



Report on the variability of nitrogen uptake in diverse *B. napus* germplasm

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Background

The majority of oilseed rape (OSR) grown in the UK is winter OSR, although a small amount of spring OSR is also grown. The area under cultivation is increasing due in part by the increased use as a feedstock for biodiesel production. Current varieties of OSR generally require high levels of nitrogen (N) fertiliser to maximise yields, higher than almost any other arable crop. This has several consequences:

1. Questionable greenhouse gas emission benefits for use as a biodiesel, compared with fossil fuels, due to the energy used in the production of the N fertiliser
2. The increase in cultivation causes increased pressure on the environment due to residues being converted into potent greenhouse gases such as nitrous oxide or through nitrate leaching into rivers

These negative environmental impacts may be reduced by improving the N use efficiency (NUE) of OSR production. One way that this may be achieved is through genetic improvement, i.e. breeding. However, historically most breeding has been geared to produce varieties that perform optimally in high input trialling conditions with little consideration to their performance at low N fertiliser inputs. This situation is now beginning to change with increased research funding in this area.

One significant project is the Defra-LINK project 'Breeding oilseed rape with a reduced requirement for nitrogen fertiliser (LK0979; referred to below as the NUE LINK project)'. Two principal aims of this project are the identification of germplasm with a low requirement for N fertiliser and the development of methods that will enable breeders to select new varieties with an even lower requirement. The germplasm screens have focussed on trialling a panel of existing varieties and mapping populations under varying N fertiliser treatments in the field and assessing yield together with a number of other traits. NUE is a complex trait that can be divided into component traits. As many potential component traits are not amenable to measurement in the field, additional glasshouse-based screens will also be performed.

It is generally considered that specific crop types, such as winter OSR, have a restricted genetic base compared with that available for the whole species as represented by genetic resource collections in genebanks. Winter OSR is one of the *Brassica napus* crops that also include spring OSR, Swedes, and a range of forage, fodder and salad kales. These are a potentially rich source of beneficial alleles for OSR improvement. However, accessing this wider genetic variation is problematical for breeders due to the difficulties in identifying favourable material in the extensive genetic resource collections, the lack of experimental characterisation available on this material and the challenges of reconstituting a marketable variety from a genetically wide cross. We are addressing these problems by generating a *B. napus* Diversity Fixed Foundation Set ([BnaDFFS](#)). This can be defined as 'an informative set of genetically fixed (true-breeding) lines that represent a structured sampling of diversity

across the *B. napus* gene pool'. The BnaDFFS provides a focus for the genetic and phenotypic analysis of traits which can subsequently be transferred into more breeder-friendly material through pre-breeding activities.

N is taken up by roots in a number of chemical forms, most notably as nitrate, nitrite and ammonium. Within the plant mineral N compounds are first reduced to ammonium and then, under the action of glutamine synthetase (GS; see accompanying [report on GS](#)), incorporated into glutamine, the first organic N compound. From glutamine the diverse array of plant N-containing organic compounds are generated. Exploiting genetic variation in the uptake and assimilation of N is a potential route for generating gains in NUE.

Aims of this study

The BnaDFFS potentially contains alleles of benefit for the improved NUE of OSR, but many of the lines are not suitably adapted for meaningful screens in field trials. The aim of this study is to use a hydroponics growing system to screen the BnaDFFS for the candidate NUE component traits:

- Variation in response to different supplies of N
- Variation in the uptake and assimilation of N into shoot tissues

Included in the study are a selection of lines from the NUE LINK project which will provide a means of comparison between this study and the NUE LINK project field trials.

Materials and methods

Plant material

A total of 103 *B. napus* lines were used in this experiment. These comprised:

- 92 lines of the [BnaDFFS](#) (1 in common with the NUE LINK project set),
- 9 lines from NUE LINK project
- 3 lines from the TNDH mapping population

The lines from the LINK project were included in order to enable a comparison to be made between the field trial results being performed in the LINK project and the results obtained here to provide information on how directly transferable these results are to normal cultivation practices.

Experimental design

The experimental set-up consisted of two glasshouse compartments at Warwick HRI, Wellesbourne, equipped with Nutrient Film Technique (NFT) facilities (see Broadley et al 2003 for a description). The NFT facilities are an arrangement of troughs through which a film of nutrient is continuously supplied from 200 l tanks. These enable plants to be grown hydroponically with a defined nutrient supply. Each glasshouse compartment contained two benches and each bench possessed 8 troughs. The troughs were plumbed so that four on one side of a bench were connected to one 200 l tank containing one nutrient solution and the other four were connected to a second tank containing the other nutrient treatment.

Either three or four plants per line were grown for each of the two treatments with a total of 640 experimental plants. The plants were arranged in a computer optimised design, blocking for bench, and row and column within benches. The variety Winner was used as guard plants at the end of the rows.

Nutrient solution recipe

A modified Hoagland's solution was used with the concentration of N fixed at either 2 mM (low N treatment) or 8 mM (high N treatment). 10% of the N was supplied as ammonium.

Table 1. Nutrient solution composition

Nutrient	Concentration in low N solution	Concentration in high N solution
Ca(NO ₃) ₂	0.8 mM	3.2 mM
NH ₄ NO ₃	0.2 mM	0.8 mM
K ₂ SO ₄	2 mM	2 mM
KH ₂ PO ₄	1 mM	1 mM
MgSO ₄	0.8 mM	0.8 mM
FeNaEDTA	0.1 mM	0.1 mM
H ₃ BO ₃	30 µM	30 µM
MnSO ₄	10 µM	10 µM
ZnSO ₄	1 µM	1 µM
CuSO ₄	3 µM	3 µM
Na ₂ MoO ₄	0.5 µM	0.5 µM

Adjusted to pH 5.8

This results in the following nitrate, ammonium and total N concentrations:

Table 2. Breakdown of N composition of nutrient solutions.

Nutrient	Concentration in low N solution	Concentration in high N solution
Nitrate	1.8 mM	7.2 mM
Ammonium	0.2 mM	1.6 mM
Total N	2 mM	8 mM

Plant husbandry

The experiment was initiated in mid September 2007 so the day length conditions experienced in the glasshouse would corresponded closely to that experienced by seedlings of autumn sown winter oilseed rape. Plants were not given any supplementary lighting, so received natural daylight only.

Day 1. Seeds were chitted in Petri dishes in an incubator at 15°C in the dark for two days.

Day 3. The most uniform looking seeds for each line were transferred to rockwool blocks (36x36x40mm) soaked in borehole water on plastic trays and placed in a glasshouse compartment set to heat at 13°C and vent at 15°C (day and night).

Day 14. The glasshouse temperature set points were raised by 2°C so that they heated at 15°C and vented at 17°C.

Day 16. The seedlings in the rockwool blocks were watered with half strength mineral solution.

Day 19. The rockwool blocks were transferred to their assigned positions in the NFT apparatus and feeding continued with half strength nutrient solution. The daytime temperature set points were adjusted to heat at 14°C and vent at 17°C and the night set points to heat at 12°C and vent at 15°C.

Day 33. Half strength nutrient solution was replaced with full strength solution.

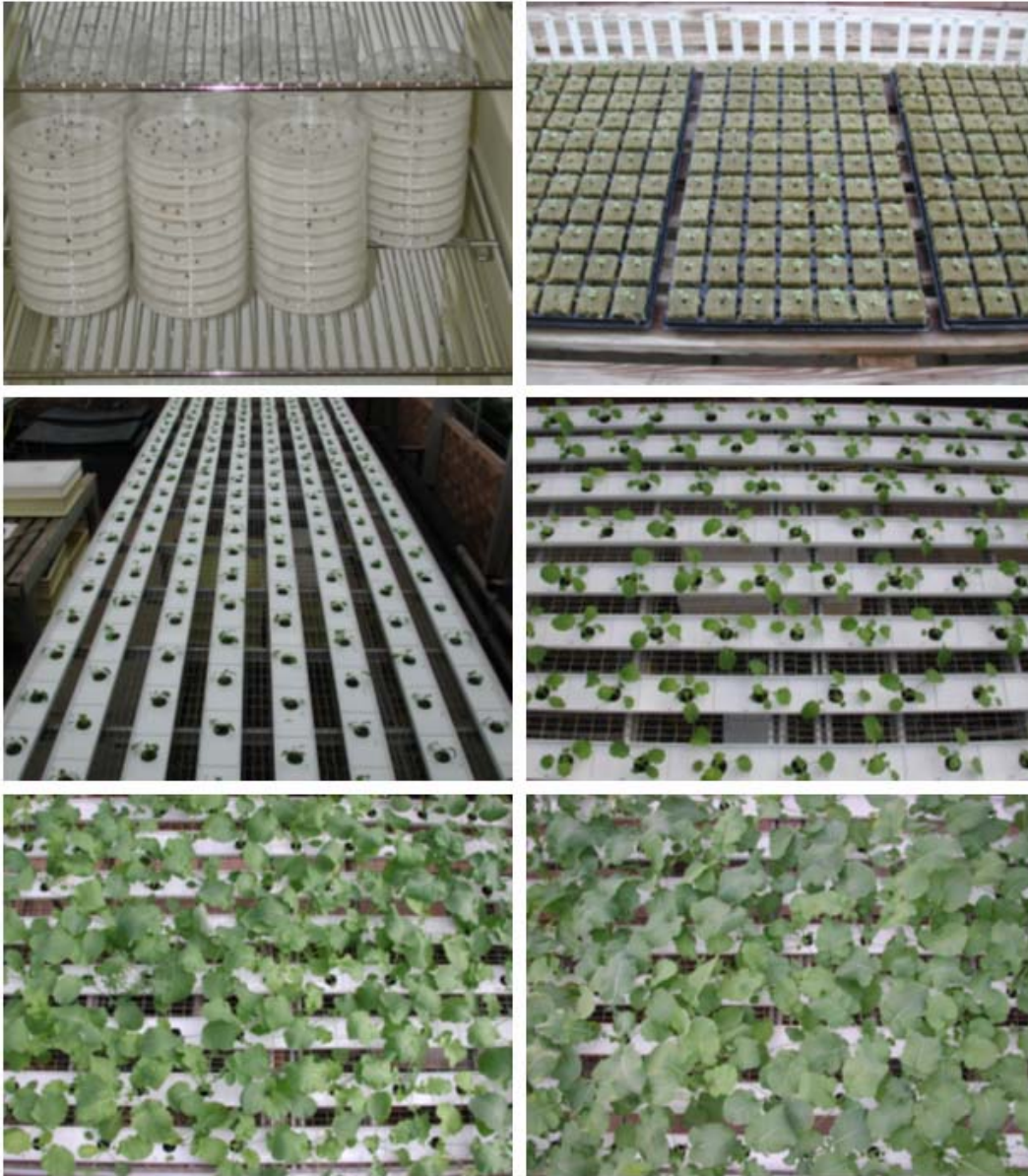
Day 56. Plants in GH 1 harvested.

Day 57. Plants in GH 2 harvested.

Harvesting occurred prior to any visual signs of floral bud initiation.

Throughout the experiment the pH and nutrient concentrations in the tanks were regularly monitored and adjusted back to the required levels as nutrients were consumed. The N concentration in the high N treatment ranged down to 6.8 mM while the low N treatment ranged down to 0.27 mM.

Figure 1. Photographs taken at different stages of the experiment. From left to right and top to bottom: Seeds being chitted, seedlings in rockwool blocks at day 12, seedlings in the NFT troughs on day 29, plants at day 37, plants at day 50, plants on the day of harvest (day 57).



Plant harvesting and mineral analysis

Shoot tissue was harvested by cutting the base of the stems with a knife. Harvested tissue was placed in a labelled paper bag and weighed to determine the fresh weight. The tissue was dried in an oven at 80°C for 5 days, the dry weight was recorded and the leaf tissue ground to a powder using a rotary grinder (Glen Creston Ltd, Middlesex) witted with 1.3mm mesh.

Mineral analysis was performed at the dedicated mineral analysis laboratory at Warwick HRI. Total organic N content was determined after Kjeldahl digestion and measured by inductively coupled plasma optical emission spectroscopy. A range of other minerals were analysed at the same time. The nitrate content of the shoot tissue was determined by flow injection analysis of aqueous extracts.

Data analysis

Statistical analysis was performed using GenStat Release 10.1. REML (REsidual Maximum Likelihood) analysis was performed on the data and estimated means and standard errors (SE) determined. Wald tests were performed to evaluate the significance of interactions between component experimental parameters.

Results

- [Download Excel workbook of the results](#)

Roots are very efficient at taking up available N so it was unclear to start with what difference would be observed between the high and low N feed solutions. However, monitoring of the nutrient levels in the feed during the experiment showed that N in the low N solution dropped to nearly zero at times, but remained much higher in the high N solution. This indicates that the low N solution was indeed providing a more limiting N supply than the high N solution.

Variation in plant growth

The fresh weight (FW) of the harvested shoots was included in the measurements, however, there are a number of experimental variables, such as time of day of harvest and length of time after harvest that the weight was recorded, that can affect the fresh weight. The harvested shoot dry weight (DW), which avoids these issues, is therefore the better measure of plant growth. Figures 2 and 3 show the variation in DW of each line from the high and low N treatments, respectively. A considerable range in variation was observed with 4.05-fold at high N and 3.4-fold at low N; each crop type is dispersed across this range. The lines from the Nitrogen use efficiency LINK project are generally clustered around the middle of the distribution, except for FD502 which was the most vigorous line in both treatments and Tapidor DH which was one of the slowest growing lines. Tapidor DH is derived from the variety Tapidor and is the female parent of the [TNDH and TVSL populations](#). The three TNDH lines behave differently and demonstrate that the TNDH population segregates for this trait. In general there was a high correlation between the relative shoot growth of the different lines at each N treatment (Figure 4; $R^2 = 0.73$). Two lines are particularly notable for generating more biomass at low N than at high N; they are the spring OSR varieties Marinka S1 and Karat S1.

Figure 2. Average dry weight at high N. The lines marked by black dots correspond to the lines from the NUE LINK project.

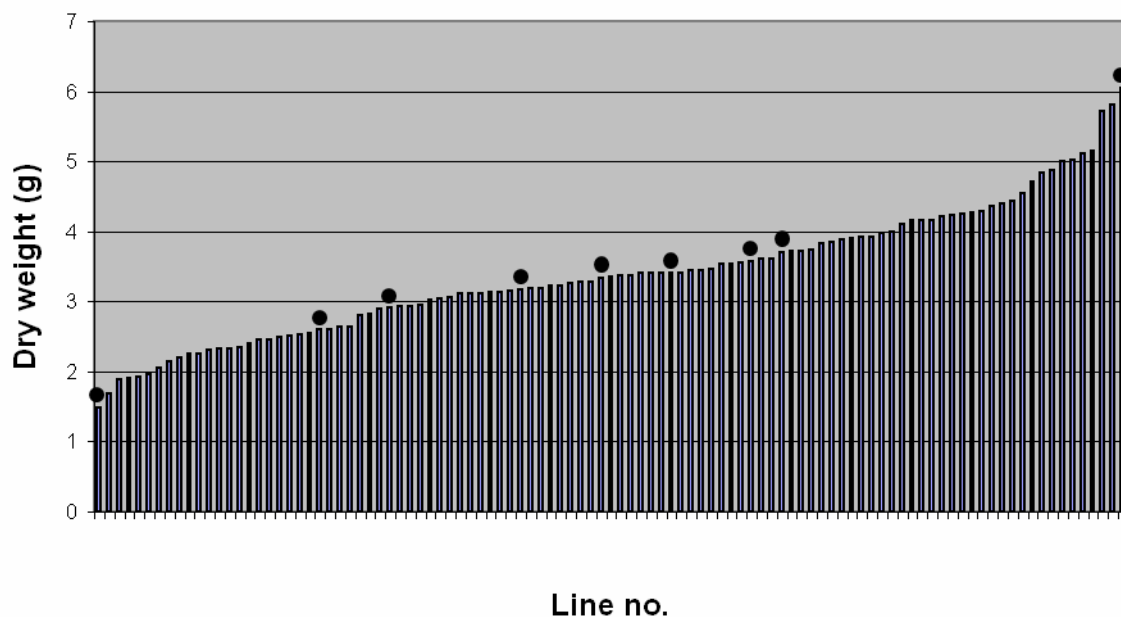


Figure 3. Average dry weight at low N. The lines marked by black dots correspond to the lines from the NUE LINK project.

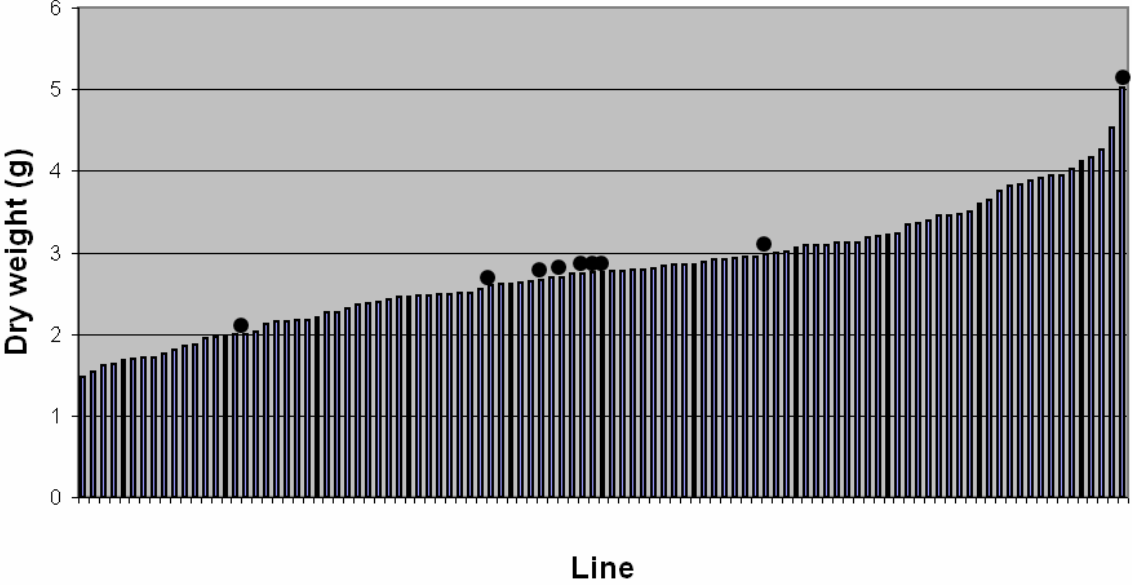
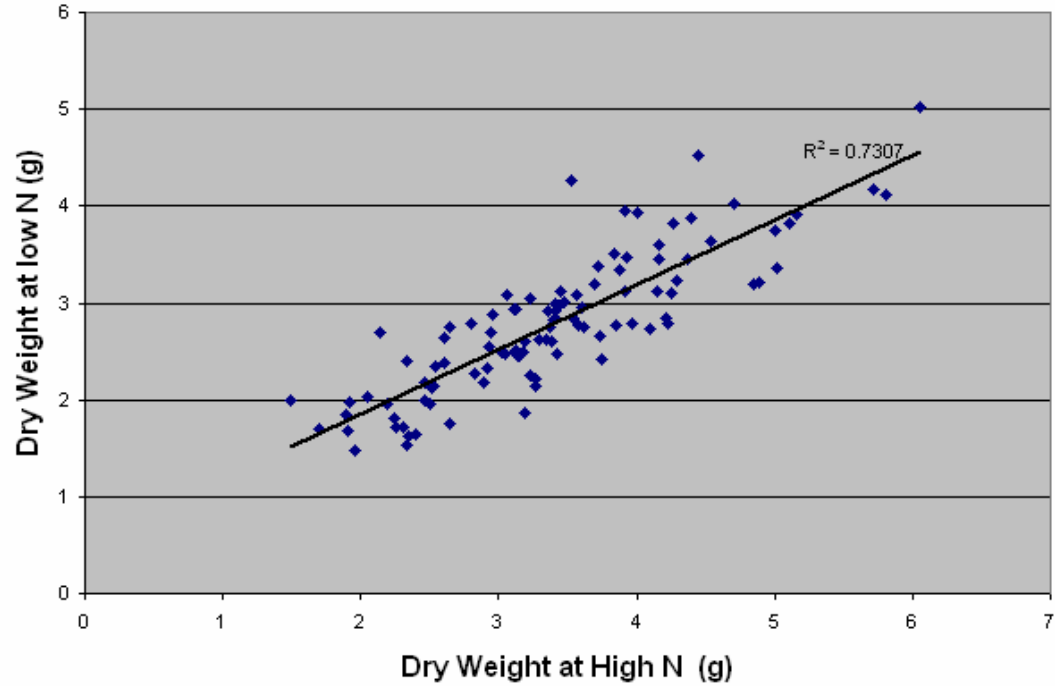


Figure 4. Scatter plot comparison of DW at high and low N for each line.



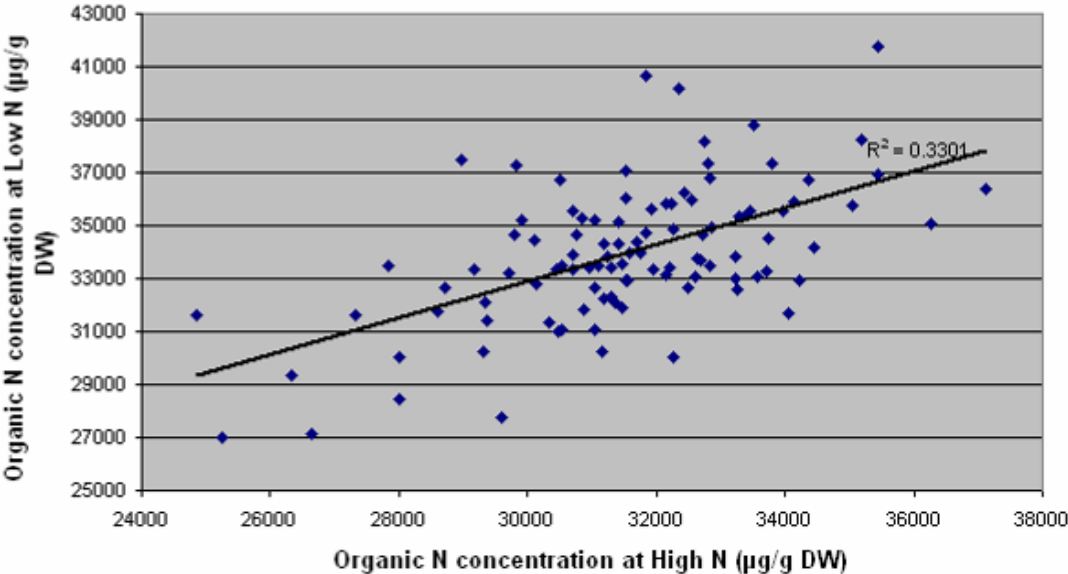
Variation in organic N content

For each of the minerals studied the analysis can be interpreted in terms of the concentration in the shoot tissue and the total amount taken up after taking into account the total shoot growth, as measured by the dry weight. The following descriptions will focus on the shoot mineral concentrations.

The total amount of N taken up by a plant is a combination of mineral N and organic N. Figure 5 shows a comparison the shoot organic N concentration in plants from the high and low N treatments. In both sets of data the range covers about a 1.5-fold difference, with slightly lower levels on average in the low N plants, demonstrating that there is considerable genetic variation in this trait. In addition, there is also much variability in how the lines

differentially respond to the two treatments ($R^2 = 0.33$). Both the NUE LINK project lines and the three TNDH lines were widely differing in their behaviour.

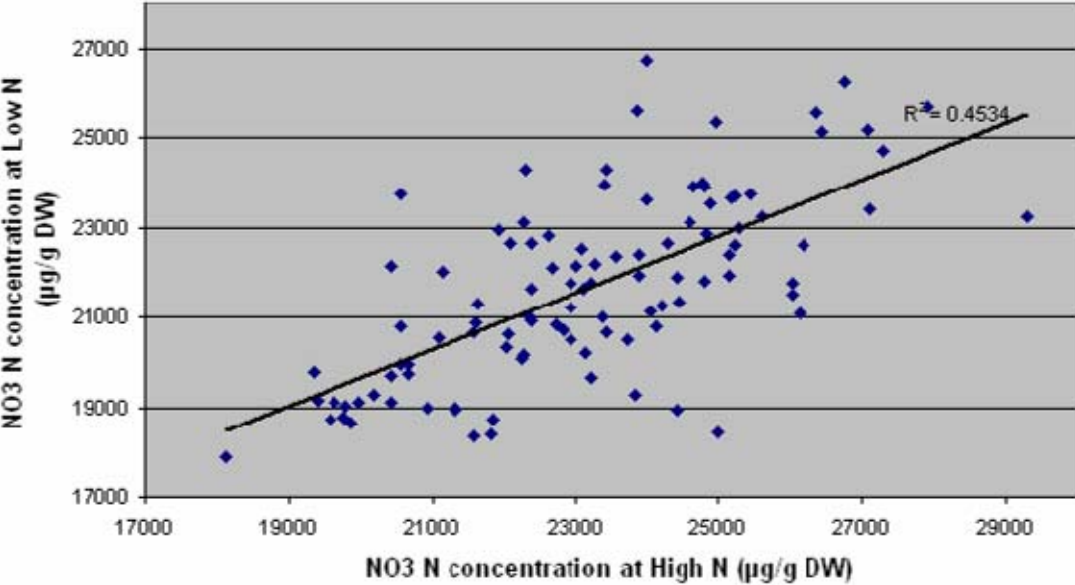
Figure 5. Comparison of the organic N shoot concentrations at high N vs low N.



Variation in shoot nitrate N content

Overall, mean nitrate concentrations were similar but slightly lower than the organic N concentrations. The levels were spread over a 1.61-fold range in the high N treatment and 1.5-fold at low N (Figure 6). The winter forage rape Winfred S2 was the lowest nitrate accumulator in both treatments, while Laugabolsrofa S1 was the highest at high N and Couve Nabica DH2 the highest at low N. There was a large line-to-line differential response between the two treatments resulting in a modest correlation ($R^2 = 0.45$). The NUE LINK project lines again showed considerable difference in their response, but the three TNDH lines were less variable.

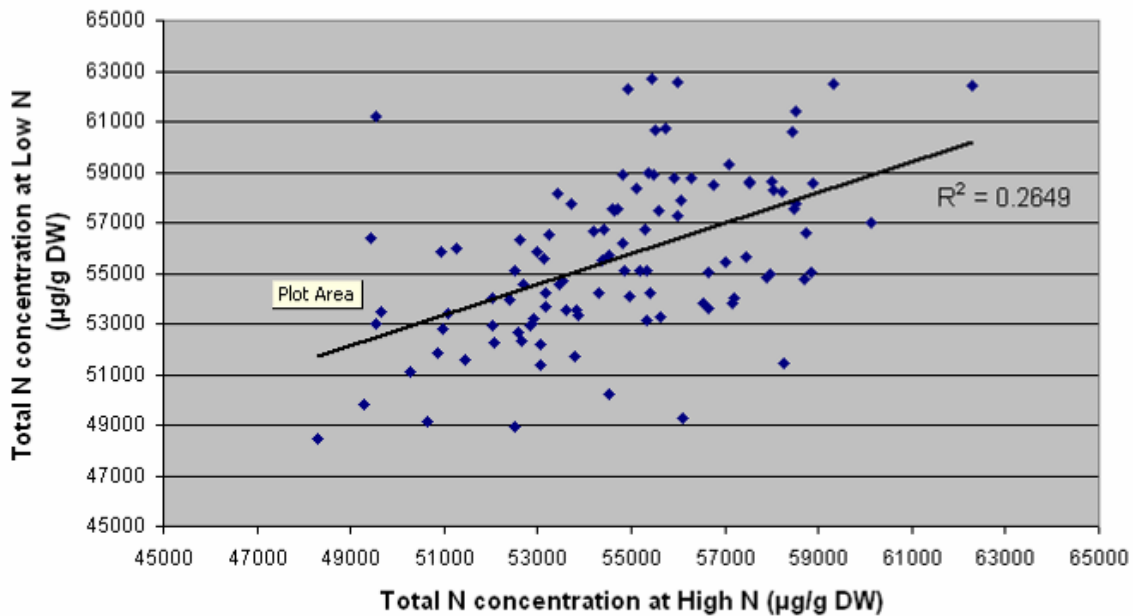
Figure 6. Comparison of the nitrate (NO₃) N shoot concentrations at high vs low N.



Variation in total shoot N content (organic N + nitrate N)

When the combined values of the organic N and the nitrate N are examined the range of variation between the lines was smaller, at about 1.3-fold for both of the treatments and the maximum, minimum and average values were also similar. However, this was not reflected at the individual line level where different lines responded considerably differently to the two treatments. Figure 7 shows that a number of lines had similarly high levels of total N accumulation in the low N treatment but widely differing values at high N. Under both the low and high N treatments the NUE LINK project lines exhibited less variation compared to the DFFS lines.

Figure 7. Comparison of the total shoot N concentration at high vs low N.



As noted above, the NUE LINK project line FD502 generated the greatest shoot biomass in each treatment. Interestingly, however, it also possessed some of the lowest shoot total N concentrations, although in total it did take up more N than almost any other line. Thus this line appears to use N very efficiently, at least for vegetative growth.

Variation in the content of other minerals

The shoot content of a number of other minerals was also determined and for each one there was considerable genetic variation, ranging from 1.36 fold-difference for potassium (K) in the low N treatment to 2.05-fold difference for Manganese (Mn) in the low N treatment. Figures 8 and 9 show the pairwise relations between each of the traits for which the correlation coefficients are given in Table 3. From this a number of significant correlations are evident. Of the minerals the strongest correlations are between Calcium (Ca) and Magnesium (Mg). This has been reported previously by Broadley et al (2008) but it is interesting that the correlation is stronger in the low N treatment than the high N treatment.

Figure 8. Pairwise scatterplot comparisons between traits (mineral concentrations or shoot weights) from lines given the high N treatment.

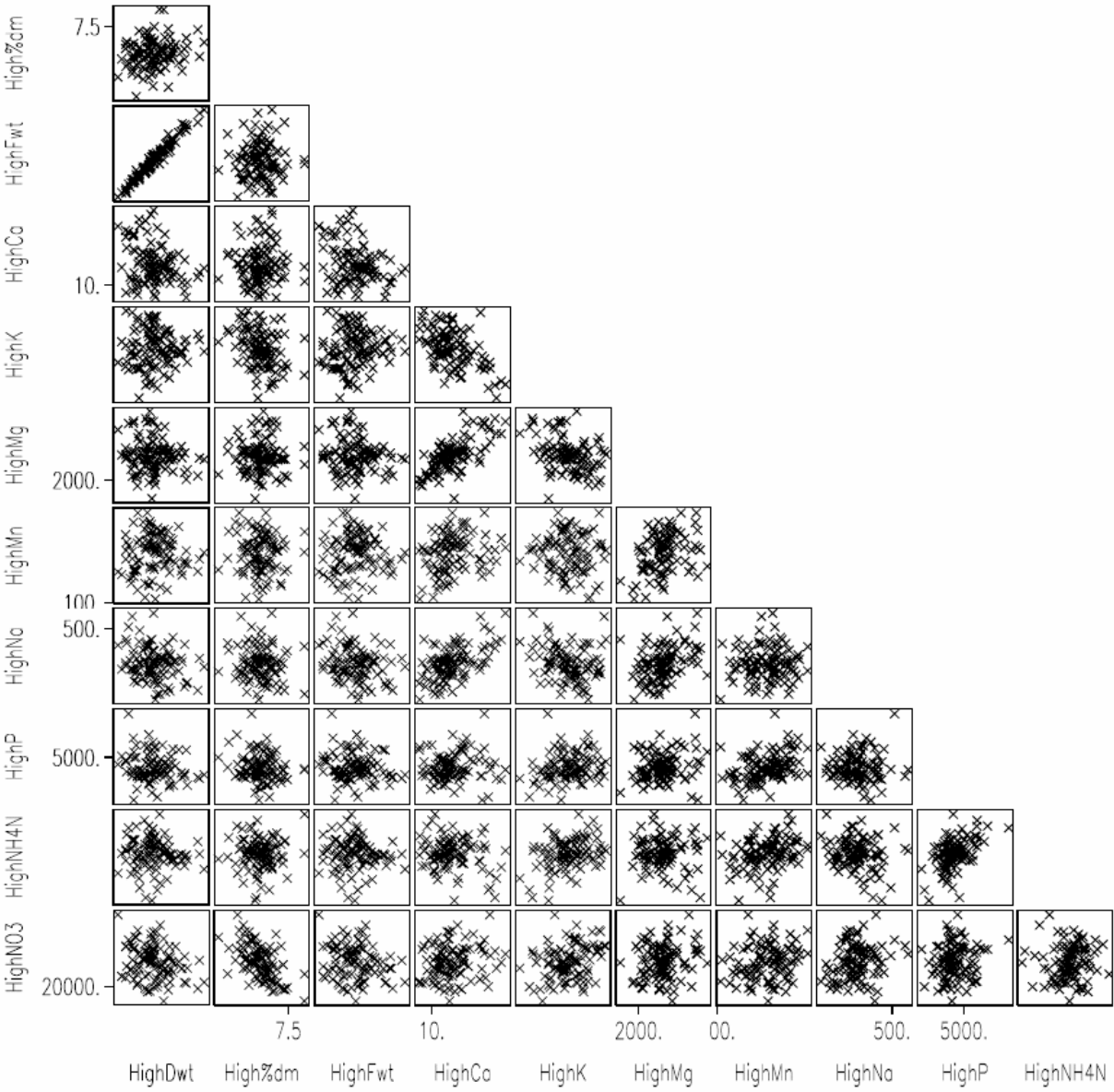


Figure 9. Pairwise scatterplot comparisons between traits (mineral concentrations or shoot weights) from lines given the low N treatment.

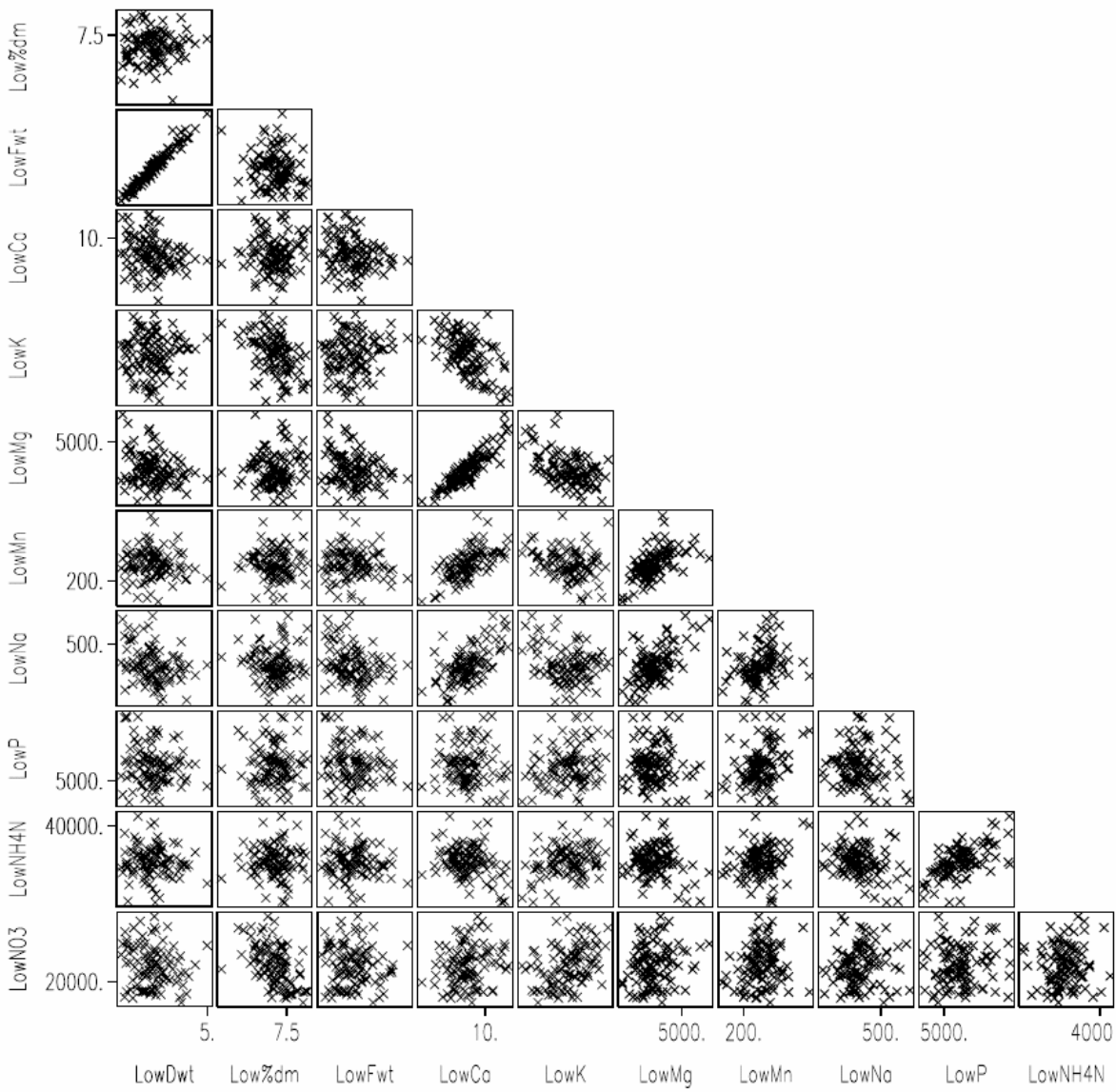


Table 3. a) correlation coefficients for interactions between the different measurements in the high N treatments; b) as for a) but for the low N treatment. The 5% significance level is 0.19 and the 1% significance level is 0.25.

a)

High Dwt	1										
High %dm	0.208	1									
High Fwt	0.981	0.026	1								
High Ca	-0.271	0.044	-0.284	1							
High K	0.028	-0.343	0.092	-0.535	1						
High Mg	-0.145	0.014	-0.15	0.713	-0.375	1					
High Mn	0.008	-0.036	0.014	0.232	-0.047	0.34	1				
High Ila	-0.234	-0.093	-0.217	0.548	-0.214	0.371	0.03	1			
High P	-0.18	-0.155	-0.148	0.078	0.193	0.186	0.363	0.009	1		
High Organic II	-0.071	0.044	-0.083	-0.205	0.37	0.014	0.296	-0.196	0.501	1	
High IIO3 II	-0.262	-0.56	-0.175	0.183	0.315	0.187	0.159	0.209	0.146	0.061	1
	High Dwt	High %dm	High Fwt	High Ca	High K	High Mg	High Mn	High Ila	High P	High Organic II	High IIO3 II

b)

Low Dwt	1										
Low %dm	0.051	1									
Low Fwt	0.976	-0.133	1								
Low Ca	-0.188	0.083	-0.214	1							
Low K	0.05	-0.445	0.146	-0.529	1						
Low Mg	-0.223	0.082	-0.241	0.894	-0.495	1					
Low Mn	-0.046	0.05	-0.078	0.631	-0.266	0.586	1				
Low Ila	-0.302	-0.067	-0.284	0.597	-0.27	0.59	0.285	1			
LowP	-0.154	-0.014	-0.143	-0.076	0.237	-0.033	0.312	-0.047	1		
Low Organic II	-0.079	0.003	-0.074	-0.278	0.309	-0.206	0.223	-0.279	0.616	1	
Low IIO3 II	-0.144	-0.502	-0.052	0.195	0.355	0.19	0.149	0.06	0.092	0.07	1
	LowDwt	Low%dm	LowFwt	LowCa	LowK	LowMg	LowMn	LowIla	LowP	Low Organic II	Low IIO3 II

Conclusions

- There is extensive genetic variation in the BnaDFFS for the rate of vegetative shoot growth at the two N treatments
- There is extensive variability for nitrate accumulation and the assimilation into organic N.
- The genetic variation in the BnaDFFS was more extensive than within the NUE LINK project lines and the three TNDH lines
- The results for the NUE LINK project lines will be used to compare with the field trial results of these lines and will give an idea to what extent these results can be interpreted with respect to the field trial data.
- The segregation between the parents of the TNDH population and the three TNDH lines show that this population will segregate for all the traits examined. This appears to be true for the other mapping populations whose parent lines are included in the BnaDFFS

References

Broadley et al (2003) Variation in the shoot calcium content of angiosperms. J. Exp. Bot. 54:1431-1446. DOI: [10.1093/jxb/erg143](https://doi.org/10.1093/jxb/erg143)

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