties at LN flowering time correlated to NutE (R^2 =0.3288, p-value = 0.0103). In contrast, for older varieties at LN and modern varieties at HN, no relationship was detected between flowering time and any other trait.

Neither under LN, nor HN a significant correlation could be detected between NupE and NutE. Closer investigation of individual accessions reveals that different strategies can confer specific advantages in achieving a high NUE. Accessions with best NUE show a more balanced contribution of NupE and NutE rather than extreme efficiency for one or the other (Figure 3). Correlations to NUE are much lower for NupE (R^2 = 0.13 for LN and R^2 = 0.24 at HN) than those for NutE (R^2=0.59 and R^2=0.73 for LN and HN respectively), indicating that within the diversity panel superiority in NutE was more relevant for total NUE than NupE (Stahl et al., 2016).

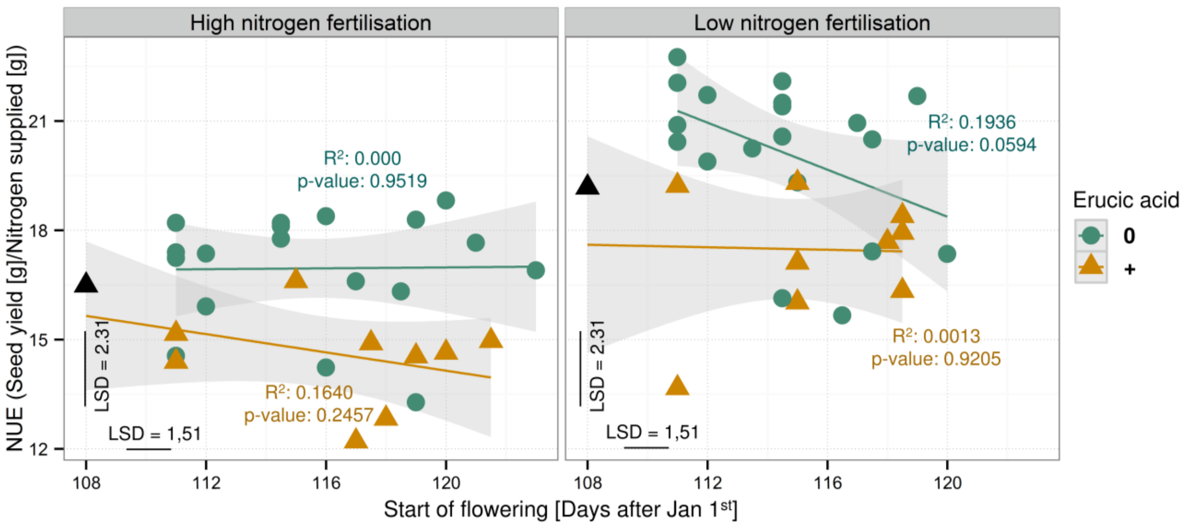

Figure 2: Correlation of nitrogen use efficiency (NUE) with flowering time at low nitrogen (right) and high nitrogen fertilisation (left). Grey shaded areas depict 95% confidence interval. Cultivar Olimpiade is marked with black triangles (Stahl et al., 2016).

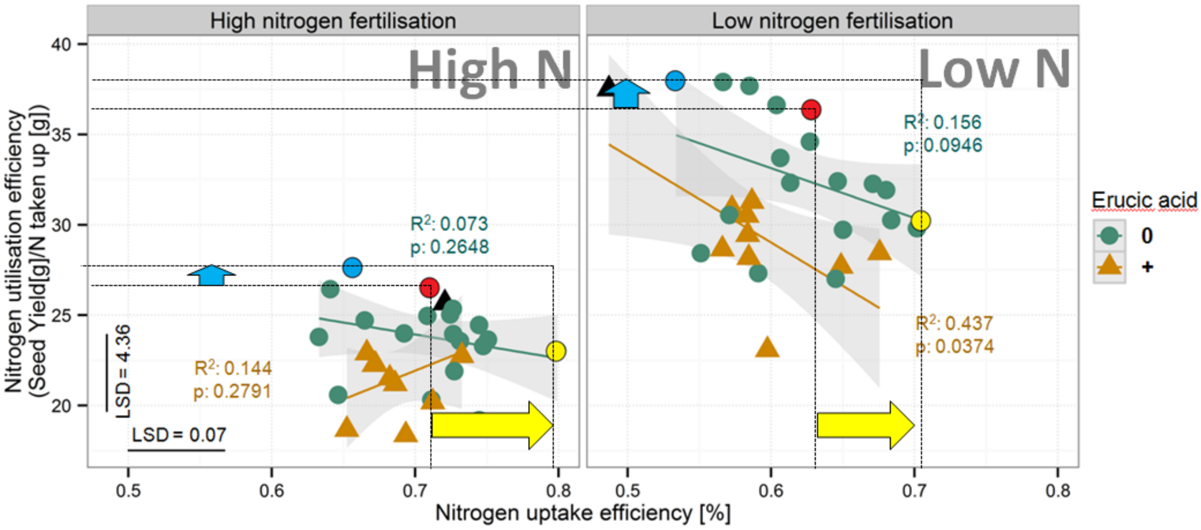

Figure 3: Relationship of N utilisation efficiency (NutE) and N uptake efficiency (NupE) at high (left) and low N (right) fertilisation. Within each N level extreme accessions are marked as: Highest NutE, (cultivar Madrigal, blue), highest NupE (cultivar Aragon, yellow), highest total NUE (red). Arrows indicate theoretical potential to improve best NUE accessions for NupE (yellow) and NutE (blue). Cultivar Olimpiade is marked with black triangles (Stahl et al., 2016).

Container experiment:

Seed yield measured in container show good correlations with seed yield measured at three field locations (Hohmann et al., 2016). Phenotyping of the root system indicated a broad variation between the genotypes in regard to their root length, biomass and morphology. Significant differences in root biomass per container were observed between the genotypes, with a variation of 56.68 g for LN and 101.3 g for HN, respectively. Although root biomass did not correlate to seed yield (LN: R^2=0.020; HN: R^2=0.010), a significant correlation was detected between root biomass and the aboveground biomass. In fact, a stronger correlation was detected at LN (R^2=0.558, p-value <0.001) than at HN (R^2=0.245, p-value = 0.0054). Although LN was not limiting for seed yield, most genotypes differed in root development between the two N treatments, although the extent and direction of the reactions varied strongly. Cultivars Gross Lüsewitzer and Beluga, for example, showed significant 1.97-fold and 1.86-fold increases in root biomass between LN and HN, respectively. The opposite reaction was observed in cultivar Major, which increased its root biomass 1.7-fold under LN conditions (Bouchet et al., 2016).

Comparison of root biomass data gathered at the adult stage in the container system with root biomass weights measured from 28 DAS in the hydroponic system (not shown) suggest that data collection on very young plants, from artificial, hydroponic cultivations systems, are not suitable to estimate the performance of a cultivar under field-like growth conditions. Moreover, images of partially washed roots from container-grown plants indicate huge differences between genotypes for the penetration of the soil by root branching and fine rooting. For example, cultivars

Mestnij, Wotan and Expert produced very low quantities of fine roots, whereas Dippes, Darmor and Gross Lüsewitzer were characterized by very dense soil penetration with fine roots (Figure 4).

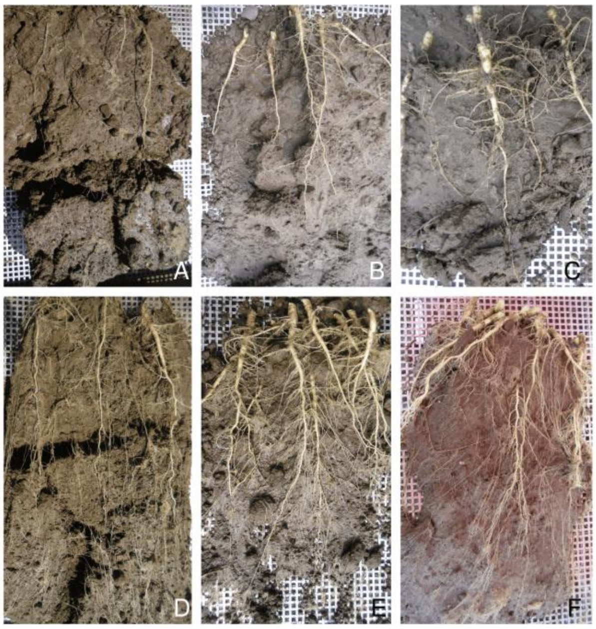

Figure 4: Different soil penetration of roots in soil profile. Accessions Mestnij (A), Wotan (B), Expert (C), differ drastic from Dippes (D), Darmor (E), Gross Lüsewitzer (F) in their root morphology. Images were taken in the low nitrogen fertilisation treatment (Stahl, 2016).

Discussion:

There is a strong empirical evidence for breeding progress towards NUE under both, high and low NFL. In Addition, in most environments the average seed yield of hybrid varieties outperformed open-pollinated varieties. Surprisingly yield differences between HN and LN were small in almost all environments across both years, suggesting a certain potential to reduce N fertilizer inputs without dramatically yield losses.

Cultivation of oilseed rape on a large-scale container platform has proven to have good field-transferability. This system can be used to phenotype multiple plant tissues for physiological parameters. Phenotyping of the root system provided first insights into genetic variation for root biomass and degree of fine rooting at the adult growth stage, as well as the response to divergent nitrogen fertilisation levels. Earlier flowering and higher leave nitrogen concentration seem to be key traits for enhanced NUE.

Conclusion:

Collectively, the results of this study suggest a considerable scope for further NUE improvement in oilseed rape by targeted combination of contributing factors in new, high-yielding varieties.

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