

Improving Nitrogen use efficiency of Winter Oilseed Rape (*Brasica napus* L.)

Alison Fraser

Supervisor – Martin Broadley

Acknowledgments

MR Broadley[1], NS Graham[1], T Alcock[1] PJ White[3], K Storer[2], P Berry[2], L Wilson[1], M Nightingale[4], R Hayden[1]

[1] *University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK*

[2]*ADAS, High Mowthorpe, Duggleby, Malton, North Yorkshire, YO178BP, UK*

[3]*The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK*

[4]*Elsoms Seeds Ltd Splading, Lincolnshire, PE11 1QG, UK*

This work was supported by the Biotechnology and Biological Sciences Research Council [grant number BB/L002124/1], (BBSRC, UK), Renewable Industrial Products from Rapeseed (RIPR) Programme.

Abstract

Nitrogen (N) fertilisers are used widely to improve winter oilseed rape (WOSR) yields. However, N is an expensive input and has environmental effects including greenhouse gas (GHG) emissions and nitrate leaching. Significant genetic variation in N use efficiency (NUE) has been reported among WOSR genotypes, and this can potentially be exploited by plant breeders. OSR yields are highly variable. They are affected by genetics, environment and agronomic practice as well as the interactions between them. Trials comparing NUE of diverse genotypes have shown that cultivar yields are ranked differently at high N and low N rates. If the interaction between genotype and N supply is highly significant but the correlation between low N and high N yields is low then it is possible to select genotypes with adaptations to low N. Better management of N application, with precise application rates dependent on genotype and environment, has the potential to improve NUE. In order to calculate the optimum N rate it is necessary to know both yield potential of the site and N requirements of the genotype, assuming the intention is to reduce N applications whilst maintaining yield (or to increase yields whilst maintaining N application). Therefore, understanding genotype performance over multiple environmental and management conditions is essential.

A field trial has been conducted as part of the Renewable Industrial Product for Rape (RIPR) project. Data was collected over two sites and two years. The field study characterised the genetics of NUE traits of WOSR in a structured association panel, testing 84 genotypes types at low (60 kg N ha^{-1}) and high (300 kg N ha^{-1}) N rates. 32 traits were measured and analysed including: N tissue analysis; phenology dates; and yield. A physiological model of N-acquisition and N-utilisation in WOSR has been developed and validated using an older field data set. The model is physiologically accurate, describing measurable varietal traits and how they impact on dry-matter production, N remobilisation and yield potential. The model has been used to identify novel traits for use in breeding strategies to: improve NUE; inform the optimal use of N fertilisers; and identify crop traits and trait combinations for increasing seed yield at low N of WOSR. Associative transcriptomic analysis (Harper et al., 2012) was conducted with the RIPR field trial data to explore the relationship between genotype and phenotype for NUE and flowering score.

Field trial results from the RIPR WOSR diversity panel showed a large amount of genetic variation for traits, yields and response to N. Significant relationships have been seen between yield formation at low N with: early flowering; post-flowering N uptake; stem N remobilisation; mid-flowering N concentration; maturity chaff N concentration; seed N concentration; height; and establishment.

However, these traits have not been seen to impact yield at every trial and a lot of variation was seen for traits within and between environments. The trials described in this thesis support previous evidence that high yielding with low N optimum genotypes exist and have great potential to increase NUE. However, our results show that NUE of WOSR is a complex trait affected by genotype, environment, management and their interactions. NUE can be improved through breeding NUE efficient genotypes and by more precise application of N through increasing knowledge of N optimum based on genotype and yield potential of the field environment. There are many practical issues linked to monitoring traits and calculating N optima of genotypes over different environments. However, improved NUE of UK WOSR would have a positive effect on GHG emissions, the aqueous environment and farm economics. A simple process based model was used to identify novel traits for use in breeding strategies to improve NUE and identify crop traits and trait combinations to increase seed yield at low N of WOSR. The model has been used to provide a good estimate of yield potential of ten varieties in low N supply trials. The model did not accurately predict yield across all environments tested. Sensitivity analysis showed agreement across important traits between the model and published field studies. The model simulations showed the most important varietal traits for either reducing N requirement whilst maintaining current commercial yields or increasing yield at low N supply are: greater post-anthesis uptake; efficiency of N acquisition from fertiliser; days for seed filling; and N remobilisation from pods. Several promising candidate genes, some previously shown to influence or respond to NUE and flowering score in *Brassica napus*, have been selected using associative transcriptomic analysis. The next step would be to conduct experiments in *A. thaliana* T-DNA knockouts to confirm the level of influence.

Contents

Acknowledgments 1
Abstract 3
List of figures 8
List of tables 13
Acronyms 15
Chapter 1. Introduction 16
1.1. Motivation 16
1.2. Scientific hypotheses 17
1.3. Experimental outline 17
2. Background 19
2.1. Current status 20
2.1.1 Oilseed rape production 20
2.1.2. N requirements of WOSR 21
2.1.3. Environmental impact of N fertiliser 25
2.1.4. NUE definitions and example 26
2.2. Improving NUE efficiency of WOSR 27
2.2.1. Traits linked to improved N uptake 27
2.2.2. Traits linked to improved N utilisation 29
2.2.3. Improve yield through crop management 30
2.2.4 N uptake mechanisms and the form(s) of N taken up 31
2.3. Breeding NUE genotypes; practical approaches 33
2.3.1. Modelling 34
2.3.2. Developing breeding screens for NUE 35
2.3.3. The use of genomics and transcriptomic approaches for genetic improvement of NUE. 35
Chapter 3. Characterising the genetic variation of NUE traits in a WOSR diversity panel 41
3.1 Introduction 41
3.2. Materials and methods 43
3.2.1. Experiment sites 43
3.2.2. Germplasm 43
3.2.3. Trial management 43
3.2.4. Measurements 45
3.2.5 Methods; Digestion of plant material for total N analysis 47
3.2.6. Methods: Unmanned aerial vehicles (UAV) image data collection, Image analysis, and scoring 48
3.2.7 Statistics 49
3.3. Results 50
3.3.1 Yields 50
3.3.2 HM14 site analysis 52

3.3.3. Yield response between LN and HN plots 2014-2015 54
3.3.4. HYLO classification; N response groups 58
3.3.5. Trait analysis; N uptake and partitioning 59
3.3.6. N utilisation and remobilising 62
3.3.7. Phenology; Establishment, flowering dates and maturity score trait ranges 63
3.3.8. Relationship between yield at LN, NUE and morphological traits 65
3.3.9. Relationships between field yield (t ha-1) at LN and morphological traits 71
3.3.10. Trait analysis at LN of HYHL v HYHO types 73
3.3.11. Trait analysis of ASSYST v elite types 75
3.3.12. Trait analysis by ranking of key genotypes 76
3.3.13. UAV Imaging analysis: full field and plot images 88
3.4. Discussion 90
3.4.1. Yields and NUE 90
3.4.2 Mitigating N loss 92
3.4.3. N uptake and utilisation 92
3.4.4. Phenology 92
3.4.5. Trait analysis 93
3.4.6 How good does a relationship need to be for a trait to be useful in selecting for NUE or yield? 94
3.4.7. Practicalities of NUE screening for breeding 94
3.5. Conclusions 96
Chapter 4. A physiological model of N-acquisition and N-utilisation in WOSR
4.1. Introduction 97
4.2. Methods 99
4.2.1 Model schematic and parameter 99
4.2.2. Detailed development of the model 101
4.2.3. Data used for model testing 103
4.2.4 Statistical analysis of model evaluation 104
5.2.5. Sensitivity analysis 104
4.3. Results 105
4.3.1. Model evaluation 105
4.3.2. Sensitivity analysis 106
4.3.3. RIPR diversity field trial results 108
4.4. Discussion 111
4.4.1. Model performance initial development 112
4.4.2 Which parameters vary among genotypes and should be varied in models for NUE? 113
4.4.3 Simulation study trait analysis 114
4.4.4 Model limitations 115
4.4.5 Utility 116
4.4.6 How useful is this model? 117

Chapter 5. Identification of Candidate Genes for traits linked to field NUE and flowering using field data of WOSR by association genetics 120
5.1 Introduction 120
5.1 Methods 122
5.2.1. Material 122
5.2.2 Transcriptome Sequencing and Population Structure Analysis 122
5.2.3 Associative Transcriptomics 123
5.2.4 Candidate Gene Identification 123
5.3. Results 124
5.3.1 Associative transcriptomics suggests potential genes that could be markers for NUE in field trials. 124
5.3.2. Associative transcriptomics analysis of potential genes that could be markers for flowering date at low N in field trials. 127
5.4 Discussion 131
Chapter 6. Discussion 134
6.1 Project outline and background 134
6.2. There is potential to breed genotypes that are consistently high yielding under low N conditions. 135
6.3. Genetic variation for N optimum and yield at low N for modern WOSR genotypes is high and there is potential for improving NUE through better matching N requirements to genotypes. 137
6.4. The phenological development of WOSR significantly impacts yield, N-optimum, and these are impacted by N-uptake and N-utilisation. Identifying key traits that can be used to predict crop growth at low N and develop simple predictive models. 138
6.5. . These diversity sets can be used to define phenotypic and genetic markers that are indicators of NUE. 142
6.6. Practical approaches for calculating N optimum. 144
6.7. Environmental and economic impact of fertiliser 145
6.8. Methods for phenotyping diversity trials 147
6.9. Conclusions 149

Bibliography 150
Appendices 170

List of figures

Figure 1. UK WOSR yield (tonnes/hectare) and production (tonnes) for 2009-2013. Source: HGCA Oilseed rape growth guide, 2013 20

Figure 2. WOSR yields (t ha^{-1}) for farms (x) and recommended list trials (●). Source: HGCA, Oilseed rape growth guide, 2013. 21

Figure 3. WOSR N dynamics over the crop cycle. The figure shows relationships between plant growth; N dynamics; and yield elaboration over the crop cycle. N uptake is represented by blue arrows whose width indicates the relative amount of N absorbed at a given time point. N losses are shown with *black arrows* whose width indicates the relative amount of N lost at a given time point. Image used with permission. Source: Bouchet et al., 2016**.** 23

Figure 4. A theoretical comparison of corresponding N applied and crop yield (t ha^{-1}) (red lines) and NUE (kg/kg) (blue lines). A typical genotype response is shown in solid lines, compared to genotypes with a different N response shown with the dashed lines for A) high yielding genotype and B) N efficient genotype. The optimal N rates are indicated by the vertical dashed lines. Theoretical data source (Storer et al., 2018) 26

Figure 5. The determination of crop N uptake (kg/ha) off WOSR over time split by tissue type: Seed, Excess pod, Pod + Flower, Excess stem, Stem and Leaf. Figure source Stafford (1996). 29

Figure 6. Photographs showing black-grass issues with trials at Deeping April 2014. 45

Figure 7. Images showing plot establishment at HM14. 46

Figure 8. Images showing m^2 sampling methods at HM14 and OM15. 46

Figure 9. UAV aerial images from the RIPR trials. 49

Figure 10. Boxplots for (A) seed yield (t ha) and (B) NUE (%) at LN for; DP14, DP15, HM14 and OM15. Each point represents a genotype mean n=3. 50

Figure **11**. Boxplot for seed yield (t ha) at LN of ASSYST (A) and ELITE (E) types for; DP14, DP15, HM14 and OM15. 51

Figure 12. Graph to show yield (t ha^{-1}) at Low N (blue) and High N (orange) of genotypes at the site HM14. Means of genotype show n=3. 53

Figure 13. Graph to show yield (t ha^{-1}) at Low N (blue) and High N (orange) of elite genotypes at the site HM14 trial site. Means of genotype show n=3. Bars show standard error. 54

Figure 14. Boxplot for seed yield (A) and NUE (B) for HN and LN treatments at; DP14 and HM14. 54

Figure 15. Boxplot for % yield gain of plots with HN treatment than LN treatment at; DP14 and HM14. 55

Figure 16. Boxplots of seed yield at LN (t ha^{-1}) for (A), yield at HN (t ha^{-1}) and Yield gain of HN / LN (%) (C) at; DP14 and HM14. A = ASSYST genotypes. E = Elite genotypes. 55

Figure 17. Relationship for seed yield (t ha^{-1}) for HN and LN plot treatment at; A) DP14 and B) HM14. Each point represents a genotype mean n=3. Linear regression analyses grouped by experiment: DP14 r^2 = 0.48; y = 0.90x + 0.94; HM14 r^2 = 0.41; 0.74x + 1.465. 56

Figure 18. Relationship for yields (t ha^{-1}) 2014-2015 at LN and HN for DP14 and HM14. Red points show ASSYST (A) genotypes, blue points show ELITE (E) genotypes. Each point represents a genotype yield mean (n=3). Linear regression analyses grouped by experiment. 56

Figure 19. Regression for trials of yields (t ha^{-1}) at HN and LN. Linear regression analyses grouped by experiment: LN (r^2=0.61, p < .0001), HN (r^2=0.60 p < .0001). 57

Figure 20. Yield gain (% HN/LN) between HM14 and DP14. 57

Figure 21. Yields at HN and LN (t ha^{-1}). Each point is the mean yield from two sites HM14 and DP14. Genotypes have been categorised into; 'HYLO' (High Yield Low Optima) (green), 'HYHO' (High Yield High Optima), 'LYLO' (Low Yield Low Optima) and 'LYHO' (Low Yield Low Optima). 58

Figure 22. Yields (t ha^{-1}) at HN and LN at (A) DP14 and (B) HM14. Each point is the mean yield from two sites HM14 and DP14. Each point represents a genotype mean n=3. Genotypes have been categorised into; 'HYLO' (High Yield Low Optima) (green), 'HYHO' (High Yield High Optima), 'LYLO' (Low Yield Low Optima) and 'LYHO' (Low Yield Low Optima). 59

Figure 23. Boxplots for LN plots of mid-flowering leaf and stem tissue for: A) dry weight (t ha^{-1}); B) tissue N (%); and C) total N (kg/ha); for leaf and stem tissue at DP14, DP1, HM14 and OM15. Each point represents a genotype mean n=3. 60

Figure 24. Boxplots for LN plots of maturity (MT) chaff, seed and stem tissue for: A) dry weight (t ha^{-1}); B) tissue N (%N); and C) Maturity total N (kg/ha); at DP14, DP15, HM14 and OM15. Each point represents a genotype mean n=3 apart from DP14 which has a genotype mean n=3 apart from DP14 which has a genotype mean off n=2. 61

Figure 25. Mean N uptake across all *genotypes* grown at LN for (A) DP14, (B) DP15, (C) HM14, and (D) OM15. Leaf (blue), stem (orange), chaff (grey), and seed (yellow). 61

Figure 26. Boxplots of N utilisation of seed (A) and N utilisation of biomass (B) at LN for; DP14, DP15, HM14 and OM15. 62

Figure 27. Boxplots for LN plots of (A) N uptake after flowering (kg/ha) and (B) stem N remobilisation (kg/ha) at; DP14, DP15, HM14 and OM15. 62

Figure 28. Flowering date (days after 1st April) at HM14 (A) and OM15 (B) site. 63

Figure 29. Aerial view RGB plot images from a showing variation in crop development at DP14 on A) 19/11/2014 B) 27/04/15 C) 20/05/15 and D) 12/06/15. 63

Figure 30. Example of extreme differences in flowering dates from genotypes in the RIPR trials. 64

Figure 31. Inter-trait phenotypic pearsons correlations at LN fertilisation for: (A) mid-flowering (MF); (B) maturity (M); and (C) score data. Trait data is mean from 53 genotypes grown at 4 trial sites: DP14; DP15; HM14; and OM15. Colours indicate the strength of correlation. 65

Figure 32. Inter-trait phenotypic correlations at LN fertilisation for data. Trait data is mean from 53 genotypes grown at 4 trial sites: DP14; DP15; HM14; and OM15. Colours indicate the strength of correlate. P values are shown in Table 6. 66

Figure 33. Inter-trait phenotypic correlations at LN fertilisation for maturity data. Trait data is mean from 53 genotypes grown at 4 trial sites: DP14; DP15; HM14; and OM15. Colours indicate the strength of correlate. P values are shown in Table 6. 66

Figure 34. Regression for yield at LN with (A) establishment (1-5), (B) height (cm), (C) flower score (1-10) and (D) maturity score. Values are means from all trials n=4. Linear regression analyses; (A) Establishment, $y = 0.25x + 0.89$, $r^2=0.15$, (B) Height, $y = 0.01x + 0.57$, $r^2=0.06$, (C) Flower score $y = -0.13x + 2.37$, $r^2=0.14$; (D) Maturity score $y = 0.17x + 0.72$, $r^2=0.14$; 71

Figure 35. Regression for yield at LN with (A) maturity stem % N, (B) maturity chaff % N, and (C) maturity seed % N. Values are means from all trials n=4. Linear regression analyses; (A) maturity stem % N, $y = -1.03x +2.27$, $r^2=0.04$; (B) maturity chaff % N, $y = 0.98x + 1.17$, $r^2=0.07$; (C) maturity seed % N, $y = -0.94x + 4.47$, $r^2=0.14$. 71

Figure 36. Regression for yield at LN with (A) mid-flowering total plant N (kg/ha), (B) maturity total plant N (kg/ha) (C) N uptake post athesis (kg/ha) and (D) stem N remobilisation (kg/ha). Values are

means from all trials n=4. Linear regression analyses; (A) MFTNP y = 0.01x + 1.45, r^2=0.49; (B) MTNP= y = 0.02x + 0.34, r^2=0.69; (C) NUPMF = y = 0.01x + 1.73, r^2=0.22, (D) SNR = y =0.01x + 1.52, r^2=0.06. 72

Figure 37. Boxplots for trait analysis of HYHO and HYLO types for Yield (t ha) at LN. Values are from both trials DP14 and HM14. ** and *** above boxes represent significance at P < 0.05 and P < 0.01 respectively, for t-test comparing HYHO and HYLO types. 73

Figure 38. Boxplots for trait analysis of LN plot differences between ASSYST and elite types at: DP14; DP15; HM14; and OM15 for: (A) Mid-flowering total N (kg/ha); (B) MT total N (kg/ha); (C) N uptake after flowering (kg/ha); (D) Establishment (1 low -10 high); (E) Flower score (1 early - 10 late); (F) Maturity score (1 low -10 high); (G) N seed utilisation (kg N / kg seed), (H) N biomass utilisation (kg/kg); (I) N seed concentration (%); (J) Maturity seed total N (kg/ha). 75

Figure 39. Distribution of genotypes according to the average ranking across sites and years for NUE and N Utilisation of seed across the four sites. An increase in rank value represents an increase in NUE and N Seed utilisation efficiency of the genotype for crops grown low N supply. 77

Figure 40. Relationship between UAV GAI assessment and field establishment score at: A) DP14; and B) HM14. Each point represents a mean genotype n=3. 88

Figure 41. Boxplots for UAV GAI assessment at DP14 and HM14 in: A) November; and B) March. Each point represents a mean genotype n=3. Lines fitted by linear regression. 89

Figure 42. Relationship with DP14 and HM14 sites for: A) November GAI; B) March GAI; and C) over-winter GAI loss (%). Each point represents a mean genotype n=3. 89

Figure 43. Boxplots for over-winter GAI loss at DP14 and HM14 presented as % GAI lost between November and March assessment. Each point represents a mean genotype n=3. 89

Figure 44. Schematic of representation of N uptake, N partitioning and crop growth of OSR as described in model. Solid arrows represent N uptake and dotted arrows represent remobilisation. 99

Fig 45. Relationship between seed N content and seed N concentration; y=0.009x – 2.05; R^2=0.74, P<0.001). Data from nine experiments described in Berry and Spink (2009). 103

Fig 46. Relationship for observed field and predicted yields of; (A) field trials; RM07 (+), RM08 (X), HM07 (Δ), HM08 (O) and (B) genotype mean values across 4 sites. 105

Fig 47. Sensitivity assessment of environmental, N-acquisition and N-utilisation model parameters at 0 N kg/ha fertiliser for: a) Environmental, b) N-acquisition and c) N-utilisation. The ratio (0 to 1)

represents the normalised crop genetic and environmental range of each parameter. Table 13 for values. 106

Fig 48. Sensitivity assessment of environmental, N-acquisition and N-utilisation model parameters at 200 N kg/ha fertiliser for: a) Environmental, b) N-acquisition and c) N-utilisation. The ratio (0 to 1) represents the normalised crop genetic and environmental range of each parameter. Table 13 for values. 106

Fig 49. Simulation analysis of environmental and genotype parameters. The solid line shows predicted yields using the model default values. The grey area shows predicted yields using the full range of parameter values for: (A) ranges of environmental parameters, (B) ranges of genotype parameters and (C) range of environmental and genotype parameters. 107

Figure 50. Relationship for observed field yields (t ha^{-1}) from the RIPR trials and model predicted yields (t ha^{-1}) of all genotypes across all environments. The solid line is a 1:1 relationship. 108

Figure 51. Relationship between observed field yields (t ha^{-1}) from the RIPR trials and model predicted yields (t ha^{-1}) of genotypes, separated as: (A) ASSYST types; (B) elite types; (C) 2014-2015 trials; and (D) 2015-2016 trials. Solid lines are 1:1 relationships
109

Figure 52. Relationship for observed field yields (t ha^{-1}) from the RIPR trials and model predicted yields (t ha^{-1}) of genotypes at sites: (A) 2014-2015 ASSYST; (B) 2014-2015 elite; (C) 2014-2015 ASSYST; and (D) 2015-2016 elite. The solid lines are 1:1 relationships. 110

Figure 53. Relationship for observed field yields (t ha^{-1}) from the RIPR trials and model predicted yields (t ha^{-11}) of genotypes across sites. Values are mean from across 4 trial sites. Elite genotypes are shown as (+) and ASSYST as (Δ). The solid line is a 1:1 relationship 111

Figure 54. –log10 P values of SNPs and GEMs associated with NUE (A,B, respectively) in order of markers within the B. napus pan-transcriptome. The data used is mean values from across all trials. Each dot represents a SNP or a GEM. The X-axis shows the genomic locations and the Y axis shows the association level. Markers of alternating chromosomes coloured black and red to distinguish between linkage groups. 125

Figure 55. –log10 P values of SNPs and GEMs associated with NUE for mean data from all trials (A,B, respectively), DP14 (C,D), DP15 (E,F), HM 14(G,H) and OM15 (I,J) in order of markers within the B. napus pan-transcriptome. Each dot represents a SNP or a GEM. The X-axis shows the genomic locations and the Y axis shows the association level. Markers of alternating chromosomes coloured black and red to distinguish between linkage groups. 126

Figure 56. –log10 P values of SNPs and GEMs associated with flowering (A,B, respectively) in order of markers within the B. napus pan-transcriptome. The data used is mean values from across all trials

Each dot represents a SNP or a GEM. The X-axis shows the genomic locations and the Y axis shows the association level. Markers of alternating chromosomes coloured black and red to distinguish between linkage groups. 127

Figure 57. –log10 P values of SNPs and GEMs associated with flowering for mean data from all trials (A,B, respectively), DP14 (C,D), DP15 (E,F), HM 14(G,H) and OM15 (I,J) in order of markers within the B. napus pan-transcriptome. Each dot represents a SNP or a GEM. The X-axis shows the genomic locations and the Y axis shows the association level. Markers of alternating chromosomes coloured black and red to distinguish between linkage groups. 128

Figure 58. Time line and outputs of NUE WOSR projects; DEFRA LINK: LK0979, HYLO:IF01110 and RIPR projects (BB/L002124/1) 134

Figure 59. Opt-plots. 5 rates can be tested on the same plot. 145

List of tables

Table 1. Overview of development and growth of WOSR. Codes are HB (Harper and Berkenkamp, 1975) and SM (Sylvester-bradley and Makepeace, 1984.) 22

Table 2. Sowing dates, soil details and N fertiliser treatments of field experiments. 44

Table 3. Colorimetric salicylic acid method reagent details 47

Table 4. Dates of UAV assessments at DP14 and HM14 sites. 48

Table 5 Mean, Minimum and Maximum yields (t ha^{-1}), NUE (kg/kg) of field sites: HM14; DP14; OM15; and DP15. Each point represents a genotype mean n=3, apart from DP14, n=2 50

Table 6. Phenotypic trait correlations with yield at LN fertilisation. Trait data used for Pearson correlation is mean for each of 59 genotypes grown at 4 trial sites: DP14; DP15; HM14; and OM15. P number is shown.

Table 6. Phenotypic trait correlations with yield at LN fertilisation. Trait data used for Pearson correlation is mean for each of 59 genotypes grown at 4 trial sites: DP14; DP15; HM14; and OM15. P number is shown. 68

Table 7. Pearson correlation results of relationship between yield at LN, NUE and morphological traits for all trials: HM14; DP14; OM15; DP15. p number shown. 69

Table 8. Mean NUE and N seed utilisation of genotypes and average rank order (1-52) across four sites. SD is the standard deviation of the rank. Genotypes ranked from lowest to highest. 79

Table 9. Mean NUE and N seed utilisation of genotypes and average rank order (1-52) across four sites. SD is the standard deviation of the rank. 81

Table 10. Mean Yield (t t ha^{-1}); NUE (kg/kg); and N seed utilisation of genotypes and average rank order across four sites. Low ranking indicates lower amounts and high indicates the higher amounts. SD is the standard deviation of the rank.

Table 11. Average rank order across four sites for mid-flowering: stem and leaf dry weights; tissue N concentration; and total N. Low ranking indicates the lower and high ranking indicates the higher weights. SD is the standard deviation of the rank. 8

Table 12. Average rank order across four sites for maturity: stem, chaff and seed - dry weights; tissue N concentration; and total N amount. Low ranking indicates the lower and high ranking indicates the higher amounts. SD is the standard deviation of the rank. 85

Table 13. Model inputs: parameters, nomenclature, units, genotype ranges, default values and sources. Range for crop traits is a genotype range under low N conditions. † Where no range is provided data were unavailable. 99

Table 14. N treatments of low N supply field data (Berry et al., 2010), Soil mineral N and Crop N at GS 3.1. 104

Table 15. Summary of candidate genes selected from associative transcriptomics outputs for NUE. Putative functions obtained from The Arabidopsis Information Resource (Huala et al., 2001). 129

Table 16. Summary of candidate genes selected from associative transcriptomics outputs for flowering. Putative functions obtained from The Arabidopsis Information Resource (TAIR; Huala et al., 2001). 130

Table 17. Summary table of traits linked to yield at low N. The project (LK0979) is sponsored by Defra through the Sustainable Arable LINK Programme. HYLO project was run by ADAS. The HYLO project refers to (IF01110) this is for the development of appropriate variety testing methodology for assessing N requirements of new varieties in trials undertaken for national listing. 141

Table 18. Figures to show economic value of less fertiliser. 146

Acronyms

Ammonia gas	NH_3
Ammonium	NH_4^+
Associative transcriptomics	AT
Break even ratio	BER
Carbon dioxide	CO2
Deoxyribonucleic acid	DNA
Gene-expression markers	GEMs
Genome-wide associations	GWAS
glutamine synthase	GS
Glutamine oxoglutarate aminotransferase	GOGAT
Greenhouse gas	GHG
High Affinity Transport Systems	HATS
High Yield High Optima genotypes	HYHO
High Yield Low Optima genotypes	HYLO
Light use efficiency	LUE
Linkage disequilibrium	LD
Low Affinity Transport Systems	LATS
Low Yield High Optimum genotypes	LYHO
Low Yield Low Optima genotypes	LYLO
N Harvest Index	NHI
Nitrate	N03-
Nitrate reductase	NR
Nitrite	N02-
nitrite reductase	NiR
Nitrogen	N
Nitrogen vulnerable zones	NVZ
Nitrous oxide	N_2O
Oilseed rape	OSR
Photosynthetically active radiation	PAR
Quantitative trait locus	QTL
Radiation use efficiency	RUE
Single nucleotide polymorphisms	SNPs
Unmanned Aerial Vehicles	UAVs
Winter oilseed rape	WOSR

Chapter 1. Introduction

1. Motivation

N fertilisers are used widely to improve WOSR yields. However, N is an expensive input and has environmental effects including greenhouse gas emissions and nitrate leaching. OSR grown in the UK has been estimated to have a NUE of 10 kg seed DM/kg N compared with 21, 25 and 69 kg DM/kg N for spring malting barley, feed winter wheat and sugar beet respectively (Sylvester-Bradley and Kindred, 2009). This low NUE is due in part to the high energy requirement of seed oil production, however it can also be attributed to current genotypes inefficient uptake and utilisation of N (Mendham et al., 1981).

OSR yields are highly variable; they are affected by genetics, environment and agronomic practice as well as the interactions between them (Dresboll et al., 2014, Bradbury et al., 1993). Trials comparing NUE of genotypes have shown yield and cultivar are ranked differently at high N and low N rates (Kessel et al., 2012; Nyikako et al., 2014; Erley et al., 2011, Berry et al. 2010). If the interaction between genotype and N supply is highly significant but the correlation between low N and High N plants is low then it is possible to select genotypes with adaptions to low N. This variation can be exploited by plant breeders

The ability to use genotypes for bioinformatics studies and computational modelling to understand yield potentials and interactions with the environment to understanding the genetic control of traits will dramatically affects breeding programs as it is a way to propose new N-efficient cultivars (Snowdon et al., 2012). Simple models and transcriptomic genetic techniques can be used to better understand genetics of key traits and identify predictive markers for NUE and/or key traits.

This project is part of the RIPR project. The principal aim of the project is to understand the genetic control, in rapeseed, of the accumulation of compounds representing emerging bio-refining opportunities and fertilizer use efficiency. The wider project aims to integrate existing knowledge and new field data to identify and fill important genotype knowledge gaps.

1.2. Scientific hypotheses

Specifically, this thesis addresses the scientific hypotheses listed below:

- Modern breeding has improved yield for WOSR under high N fertiliser conditions but has not directly improved crop NUE at LN. There is potential to breed genotypes that are consistently high yielding under low N conditions.

- The phenology development of WOSR significantly impacts yield, N-uptake and N-utilisation. Defining key traits, and their interactions can be used to predict crop growth at low N and develop simple predictive models.

- Analysis of older genotypes within a diversity trial will be beneficial for better nutrient use, since they might have comparative advantages in adaptation to low input systems. These diversity sets can be used to define phenotypic and genetic markers that are indicators of NUE.

1.3. Experimental outline

A field trial has been conducted as part of the renewable industrial product for rape (RIPR) project. The trial was conducted over two sites and two years;

Characterising the genetics of NUE traits of WOSR in a structured association panel; 84 genotypes types, 2 N rates, a low (60 kg N ha^{-1}) and a high (300 kg N ha^{-1}). 32 traits were measured and analysed including; N tissue analysis, phenology dates, and yield.

Objectives of field study were to study

(1) yield response to low and high N

(2) traits determining high yield under low N conditions

(3) trait ranges

(4) compare traits from, high yielding low optima HYLO and high yielding high optima HYHO genotypes.

A physiological model of N-acquisition and N-utilisation in WOSR was developed, validated and used for simulation studies. The model can be used to identify novel traits for use in breeding strategies to improve NUE, inform the optimal use of N fertilisers and identify crop traits and combinations of traits for both increasing seed yield at low N of WOSR.

As part of the RIPR project, initial transcriptomic analysis has been conducted (jointly with consortium members) with the data from the field phenotype data. Associative transcriptomics, used the transcriptome sequencing to identify and score molecular markers representing variation in both gene sequences and gene expression, and correlate this with trait variation. From the RIPR trial we have 32 phenotypes that could be analysed. I identified potential marker genes for NUE and Flowering score in the field.

2. Background

Sustainable crop production means protecting the environment and human health while maintaining or increasing crop yield, economic viability, and nutritional quality. Nitrogen (N) fertilisers are used to improve crop yields, but the costs are becoming better recognised, both due to market forces and realisation of their environmental effects including, greenhouse gas (GHG) emissions and NO^-_3 leaching (Kindred et al., 2008). Although progress has been made to optimise N fertilisation, more than half of worldwide N applied to crops is lost to the environment (Lassaletta et al., 2014). Improving N use efficiency (NUE) of crop production is crucial to reducing reliance on N fertilisers in arable cropping. Global N efficiency of oil crop production needs to improve from an estimated 43% to 70% to meet 2050 food security and environmental goals (Zhang et al., 2015). Sustainable agriculture will require an increase in knowledge through developed technologies (remote sensing, crop genetics) and human application through farm management. There is great potential to improve NUE and lower optimal N rate of winter oilseed rape (WOSR) at an individual crop level and a cropping system level. Significant genetic variation in N requirements has been reported among WOSR genotypes meaning there is an opportunity for focused breeding of N efficient genotypes (Berry et al., 2010).

This introduction will review;

1) Current status;
    (1) OSR production
    (2) N requirements of WOSR
    (3) environmental consequences of fertiliser N
    (4) NUE definitions and examples
2) Potential improvement of WOSR NUE through;
    (1) N uptake
    (2) N utilisation
    (3) farm management.
3) Practical approaches and methods;
    (1) crop models,
    (2) breeding screens for NUE
    (3) genetics

2.1. Current status

2.1.1 Oilseed rape production

OSR is a crop of major economic importance, mainly grown for its oil-rich seeds, but it also produces a wide range of products including foods, cosmetics, plastics, energy and animal feed. OSR is the third most important oilseed crop worldwide after perennial oil, palm oil and soybean (Food and Agriculture Organization of the United Nations, 2013) with significant areas of production in Europe, Canada, China, and India. Inside Europe WOSR is by far the most important oil crop and is typically the most profitable break crop. In 2016 the area planted in the UK was 675,000 hectares (DEFRA, 2016) and the area planted in 2018 was 583,000 hectares (DEFRA, 2018).

WOSR was introduced into the UK in the 1970s making it a relatively new crop. Yields of WOSR have increased slowly since the early 1980s, with a long-term mean of 3.1 t ha^{-1} (Fig 1). There is massive potential to increase WOSR yields in the UK - the UK long-term yield is significantly lower than the recommended list yield (Fig 2), showing current field grown WOSR is not reaching its potential. WOSR yield loss occurs as a result of weather conditions, crop establishment, weed burden, pest attack, disease control, poor fertilisation, and non-current genotype choice. The biggest threats to WOSR yields in the UK are: cabbage stem flea beetle; Light leaf spot; and Turnip yellow virus (HGCA, 2013)

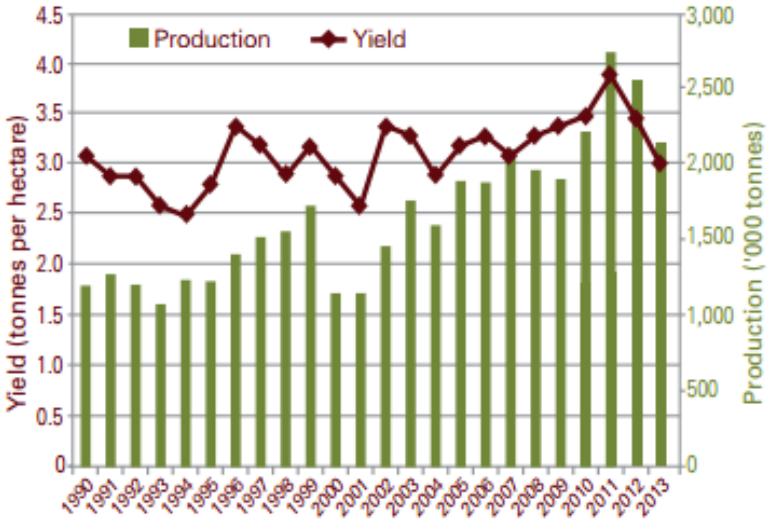


Figure 1. UK WOSR yield (tonnes/hectare) and production (tonnes) for 2009-2013. Source: HGCA Oilseed rape growth guide, 2013

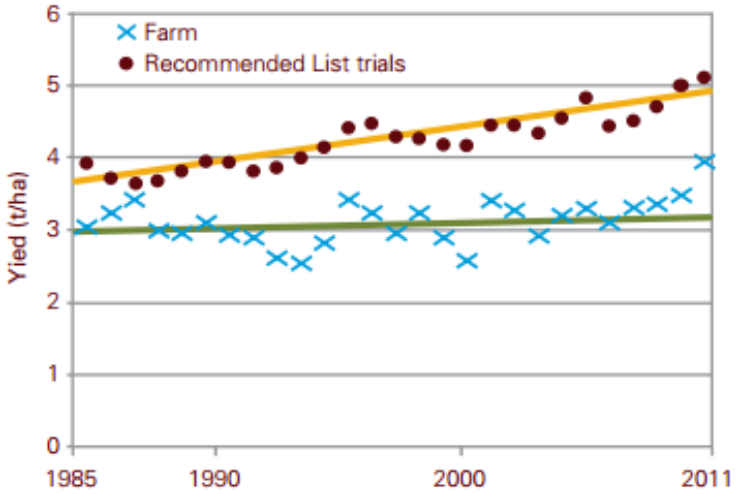


Figure 2. WOSR yields (t ha^{-1}) for farms (x) and recommended list trials (●). Source: HGCA, Oilseed rape growth guide, 2013

2.1.2. N requirements of WOSR

WOSR has high N requirement: in the UK and Europe, N is the biggest variable cost for WOSR production (Berry et al., 2008). In the UK, WOSR receives a mean of 184 kg N/ha^{-1} and has a long-term mean seed yield of 3.1 t ha^{-1} at 91% dry matter (HGCA, 2013). Comparatively, winter wheat receives 184 kg of fertiliser N and has a mean yield of 8 t ha^{-1} at 85% dry matter (Berry et al., 2010). The high N requirement of oil crops relative to seed yield is due to both the high energy requirement of seed oil production and current genotypes' inefficient uptake and utilisation of N. N fertiliser rates in the UK are predicted using national guidelines (e.g., Defra, 2017), and are generally chosen to maximise profitability for the grower. The economically optimal N rate is when any further increase in N application rate will result in higher fertiliser costs than the value of the additional yield. This means that the optimum N is dependent on the price of the seed and N fertiliser and is expressed as the breakeven ratio: the amount of seed (kg) required to pay for one kg of fertiliser.

The N demand of WOSR varies with developmental stage - demand is high during vegetative growth and decreases during the reproductive phase, which corresponds to remobilisation of reserves. WOSR is typically sown in late August or early September. The crop overwinters; flowers in spring; and is harvested in late summer - the cycle lasting about 320 days (Table 1). Figure 3 shows the growth phase of WOSR and corresponding N demands. The figure shows relationships between plant growth; N dynamics; and yield over the crop cycle. In the UK most sites contain sufficient soil N for autumn growth, meaning N fertiliser is not required. By the end of November, a well performing crop could take up approx. 70 N kg/ha^{-1}, making this the most effective crop at utilising N from previous crop residues. Through February and March, as demand for N increases and before fertilisers are applied, soil N supply should be assessed. There is a large early demand for N in April. Delaying

spring N fertiliser application so that it coincides with this demand can increase yield if there is a risk that the canopy may become over large (Berry and Spink, 2009). However, shortage of N at this point will accelerate stem elongation reducing total yield potential. After petal fall, to maximise yield, the pods need to be provided with adequate N for both photosynthesis and to supply N to the developing seed for protein. By this stage, the crop should have taken up over 200 N kg/ha^{-1} (AHDB, 2009).

Table 1. Overview of development and growth of WOSR. Codes are HB (Harper and Berkenkamp, 1975) and SM (Sylvester-bradley and Makepeace, 1984.)

Development	**Code HB**	**Code SM**	**Growth**
Sowing	0	0	
Emergence	1	0.8	Expansion of cotyledons; growth of taproot.
Leaf production	1.1 2.1 2.8	1 1.2	Establishment of root system and expansion of leaves; interception of solar radiation; photosynthesis; increased leaf dry weight.
Inflorescence initiation (vernalisation and photoperiod response)			
Stem elongation (photoperiod responses)		2 2.2	Stem dry weight increases; stem photosynthesis commences; reserves laid down.
		3 3.9	
Flowering (pollination, seed set)		4.1 4.9	Leaf area and root extension close to maximum. Flowers shade leaves; young pods
Pod development (pod and seed abortion, final numbers determined)		5.1 5.9	Pod and stem photosynthesis replaces declining leaf area as leaves senesce. Pod walls reach maximum size and seed growth commences.
Seed development (formation of embryo, storage cells)	5.1 5.4	6.1 6.9	Seed growth with assimilate from leaves, stems, pods; Oil and protein synthesis and storage.

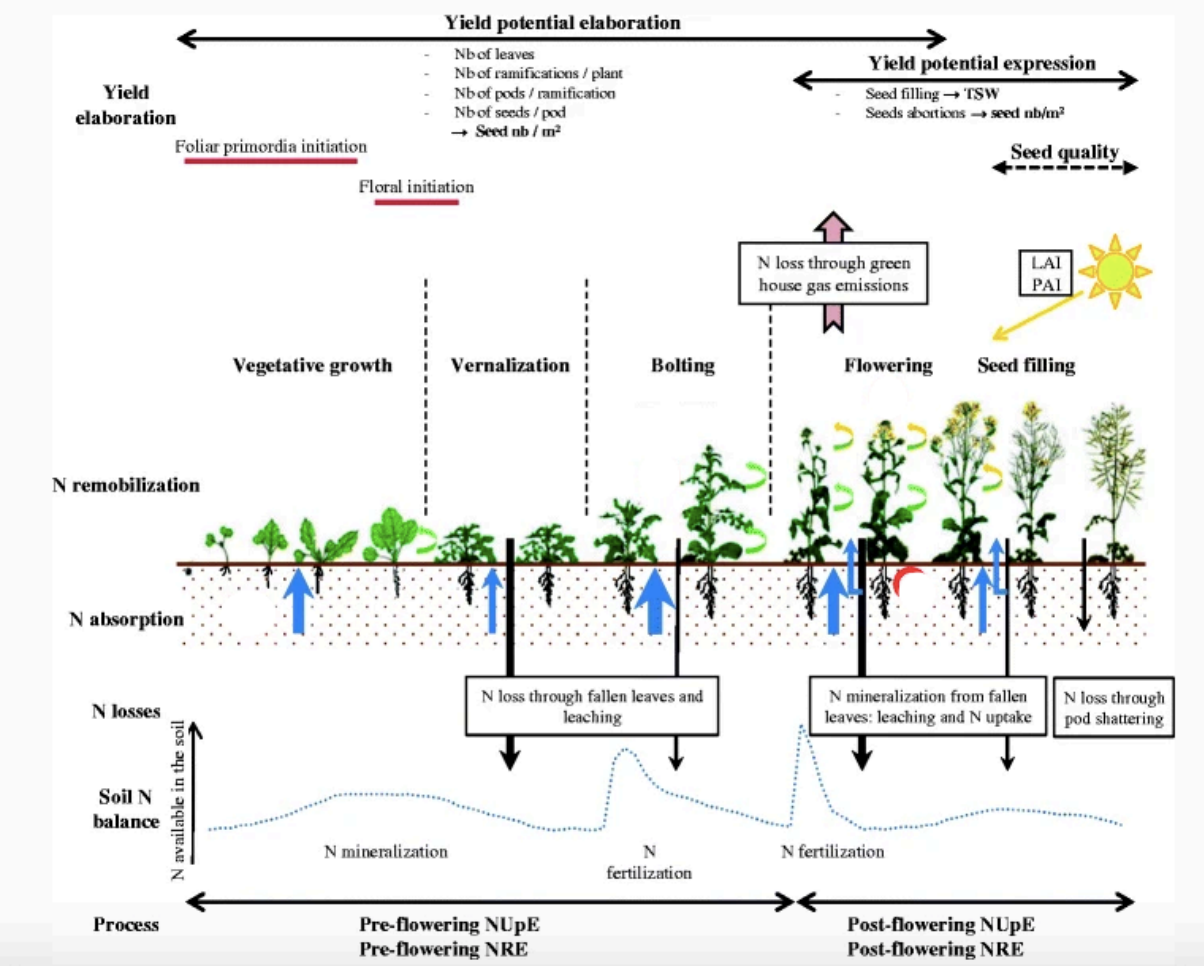


Figure 3. WOSR N dynamics over the crop cycle. The figure shows relationships between plant growth; N dynamics; and yield elaboration over the crop cycle. N uptake is represented by blue arrows whose width indicates the relative amount of N absorbed at a given time point. N losses are shown with *black arrows* whose width indicates the relative amount of N lost at a given time point. Image used with permission. Source: Bouchet et al., 2016.

The form of N in the soil affects both the availability of N for crop demand and how much N is leached. N mineralisation is the process by which N is broken down by microbes and released in mineral forms that are utilised by crops, including ammonium (NH_4^+) and nitrate (NO_3^-) - these are the forms of N that is commonly taken up by the roots, Mineralisation therefore has a direct impact on N availability in the soil and how much N can be taken up by WOSR. The amount of mineralisation depends on the recent history of cropping; organic manure; moisture; and temperature. For example, the amount of organic N mineralised for WOSR has been shown to be larger on organic and peaty soils where organic manures have been used for many years or where N-rich, organic material is ploughed into the soil (AHDB, 2009). It has also been shown that less mineralisation occurs when soil temperatures are lower than 4*C (AHDB, 2009). Higher soil temperature supports mineralisation in early autumn that can contribute to the nitrogen requirement of an autumn-sown crop. Mineralisation will drastically reduce over winter but late harvested crops will utilise more mineralised N than crops which are harvested in mid- or late-summer.

The three main pathways of N loss in agriculture are NO^{-}_{3} leaching; denitrification; and volatilisation. These pathways are all affected by the form of N. Most transformations in the N cycle are the result of microbial activity that is sensitive to the environment. In this section we will discuss N transformations in the soil and how these may relate to N availability and N uptake efficiency.

Nitrification is the biological oxidation of ammonia (NH_3) or NH_4^+ to nitrite (NO_{2-}) followed by the oxidation of the NO_{2-} to NO^{-}_{3}. The negatively charged NO^{-}_{3} does not attach to soil particles and this soluble form will be leached with water. For all crops, leaching depends on precipitation differences across years and sites (AHDB, 2009). This soluble form of N will increase leaching during periods of low crop uptake and therefore risk is highest in the early spring due to higher rainfall and slow crop growth. Therefore losses due to leaching can be managed by putting limitations on the amount of N that can be applied at high risk times. Leaching is more rapid on light sandy soils compared to deep clay or silt soils which are less free draining and therefore more retentive of NO^{-}_{3} (Vinten et al, 1994).

Denitrification occurs when soil is saturated by water and microbes that would normally use oxygen to decompose organic matter use NO^{-}_{3} instead. The NO^{-}_{3} is converted to gas forms of N and released to the atmosphere including nitrous oxide (N_2O) which is a major GHG. Denitrification is most significant in wet and warm soils where there is a supply of NO^{-}_{3} after harvest or where there has been a recent N application and there is organic matter for the microbes to feed on. In agricultural systems, N_2O emissions have been seen to increase rapidly in the weeks following N fertiliser application (Ambus et al., 1993, Liu et al., 2005), and emissions have shown variation on a diurnal scale (Shurpali et al., 2016). Measurements from soil under WOSR have shown considerable variation in the magnitude of N_2O fluxes, ranging from < 40 µg m-2 h-1 (Barton et al., 2010) to over 2000 µg m-2 h-1 (Drewer et al., 2011). Keane et.al. (2018) found strong evidence to suggest that photosynthetically active radiation (PAR) rather than soil temperature drove diurnal N_2O flux variation in WOSR field trials.

Volatilisation of N in the NH_3 form occurs from surface applied manure and some fertilisers. The N found in urea-containing fertilisers and animal manure converts to NH_3 and is lost into the atmosphere if it is exposed on the surface of soil. There is a higher risk of volatilisation after use of organic fertiliser that is not immediately incorporated by cultivation. If urea or manure is not exposed to air the NH_3 converts to NH_4^+, which is held to the soil particles.

2.1.3. Environmental impact of N fertiliser

N fertilisers have negative environmental consequences due both to the energy intensive production and to damage caused by N loss into the environment. These environmental factors impact the efficiency and to some extent the viability of WOSR for food production and biodiesel. The invention and up-scaling of the Haber-Bosch process in the early 1900s allowed manufacturing of affordable mineral N. The process now produces 450 million tonnes of N fertiliser per year; this uses three – five percent of the world's natural gas production (Vaclav et al., 2004). If crops are over-fertilised, there will be N surpluses; if the crop is under-fertilised the reduced N results in loss of yield, lowering land productivity. If WOSR prices are high, some management decisions will make sense from an economic point of view but will not reduce N surpluses.

Five critical environmental problems associated with N overuse are: water quality; air quality; GHG emission; biodiversity loss; and soil quality. NO^{-}_{3} is highly water soluble, leaching into rivers, lakes and oceans and causing eutrophication which has massive impacts on biodiversity. Air quality is affected by emissions of N oxides or volatile NH_3 (Venterea et al., 2012). GHG emissions from agriculture accounted for 9% of UK emissions in 2010 (HGCA, 2010). Field and laboratory experiments and 43 surveys with a range of N pollutants have shown that long term elevated N deposition, related to agricultural waste, is linked to reduced plant biodiversity (Dupre, et al. 2010, Maskell, et al. 2010). Excess N impacts plants through direct toxicity; soil acidification;nutrient imbalances; and interspecific competition (Dise, et al. 2011).

Mahmuti et al. (2009) predicted that fertiliser N application could be responsible for over 79% of the GHG emissions associated with WOSR production. Both industry and government have highlighted the importance of improving crop N efficiency. The agriculture industry has signed up to the GHG action plan 2008, committing to the reduction of carbon emission in UK agriculture by 3 million tonnes of CO2 by 2020. Smaller carbon footprints are associated with efficient crop production. Large research projects investigating and generating genetic diversity for NUE in rapeseed have been recently completed or are currently in progress in France (RAPSODYN: www.rapsodyn.fr); Germany (PreBreedYield); and the UK (NUECrops). In 2016 the EU N Expert Panel proposed that environmental regulation of agriculture should be based on nutrient balance accounting to prove crop N use efficiency. UK legislation to minimise the risk in N vulnerable zones (NVZ) limits the amount of fertiliser N that can be applied to OSR to 310 N kg/ha (DEFRA. 2017). German regulations limit annual N-balance (N applied minus N removed by harvest) to a three year mean of 60 kg/ha. The N-balance measured after winter OSR usually exceeds this limit and is frequently above 100 kg/ha (Henke et al., 2007). N-balance can differ sharply from site to site (Sieling et al., 2010); it is affected by the environment and the significant genetic variation in N response (Berry et al., 2010; Stahl et al., 2016). In wet soils, N shows increased leaching, whereas in cool, moist and dry soils N mineralisation can be inhibited. It is particularly important to understand the risk of N_2O fluxes for OSR because it is a major GHG gas. Keane et.al. (2018) found that in field trials in the UK high N_2O emissions were

seen across all treatments, even at 50% of the management applied N rate, demonstrating how important N_2O gas is for crop GHG balance. The study concluded that due to these high levels of N_2O fluxes WOSR may not be a suitable crop for bio-fuel.

2.1.4. NUE definitions and example

The definitions used for N derived traits in this study are based on Moll et al., (1982). NUE is defined as harvestable dry matter yield (in kg/ha) divided by the amount of N available in soil and fertiliser (also in kg/ha). NUE may be considered as the direct increase in seed yield per additional unit of N, or the ability to convert N to seed yield by dividing the difference of seed yield between two N nutrition regimes by the N supplied (that defines the agronomic efficiency) (Good et al., 2004; Rathke et al., 2006). NUE can be split into two components, namely N uptake efficiency and N utilisation efficiency. N uptake efficiency is the proportion of N absorbed from the soil. N utilisation is the efficiency with which the absorbed N is converted into available yield. N Harvest Index (NHI) is defined as the ratio of N present in the seed to total plant N content. NHI is a measure of N translocation efficiency.

All else being equal, the highest efficiency of N fertiliser use is achieved with the first increments of added N. Yield response to N typically shows a rapid increase in yield at low N rates, followed by a levelling off, and often by a reduction at super-optimal N rates due to factors such as lodging and disease (Mendham et al., 1981). NUE is a complex trait affected by genotype, environment, and management. The optimal N rate is the rate beyond which any further increase in N will result in greater N fertiliser costs than added financial value. The ideal N efficient genotypes will have high yields but low N optimum. In this study, these are referred to as High Yield Low Optima genotypes (HYLO). A diverse panel also included 'HYHO' (High Yield High Optima), 'LYLO' (Low Yield Low Optima) genotypes and "LYHO" (Low Yield High Optimum).

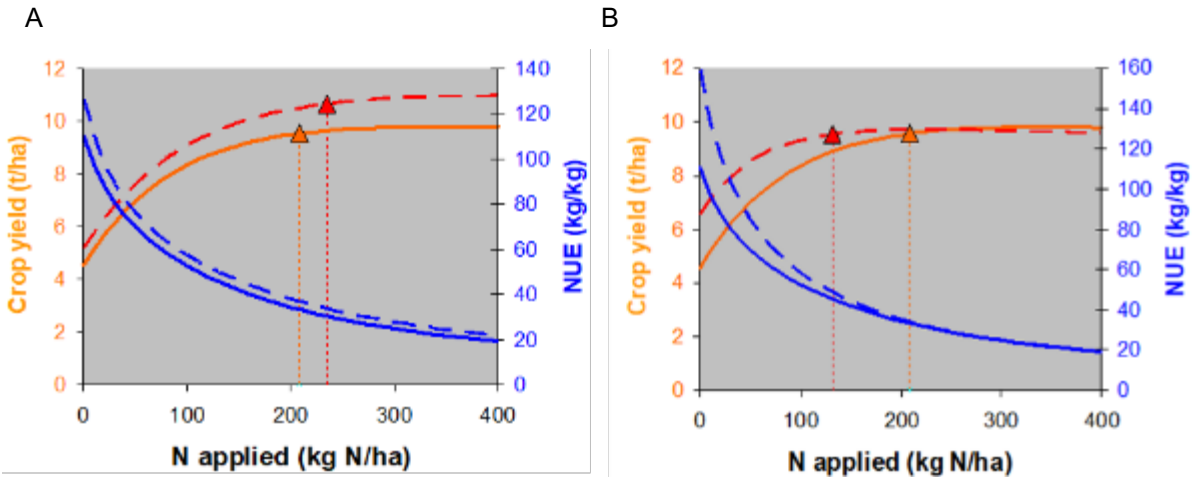


Figure 4. A theoretical comparison of corresponding N applied and crop yield (t ha^{-1}) (red lines) and NUE (kg/kg) (blue lines). A typical genotype response is shown in solid lines, compared to genotypes with a different N response shown with the dashed lines for A) high yielding genotype and B) N efficient genotype. The optimal N rates are indicated by the vertical dashed lines. Theoretical data source (Storer et al., 2018)

Fig 4A compares a standard response (solid line) to a genotype that is higher yielding (dashed line). The higher-yielding genotype has improved NUE at all N applications. However, as the higher yielding genotype also has a higher N optimum the NUE at optimum is unchanged compared to the standard genotype. Fig 4B compares a standard response (solid line) to a genotype that is HYLO. The HYLO genotype has a steeper increase in yield at low N rates. In this example, an application of 200 N kg/ha fertiliser results in a minor difference in yield and NUE between genotypes. However, at the optimum N rate, the HYLO genotype (dashed line) shows a substantial increase of NUE compared to the standard genotype. In this hypothetical example, the NUE of the HYLO genotype is 47% compared to 34% of the standard genotype. These optima are calculated using the economic optimum.

2.2. Improving NUE efficiency of WOSR

Improved WOSR NUE and lower optimal N rates can be achieved by taking advantage of variation in genotype N requirements and improved crop management practices. Traits affecting both WOSR yields and NUE have been studied in detail throughout the crop cycle (Mendham et al., 1981; Diepenbrock et al., 2000; Berry et al., 2006). Studies have estimated that only 30 – 50% of applied N is utilised by WOSR (Berry et al., 2006, Diepenbrock et al., 2000, Sylvester-Bradley and Kindred, 2009, Mendham et al., 1981). Trials comparing NUE of genotypes have shown that yield and cultivar are ranked differently at high N and low N environments (Berry et al., 2010; Erley et al., 2011; Kessel et al., 2012; Nyikako et al., 2014; Hui et al., 2017; Stahl et al., 2017). NUE has been shown to correlate more strongly with N uptake in low N environments and more strongly with N utilisation efficiency in high N environments (Nyikako et al., 2014; Berry et al., 2010a; Kessel et al., 2012). Intensive agriculture practices and breeding of WOSR has been carried out under non-limiting N supply. Plant breeders have selected genotypes that performed well under optimum N supply.

The development of HYLO genotypes is crucial in order to meet environmental N regulations and to ensure profit for farmers. In this section we will discuss the increasing efficiency of WOSR NUE in WOSR through improved;

(1) N uptake
(2) N utilisation
(3) crop management
(4) N uptake mechanisms

2.2.1. Traits linked to improved N uptake

Several field studies report that NUE has stronger links to N uptake efficiency than to N utilisation efficiency in low N environments (Berry et al., 2010; Schulte auf´m Erley et al., 2011; Nyikako et al. 2014). This suggests that the enhancement of WOSR performance under low N environments requires the improvement of N absorption. A large proportion of N is taken up during the vegetative and pre-flowering stages (Fig 5), therefore strong establishment and early root development are

crucial. N absorption depends on soil N reserves; environmental conditions; and volume of soil investigated by roots linked to the capacity for N absorption.

The literature on below-ground traits is limited, and not all available research directly relates to yield (White et al., 2013). N uptake in WOSR is affected by soil composition; mineralisation; root physiology (Ulas et al., 2012); and the sink-source relations of the reproductive phase (Rossatto et al., 2001). Studies have assessed genetic variation of root mass, root length and root-shoot ratio in commercial WOSR genotypes in both field and lab conditions (Kamh et al., 2005, White et al., 2013, Bouchet et al., 2016, Stahl et al., 2017). Greater importance of root length, compared to root biomass, was seen by (Kamh et al., 2005). Genetic diversity for root architecture was assessed in WOSR genotypes - the more N-efficient genotypes showing higher fine root proportion and increased root length (Bouchet et al., 2016, Stahl et al., 2017). Increasing root surface by improving fine root density was considered as a possible strategy in wheat (White et al., 2013). It has been reported that OSR will take up the total amount of soil mineral N (SMN) that is available to the roots (AHDB, 2009). The actual efficiency with which SMN is recovered is likely to be less than 100% (and might typically be closer to 60%), however, additional soil N becomes available for uptake during the growing season (often through mineralisation of crop residues and soil organic matter) (AHDB, 2009).

N residues refers to the amount of mineral N (NO_3^- and NH_4^+) left after harvest. This is affected both by management and by how much N the crop has taken up during growth. Residues will be larger than average when yields are unusually small due to environmental factors (disease or drought) - the vast majority of these residues will re-enter the soil. OSR has high residues and this can result in fast mineralization and high N leaching potential in the subsequent season. In OSR field trials Malagoli et al. (2005) observed N losses from the crop of up to 45 kg N ha^{-1} due to leaf losses between stem elongation and harvest.

Several field studies have shown that post flowering N uptake efficiency has a significant effect on total NUE and is the most important factor to discriminate between genotype for yield (Berry et al., 2010a; Schulte auf'm Erley et al., 2011; Ulas et al., 2013). Wiesler et al. (2001) concluded, as part of an ideotype study, that an “alternative ideotype” showing a higher N uptake efficiency during the reproductive stage would have better prospects for improving global NUE of WOSR. Flowering is associated with a massive change in the source-sink relationship, and it is likely that the majority of N taken up after flowering would go straight to seed yield (Fig 5).

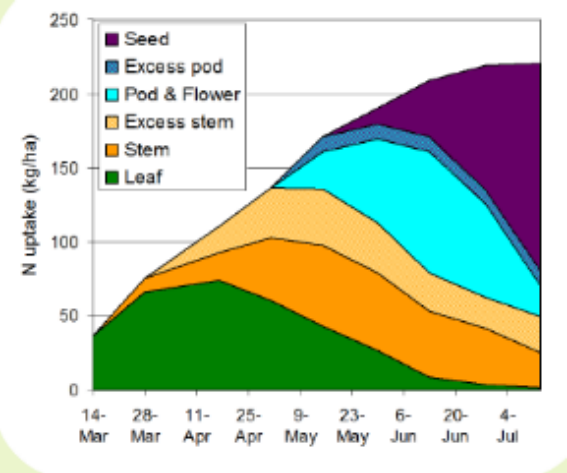


Figure 5. The determination of crop N uptake (kg/ha) off WOSR over time split by tissue type: Seed, Excess pod, Pod + Flower, Excess stem, Stem and Leaf. Data from Stafford (1996).

2.2.2. Traits linked to improved N utilisation

Traits linked to N utilisation efficiency include: late leaf senescence (Ulas et al., 2013; Kessel et al., 2012, Malagoli et al., 2005); early flowering (Stahl et al., 2016); N remobilisation from leaf and stem; and seed N content (Berry et al., 2010a). Low NUE of WOSR has been significantly associated with the weak N remobilisation that occurs in leaves during vegetative development (Dejoux et al., 2000; Malagoli et al., 2005, Aufhammer et al., 1994; Desclos et al., 2009), mainly due to the loss of N-rich leaves (Schjoerring et al., 1995; Dreccer et al., 2000; Rossato et al., 2001) stem N storage and low seed protein:high seed oil contents (Nyikako et al., 2003, Stahl et al., 2017).

The importance of N remobilisation to yield is more confused than uptake. Seed N utilisation has no correlation to yield at low or high N (Schute et al., 2010). Late leaf senescence potentially leads to better assimilate supply by pods and roots and increased N uptake (Ulas et al., 2013; Nyikako et al., 2013; Kessel et al. 2012). Gombert et al. (2010) found that more than 70% of pod N is derived from N remobilisation. However, Koeslin-Findeklee et al., (2014) found that greater NUE of hybrids was not related to delayed senescence, but it was related to N uptake pre maturity and to efficient N utilisation. Rossato, et al. (2001) reported losses of 15% of N - the equivalent of up to 45kg/ha. Berry et al. (2010) did not find a significant relationship between delayed senescence and N remobilisation from leaves. Several studies have found no difference in N remobilisation of leaves at high and low N (Rossato, et al., 2001; Malagoli et al., 2005). This suggests a sink limitation rather than a source limitation. Koeslin-Findeklee, (2015) found that an N efficient cultivar (Apex) had delayed leaf senescence under limited N supply, but this trait was not linked to increased N remobilisation.

Residual stem N has higher genetic variation and is therefore an important pool of N (Ulas et al., 2013; Bouchet et al., 2016; Berry et al., 2010a). The residual N left in the stem was found to be much higher than N lost in leaves. Significant N losses are also seen due to early pod shattering before harvest or early harvest. Seed pods are fragile, and seed loss can significantly impact yield if bad

weather occurs when the crop is most vulnerable. Even in good weather, yield loss of up to 25% has been reported when harvest operations are late (Bruce et al., 2002).

2.2.3. Improve yield through crop management

There is potential to improve WOSR yields through both breeding and effective crop management. Genotypes that have higher yields will improve NUE at all N application rates (Fig 4A). Over the last 25 years, breeding has successfully increased WOSR yields (Fig 2). The amount of N fertiliser required to produce 1 ton of WOSR has dramatically declined (Stahl et al, 2016). In the UK, plant breeders have increased the potential yield of new WOSR genotypes by 0.05 t ha^{-1} per year (Berry and Spink, 2006). Recommended list genotypes have a reported yield potential of approx. 6.5 t ha^{-1} (HGCA, 2015), but current UK farm yields have much lower long-term mean of 3.1 t ha^{-1}

Fundamentally, yield gaps are caused by deficiencies in crop growth environments that are not addressed by management processes. Yield limiting factors could include:

(1) inadequate disease control
(2) sulphur deficiency
(3) slow adoption of new genotypes
(4) sub-optimal N fertiliser.

Though HGCA recommends all OSR crops receive sulphur, in 2016 only 70% of crops were treated (DEFRA, 2017). Adoption of new genotypes is often a financial decision based on predicted markets. The mean amount of N fertiliser applied in the UK has fallen from approx. 270 N kg/ha^{-1} in 1983 to the 2017 level of 184 kg N/ha^{-1} (HGCA, 2017). This decrease was due to increased costs of N and to legislation aimed at reducing N leaching to water courses. Optimum N management requires precise rates and optimum timings. The aim is to improve N management through various complementary fertilisation strategies and soil management regimes such as precision fertilisation through split fertiliser (Hirel et al., 2007) or placement strategies. Alongside precise fertiliser applications, accurate disease matching to site and genotype requirement will help to adapt management practices to minimise excessive N-balances while achieving optimum yields.

N fertiliser is applied in different forms, and the form has an effect on uptake and utilisation. Since soils differ in their capacity for nitrification or denitrification (Bateman & Baggs, 2005), fertiliser type can affect consequential N_2O fluxes (Dobbie & Smith, 2003; Zhang et al., 2014), and therefore NUE. Ammonium nitrate (33.5-34.5% N) is the main form of N used in the UK - the NO^-_3 is immediately available for crop uptake and the NH_4^+ can be taken up directly but is also quickly converted to NO^-_3 by soil microbes. A common alternative is urea (46% N) which is more affordable but as discussed previously this has a high risk of N loss through volatilisation - typically around 20%. To be available to be taken up by plants first urea-N must first be converted to NH_4^+ by the enzyme urease that occurs in all soils. This process usually occurs quickly and does not significantly delay the availability of N for crop uptake. Solutions based on urea alone will contain no more than 18% N

because at low ambient temperatures urea crystallises out of solution. Foliar N is applied to some WOSR in the UK - estimated at <10% of crop in 2011 (White et al., 2012). The advantage of foliar N is that it can be applied at a later growth stage than soil applied N. A study of the effectiveness of foliar applied N in *Brassica napus* found that there was a significant response of foliar N at 3 of 4 sites, even when applied to plots that had received optimum N (White et al., 2015). However, there was a large variation in response, probably due to environmental conditions they concluded: "This indicates that foliar N could be used if conditions were predicted to be dry for soil applied N to be taken up efficiently. However, it should be emphasised that foliar N yield responses also occurred falling optimal or sub-optimal rates of soil applied N (which included late applications at yellow bud)."

2.2.4 N uptake mechanisms and the form(s) of N taken up

Uptake mechanisms depend on both maximal crop growth rate and the external N concentration and forms. N utilisation by plants involves several steps including uptake, assimilation, translocation, recycling and remobilisation. The critical N concentration is defined as the minimum tissue N concentration required for maximum above-ground biomass at a particular time during vegetative growth (Colneme et al.,1998**)**. NO^-_3 and NH_4^+ are the main inorganic N sources for WOSR. As the requirement of N for OSR is higher than for other crops it has a high capacity to take up NO^-_3 from the soil (Laine et al., 2006) and store it in vegetative parts. It is thought that NO^-_3 transport activity is finely tuned by the plant demand (the amount of N required to sustain growth under environmental conditions). N assimilation is the formation of organic N compounds like amino acids from inorganic N compounds present in the environment. For most plants, some NO^-_3 taken up by the roots is assimilated into the roots, but the larger part is transported to the shoot, where it is first reduced to nitrite by nitrate reductase (NR) in the cytoplasm and then further to NH_4^+ by NR and nitrite reductase (NiR) in the plastids and by glutamine synthase (GS) in the plastids and cytoplasm. NH_4^+ taken up from the soil (or that has been converted) is incorporated into amino acids via GS and the glutamine-2-oxogultarate aminotransferase pathway.

Many studies during the past decade have advanced the understanding of the regulation of N use in plants by identifying transporters for NO^-_3 and NH_4^+, along with the functions of plant-specific sensors and transcription factors. When considering N uptake efficiency in a wide range of N conditions, molecular and physiological studies have shown the importance of the NO^-_3 and NH_4^+ High Affinity Transport Systems (HATS) and Low Affinity Transport Systems (LATS) in the control of N uptake (Hirel et al, 2001, Orsel 2002, Miller et al., 2007;). These have been shown to coexist in plants and to act in co-ordination to take up NO^-_3 from the soil solution and distribute it within the whole plant (Daniel-Vedele et al., 1998). Molecular studies have also shown that the multiple gene families encoding putative transporters are present for both NO^-_3 and NH_4^+ transport (Gansel, 2001). Where soil NH_4^+ concentration is low, the HATS system functions at lower concentrations and the LATS system acts at high concentrations and is thought to be controlled by external amino acids, allowing

the crop to store N. These pathways are controlled by the maximum crop growth rates and external N concentrations.

It is necessary to understand the genetic controls of NO_3^- and assimilation at different N levels in relation to root and shoot development (Hirel et al., 2007) There is vast potential for studying the regulation of genotypes under low or high N inputs. In Maize it was found that the inducible NO_3^- transport system could be a physiological marker for adaption to low N inputs (Quaggiotti et al., 2003). NUE of OSR was increased by increasing the Glutamine oxoglutarate aminotransferase (GOGAT) and GS activities by a transgenic method (Seiffer et al., 2004). Two transporters in arabidopsis (NRT2.1 and NPF6.3/NRT1.1), as well as their protein partners (NAR2, CBL1, CBL9, CIPK23, ABI2), are involved in NO_3^- transport and sensing (Noguero et al., 2016). Vacuolar transporters are involved in NO_3^- accumulation and consequently impact on NUE, as observed in rapeseed for the transporter *BnNRT1.5* (Han et al., 2016). This means that understanding the regulatory factors interacting with NO_3^- transporters (as well as other transporters), is required to understand how NO_3^- influences plant growth and development. It has been shown that NO_3^- is also a signal molecule involved in the control of many physiological processes; plant growth; and crop yield (Krapp et al., 2014, Crawford et al., 1995). As a signal molecule it can also induce the expression of a number of genes implicated in NO_3^- transport and assimilation (Medici et al., 2015) NO_3^- participates in the regulation of lateral root development and architecture (Ruffel et al., 2011, 2016); leaf development (Chiu et al., 2004); flowering induction (Castro Marin et al., 2011); and seed dormancy (Alboresi et al., 2005). In WOSR it has been shown that NO_3^- transport activity is repressed post-flowering (Bouchet et al, 2016). Plant levels of amino acids can also indicate N status and could be involved in the expression of transporters (Miller et al., 2007).

The different forms of N can affect the physiological and metabolic processes of plants, such as nutrient uptake; enzyme activity; photosynthesis rate; respiration rate; water balance; and signalling pathways; and therefore eventually influence plant growth and crop yield (Masclaux-Daubresse et al., 2010). Plants adapted to low pH and reducing soils, as found in mature forests or arctic tundra, tend to take up NH_4^+ or amino acids; whereas plants adapted to higher pH and more aerobic soils prefer NO_3^- (Maathuis, 2009). Guo (2019) showed that in wheat a high N rate increases plant biomass under NO_3^- nutrition, but reduces biomass under NH_4^+ nutrition, indicating the negative effects of pure NH_4^+ nutrition on growth. When comparing NH_4^+ to NO_3^-, biomass was depressed by 54% or 85% for low or high N rate.

Tang (2019) found that N accumulation, and root architecture of two OSR cultivars (Xiangyou 15 (high N efficiency) and 814 (low N efficiency), were inhibited under NH_4^+ nutrition, although both cultivars grew normally under NO_3^- nutrition. The results provided insight into the regulatory mechanisms of some candidate genes, which can reveal the molecular mechanisms of decreased WOSR growth in response to a high concentration of NH_4^+ as the sole N source. In further molecular studies changes were investigated in the roots of the plants subjected to N-free control; NO_3^-; and NH_4^+ treatments at

the seedling stage. Differentially expressed genes were identified such as N transporters and genes involved in N assimilation. *CESA* genes related to cellulose synthase were also mostly downregulated in the NH_4^+ treatment group. This down regulation of genes related to crucial metabolic pathways resulted in inhibition of WOSR growth.

Assimilation efficiency of different forms and tissues will also impact on crop NUE. N assimilation is the formation of organic N compounds like amino acids from inorganic N compounds present in the environment, NO_3^- assimilation efficiency has been shown to be significantly increased in shoots compared to roots (Tang et al., 2013, Smirnof and Stewart., 1985). Wang et al (2009) studied the importance of NR and GS in N assimilation and the regulation mechanisms of gene expression in seedlings of a *Brassica napus*. It was found that NR was higher in root and shoots of efficient types when grown in low N conditions. The results support the suggestion that NR plays an indirect role in regulating the absorption of NO_3^- uptake and accumulation. GS was also found to be high in the leaves but not in the roots - the hypothesis is that this is an effect of higher remobilisation of the N efficient type. There are also suggestions that the feedback regulation may occur post translationally modifying NR and GS protein levels. Photorespiration has been reported to be necessary for optimal rates of NO_3^- assimilation (Bloom, 2015); therefore maintaining photosynthesis and enhancing the reassimilation of photorespiratory NH_3 in relatively low-N-content leaves at the grain-filling stage is a potential avenue for improving NUE in agriculture. It has also been shown that expression of genes relating to N metabolism are regulated by NO_3^- and N metabolites as well as by N deficiency (Cai et al., 2010)

2.3. Breeding NUE genotypes; practical approaches

The long-term goal of improving NUE of all crop production is to use biotechnology and crop breeding alongside best N management. The development of the ideotype concept (Rasmusson, 1991) has led to breeding and crop physiology research to focus on identifying simple crop characters which influence the physiological processes determining final seed yield. However, some of the strategies proposed are conflicting; the quantitative effects of crop traits on seed yield were often not clearly identified; and physiological and genetic interactions between characters often remain unclear.

Genetic and computational methods have the potential to dissect the genetics of quantitative traits but require field phenotyping of large diversity trials (Araus and Cairns et al., 2014, Hawkesford, 2014). Over the last ten years field studies have been conducted that have analysed WOSR N response (Stahl et al., 2017; Kessel et al., 2012; Nyikako et al., 2014; Erley et al., 2011, Berry et al., 2010). Recent reviews and studies have highlighted the need for data collected from trials that include a large phenotypic dataset of highly genetically diverse material (Hawkesford et al., 2014; Stahl et al., 2017; He et al., 2017; Storer et al., 2018). Refining phenotyping, by conducting field trials to pinpoint the exact step where WOSR yield component is modified under N limitation, is probably critical to

differentiate between NUE mechanisms. Genomics approaches are particularly useful when dealing with complex traits which have a multi-genic nature and important environmental influence.

There are key traits linked to WOSR yield potential and NUE that are beneficially monitored in breeding programs (Berry et al., 2006). There is a need to produce and assess practical methods for phenotyping. In this section, we will discuss fundamental methods and practical approaches of using information from field trial data to improve NUE of WOSR through;

(1) Crop model approaches;
(2) Breeding-pipeline methods
(3) Genetic analysis

2.3.1. Modelling

Mathematical models are increasingly being used in research; farm management; policy analysis; and production forecasting. High-quality models will allow future genotypes to be chosen according to the cropping system in which they will be grown (Jeuffroy et al., 2002). Crop models can be used as a valuable tool for;

(1) simulate the effect of agronomic practices on yield, quality and the environment
(2) quantify the individual and combined effect of traits on N requirement
(3) define the traits of NUE crop ideotypes

Crop yields are affected by genetics, environment, and agronomic practice as well as by the interactions between them (Dressboll et al., 2014, Bradbury et al., 1993). Process-based crop physiological models have mainly been used as research tools to integrate knowledge of crop functioning, by the analysis of field experiments and the evaluation of crop trait impact. Models allow a broad set of hypotheses to be tested simultaneously. Individual and combined trait sensitivity analysis can be used to identify important traits and trait combinations that will minimise N fertiliser requirement for the least reduction in yield. Development and introduction of a new crop genotype is a long process which can take 5-15 years. A model calibrated and evaluated can feasibly be applied by breeders to save money and time in distinguishing NUE between new genotypes. The level of detail involved in the crop model is a balance between usability and the precision required.

Current process-based models use the concepts of radiation interception use efficiency (RUE) or more complex photosynthetic models to predict growth. Growth is then used to calculate dry matter production rates using estimated rates of photosynthesis. Models have been used to understand physiological features in depth. There are examples of WOSR models for roots (Malagoli et al., 2009); senescence (Gabrielle, 1998b); flowering dates (Habekotte, 1997b); and organ area indices (Muller et al., 2006). Modelling the N demand during crop growth is one of the tools to support precision fertilisation to adjust application timing and placing of N based on measurements of plant material (such as petiole tests); measurements of soil conditions (available N); or both (Sibley et al., 2009,

2010; Schröder 2014); together with predictions of future gains in dry matter production (De Koeijer et al., 1999). Many WOSR yield models exist, reviewed in chapter 4.

2.3.2. Developing breeding screens for NUE

Field studies of NUE have focused on investigating yield and genotype difference. However, refining phenotyping by conducting field trials to pinpoint the exact step where WOSR yield is modified under N limitation is critical to help differentiate between NUE mechanisms. Methods of rapidly identifying breeding lines with lower N requirement are required to improve yield at low N (Berry et al, 2011b). N response is not currently identified through the UK testing systems. It is likely that plant breeders would favour N efficient types for the marketplace if testing systems were available to assess fertiliser N requirements, along with the other important agronomic traits (e.g., yield, quality, disease, and lodging resistance). N fertilisers are becoming more expensive relative to grain, but fertiliser costs are still small relative to the value of their full effects; growers will favour N efficient genotypes only if yield is not compromised.

For testing NUE of new genotypes one solution would be to include a low N environment within the breeding programmes. This would be effective if genotypes with a higher yield at low N have a greater NUE at the economic N fertiliser application rate through achieving a similar yield at low N rate (Sylvester- Bradley and Kindred, 2009). However, Storer et al. (2018) found that two N levels did not allow determination of optimum N. It is not possible to draw reliable conclusions about which variety has a lower N requirement from experiments with two N levels. However as described in Fig 4 this approach does not measure the steepness of the N effect and could result in misleading varietal assessment. Another solution is to identify traits which indicate high yield performance in a low N environment. There are key traits linked to WOSR yield potential relating to NUE and hence are beneficial for monitoring in breeding programs (Berry et al., 2006). Increasing our understanding of which traits are linked to NUE and are useful to measure is essential if an ideotype approach is taken. Crop traits are time-consuming to score as part of large field trials. Development of high throughput phenotyping is required. These technologies include root screens; markers; remote sensing; Unmanned Aerial Vehicles (UAVs); automated assessment systems; and satellite data. Many studies have used visible spectrum and infra-red spectrum to assess vegetative indices and N content (Hunt et al., 2010; Deery et al., 2014).

2.3.3. The use of genomics and transcriptomic approaches for genetic improvement of NUE.

Understanding the relationship between genotype and phenotype is an important tool in plant breeding. Unravelling the genetic control of the traits contributing to the yield of WOSR grown under low N input is a way to propose new N-efficient WOSR cultivars and improve NUE. Whole plant molecular physiology approaches are required to understand the regulation of N uptake and utilisation, while determining how these change during crop development and across different fertiliser treatments. Genomics is the sequencing and analysis of an organism's genome, where the genome is

the entire DNA content that is present within one cell of an organism. Two methods commonly used in genomics are quantitative trait locus (QTL) mapping and genome-wide associations (GWAS). QTL analysis is performed in constructed bi-parental populations using contrasting parents, therefore only one recombination event is recorded in the population. GWAS considers the use of natural populations or panels with diverse cultivars with the purpose of recording more recombination events that contributes to a higher resolution to find regions associated to traits.

A QTL is a region of DNA which is associated with a particular phenotypic trait, this can vary in degree and is often found on different chromosomes and can be attributed to polygenic effects, i.e., the product of two or more genes and the environment. QTLs allow the identification of existing favourable alleles of relatively small effect, which have frequently remained unnoticed and have not actively been included in the gene pool used for breeding. QTLs have been used to determine regions which could be a good candidate for introducing stable seed yield and oil yield traits into rapeseed varieties in a breeding program. Previous analyses identified several QTLs for seed oil content (Gül 2012; Delourme et al. 2006; Zou et al. 2010); seed yield (Shi et al., 2009, Chen et al., 2007); plant height (Basunanda et al., 2010); thousand seed weight (Basunanda et al., 2010; Ding et al. 2012); number of seeds per area (Ding et al. 2012); and flowering time (Honsdorf et al. 2010). In multiple studies, many of the chromosomes containing overlapping yield-related QTL appeared to correspond, with prevalent QTL hotspots being consistently found on chromosomes N01/N11, N03/N13, N05/N15, N07/N16, N12 and N10/N19 (Basunanda., 2010).

In the context of N stress, QTLs for NUE studies have been conducted in OSR (Gül 2012, Miro 2010, Bouchet et al., 2014). Bouchet (2014) set up a study with the objective of introducing genomic regions involved in NUE under low N fertilisation conditions in the new varieties. The study did not highlight QTLs specific to low N conditions; however, 11 regions were found to be stable across N conditions and/or years of trial and could be used for further studies. The work found that R-A5 is of particular interest as a dense QTL area which was consistent at a low and a high N conditions and two out of the three sites. Genomic regions involved in response to grain yield selection at high and low N fertilization in maize have been highlighted. The studies utilised bi-parental populations to identify the genetic basis of NUE. Most QTLs were located on chromosomes A02, A06, A07, C03, C06 and C09. However these were only detected in a single-year environment.

There are limitations with QTL mapping - the data obtained is often from wide crosses, in spite of the fact that they have generated relatively robust QTL information datasets. These may not be relevant in developing modern crops, and diverse elite genetic materials, which are well-adapted and commercially relevant for specific interests are more useful to identify allelic variation for superior NUE. In contrast GWAS association mapping exploits ancestral recombination events that occurred throughout the evolutionary history of the populations and takes into account all the segregating alleles present in the population. WOSR is an allopolyploid species and has a complex genome structure, with both homologous and non-homologous exchange between the A- and C-subgenomes.

The relatively short domestication period (400–500 years) and a double bottleneck created during the selection for low erucic acid and low glucosinolates while breeding for modern WOSR has led to some narrow genetic diversity in modern *Brassica napus* traits (Bus et al. 2011). Therefore, the use of genetically diverse panels from different geographic origins and breeding status in association mapping studies to identify useful new genetic variation is potentially very powerful.

High-throughput genomics technologies offer great possibilities for gene discovery; complex trait analysis by GWAS global gene expression analyses; genomic selection; and predictive breeding strategies. The use of association panels provides a higher mapping resolution since it exploits all the ancestral recombination events present giving it the power of associative mapping to discover marker–trait associations in genetically diverse panels or populations (Soto-Cerda and Cloutier 2012). This approach detects associations between crop phenotypes and markers on the basis of linkage disequilibrium (LD). Linkage disequilibrium refers to the non-random association of alleles at two or more loci in a general population. When alleles are in linkage disequilibrium, haplotypes do not occur at the expected frequencies. Associative mapping offers a high-resolution power when there is large genotypic diversity and low LD for the germplasm being investigated. Because of its resolution power and the possibility to study various regions of the genome simultaneously without the construction of mapping populations, associative mapping is a useful option for studying agronomic traits, considering that most agronomic traits are multigenic with underlying genes of independent inheritance.

A DNA polymorphism is any difference in the nucleotide sequence between individuals. These differences can be single base pair changes deletions; insertions; or even changes in the number of copies of a given DNA sequence. Single nucleotide polymorphisms (SNPs) are the most common type of DNA polymorphism - these are a variation at a single position in a DNA sequence between individuals. Markers based on SNPs have been the main focus of molecular genetics during the recent years due to their abundance in the genomes and their amenability for high-throughput detection formats. By greatly reducing the cost and time of genotyping, high-throughput genomics have made a large number of markers routinely available. The extensive application of the *Brassica* Illumina Infinium 60K SNP array, based on next-generation sequencing technology, has greatly accelerated the process of genetic architecture dissection, and high-density genetic linkage maps based on SNPs have greatly improved the efficiency and precision of detected QTL (Trick et al. 2009).

GWAS has been used in *Brassica napus* to identify genes underlying control of seed glucosinolate content (Lu et al., 2014); calcium and magnesium content (Alcock et al., 2017); branching (Luo et al., *2015*); and anion homeostasis (Koprivova et al., 2014). Xu et al. (2017) identified 35 QTLs for flowering time, one being an orthologous to A. thaliana CONSTANS, an important gene in the photoperiod flowering pathway. Luo et al., (2015) identified nine SNPs for harvest index and seed yield per plant that explained 3.42% of the phenotypic variance in the former trait. Recently, Luo et al

(2015) reported six SNPs on A03 associated with rapeseed branch number using GWAS. However, despite the studies on rapeseed branching, the basis for genetically controlling branching morphogenesis has not been fully elucidated for rapeseed, and few genes have been predicted in the QTL intervals. The study concluded that better understanding of the genetic determinants for rapeseed branching is needed.

Transcriptomics is the study of the complete set of RNA transcripts produced by the genome at any one time. It is specifically focused on how transcript patterns are affected by development, disease, or environmental factors such as growth stage, stress and hormones. Associative transcriptomics (AT) is the transcriptome-based equivalent of GWAS. RNA sequencing, a high-throughput sequencing technology, is the best approach and has replaced microarray even in model plants like rice and Arabidopsis, which have high-quality whole-genome sequence information available. AT captures a snapshot in time and shows the total transcripts present in a cell. It is therefore dynamic and a good representative of the cellular state. Messenger RNA (mRNA) serves as a transient intermediary molecule in the information network, whilst non-coding RNAs perform additional diverse functions. The key aims of AT are described by Wang (2009) as being, "to catalogue all species of transcript, including mRNAs, non-coding RNAs and small RNAs; to determine the transcriptional structure of genes, in terms of their start sites, 5′ and 3′ ends, splicing patterns and other post-transcriptional modifications; and to quantify the changing expression levels of each transcript during development and under different conditions."

AT has emerged as a particularly popular approach for identification of differentially expressed genes under environmental change (Wang et al., 2009). Measuring the expression of an organism's genes in different tissues; stress conditions; growth stage; or time points provides important information on how genes are regulated. An advantage of using transcriptomics RNA over genotypic DNA sequences in association studies is the ability to develop markers based on both SNPs and transcript abundance (gene-expression markers; GEMs; Harper et al., 2012). Choosing to focus on characterising the transcriptome at a particular growth stage, often juvenile, provides a defined genome compartment with both the greatest functional significance and the greatest potential for analysing cross-species collinearity based upon sequence similarity. For *Brassica napus*, the genetic basis of salt tolerance (Yong et al., 2014); root length (Dun et al., 2016); dormancy; and the effects of night temperature on storage lipids (Zhou et al., 2018) have all been studied using RNA-sequencing data.

Factors that impact the effectiveness of AT studies include the specific samples used (e.g. stress vs. developmental series); method of data transformation (e.g. fold change vs. absolute expression values); and the procedures and parameters used to define co-expression. Optimising the use of co-expression based approaches requires consideration of the pathway of interest; expression dataset; and clustering algorithm. The quality of the data is important to uncover genes and response pathways, it is best to use a specific stress dataset instead of a collection that includes potentially

uninformative experiments. It is also very important to consider the growth stage tested. Often the age set used is juvenile, but although this is the most uniform for screening, genes may not be expressed that would be at a later date (Bus., et al., 2011). Another issue is being able to efficiently, intelligently and without bias be able to identify genes with effects on fitness from a long list of candidate genes. In an association analysis, the population structure can produce a strong association between non-linked loci due to genetic drift, population stratification and natural selection. The awareness of the demand for gene functional analyses is growing, and much-needed progress is being made to expand gene functional analyses beyond model organisms to target specific genes of interest in a wider range of species (e.g. CRISPRi) (Larson et al., 2013; Qi et al., 2013), and manipulate the expression of those genes at specific times and under specific conditions. Combining association mapping and a linkage analysis not only avoids the false positives from associated loci due to high LD, but can also facilitate fine mapping of a target region with a large QTL interval (He et al., 2017b).

AT has been criticised as an inappropriate method to identify genes with large impacts on adaptive responses to the environment, because genes that have a large impact on fitness are rare and often large changes in gene expression do not necessarily equate to a large effect on fitness (Feder and Walser, 2005). AT has a tendency to focus on genes that are highly conserved and undergo large fold-changes, and it may miss regulatory genes that undergo moderate fold-changes. Such genes can play major roles in environmental adaptation. For example, hub genes are less likely to be detected through AT screens because these genes have stable expression, instead influencing fitness through post-translational changes in protein activity (Lehner, 2008; MacNeil and Walhout, 2011). In contrast, AT is best-suited to interrogate the downstream effectors of biological responses because these genes tend to have high expression variability (Gracey, 2007). AT has also been criticised as mRNA abundance is an unreliable indicator of protein level (Greenbaum et al., 2003; Tian et al., 2004). Even with the significant developments in the technologies used to quantify protein abundance, protein identification and quantification still lags behind the high-throughput experimental techniques used to determine mRNA expression levels.

In summary, AT can be used for revealing candidate mRNA species whose abundance and phenotypes as proteins require further study. As explained by Evans (2015) “As transcriptomics infiltrates new areas of research and more investigators consider integrating this technology into their research programs, knowledge of these biases, in addition to the substantial benefits offered by transcriptomics, is critical to determining the types of experimental questions that transcriptomics can be directed toward, improving experimental designs and accurately interpreting transcriptomic data.”

2.4. Conclusion

Sustainable crop production means protecting the environment and human health while maintaining or increasing crop yield, economic viability, and nutritional quality. WOSR has high N requirement, in the UK and Europe. Studies have estimated only 30 – 50% of applied N is utilised by WOSR (Berry et al., 2006, Diepenbrock et al., 2000, Sylvester-Bradley and Kindred, 2009, Mendham et al., 1981). The ideal N efficient genotype will have high yields but low N optimum (HYLO types). The development of HYLO genotypes is crucial in order to meet environmental N regulations and to ensure profit for farmers. Improved WOSR NUE and lower optimal N rates can be achieved by taking advantage of variation in genotype traits associated with N requirements of N uptake and N utilisation. An approach that integrates genetic, physiological and agronomic studies of whole plant N responses will be required to understand the regulation of NUE and highlight targets for breeders. Traits affecting both WOSR yields and NUE have been studied in detail throughout the crop cycle (Mendham et al.,1981; Diepenbrock et al., 2000; 1993, Berry et al., 2006). Current genetic and computational methods have the potential to dissect the genetics of quantitative traits (Cairns et al., 2014, Hawkesford, 2014). Recent reviews and studies have highlighted the need for data collected from WOSR field trials that include a large phenotypic dataset of highly genetically diverse material (Stahl et al., 2017; He et al., 2017, Storer et al., 2018).

Chapter 3. Characterising the genetic variation of NUE traits in a WOSR diversity panel

Contributor's statement

Field trial genotypes, rates and design chosen by RIPR project consortium members. Field trial assessment; Mark Knightingale (Elsoms), Alison Fraser, ADAS technician and UoN staff. Field trial sampling; Alison Fraser and Rory Hayden (UoN), ADAS technicians. Kjeldahl analysis; Alison Fraser and Lolit Wilson (UoN).

3.1 Introduction

N fertilisers are widely used to improve WOSR yields, but their application has both environmental and financial consequences. In Europe, N is the biggest variable cost for WOSR production (Defra, 2008), and N is a significant source of water and air pollution (Kindred et al., 2008). The high N requirement of oil crops is due in part to the high biochemical energy demand of seed oil production, but it can also be attributed to current genotypes' inefficient uptake and utilisation of N (Berry et al., 2006; Diepenbrock et. al., 2000; Mendham et al., 1981). Sylvester-Bradley and Kindred (2009) found only 30 – 50% of applied N is utilised by WOSR.

NUE is defined as harvestable dry matter yield / the amount of N available in soil and fertiliser, reported in kg/ha dry matter yield per kg/ha N supply. Definitions are based on Moll et al. (1982) and have been described in the introduction (section 2.1.4). There is great potential to improve NUE of WOSR through taking advantage of the significant genetic variation reported in N requirements between genotypes. The ideal N efficient genotype will have high yield but low N optimum (HYLO type). The development of HYLO genotypes is crucial in order to meet environmental N regulations and to ensure profit for farmers. Many field trials have shown yield and genotype are ranked differently under HN and LN conditions (Berry et al. 2010; Erley et al., 2011; Kessel et al., 2012; Nyikako et al., 2014; Bouchet et al., 2014; He et al., 2017; Stahl et al., 2017). Since the results suggest that genotype and N supply are not well correlated, cultivars selected for high yield under HN conditions may not be the highest yielding under LN conditions.

Understanding which are the key traits to measure, and developing efficient data collection techniques, are critical to help further advancement in breeding. Field data is required from diversity populations for N and environment response of traits during the full course of crop development. Traits linked to NUE include: late leaf senescence (Ulas et al., 2013; Kessel et al., 2012); post flowering N uptake (Berry et al., 2010); early flowering (Stahl et al., 2016); stay-green traits associated

with optimized post-anthesis N remobilization (Derkx et al., 2012; Hawkesford, 2014); and / or increased post-anthesis N-uptake (Bogard et al., 2011; Gaju et al., 2014). High N utilisation efficiency has also been associated with greater N remobilisation from the stem to the seed after flowering (Girondé et al., 2015); and genetic improvement in NUE has been associated with greater N remobilisation within the plant (Bouchet et al., 2016). High N utilisation efficiency has been associated with lower seed protein concentration (Schulte auf'm Erley et al., 2007; Koeslin-Findeklee et al., 2014) and higher seed oil concentration (Stahl et al., 2017).

Over the last decade, increasing numbers of field trials have studied WOSR yield in response to LN environments (Berry et al., 2010; Erley et al., 2011; Kessel et al., 2012; Nyikako et al., 2014; He et al., 2017a; Stahl et al., 2017). Kessel et al. (2012) evaluated 36 genotypes in the vegetation period 1997–1998. This study included 3 hybrids; 6 resynthesized lines; 8 old cultivars; and 19 modern cultivars. They found that modern cultivars and hybrids outperform older genotypes and resynthesized lines at LN. Berry et al. (2010) studied 10 genotypes at LN over 4 environments. They found significant correlation between NUE and post-flowering N uptake; and that increasing sink capacity (seeds/m^2) was important for increasing both N uptake efficiency and N utilisation efficiency. Stahl et al. (2017) studied 30 genotypes registered between 1989 and 2014, including hybrids and elites. Changes in seed yield and seed quality traits were studied over a 2-year experiment in 10 environments across Germany. The study found a surprisingly low yield gap between HN and LN fertilisation, promising further N fertiliser-saving potential through N optimisation. The study showed that breeding at HN had increased yield in both HN and LN systems. He et al. (2017a) studied 50 genotypes at two N rates over one year of field trials. In this trial they collected data for OSR growth and N content. They found that over one year, genotypes showed similar underlying sub-traits linked to N utilisation. Seed number per silique; harvest index; and N harvest index were significantly correlated with N utilisation under contrasting N conditions.

Conducting crop studies in controlled environments can significantly reduce experimental costs; experimental period; and environmental variation. However, it is essential to assess crop traits in the target field environment. Phenotype relationships that are significant in glasshouse trials may not be significant in field studies. He et al., (2017a) compared 50 OSR genotypes, grown under low and high N application rates in simplistic (pot) and complex (field) growth conditions. In a single year they showed that some genotypes performed consistently across these environments. However, they concluded that further field trials are necessary to confirm the importance of these traits in varied environments. It is essential to assess whether or not NUE traits are robust over a range of environments. Hohmann et al. (2016) developed a method using large refuse containers (120 L 'wheelie bins') which allow detailed phenotyping of small field-crop populations under semi-controlled growth conditions. Diverse WOSR cultivars were grown at field densities throughout the crop lifecycle, in different experiments over 2 years. The studies showed that the container system proved suitable for detailed studies of stress response physiology and performance in pre-breeding populations

This study reports the most thorough assessment of field-based data generated to date for trait evaluation of a WOSR diversity panel tested at two widely different N fertilisation levels. Genotypic variation in NUE has been studied for 85-120 genotypes (dependent on site) of WOSR at low (60 kg N ha^{-1}) and high (300 kg N ha^{-1}) N application rates at 2 sites over two years: 2014-15 (Bessingby, East Yorkshire and Deeping, Lincolnshire); and 2015-16 (Ompton, Nottinghamshire and Deeping, Lincolnshire). The genotypes are from the ERANET-ASSYST consortium diversity population (Bus et al. 2011, 2014; Körber et al., 2012, 2015) plus elite commercial genotypes previously identified with high and low NUE (Berry et al., 2010). In total, 32 phenotypic measures were measured including yield; phenology; and N uptake & biomass of partitioned tissue at mid-flowering and maturity. The study aims to phenotype a diverse WOSR population and:

(1) analyse response to LN and HN fertiliser treatments;
(2) identify traits determining high yield under LN conditions;
(3) quantify trait ranges;
(4) compare and contrast genotype groups: elite v ASSYST; HYLO v HYHO;
(5) introduce practical ideas for improving NUE assessment within a breeding pipeline.

3.2. Materials and methods

3.2.1. Experiment sites

Field experiments were carried out at two sites in the UK over two years:

2014/15 ADAS High Mowthorpe (HM14) (0.5◦W, 54.1◦N),
on a shallow silty clay loam overlying chalk (Wold series);
Elsoms Deeping (DP14) (2.6◦W, 52.1◦N), on a silty clay loam (Bromyard series);
2015/16; ADAS Ompton (OM15) (1.0◦W, 53.2◦N), on a clay loam (Wold series);
Elsoms Deeping (DP15) (2.6◦W, 52.1◦N), on a silty clay loam (Bromyard series).

3.2.2. Germplasm

Genotypes representing a diverse genetic response to N were selected from elite WOSR and the ASSYST diversity panel (Bus et al. 2011, 2014; Körber et al., 2012, 2015). For HM14, DP14 and DP15 trials, 19 elite WOSR and 64 ASSYST genotypes were trialled (Appendix Table 1). At OM15 site an alternative selection of genotypes from the ASSYST diversity panel (n=100) and elite WOSR (n=20) were trialled, of which 58 genotypes selected were the same as the other trials (Appendix Table. 1). These were selected from the ERANET-ASSYST consortium diversity population (Bus et al., 2011, 2014; Körber et al., 2012, 2015).

3.2.3. Trial management

In the 2014-2015 trials (HM14 and DP14), germplasm was grown at LN and HN fertiliser rates (Table 2). The 2015-2016 trials (OM15 and DP15) germplasm were grown at LN fertiliser rate only. The HN

fertiliser treatment was intended to replicate commercial practice and was calculated using methods described in AHDB. (2009), from measurements of the amount of mineral N in the soil and the N content of the crop in March just prior to the start of stem extension (GS 3,1; Sylvester- Bradley and Makepeace, 1984). Each N treatment was applied in two equal splits with the first split applied between GS 3,1 and GS 3,3 and the second split applied 2–3 weeks later when the stem was extending, GS 3,5 to GS 3,6. All N fertiliser was applied as liquid application of ammonium nitrate (34.5% N). Sulphur was applied at 60 - 75kg/ha SO3 to WOSR as potassium sulphate (18% S) in mid-March. All other crop inputs including pest, weed and disease control; and potassium phosphate; were applied at levels to prevent non-N nutrients, weeds, disease or pests from limiting yield. Details of N inputs, sowing and harvest dates can be found in Table 2. Each treatment and genotype combination was replicated three times. The HM14 and OM15 trial used a split-plot randomised block alpha design with N as the main plot treatment and genotype as the sub plot treatment (Appendix, Figure 1). The DP14 and DP15 experiment was split into two sides, HN and LN, these were split into 3 blocks with plots randomised within each. Plots measured 12m x 2m at DP14, HM14 and DP15. Plots measured 2.5m x 3m at OM15. WOSR was sown at 60 seeds / m². There were issues with black-grass in Block 5 and 6 of the Deeping trial in 2014-2015 and data from these blocks were omitted (Fig 6).

Table 2. Sowing dates, soil details and N fertiliser treatments of field experiments.

Sites	Year	Sowing date	Harvest date	SMN GS 3,1 (kg/ha)	Crop N GS 3,1 (kg/ha)	LN F TRT (kg/ha)	HN F TRT (kg/ha))
HM14	2014/2015	07-09-14	15-07-15	74	77	0	130
DP14	2014/2015	07-09-14	12-07-15	54	31	60	225
OM15	2015/2016	07-09-15	06-07-16	23	50	0	N/A
DP15	2015/2016	03-09-15	20-07-16	28	60	0	N/A



Figure 6. Photographs showing black-grass issues with trials at Deeping April 2014.

3.2.4. Measurements

The following traits were measured under LN conditions at all sites: establishment score; plant count; flowering date; N uptake & biomass of partitioned tissue at mid-flowering; height at late flowering; N uptake & biomass of partitioned tissue at harvest; disease; time of maturity; lodging; and yield. In the 2014-2015 trials, yield was collected from both HN and LN conditions. In the 2015-2016 trials, yield was collected from LN conditions only.

Establishment assessment was done on all trial sites once plots had emerged, at around the 2-3 true leaf stage (Fig 7). Plots were assessed on a scale of 1-5, where 1 = worst established plots, and 5 = best established plots. Plant counts were assessed as plants / m², as a mean of 3 replicates per plot. At HM14 and OM15 flowering was visually assessed every 2 days during flowering, estimating the percentage flowering of each plot. Plots were also given a score from 1- 9, where 1 represented early flowering and 9 late flowering. At DP14 and DP15 trial flowering was assessed and scored at three time points and given a flower score on the same 1- 9 scale. At all sites crop height was measured at the end of flowering by measuring the height from soil surface to the top of the terminal raceme.

Crop biomass and N content were assessed at mid-flowering (GS 4,5) and at seed maturity (GS 5,9). At HM14 and OM15 a quadrat sample (1m x 1m at HM14, 0.5m x 0.5m at OM15) was collected from each plot 14 days after the plot reached 49% flowering. At maturity harvest (GS 5,9) a quadrat sample was collected on a single date, just prior to harvest (Fig 8). Following sampling, a random 20% sub-sample (by weight) of plant material was taken for measurements. At both DP14 and DP15 a sample at GS 4,5 and GS 5,9 was taken of three plants randomly selected from the middle of the plots. GS 4,5 sampling was done on a single date when the majority of plots were 50% flowering and GS 5,9 was on a single date, just prior to harvest. At GS 4,5, the fresh weights, dry weights and N

concentration of the stems and leaves were measured. At GS 5,9, the dry weights and N concentration of the stems, pod walls and seeds were measured. The plant dry weight was recorded after drying at 80◦C for 48 h. The N concentration of the partitioned tissue was measured using the Kjeldahl method.

At sites HM14 and OM15, lodging was defined as stems leaning at an angle of at least 10◦ from the vertical. Close to the time of harvest the proportion of the plot area that was lodged was visually assessed to the nearest 0.05 on a 0-1 scale. At sites DP14 and DP15, lodging was scored from 1-9, where 1 had lower levels of lodging and 9 had higher amounts of lodging. Due to the difference in scoring for lodging between sites this data was not included in the trait analysis. At all sites, disease was scored from 1-9 where 1 had lower levels of disease and 9 had higher levels of disease; and maturity was scored from 1-9 where 1 had early maturation and 10 had later maturity date.

A small plot combine harvester was used to determine the seed yield from an individual plot area of at least 48m². Thousand seed weight was measured on a representative seed sample of 5 g. The seed was counted using a Numigral seed counter (Villeneuve-La- Garenne, France), and the weight corrected to 0% moisture using a Dickey John GAC 2000 grain analysis computer (Church Industries, Minneapolis).

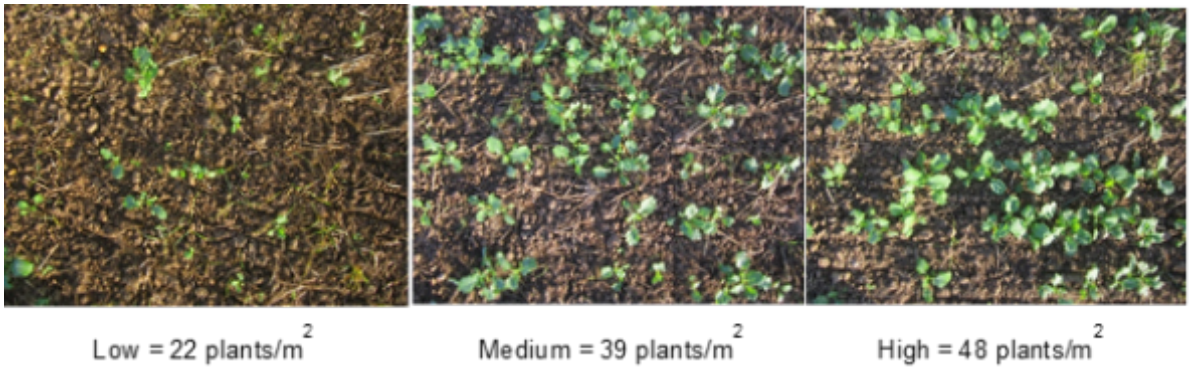


Figure **7**. Images showing plot establishment at HM14.



Figure 8**.** Images showing m^2 sampling methods at HM14 and OM15.

3.2.5 Methods; Digestion of plant material for total N analysis

Total Kjeldahl N is the sum of organic N, ammonia (NH_3), and ammonium (NH_4^+) in the chemical analysis of plant material. The Kjeldahl system comprised a heating platform with a 42-vessel digestion unit; a scrubber unit with a 20% Sodium hydroxide neutraliser; and a recirculating water pump (VELP, Germany). Dried tissue subsamples (~0.1 g) were digested in 8 mL 95% sulphuric acid H2SO4 (Fisher Scientific UK Ltd, Loughborough, UK) with a selenium mix catalyst tablet (Sigma-Aldrich, Dorset, UK). Kjeldahl settings were used as follows: temp = 390 C; water pump pressure = 1.5 bar; digestion time = 120 minutes. Following digestion, each sample was made up to a final volume of 50 mL by adding 11 mL of de-ionized water and transferring to a falcon tube.

Three operational blanks were included in each Kjeldahl digestion run. Initially, blank samples of certified reference material (CRM) (Tomato leaf SRM 1573a, NIST, Gaithersburg, MD, USA) were included approximately every third digestion run. In later runs, laboratory reference material (LRM), from pooled / freeze-dried WOSR leaves, was included instead. Over the 7000 N samples tested, the mean recovery of sample standard tissue was recorded at 95%.

The concentration of plant tissue total N was obtained using a microtiter plate reader using the colorimetric salicylic acid method (Youngs, 1975); for reagent details see Table 3. Digested plant tissue was compared to a set of freshly prepared $(NH_4)_2SO_4$ standards, prepared at 200ppm using a blank digested matrix solution and diluted as a series of standards as required. Samples were run on 96 well plates; 25 µl volume of sample and 25 µl deionised H2O (dH_2O) in each well with three replicates for each sample. Microtiter plate settings were as follows: absorbance = 660 nm; temp = 24°C; developing time = 40 mins. Absorbance was read against the standard curve to give ppm N, and back-calculated to give g/kg plant tissue.

Table 3. Colorimetric salicylic acid method reagent details

Reagent name	**Reagent**	**Volume per well**
Salicylic acid solution	Sodium salicylate 6.8g Sodium citrate 5g Sodium tartrate 5g Sodium nitroprusside 25 mg made up to 100ml with dH_2O	150 µl
Bleach solution	Sodium hydroxide 6g Sodium hypochlorite 0.2ml made up to 100ml with dH_2O	150 µl

3.2.6. Methods: Unmanned aerial vehicles (UAV) image data collection, Image analysis, and scoring

Photographic images were collected and simple quantitative image analysis conducted for crop establishment; early spring growth; over-winter loss; and crop phenology. Data were collected from 16 flights; 8,000 plot photographs were collected in total (Table 4). The photographs provided a simple red green blue (RGB) image for analysis. UAV image data was collected using a 14-megapixel digital camera attached to a hobbyist commercial motor rotor, DJI vision plus version 2. The RGB sensor was mounted on a gimbal that maintained a near-nadir camera position. Table 4 contains dates of UAV data collection. The UAV recorded full site and plot level data (Fig 9). The full field trial images were captured at a flight height of 150m. Six images were taken in total to cover the full trial area. Images were taken at 20% overlap in order to be combined (mosaicking) as a single image (Adobe CS6). Plot-level images were captured at a flight height of 30m. The UAV was held stationary when images were taken to avoid blurring.

The green area index (GAI) describes the size of the WOSR canopy and is represented on a scale from 0-4. The GAI was calculated in image analysis software by working out the proportion of green pixels in an image, where a pixel is considered green if its hue is within the range 60–180°. The flowering and maturity images were also scored manually by a field trial assessor. When required, and before image analysis, colour correction software (Curve) within Adobe Photoshop 8 was used to normalise blue/red imbalances between pictures caused by differing light conditions. Patches of soil were used as control units between images. Here we will present and compare UAV and standard on-site trial assessments collected from 2 sites during the 2014-2015 season.

Table 4. Dates of UAV assessments at DP14 and HM14 sites.

	DP14	HM14
1	19/11/2014	10/11/2014
2	25/02/2015,	10/03/2015
3	20/04/2015	18/04/2015
4	27/04/2015	26/04/2015
5	26/05/2015	27/05/2015
6	12/06/2015	17/07/2015
7	29/06/2015	N/A
8	17/07/2015	05/07/2015



Figure 9. UAV aerial images from the RIPR trials.

3.2.7 Statistics

Analysis of variance was carried out for a split-plot randomised plot design of yield data from HM14. The effect of the N rate, and Genotype were tested. Due to plot design and trial failure it was not possible to conduct ANOVA at the other sites.

Linear regression analysis was done for the 59 genotypes which were grown across all four sites. The mean, min and max results of all traits are presented as box plots across all 4 sites. Regression was used to assess correlation of trait parameters within sites and between sites, using yield at LN as the dependent variable. Pearson's correlation coefficient was calculated for correlations between mean yield and genotype trait parameters at LN. T-tests were conducted between genotype N response groups; HYLO v HYHO, and elite v ASSYST.

Further investigation was done through ranking for each site-year. Genotype traits were ranked in descending order of NUE and N seed utilisation. The average ranking and its standard deviation where then calculated. The standard deviation then offered a measure of the stability of a variety

relative to the others across sites and years. Genstat was used for ANOVA of the High Mowthrope site data. R statistics was used for all other statistical analyses (R Studio Team, 2015).

3.3. Results

3.3.1 Yields

Table 5 Mean, Minimum and Maximum yields (t ha^{-1}), NUE (kg/kg) of field sites: HM14; DP14; OM15; and DP15.

		HM14	DP14	OM15	DP15
Yield at LN (t ha^{-1})	Mean	3.04	2.35	0.84	1.02
	Min	1.33	0.73	0.35	0.3
	Max	4.68	3.5	1.56	1.64
Yield at HN (t ha^{-1})	Mean	3.70	2.87	N/A	N/A
	Min	1.81	1.07	N/A	N/A
	Max	5.31	4.39	N/A	N/A
N use efficiency at LN fertiliser (kg/kg)	Mean	20.13	15.97	11.37	11.6
	Min	8.81	2.72	2.9	3.38
	Max	30.97	30.74	21.31	18.61
N use efficiency at HN fertiliser (kg/kg)	Mean	13.18	9.30	N/A	N/A
	Min	6.43	3.47	N/A	N/A
	Max	18.90	14.20	N/A	N/A

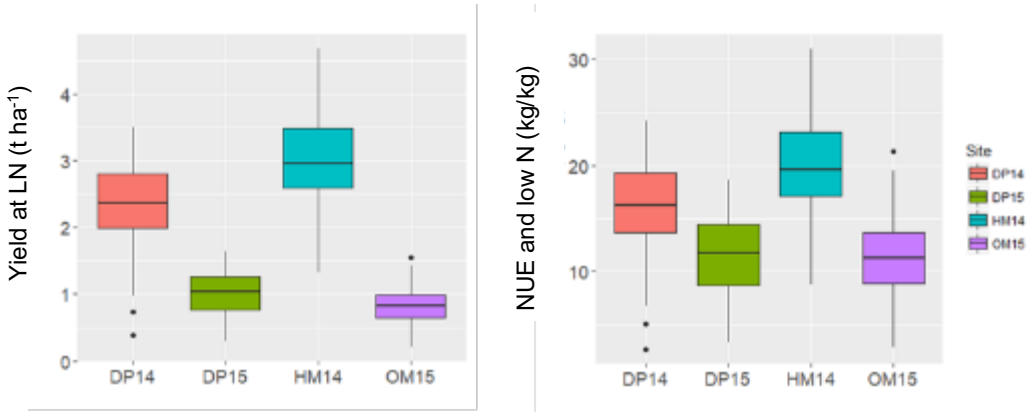


Figure 10. Boxplots for (A) seed yield (t ha^{-1}) and (B) NUE (%) at LN for; DP14, DP15, HM14 and OM15. Each point represents a genotype mean n=3.

The genotype yields at LN had an extremely large range at all sites (Fig 10A) (Table 5). For LN plots the difference in yield between highest yielding and lowest yielding genotype was 3.35 t ha^{-1} at HM14;

2.77 t ha^{-1} at DP14; 1.21 t ha^{-1} at OM15; and 1.34 t ha^{-1}at DP15 (Fig 10). The 2014-2015 trials were higher yield at LN than the 2015-2016 trials at LN (p<.0001). Mean LN yields (t ha^{-1}) across all genotypes were 3.05 t ha^{-1} at HM14; 2.35 t ha^{-1} at DP14; but only 0.84 t ha^{-1} at OM15; and 1.02 t ha^{-1} at DP15.

The yields in 2015-2016 trials were extremely low, due to N availability; management issues; and seasonal environmental conditions. The OM15 site had the lowest combined soil (23 kg/ha) and crop mineral N (50kg/ha) in early spring (Table 2). There were issues with weed control affecting site yield potential in OM15 (gallium) and DP15 (black-grass) (Fig 6). Poor yields in this season were seen across the country, leading to a national yield estimate of 3.0-3.2 t ha^{-1}; an 11-17% decrease on the five year average 3.6 t ha^{-1} (AHDB, 2016).

NUE is calculated as the amount of gross yield per kg of N supply available in soil and crop (GS 3.1) plus N fertiliser application. There was a very wide range of NUE at all sites (Fig 10B). Mean NUE across all genotypes at LN were 20.13 kg/kg at HM14; 15.97 kg/kg at DP14; 11.6 kg/kg at OM15; and 11.37 kg/kg at DP15. The Mean NUE was lower at DP14 than HM14 due to a higher fertiliser N application of 60 kg/ha on the LN plots at the HM14 site (Table 5).

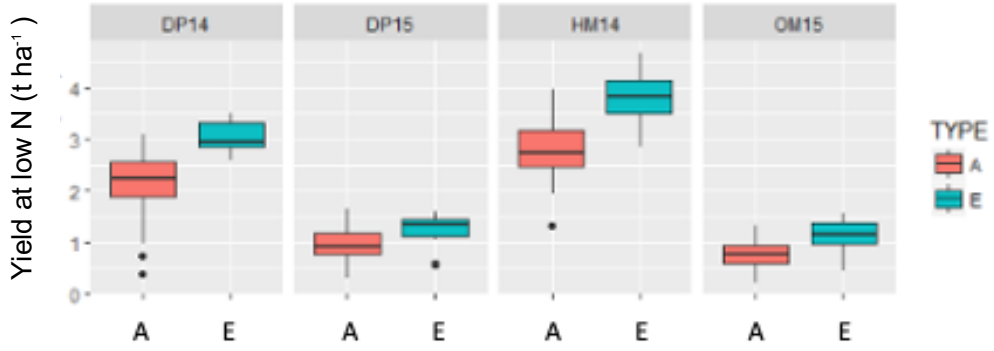


Figure 11. Boxplot for seed yield (t ha^{-1}) at LN of ASSYST (A) and ELITE (E) types for; DP14, DP15, HM14 and OM15.

The majority of ASSYST genotypes showed healthy establishment and growth with some achieving yields similar and higher than current elite lines (Figure 11). This was impressive considering some of the population had not previously been grown in the UK. At the 2014-2015 sites only 2 ASSYST genotypes had extremely poor health (Chinese 3 and Chinese 5); these were excluded from the analysis,and were not included in the 2015-2016 trials.

3.3.2 HM14 site analysis

Split-plot ANOVA for yield based on genotype and fertiliser treatment was conducted at HM14. Due to the trial design and plot failure this was the only trial suitable for ANOVA to be conducted. When considering all genotypes there was no significant impact of N fertiliser on yield (P.0.02, LSD = 0.44). There was a significant effect of genotype on yield (P < 0.001, LSD = 0.85). There was no significant impact of the interaction variable N x genotype (P= 0.527, LSD = 1.21). When considering elite types there was a significant impact of N fertiliser on yield at HM14 (P = 0.008, LSD = 0.287). There was no significant effect of genotype on yield (P = 0.828, LSD = 1.19), nor was there a significant impact of N x genotype (P= 0.826, LSD = 1.21). When considering ASSYST types only there was no significant impact of N fertiliser on yield (P = 0.36, LSD = 0.53). There was a significant effect of genotype on yield (P < 0.001, LSD = 0.72), but there was no significant impact of N x genotype. Fig 12 shows show yield (t ha^{-1}) at Low N (blue) and High N (orange) of genotypes at the site HM14. Each bar is a mean of 3 replicates and the genotypes are ordered by genotype yield at High N. The results show a wide range of yields at both fertiliser treatments. We also saw a wide range in response to N.

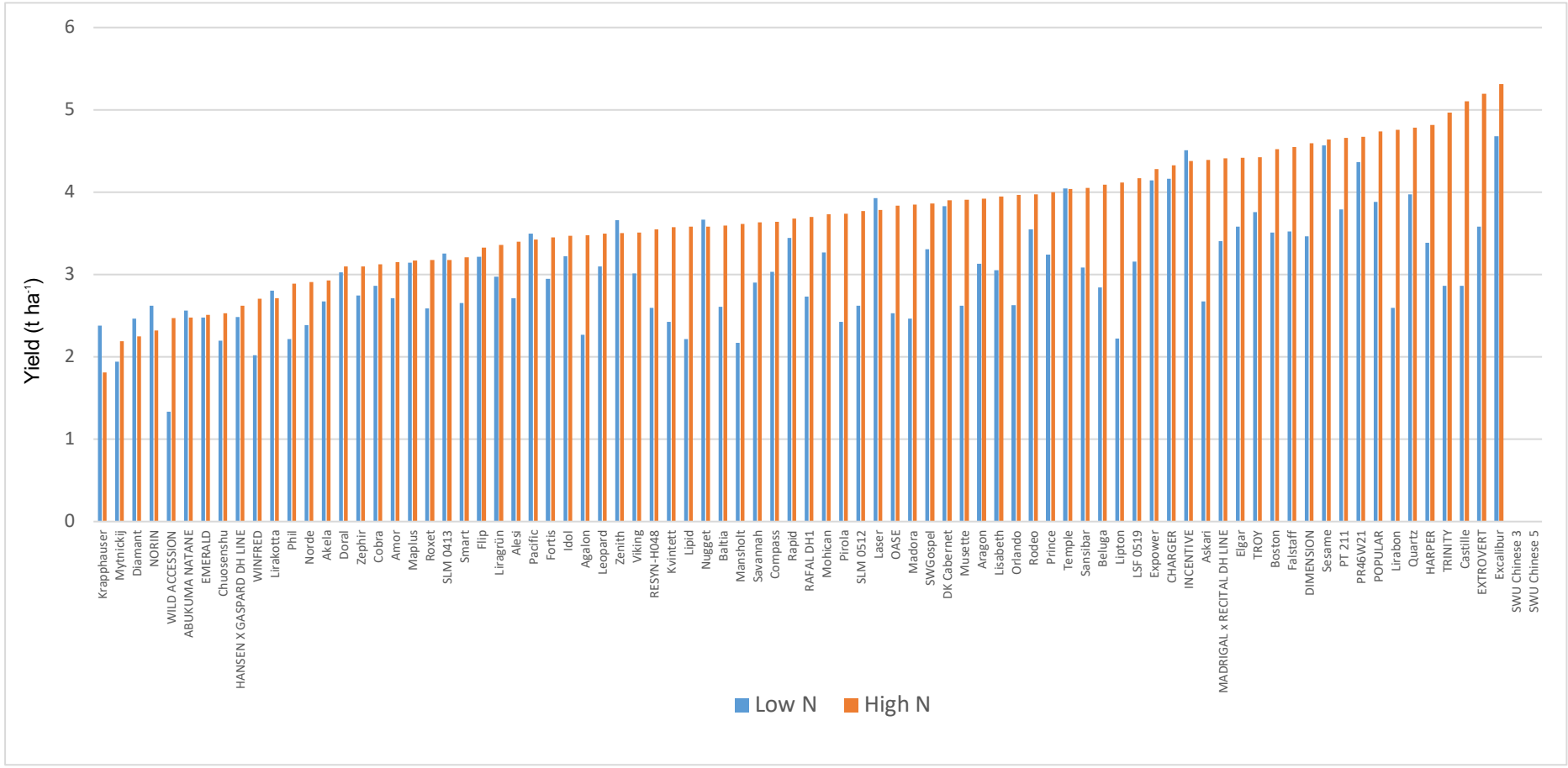


Figure 12. Graph to show yield (t ha^{-1}) at Low N (blue) and High N (orange) of genotypes at the site HM14. Means of genotype show n=3.

Fig 13 shows yield (t ha^{-1}) at LN (blue) and HN (orange) of elite genotypes at the site HM14 trial site. n=3 and standard errors are shown. The genotypes are ordered based on yield at HN. The mean yield results of DK cabernet, Temple, Expower, Charger, Incentive, and Sesame showed very little difference between yield at low N and yield at HN. The biggest difference in yield was seen for Harper, Trinity, Castille and Extroverts. For some elites, no effect of N rate was seen.

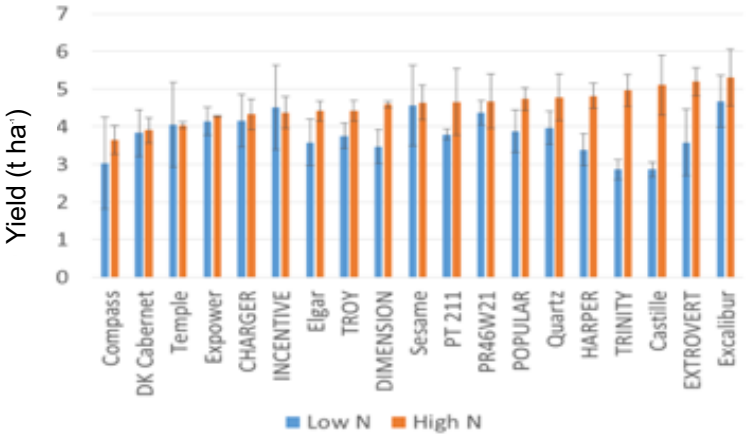


Figure 13. Graph to show yield (t ha^{-1}) at Low N (blue) and High N (orange) of elite genotypes at the site HM14 trial site. Means of genotype show n=3. Bars show standard error.

3.3.3. Yield response between LN and HN plots 2014-2015

LN and HN seed yield data were collected in the 2014-2015 trials (DP14 and HM14). With both HN and LN data this allowed the analysis of genotype response to N. Across all genotypes the HN fertiliser rate (HN-LN) increased yield, by 0.76 t ha^{-1} at HM14 ($p < .0001$) and 0.51 t ha^{-1} at DP14 (Fig 14BA). Although N treatment increased yield there was greater NUE for crops grown at LN than HN at HM14 ($p < .0001$) (14B).

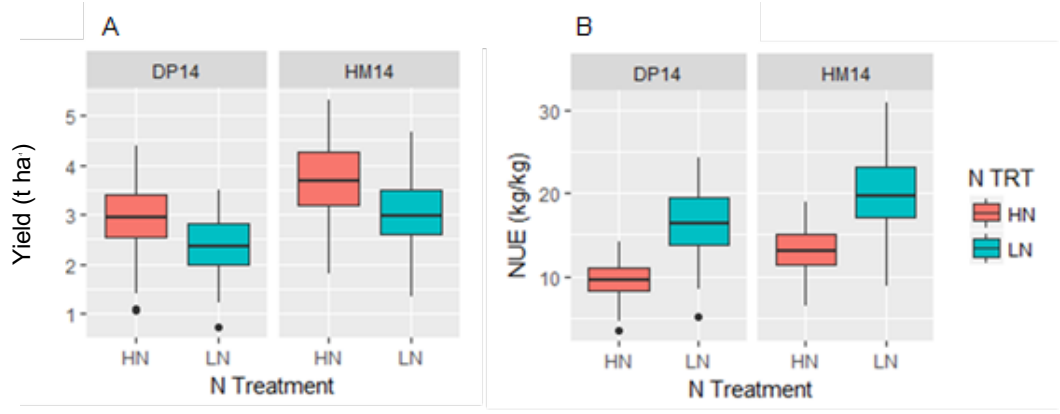


Figure 14. Boxplot for seed yield (t ha^{-1}) (A) and NUE (B) for HN and LN treatments at; DP14 and HM14.

There was a large range of N response values at both sites (Fig 14). The mean % yield gain of N treated plots compared to LN treated plots was 16.6% at DP14 and 15.6% at HM14 (Fig 15). The most extremely affected genotypes had plot losses of 50% at HM14 and 48% at DP14, representing yield loss of 2.0 t ha^{-1} and 2.4 t ha^{-1} respectively. The least affected plots showed no yield gain between N treatments, with some genotypes even showing an increase in yield at the LN plots.

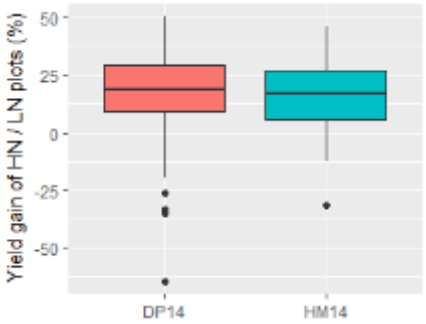


Figure 15. Boxplot for % yield gain of plots with HN treatment than LN treatment at; DP14 and HM14.

At both sites, elite genotypes were higher yielding than ASSYST genotypes at LN (DP14, $p < .0001$; HM14, $p < .0001$) and at HN (DP14 $p < .0001$; HM14 $p < .0001$) (Fig 16). There was no significant difference between ASSYST and elite types in the HN/LN yield gain (%) (Fig 16C). ASSYST types had an increased range of yield and response to N than elite types (Fig 14 A,B). It is important to note that there was a wide range of yield at LN and response to N for the modern elite types.

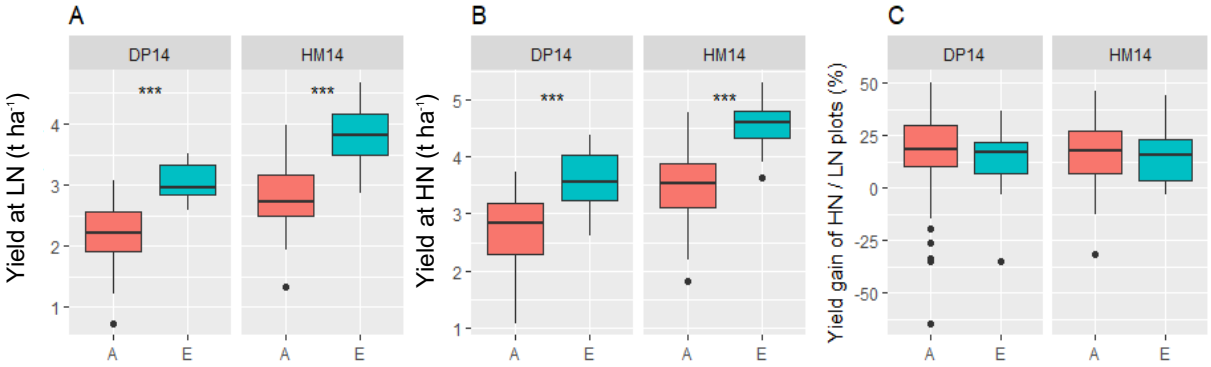


Figure 16. Boxplots of seed yield at LN (t ha^{-1}) for (A), yield at HN (t ha^{-1}) and yield gain of HN / LN (%) (C) at; DP14 and HM14. A = ASSYST genotypes. E = Elite genotypes.

Linear analysis across all genotypes showed a significant relationship between HN and LN yield at both HM14 ($r^2 = 0.41$, $p < .0001$) and DP14 ($r^2 = 0.48$; $p < .0001$) (Fig 17). This suggests yields at HN can be used to predict yield at LN. However, the relationship is affected by genotype category - ASSYST or elite (Fig 18). The relationship was positive for ASSYST types at both HM14 ($r^2 = 0.27$, $p < .0001$) and DP14 ($r^2 = 0.45$; $p < .0001$). Elite types gave no significant relationship between LN and HN at either site. Since elite genotype and N supply do not interact, cultivars selected for high yield

under HN conditions may not be the highest yielding under LN conditions. However, at both sites elite genotypes were higher yielding than ASSYST types at LN (DP14, p < .0001; HM14, p < .0001) and HN (DP14 p < .0001; HM14 p < .0001) (Fig 11).

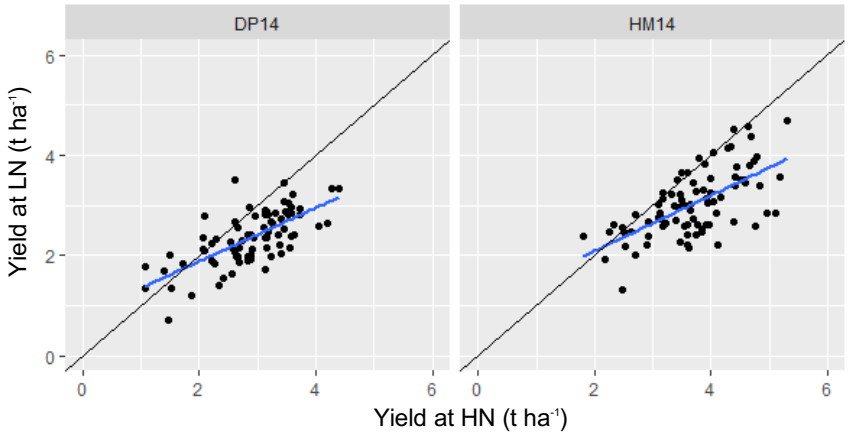


Figure 17. Seed yield (t ha^{-1}) for HN and LN plot treatment at; A) DP14 and B) HM14. Each point represents a genotype mean n=3. Linear regression analyses grouped by experiment: DP14 r^2 = 0.48; y = 0.90x + 0.94; HM14 r^2 = 0.41; 0.74x + 1.465.

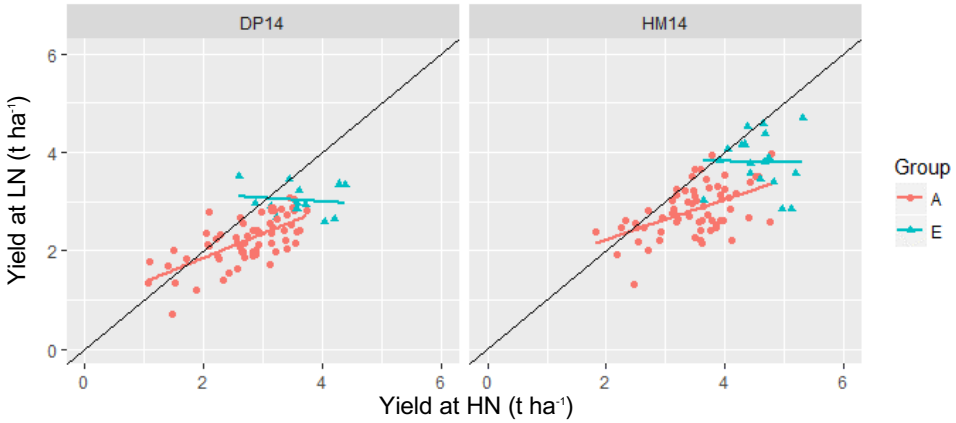


Figure 18. Yields (t ha^{-1}) 2014-2015 at LN and HN for DP14 and HM14. Red points show ASSYST (A) genotypes, blue points show ELITE (E) genotypes. Each point represents a genotype yield mean (n=3). Linear regression analyses grouped by experiment:

In the 2014-2015 trials there was a positive linear relation for genotype yield between DP14 and HM14 when measured at LN (r^2=0.61, p < .0001), HN (r^2=0.60 p < .0001) (Fig 19). There was not a positive linear relationship for the proportional loss of yield gain between sites (r^2=0.012, p = NS) (Fig 20). The non-significant relationship between genotype and % yield gain (HN/LN) between environments suggests that environment has an impact on N response for genotypes. The observed linear relationship of ASSYST genotypes between yields at high N and low N (Figure 18) could be due to the wider range of yields, due in turn to the inclusion of older and more diverse genotypes

having other yield limiting factors (disease susceptibility, phenology, physiology) with a greater impact on yield than fertiliser efficiency.

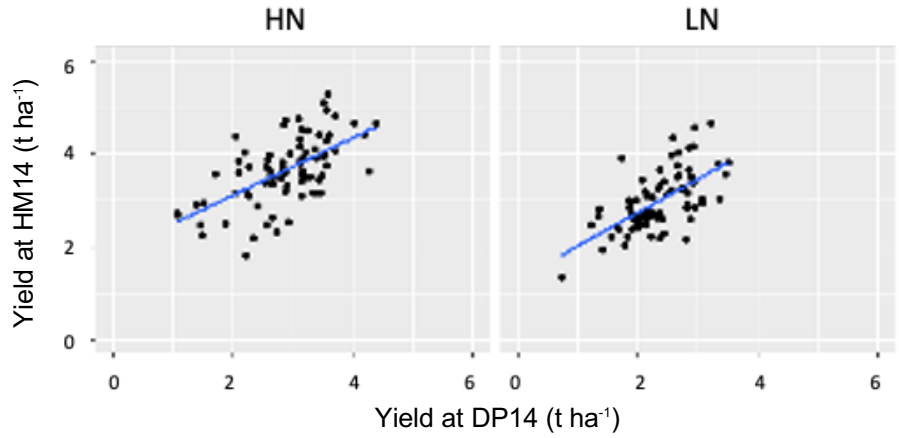


Figure 19. Regression for trials of yields (t ha^{-1}) at HN and LN. Linear regression analyses grouped by experiment: LN (r^2=0.61, p < .0001), HN (r^2=0.60 p < .0001).

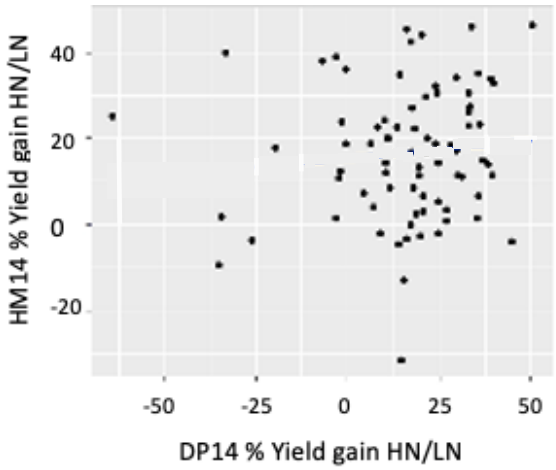


Figure 20. Yield gain (% HN/LN) between HM14 and DP14.

3.3.4. HYLO classification; N response groups

In this section the genotypes from the 2014-2015 trials have been analysed based on N response. These trials included genotype outliers with high yields but low N requirements. For data analysis we have grouped genotypes by yield and response to N. Genotypes with high yields but low N requirements are referred to as 'HYLO' (High Yield Low Optima) types. The diverse panel also included 'HYHO' (High Yield High Optima) and 'LYLO' (Low Yield Low Optima) genotypes. This category system is fully described, and examples are given in the introduction (2.1.4). At DP14 genotypes were classified as HYLO if they had a yield higher than 2.5 (t ha^{-1}) at LN and HN; as HYHO if they had a yield higher than 2.5 (t ha^{-1}) at LN but lower than 2.5 (t ha^{-1}) at HN; and as LYLO if they had a yield less than 2.5 (t ha^{-1}) at LN and HN. At HM14 the same grouping was used but the cut off yield was 3 (t ha^{-1}). The split of these categories are broad. Some of the genotypes categorised as HYLO and HYHO had very similar responses to N. It is genotypes at the most extreme of each group that will be the most interesting to understand differences between.

The same categories will later be used to compare trait differences between groups (section 3.3.11.). The ideal genotype will have high yield but low N requirements - HYLO. This needs to be replicable over environments and year. When comparing genotypes between sites several lines stood out as being HYLO and HOHO across both sites. HYLO: Charger; DK Cabernet; ExPower; Idol, Pacific; Rodeo; PR46W21; Troy; Harper; and Zenith. HYHO: Agalon; Orlando; Pirola; Lipton; SLM 0512; Zephir; Mussette; and Liragrun. These groupings will be discussed further in section 3.3.13.

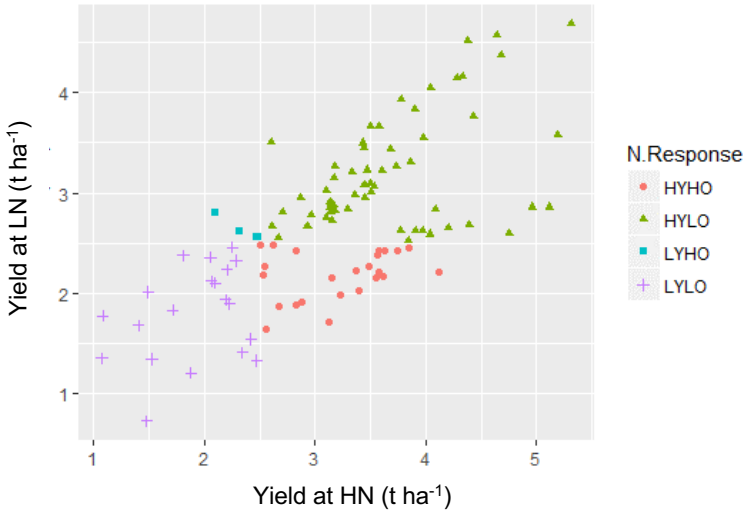


Figure 21. Yields at HN and LN (t ha^{-1}). Each point is the mean yield from two sites HM14 and DP14. Genotypes have been categorised into; 'HYLO' (High Yield Low Optima) (green), 'HYHO' (High Yield High Optima), 'LYLO' (Low Yield Low Optima) and 'LYHO' (Low Yield Low Optima).

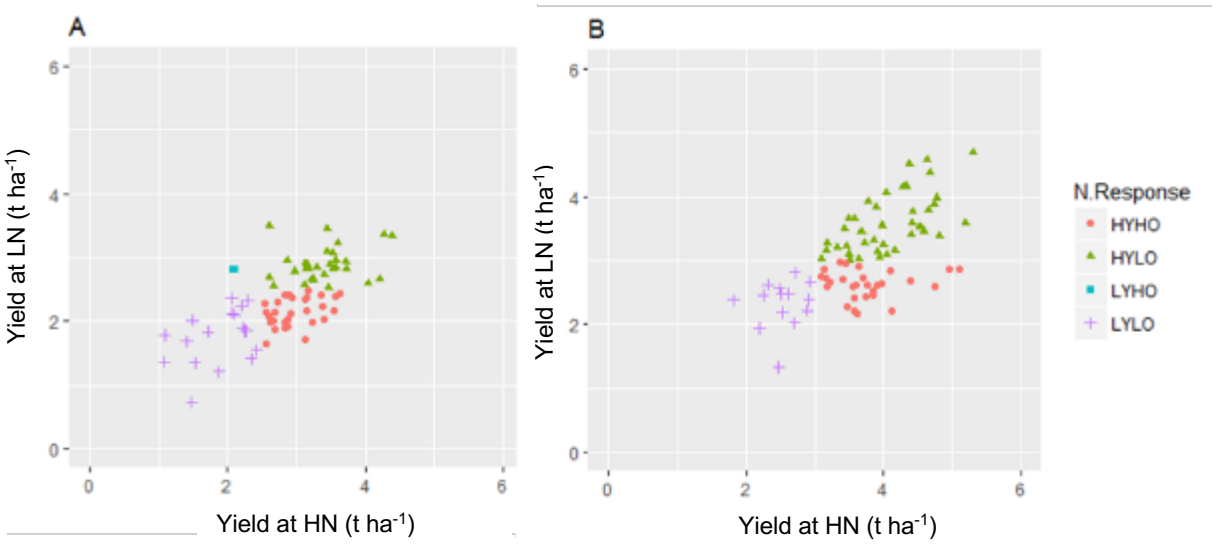


Figure 22. Yields (t ha^{-1}) at HN and LN at (A) DP14 and (B) HM14. Each point is the mean yield from two sites HM14 and DP14. Each point represents a genotype mean n=3. Genotypes have been categorised into; 'HYLO' (High Yield Low Optima) (green), 'HYHO' (High Yield High Optima), 'LYLO' (Low Yield Low Optima) and 'LYHO' (Low Yield Low Optima).

3.3.5. Trait analysis; N uptake and partitioning

There were large genotype ranges for tissue dry weight (DW); tissue N concentration; and total N at mid-flowering (Fig 23); and maturity (Fig 24); at all sites (Appendix table 2). Genotype partitioned tissue means and ranges were comparable between sites. Notable differences between sites were: HM14 and OM15 had higher % N content for leaf tissue; HM14 had higher % N; and OM15 had lower total N for mid-flowering stem. Leaf tissue had a higher variation in mid-flowering N (%) than stem mid-flowering N (%). Stem tissue had increased variation in MF dry weight than leaf tissue. Although not assessed, leaf size, colour and shape were also dramatically different. At maturity the vast majority of N was in the seed (Fig 24). Therefore, variation in seed N and biomass of seed had the most impact of total crop N. Across all sites Seed % N varied between 2% - 3.8%. Seed at HM14 had the highest seed N concentrations and seed at OM15 had the lowest.

Temporal patterns of N uptake and partitioning (Fig 25) broadly agreed with previous studies (Berry, 2010; Dreccer 2005). The 2015 trials sites (DP15 and OM15) showed crop N content apparently decreasing between GS 3,1 and GS 4,5 (Fig 25). OM15 had very low mean uptake at maturity of just 40 kg/ha. The apparent decrease in crop N content between stem extension and flowering is probably due to a combination of: N loss in shed leaves; very dry soil conditions during stem extension which restricted N uptake; and resulting from the low seed production of the trials. This loss has been seen in previous field trials (Berry et al., 2010). DP14 and HM14 had high proportional allocation of N to the seed with means of 84% and 92% of N redistributed to the seed. DP15 and OM15 had much lower

proportion of maturity N in the seed with 50% and 45% respectively. DP15 had a higher proportion of maturity N in the stem than the other trials with 35% of total N.

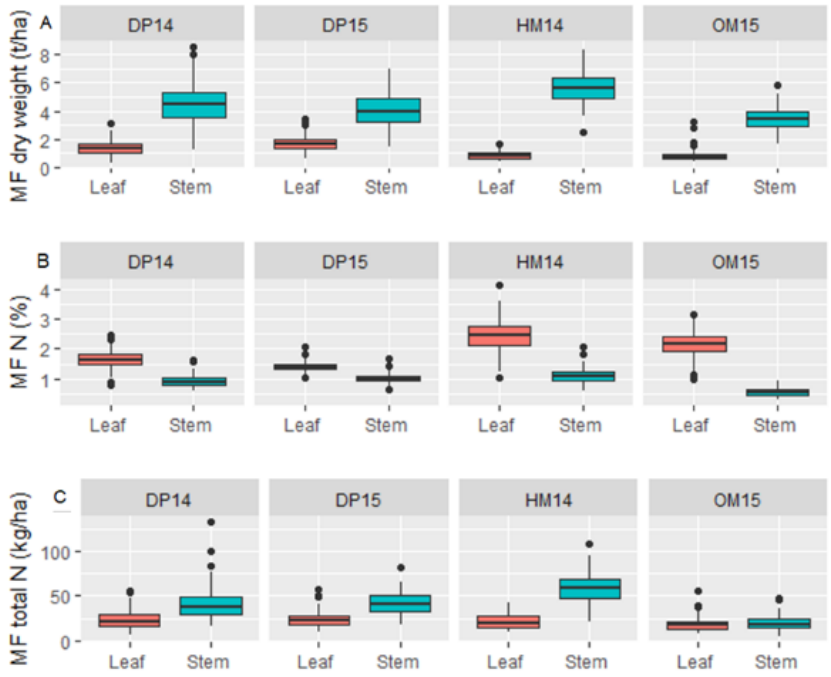


Figure 23. Boxplots for LN plots of mid-flowering leaf and stem tissue for: A) dry weight (t ha^{-1}); B) tissue N (%); and C) total N (kg/ha); for leaf and stem tissue at DP14, DP1, HM14 and OM15. Each point represents a genotype mean n=3.

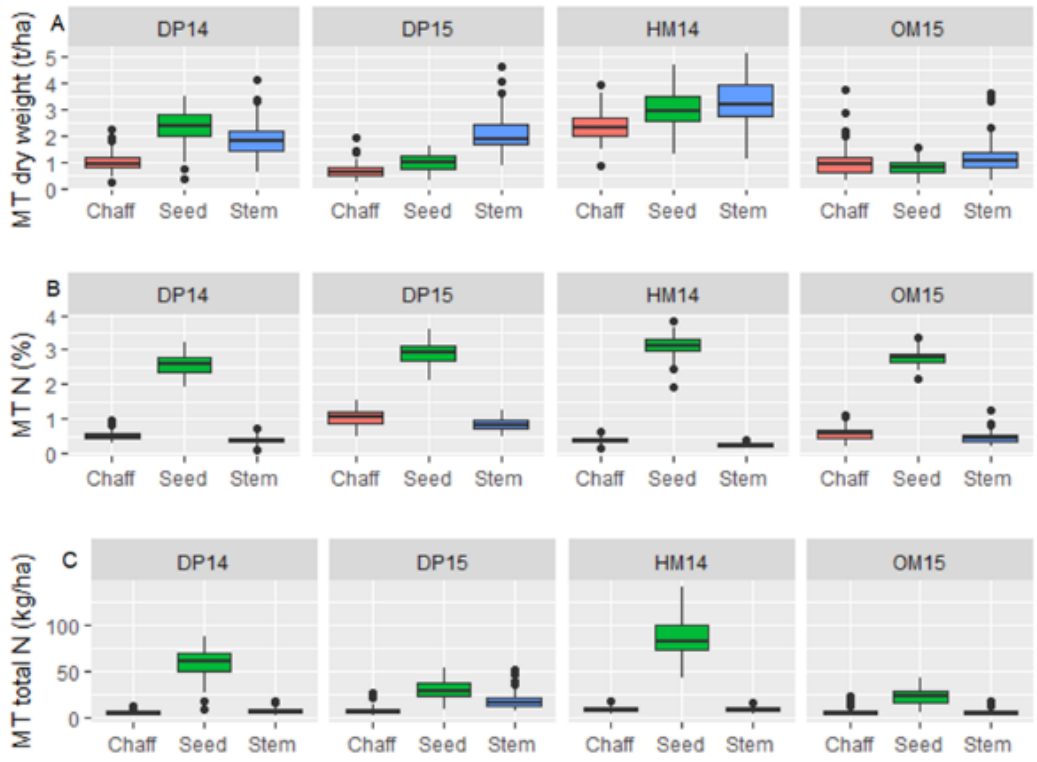


Figure 24. Boxplots for LN plots of maturity (MT) chaff, seed and stem tissue for: A) MT dry weight (t ha^{-1}); B) MT tissue N (%N); and C) MT total N (kg/ha); at DP14, DP15, HM14 and OM15.

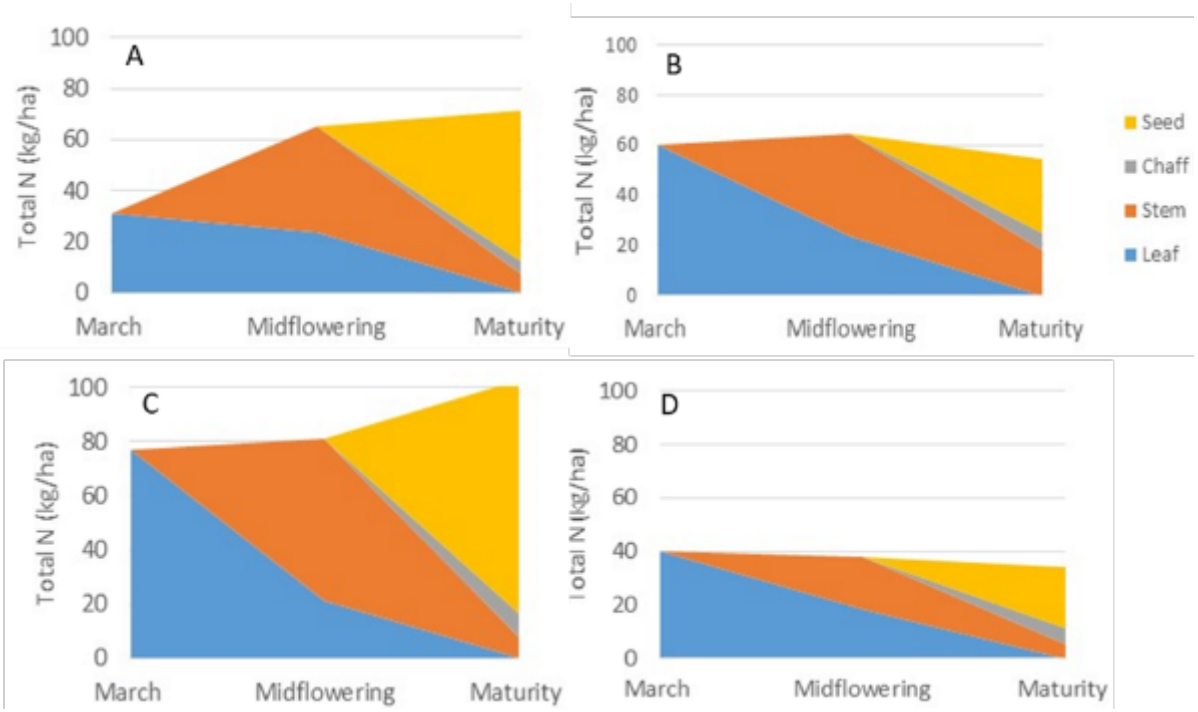


Figure 25. Mean N uptake (kg/ha) across all *genotypes* grown at LN for (A) DP14, (B) DP15, (C) HM14, and (D) OM15. Leaf (blue), stem (orange), chaff (grey), and seed (yellow).

3.3.6. N utilisation and remobilisation

N utilisation calculations show how efficiently N is converted to seed yield (Figure 26A) and biomass (Figure 26B) in the trials. N utilisation at LN of seed production and biomass production was calculated by dividing yield (kg/ha) and biomass (kg/ha) respectively by crop total N at maturity. Across all sites, N utilisation of seed production was 27.05 and biomass utilisation was 79.35. N utilisation of seed was lowest at DP15; this was due to low yields relative to N uptake. N utilisation was higher in the 2014 trials than in the 2015 trials (Fig 26A). HM14 and OM15 showed higher N utilisation of biomass than DP14 and DP15 (Fig 26B). N uptake after flowering relates to both N uptake (from the environment) and N utilisation (from the crop). For these traits there was an extremely large range at all sites (Fig 27). The mean value was positive at all sites but many genotypes had a negative value. This loss can be explained by leaf senescence and seed loss due to shattering. Stem N remobilisation is the amount of stem N at mid-flowering minus the amount of N at maturity - essentially how much stem N is remobilised by the plant. This value was highest at HM14 (48 kg/ha). At HM14 the total N in chaff and stem (non-remobilised N) varied by 40 kg/ha^{-1} between genotypes.

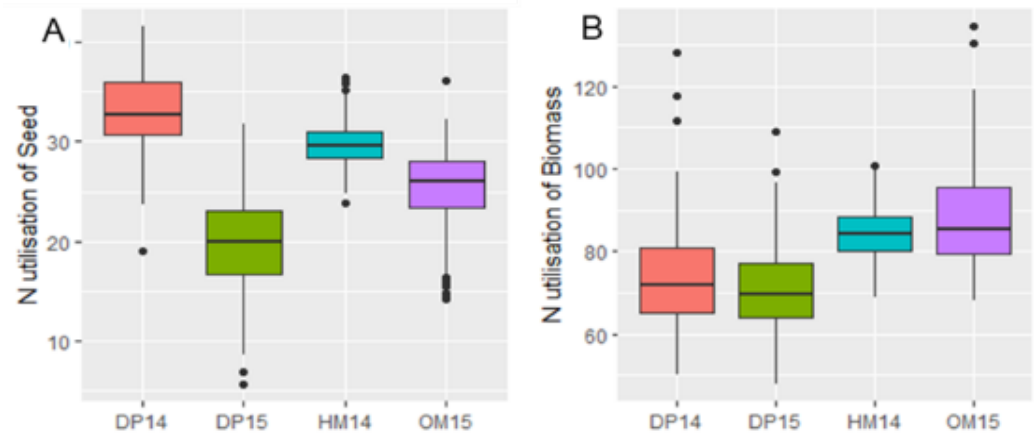


Figure 26. Boxplots of N utilisation of seed (kg/ha) (A) and N utilisation of biomass (B) (kg/ha) at LN for; DP14, DP15, HM14 and OM15.

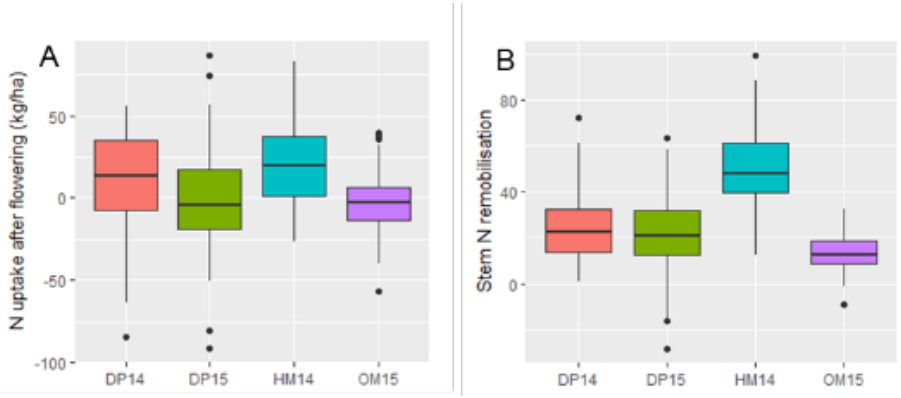


Figure 27. Boxplots for LN plots of (A) N uptake after flowering (kg/ha) and (B) stem N remobilisation (kg/ha) at; DP14, DP15, HM14 and OM15.

3.3.7. Phenology; Establishment, flowering dates and maturity score trait ranges

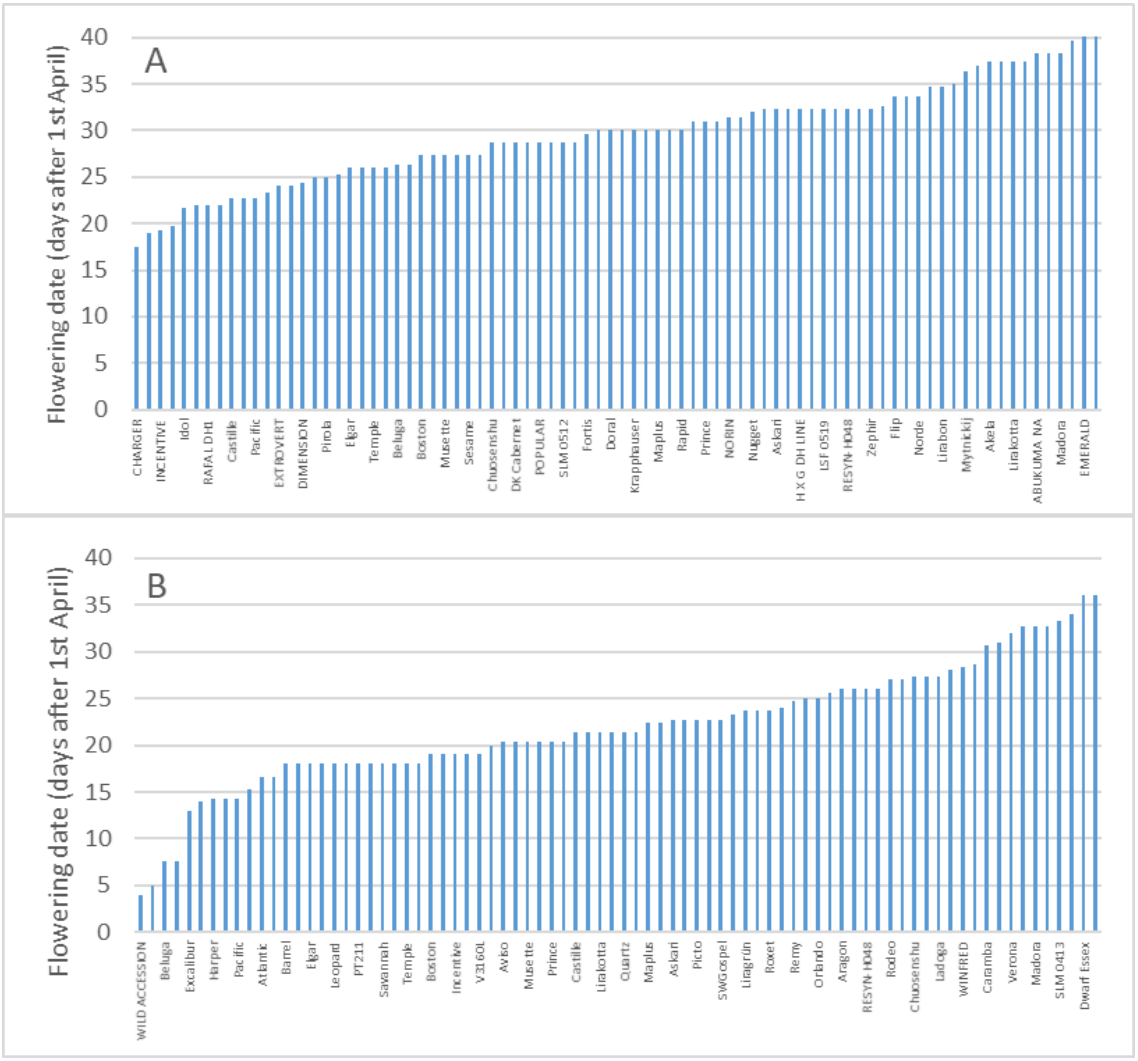


Figure 28. Flowering date, date until first flower (days after 1st April) at HM14 (A) and OM15 (B) site.

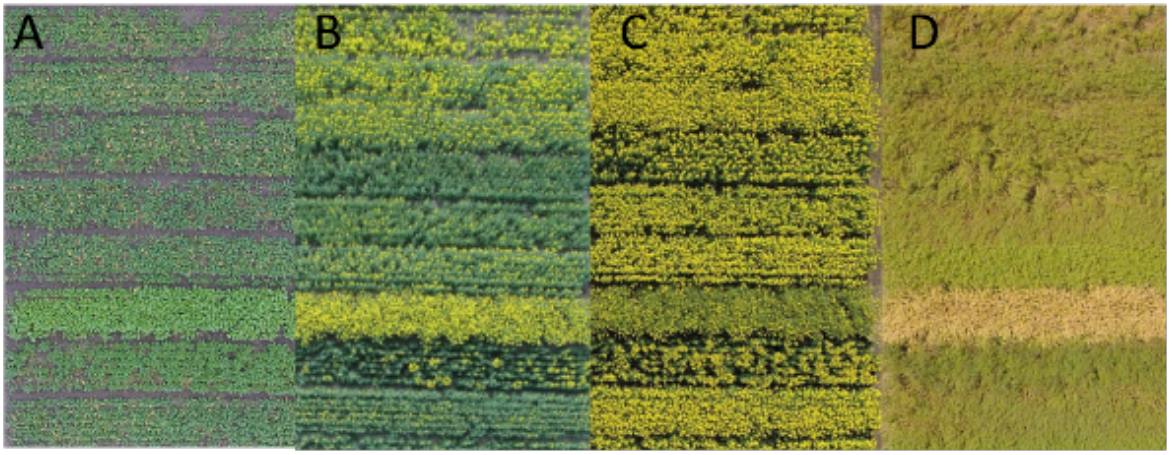


Figure 29. Aerial view RGB plot images from a showing variation in crop development at DP14 on A) 19/11/2014 B) 27/04/15 C) 20/05/15 and D) 12/06/15.



Figure 30. Photo example of extreme differences in flowering dates from genotypes in the RIPR trials.

Substantial differences were observed for establishment; flowering; and maturity scores. Across all sites plant counts ranged between 10-59 plants m^2. Although there was a wide range, the majority of genotypes were above 35 plants m^2. Establishment was assessed in March as a score on a 1 – 9 scale.

Flowering dates were assessed thoroughly at the HM14 and OM15 trials (Fig 28). At these sites flowering was visually assessed every 2 days during flowering, estimating the percentage flowering of each plot (Fig 30). This allowed for more in depth analysis of flowering from these trials. There was a wide range of flowering dates; at HM14 there was a 26.6 day range, with the earliest flowering 17.5 days after April 1st and the latest 40 days. There was a larger range and earlier flowering at OM15 of 36 days, with the earliest flowering 4 days after April 1st and the latest 36 days.

At all sites maturity was scored from 1-9 where 1 had an early maturation date and 9 had a late maturity date. The trials had genotypes with scores throughout this range. A drone image (Fig 29) gives some indication of the extreme differences in maturity. The average score for each trial site was 6.22, (HM14); 7.46 (DP14); 7.33 (OM15); and 6.68 (DP15).

3.3.8. Relationship between yield at LN, NUE and morphological traits

Understanding trait relationship to yield and seed N utilisation at LN is essential for understanding NUE. Yield is an absolute value of production, and the trait obviously of highest value to farmers. Seed N utilisation is an indicator of how efficiently N is used. Table 6 reports correlations across sites of morphological and phenology traits and yield at LN. Here we have shown the inter-trait phenotypic correlations of mean trait data across all sites separated as: Mid-flowering (Fig 31), Maturity (Fig 32); and score data (Fig 33). Pearson's correlation coefficient is a test statistic that measures the statistical relationship, or association, between two continuous variables. It is known as the best method of measuring the association between variables of interest because it is based on the method of covariance. Table 7 shows Pearson correlation with yield at LN at all the different trial sites.

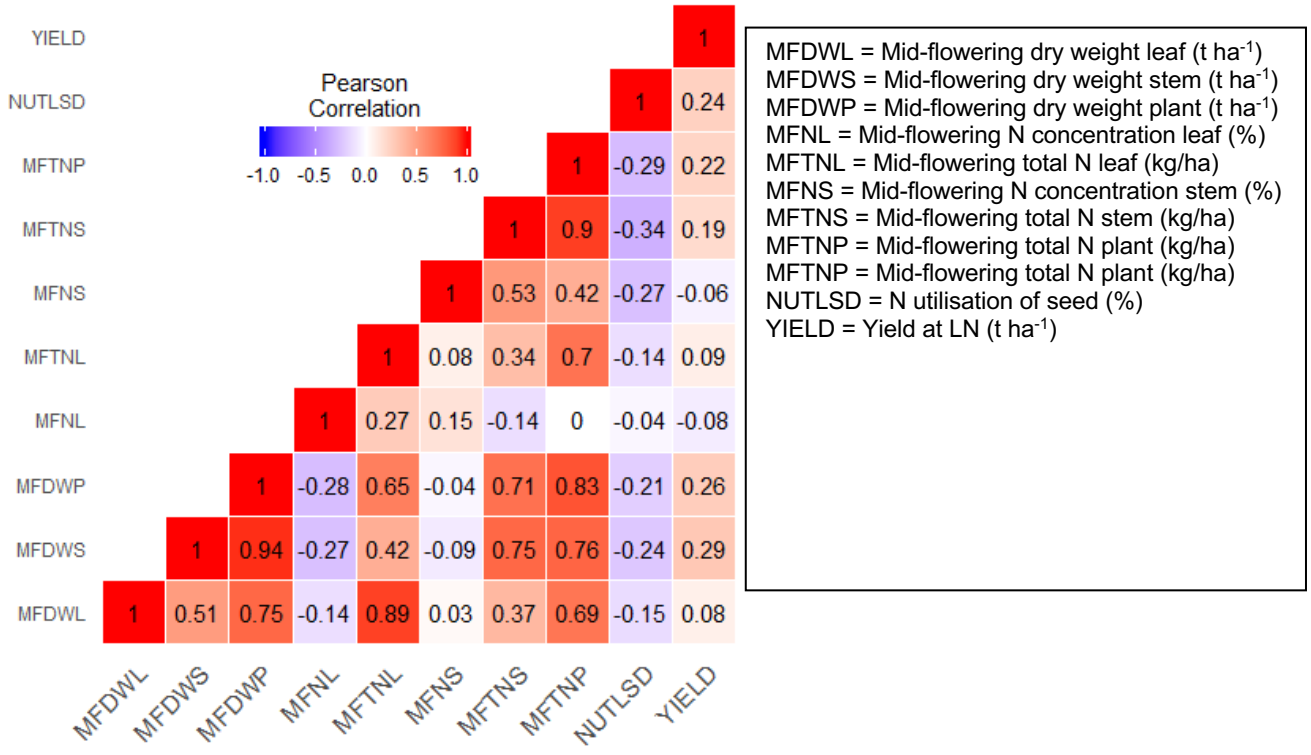
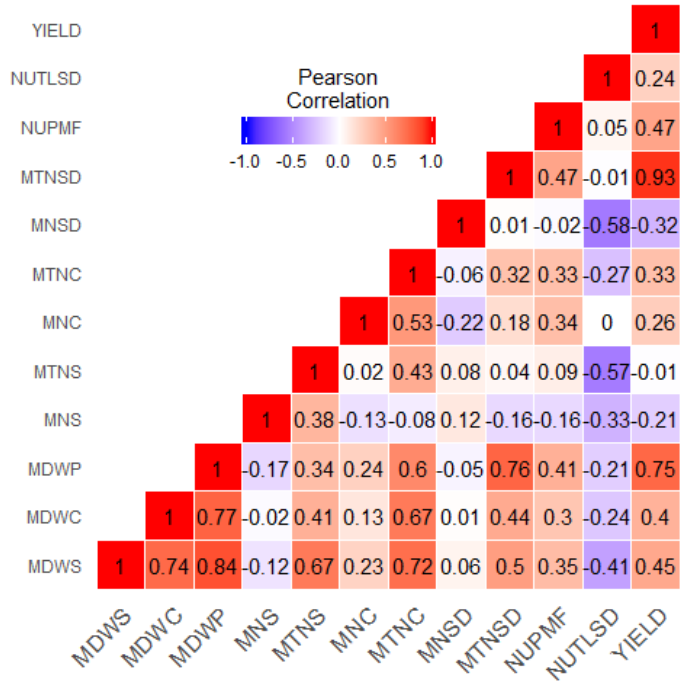
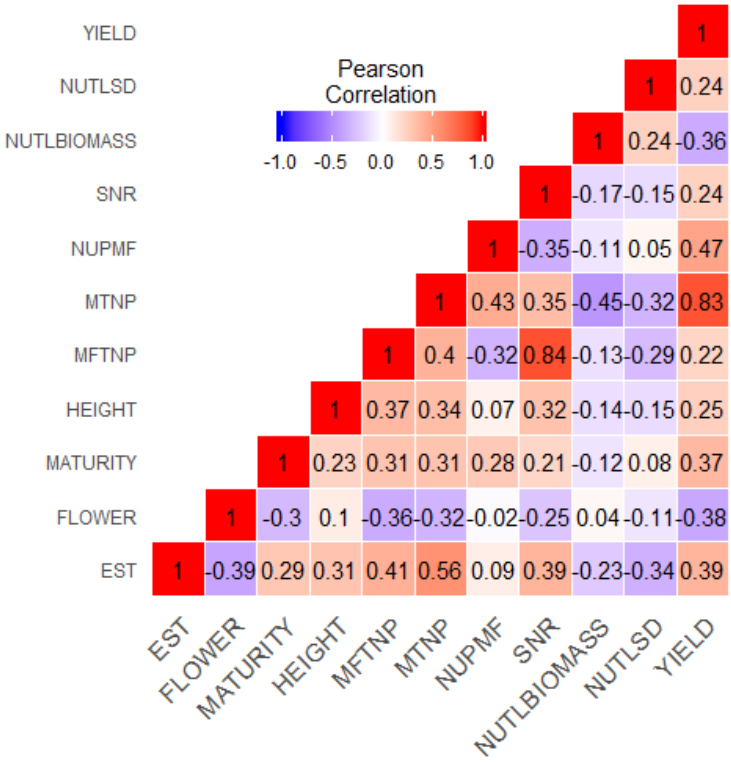


Figure 31. Inter-trait phenotypic pearsons correlations at LN fertilisation for: (A) mid-flowering (MF); (B) maturity (M); and (C) score data. Trait data is mean from 53 genotypes grown at 4 trial sites: DP14; DP15; HM14; and OM15. Colours indicate the strength of correlation. P values are shown in Table 7.



MDWS = Maturity dry weight stem (t ha^{-1})
MDWC = Maturity dry weight chaff (t ha^{-1})
MDWP = Maturity dry weight plant (t ha^{-1})
MNS = Maturity N concentration stem (%)
MTNS = Maturity total N stem, (kg/ha)
MNC = Maturity N concentration chaff (%)
MTNC = Maturity total N chaff (kg/ha)
MNSD = Maturity N concentration seed (%)
MTNSD = Maturity total N seed (kg/ha)
NUPMF = N uptake from mid-flowering (kg/ha)
NUTLSD = N utilisation of seed (%)
YIELD = Yield at LN (t ha^{-1})

Figure 32. Inter-trait phenotypic correlations at LN fertilisation for maturity data. Trait data is mean from 53 genotypes grown at 4 trial sites: DP14; DP15; HM14; and OM15. Colours indicate the strength of correlate. P values are shown in Table 7.



EST = Establishment
FLOWER = Flower score
MATURITY = Maturity
HEIGHT = Height
MFTNP = Mid-flowering total N plant (kg/ha)
MTNP = Maturity total N plant (kg/ha)
NUPMF = N uptake from mid-flowering (kg/ha)
SNR = Stem N remobilisation (kg/ha)
NUTLBIOMASS = N utilisation of biomass (%)
NUTLSD = N utilisation of seed (%)
YIELD = Yield at LN (t ha^{-1})

Figure 33. Inter-trait phenotypic correlations at LN fertilisation for score data. Trait data is mean from 53 genotypes grown at 4 trial sites: DP14; DP15; HM14; and OM15. Colours indicate the strength of correlate. P values are shown in Table 6.

Table 6. Phenotypic trait correlations with yield at LN fertilisation. Trait data used for Pearson correlation is mean for each of 59 genotypes grown at 4 trial sites: DP14; DP15; HM14; and OM15. P number is shown.

	R	P
Establish score (1-9)	0.39	0.0044 (**)
Flowering score (1-9)	-0.38	0.0059 (**)
Maturity score (1-9)	0.37	0.0068 (**)
Height (1-9)	0.25	0.0706
N utilisation biomass (kg/kg)	-0.36	0.0093 (**)
Mid-flowering dry weight leaves (t ha^{-1})	0.08	0.5813
Mid-flowering dry weight stem (t ha^{-1})	0.29	0.038 (*)
Mid-flowering dry weight plant (t ha^{-1})	0.26	0.0675
Mid-flowering N concentration leaf (%)	-0.08	0.5854
Mid-flowering total N leaves (kg/ha)	0.09	0.5045
Mid-flowering N concentration stem (%)	-0.06	0.6489
Mid-flowering total N stem (kg/ha)	0.19	0.1733
Mid-flowering total N plant (kg/ha)	0.22	0.1156
Maturity dry weight stem (t ha^{-1})	0.45	0.0009 (***)
Maturity dry weight chaff (t ha^{-1})	0.4	0.0033 (**)
Maturity dry weight plant (t ha^{-1})	0.75	< 0.001 (***)
Maturity N concentration stem (%)	-0.21	0.1376
Maturity total N stem (kg/ha)	-0.01	0.9347
Maturity N concentration chaff (%)	0.26	0.0605
Maturity total N chaff (kg/ha)	0.33	0.0187 (*)
Maturity N concentration seed (%)	-0.38	0.0062 (***)
Maturity total N seed (kg/ha)	0.93	< 0.001 (***)
N uptake post mid-flowering (kg/kg)	0.47	0.0005 (***)
Maturity total N plant (kg/ha)	0.83	< 0.001 (***)
N utilisation seed (kg/kg)	0.24	0.0815

When considering mean data across all sites (Table 6), the traits correlating significantly with yield at LN are: Maturity total N of seed (R=0.93, p < .0001 ***); Maturity total N of plant (R=0.83, p < .0001 ***); Maturity dry-weight of plants (R=0.75, p < .0001 ***); N uptake post mid-flowering (R=0.47, p < .0005 ***); Maturity dry-weight of stem (R=0.45, p= 0.0009 ***); Maturity dry-weight of chaff (R=0.4, p = .0033 **); maturity score (R=0.37, p = .0068 **); flower score (R=-0.38, p = 0.0059 **); N biomass utilisation (R=-0.36, p = 0.0093 **); maturity total N chaff (R=0.33, p = 0.0187 *); maturity N concentration of seed (kg/kg) (R=-0.38, p = 0.0062 **); mid-flowering dry weight stem (t ha^{-1})

(R=-0.29, p = 0.038 *); and mid-flowering dry weight plant (t ha^{-1}) (R=0.26, p = 0.0675 *). Traits significantly related to seed N utilisation are: maturity N concentration of seed (R= -0.58, p = 0.0093 **); maturity total N stem (R= -0.57, p = 0.0093 **); maturity dry weight stem (t ha^{-1} (R= -0.41, p = 0.0093 **); establishment (R=-0.37, p = 0.0093 **), mid-flowering total stem N (R= -0.34, p = 0.0093 **); maturity N concentration of stem (R=-0.33, p = 0.0093 **); maturity total N plant (R= -0.32, p = 0.0093 **); and mid-flowering total N of plant (t ha^{-1}) (R=0.29, p = 0.0093 **).

Considering phenology and relationship to yield there were significant findings from the RIPR trial. Establishment had greater interaction with yield than with seed N utilisation. Late flowering has a negative relationship with yield (R= -0.38, p = 0.0059 **) when considering trial mean and at each individual trial: (HM14 (R=-0.40, p = 0.0002 ***); DP14 (R=-0.27, p = 0.0021 **); OM15 (R=-0.22, p = 0.05 *); and DP15 (R=-0.30, p = 0.005 **) (Table 7). Maturity score had a positive relationship with yield for mean rates (R= 0.37, p = 0.0068 **); however, when considering trial mean and individual trials there was no significant association with yield at HM14 (Table 7).

At mid-flowering (Fig 31), stem dry weight had stronger association with yield at LN than total dry weight of plants. Dry weight of leaf tissue had no association with yield at LN. Mid-flowering N concentration of stems and total stem N had a negative association on N seed utilisation. Stem N concentrations linked to mid-flowering total plant N (R= 0.38, p < 0.001**).** Maturity partitioned dry tissue had positive association with yield and negative with seed N utilisation. Total stem N had no association with yield but had negative association to seed utilisation (R = -0.57, p<0.001). N concentrations of seeds had a negative association with yield (R = -0.32, p = 0.0062). N uptake post mid-flowering had no association on seed utilisation. Crop N uptake at maturity was associated with a greater percentage of variation (R = 0.83, p <0.0001) in seed yield at LN amongst genotypes than seed N utilisation efficiency (R = - 0.32, p= 0.023) (Fig 33). N uptake post flowering had significant relationship with yield at LN (r=0.47, p = 0.0005) and this was seen across all sites: HM14 (R =0.63, p= 0.0002); DP14 (R =0.41, p=0.0002); OM15 (R = -0.35, p = 0.0001); and DP15 (R =0.31 p = 0.0057). Due to the difference in scoring for lodging between sites this data was not included in the trait analysis.

Table 7. Pearson correlation results of relationship between yield at LN, NUE and morphological traits for all trials: HM14; DP14; OM15; DP15. p number shown.

	DP14	DP14	DP15	DP15	OM15	OM15	HM14	HM14
	R	p	R	p	R	p	R	p
Establishment (1-9)	0.19	0.0956 (*)	0.26	0.0176 (*)	0.12	0.1866	0.35	0.0014 (**)
Flowering score (1-9)	-0.34	0.0021 (**)	-0.30	0.005 (**)	-0.22	0.0511 (*)	-0.40	0.0002 (***)
Maturity (1-9)	0.25	0.025 (*)	0.50	0 (***)	0.21	0.0268 (*)	0.09	0.4279
Height (cm)	0.32	0.0042 (**)	0.40	0.0002 (***)	0.40	0.0002 (***)	0.22	0.051 (*)
N uptake post mid-flowering (kg/ha)	0.41	0.0002 (***)	0.31	0.0057 (**)	0.35	0.0001 (***)	0.63	0 (***)
N utilisation biomass	-0.44	0 (***)	-0.14	0.202	-0.19	0.0435 (*)	-0.01	0.9467
N utilisation seed	0.56	0 (***)	0.66	0 (***)	0.32	0.0006 (***)	0.25	0.0254 (*)
Mid-flowering dry weight leaves (t ha^{-1})	0.07	0.5375	0.12	0.2872	0.02	0.83	0.46	0 (***)
Mid-flowering dry weight stem (t ha^{-1})	0.15	0.1962	0.14	0.1934	0.43	0 (***)	0.27	0.0125 (*)
Mid-flowering dry weight plant (t ha^{-1})	0.14	0.2216	0.16	0.1530	0.36	0 (***)	0.32	0.0029 (**)
Mid-flowering N concentration leaf (%)	0.03	0.8065	-0.02	0.8399	0.19	0.0416 (*)	-0.16	0.1503
Mid-flowering total N leaves (kg/ha)	0.06	0.6018	0.13	0.2338	0.15	0.118	0.24	0.0316 (*)
Mid-flowering N concentration stem (%)	0	0.9885	-0.18	0.0985 (*)	-0.18	0.0558 (*)	-0.15	0.1832

Mid-flowering total N stem (kg/ha)	0.11	0.3418	0.04	0.7332	0.18	0.0505 (*)	0.16	0.1631
Mid-flowering total N plant (kg/ha)	0.09	0.4064	0.10	0.3522	0.25	0.0078	0.23	0.0366 (*)
Maturity dry weight stems (t ha^{-1})	0.25	0.0247 (*)	0.08	0.4661	0.39	0 (***)	0.82	0 (***)
Maturity dry weight chaff (t ha^{-1})	0.09	0.4449	-0.07	0.5560	0.17	0.065 (*)	0.69	0 (***)
Maturity dry weight plant (t ha^{-1})	0.68	0 (***)	0.35	0.0013 (**)	0.53	0 (***)	0.89	0 (***)
Maturity N concentration stem (%)	-0.09	0.4504	-0.54	0 (***)	-0.40	0 (***)	-0.14	0.2092
Maturity total N stem (kg/ha)	0.14	0.2073	-0.25	0.02 (*)	0.14	0.1401	0.43	0 (***)
Maturity N concentration chaff (%)	-0.15	0.1810	0.22	0.0466 (*)	-0.03	0.7181	-0.03	0.7831
Maturity total N chaff (kg/ha)	-0.01	0.9545	-0.02	0.8297	0.17	0.0755 (*)	0.48	0 (***)
Maturity N concentration seed (%)	-0.12	0.2714	-0.09	0.4393	-0.09	0.3624	-0.20	0.0765 (*)
Maturity total N seed (kg/ha)	0.89	0 (***)	0.92	0 (***)	0.94	0 (***)	0.87	0 (***)
Maturity total N plant (kg/ha)	0.87	0 (***)	0.46	0 (***)	0.76	0 (***)	0.82	0 (***)

3.3.9. Relationships between field yield (t ha^{-1}) at LN and morphological traits

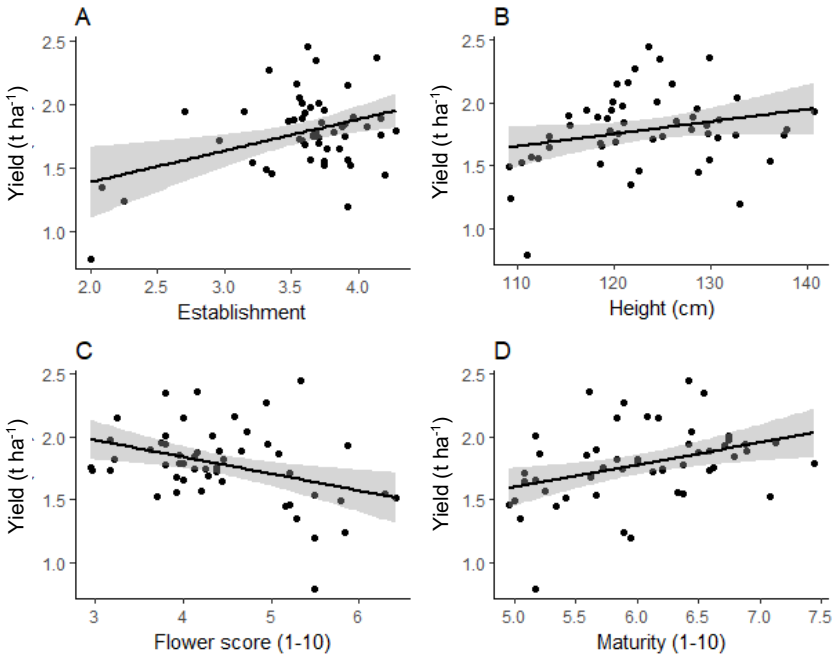


Figure 34. Regression for yield at LN with (A) establishment (1-5), (B) height (cm), (C) flower score (1-10) and (D) maturity score. Values are means from all trials n=4. Linear regression analyses; (A) Establishment, y = 0.25x + 0.89, r^2=0.15, (B) Height, y = 0.01x + 0.57, r^2=0.06, (C) Flower score y = -0.13x + 2.37, r^2=0.14; (D) Maturity score y = 0.17x + 0.72, r^2=0.14;

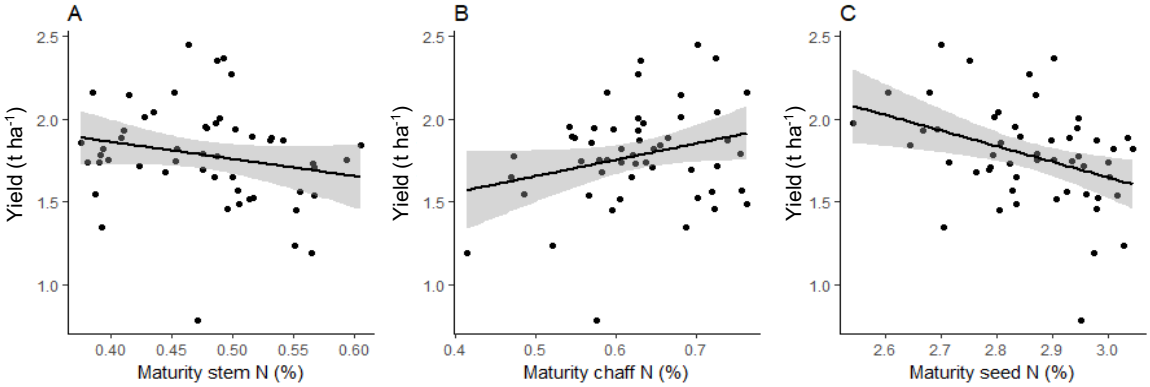


Figure 35. Regression for yield at LN with (A) maturity stem % N, (B) maturity chaff % N, and (C) maturity seed % N. Values are means from all trials n=4. Linear regression analyses; (A) maturity stem % N, y = -1.03x +2.27, r^2=0.04; (B) maturity chaff % N, y = 0.98x + 1.17, r^2=0.07; (C) maturity seed % N, y = -0.94x + 4.47, r^2=0.14.

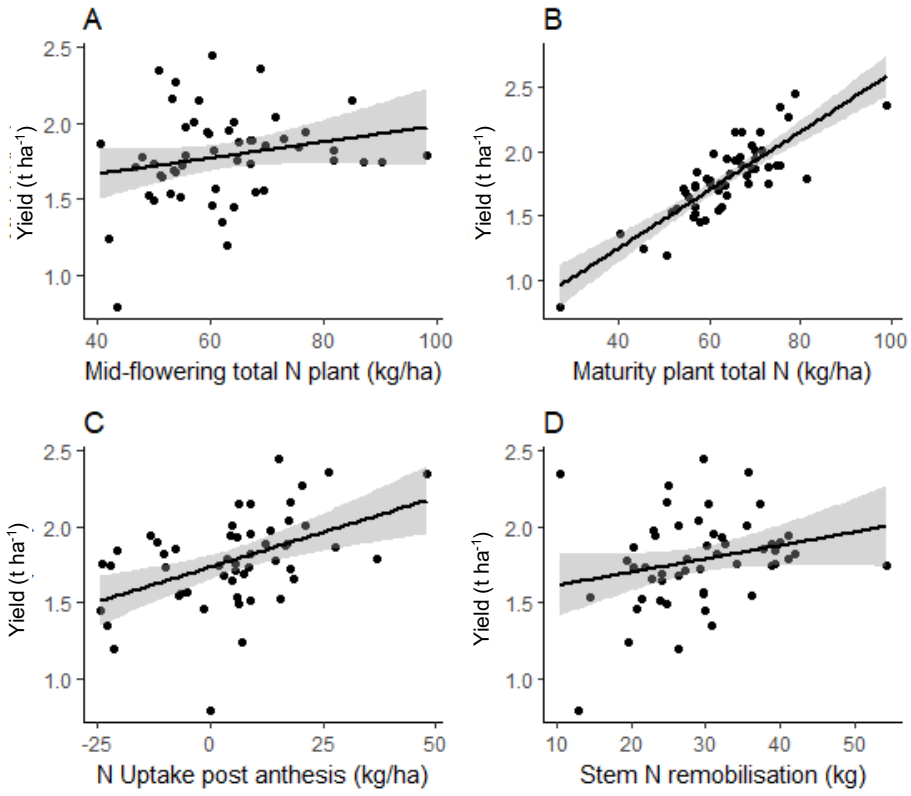
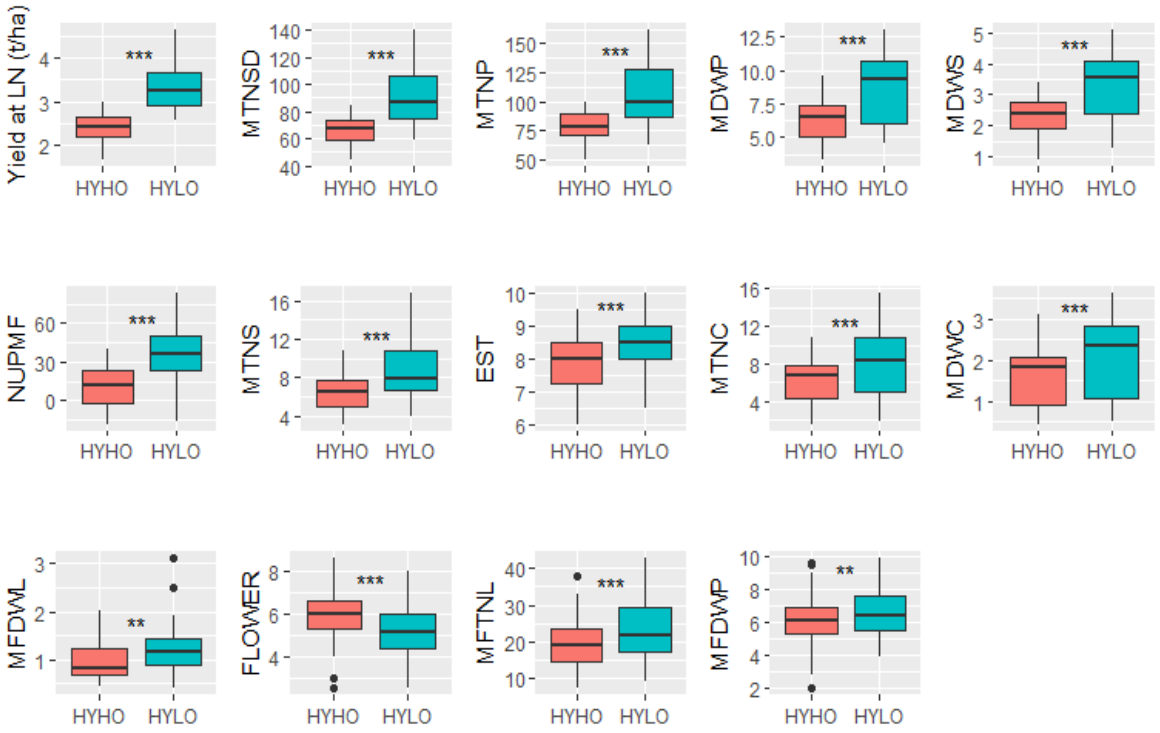


Fig 36. Regression for yield at LN with (A) mid-flowering total plant N (kg/ha), (B) maturity total plant N (kg/ha) (C) N uptake post anthesis (kg/ha) and (D) stem N remobilisation (kg/ha). Values are means from all trials n=4. Linear regression analyses; (A) MFTNP y = 0.01x + 1.45, r^2=0.49; (B) MTNP= y = 0.02x + 0.34, r^2=0.69; (C) NUPMF = y = 0.01x + 1.73, r^2=0.22, (D) SNR = y =0.01x + 1.52, r^2=0.06.

Linear regression analysis can be used to calculate how much yield increase is linked to each individual trait (Fig 34-36). These values can then be used to calculate financial value of each trait and the trait combinations can be analysed within models to understand the value of trait combinations. Some examples: linear regression analysis of LN traits showed that 1 point earlier flowering score (representative of 2-3 days earlier flowering) resulted in a yield increase of 0.42 t ha^{-1}; decreasing seed N content by 0.5% increased yield by 0.47 t ha^{-1}; an increase of crop height by 10cm resulted in increase of yield of 0.1 t ha^{-1}.

3.3.10. Trait analysis at LN of HYHL v HYHO types



MTNSD = Maturity total N seed (kg/ha) MTNP = Maturity total N plant (kg/ha) MDWP = Maturity dry weight plant (t ha^{-1}) MDWS = Maturity dry weight stem (t ha^{-1}) NUPMF = N uptake from mid-flowering (kg/ha) MTNS = Maturity total N stem, (kg/ha) EST = Establishment	MTNC = Maturity total N chaff (kg/ha) MDWC = Maturity dry weight chaff (t ha^{-1}) MFDWL = Mid-flowering dry weight leaf (t ha^{-1}) FLOWER = Flower score (1-10) MFTNL = Mid-flowering total N leaf (kg/ha) MFDWP = Mid-flowering dry weight plant (t ha^{-1})

Figure 37. Boxplots for trait analysis of HYHO and HYLO types for Yield (t ha) at LN. Values are from both trials DP14 and HM14. ** and *** above boxes represent significance at P < 0.05 and P < 0.01 respectively, for t-test comparing HYHO and HYLO types.

Analysis of genotypes that were responded differently to N is important to identify the reasons why they are outliers i.e. efficient or inefficient at using N. The N response type categories were assessed for trait differences using t-tests (Fig 37). HYLO types had high yield at HN plots and LN plots. HYHO had high yield at HN but low yields at LN plots (Fig 37).

HYLO genotypes showed increased values against HYHO types for traits linked to: seed yield at low N ($p<0.001$ at both sites); maturity total N Seed ($p<0.001$ at both sites); maturity N plant ($p<0.001$ at both sites); and maturity dry weight of plant ($p<0.001$ at both sites). They also had increased yield for other traits: maturity dry weight of stems; N uptake post mid-flowering; maturity total N of stems;

establishment; maturity total N of chaff; maturity dry weight of chaff; mid-flowering dry weight of leaves; flowering score; mid-flowering total N of leaves; and mid-flowering dry weight of plants. Some of these traits also matched with traits linked to seed utilisation efficiency at low N (Fig 31-33), including: maturity dry-weight of stems; maturity total N of stems; establishment; maturity total N plant; and mid-flowering total N plant. Interestingly the HYLO analysis showed an effect of mid-flowering leaf tissue for dry-weight and total N content. These traits were not linked to yield when considering all genotypes. These genotypes will further be analysed using ranking in section 2.3.9.

3.3.11. Trait analysis of ASSYST v Elite types

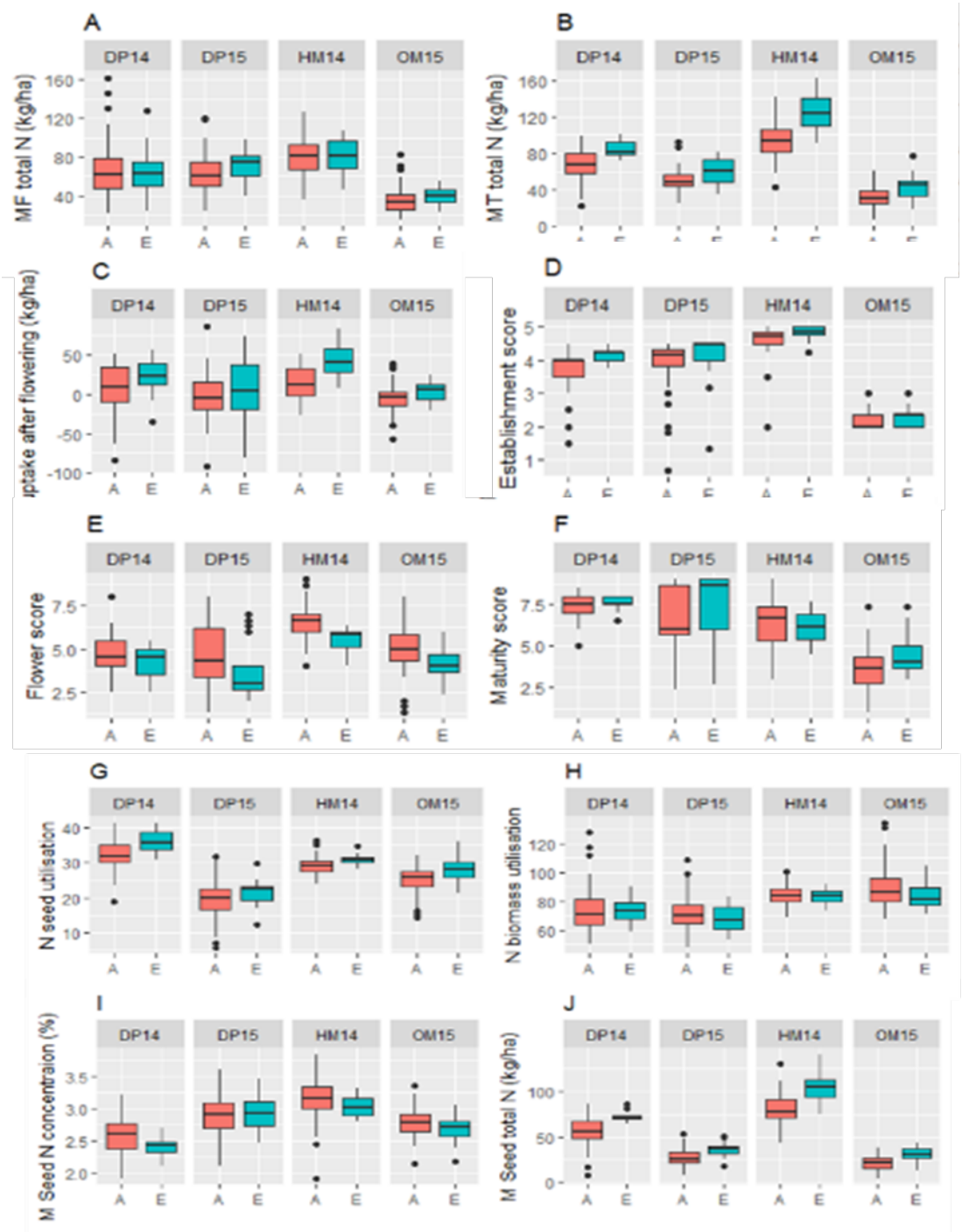


Figure 38. Boxplots for trait analysis of LN plot differences between ASSYST and elite types at: DP14; DP15; HM14; and OM15 for: (A) Mid-flowering total N (kg/ha); (B) MT total N (kg/ha); (C) N uptake after flowering (kg/ha); (D) Establishment (1 low -10 high); (E) Flower score (1 early - 10 late); (F) Maturity score (1 low -10 high); (G) N seed utilisation (kg N / kg seed), (H) N biomass utilisation (kg/kg); (I) N seed concentration (%); (J) Maturity seed total N (kg/ha).

Mean min and max trait values of ASSYST and elite types at all sites are shown in Appendix Table 3. Elites and ASSYST types were analysed individually at each site because there were only 5 elite types which were grown at every trial site. For all traits there was a higher range of values for ASSYST diversity types than elite types (Fig 38). However, elite types also had a very large range of traits.

T-tests were conducted to show differences between traits of the groups; p-values are shown in in Appendix Table 3. The T-tests have shown significant differences between elite and ASSYST genotypes at all sites for: When considering plant growth and development, elite types had stronger establishment (Fig 38 D) (DP14, p=0.01; DP15, NS; HM14, p = <0.001 and OM15, P = NS) and earlier flowering (Fig 38 E) (DP14, p= NS; DP15, p=0.08, HM14 p = <0.001 and OM15 p =0.01) but no difference was seen for maturity score (Fig 38 F). Elite types did not have increased N content mid-flowering or at maturity. For partitioned tissue there were no differences between N concentrations apart from seed N% for which elite genotypes had lower seed N values. This lower N could be due to modern breeding for lower protein types. For total N tissue, differences were seen for maturity tissue (Fig 38.B) ($p<0.001$ at all sites) but not mid-flowering (Fig 38.A). From biomass data only dry weight of seed ($p<0.001$) and stem at maturity ($p<0.001$) showed a significant difference Mean trait values, min, max of ASSYST and elite types at all sites are shown in Appendix Table 3.

3.3.12 Trait analysis by ranking of key genotypes

The interaction between site-year and genotype on NUE and N seed utilisation was investigated further by examining changes in relative ranking of genotypes between years. Traits were ranked in descending order of NUE and Seed N utilisation efficiency for the 59 genotypes grown at all of the sites (Table 8). The average ranking and its standard deviation were then calculated. With this data we can analyse the consistency of potentially useful genotypes by using their ranking across sites and years.

Genotypes were identified that had both high utilisation and efficiency (Fig 39). Pacific, Compass, Excalibar, Orlando and Rodeo all had high ranking for NUE and Seed N utilisation efficiency. Emerald, Viking, smart and Kvintett all had a mean low ranking across sites for NUE and Seed N utilisation efficiency.

PT211, Elgar, Pacific and Aragon all had high ranks for NUE and a standard deviation of less than 10. Emerald had a low rank for NUE with a standard deviation of less than 10. Castille, Harper and Rodeo had high Seed N utilisation with a standard deviation of less than 10. SLM413, Idol, Agalon, Kvintett had a lower Seed N utilisation score with a lower standard deviation

The standard deviation of the ranking allowed the ability to select genotypes that had a higher measure of stability across sites. The standard deviations of ranks had an extremely wide range for some traits and genotypes (Table 8). There is the additional fact that we are working with ASSYST genotypes, not bred to be grown consistently throughout different environments, hence with increased trait variation.

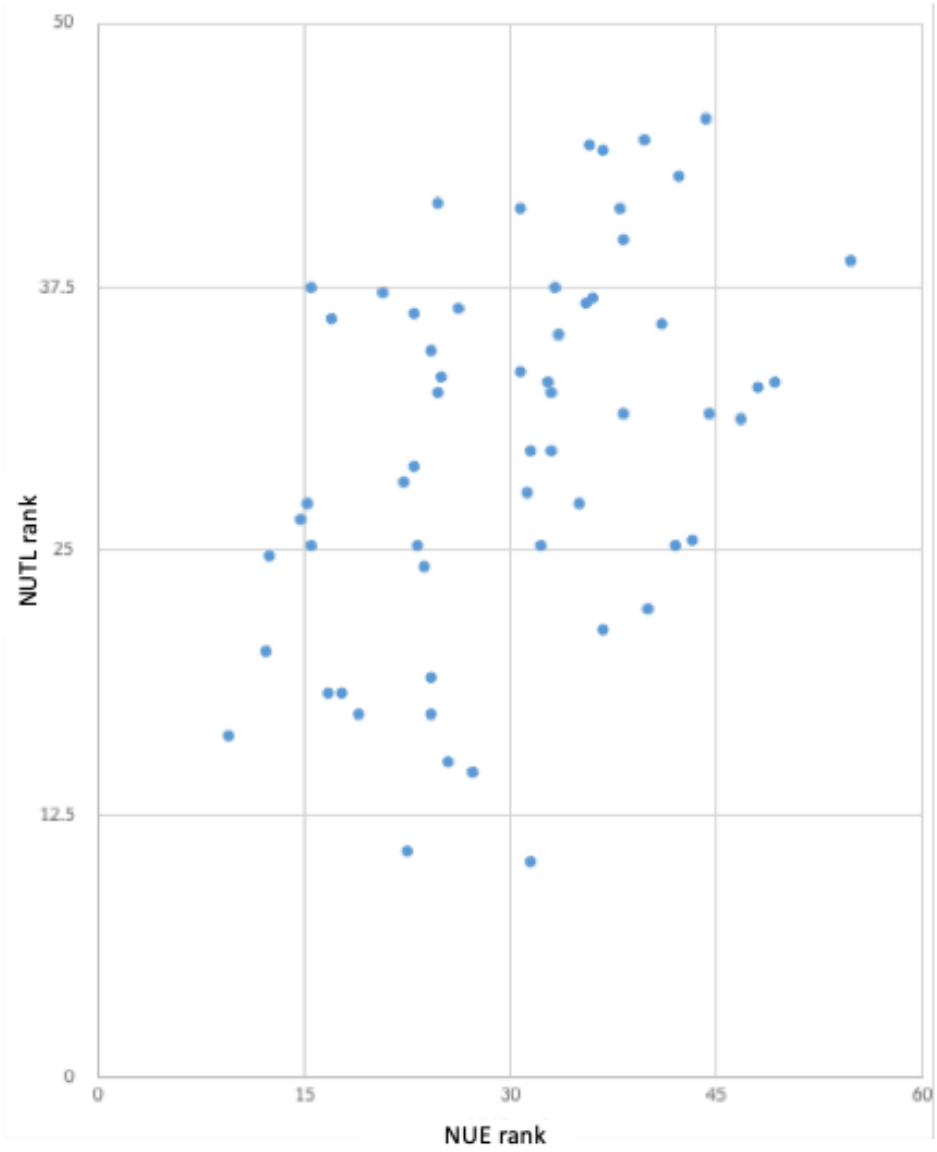


Figure 39. Distribution of genotypes according to the average ranking across sites and years for NUE and (NUTKSD) N Utilisation of seed across the four sites. An increase in rank value represents an increase in NUE and N Seed utilisation efficiency of the genotype for crops grown low N supply.

Table 8. Mean NUE and N seed utilisation of genotypes and average rank order (1-52) across four sites. SD is the standard deviation of the rank. Genotypes ranked from lowest to highest.

Genotype	Mean NUE (kg/kg)	Av Rank	SD rank	Genotype	Mean SD N utilisation (kg/kg)	Av Rank	SD Rank
Emerald	17.9	9.5	6.0	HANSEN X GASPARD	24.9	10.3	6.1
Viking	23.9	12.3	12.8	Smart	25.8	10.8	11.9
Chuosenshu	18.5	12.5	9.3	SLM 0413	26.8	14.5	10.5
Mansholt	23.3	14.8	8.1	Idol	25.7	15.0	9.5
Wild Accession	17.3	15.3	27.8	EMERALD	25.0	16.3	14.9
Amor	26.9	15.5	9.0	Leopard	24.3	17.3	15.8
WINFRED	21.3	15.5	17.3	Maplus	26.8	17.3	13.1
Agalon	19.6	16.8	14.5	Kvintett	26.0	18.3	10.1
FORTIS	26.0	17.0	17.0	Agalon	26.2	18.3	7.8
Kvintett	22.1	17.8	13.5	Askari	23.8	19.0	22.2
Leopard	23.9	19.0	14.1	Viking	25.0	20.3	20.4
Oase	27.6	20.8	6.8	Liragr□n	25.0	21.3	16.6
Lirakotta	21.1	22.3	9.9	RESYN-H048	26.4	22.3	9.5
Smart	22.7	22.5	15.4	RAFAL DH1	28.5	24.3	22.6
Musette	27.9	23.0	17.1	Chuosenshu	25.3	24.8	19.2
Zephir	29.4	23.0	12.5	WINFRED	26.2	25.3	18.5
SLM 0512	28.6	23.3	19.4	Aragon	27.4	25.3	6.3
RAFAL DH1	22.6	23.8	17.9	SLM 0512	27.6	25.3	4.8
Askari	23.5	24.3	18.8	Beluga	27.8	25.3	10.2
Maplus	25.8	24.3	14.3	Sesame	27.7	25.5	14.4
Prince	26.2	24.3	16.3	Mansholt	26.8	26.5	7.8
Castille (E)	26.2	24.8	12.0	Wild Accession	19.9	27.3	25.5
NORIN	27.3	24.8	19.6	Lipton	27.7	27.3	12.7
LSF 0519	26.2	25.0	9.4	Savannah	28.4	27.8	20.2
Idol	26.3	25.5	9.3	Lirakotta	26.2	28.3	19.3
Roxet	28.2	26.3	14.2	Zephir	28.4	29.0	21.5
SLM 0413	27.0	27.3	11.7	Boston	27.5	29.8	16.6
Harper	33.3	30.8	21.1	Madora	28.3	29.8	17.2
Madrigal x recital	29.1	30.8	18.0	Temple	28.2	31.3	17.9
Savannah	29.7	31.3	15.0	Rapid	27.1	31.5	26.9

Hansen x gaspard	21.2	31.5	19.4	Trinity	29.4	31.5	21.5
Madora	21.7	31.5	18.0	PR46W21	27.3	32.5	15.3
Beluga	23.1	32.3	9.2	NORIN	28.3	32.5	26.3
SWGospel	30.3	32.8	13.9	Quartz	22.5	32.8	22.6
Boston	26.1	33.0	14.0	SWGospel	26.7	33.0	16.8
PR46W21	33.3	33.0	20.9	Elgar	31.6	33.0	19.5
Charger	33.0	33.3	24.0	LSF 0519	25.9	33.3	20.5
DK Cabernet	33.1	33.5	18.2	Madrigal x recital	30.0	33.5	23.9
Lipton	25.3	35.0	20.9	Prince	28.0	34.5	15.8
Ex Power	36.5	35.5	17.8	DK Cabernet	28.3	35.3	14.2
Orlando	27.9	35.8	13.3	Troy	29.0	35.8	16.9
Laser	29.6	36.0	22.0	FORTIS	31.3	36.0	23.6
Compass	32.2	36.8	9.5	Musette	27.0	36.3	19.5
Liragrn	22.3	36.8	6.8	Roxet	28.7	36.5	11.2
Nugget	29.2	38.0	20.7	Ex Power	30.7	36.8	13.5
Falstaff	30.6	38.3	12.1	Laser	30.5	37.0	12.7
Rapid	27.0	38.3	19.5	Oase	30.8	37.3	18.2
Excalibur	33.8	39.8	21.0	Amor	30.1	37.5	12.7
RESYN-H048	26.1	40.0	19.2	Charger	30.4	37.5	11.5
Troy	31.6	41.0	16.0	PT211	28.2	38.8	18.9
Aragon	26.9	42.0	11.0	Falstaff	31.0	39.8	17.3
Rodeo	30.0	42.3	15.9	Nugget	23.1	41.3	25.2
Sesame	33.8	43.3	16.6	Harper	30.1	41.3	3.9
Pacific (E)	30.0	44.3	7.6	Castille	29.0	41.5	5.8
Trinity (E)	32.2	44.5	13.6	Rodeo	30.3	42.8	11.6
Temple (E)	35.0	46.8	15.2	Compass	29.4	44.0	17.0
Quartz	33.3	48.0	13.7	Orlando	29.6	44.3	13.5
Elgar (E)	33.1	49.3	9.5	Excalibur	31.2	44.5	18.3
PT211 (E)	34.6	54.8	3.4	Pacific	32.9	45.5	14.2

The same ranking process was conducted for: mid-flowering and maturity biomass; partitioned N concentration; dry weight; and total N data, for the 59 genotypes grown at all of the sites. We were unable to rank traits that were scored (Establishment, Flowering and Maturity) because many of the score results were repetitive and not suitable for ranking in a way that would be relevant across sites. The ranking was used to highlight genotypes that stood out as being low or high ranking with a tight standard deviation (Table 9).

Table 9. Genotypes with a high or low ranking order across four sites with a mean rank of <15 being classified as low and a mean rank of > 45 classified as high.

	High ranking genotypes	Low ranking genotypes
Mid-flowering dry weight of leaves (t ha^{-1})	Sesame, M x R and Leapord	Resyn-Ho48, Amor and Winfred
Mid-flowering dry weight stem (t ha^{-1})	Askari	Amor
N concentration at mid-flowering stem (%)	Beluga, LSF 0519	PT211 and Excalibur
N concentration at mid-flowering leaf (%)	Askari, Castille, Viking	Charger
Mid-flowering total stem N (kg/ha)	Compass, Beluga and Norin	Leopard, M x R, Roxet, SLM 0413, Winfred
Mid-flowering total leaf (kg/ha)	PT211 and SLM 0512	Wild accession, Temple, Agalon, Winfred, RESN-H048
Maturity dry weight stem (t ha^{-1})	Elgar	Winfred and Orlandov
Maturity dry weight chaff (t ha^{-1})	Liragrun, Compass, Aragon, Troy and Ex power	RAFAL DH1, Amor, Winfred and Kvintett,
Maturity N concentration stem (%)	Kvintett, RESYN H048, Smart, Leopard, Amor, fortis	Excalibur, Rodeo, Harper, Madora
Maturity N concentration chaff (%)	. H x G, Quartz, Idol, Agalon and sesame	Emerald and Mansholt
Maturity N concentration seed (%)	Mansholt, SLM 0413, and Smart	na
Maturity total N stem (kg/ha)	PR46W21, PT211 and Leopard	Orlando
Maturity total N chaff (kg/ha)	Ex power, Idol, Sesame, Rodea and PR46W21	Manshot, and Winfred
Maturity total N plant (kg/ha)	Compass, Quartz, PT211 and Elgar	Lirakotta, Winfred Emerald, RAFAL
Maturity total N seed (kg/ha)	Quartz, Ex power, sesame, Excalibur, DK cabernet, Temple, Harper, PT211 and Elgar.	WILD ACCESSION, Lirakotta, WINFRED, emerald, Viking, Kvintett and Agalon.

In section 3.3.4 genotypes were highlighted that were HYHO and HYLO at all trial sites. Using the same ranking as presented in Table 8 the differences between the HYHO and HYLO types were

analysed (Tables 10-12). With this data we can analyse the consistency of potentially useful genotypes by using their ranking across sites and years. We chose to use the HYHO and HYLO genotypes because understanding the difference between these groups will be key for future breeding of high yielding efficient types.

For Yield and NUE, HYHO types were lower in the mean ranking of the genotypes than HYLO types. Agalon in particular was lowest ranking for both yield at LN and NUE. Harper, DK cabernet and Expower were the highest rank for yield (Table 10). The same was seen for N utilisation of biomass - HYHO types were generally low ranked and HYLO were highest ranked. However, for N utilisation of seed, HYLO and HYHO types were distributed throughout the mean rank distribution. For N uptake post flowering, HYHO types were the lowest ranking and HYLO were the highest ranking. For Stem N remobilisation, HYHO types were generally lower ranking and HYLO ranking highest. However the lowest ranking (Troy) was HYLO and the highest ranking (SLM 0512) is HYHO.

When considering mid-flowering data, several differences in genotype ranking were seen between the traits measured (Table 11). For the dry-weight of leaves, HYHO types were ranked lower than HYLO types. Pacific, Harper, Expower, Musette and Zephir had high rankings and tight standard deviations. For the DW of stems, HYHO types were generally lower (Agalon) and HYLO was at the top (SLM 0512) of this ranking but there was less difference in the ranking between the two types when compared to the weight of leaves. For stem % N content, Expower and DK cabernet are HYLO types and had high stem N content and tight standard deviations; other genotypes all had high standard deviation for ranking. For the N content (%) of leaves, HYLO genotypes Charger and Troy were the lowest ranked with small standard deviations. A HYHO genotype Pacific was the highest ranked genotype with a rank mean of 45; however, SL0512 was ranked second which was classified as a HYLO type. When considering the total N of leaves, a HYHO type (Agalon) was the lowest ranked and a HYHO type (SLM 0512) was the highest ranked. When considering the total N of stem, HYLO types were generally higher in the rankings; none of the HYLO or HYHO genotypes selected were the lowest ranking (below mean rank 15) or highest ranked (above mean rank 15).

The ranked maturity data of traits for HYLO types is shown in Table 12. For maturity DW of stems; chaffs; and total plant, HYHO types were lower ranked and HYLO were higher ranked. Liragrun was an exception; this genotype is a HYHO genotype that is ranked highly for dry-weight of chaff. For stem and chaff weights, Troy and Export HYLO types were highly ranked with tight SD. For whole plant weights, HYLO types PR46W21 and DK cabernet were amongst the highest ranking genotypes; Agalon and Orlando were the lowest ranked. Considering N concentrations at maturity for chaff, HYLO and HYHO types were distributed throughout the ranking. Agolon (HYHO) was the highest ranked but Idol, a HYLO type, was in second place. Charger had a low ranking with a tight

distribution. For N concentrations at maturity for stems, Rodeo and Harper HYLO genotypes were the lowest ranking. None of the genotypes had a high mean ranking (over 45 in the ranks) but PR46W21 was the highest ranked. For N concentrations of seeds, Pacific (HYLO) had a low mean ranking. For maturity total N of stems, HYLO types were low ranking and HYLO where high ranking. Orlando (HYHO) had the lowest ranking for MTNS and MTNC. For total stem N PR46W21, DK cabernet and Idol had high mean ranks. For maturity total N of chaff, HYLO types were the highest ranking across sites.

Table 10. Mean Yield (t ha^{-1}); NUE (kg/kg); and N seed utilisation of genotypes and average rank order across four sites. Low ranking indicates lower amounts and high indicates the higher amounts. SD is the standard deviation of the rank.

Yield t ha^{-1}				NUE kg/kg			
VARIETY	Rank mean	SD		VARIETY	Rank mean	SD	
Agalon	14.75	13.6	HYHO	Agalon	16.8	14.5	HYHO
Zephir	21	12.5	HYHO	Zephir	23.0	12.5	HYHO
Liragr□n	26	15.7	HYHO	Musette	23.0	17.1	HYHO
Lipton	28.25	18.1	HYHO	SLM 0512	23.3	19.4	HYHO
Musette	29.5	15.8	HYHO	Idol	25.5	9.3	HYLO
Orlando	30.5	15.2	HYHO	Harper	30.8	21.1	HYLO
SLM 0512	32.25	15.6	HYHO	PR46W21	33.0	20.9	HYLO
Idol	32.75	13.2	HYLO	Charger	33.3	24.0	HYLO
Charger	34	22.6	HYLO	DK Cabernet	33.5	18.2	HYLO
Pacific	36.75	11.3	HYLO	Lipton	35.0	20.9	HYHO
PR46W21	40.5	11.1	HYLO	Ex Power	35.5	17.8	HYLO
Rodeo	42.25	11.0	HYLO	Orlando	35.8	13.3	HYHO
Troy	44.25	9.5	HYLO	Liragr□n	36.8	6.8	HYHO
Harper	48	10.5	HYLO	Troy	41.0	16.0	HYLO
DK Cabernet	50.5	4.8	HYLO	Rodeo	42.3	15.9	HYLO
Ex Power	51.5	10.5	HYLO	Pacific	44.3	7.6	HYLO
N utilisation biomass (kg/kg)				N utilisation seed (kg/kg)			
VARIETY	Rank mean	SD		VARIETY	Rank mean	SD	
Orlando	19.75	16.8	HYHO	Idol	15.0	9.5	HYLO
Zephir	21	9.1	HYHO	Agalon	18.3	7.8	HYHO
Agalon	22	6.4	HYHO	Liragr□n	21.3	16.6	HYHO
Idol	24.5	23.4	HYLO	SLM 0512	25.3	4.8	HYHO

DK Cabernet	24.75	12.1	HYLO	Lipton	27.3	12.7	HYHO
Charger	25.25	11.1	HYLO	Zephir	29.0	21.5	HYHO
Harper	26	19.4	HYLO	PR46W21	32.5	15.3	HYLO
PR46W21	26.25	8.8	HYLO	DK Cabernet	35.3	14.2	HYLO
SLM 0512	30.75	9.6	HYHO	Troy	35.8	16.9	HYLO
Musette	33.25	15.5	HYHO	Musette	36.3	19.5	HYHO
Rodeo	33.75	16.7	HYLO	Ex Power	36.8	13.5	HYLO
Lipton	34.25	17.3	HYHO	Charger	37.5	11.5	HYLO
Ex Power	36.75	13.9	HYLO	Harper	41.3	3.9	HYLO
Pacific	37.5	15.1	HYLO	Rodeo	42.8	11.6	HYLO
Troy	39.5	24.8	HYLO	Orlando	44.3	13.5	HYHO
Liragr□n	40.25	15.0	HYHO	Pacific	45.5	14.2	HYLO
N uptake post MF (kg/kg)				Stem N remobilisation (Kg)			
VARIETY	Rank mean	SD		VARIETY	Rank mean	SD	
SLM 0512	17.5	19.8	HYHO	Troy	10.5	10.6	HYLO
Orlando	27	12.9	HYHO	Liragr□n	20.5	8.7	HYHO
Musette	28	19.9	HYHO	PR46W21	22.5	4.7	HYLO
Agalon	28.25	13.3	HYHO	Musette	26	23.8	HYHO
Lipton	28.25	13.0	HYHO	Lipton	26.25	9.9	HYHO
Harper	29.5	19.6	HYLO	Agalon	27	13.5	HYHO
Pacific	30.25	19.8	HYLO	Zephir	27	23.8	HYHO
DK Cabernet	33.5	17.4	HYLO	Rodeo	27.25	15.8	HYLO
Idol	34.5	19.7	HYLO	Charger	29.5	18.6	HYLO
Zephir	37.75	11.6	HYHO	Orlando	30.75	9.9	HYHO
Liragr□n	38.25	11.4	HYHO	Ex Power	32.25	19.1	HYLO
Charger	38.75	9.9	HYLO	DK Cabernet	34.75	10.8	HYLO
Rodeo	40.5	14.4	HYLO	Idol	35	14.3	HYLO
PR46W21	41.75	11.8	HYLO	Harper	39.75	17.7	HYLO
Ex Power	42	11.6	HYLO	Pacific	40	12.4	HYLO
Troy	46	11.1	HYLO	SLM 0512	40.5	19.6	HYHO

Table 11. Average rank order across four sites for mid-flowering: stem and leaf dry weights; tissue N concentration; and total N. Low ranking indicates the lower and high ranking indicates the higher weights. SD is the standard deviation of the rank.

Mid-flowering dry weight leaves (t ha^{-1})				Mid-flowering dry weight stems (t ha^{-1})			
VARIETY	Rank mean	SD		VARIETY	Rank mean	SD	
Musette	16.5	9.3	HYHO	Agalon	17.75	13.2	HYHO
Zephir	17.25	7.6	HYHO	Lipton	18.25	9.0	HYHO
Liragr□n	18.5	15.3	HYHO	Charger	21.25	10.8	HYLO
Agalon	18.75	16.8	HYHO	Zephir	25.5	16.6	HYHO
Lipton	26.25	23.1	HYHO	Troy	25.75	18.5	HYLO
DK Cabernet	26.25	8.8	HYLO	Liragr□n	27.75	14.2	HYHO
Orlando	29.75	17.7	HYHO	DK Cabernet	28.25	8.1	HYLO
Charger	29.75	20.0	HYLO	Musette	30.75	9.4	HYHO
PR46W21	33.75	18.6	HYLO	PR46W21	30.75	15.2	HYLO
Rodeo	36	12.0	HYLO	Ex Power	31.25	11.1	HYLO
SLM 0512	38.75	11.3	HYHO	Rodeo	32.25	16.5	HYLO
Ex Power	42	10.4	HYLO	Idol	36	23.0	HYLO
Harper	43.5	10.2	HYLO	Pacific	37	21.2	HYLO
Troy	46	12.5	HYLO	Orlando	39.25	23.2	HYHO
Idol	46	11.3	HYLO	Harper	47	7.5	HYLO
Pacific	50.75	8.9	HYLO	SLM 0512	49.5	9.3	HYHO
Mid-flowering N concentration leaves (%)				Mid-flowering N concentration stem (%)			
VARIETY	Rank mean	SD		VARIETY	Rank mean	SD	
Charger	11.5	6.4	HYLO	Orlando	16	16.4	HYHO
Troy	13.5	11.3	HYLO	Rodeo	19	16.7	HYLO
PR46W21	16.5	21.6	HYLO	Idol	23	21.4	HYLO
Idol	18.5	7.3	HYLO	Liragr□n	23.25	19.2	HYHO
DK Cabernet	19.25	9.1	HYLO	Musette	23.25	21.4	HYHO
Liragr□n	22.25	19.6	HYHO	PR46W21	23.75	15.3	HYLO
Orlando	22.5	14.5	HYHO	Troy	24	16.9	HYLO
Musette	25	17.1	HYHO	Harper	25.25	19.6	HYLO
Zephir	25.25	20.3	HYHO	Zephir	25.5	18.4	HYHO
Agalon	26	11.4	HYHO	SLM 0512	25.5	14.0	HYHO
Rodeo	30.75	14.5	HYLO	Pacific	30.75	17.7	HYLO
Harper	32	9.4	HYLO	Charger	33.75	19.2	HYLO
Ex Power	32.25	21.4	HYLO	Lipton	34.75	14.2	HYHO

Lipton	34.75	21.4	HYHO	Agalon	35.75	17.8	HYHO
SLM 0512	44.25	7.3	HYHO	DK Cabernet	44.25	6.2	HYLO
Pacific	45	12.0	HYLO	Ex Power	47.25	10.6	HYLO
Mid-flowering total N of leaves (kg/ha)				Mid-flowering total N of stems (kg/ha)			
VARIETY	Rank mean	SD		VARIETY	Rank mean	SD	
Agalon	12.25	8.9	HYHO	Troy	19.5	12.7	HYLO
Liragr□n	20	14.0	HYHO	Agalon	20.25	10.0	HYHO
Zephir	20.25	13.2	HYHO	Rodeo	20.25	16.8	HYLO
Rodeo	22.75	10.2	HYLO	Orlando	21.75	11.6	HYHO
Musette	22.75	12.7	HYHO	Idol	22	15.3	HYLO
Charger	24.5	22.1	HYLO	Liragr□n	22.5	7.5	HYHO
PR46W21	25	16.2	HYLO	Lipton	23.75	12.1	HYHO
DK Cabernet	25.5	11.9	HYLO	Zephir	24	16.2	HYHO
Lipton	26.5	21.8	HYHO	Musette	25.5	18.2	HYHO
Idol	27.75	17.3	HYLO	PR46W21	27	8.5	HYLO
Harper	31.25	20.5	HYLO	Pacific	28.75	21.9	HYLO
Ex Power	32	20.2	HYLO	Harper	29	20.9	HYLO
Troy	36	5.9	HYLO	Charger	29.5	12.8	HYLO
Orlando	36.5	12.4	HYHO	Ex Power	33.5	21.5	HYLO
Pacific	38.75	21.8	HYLO	DK Cabernet	35.75	10.8	HYLO
SLM 0512	41.75	10.2	HYHO	SLM 0512	40.25	20.9	HYHO

Table 12. Average rank order across four sites for maturity: stem, chaff and seed - dry weights; tissue N concentration; and total N amount. Low ranking indicates the lower and high ranking indicates the higher amounts. SD is the standard deviation of the rank.

Maturity dry weight stem (t ha^{-1})				Maturity dry weight chaff (t ha^{-1})			
VARIETY	Rank mean	SD		VARIETY	Rank mean	SD	
Orlando	11.0	3.6	HYHO	Zephir	16.3	4.3	HYHO
Agalon	16.0	18.3	HYHO	Agalon	17.8	18.8	HYHO
Zephir	17.3	10.6	HYHO	Orlando	18.8	5.7	HYHO
Lipton	20.0	11.0	HYHO	Musette	21.3	6.1	HYHO
Musette	27.0	19.1	HYHO	Charger	27.8	27.5	HYLO
Pacific	29.3	16.2	HYLO	SLM 0512	28.8	14.5	HYHO
Charger	31.8	23.8	HYLO	DK Cabernet	29.0	16.0	HYLO

Harper	34.5	11.6	HYLO	Pacific	35.5	10.7	HYLO
Liragr□n	37.0	3.7	HYHO	PR46W21	38.5	9.9	HYLO
SLM 0512	37.5	13.7	HYHO	Lipton	39.3	14.7	HYHO
Rodeo	39.5	12.1	HYLO	Harper	40.3	9.9	HYLO
Idol	42.0	11.2	HYLO	Rodeo	41.8	11.8	HYLO
PR46W21	44.3	15.0	HYLO	Idol	42.5	15.3	HYLO
DK Cabernet	44.5	10.2	HYLO	Liragr□n	45.3	11.6	HYHO
Ex Power	45.0	15.2	HYLO	Troy	50.0	7.4	HYLO
Troy	47.5	12.5	HYLO	Ex Power	51.5	9.9	HYLO
Maturity N concentration of seed (%)				Maturity N concentration of stem (%)			
VARIETY	Rank mean	SD		VARIETY	Rank mean	SD	
Pacific	14.5	12.3	HYLO	Rodeo	11.0	3.7	HYLO
DK Cabernet	21.3	14.7	HYLO	Harper	11.8	1.7	HYLO
Ex Power	21.5	16.3	HYLO	Orlando	14.5	14.8	HYHO
Liragr□n	23.5	23.7	HYHO	Musette	20.0	13.0	HYHO
Rodeo	23.5	16.1	HYLO	SLM 0512	22.3	17.0	HYHO
Troy	24.5	20.4	HYLO	Zephir	22.3	11.7	HYHO
Charger	25.5	16.1	HYLO	Troy	22.8	21.9	HYLO
Orlando	27.5	16.7	HYHO	Ex Power	25.8	16.9	HYLO
Lipton	27.8	20.3	HYHO	Pacific	26.0	18.5	HYLO
Musette	28.0	21.6	HYHO	Charger	27.5	20.2	HYLO
PR46W21	28.0	16.4	HYLO	DK Cabernet	30.3	15.7	HYLO
Agalon	29.0	15.6	HYHO	Lipton	35.0	10.5	HYHO
SLM 0512	29.0	6.4	HYHO	Agalon	36.3	21.7	HYHO
Harper	32.8	17.0	HYLO	Liragr□n	37.0	18.7	HYHO
Idol	36.0	16.0	HYLO	Idol	39.5	21.2	HYLO
Zephir	40.3	14.6	HYHO	PR46W21	40.3	13.7	HYLO
Maturity N concentration of chaff (%)				Maturity total N Seed			
VARIETY	Rank mean	SD		VARIETY	Rank mean	SD	
Charger	16.0	13.6	HYLO	Agalon	14.3	13.2	HYHO
Pacific	23.0	10.2	HYLO	Liragr□n	23.5	14.1	HYHO
Harper	24.0	16.8	HYLO	Musette	24.3	10.8	HYHO
Troy	27.5	18.0	HYLO	Zephir	24.3	18.7	HYHO
Lipton	27.8	21.8	HYHO	Lipton	26.8	18.7	HYHO
Liragr□n	29.5	1.0	HYHO	Orlando	30.3	16.0	HYHO
SLM 0512	29.5	14.6	HYHO	Pacific	34.0	8.9	HYLO

Orlando	30.8	10.0	HYHO	SLM 0512	35.3	13.9	HYHO
Musette	33.0	22.5	HYHO	Rodeo	36.5	12.3	HYLO
DK Cabernet	34.3	25.3	HYLO	Charger	37.0	23.1	HYLO
Ex Power	38.8	12.3	HYLO	Idol	38.0	13.0	HYLO
PR46W21	39.3	15.7	HYLO	Troy	38.8	5.9	HYLO
Rodeo	39.8	18.9	HYLO	PR46W21	39.0	12.1	HYLO
Zephir	41.8	18.7	HYHO	Ex Power	45.5	9.5	HYLO
Idol	43.8	9.8	HYLO	DK Cabernet	47.0	9.2	HYLO
Agalon	46.0	9.1	HYHO	Harper	49.5	8.9	HYLO
Maturity total N of stem (kg/kg)				Maturity total N of chaff (kg/kg)			
VARIETY	Rank mean	SD		VARIETY	Rank mean	SD	
Orlando	8.0	5.4	HYHO	Orlando	18.0	3.3	HYHO
Zephir	15.3	15.1	HYHO	Charger	23.0	19.4	HYLO
Agalon	22.8	9.6	HYHO	Musette	27.3	10.8	HYHO
Musette	24.0	19.8	HYHO	SLM 0512	27.3	8.7	HYHO
Charger	24.5	20.1	HYLO	Zephir	27.5	15.0	HYHO
Harper	26.3	10.2	HYLO	Agalon	29.3	17.4	HYHO
Lipton	26.8	12.8	HYHO	Pacific	30.3	12.3	HYLO
Pacific	27.5	5.8	HYLO	DK Cabernet	31.0	21.6	HYLO
Rodeo	31.0	8.2	HYLO	Lipton	35.3	11.6	HYHO
SLM 0512	32.0	14.0	HYHO	Harper	36.3	13.2	HYLO
Ex Power	37.5	20.2	HYLO	Liragr□n	41.0	10.2	HYHO
Liragr□n	39.3	13.7	HYHO	Troy	41.5	19.8	HYLO
Troy	40.5	21.7	HYLO	PR46W21	43.0	14.7	HYLO
Idol	42.8	14.2	HYLO	Rodeo	45.3	8.1	HYLO
DK Cabernet	43.8	9.7	HYLO	Idol	48.0	11.4	HYLO
PR46W21	44.0	13.9	HYLO	Ex Power	55.5	1.9	HYLO

3.3.13. UAV Imaging analysis: full field and plot images

The low-cost UAV successfully imaged the RIPR field trial at full site (Fig 9) and plot scale. The images allowed an assessment and record to be made of trial health and trial consistency across the full site. At the DP14 site (image not shown), we clearly visualised and assessed black-grass in the 6th block and patchy growth – possibly due to underground piping common in the area. In HM14 bird damage in March could be seen from the UAV images and preventative action was taken. The plot images had a resolution of 10 cm. These plot images are of high enough quality for image analysis using Image J software. The plot images showed that crops had a dramatic change in colour and appearance during phenology of WOSR at flowering; initial pod development; and maturity.

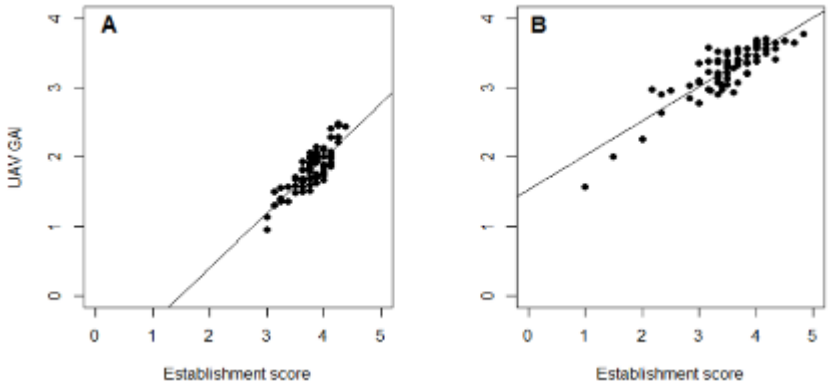


Figure 40. Relationship between UAV GAI assessment and field establishment score at: A) DP14; and B) HM14. Each point represents a mean genotype n=3.

There was a strong relationship between establishment score and UAV assessed GAI from November (Fig 40), at both DP14 (r^2=0.67, p< .0001) and HM14 (r^2=0.74, p< .0001). This shows that we can score establishment variation in large field trials using an RGB sensor on a UAV system. Field trial establishment data used to evaluate the image analysis covered a wide range and included both environmental and genotype variation thus allowing thorough testing of the UAV image analysis. Crop establishment was scored in October at both sites: DP14 had a mean establishment score of 3.81 with a range of 3 – 4.37; HM14 has a mean score of 3.49 with a range of 1 – 4.8. Assessing data, the scales are not the same, so a 1:1 relationship was not expected. Establishment scores were higher and had a tighter range at DP14, but GAI was higher at HM14 (Fig 41). This could be due to different scales of establishment and GAI, or to differences in establishment scoring between sites. It is not an important point as the ranking of genotype within the site is the most important factor for the diversity trial.

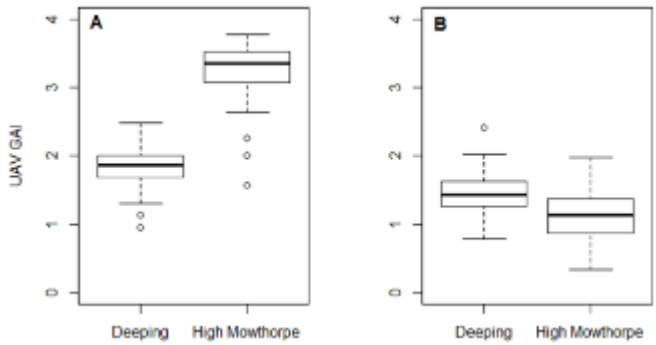


Figure 41. Boxplots for UAV GAI assessment at DP14 and HM14 in: A) November; and B) March. Each point represents a mean genotype n=3.

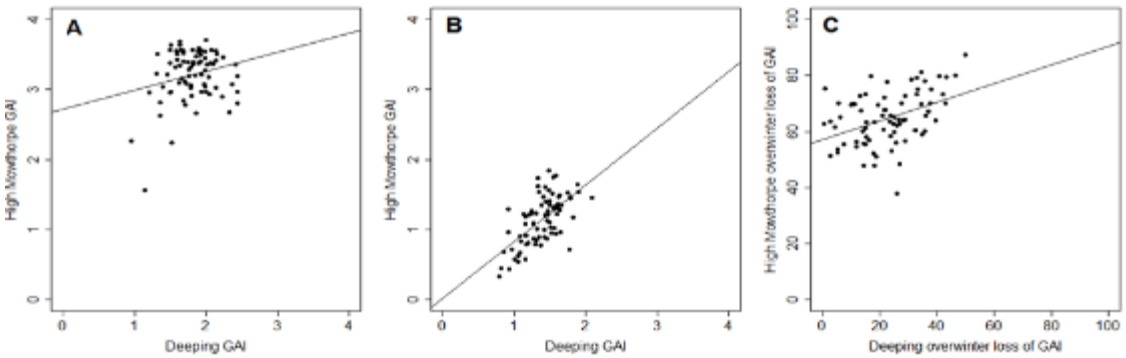


Figure 42. Relationship with DP14 and HM14 sites for: A) November GAI; B) March GAI; and C) over-winter GAI loss (%). Each point represents a mean genotype n=3. Lines fitted by linear regression.

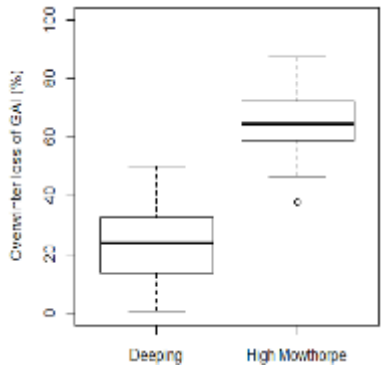


Figure 43. Boxplots for over-winter GAI loss at DP14 and HM14 presented as % GAI lost between November and March assessment.

GAI data from November and March was used to assess site and genotype differences of establishment (Fig 41) and over-winter crop health (Fig 43). In November, GAI was higher at HM14 (mean GAI 3.26) than DP14 (mean GAI 1.83). HM14 had higher initial growth in autumn; this was probably due to higher September soil N (HM14, 74 kg N kg/ha; DP14, 54 N kg/ha). In March, GAI was similar at HM14 (mean GAI 1.13) and DP14 (mean GAI 1.44). Comparing genotype GAI between sites (Fig 42), there was a strong relationship in March (r^2=0.40, p < .0001) than in November (r^2=0.05, p = NS), meaning genotype had more of an impact on crop health in March than November. Over-winter health was assessed as a % of GAI lost between November and March (Fig 43). Overwinter loss of WOSR is common, and is referred to as loss of soft growth, but it can have an impact on NUE. HM14 suffered a mean GAI loss of 64.8% loss, much higher than DP14 which had a mean loss of 23%. At both sites, there was a high range of genotype response.

The UAV images in this study clearly distinguished between flowering and maturity developmental stage. There is potential to score flowering and maturity using image analysis by assessing the proportion of yellow and brown respectively. Within this project we did not develop the image analysis techniques but we did score UAV images manually (data not included). Image analysis could be

developed to compare and use the RGB images; image scores; and field trial assessment. A limitation of this approach to assessment is that trialists are assessing the top of view of the crop only, which inevitably means % flowering or pod cover cannot be calculated accurately.

3.4. Discussion

3.4.1. Yields and NUE

Despite working with old and foreign genotypes, and very LN conditions, the majority of plots showed successful germination and healthy growth. Increasing yield at LN is highly effective at improving NUE and reducing the negative effects on the environment (Burney et al. 2010). This study showed an incredibly wide range of yields and NUE for genotypes at both LN and HN (Fig 10). It also showed large differences in Yield N response (Fig 16). The difference in fertiliser N (FN) applied between LN and HN plots was substantial – in the 2014-2015 trials 180 kg/ha additional FN at HM14 and 160 kg/ha additional FN at DP14. In these trials some genotypes showed no yield loss between FN treatments, while some genotypes suffered 50% loss of yield (Fig 15). It was very surprising that some genotypes applying less N than currently applied.

A WOSR field trial study (Berry et al., 2010) has shown an interaction between genotype and N supply for yield. A Stahl (2017) found a strong increase in yield and NUE for both HN and LN, with a high correlation between the treatments. Bouchet et al. (2014) found small G × N interactions when FN differences between treatments of 80–90 kg N ha−1.

The linear analysis in this study done between genotype and yield at HN and LN was significant at both 2014-2015 sites (Fig 17): HM14 ($r^2 = 0.41$, $p < .0001$); and DP14 ($r^2 = 0.48$; $p = < .0001$). However, the relationship was affected by genotype category. ASSYST types showed a positive relationship between N treatments ($r^2 = 0.27$, $p < .0001$); and DP14 ($r^2 = 0.45$; $p < .0001$). Elite types showed no significant relationship between LN and HN at either site (Fig 18). This apparent difference between the groups may simply reflect the relatively narrow range of yields seen for the elite genotypes. In fact, both groups show a similar range of (HN – LN) yield differences (Fig 16). The observed linear regression of ASSYST genotypes between yield at HN and LN yield could be due to the wider range of yields, due in turn to the inclusion of older and more diverse genotypes having other yield limiting factors (disease susceptibility, phenology, physiology) with a greater impact on yield than fertiliser efficiency. Stahl et al., 2017; and Kessel et al. 2012, rejected the hypothesis that cultivation of older genotypes could be beneficial for better nutrient use, since they might have comparative advantages in adaptation to low input systems.

Over the last few decades, breeders have selected and maintained growing environments to mimic farming practice, in order to select genotypes for the target environment. Since elite genotype and N supply does not have a linear relationship in this study, cultivars selected for high yield under HN conditions may not be the highest yielding under LN conditions. However, the modern elite genotypes

in this study achieved significantly higher yields at LN than older ASSYST types (Fig 11). Therefore, modern breeding at HN fertiliser rates has resulted in genotypes that are higher yielding at LN. The results of our study should encourage breeders to select genotypes directly at LN fertiliser conditions, in order to speed up the NUE breeding process. Although breeding for yield at HN does typically increase yield at LN, it is more likely due to key yield improving traits (weed resistance, seed filling) rather than improving NUE directly. The evidence is that we found no significant difference for yield loss between N treatments for elites and ASSYST types at DP14 or HM14 (Figure 16). Therefore the increased yield at LN of elites over ASSYST types was probably due to improved breeding of other features.

3.4.2 Mitigating N loss

The ultimate yardstick for mitigating N losses is to create a sustainable WOSR production system which requires less N inputs but still produces high yields. As we have already highlighted, improving yield is highly effective at increasing NUE and reducing the negative effects on the environment through ghg emissions. When breeding new NUE lines, maintaining high yields at LN is critical. Due to relatively low LN fertiliser costs and cost of land use to the environment, even a relatively small loss in yield, due to under fertilisation or management, has a big impact on NUE. Optimal fertilisation must ensure that the current rapeseed genotypes reach their full yield potential. Overuse of N fertiliser does cause an N surplus, which (as discussed) has an environmental cost. But loss of yield due to under fertilisation has substantial impacts when considering land use change (Don et al., 2011). These facts only highlight how breeding for yield has great financial and environment benefits. The range of proportional yield loss between LN and HN plots in this study suggests a significant variation in optimum N for current elite genotypes, with some elite genotypes showing no loss between HN and LN.

Better management of N application, with precise application rates dependent on genotype, could improve NUE. The large variation in yield at low N for elite types suggests immediate potential for improving NUE and increasing yield by recommending particular genotypes for areas which have legal limits for N application. These new studies support previous evidence (Berry, 2010; Storer 2018; Stahl 2017) that HYLO type genotypes exist, and that for these varieties, in all environments, N dosage of much less than the standard 180kg/ha rate is sufficient to produce the yield levels usually achieved in farming practice. However, to make these genotypes viable for the market, confirmation of effective NUE over multiple years and environments is essential.

There were several elite types that were in the HYLO group and were highly ranked for Yield and low optimum. These include: Pacific; Trinity; Temple; Quartz; Elgar; and PT211. There were also elite types that were high ranked for seed N utilisation: Compass; Orlando; Excalibur; and Pacific. These genotypes had a tight standard deviation within the mean ranking suggesting that the effects are consistent across sites. Further investigation and understanding of these interactions across

environments would be useful for both breeding NUE traits and for reducing N input through variety choice.

3.4.3. N uptake and utilisation

The wide range of NUE and other traits at low N suggests that there are potentially useful phenotypes for NUE available within the diversity panel. N uptake was significantly linked to LN yield at mid-flowering and maturity. At LN, N-uptake at maturity has a stronger relationship to yield than to seed utilisation. The results confirm that increasing N uptake is key for NUE and the production of high yielding genotypes at LN. Seed N concentrations had significant negative relationship for yield at LN, and were significantly lower for HYLO than HYHO genotypes. This suggests that if seed require lower N concentration, greater yield can result. This could be an indirect effect – new genotypes have lower seed N and increased yields.

There were significant associations between yield at LN and lower stem N (%) and seed N (%) (Fig 32). Stem % N concentration at maturity was negatively correlated with yield, but Stem DW had a positive relationship with yield (Fig 32). There is potential for Stem N remobilisation to be utilised in future ideotype work.

3.4.4. Phenology

It is important to understand how genotype differences in late N uptake are related to phenology traits such as: root activity or root growth; canopy traits at flowering such as leaf photosynthesis; or the GAI produced per kg of N uptake. Yield phenology is greatly affected by genotype and by environment. During the period from sowing until the onset of flowering, yield potential is set by the number of flower buds and leaf area index at onset of flowering (Mendham et al., 1981; Jullien et al. 2011). Conditions during flowering are particularly important for pod formation and seed set (Jullien et al. 2011; Habekotté, 1993), and the length of the seed filling phase partly determines assimilate availability for seed filling (Mendham et al., 1981). The stay-green phenotype in WOSR can increase time for NupE during the reproductive stage (Diepenbrock 2000; Rathke et al. 2006), and this study suggests it is a source of genetic variation for enhancement of NUE. Besides, late N remobilization may lead to greater N losses through a combination of leaf loss and a high N/C ratio in stems at harvest (Kaiser et al. 1998; Baggs et al. 2000).

In this study seed yield at LN was significantly linked at all sites to: establishment score; early flowering; and maturity score (Fig 31-33). These links were also significantly apparent when looking at these traits across all environments (Table 7). There were significant differences between HYHO and HYLO types for establishment and early flowering, but no difference between them for maturity (Fig 37). In our study we saw that that flowering was significantly earlier in the 2015-2016 trials than in the 2014-15 trials. However, we did not collect flowering data at HN; Stahl et.al (2016) studied flowering at HN and LN in outdoor containers and found a relationship between flowering date at LN and HN.

This study shows the value in monitoring establishment, flowering and maturity during breeding as an indicator of increased NUE. These traits are affected by environmental conditions but relationships with yield at LN were seen to be consistent across trials sites (Table 7). Linear regression analysis of LN traits showed that 1 point earlier in flowering score (representative of 2-3 days earlier flowering) would result in financial gain of £32.40/ha; £41 for establishment; and £52 for maturity score.

3.4.5. Trait analysis

The assessment of N uptake and partitioning of genotypes from a diversity panel showed a very wide range in traits. The diversity panel had greater variation in all traits than elite types (Appendix, Table 3), and this variation is critical to aid understanding of trait relationships with yield and NUE. In principle, working with a diversity panel allows assessment and pinpointing of N utilisation from a wider range of traits. This trait range information can be used to determine the phenotypic diversity in the field, and the interrelationships in the field population. This can also inform further studies; production of process based crop models; guidance for key traits in LN breeding trials; and subsequent studies to dissect the genetic bases and identify markers in traits relevant for crop improvement.

Within this work we have analysed trait relationship to yield at LN. The analysis showed that WOSR populations showed the same traits linking to NUE across a range of environmental conditions. The traits significantly linked to yield at LN were: Maturity total N of seeds; Maturity total crop N; Maturity dry weight of crop; N uptake post flowering; Maturity dry weight of stem; Maturity dry weight of chaff; Maturity score; Flower score; N biomass; Maturity total N of chaff; N concentration of seed; Mid-flowering Stem weight; and Maturity N concentration of chaff.

As described in the methods section, there were serious issues with environment and management in the 2015-2016 trials. In this study we found that N uptake post mid-flowering significantly correlated with yield at LN and was significantly increased for HYLO types. There was a very large range of N uptake post mid-flowering. At HM14 some types showed a 100 kg/ha uptake between mid-flowering and maturity. N uptake post flowering were positively correlated with maturity N concentration of chaff and negatively correlated with maturity N concentration of stem, suggesting possibly increased remobilisation and sink strength. However, this trait is not in practice easy to identify and define as part of a breeding trial. Leaf loss is very difficult to mark and score. It may be that other traits correlating with N uptake post flowering can be used as indicators.

Future work could assess and understand how genotypic differences in late N uptake are related to differences in root activity or root growth; or to canopy traits at flowering such as leaf photosynthesis or the GAI produced per kg of N uptake. He (2017) suggested seed number per silique may be a good indicator for rapid selection of N efficient genotypes. We did not measure this trait. However, we did find that dry weight or total N of siliques (chaff) and stems were significant predictors of yield at

LN. These traits both had significant correlations with yield at LN and values that were significantly higher at LN for genotypes which had lower N optima.

3.4.6 How good does a relationship need to be for a trait to be useful in selecting for NUE or yield?

NUE and yield are the final outcomes of complex cropping systems with Genotype × Environment × Management (G × E × M) interactions (Dresbøll and Thorup-Kristensen, 2014; Thorup-Kristensen and Kirkegaard, 2016). Significant p values over multiple trials indicate that relationships are potentially useful for prediction of NUE and yield. We have highlighted above the traits that are significantly linked to yield at LN across all trial sites. Individual genotypes that had different response to N were analysed by t-test with HYLO and HYHO data and also analysed by ranking of genotypes. Due to the trial design and failure, ANOVA across sites was not possible. In this analysis environmental effect was considered through ranking of genotypes for key traits across environments (Table 10). This will be discussed in greater detail in Chapter 6.

3.4.7. Practicalities of NUE screening for breeding

Methods of rapidly identifying breeding lines with lower N requirement are needed to improve NUE in breeding programs (Berry et al., 2010). Differentiating genotypes for NUE will require refined phenotyping in field trials to pinpoint the exact step where WOSR yield component is modified by N. As described above, analysis of data from these trials and further characterisation of HYLO genotypes have highlighted key traits linked to NUE providing promise towards further N fertiliser saving potential. I believe this study demonstrates that consistently including a LN environment within breeding programmes and trial schemes would increase our trait knowledge and give a strong indication of yield potential at LN. Genotypes with a greater yield at LN are likely to have a greater NUE at the economic N fertiliser application rate through achieving a similar yield with a lower economic N rate (Sylvester- Bradley and Kindred, 2009).

In this study, the collection of partitioned tissue and N analysis allowed us to refine phenotyping, model validation, and genome analysis by increasing our understanding of the range of N partitioning and traits under N limitation. However, such large studies are expensive and labour intensive. For example, N uptake post mid-flowering was significantly linked to NUE at all trial sites, but this assessment is very time consuming. A thorough assessment of remobilisation would need to include N content of fallen leaves. This study suggests the alternative, and perhaps easier, analysis of stem residues as an indicator of N remobilisation - in our study there was a negative relationship with this trait at all sites.

Another proposed solution to identify traits which might indicate high yield performance in a LN environment, but which are exhibited in higher N environments typically used within breeding and testing systems (Berry et al., 2010). As LN and HN yields of elites did not correlate, this approach will

be challenging. This study did not assess traits at HN; however, key traits that improved yield at LN are significantly linked to yield at HN.

UAV technology is becoming increasingly affordable and its use in agriculture is an active and current research topic (Yang et al., 2017; Deery et al., 2014). UAVs can collect fine-scale vegetation data over large areas. We collected image data from a low-cost hobbyist UAV as part of the RIPR diversity trial. The system trialled cost approx. £700. RGB sensors are very affordable; have relatively low storage requirements; and many image analysis techniques already exist for their use. Green area index (GAI) is commonly used as a growth measurement scale for canopy management. The GAI is assessed using the visible spectrum as the ratio of green leaf and stem to the area of the ground on which the crop is being grown. Many studies have used the visible spectrum to assess vegetative indices (Hunt et al., 2010). Recent studies have shown that a UAV system is capable of capturing field variability and to detect subtle differences in N stress (Zaman-Allah M et al. 2015). N status in WOSR has successfully been assessed using UAV multispectral images (Shishi Liu et al., 2018). Han et al. 2017 have conducted studies mapping above-ground biomass of WOSR using high spatial resolution satellite data.

3.5. Conclusions

For a more precise and direct selection of even more efficient genotypes, a better understanding of genotypic × environmental × management interaction and physiological determinants of NUE are essential targets for future research. This study has presented and assessed trait variation at LN of a genetically diverse population over two years and two environments. The results from field trials of a WOSR diversity panel showed a substantial genotypic trait variation. The 2014-2015 trials included genotypes with high yields and LN requirements. Genetic variation in traits and yield revealed that genetic improvement through modern breeding techniques in conjunction with reduced N fertiliser inputs has massive potential to increase NUE of WOSR. It is also clear that there already exist genotypes with a wide range of N requirements that could be utilised in farming practice. Significant relationships have been seen between yield at LN and key traits that are consistent over all environments. These traits include: post-flowering N uptake; seed N %; and early flowering.

Improvements in NUE may be maximised by combining N efficient genotypes with optimal fertiliser N timings (Berry and Spink, 2009); optimal inputs of other nutrients; appropriate cultivations; and effective disease control. Genetic improvements should reduce the reliance on fertiliser inputs, but yield improvements will play a leading role in sustainable crop production. Determining the phenotypic diversity in these traits in this population, and their interrelationships, could inform subsequent studies to dissect the genetic bases and identify markers in traits relevant for crop improvement.

Chapter 4. A physiological model of N-acquisition and N-utilisation in WOSR

Collaboration work statement

Work in this chapter is the refining, simplifying and testing the limits of an initial model developed by P Berry. The data used to validate the model is from field trials that have been published (Berry et al., 2010.)

4.1. Introduction

Precise management of N fertiliser application is required in order to maximise farm profitability and reduce negative environmental effects, including GHG emissions and nitrate leaching (Kindred et al., 2008). N fertiliser rates in the UK are generally chosen to maximise profitability for the grower. They are predicted by national guidelines (e.g. Defra, 2017), and the economically optimal N rate is that for which any further increase will result in greater N fertiliser costs than the value of the additional yield produced. Matching fertiliser application rates to site and genotype requirement will help to adapt management practices to minimise excessive N-balances while achieving optimum yields. The development of new N use-efficient OSR genotypes with high yield is crucial in order to meet environmental N regulations and ensure profit for farmers.

The relationship between applied N and yield in OSR is usually typified by a rapid increase in yield at low N rates, followed by a levelling off for the yield response, and often a reduction at super-optimal N rates due to factors such as lodging (Mendham et al. 1981). This response to N can be represented by a linear plus exponential relationship between N fertiliser rate and seed yield (George et al., 1984). The challenge in managing N and estimating the optimum N fertilisation rate comes from the complex interactions that exist in the dynamic soil-plant-atmosphere system and uncertainty in weather (Havlin et al., 2005) These processes are always changing throughout the season and are related to many factors. Identifying where breeding can be improved is limited by the ability to identify strong efficient genotypes and best management practice in particular environments (Hammer and Jordan, 2007).

Few published OSR yield models have considered a range of N supply when predicting yields. Existing models share the approach that potential growth is estimated from the product of absorbed photosynthetic active radiation (PAR) and light use efficiency (LUE), assuming the product is either constant or changes with development stage. Of the models available, some consider yield under specific N conditions (e.g. optimal N rate, Habekotté et al., 1997a, 1997b; or three N rates, Gabrielle et al., 1998a, 1998b). Habekotté (1997a) developed an empirical model, BRASNAP-PH, that successfully predicted early growth; onset and end of flowering; and maturity of WOSR in relation to temperature and photoperiod. The model was validated with a single genotype at various locations in

Western Europe. Böttcher et al. (2016) have described WOSR development using a combination of differential and conversion equations based on the structure of the BRASNAP-PH model (Habekotté, 1997a). This was able to reproduce the whole OSR cycle for a wide range of years; locations; sowing dates; and genotypes (Böttcher et al., 2016). The Agricultural Production Systems Simulator (APSIM) OSR module has produced consistently good yield predictions in multiple environments in Australia, although N was not a limiting factor at most sites (Robertson et al., 1999). Hoffman et al. (2015) successfully adapted the APSIM model to predict yield under limited N and water supply at multiple North German locations. However, a recent assessment of the APSIM model recommended further refinement of model parameters affecting response to N fertiliser (Robertson et al. 2016).

Few of the published simulation models for WOSR are able to simulate crop growth and yields in different environmental conditions involving N and water limitations (Yin et al., 2017). Böttcher (2020) developed and evaluated a plant growth model for WOSR, based on allometric partitioning, and tested the capability of the model for estimating crop growth, development and yield of WOSR under different environmental conditions and N treatments. The model response was validated with data obtained throughout Europe and was judged suitable to be applied for supporting optimisation of management strategies, climate change studies and future breeding programs. The model simulates crop growth on a daily basis from sowing until maturity. The study highlighted the lack of good data for harvest index and canopy data for later growth stages; this is a principal problem for high yielding OSR crops, which can have a very vigorous growth especially under high N supply. The authors also suggested the model could be simplified or made more complex depending on field application. To our knowledge, no models have been published which can explain genotype differences in yield productivity under low N, or identify genetic trait combinations to minimise N fertiliser requirement whilst maintaining or increasing yield.

Several genotype traits have been associated with the crop N requirement of OSR. Considering N acquisition efficiency (kg of N taken up per kg of N supply), initial root growth is important for early establishment (Mendham et al. 1981), and continuing root growth after stem extension is believed to be linked to continuing N uptake (Kamh et al., 2005; White et al., 2015). In low N field trials, high yields have been attributed to continuing acquisition of N after flowering (Berry et al., 2010a). A green area index (GAI) of about 3.5 during flowering has been associated with maximum production of seeds/m² (Lunn et al. 2001). Large canopy size would be expected reduce radiation use efficiency and result in surplus N remaining in the plant. Considering utilisation traits, genotypes with early flowering time and delayed senescence should have an extended period of photosynthetic capacity for seed filling and nutrient mobilisation from source to sink (Kamh et al., 2005; Schulte auf'm Erley et al., 2007). Stahl et al. (2016) recently found that in a low N environment, early flowering was associated with high N utilisation efficiency (kg of seed yield per kg of N taken up) and with low N residues in stems.

This chapter first describes a simplified model of yield formation under a range of N supply values, which takes account of N acquisition and utilisation in relation to dry-matter production. The model is tested with field data from two years; two sites; and 10 genotypes. The model is then used to identify genotype traits and trait combinations which can reduce N requirement whilst maintaining or increasing yield. Next, the model is tested with field data from RIPR trials with data from two years; two sites; and 85-120 genotypes (Chapter 3). Differences between trials and genotypes have been assessed. Rather than building on existing models, a new model has been generated and described, in order that the focus of development is minimising input trait data required while still successfully predicting the effect of genotype traits on yield under a range of N supply environments. Many traits have been identified as being important for N requirement, but this model has the potential to quantify the combined effect of multiple traits on N requirement. This information can be used for development of new N use-efficient OSR genotypes within breeding trials.

4.2. Methods

4.2.1 Model schematic and parameter

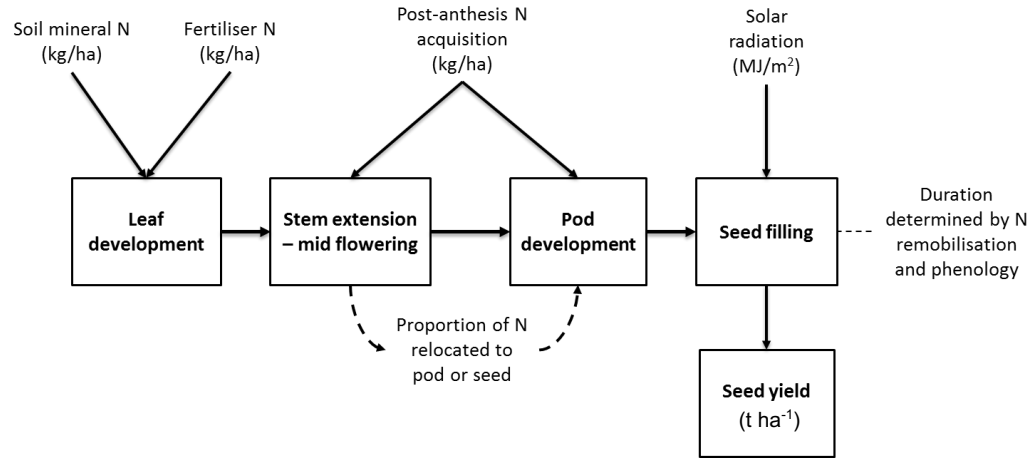


Figure 44. Schematic of representation of N uptake, N partitioning and crop growth of OSR as described in model. Solid arrows represent N uptake and dotted arrows represent remobilisation.

Table 13. Model inputs: parameters, nomenclature, units, genotype ranges, default values and sources. Range for crop traits is a genotype range under low N conditions. † Where no range is provided data were unavailable.

Parameter	Nomen-clature	Unit	Range*†	Default value	Source
Total crop N content at the point of stem extension	x	kg/ha	59-73	64	Berry et al. (2010)
Soil mineral N (SMN) at stem extension	y	kg/ha	30 to 90	40	Defra (2010)

Fertiliser N applied	z	kg/ha	0 to 400		
Crop acquisition of SMN between stem extension and flowering	ye	proportion	0.5-1.0	0.8	Berry et al. (2011a,b)
Crop acquisition of fertiliser N between stem extension and flowering	ze	proportion	0.5-0.7	0.6	Berry et al. (2011a,b)
Crop specific N content of leaf + stem area at mid flowering	η	g of N/m^2 tissue	4.7	4.7	Mendham et al. (1981)
Crop post anthesis N acquisition	ν	kg/ha	0-34	6	Berry et al. (2010)
Proportion of crop leaf N & stem N that could be relocated to pods or seeds	$a\nu r$	proportion	0.67-0.82	0.75	Berry et al. (2011b)
Pod specific crop N required for photosynthesis + structure	σ	g of N/m^2	7.5	7.5	Author estimate
Proportion of crop pod N that can be relocated to seeds	φr	proportion	0.67-0.82	0.75	Berry et al. (2011b)
Extinction coefficient of canopy post-anthesis	κ	dimensionless	0.54-0.66	0.60	Gabrielle et al. (1998)
Radiation Use Efficiency of crop canopy	Δ	g/MJ PAR	1.17-1.43	1.30	Mendham et al. (1981)
Variety factor for variation in Seed N concentration	Λ	%	0-0.16	0.08	Berry and Spink, (2009)
Daily incident light during seed filling	δ	MJ/m^2 PAR	8-12	9	
Duration from start of seed filling to natural senescence	υ	days	40-50	45	Mendham et al. (1981)

The model consists of four modules: leaf development to the start of stem extension; stem extension to mid-flowering; pod development; and seed filling (Fig. 44). Crop N uptake before flowering is driven by the supply of N from the soil and fertiliser and the efficiency at which the genotype takes up these N sources. N uptake post-flowering is driven by genotype differences in N demand. The proportion of N taken up by flowering which can then be remobilised to the developing pods is also dependent on

genotype factors. The amount of N available for pod development is used to estimate the GAI of the canopy during seed filling, which together with assumptions about the canopy extinction coefficient and radiation use efficiency determines the rate of seed dry matter growth. Genotype differences in the seed N concentration then determine how quickly N must be remobilised from the pod walls and stems to the seed, the duration of seed filling being limited either by the amount of N available for seed growth or by a specified maximum duration for seed growth. Model inputs are: environmental parameters; soil N supply; and solar radiation; together with a range of crop characteristics associated with the capture and utilisation of N and solar radiation. Table 13 presents: nomenclature; genotype ranges; default values; and data source for each model parameter. The model has been developed in the R language environment for statistical computing (R Core Team, 2015).

4.2.2. Detailed development of the model

Leaf development up to the start of stem extension

Total N acquired by the crop at the start of stem extension (x, kg/ha) is dependent on the genotype and environmental conditions. The model does not distinguish between fertiliser N and soil mineral N (SMN) uptake at this point, although it is likely that the majority of the N taken up will be SMN from the previous crops. As the first model inputs are at the start of stem extension the model does not consider autumn establishment. However, within the model crop, factors affecting N taken up at the start of stem extension will encompass: establishment, environmental, and genotype differences that will be relevant to yield.

Stem extension to mid-flowering.

The model estimates crop N acquisition at mid-flowering (a, kg/ha) using Equation 1, in which y is the soil mineral N (SMN) at the start of stem extension; z is the amount of N fertiliser applied; and (ye) and (ze) are genotype efficiencies of N acquisition from SMN and fertiliser N respectively. A maximum limit of N acquisition of 350 kg/ha is set. This maximum value is explained by the physiological limit for daily N acquisition of 3 kg/ha (Berry and Spink, 2009).

The crop N content at mid-flowering (a, kg/ha), and specific N content of the combined leaf and stem tissue (η, g of N /m^2 of tissue) (Mendham et al., 1981), are used to estimate GAI at mid-flowering (Equation 2).

$$a = x + y(ye) + z(ze) \quad \text{(Equation 1)}$$

$$\text{GAI} = (a / \eta) / 10 \quad \text{(Equation 2)}$$

Pod development

The amount of N available for pod growth (φ, kg/ha), is calculated using Equation 3 in which: (a) is crop N content at mid-flowering; (ν, kg/ha) is post anthesis N acquisition; and ($a\nu r$) is the proportion of leaf N and stem N at flowering that could be relocated to pods and seeds. The pod area index (βp) is calculated using Equation 4 in which σ is the pod specific N. Pod area index (βp) represents the biomass of pods.

$\varphi = (a + \nu)*a\nu r$ (Equation 3)

$\beta p = (\varphi / \sigma) / 10$ (Equation 4)

Seed filling

Total N available for seed filling (Ω) (kg/ha) is calculated using Equation 5, in which φr is the proportion of pod N that can be relocated to seeds.

$\Omega = \varphi * \varphi r$ (Equation 5)

Proportion of light intercepted by pods (ρ), is calculated using Equation 6, in which κ is the extinction coefficient of canopy post-anthesis.

$\rho = 1 - EXP(-\kappa * \beta p)$ (Equation 6)

Daily biomass accumulation of seed from current photosynthesis (ω, t ha^{-1}), is calculated by equation 7 using: the canopy radiation use efficiency (RUE, Δ); daily incidence of light (δ); and proportion of light intercepted by pods (ρ). The default value for radiation use efficiency is set at 1.3 g/MJPAR, based on measurements of dry matter accumulation during seed filling (Mendham et al., 1981). Note this RUE value is lower than typical for C3 plants because it takes account of the high energy cost of accumulating oil rich seed biomass. This model assumes a seed oil concentration of 40 g oil per 100 g seed biomass, measured at 100% dry matter.

$\omega = \Delta * \delta * \rho / 100$ (Equation 7)

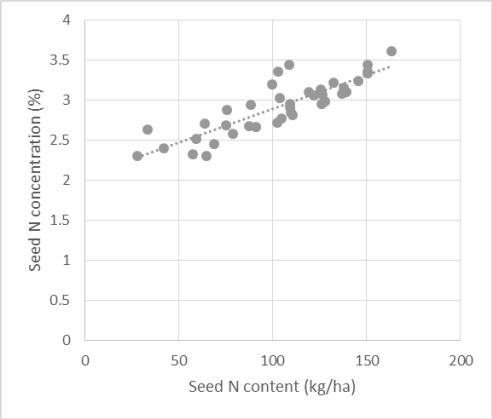


Figure 45. Relationship between seed N content and seed N concentration; y=0.009x – 2.05; R^2=0.74, P<0.001). Data from nine experiments described in Berry and Spink (2009).

N content of seed (τ) (%) is estimated from Equation 8 where Λ is genotype factor for seed N and Ω is the total N supply available for seed filling (kg/ha). This relationship was developed from data published in Berry and Spink (2009) (Fig. 45). N withdrawn from the pod wall to the seed during one day (λ) (kg/ha) is calculated using Equation 9 in which τ is the N concentration of seed (%) and ω is the daily biomass accumulation of seed from photosynthesis (t ha^{-1}).

$$\tau = 1.9 + \Lambda + \Omega * 0.009 \qquad \text{(Equation 8)}$$

$$\lambda = \omega*(\frac{\tau}{100})*1000 \qquad \text{(Equation 9)}$$

The model calculates the number of days (o) required for relocation of available translocatable N from the pod walls to the seed using Equation 10 in which Ω and λ are total N available for seed filling and N withdrawn from pod wall in one day respectively. The user inputs the number of days from start of seed filling until crop senescence that would be expected in non N limiting conditions (υ). The duration of seed filling is then taken as either the number of days for all available N to be relocated to the seed or the seed filling period under non N limiting conditions - whichever is shorter. Seed yield (θ) (t ha^{-1}) is then calculated using Equation 11.

$$o = \frac{\Omega}{\lambda} \qquad \text{(Equation 10)}$$

$$\theta = \mathrm{IF}(o < \upsilon, o*\omega, \upsilon*\omega) \qquad \text{(Equation 11)}$$

4.2.3. Data used for model testing

Table 14. N treatments of low N supply field data (Berry et al., 2010), Soil mineral N and Crop N at GS 3.1.

Site	Year	Soil mineral N at GS 3,1 (kg/ha)	Crop N at GS 3,1 (kg/ha)	N fertiliser rate (kg/ha)
High Mowthorpe (HM07)	2006/7	14	37	80
Rosemaund (RM07)	2006/7	20	137	0
High Mowthorpe (HM08)	2007/8	38	34	0
Rosemaund (RM08)	2007/8	63	69	0

The model has been tested using field data from low N supply trials (Berry et al., 2010). Field experiments were carried out at two sites in the UK in 2006/7 and 2007/8: ADAS Rosemaund (2.6°W, 52.1°N) (RM07 and RM08) on a silty clay loam (Bromyard series); and ADAS High Mowthorpe (0.5°W, 54.1°N) (HM07 and HM08) on a shallow silty clay loam overlying chalk (Wold series). The same experiment was conducted each year at each site including 10 genotypes (Canberra, FD502, Grizzly, Lioness, NK Bravour, PR45D01, Royal, Sun, Tapidor and Winner) grown using either zero N fertiliser or a low rate of N fertiliser (Table 14). Duration from start of seed filling to natural senescence; Canopy radiation use efficiency; and genotype factor for Seed N input were not measured, so were set to default values (Table 13).

The model was further tested using the RIPR trial data. The data and the methods described for collecting the data can be found in chapter 2.

4.2.4 Statistical analysis of model evaluation

Observed yield data was compared with model predicted values. To assess the goodness of fit of the simulated—measured comparisons the root mean square error (RMSE) between predicted and observed data was calculated as follows:

$$\text{RMSE} = \left[\sum \frac{(O - P)^2}{n} \right]^{0.5} \qquad \text{RMSE = (Equation 12)}$$

In equation 12 O and P are the paired observed and predicted data and n is the total number of observations. Additionally, for comparison, the traditional r^2 regression statistic (least-squares coefficient of determination) forced through the origin was calculated).

5.2.5. Sensitivity analysis

Sensitivity analysis was conducted of the impact of each model input on yield at fertiliser N rates of 0 and 200 kg/ha. Each input parameter was varied about its extreme range as defined in Table 13, whilst all other model inputs were kept the same using their default values. This exercise was repeated for all combinations of all traits and environmental factors to define the full output range under all possible crop type and environment combinations. The N fertiliser rates used were 0 – 420 kg/ha in 60 kg/ha intervals. This tested over 3.8 million parameter combinations at each fertiliser rate.

4.3. Results

4.3.1. Model evaluation

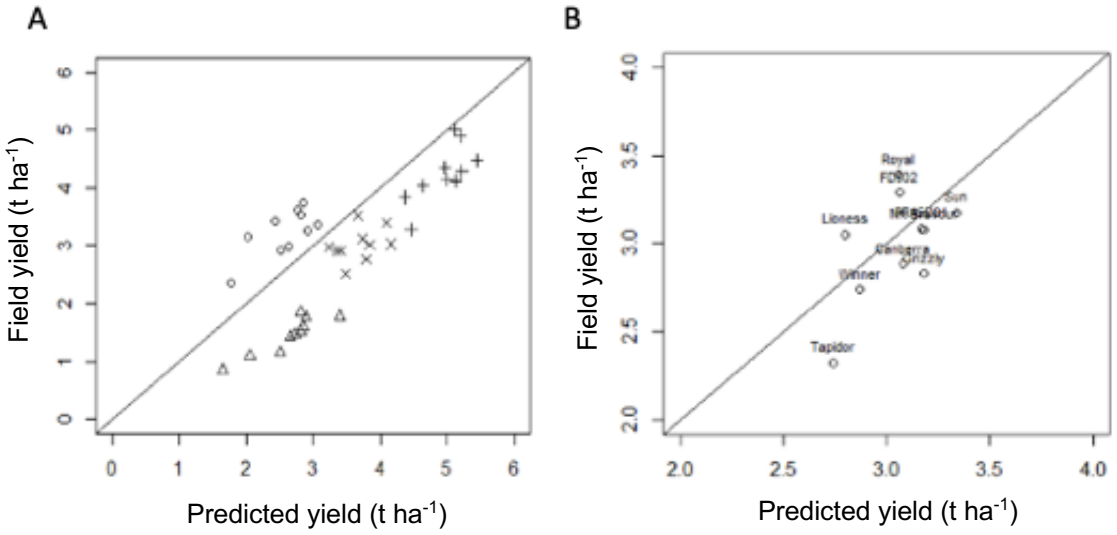


Figure 46. Relationship for observed field and predicted yields of; (A) field trials; RM07 (+), RM08 (X), HM07 (Δ), HM08 (O) and (B) genotype mean values across 4 sites.

Field trial data used to evaluate the model covered a wide range of yields (0.85 t ha^{-1} – 5 t ha^{-1}) and included both environmental and genotype variation. The data therefore allowed thorough testing of the model. When considering all data at harvest (Fig. 46), observed grain yields matched predicted yield with an RMSE of 0.57 t ha^{-1} against an observed mean of 2.99 t ha^{-1} (r^2=0.78). The r^2 indicates a good relationship between genotype field yields and predicted yields. For the Rosemaund site over both years, observed grain yields matched predicted ones with an RMSE of 0.46 t ha^{-1} against an observed mean of 3.63 t ha^{-1} (r^2=0.80). At HM08, predicted yields were underestimated but there was a strong relationship between observed and predicted yields, with an RMSE of 0.81 t ha^{-1} against an observed mean of 1.45 t ha^{-1} (r^2=0.81). In the 2007 trial the model slightly overpredicted yields. The observed grain yields matched predicted ones with an RMSE of 0.47 t ha^{-1} against an observed mean

of 3.24 t ha^{-1} (r^2=0.423). When considering mean values of genotype inputs across sites there was a relationship of r^2=0.32 suggesting the model has an ability to predict genotype response to N over multiple sites.

4.3.2. Sensitivity analysis

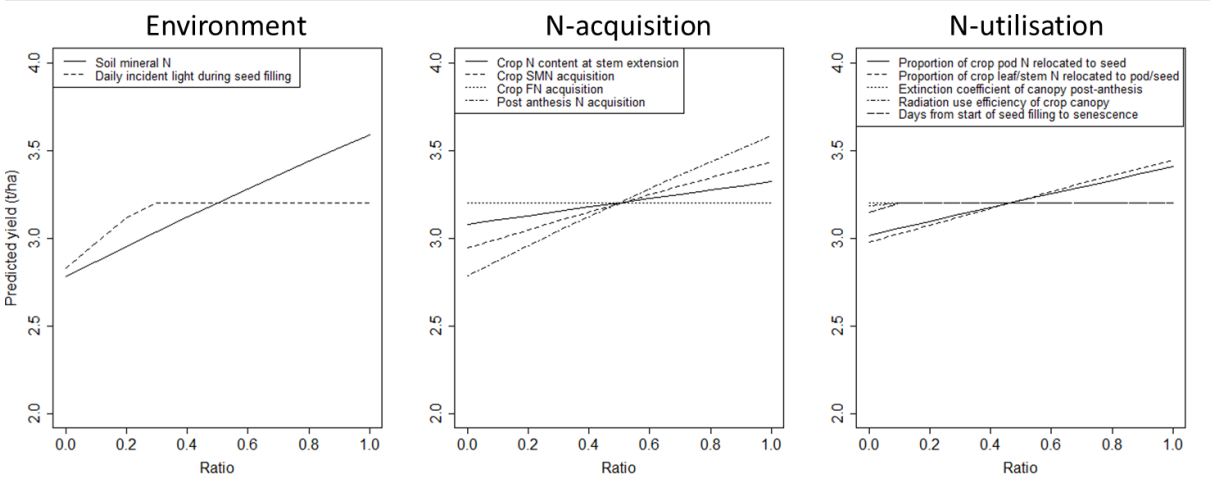


Figure 47. Sensitivity assessment of environmental, N-acquisition and N-utilisation model parameters at 0 N kg/ha fertiliser for: a) Environmental, b) N-acquisition and c) N-utilisation. The ratio (0 to 1) represents the normalised crop genetic and environmental range of each parameter. Table 13 for values.

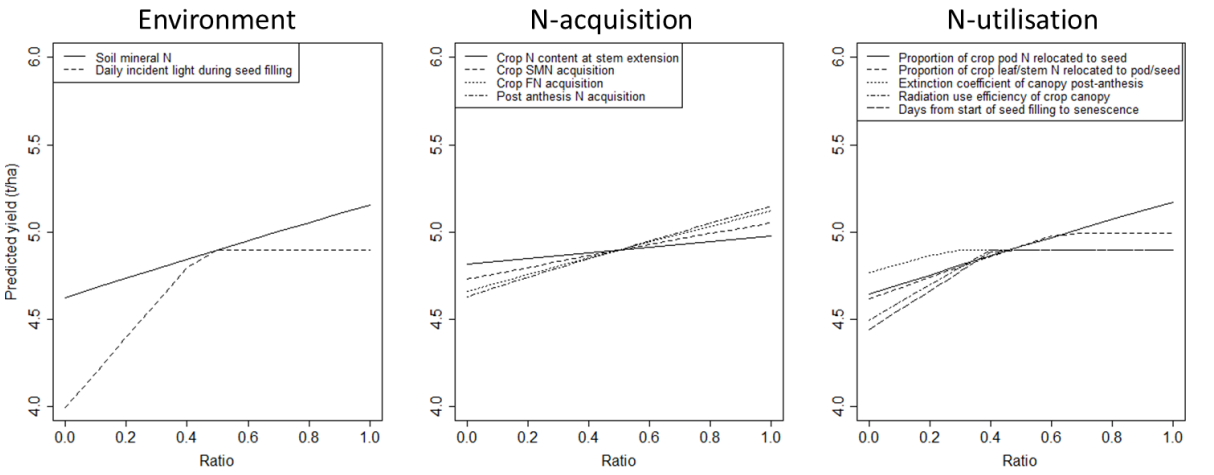


Figure 48. Sensitivity assessment of environmental, N-acquisition and N-utilisation model parameters at 200 N kg/ha fertiliser for: a) Environmental, b) N-acquisition and c) N-utilisation. The ratio (0 to 1) represents the normalised crop genetic and environmental range of each parameter. Table 13 for values.

Sensitivity analysis for individual traits and inputs shows that different environmental, N-acquisition and N-utilisation factors are most influential for yield at 0 N fertiliser (Fig. 47) compared with yield at 200 kg/ha N (Fig. 48). N-acquisition traits are more important for yield when N is limited. Conversely, N-utilisation traits are more influential on yield at 200 kg/ha applications when N-acquisition is less of

a limiting factor to crop growth. At 0 N fertiliser the most influential model input was the environmental parameter SMN (yield effect of 0.81 t ha^{-1}) followed by the genetic traits: post-anthesis N acquisition (0.79 t ha^{-1}); SMN acquisition efficiency (0.49 t ha^{-1}); and crop establishment through to autumn (0.24 t ha^{-1}). At 200 kg N/ha the most influential model input was the environmental parameter daily light incidence (yield effect of 0.9 t ha) followed by the genetic traits: days for seed filling (0.56 t ha^{-1}); N remobilisation from pods (0.53 t ha^{-1}); post-anthesis N acquisition (0.52 t ha^{-1}); SMN (0.52 t ha^{-1}); and fertiliser N acquisition efficiency (0.46 t ha^{-1}). N-acquisition was affected most by post-anthesis N acquisition at both 0 N fertiliser and 200 kg/ha fertiliser N, improving yields by 0.79 t ha^{-1} and 0.52 t ha^{-1} respectively. Considering N-utilisation traits, the proportion of both pod N and stem/leaf N that can be remobilised was important within the model at both 0 fertiliser N and 200 kg/ha fertiliser N. At 200 kg/ha days from start of seed filling to natural senescence was the most important utilisation trait (0.56 t ha^{-1}) - the effect of this trait is limited by the amount of N available for seed filling. Genotype variation in seed N concentration was the only trait that reduced yield at the top of the trait range as a result of higher N requirement of seeds. It was influential on yield at both 0 N fertiliser and 200 kg/ha^{-1} fertiliser N, having a yield effect of 0.19 t ha^{-1} and 0.46 t ha^{-1} respectively.

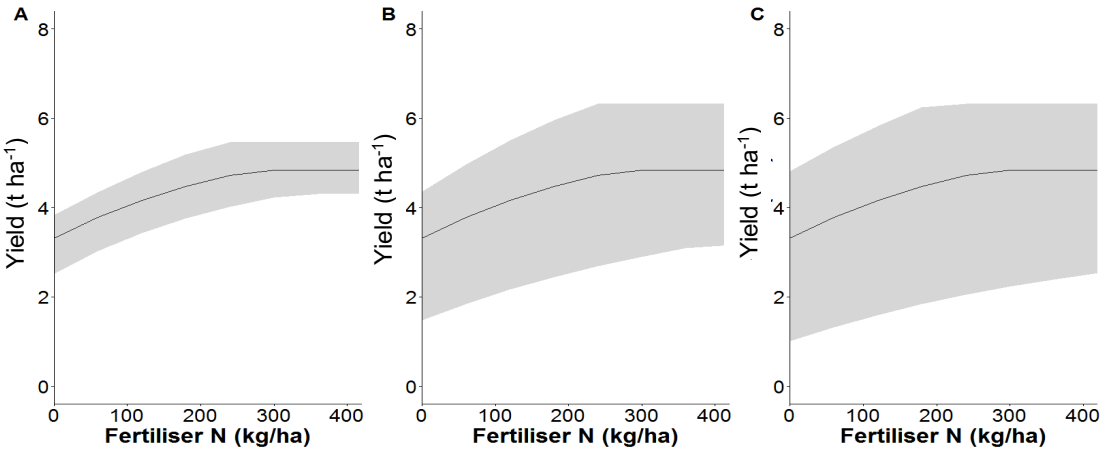


Figure 49. Simulation analysis of environmental and genotype parameters. The solid line shows predicted yields using the model default values. The grey area shows predicted yields using the full range of parameter values for: (A) ranges of environmental parameters, (B) ranges of genotype parameters and (C) range of environmental and genotype parameters.

The range of yields shown by the sensitivity analysis (Fig. 49) are typical of UK yields (1-6 t ha^{-1}). This large yield range is due to environmental, management and genotype differences and highlights the plasticity of OSR yields. The predicted relationship (Fig. 49) between applied fertiliser N and yield in OSR is typical of that seen in field experiments and is defined by a rapid increase in yield at low N rates, followed by a levelling off of the yield response at higher rates. Variation in the genotype traits lead to a wider range of yield (1.6-6.4 t ha^{-1}) than variation of environmental inputs (2.5-5.2 t ha^{-1}) illustrating that genotype traits have a great potential to influence on yield. It should be however recognised that this model does not include all possible environmental factors (e.g. water supply).

With all traits considered there was an additive effect on yield ranges when considering both genotype and environmental ranges (Fig. 49C).

With 0 N fertiliser the maximum yield potential was estimated to be 4.8 t ha, whereas at 200 kg/ha N fertiliser the yield potential was 6.4 t ha (Fig. 49). To achieve 4.8 t ha^{-1} with 0 fertiliser N the following genotype traits were set at the top of their range as defined in Table 13: Total crop N content at the point of stem extension; Crop acquisition of SMN between stem extension and flowering; Crop post anthesis N acquisition; Proportion of crop leaf N & stem N that could be relocated to pods or seeds; and Proportion of crop pod N that can be relocated to seeds. The environmental trait SMN was set to its range maximum and daily light incidence was 12 MJ/m^2. For the remaining genotype traits there was a range of suitable values that would enable high yield including: radiation use efficiency, 1.17-1.43 g/MJ PAR (mean 1.34 g/MJ PAR); duration from start of seed filling to natural senescence, 40-50 days (mean 46.4 days); extinction coefficient of canopy post-anthesis, 0.54-0.66 (mean of 0.6).

4.3.3. RIPR diversity field trial results

The data from the diversity trials (Chapter 3) has allowed testing of the model with a larger diversity set. This data was taken from two sites over two years. The field trial dataset used to evaluate the model covered a wide range of yields (0.45 t ha^{-1} – 5 t ha^{-1}), and as described in this thesis also had a wide range of genotypic and environmental variation. For example, inputs for N uptake post flowering varied from -20 kg/ha to + 200 kg/ha. When considering the RIPR data the model over predicted yield. Considering all data from the RIPR trial dataset (Fig. 50) at harvest, observed grain yields matched predicted yield with an RMSE of 0.49 t ha^{-1} against an observed mean of 1.71 t ha^{-1} (r^2=0.68). The r^2 indicates an ability to predict ranking of genotype field yields.

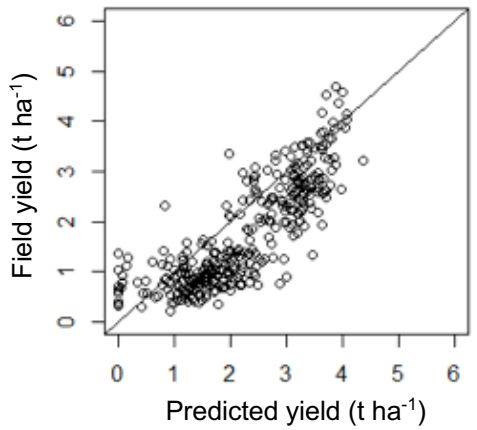


Figure 50. Relationship for observed field yields (t ha^{-1}) from the RIPR trials and model predicted yields (t ha^{-1}) of all genotypes across all environments. The solid line is a 1:1 relationship.

The primary reason for the over prediction and high RMSE is the over prediction of the 2015-2016 trial yield data (Fig 52, C, D). The model was unable to accurately predict the 2015-2016 data considering the extremely low yields in field trials. The RIPR 2015-2016 trials were lower yielding than 2014-2015 due to environmental and management factors; primarily serious black grass and galium issues limiting yield in the majority of plots. These factors have been further explained in chapter 3. Much of the yield data from the 2015 RIPR trials was below the model predictive range (Fig 49). In fact, some of the genotypes had extremely low or even 0 for the predicted yield. This was due to the crop losing a very high amount of N between stem extension and maturity so that there was no N available for seed filling in the model. The quality of data was not high enough to produce physiologically accurate results in this case.

Fig 51 shows the much stronger prediction of 2014-2015 trials than 2015-2016 trials. Considering the 2014 trials, the model showed good predictive power with a RMSE of 0.39 t ha^{-1} against an observed mean of 2.68 t ha (r^2 = 0.38). Both sites had RMSE of under 15% (DP14 (15%); HM14 (13.5%). In contrast, both 2015 sites had very poor RMSE: DP15 (46%); and OM15 (76.74%). In fact, some of the RIPR trial treatments had a predicted yield of 0.

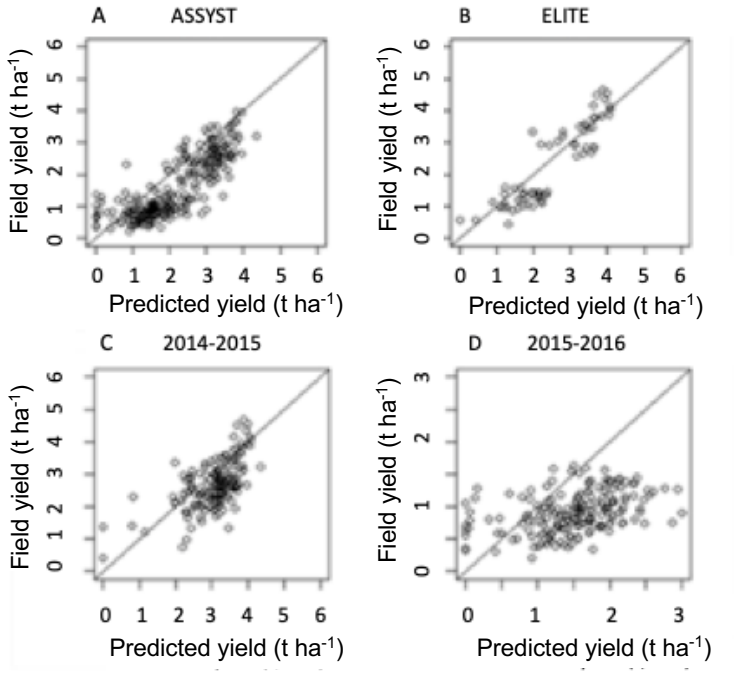


Figure 51. Relationship between observed field yields (t ha^{-1}) from the RIPR trials and model predicted yields (t ha^{-1}) of genotypes, separated as: (A) ASSYST types; (B) elite types; (C) 2014-2015 trials; and (D) 2015-2016 trials. Solid lines are 1:1 relationship.

Considering all data, elite types had a good prediction (Fig 51B) with RMSE of 0.18 t ha against an observed mean of 2.27 t ha^{-1} (r^2 = 0.81). ASSYST genotypes were less well predicted, the model over-predicting yields (Fig 15A) with RMSE of 0.56 t ha^{-1} against an observed mean of 1.59 t ha (r^2=0.66).

In 2014, elite types had RMSE of 0.10 t ha^{-1} against an observed mean of 3.48 t ha (2.87% NRMSE, r^2=0.37) (Fig 52B). For the ASSYST types RMSE was 0.50 t ha^{-1} against an observed mean of 2.49 t ha^{-1} (r^2=0.33) (Fig 52A). The predictive power of 2015 trials was very poor. For elite types RMSE was 0.45 t ha^{-1} against an observed mean of 1.17 t ha^{-1} (r^2=0.35) (Fig 52D). For ASSYST types RMSE was 0.61 t ha^{-1} against an observed mean of 0.84 t ha^{-1} (r^2=0.13) (Fig 52C).

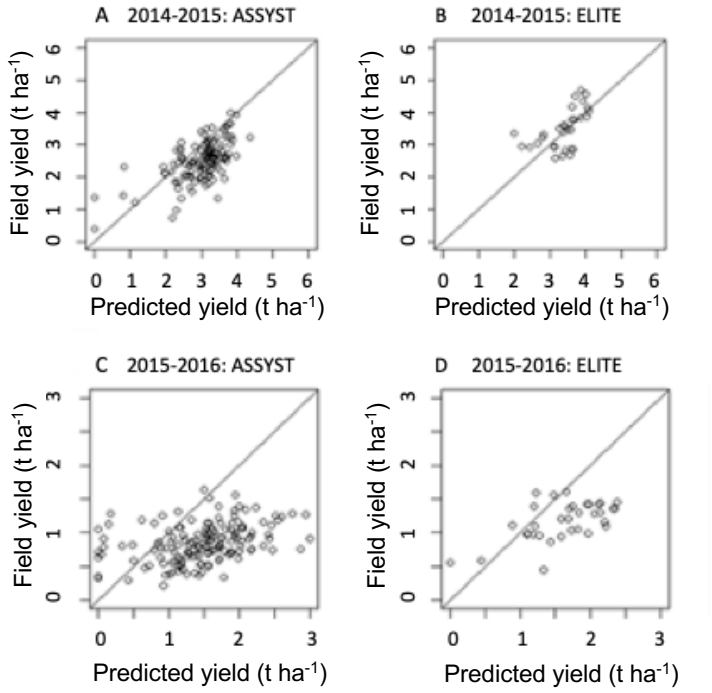


Figure 52. Relationship for observed field yields (t ha^{-1}) from the RIPR trials and model predicted yields (t ha^{-1}) of genotypes at sites: (A) 2014-2015 ASSYST; (B) 2014-2015 elite; (C) 2015-2016 ASSYST; and (D) 2015-2016 elite. The solid lines are 1:1 relationship.

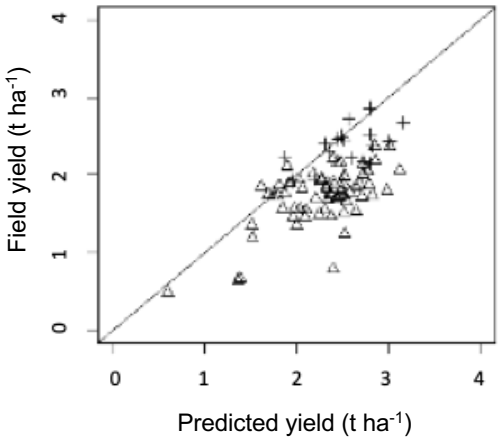


Figure 53. Relationship for observed field yields (t ha^{-1}) from the RIPR trials and model predicted yields (t ha^{-1}) of genotypes across sites. Values are mean from across 4 trial sites. Elite genotypes are shown as (+) and ASSYST as (Δ). The solid line is a 1:1 relationship

Considering mean values of genotype inputs across sites, there was a relationship of r^2=0.40 (Fig. 53). This r^2 value is higher than the sites individually, suggesting the model has an ability to predict genotype response to N over multiple sites. ASSYST types had an RMSE of 0.55 t ha^{-1} against an observed mean of 2.27 t ha^{-1} (r^2=0.39). Elite genotypes had a very tight RMSE of 0.18 t ha against an observed mean of 2.55 t ha^{-1} predicted (r^2=0.30). 12 of the elite genotypes had predicted values within 5% of actual values.

4.4. Discussion

This chapter describes a simple functioning model explaining WOSR yield productivity in relation to genotype traits under different N supply regimes. Genotype field data was used in the model development in 3 ways: identifying the most important traits; estimation of its cultivar parameters; and testing the model for decision support. We need to understand the amount, repeatability, and predictability of the genotype x environment x management relationship.

Use of models in practical applications to assist real-life challenges such as N rate guidance is limited because models typically require: (a) a large number of input parameters, which are usually not available; (b) particular skills to develop model specific input parameters and cultivar coefficients from internet databases; and (c) intensive training for use. This model has been simplified in steps to 12 parameters. Here we will discuss model performance, parameter choice, model limitations, utility and how the model can be useful.

4.4.1. Model performance initial development

The evaluation of crop models is performed through analysis of their predictive quality by comparing observed versus simulated output data (Asseng et al., 2003; Houles et al., 2004). The model performance was tested using two data sets. The first is previously published (Berry et al., 2010), of elite genotypes; and the second is from the RIPR diversity trials. Testing using the previously published (Berry et al., 2010) elite genotype data has shown a good relationship between model-predicted and observed field yields of genotypes for individual sites; and to a lesser extent across sites. The ability of the model to predict the correct ranking of yield at low N efficiency of 10 genotype across 4 environments is good given that the original testing data only included information for 7 of the 12 genotype inputs.

The second data set from the RIPR trials included additional parameters for N content at stem extension and flowering dates. In the original model validation and simulations data a parameter that was not available for testing was N content at the point of stem extension uptake (value set to default 54 kg/ha). Considering average data across sites, elite genotypes had a very strong RMSE of 0.18 t ha^{-1} against an observed mean of 2.55 t ha^{-1} predicted (r^2=0.30). This was a better RMSE than seen with the initial model validation. This indicates that this model has strong potential for use as a breeding tool for predicting elite genotype yield rankings over multiple field trials.

The model had less strong prediction for the ASSYST diversity panel genotypes than for the elite types. An explanation is that the older types are likely to have other yield limiting factors that are not considered in the model development. The model was validated and developed with data from modern elite types. Elite types have been bred to be consistently high yielding across different environments so they are likely to be less impacted by environmental factors than genotypes from a diversity panel. This may demonstrate that the model does not have enough parameters to successfully predict yields when considering other variables not directly related to N uptake.

The model was unable to predict yields for the 2015-2016 RIPR trials (Fig 51D). In this trial the yields were lower than the predictive range and as a result the model over-predicted yields. However, the field data in this trial were in many ways unsatisfactory, and unrepresentative of real farm yield. This was due to additional extreme weed and disease pressure. It is highly unlikely that data comparable to this would be included in breeding trials. Considering the extreme issues considered the fact that the model could rank the ASSYST types in a sensible order shows the parameters chosen were effective.

The model presented has been highly simplified and only contains 12 parameters. Dynamic cropping system simulation models such as Agricultural Production Systems simulator (APSIM; Holzworth et al., 2014); DSSAT (Jones et al., 2003); CropSyst (Stockle et al., 2003); and others, have been used to investigate soil-crop-weather dynamics. Our simplified model does not consider these factors, the only environmental factors being light radiation and SMN at stem extension. Many environmental and

management features that are known to impact yield are not considered, for example soil N mineralisation and how the different forms of N are related to uptake. The challenge in managing N and estimating the optimum N fertilisation rate comes from the complex interactions that exist in the dynamic soil-plant-atmosphere system and also uncertainty in weather (Havlin et al., 2005). These processes are always changing throughout the season and are related to many weather and management factors. Limitations of the model will be further discussed in section 4.4.5.

It is difficult to compare prediction analysis with other physiological models available. Firstly, the physiological models available have been generated with a small number of genotypes and their main purpose is not to distinguish between genotype N use. The models that do take N into account are complex dynamic models with many parameters. Development of the HUME-OSR model by Böttcher, (2020) used data from Berry et al., 2010 as part of its validation. They tested their model with 9 data sets and found that seed yield prediction accuracy varied widely between the single data sets and locations, with a high accuracy for the data sets from two sites, one being the Berry et al data. For Berry et al., the study found a RMSE of 0.73; relative RMSE of 18%; and model efficiency of 0.54. This is well aligned with the results from the model described in this chapter.

4.4.2 Which parameters vary among genotypes and should be varied in models for NUE?

Dynamic crop models generally include a large number of parameters - up to 300 in some cases. While the use of genotypic traits in models is common, how the traits are chosen is not always described in the literature. It would be inappropriate and not practical to try to adapt all of them to the range of genotypes studied; instead analysis can be achieved after selecting a small number of parameters for which genotypic variability has already been observed or assumed. This method has been used for the adaptation of the Azodyn crop model to various wheat cultivars (Barbottin 2004; Makowski et al. 2006), and to build the SUNFLO model for sunflower (Casadebaig et al. 2011). Boote et al. (2003) proposed to analyse the sensitivity of the model to its parameters, in order to identify those to modify as they have a clear effect on the outputs.

Parameters that are varied for genotypes in our model were based on crop leaf area and crop development. We chose parameters that from the literature that had been shown to have an impact on NUE (Table 13). Seed filling takes place during pod growth and N remobilisation (Diepenbrock, 2000), which makes it one of the most important stages for yield formation. Therefore, focus was applied to representing N dynamics within the OSR crop; including senescence and translocation processes. In LINTULBRASNAP, this is estimated from: the accumulation and redistribution of carbohydrates; maximal growth rate per individual seed; and seed density (Habekotte, 1997a). In contrast, CERES-RAPE simulates the partitioning of assimilates among pod walls and seeds by an allometric approach (Gabrielle et al., 1998b). In our model N uptake post mid-flowering was used to understand N utilisation.

The choice of parameters is even more involved, as the results of our sensitivity analysis showed that a range of key parameters have an impact on yield. Parameters which have a larger impact could also have indirect effects on other parameters (Jeuffroy et al., 2014). For example, in our model the range of N uptake and remobilisation had very high variation between genotypes and was shown to affect yield at low N. If N uptake before flowering was already high this will have less of an impact because N was less of a limiting factor for flowering period and radiation use efficiency.

4.4.3 Simulation study trait analysis

Sensitivity analysis of models can increase our understanding of G x E x M. As described by Jeuffroy (2014): “Moreover, on-going progress in sensitivity analysis methods could benefit the determination of genotype-dependent parameters in models. Few methods are available nowadays to run sensitivity analysis on complex models by taking account of relationships between parameters or by using dynamic variables (Lamboni et al., 2008). When genotype-dependent parameters can be easily recorded by people choosing cultivars, it will be necessary to create a method for building cultivar typology in order to identify those best suited to given environments and crop management systems.”

Once validated with field data, the model has been used to improve our understanding of how simple to measure traits affect N dynamics. Over time, this may allow us to answer questions, relating to varietal differences, which cannot be addressed with field research due to time and cost constraints. We have evaluated the possibility of designing crop ideotypes alongside modelling for yield and low N; and field data evaluation. We simulated indicators of crop performance (grain yield) with a previously developed and validated model (Berry et al., 2010).

The individual and combined trait sensitivity analysis can be used to identify important traits and combinations which will minimise N fertiliser requirement with least reduction in yield. The sensitivity study of all traits showed a yield potential of 4.8 t ha^{-1} at 0 N fertiliser, which is greater than the current UK mean yield. It also showed that the most important traits for yield at low N were: post-anthesis N acquisition; and SMN acquisition efficiency. Field studies have concluded that N acquisition is more important than utilisation traits at low N (Kessel et al., 2012; Berry et al., 2010). Post-anthesis N acquisition has also been reported as an important trait for increasing yield at low N supply (Berry et al., 2010). The most important traits for yield at high N supply were: days for seed filling; N remobilisation from pods; post-anthesis N acquisition; and fertiliser N acquisition efficiency. Extended flowering time has been seen as an important trait for increased NUE (Stahl et al., 2016). The most influential trait (post-anthesis N uptake) caused a 0.8 t ha^{-1} yield effect at 0 kg fertiliser N/ha when tested between its maximum and minimum value. However, varying all the traits resulted in a yield range of 2.4 t ha^{-1} (Figure 48B). This suggests that to achieve substantial improvements in yield at low N supply it will be necessary to breed for multiple traits simultaneously. However, although each individual trait value is physiologically possible the combined traits of this NUE genotype may not be achievable.

The model can be used to estimate the value of traits in terms of the amount of N fertiliser required to achieve a particular yield. For example, at 200 kg N/ha, the model predicts a yield of 4.38 t ha^{-1} using default model parameters. If late N acquisition is increased from its default value of 6 kg N/ha to its maximum value of 34 kg N/ha, then the amount of N fertiliser required to achieve the same yield of 4.38 t ha^{-1} falls to 138 kg N/ha. If fertiliser N efficiency is increased from its default value of 0.6 to its maximum value of 0.7, then the amount of fertiliser required to reach the default yield falls to 172 kg N/ha. If SMN efficiency is increased from its default value of 0.8 to its maximum value of 1, then 180 kg N/ha is required to reach default yield. If days for seed filling is changed from its default value of 45 days to its maximum value of 50 days, increasing time available for N remobilisation and hence utilisation by the seed, then the amount of fertiliser required to achieve the same yield of 4.38 t ha^{-1} falls to 169 kg /ha. If all of the above traits are improved simultaneously then the amount of fertiliser required to achieve the same yield of 4.38 t ha^{-1} falls dramatically to 48 kg /ha.

4.4.4 Model limitations

In both the initial validation and the RIPR trial work with the model our validations have been limited to two sites over 2 years, so the same varieties have not been robustly tested over multiple years. Evaluating a model against long-term data is critical when the model is to be used for N management. This is because processes such as N mineralization require several years for sufficient evaluation (Körschens, 2006) and can differentially affect N response among years. One factor that may compensate for this is the fact that modern elite WOSR genotypes that have been used in the initial validation have been bred to be consistently high yielding across different UK environments.

The primary purpose of this model is to predict the effect of genotype traits on yield under a range of N supply within a specific environment. The model only includes two environmental parameters: light availability; and Soil mineral N; and it does not take other known traits into account, for example: response to temperature; response to moisture. Additional environmental parameters would increase predictive accuracy, for example to account for: water stress; temperature; or yield reduction at super-optimal N rate due to lodging. However, including data for these environmental factors would result in a larger and more complex model, which would probably reduce its utility.

The model starts with total N acquired by the crop at the start of stem extension. The model does not distinguish between fertiliser N and SMN uptake at this point, although it is likely that the majority of the N taken up will be SMN from the previous crop(s). As the first model inputs are at the start of stem extension, the model does not consider autumn establishment. These effects have been modelled by (Ulas et al., 2012), but for large field trial studies collection of the relevant parameters is not practicable. Soil N at stem extension (kg/ha) is measured and input into the model and we have differentiated between the efficiency of soil N uptake efficiency and fertiliser N uptake. Within the model, crop N taken up at the start of stem extension will encompass prior establishment, environmental and genotype differences that will be relevant to yield.

Yield prediction in this model is in the most part based on genotype N uptake and partitioning. These are very complicated traits, dependent on many interacting factors: soil N availability; rotation information; form of N; other nutrient availability; soil composition; weed pressure; and disease. Considering N uptake and partitioning in the crop, we have not differentiated between form of N, but we do know that this this does have an impact. Sulphur deficiency in oilseed rape is increasingly becoming an issue in European agriculture. Sulphur (S) produces amino acids essential for plant growth, and S deficiency results in depression of nitrate reductase resulting in nitrate accumulation. High N levels can induce or aggravate S deficiency in oilseed rape effecting yield (Zhao et al., 1993; Abdallah et al., 2011). Understanding how N rate affects S efficiency is necessary to maximise yields (DEFRA., 2017). There is currently little field trial data for S uptake and utilisation in the literature, although some key parameters are available (Zhao et al., 1993; Abdallah et al., 2011). Models exist for Sulphur in WOSR, including SuMoToRI, which predicts growth and Sulfur allocation and partitioning in WOSR until the onset of pod formation (Poisson et al., 2018). Linking sulphur models to NUE models together in the future will be crucial. From this data we do not have the data to be able to provide oil content information as part of the production. This will be a very important factor for breeding and should be considered for future development. From a management perspective this model does not take into account its insertion in the cropping system, which can have an effect on yields. Although soil N is measured and used in our modelling, other nutrient, rotation and soil quality traits are not considered.

These limitations can mean that this model cannot easily identify traits for N because it does not simulate all the effects of the cropping system. It may be that it has been simplified too far for the ideotype data to be useful. However, the validation has shown that even when considering only these limited inputs the model has predictive power within and across sites. One explanation for this success is that the impact of environmental variables on yield is considered automatically through the crop N content at mid-flowering and maturity - this trait is measured as whole plant N. Bänziger et al. (2006) concluded that it is important to choose breeding environments similar to the target ones. For example, phenotyping trials are often sown on deep uniform soils, to minimise experimental error, and these are often more fertile than the soils encountered on commercial farms.

4.4.5 Utility

The model trait analysis has identified several key traits to improve NUE but measuring these traits within breeding programs will lead to practical challenges. In selecting model parameters, it is important to consider ease of measurement of these parameters in breeding or advisory trials. This is necessary to guarantee their use. The model described in this chapter has only 12 genotype inputs and the sensitivity analysis indicates that these could be further reduced by simplifying extinction coefficient to a default mid-range value, since the model output was relatively insensitive to its variation.

To maintain and improve model efficiency, Zhang et al. (2006) suggested that cultivars could be pooled into classes with similar phenotypes. However, the report stresses that this can introduce difficulties. “Such a classification should facilitate the quick identification of the group to which a new variety belongs and hence the prediction of its response in various environments. However, concerning the response to abiotic stresses (water, nitrogen and temperature), the characterisation of genotypic differences is more difficult to achieve in regular field trials as likely environmental conditions (e.g. soil water or nutrient content) are difficult to record at variety level because of intra-specific differences in soil resource use with time.”

N acquisition inputs require tissue analytics not typically carried out in field trials due to sampling time and cost. However, crop N uptake at the start of stem extension can be estimated easily and reasonably accurately using green area index (GAI) as a proxy measure (Berry et al., 2011a). However, these remote sensing techniques are less likely to be useful after the start of stem extension when full canopy cover is achieved, which means they are less likely to be useful for estimating post-anthesis N uptake. N remobilisation efficiency from pod, stem and leaf can be estimated using pod green area index or tissue biomass (m^2) and NHI, thus avoiding multiple tissue N analyses. Monitoring exact dates for end of flowering and maturity to determine seed filling duration involves visiting and scoring trial sites every 2-3 days during flowering and seed filling. This is time consuming but there is potential for using scoring methods instead of exact dates or using remote sensing tools such as unmanned aerial vehicles or satellite data for large trials.

4.4.6 How useful is this model?

In Europe, and globally, there are some areas where N application is limited and regulations may be tightened in the future. In these conditions, advisors and growers will need to consider both: applying N based on genotype requirement; and using genotypes that are adapted for yield at low N to maintain performance. Here we present a physiologically robust model approach, which uses the N content per unit leaf area as a key driver of N demand. This model has the potential to be a tool to help breeding programs and technical advisors identify genotype characteristics for selection or cultivars to be used in specific environments. It can be used to explore the genetic underpinning of complex traits. We have already discussed the limitations and utility of the model but how can the model be useful?

Expected changes in hybrid rank due to such G × E interactions, along with the complex effects of management on yield, increase the need to match crop design to specific sites and seasons. The value of targeted crop design depends on the diversity of traits available in commercial hybrids and the availability of a skilful seasonal climate forecast to allow farmers to match hybrids and management to prevailing and expected seasonal conditions. Our model allowed the ranking of genotypes grown under low N across environments but was not able to predict optimum N from the model. Further evaluation should be done using datasets with all of the relevant genotype inputs and

a range of N rates. The use of models as decision-making tools does not necessarily require a high predictive quality. An observed fit of medium quality could be compatible with a good decision quality as, for example, when ranking nitrogen strategies (David et al., 2004, 2005) or segregating wheat batches according to protein concentration predictions (Le Bail and Makowski, 2004). In general our model has proved to be helpful to analyse the results of a given trial in a characterised environment.

Measured data for environmental variables is scarce in breeding trials but crop simulation models can be used to generate covariates which could be effective for analysing differential responses of genotypes to environmental factors (Asseng and Turner, 2007). The ideal N management tool would consider how agronomic practices such as date of application and form of N application would impact yield in different environments. We have already discussed how using a model to support cultivar management requires that the model be usable across a wide range of environmental conditions and also that it does not need too many parameters and inputs. We have presented initial testing results for a simulation approach which may offer a time- and cost-effective alternative to experiments for genotype evaluation and management. The potential of a process-based crop model to be an effective tool for ideotype breeding is because the model's purpose is to describe the causal relationships between crop growth and factors driving them. After validating the model we were able to conduct simulation studies with the parameters that highlighted traits with potential for breeding genotypes that are high yielding at low N.

In this study we compared the simulated results with the experimental data (independently of those used for model parameterisation) but we did not compare the results with other models due to the complexity of available model data. Our model had errors in environmental prediction but was able to give good prediction of the relative ranking of genotypes. Similar results were seen with the Azodyn model which under-estimates the values of environmental variance (Jeuffroy et al., 2014), but with a good prediction of the ranking of genotypes. The choice of model can be based on the requirement of the users, who generally compare genotypes on the average ranking across all environments (Lecomte et al., 2010).

As well as evaluation of the prediction of crop models, it is essential to assess their ability to make good decisions, i.e. to achieve the goals of the potential users. Because of the small number of genotypic parameters and inputs and their easy measurement in field conditions, this model has potential to be used as a genotype evaluation tool. The model should also be able to answer the main questions asked by the growers better than their own tools usually do. Genotype performance is usually assessed through multi-environment trials comparing a variable number of genotypes grown in a wide range of soils, climatic conditions and cropping systems. Due to cost constraints and market pressures new cultivars are generally tested for relatively few years before they are released to farmers. The breeding trials do not allow the exploration of all the variability encountered in farmers' fields. They are also generally only tested at high field N conditions. Being able to highlight important phenotypes that are affected by N in models can allow the breeders to make better decisions to

identify the situations at low N in which a genotype type is promising or should be avoided. This model has the potential to facilitate the enlargement of the range of conditions in which the response of the genotypes can be tested.

It is difficult to assess if this model is a better tool for predicting yield than methods previously used by growers. The vast majority of breeding trials and therefore yield models are done at field rate N. At the moment it is not clear if our model has been able to improve on this. It has been able to show that genotype trait parameters were usable to rank elite cultivars over 4 environments. An alternative to developing methods of measuring each individual trait is to develop experiments which efficiently measure genotypic response to N supply so that the economically optimum N rate can be directly measured. These experiments may only be appropriate for the later stages of a breeding programme when there is sufficient seed to plant sufficiently large experimental plots. However, analysis of individual traits will probably remain important during the early phases of a breeding programme.

4.5. Conclusions

We have developed a model of WOSR yield formation under a range of N supply rates which takes account of N acquisition and utilisation in relation to dry-matter production. The model is simplified, having limited inputs while aiming to remain physiologically accurate; describing measurable genotype traits and how they impact on dry-matter production, N remobilisation and yield potential.

Sensitivity analysis showed agreement between important traits within the model and published field studies. The most important genotype traits for either reducing N requirement whilst maintaining current commercial yields or increasing yield at low N supply are greater post-anthesis uptake; efficiency of N acquisition from fertiliser; and days for seed filling and N remobilisation from pods. Breeders should be selecting for these traits in order to reduce N requirement and maintain yield.

The model, once developed and tested further, will allow prediction of genotype yield ranking and predict the optimum combination of traits for environments with different N supplies. At the moment the model has only been validated at low N. The model successfully predicted and gave a good indication of ranking of yield of elite genotypes over 2 sites over 2 years. The results also suggest that when considering a diverse panel, predicting yield is not possible by monitoring N-acquisition and utilisation alone. This is not unexpected as yield is more complicated – other factors must be involved. The primary purpose of this model is to predict the effect of genotype traits on yield under a range of N supply values within a single environment. We were able to predict the effect of genotype traits on yield under a range of N supply through a range of environments, including extreme genotypes included in the RIPR trials. Finally, the analysis of the limitations for wider use of crop models in variety breeding and assessment addresses some major questions for future research have been identified.

Chapter 5. Identification of Candidate Genes for traits linked to field NUE and flowering using field data of WOSR by association genetics

Collaboration work statement

R script was developed by (Lipka et al., 2012). Plant tissue extraction was done by Lolit Wilsoon. Samples were run by Alison Fraser and Thomas Alcock. Candidate Gene identification was done by Alison Fraser.

5.1 Introduction

Understanding the relationship between genotype and phenotype is an important tool in plant breeding. Genomics approaches are particularly useful when dealing with complex traits, which have a multi-genic nature and important environmental influence. Genomic tools are thus facilitating the detection of quantitative trait locus (QTL) and the identification of existing favourable alleles which have frequently remained unnoticed and have not actively been included in the gene pool used for breeding. In recent years, genome wide association study (GWAS), also known as association mapping based on linkage disequilibrium, have aimed to identify genetic variants linked to traits. GWAS has been recently used in *Brassica napus* to identify genes underlying control of seed glucosinolate content (Lu et al., 2014), calcium and magnesium content (Alcock et al., 2017) and anion homeostasis (Koprivova et al., 2014). Xu et al. (2017) identified 35 QTLs for flowering time, one being orthologous to A. thaliana CONSTANS (CO), an important gene in the photoperiod flowering pathway. Luo et al. (2015) identified nine SNPs for harvest index and seed yield per plant that explained 3.42% of the phenotypic variance in the former trait. Bouchet et al. (2014) analysed genetic control of yield at low and high N. Eleven regions were found to be stable across N conditions. R-A5 was a dense QTL area consistent in both N conditions.

GWAS uncover QTLs or genes from natural populations, and have the advantage of higher resolution and greater cost-effectiveness relatively due to screening a larger effective number of recombination events than are accessible in moderate size bi-parental segregating populations. But most crops are polyploid, making it difficult to identify the required markers and to assemble a genome sequence to order those markers. To compensate for this associative transcriptomics, has been developed which uses transcriptome sequencing to identify and score molecular markers representing variation in both gene sequences and gene expression, and correlate this with trait variation. ATs (Harper et al., 2012)

focuses on the analysis of transcribed sequences (mRNA-seq) across diversity populations to identify high-resolution loci influencing complex traits. An advantage of using RNA over DNA sequences in association studies is the ability to develop markers based on both single-nucleotide polymorphisms (SNPs) and transcript abundance (gene-expression markers; GEMs; Harper et al., 2012). Gene expression levels may be particularly important in the control of traits in polyploid species in which gene duplication may have led to unequal expression (Adams et al., 2003).

In this chapter we have used AT analysis (Harper et al., 2012) with the RIPR field trial data to explore the relationship between genotype and for the phenotype NUE and Flowering score. From the RIPR trial data we have phenotype data for 32 phenotypes across 2 environments over 2 years that can be analysed. We chose to look into NUE and flowering in this thesis because NUE is a direct score of N efficiency and directly impacting on yield at low N. Flowering score had a very wide range in score. We also found that early flowering was related to yield at low N at all sites.

Flowering time genes have a strong influence on successful reproduction and life cycle adaptation. However, their regulation is highly complex and only well understood in diploid model systems. For crops with a polyploid background from the genus *Brassica*, data on flowering time gene variation are scarce, although indispensable for modern breeding techniques like marker-assisted breeding. The expression of FT is negatively regulated by the transcriptional repressor FLOWERING LOCUS C (FLC) in interaction with other genetic factors from the vernalisation pathway, and positively regulated via the transcriptional activator CONSTANS (CO) in interaction with genetic factors from the photoperiod pathway and the circadian clock (Srikanth and Schmid, 2011; Blümel et al., 2014).

Flowering time involves a variety of genes and is regulated by internal signals (Jack et al.,2004). Environmental factors play additional key roles in stimulating internal regulatory factors. Switching the shoot apical meristem (SAM) from vegetative to reproductive growth is controlled by both environmental and endogenous signals, and this process is affected mainly by photoperiodic promotion, autonomous enhancement, flowering inhibition, vernalization (the acceleration of flowering by low temperatures and the suppression of specific genes), and gibberellin induction. These factors act on the inflorescence/floral meristem in combination, resulting in the conversion of the apical meristem to the inflorescence/floral meristem. The inflorescence/floral meristem then forms the floral organs by activating the expression of floral organ-specific genes. The A. thaliana flowering-time networks comprise a total of 306 genes, and most of these are members of multigene families. Flowering-time genes have been studied widely in *Brassica napus*, and several genes involved in flowering time, such as Flowering Locus C, CONSTANS, Protein FLOWERING LOCUS T , and Protein FRIGIDA, have been identified (Schiessl et al., 2017, Schranz et al., 2002; Zhang et al., 2015; Razi et al., 2008)

N absorption by plants is comprised of three major steps: uptake, assimilation and remobilization. NUE is the product of N uptake efficiency (NUpE) and N utilization efficiency (NUtE).
N can be taken up either as NO^{-}_{3} or NH_{4}^{+} directly from the soil through roots. NH_{4}^{+} is moved into intracellular compartments by the ammonium transporter (AMT) and, then converted through the GS/GOGAT pathway into a variety of organic molecules such as amino acids for plant growth. The process of resulting molecules derived from ammonia via the GS/GOGAT cycle can be as part of primary N assimilation (Oaks, 1994).

Recently, Luo et al (2015) reported six SNPs on A03 associated with rapeseed branch number using GWAS. However, despite the studies on rapeseed branching, the basis for genetically controlling branching morphogenesis has not been fully elucidated for rapeseed, and few genes have been predicted in the QTL intervals. The study concluded that better understanding of the genetic determinants for rapeseed branching is needed.

Not all genotypes in our study that were part of the RIPR population have been transcriptome sequenced so genotype numbers for analysis were limited. In the field trials we had accession numbers of; DP14 (68), DP15 (68), HM14 (67) and OM15 (88). Fifty three genotypes were grown across all trial sites and had mean data available to be analysed. These are small populations which must be taken into account as a limitation. In comparison, Alcock et al. (2017) used mineral data analysis of 383 accessions. However, Lu et al. (2014) utilised panels off 84 genotypes in *Brassica napus* and despite the relatively small population sizes, a number of loci associated with seed glucosinolate concentrations were identified. This is a unique opportunity to use associate transcripts on data collected in the field under multiple environmental pressures under low N conditions. The specific objective being to lay a foundation off analysing the genetic composition of genotype data in the RIPR trial and highlight promising candidate genes for NUE and flowering.

5.2 Methods

5.2.1. Material

This study uses the field trial data from the RIPR trial. Methods for collecting this data and results from the phenotype analysis are described in Chapter 2. Renewable Industrial Products from Rapeseed (RIPR) diversity population of inbred lines of *Brassica napus* genotypes were used. These were developed from the ERANET-ASSYST consortium diversity population (Bus et al., 2011, 2014; Körber et al., 2012, 2015).

5.2.2 Transcriptome Sequencing and Population Structure Analysis

Extraction of RNA, quality checking and Illumina transcriptome sequencing were carried out as described by He et al. (2016). Plants were sown on Levington professional F2 compost and grown in long day (16/8 h, 20 °C/14 °C) glasshouse conditions. Second true leaves from each of four plant

replicates per accession were harvested when they reached ~ 3cm in diameter, as close to the mid-point of the light period as possible. Leaves were harvested separately for processing as individual replicates or pooled when processed as a single sample per accession and immediately fromzen in liquid nitrogen. Frozen leaf samples were stores at -80 °C. Pooled frozen leaf samples were ground in liquid nitrogen. RNA was extracted using the manufacturer's instruction for Omega Biotek EZNA Plant RNA Kit. RNA-seq data from each accession was mapped using methods described by Bancroft et al. (2011) onto ordered *Brassica* A and C genome-based pan-transcriptomes developed by He et al. (2015).

Transcriptome sequencing was performed by the Earlham Institute (formerly The Genome Analysis Centre; Norwich, United Kingdom). Across the 383 accession panel, 46,307 single SNPs and 309,229 hemi-SNPs were detected and scored of which 256,397 SNPs had a population second allele frequency (saf) > 0.01. Transcript abundance was quantified and normalized as reads per kb per million aligned reads (RPKM) for each accession for 116,098 coding DNA sequence (CDS) models of the pan-transcriptome reference. Significant expression (mean > 0.4 RPKM) was detected for 53,889 CDS models. Inference of population structure by Q-matrix was obtained by Population Structure Inference using Kernel-PCA and Optimization (PSIKO; Popescu et al., 2014).

5.2.3 Associative Transcriptomics

Associative transcriptomics was performed using SNPs, Q-matrix and trait data in a compressed mixed linear model approach (Zhang et al., 2010) implemented in the GAPIT R package (Lipka et al., 2012) in R 3.2.0 (R Core Team, 2015). The association analysis between gene expression markers (GEMs) and traits was performed by using fixed effect linear modelling in R with RPKM values and Q-matrix data as the explanatory variables and trait score the response variable using custom designed scripts (Harper et al., 2012; Havlickova et al., 2017). Coefficients of determination (r^2), constants and significance values were calculated for each regression. Manhattan plots were generated using graph functions in R. SNPs with low second allele frequency (<0.01) were filtered from the dataset prior to generating plots. In total 256,397 SNPs and 53,889 GEMs were plotted. False discovery rate (FDR; Benjamini and Hochberg, 1995) and Bonferroni (Dunn, 1961) corrections were used to set significance thresholds at $P < 0.05$. Due to sequence similarity between B. napus A and C genomes, assignment to a specific genome was not possible for all SNP markers; such markers are plotted in grey and appear in both positions on Manhattan plots.

The predictive power of the best GEMs and SNPs were assessed using a ‘take-one-out’ approach (Harper et al., 2016) whereby each accession is removed from the SNP or GEM analysis in turn. An in-house r script was performed with adaptation from Harper et al., (2016), with a modification of incorporating all SNPs and GEMs above Bonferroni lines. When permutations finish, an R^2 value is calculated from predicted trait values regressed against the observed trait values, which indicates the predictive power of the top selected GEMs and SNPs.

5.2.4 Candidate Gene Identification

Ordered pan-transcriptome data based on *Brassica* A and C genomes from B. rapa, B. napus, and B. oleracea CDS gene models (He et al., 2015) were used to identify candidate genes. Candidate genes were selected based on Arabidopsis thaliana annotated functions of *Brassica* orthologs within estimated linkage disequilibrium (LD) decay of significantly associated markers (around 1–2 cM on average; Ecke et al., 2010. A. thaliana functional information were obtained from The Arabidopsis Information Resource (TAIR; Huala et al., 2001).

Candidate genes were selected based on uniform peaks across trial sites. When considering the trait visually determined association peaks on Manhattan plots across all trials were determined. In Manhattan plots, genomic coordinates are displayed along the X-axis, with the negative logarithm of the association p-value for each single nucleotide polymorphism (SNP) or GEMs displayed on the Y-axis. Because the strongest associations have the smallest P-values (e.g., 10−15), their negative logarithms will be the greatest (e.g., 15). Each dot represents a SNP of a GEM, with the X-axis showing genomic location and Y-axis showing association level. When considering the Y axis each band represents a different part of the genome. The line in the middle of the diagram splits the A genome and the C genome. Markers of alternating chromosomes are coloured black and red to distinguish between linkage groups. To identify candidate loci, SNPs and GEMs were used separately in analyses. SNPs represent areas where there was an effect on genes. GEMs represent areas where gene expression was different. A peak means that there is an association between. Fundamental unit for reporting effect sizes is the odds ratio. The odds ratio is the ratio of two odds, which in the context of GWAS studies are the odds of case for individuals having a specific allele and the odds of case for individuals who do not have that same allele. In our figures each chromosome is distinguishable because they are coloured red and black alternatively. There is a line down the middle that separates chromosome A from chromosome C.

5.3. Results

5.3.1 Associative transcriptomics suggests potential genes that could be markers for NUE in field trials.

The association analysis did not identify any SNPs of GEM loci that was more than the bison confidence level at any of the sites. We focused the candidate gene assessment of NUE with the mean data from 4 environments (Fig 54) In this work when considering NUE visually determined association peaks on Manhattan plots peaks were observed on chromosomes A1, A2, A3, A9, C3, C4, C5 and C9 (Figure 54A). The most well defined peaks were located on chromosomes A9 and C9.

Candidate genes were selected based on direct GEM hits as described above. Some of the candidate genes for NUE are summarised in table 15. Putative functions were obtained from The Arabidopsis Information Resource (TAIR; Huala et al., 2001). These candidate genes were not above the bison confidence significance threshold. However, the SNP and GEM associations for NUE were similar when considering all environments; DP14, DP15, HM14 and OM15 (Fig 55). This suggests that there are some key associations and gene expression in common across environments.

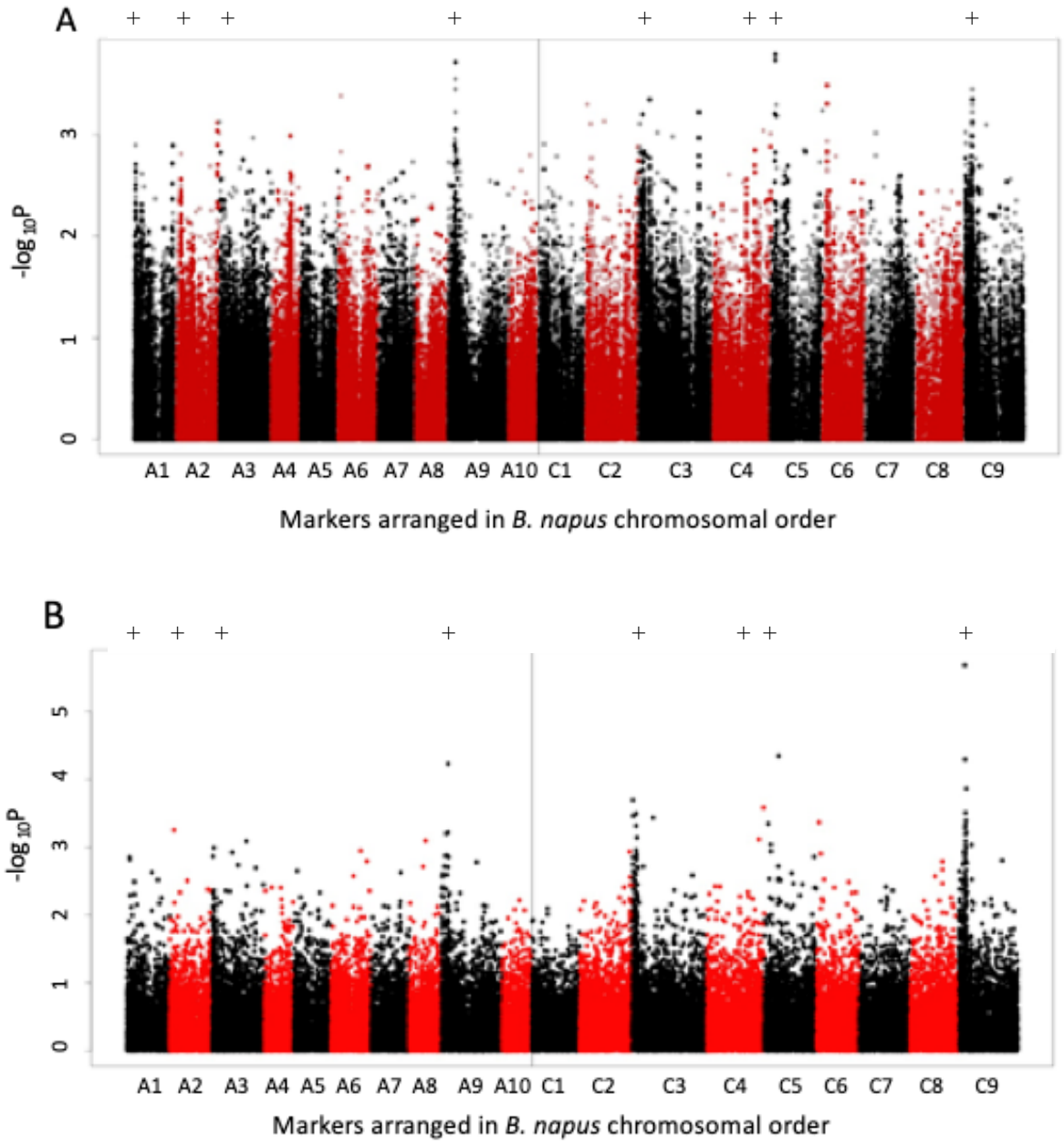


Figure 54. –log10 P values of SNPs and GEMs associated with NUE (A,B, respectively) in order of markers within the B. napus pan-transcriptome. The data used is mean values from across all trials. Each dot represents a SNP or a GEM. The X-axis shows the genomic locations and the Y axis shows the association level. Markers of alternating chromosomes coloured black and red to distinguish between linkage groups. Symbol + show the peaks described in the text. These are not significant peaks.

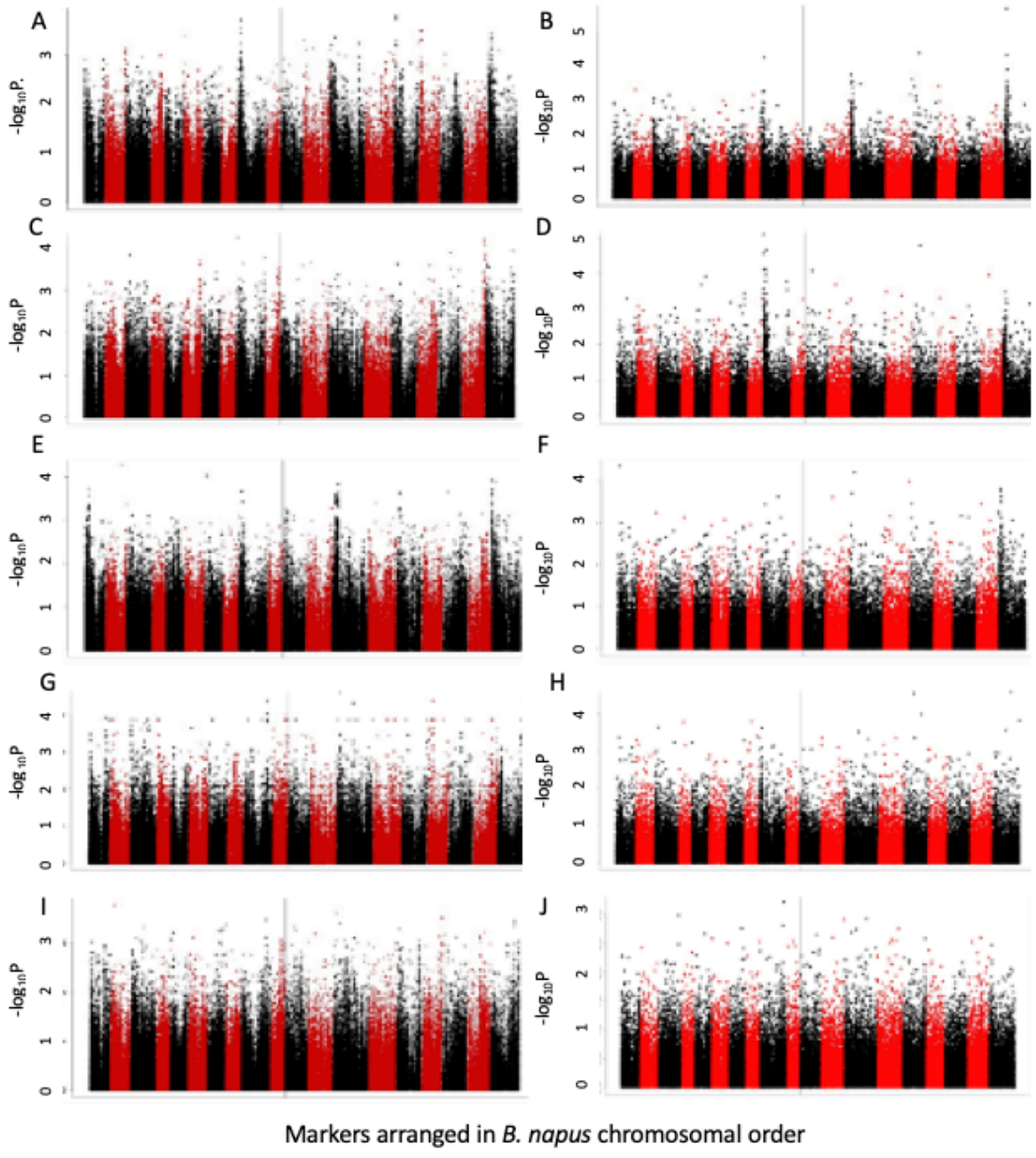


Figure 55. –log10 P values of SNPs and GEMs associated with NUE for mean data from all trials (A,B, respectively), DP14 (C,D), DP15 (E,F), HM 14(G,H) and OM15 (I,J) in order of markers within the B. napus pan-transcriptome. Each dot represents a SNP or a GEM. The X-axis shows the genomic locations and the Y axis shows the association level. Markers of alternating chromosomes coloured black and red to distinguish between linkage groups.

6.3.2. Associative transcriptomics analysis of potential genes that could be markers for flowering date at low N in field trials.

When considering flowering, like NUE, the Manhattan plots showed similar peaks for GEMs and SNPS and similarities across all sites; DP14, DP15, HM14 and OM15 (Figure 57). Similar patterns across all trial sites suggests some key associations in common. For flowering when considering mean data across sites (Figure 56A), association peaks on Manhattan plots for all trials were observed on regions of chromosomes A3, A6, A10, C3, C4, C5, C7, and C8. Some candidate genes for flowering are summarised in table 16.

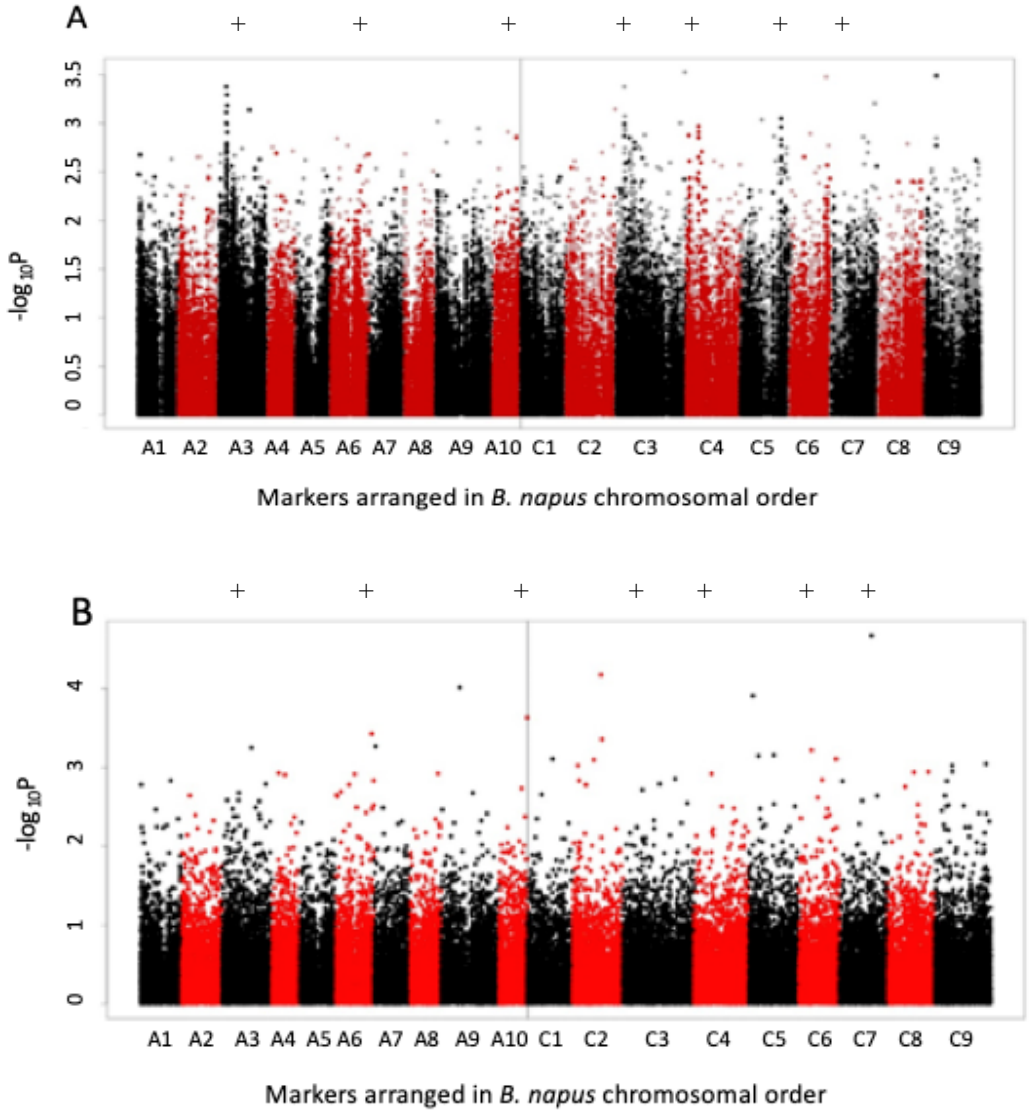


Figure 56. –log10 P values of SNPs and GEMs associated with NUE (A,B, respectively) in order of markers within the B. napus pan-transcriptome. The data used is mean values from across all trials Each dot represents a SNP or a GEM. The X-axis shows the genomic locations and the Y axis shows the association level. Markers of alternating chromosomes coloured black and red to distinguish between linkage groups. Symbol + show the peaks described in the text. These are not significant peaks.

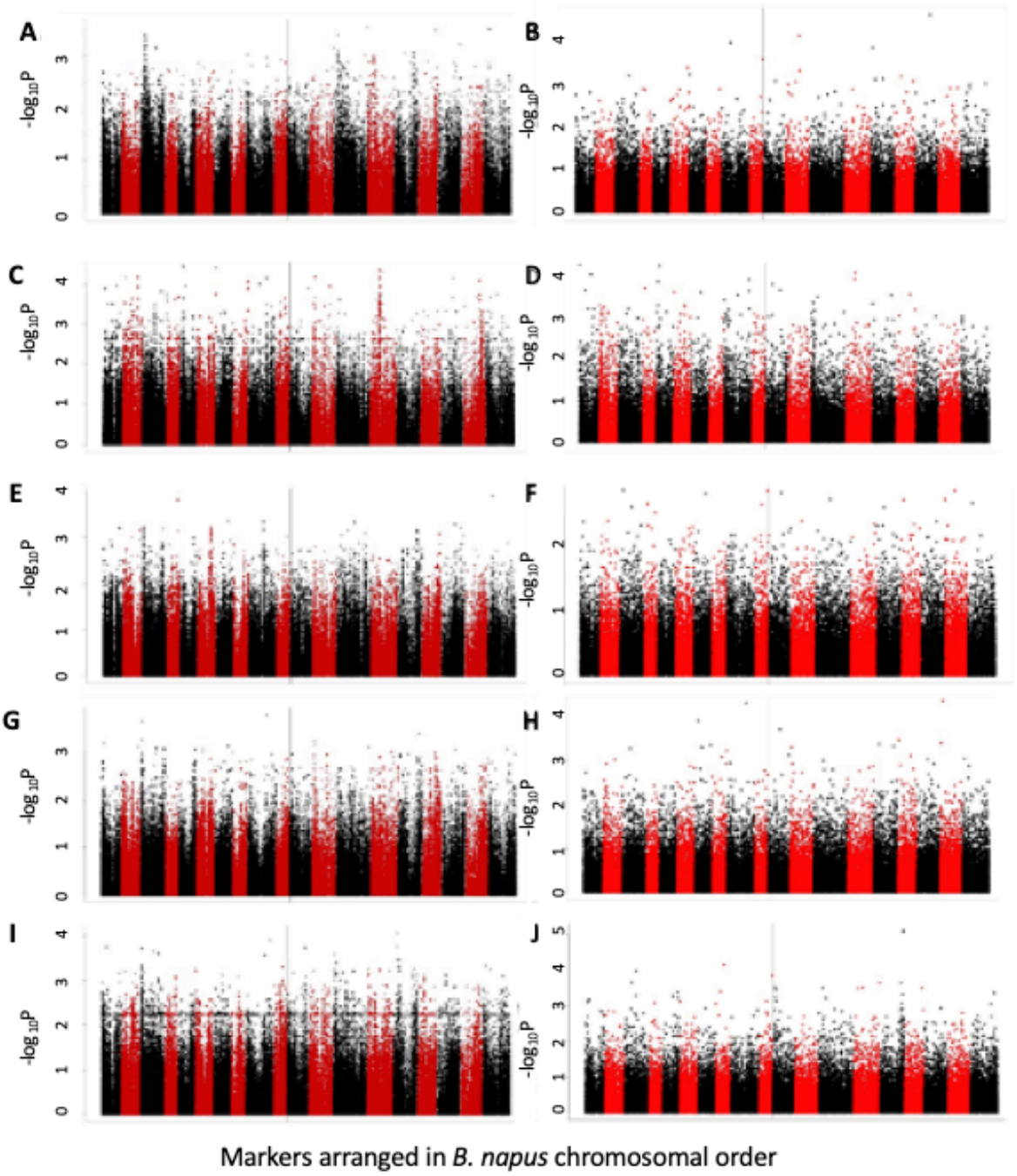


Figure 57. –log10 P values of SNPs and GEMs associated with flowering for mean data from all trials (A,B, respectively), DP14 (C,D), DP15 (E,F), HM 14(G,H) and OM15 (I,J) in order of markers within the B. napus pan-transcriptome. Each dot represents a SNP or a GEM. The X-axis shows the genomic locations and the Y axis shows the association level. Markers of alternating chromosomes coloured black and red to distinguish between linkage groups.

Table 15. Summary of candidate genes selected from associative transcriptomics outputs for NUE. Putative functions obtained from The Arabidopsis Information Resource (TAIR; Huala et al., 2001).

Chromosome	Candidate genes	A. thaliana	Description
C09	Leo	AT5G61150.2	An RNA polymerase-associated *protein* LEO1
A09	Glutathione synthetase	AT5G27380.1	The second enzyme in the glutathione (GSH) biosynthesis pathway. It catalyses the condensation of gamma-glutamylcysteine and glycine, to form glutathione.
A02	High affinity nitrate transporter 2.7	AT5G14570.1	Involved in high-affinity nitrate transport. Has been shown to Control nitrate content in seeds of *A. thaliana* (Chopin F., 2007). N content of seeds was shown to be significant to NUE.
C09 + A03	Ubiquiutin or ubiquitin signals	AT5G62990.1	*Ubiquitin* involved in carbon and *nitrogen* signalling.
C02	NAD(P)-linked oxidoreductase	AT5G62420.1	Protein – Nitrogen cycle
A03	Glu-AdT	AT1G48520.1	Classified as a glutamine amidotransferase, enzymes that catalyze amide nitrogen transfer from glutamine to acceptor substrates.
C04	UDP glycosyltransferases	AT2G30150.1	A superfamily of enzymes that catalyzes the addition of the glycosyl group from a UTP-sugar to a small hydrophobic molecule.
A09	Glycin rich proteins	AT5G27380.1	Has been shown defence against stress potentially cause by low N conditions.

Table 16. Summary of candidate genes selected from associative transcriptomics outputs for flowering. Putative functions obtained from The Arabidopsis Information Resource (TAIR; Huala et al., 2001).

Chromosome	Candidate genes	A. thaliana	Description
A03	UBX domain-containing protein	AT4G00752.1	This new unexpected level of regulation for p97/CDC48 was demonstrated to be critical in vivo as pux1 loss-of-function mutants display accelerated growth relative to wild-type plants. A role for AtCDC48 and PUX1 in regulating plant growth.
C06	ATP-dependent protease La domain protein	AT2G25740.1	Key gene on C5 carertonids biosytntheis – genes involded in absorbing light energy for use in photosynthesis,
A10	Sulfoquinovosyldiacylglycerol	AT5G01220.1	Major SQDG species (34:3, 34:2 and 36:6) showed a transient decrease during the mid-stage of flower development.
A06	Beta HLH protein 71	AT5G46690.1	These results indicate that PAR1–PRE1 and PAR1–PIF4 heterodimers form a complex HLH/bHLH network regulating cell elongation and plant development in response to light and hormones. Key for vernalisation.
C02	Sterile alpha motif (SAM) domain-containing protein	AT1G70180.2	A SAM oligomerization domain shapes the genomic binding landscape of the LEAFY transcription factor. Here we focus on the LEAFY transcription factor, a master regulator of flower development in angiosperms

5.4 Discussion

This study lays a foundation off analysing the genetic composition of genotype data in the RIPR trial. This initial work showed several promising candidate genes, some genes previously shown to influence or respond to NUE and Flowering score in *Brassica napus.* None of the candidate genes were above the bonferroni corrected significance threshold. However, some were close, there were evident peaks and candidate genes that were consistent across field trials. Products were not measured, RNA transcripts were measured.

We identified some peaks with genes that are associated with N assimilation and N uptake pathways. These are outlined in table 15. Glutamine synthetase (GS) was identified on A09. GS plays critical roles in N metabolism. However, the exact relationship between GS and NUE remain unclear. Zhang et al (2017) selected and compared two wheat cultivars, YM49 and XN509, which were identified as N-efficient and N-inefficient genotypes, respectively. In the study, they showed that *TaGS1* was highly expressed post-anthesis, and *TaGS2* was highly expressed pre-anthesis in N-efficient genotype compared to N-inefficient genotype. A high affinity nitrate transporter was found on A02. In Arabidopsis the Nitrate Transporter NRT1.7, expressed in phloem, is responsible for source-to-sink remobilization of nitrate (Fan et al., 2009). Glutamine amidotransferase was identified on A03. Classified as a glutamine amidotransferase, enzymes that catalyze amide nitrogen transfer from glutamine to acceptor substrates.

We also identified genes that are associated with signalling and response to stress. UDP glycosyltransferases (GTs) was identified on the peak A03. Increasing evidence is suggesting that glycosylation mediated by GTs plays crucial roles in plant growth and response to biotic and abiotic stresses (Bowles et al., 2006). GTs catalyze the transfer of sugar moieties from active sugar molecules to a variety of acceptor molecules, namely, hormones, lipids and some other small molecules. The formation of a glycosidic bond can change an acceptor's chemical properties and bioactivity, adjustments that are essential for the maintenance of cellular homeostasis. In addition, conjugation by GTs allows plant cells to modulate their biochemical proprieties and thus have a strong influence on their biological activity and compartmental storage (Le Roy et al., 2016). Glycin rich proteins (GRP) were found on A09. Several proteins, including the GRP superfamily, are involved in cellular stress responses and signalling (Czolpinska et al., 2018). GRPs are characterized by high glycine content and the presence of conserved segments including glycine-containing structural motifs composed of repetitive amino acid residues. The general structure of this superfamily facilitates division of GRPs into five main subclasses. Although the participation of GRPs in plant stress response has been indicated in numerous model and non-model plant species, relatively little is known about the key physiological processes and molecular mechanisms in which those proteins are engaged.

In the context of N stress, QTLs for NUE studies have been conducted in OSR (Gül 2002, Miro 2010, Bouchet et al., 2010). Bouchet (2010) set up a study with the objective to introduce genomic regions involved in NUE under low N fertilisation conditions in the new varieties. The study did not highlight QTLs specific to low N conditions; however, 11 regions were found to be stable across N conditions and/or years of trial and could be used for further studies. The work found that R-A5 is of particular interest as a dense QTL area which was consistent in both N conditions and two out of the three sites. Genomic regions involved in response to grain yield selection at high and low N fertilization in maize have been highlighted. The studies utilised bi-parental populations to identify the genetic basis of NUE. Most QTLs were located on chromosomes A02, A06, A07, C03, C06 and C09. However these were only detected in a single-year environment. In this work when considering NUE visually determined association peaks on Manhattan plots peaks were observed on chromosomes A1, A2, A3, A5, A9, C3, C4, C5 and C9 (Figure 54A). The most well defined peaks were located on chromosomes A9 and C9. Our data was collected over 2 sites and 2 years.

We identified some peaks with genes that are associated with flowering date, these are outlined in table 16. Xu et al. (2017) used a diversity panel consisting of 523 *Brassica napus* cultivars and inbred lines was genotyped with the *Brassica* 60K Illumina Infinium SNP array. The work identified 35 QTLs for flowering time, one being orthologous to A. thaliana CONSTANS, an important gene in the photoperiod flowering pathway. Beta HLH protein 71 was identified in this study on AO6. FLOWERING BHLH transcriptional activators control expression of photoperiodic flowering and a regulator CONSTANS in Arabidopsis (Ito et al., 2012). Sulfoquinovosyldiacylglycerol (SQDG) was identified on A10. Gene expression for major SQDG species showed a transient decrease during the midstage of flower development. The later increase in 34:3 species corresponds with increases in 34:6-MGDG and 34:4-PG species in stimulation of plastidic lipid biosynthesis. A high level of SQDG at the initial stage of flower development is intriguing, with no further supportive data for the increase in SQDG in this developmental stage. SQDG was a candidate gene associations for heading and anthesis dates identified by GWAS for 2 sites over 2 years in switchgrass (Taylor et al, 2018).

Previous studies of the genetic architecture of flowering time in rapeseed were based on QTL linkage mapping using traditional molecular markers, such as restriction fragment length polymorphisms, simple sequence repeats, intron polymorphisms and sequence-related amplified polymorphism markers in bi-parental populations. Many QTLs related to the flowering time of *Brassica napus* have been detected. A total of ~15 QTLs with large phenotypic effects have been mapped, and these QTLs are mainly distributed on chromosomes A2, A9, A10, C2 and C3 (Nelson et al., 2014, Wei et al., 2014). For flowering (Figure 56A), association peaks on Manhattan plots for all trials were observed on regions of chromosomes A3, A6, A10, C3, C4, C5, C7,

This study used *Brassica napus* associative transcriptomics to identify candidate genes from field trial data. RNA transcripts were measured, products were not measured. Literature and our research has shown that environmental factors and crop management influence NUE and flowering such as

planting density, photoperiod, and nutrient availability. Multi-environment testing is necessary to determine whether the effects is consistent across environments. We have analysed field phenotype data over multiple sites and 2 years. By comparing peaks across multiple sites we have been able to show that expressed loci show consistency. By using a transcriptome at a juvenile growth stage the project has defined a genome compartment with both the greatest functional significance and the greatest potential for analysing cross-species collinearity based upon sequence similarity. Juvenile leaf was used as a tissue for mRNA extraction, because it has a large number of expressing genes and uniform growth stage. However, factors that impact the effectiveness of associative transcriptomics studies include the specific samples used (e.g. stress vs. developmental series). NUE is impacted throughout the crop lifecycle for NUE. Flowering is impacted at a later stage than juvenile. It may be that gene expression at a juvenile stage where tissues are not under N stress would not reveal key genes in the flowering or NUE pathway.

AT has been criticised as an inappropriate method to identify genes with large impacts on adaptive responses to the environment because genes that have a large impact on fitness are rare and often large changes in gene expression does not necessarily equate to a large effect on fitness (Feder and Walser, 2005). AT has a tendency to focus on genes that are highly conserved and undergo large fold-changes and may miss regulatory genes that undergo moderate fold-changes. Such genes can play major roles in environmental adaptation. For example, hub genes are less likely to be detected through AT screens because these genes have stable expression, instead influencing fitness through post-translational changes in protein activity (Lehner, 2008; MacNeil and Walhout, 2011). In contrast, AT is best-suited to interrogate the downstream effectors of biological responses because these genes tend to have high expression variability (Gracey, 2007). AT has also been criticised as mRNA abundance is an unreliable indicator of protein level (Greenbaum et al., 2003; Tian et al., 2004). Even with the significant developments in the technologies used to quantify protein abundance over the past couple of years, protein identification and quantification still lags behind the high-throughput experimental techniques used to determine mRNA expression levels.

Selection of genotypes with different alleles for target genes may lead to improved ability to grow under low N conditions and act as markers for breeding. We did not test the structure and function of the candidate genes selected. The next step would be to conduct experiments in A. thaliana T-DNA knockouts to confirm the level of influence. Flowering and NUE are both complex traits with multiple genes/pathways involved. Due to the many genes involved in these pathways it is likely that any genes selected are likely to have additive effects in *B. napus*. Work could also be done to test if functions observed here and in previous studies are conserved. Selection of *B. napus* genotypes with different alleles of target genes may lead to improved ability to grow in the presence of low soil N concentrations. This was an initial genetic analysis of the RIPR phenotypes to see if the small accession number would provide useful results. There are also 30 other traits from the RIPR trials to be analysed. This study could also be investigated with samples taken at the 2 leaf stage. The data has the potential for in-depth candidate gene analysis.

Chapter 6. Discussion

6.1 Project outline and background

In this thesis we have presented results from; phenotyping diversity trials (Chapter 3), process-based model development (chapter 4) and genome analysis (chapter 5). The work in this thesis has been part of a larger consortium study – the RPR project. The principal aim of the project is to understand the genetic control, in rapeseed, of the accumulation of compounds representing emerging bio-refining opportunities and fertilizer use efficiency. The wider project aims to integrate existing knowledge and new field data to identify and fill important genotype knowledge gaps. This work has built on knowledge from two previous NUE WOSR projects, DEFRA LINK: LK0979 and HYLO:IF01110 (Fig 58). Together these three projects have run over 12 years. The field trial in this thesis used genetically diverse genotypes to characterised the genetics of NUE traits of a WOSR structured panel.

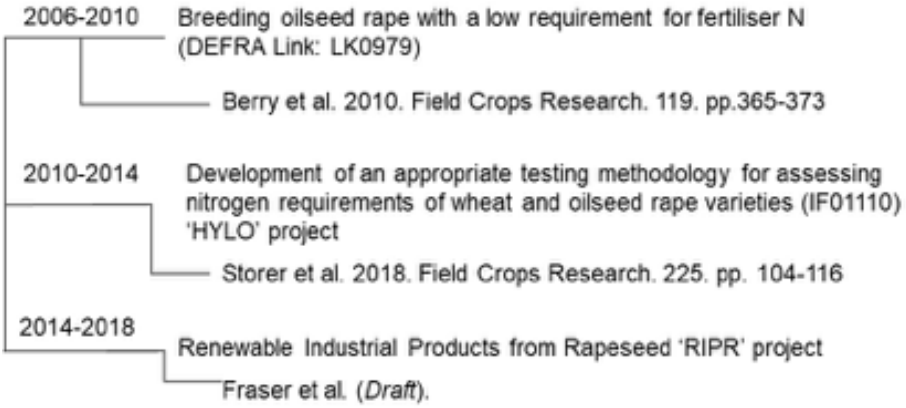


Figure 58. Time line and outputs of NUE WOSR projects; DEFRA LINK: LK0979, HYLO:IF01110 and RIPR projects (BB/L002124/1)

In this chapter the scientific questions outlined in the hypothesis will be discussed:

- Modern breeding has improved yield for WOSR under high N fertiliser conditions but has not directly improved crop NUE at LN. There is potential to breed genotypes that are consistently high yielding under low N conditions.
- The phenology development of WOSR significantly impacts yield, N-optimum, N-uptake and N-utilisation. Identify key traits, and their interactions that can be used to predict crop growth at low N and develop simple predictive models.
- Analysis of older genotypes within a diversity trial will be beneficial for better nutrient use, since they might have comparative advantages in adaptation to low input systems. These diversity sets can be used to define phenotypic and genetic markers that are indicators of NUE.

This chapter will then discuss the project in context of:

- Practical approaches to calculate N optimum
- Farmer motivation for improving NUE
- How the results can be utilised by breeders

6.2. There is potential to breed genotypes that are consistently high yielding under low N conditions.

In the RIPR field trial, some genotypes had minimal response to N, this was surprising considering the very large differences in FN applied on the treated plots. There were genotypes that were very high yielding at LFN at both HM14 and DP14; some genotypes yielding above 4 t ha^{-1} at LN. High yields at low N was seen in both the diversity panel and the elite types (Figure 10); indeed some elite types showed minimal or no response to N (Figure 13). This suggests that there is vast potential for achieving high yields while applying less N than currently is applied. This trial agreed with previous work in the finding that NUE of genotypes have shown yield and cultivar are ranked differently at high N and low N rates (Kessel et al., 2012; Nyikako et al., 2014; Erley et al., 2011, Berry et al. 2010). Modern breeding has improved yield for WOSR under high N fertiliser conditions but has not directly improved crop NUE at LN. This would mean that there is potential to breed genotypes that are consistently high yielding under low N conditions.

When breeding genotypes with high yields at LN the genotypes must be consistently high yielding at low N across environments and year. There were several elite types that were in the HYLO group and were highly ranked for: Pacific; Trinity; Temple; Quartz; Elgar; and PT211. There were also elite types that were high ranked for seed N utilisation: Compass; Orlando; Excalibur; and Pacific. These genotypes had a tight standard deviation within the mean ranking suggesting that the effects are consistent across sites.

Yield at LN fertiliser application varied drastically across sites. For the RIPR experiments the 2015-2016 sites were much lower yielding than the 2014-2015 trials. Mean LFN yields (t ha^{-1}) across all genotype were 3.05 t ha^{-1} at HM14, 2.35 t ha at DP14 but only 0.84 t ha^{-1} at OM15 and 1.02 t ha^{-1} at DP15.

Another important factor in increasing yield at LN of WOSR production is to prevent loss of yield through environmental conditions and other management factors including, genotype choice, sulphur fertilisation, disease control, and weed control. Therefore, understanding genotype performance under different management condition is essential. In our study this could be seen by the extremely different site yields of genotypes at LN and HN in the diversity RIPR trial. Sulphur fertilisation has an interaction with NUE. Sulphur produces amino acids essential for plant growth – deficiency results in nitrate reductase depressed resulting in nitrate accumulation. High N levels can induce or aggravate Sulphur deficiency in WOSR effecting yield (Zhao et al., 1993; Abdallah et al., 2011). Understanding how N rate effect Sulphur efficiency is required to maximise yields. It is important when considering plant breeding and fertiliser regimes to consider the N system as a whole rotation. If crops are highly N efficient at uptake, deep roots, root surface area, alongside N, other key nutrients may be removed from the soil affecting the next crop in the rotation. Keeping this in mind may justify focus on the importance to increase N utilisation.

The model sensitivity study of all traits (Chapter 4) showed a yield potential of 4.8 t ha^{-1} at 0 N fertiliser, which is greater than the current UK mean yield. To achieve 4.8 t ha^{-1} with 0 fertiliser N the following genotype traits were set at the top of their physiological range: Total crop N content at the point of stem extension; Crop acquisition of SMN between stem extension and flowering; Crop post anthesis N acquisition; Proportion of crop leaf N & stem N that could be relocated to pods or seeds; and Proportion of crop pod N that can be relocated to seeds. The environmental trait SMN was set to its range maximum and daily light incidence was 12 MJ/m^2. For the remaining genotype traits there was a range of suitable values that would enable high yield including: radiation use efficiency, 1.17-1.43 g/MJ PAR (mean 1.34 g/MJ PAR); duration from start of seed filling to natural senescence, 40-50 days (mean 46.4 days); extinction coefficient of canopy post-anthesis, 0.54-0.66 (mean of 0.6).

Chapter 5 shows genes that were associated with NUE at 0N across the RIPR field trails. Selection of *B. napus* genotypes with different alleles for target genes may lead to improved ability to grow under

low N conditions. We did not test the structure and function of the candidate genes selected. The next step would be to conduct experiments in A. thaliana T-DNA knockouts to confirm the level of influence. However, yield at LN along with NUE are both complex traits with multiple genes/pathways involved (Xu et al., 2017; Schiessl et al., 2017). Due to the many genes involved in these pathways it is likely that any genes selected are likely to have additive effects in *B. napus (*Feder and Walser, 2005*)*.

6.3. Genetic variation for N optimum and yield at low N for modern WOSR genotypes is high and there is potential for improving NUE through better matching N requirements to genotypes.

The RIPR field trials showed a wide range of genotype response to fertiliser N. As the optimum N for WOSR is affected by genotype and environment, and the interaction, working out accurate N optima will be a key future step in precision agriculture. Genotype yield ranking changed between N fertiliser rates, therefore highlighting importance of measuring NUE at the economic optimum. In the 2014-2015 RIPR diversity trials, yield data from HN and LN was collected and genotype response to N was analysed. In all these trials there were examples of genotypes that showed very low response to N when the FN applied was increased dramatically. At the RIPR trial genetic variation was extremely high, when considering the mean % yield gain of N treated plots compared to LN treated plots was 15.6% at DP14 and 16.6% at HM14. The most extreme genotypes had plot losses of 50% at HM14 and 48% at DP14, representing yield loss of 2 t ha^{-1} and 2.4 t ha^{-1} respectively. The least affected plots showed no yield loss between N treatments, with some genotypes even showing an increase in yield at the LN plots. However, the data needs to be considered critically as many of these genotypes were form an ASSYST panel and had low yield potential. In order for a HYLO genotype to be viable it must have high yields that are comparable or greater than the current elites

The low N response of some of the genotypes and the high yields at LN in the first year of the RIPR trial would support that there are N fertiliser saving potential by using N optimums. Stahl et al., (2017) studied 30 elite varieties registered between 1989 and 2014, including hybrids and elites. Changes in seed yield and seed quality traits were studied over a 2-year experiment in 10 environments across Germany. The study found a surprisingly low yield gap between HN and LN fertilisation promising hints toward further N fertiliser-saving potential by calculating N optima. However, the extremely low yielding trials in the diversity 2015-2016 LN plots compared to the 2014-2015 trials does not confirm this theory. Mean LN yields (t ha^{-1}) across all genotype were 3.05 t ha^{-1} at HM14, 2.35 t ha^{-1} at DP14 but only 0.84 t ha^{-1} at OM15 and 1.02 t ha^{-1} at DP15.

The large variation in yield at low N for elite types suggests immediate potential for providing an NUE at LN ranking for farmers. This could result in improved NUE and increased yields by recommending particular genotypes for areas which have legal limits for N application. These new studies support

previous evidence (Berry, 2010; Storer 2018; Stahl 2017) that HYLO type genotypes exist, and that for these varieties, in all environments, N dosage of much less than the standard 180kg/ha rate is sufficient to produce the yield levels usually achieved in farming practice. However, to make these genotypes viable for the market, confirmation of effective NUE over multiple years and environments is essential.

This different response of genotypes across year and environment highlights that being able to understand the yield potential of a site based on environmental and management decisions is important when improving NUE of WOSR. This is because it will not account for differences of soil type and annual conditions which have a massive impact on NUE, for example, the risk of nitrate leaching. Future work could be to adapt the model in chapter 4 to consider site Nitrate leaching potential. Matching each site to an N optimum would increase NUE and reduce N losses.

6.4. The phenological development of WOSR significantly impacts yield, N-optimum, and these are impacted by N-uptake and N-utilisation. Identifying key traits that can be used to predict crop growth at low N and develop simple predictive models.

Traits linked to N uptake and N utilisation were found to have a significant impact on yield. In this section we will assess the mechanisms that could improve NUE and also consider practical measures for collecting each trait. We will then summarise which traits are linked to yield at LN across multiple projects, how this information can used for modelling and consider critically which traits are key for monitoring in field trials for predicting NUE.

There was evidence from the field study that yield at LN was influenced by early development. For the RIPR trials yield at low N was linked to establishment. A large proportion of N is taken up during the vegetative stage (Berry et al., 2010). Therefore, strong establishment and early root development are crucial. Previous studies have shown that the HYLO genotypes of WOSR had much stronger N uptake and xylem loading but with weaker vacuolar N storage relative to the HYHO (lower NUE) genotypes (Han *et al.* 2016). WOSR genotypes with the more N-efficient genotypes showing higher fine roots proportions and increased root length (Bouchet et al., 2016; Stahl et al., 2017). Monitoring roots in field with soil cores is expensive and time consuming however, a high-throughput root phenotyping technique in a controlled environment showed links with field performance (Thomas et al., 2016).

At the RIPR trials N uptake at mid-flowering was linked to yield at LFN. Schulte auf'm Erley (2007) found that N uptake before flowering was substantially higher at high N compared to reduced N

supply. The study found that Genotype differences were more pronounced at high N than at low N supply (significant cultivar × N rate interaction).

Height was linked to NUE at LN at all sites across the RIPR trials. In theory plant with increased height show stronger initial establishment, using the limited N resource available during stem elongation to increase area for photosynthesis instead of over-producing foliage which is not available for photosynthesis during seed filling. Height is easy to measure, it is standard measurement included in breeding and RL trials. UAV systems can be used to automatically monitor height using lidar technology.

NUE and flowering time was linked at the RIPR trial in this thesis. Previous studies have found early flowering date linked to NUE (Müller et al., 2009; Stahl et al., 2016). The overlap between N availability of old leaves and N requirements on developing pods make the flowering and early post-flowering period a critical stage. In theory the earlier switch from the vegetative to generative phase, and therefore likely earlier start of senescence processes, can stretch the remobilisation phase, mobilise more N from source to sink organs and thus improve the N utilisation (Mendhalm et al., 1964). In study RIPR early flowering was also associated with higher seed N utilisation, although to a lesser extent than yield at LN. We did not collect flowering data from high N plots. However, Stahl et al. (2016) found a relationship with flowering at LN but not HN treatments. This observation suggests a potential to increase NUE in low input environments by modifying flowering time.

Several field studies have shown that post-flowering N uptake efficiency has a significant effect on total NUE and is the most important factor to discriminate between genotype for yield (Berry et al., 2010; Schulte auf'm Erley et al., 2011; Ulas et al., 2013). Flowering is associated with a massive change in the source-sink relationship, and it is likely that the majority of N taken up after flowering would go straight to seed yield (Mendham, 1981). Schulte auf'm Erley et al., 2011 found that N uptake between beginning of flowering and maturity was much lower than before flowering and sometimes negative, because of N losses from the above-ground plant material. For the NUE genotypes in the RIPR study N uptake post flowering was generally higher at low N for the genotypes that were HYLO but T-tests did not show a significant differences.

Stem N remobilisation was significant linked to NUE and N utilisation at most trial sites. Efficient remobilisation requires both a source (i.e. leaves, stems and siliques) that provides enough N and a sink (seeds) ready to receive the mobilized N. Phenotypic traits linked to N utilisation efficiency include: leaf senescence (Ulas et al., 2013; Kessel et al., 2012, Malagoli et al., 2005); early flowering (Stahl et al., 2016); N remobilisation from leaf and stem; and seed N content (Berry et al., 2010). Low NUE of WOSR has been significantly associated with the weak N remobilisation that occurs in leaves during vegetative development (Dejoux et al., 2000; Malagoli et al., 2005, Aufhammer et al., 1995; Desclos et al., 2009), mainly due to the loss of N-rich leaves (Schjoerring et

al., 1995; Rossato et al., 2001) optimised stem N storage and low seed protein:high seed oil contents (Nyikako et al., 2014, Stahl et al., 2017).

In the RIPR trial and the DEFRA study there was a negative relationship between seed N concentration (%) and yield at LN (t ha^{-1}). Theoretically the increase in yield could be due to sink-source relationship. If seed has less N concentration more seed yield can be produced (Stahl et al., 2017). Field trials have found that enhanced seed yield is linked to higher oil concentration, whereas both are negatively correlated to seed N concentration (Stahl, 2016). They concluded that older genotypes have been selected for yields have generally been subjected to strong selection towards increased oil yield. This study did not analyse oil contents of seeds. This is important as oil is the most valuable product from WOSR.

Table 17 summarises which traits are linked to yield at LN across multiple project. Can these traits be used to predict yield at LN? Different traits were seen to be linked at different projects. Flowering date was linked to yield at LN across all field trials. Flowering date, post-flowering N uptake, stem N concentration, height and establishment were linked at both the RIPR trials. Seed N concentration (%) and maturity were significantly linked to yield in the LK and RIPR FS1. Yields at the 2015-2016 trials were extremely low, however some of the traits linked to NUE such as early flowering and seed N content were shown to be significant. How useful these traits are is questionable because some have not been seen consistently across environments and trials. For example, seed N concentration and maturity were seen to be significant in two out of four field studies. Post-flowering N uptake was significant across all trial sites. However, this trait is difficult and time consuming to measure in field trials. When ranking traits between genotypes we showed that there was high variation however genotype at the extremes were more consistent. This effect has also been seen in other WOSR trials in the UK (Storer et al., 2018).

When working with diversity trials the usefulness of the data has to be questioned because these genotypes have a wider range of trait. This will increase the chance of a significant finding but may not be useful in modern day breeding trials. Pearson correlation has to be used carefully. When it is used with a diverse data set results can easily be over interpreted. When your values are all quite similar the noise can affect the correlation.

Table 17. Summary table of traits linked to yield at low N. ✓ means the trait has a significant positive on yield. The project (LK0979) is sponsored by Defra through the Sustainable Arable LINK Programme. HYLO project was run by ADAS. The HYLO project refers to (IF01110) this is for the development of appropriate variety testing methodology for assessing N requirements of new varieties in trials undertaken for national listing.

Trait	LK0979	HYLO	RIPR variety
Post-flowering N uptake	✓	-	✓
Flowering date	-	-	✓
Seeds/m^2	✓	-	-
Stem N remobilisation	✓	-	✓
Stem N concentration (%)	-	-	✓
Height	✓	✓	✓
Maturity	✓	✓	✓
Seed moisture content	✓	-	NA
Seed oil concentration	✓	✓	NA
Seed N concentration	✓	✓	✓
Seed glucosinolate	✓	-	NA
NUE at optimum	-	✓	NA
Establishment	-	-	✓

The aim of the model was to produce a physiologically accurate simple N-driven model which could distinguish between genotype. The model was initially developed using genotype ranges from published data (Berry et al., 2010) and ADAS reports of an elite set of data. The model showed good predictions over four sites. The sensitivity analysis of the model showed that the most important traits for yield at low N were post-anthesis N acquisition; and SMN acquisition efficiency. Field studies have concluded that N acquisition is more important than utilisation traits at low N (Kessel et al., 2012; Berry et al., 2010). Post-anthesis N acquisition has also been reported as an important trait for increasing yield at low N supply (Berry et al., 2010). The model was not able to predict yields in the RIPR trials. It has also not been tested for predicting yields at high FN rates.

Ease of measurement is critical from a breeders perspective. Height and establishment are easy to score and this measurement is often taken as standard. Seed N content can be estimated using seed quality readings commonly taken at harvest such as protein content. Work could be done to accurately correlate these reading as protein analysis is quicker and cheaper than N analysis. Flowering is relatively easy to monitor as part of a breeding trial. UAVs and image analysis can be automated to allow flowering assessed at multiple time points. In practise there are issues with early flowering

associated with crops which over-winter. There are costs associated with cold temperatures effect on pollination. Remobilisation of N is difficult and time-consuming to assess. A thorough assessment of remobilisation would need to include N content of fallen leaves. When the plant scale is considered, the N flux measurements by using isotope labelling techniques (15N) allow assessing precise N dynamics throughout the plant organs**.** Crop N uptake at the start of stem extension can be estimated easily and reasonably accurately using green area index as a proxy measure (Berry et al. 2011a). There is also potential to measure green area index and crop N content remotely using spectral reflectance indices (Muller et al. 2008). However, these remote sensing techniques are less likely to be useful after the start of stem extension when full canopy cover is achieved, which means that are less likely to be useful for estimating post-anthesis N uptake. N remobilisation efficiency from pod, stem and leaf can be estimated using pod green area index or tissue biomass (m^2) and NHI, thus avoiding multiple tissue N analyses.

6.5. These diversity sets can be used to define phenotypic and genetic markers that are indicators of NUE.

Analysis of older genotypes within a diversity trial is beneficial for better nutrient use, since they might have comparative advantages in adaptation to low input systems. These diversity sets can be used to define phenotypic and genetic markers that are indicators of NUE. Narrow elite gene pools of modern crops generally exhibit reduced genetic variation due to extensive selection for adaptation, quality and resistance traits. In principle, working with a diversity panel allows assessment and pinpointing of N utilisation from a wide range of traits. This trait range information can be used to determine the phenotypic diversity in the field, and the interrelationships in the field population. This can also inform further studies; production of process based crop models; guidance for key traits in LN breeding trials; and subsequent studies to dissect the genetic bases and identify markers in traits relevant for crop improvement. To our knowledge, RIPR reports the most thorough assessment of field-based data generated to date for trait evaluation of a WOSR diversity panel under two divergent N fertilisation levels.

Working with a diversity panel does have significant challenge. The spread of the data set can result in significant results for characteristics that may not be impactful or help when breeding elite types. Many of the genotypes grown are impacted by other yield limiting factors. For example the relationship in the RIPR trial between genotype and yield at HN v LN was affected by genotype category. ASSYST types showed a positive relationship between N treatments (Fig 18): HM14 (r^2 = 0.27, $p < .0001$); and DP14 (r^2 = 0.45; $p < .0001$). Elite types showed no significant relationsbetween LN and HN at either site. This apparent difference between the groups may simply reflect the relatively narrow range of yields seen for the elite genotypes when compared to the ASSYST. In fact, both groups show a similar range of (HN – LN) yield differences (Fig 16C). The observed relationship

of ASSYST genotypes with (HN – LN) yield difference could be due to the wider range of yields, due in turn to the inclusion of older and more diverse genotypes having other yield limiting factors (disease susceptibility, phenology, physiology) with a greater impact on yield than fertiliser efficiency. Stahl et al., 2017; and Kessel et al. 2012, rejected the hypothesis that cultivation of older genotypes could be beneficial for better nutrient use, since they might have comparative advantages in adaptation to low input systems because they did not see improve NUE in older genotype.

The RIPR trial analysed trait relationship to yield at LN. The analysis showed that WOSR populations showed some of the same traits linking to NUE across a range of environmental conditions. The traits significantly linked to yield at LN were: Maturity total N of seeds; Maturity total crop N; Maturity dry weight of crop; N uptake post flowering; Maturity dry weight of stem; Maturity dry weight of chaff; Maturity score; Flower score; N biomass; Maturity total N of chaff; N concentration of seed; Mid-flowering Stem weight. It is important to add that these traits are linked through pearsons correlations. It is a measure of their linear relationship and these can be influenced by extreme genotypes.

He et al. (2017) studied 50 genotypes at two N rates over one year of field trials. In this trial they collected data for OSR growth and N content. They found that over one year, genotypes showed similar underlying sub-traits linked to N utilisation. Seed number per silique; harvest index; and N harvest index were significantly correlated with N utilisation under contrasting N conditions.

Due to differences in traits identified across environments it seems likely that monitoring one trait alone will not be a suitable indicator of NUE. Once validated with field data, the model has been used to improve our understanding of how simple to measure traits affect N dynamics. Over time, this may allow us to answer questions, relating to varietal differences, which cannot be addressed with field research due to time and cost constraints. We have evaluated the possibility of designing crop ideotypes alongside modelling for yield and low N; and field data evaluation. We simulated indicators of crop performance (grain yield) with a previously developed and validated model (Berry et al., 2010). Ranking and optimisation methods were used on these indicators to search for GEM combinations matching ideotype specification. One conclusion from this study is that environmental variation of N response has an impact, therefore being able to test over multiple environments will be key.

This thesis also lays a foundation of analysing the genetic composition of genotype data in the RIPR trial. This initial work showed several promising associations, some genes previously shown to influence or respond to NUE and Flowering score in *Brassica napus.* None of the candidate genes were above the bonferroni corrected significance threshold. However, some were close, and there were evident peaks and candidate genes that were consistent across field trials. We identified some

peaks that are associated with N assimilation and N uptake pathways. Some of our candidate genes had been seen in previous work (Bowles et al., 2006; Xu et al., 2017). Selection of genotypes with different alleles for target genes may lead to improved ability to grow under low N conditions and act as markers for breeding. We did not test the structure and function of the candidate genes selected. The next step would be to conduct experiments in A. thaliana T-DNA knockouts to confirm the level of influence. Flowering and NUE are both complex traits with multiple genes/pathways involved. Due to the many genes involved in these pathways it is likely that any genes selected are likely to have additive effects in *B. napus*. Work could also be done to test if functions observed here and in previous studies are conserved. Selection of *B. napus* genotypes with different alleles of target genes may lead to improved ability to grow in the presence of low soil N concentrations.

6.6. Practical approaches for calculating N optimum.

As described above data from these trials, further charactering of HYLO genotypes have shown key traits linked to NUE provide promise towards further N fertiliser-saving potential. This study demonstrates that including a LN environment within the breeding programmes and trial scheme would increase our trait knowledge and give an indication of yield potential at LN as genotypes with a greater yield at LN are likely to have a greater NUE at the economic N fertiliser application rate through achieving a similar yield with a lower economic N rate (Sylvester- Bradley and Kindred, 2009, Berry et al., 2010). However, large studies are expensive and labour intensive.

Motivation for farmers will increase if regulations are tightened, or if the price of N increases relative to the prices of WOSR. Approaches are therefore required to test genetic differences in N optima in a cost effective way. There are savings associated with precise N application. Our study confirms with other studies that genotypes have different N responses across environments. Therefore, it is important to test genotypes at multiple locations. The costs of conducting full N response experiments is very large and there are practical issues with the implementation. Recommended List reports yield and crop protection requirements of new genotypes, but it has none for nutrient requirements (HGCA, 2013). Recommended List trials test for ± fungicides & ± PGR treatments. It is unlikely that it would be possible to add 7 N rates within the recommended list system to add N fertiliser or during breeding trials due to trial size and seed availability.

Possible new approaches include the use of existing or new measurements in genotype trials, altering the N rate used in genotype trials, or testing at additional N rates. If an NUE score was included in recommended lists it would vastly increase knowledge of genotype difference and encourage breeders to breed directly for this trait. Recommended trials are conducted over 14 sites and include many genotypes. Approaches are therefore required to test genetic differences in N optima in a cost effective way. This in part could be through process based modelling system. However, this study has

shown that traits are not consistent over trial sites and environments. An alternative approach is the use of Opt-plots can be used to calculate optimum fertile or pesticide rate. Inputs ‘ramped’ across micro-plots (Fig 59). Yield results can then be collected quickly using breeders’ harvesting methods.

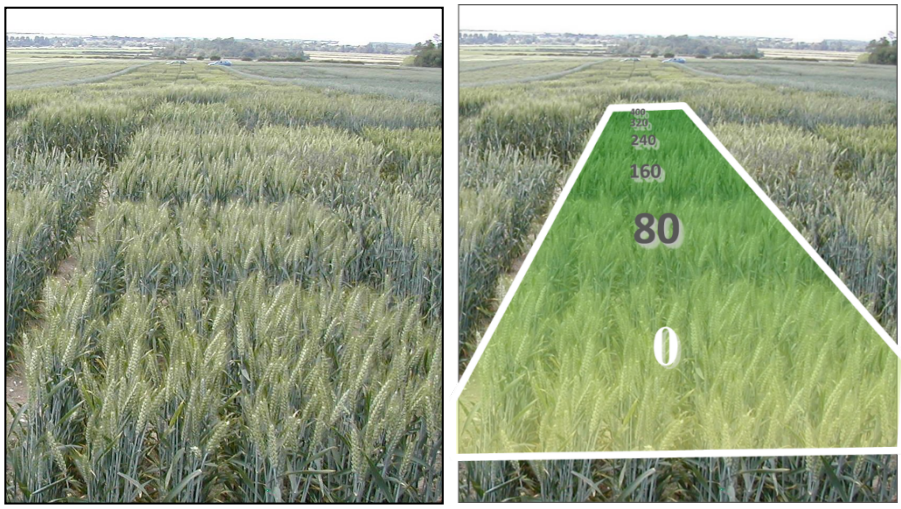


Fig 59. Opt-plots. 5 rates can be tested on the same plot.

6.7. Environmental and economic impact of fertiliser

There are both economic and environmental demand for breeding HYLO genotypes. Fertiliser N use is linked to the most critical issues associated with farming; ghg emission, land-use change, soil damage, air quality and loss of biodiversity. Globally agriculture contributes 14% to ghg emissions. A third of this is due to methane (livestock), a third due to nitrous oxide (N fertiliser). In the UK agriculture is responsible for 10% of ghg emissions and 83% of UK ammonia emissions (DEFRA, 2017). Ammonia emissions have an estimated cost of £456 cost to human health and environment from UK agriculturally produced ammonia in 2015 (data uses 2017 prices). There is already evidence that disease control in UK winter wheat crops can contribute to climate change mitigation (Berry et al., 2008).

N fertiliser has a direct part to play in the discussion of trade offs between farming productivity and the environment. If the crop is over-fertilised, there will be N surpluses, if the crop is under-fertilised the reduced N results in loss of yield, lowering land productivity. Reactive N deposition from intensive agricultural and industrial activity has been identified as the third greatest threat to global terrestrial biodiversity (Payne, et al., 2017). While the impacts of N deposition on biodiversity are widely acknowledged, their magnitude is poorly quantified. Studies have concluded that the effects of N deposition on biodiversity are severe and are unlikely to be quickly reversed (Payne et al., 2017).

Worldwide production of WOSR is 50Mt per annum (www.fas.usda.gov), worth £15000M at a price of £300/t. The main diseases that affect the UK WOSR crop are phoma stem canker (*Leptosphaeria maculans*) and light leaf spot (*Pyrenopeziza brassicae*), which cause annual losses worth more than £550M worldwide (Mahmut *et al*., 2009). Reducing NUE by crop breeding or precision agriculture has potentially large financial savings for farmers (Table 18).

Table 18. Figures to show economic value of less fertiliser.

	Default values	Improved N efficiency	Improved N efficiency
N rate to achieve 4 t ha^{-1} (kg/ha)	200	170	140
Cost of N fertiliser (£/kg)	0.75	0.75	0.75
N fertiliser cost (£/ha)	150	128	105
UK OSR area (ha)	500,000	500,000	500,000
Cost to fertilise UK OSR	£75 million	£64 million	£52.2 million

Over use of N fertilisers also has other non-direct impacts on farm management. High N fertiliser application changes the weed spectrum, increasing competitive and aggressive weeds such as cleavers and black-grass. These weeds take up N applied in early spring – being both competitors for the crop area and fertiliser. If not controlled the weeds will significantly reduce yields. However, an under fertilised crop is more prone to be overtaken by weed competition, especially from weeds developing later in the crop cycle (HGCA, 2013). Over-fertilised crops are more prone to stem based disease and powdery mildew. High N crops can have increased sap content and more prone to pests such as aphids. This may mean that higher N levels than required result in higher herbicide, fungicide and insecticide than would be required at a lower N rate. However, loss of yield due to drought, floods, unpredictable weather conditions, which may increase due to global warming, may be more consequential if N input is lowered.

DEFRA future farming aims to both (1) Improve productivity and competitiveness of agriculture and (2) preserve rural resilience and traditional farming and landscapes (DEFRA, 2019). Future policy could therefore fund the uptake of innovative technologies; new business models; skills and businesses training; continuous farmer learning; facilitating “research syndicates” to better disseminate new findings and supporting investment in a domestic workforce and automation. It is a time of change for UK agriculture policy and there is a possibility of tightened N use regulations. Motivation for farmers to improve NUE will increase if the price of N increase compared to value of WOSR decreases.

6.8 Methods for phenotyping diversity trials

Central to this study is monitoring traits of a genetically diverse population's response to N and environment during the full course of WOSR development. Current phenotyping techniques, in particular the capacity to collect in field data, is a bottleneck for further advancements in the development of crop genotypes and yield gains (Hawkesford, 2014). Phenotyping is expensive and labour intensive. Collection of cost-efficient, quality phenotyping data is essential for use in research and standard breeding programs. In this project we developed methods for UAV image analysis. We also developed a flow for collecting phenotypic N analysis for over 9000 samples. Although there are incredible new technologies with massive potential for phenotyping there is a demand for methods which are affordable, accessible and efficient to set-up and establish. The focused phenotyping methods developed during this study have allowed the production of tissue data and UAV RGB data within budget and time frame, allowing additional phenotype data to be added to the project.

When collecting phenotypic data from a diversity trial, there is a balance of quality and quantity. For large statistical and bioinformatics studies, the number of genotypes is important to provide the study power. In this study, we assessed traits with very large ranges, the data analysis included analysis of data with extreme differences. Field trial data over multiple environments have lots of variables within site and across sites, e.g., weather, management, and soil type. As discussed, genotype traits differences have to be large enough that they have significant impact over multiple environments. Therefore, significant efforts spent on measuring small, subtle difference between traits may not be an efficient use of resource. However, if phenotype data is scored inaccurately to the point that it is not be reliable or useful for analysis, then there is no point in collecting that data.

The UAV system used in the trials allowed for efficient, easy to use, and affordable collection of additional time point data for the RIPR field trials. 508 individual plots were imaged in a 12-minute flight path. It would take 4-5 hours of field technician time to collect images using a camera and tripod set up. The DJI Phantom UAV equipment was suitable for use with minimal training and retained its height and position during data collection. The UAV system can be deployed quickly and repeatedly. Although licenses are required UAVs are readily available to agronomists, researchers, and breeders. The UAV system was able to differentiate between plots in large field trials using an RGB sensor and simple image analysis techniques. There were strong relationships between GAI results from the UAV system and establishment scoring; HM14 (r^2=0.74) and DP14 (r^2=0.67). The methods used in this study can be used for future diversity field trials or breeding programs.

There are important limitations within the UAV system to consider. This simple image analysis method does not distinguish between crops and weed material. Field trials and breeding programs are conducted with the use of agronomy. This means weeds should be minimal but 100% control is

commonly not achieved. With the RGB plot images it is pronounced if weed pressure is high, so images can be manually scored for weeds and excluded if required. Methods are available that distinguish between weeds and crop (Perez et al., 2000), but the majority require more complex sensors. Cloud coverage and light conditions affect colours of images and therefore image analysis. To combat this, flights were made around midday as suggested by (Shishi et al., 2018). If image conditions varied within flights then before analysis images were normalised using the curve tool using sections of soil as key. However, the UAV image analysis does have some advantages. The method removes variability of different field trial assessors with potentially different scales.

UAV data collected can be used to refine further data models to use precision agriculture will be able to use UAV data to match nutrient supply (and other operations) to the spatial variability of crop plants. As well as phenotyping field trials the UAV data collection and analysis can allow on-field management decisions (e.g., irrigation, herbicide application) to be made mid-season. Other potential useful RGB phenotyping methods include estimates off; Height, biomass and crop counts. More complex sensors (near infrared, thermal) can score plant health and environmental conditions (Shishi et al. 2018).

6.9. Conclusions

The trial described in this thesis support previous evidence (Berry, 2010; Storer 2018; Stahl 2017) that HYLO type WOSR genotypes exist and have great potential to reduce NUE. However, our results show that NUE of WOSR is a complex trait affected by: genotype; environment; management; and their interactions. NUE can be improved through breeding NUE efficient genotypes and by more precise application of N through increasing knowledge of optimising N based on genotype and yield potential of the field environment. There are many practical issues linked to monitoring traits and calculating N optima of genotypes over different environments. However, improving NUE of UK WOSR would have a positive effect on GHG emissions, the aqueous environment and farm economics. Possible new approaches for identifying N optima include: the use of existing or new measurements in genotype trials; altering the N rate used in genotype trials; or testing at additional N rates. Opt-plots can be used to calculate optimum fertiliser or pesticide rate.

Field trial results with the RIPR WOSR diversity panel showed a large amount of variation across genotypes for traits, yields and response to N. Significant relationships have been seen between yield formation at low N with: early flowering; post-flowering N uptake; stem N remobilisation; mid-flowering N concentration; maturity chaff N concentration; seed N concentration; height; and establishment. However, these traits have not been seen to impact yield at every trial and much variation was seen for traits within and between environments. Several promising candidate genes,

some previously shown to influence or respond to NUE and flowering score in *Brassica napus*, have been selected using associative transcriptomic analysis. The next step would be to conduct experiments in A. thaliana T-DNA knockouts to confirm the level of influence.

A simple process based model was used to identify novel traits for use in breeding strategies. These could help to improve NUE and to identify crop traits and trait combinations with potential for increasing seed yield at low N of WOSR. The model has been used to provide a good estimate of yield potential of ten genotypes in low N supply trials. The model did not accurately predict yield across all environments tested but has potential for future development. Sensitivity analysis showed agreement across important traits between the model and published field studies. The most important varietal traits for either reducing N requirement whilst maintaining current commercial yields or increasing yield at low N supply are: greater post-anthesis uptake; efficiency of N acquisition from fertiliser; and days for seed filling and N remobilisation from pods.

Bibliography

Abdallah M, Etienne P, Ourry A, Meuriot F., 2011. Do initial S reserves and mineral S availability alter leaf S-N mobilization and leaf senescence in oilseed rape? Plant Science, 180: 511-520.

Adams K, Cronn R, Percifield R, Wendel J., 2003. Genes duplicated by polyploidy show unequal contributions to the transcriptome and organ-specific reciprocal silencing. Proceedings of the National Academy of Sciences, 100 (8): 4649-4654.

AHDB, 2009. Nutrient Management Guide: RB209.

Alboresi A, Gestin C, Leydecker MT, Bedu M, Meyer C, Truong HN., 2005. Nitrate, a signal relieving seed dormancy in Arabidopsis. Plant Cell Environment, 28: 500–512.

Alcock TD, Havlickova L, He Z, et al., 2017.Identification of Candidate Genes for Calcium and Magnesium Accumulation in Brassica napus L. by Association Genetics. Frontier Plant Science, 8:1968.

Ambus P, Christensen S., 1993. Denitrification variability and control in a riparian fen irrigated with agricultural drainage water. Soil Biology & Biochemistry. 25: 915–923.

Araus JL, Cairns JE., 2014. Field high-throughput phenotyping: the new crop breeding. Frontier Plant Science. 19(1): 52-61.

Asseng S, Turner NC, Botwright T, Condon AG., 2003. Evaluating the impact of a trait for increased specific leaf area on wheat yields using a crop simulation model, Agronomy Journal. 95: 10–19.

Asseng S, Turner N., 2007. Modelling Genotype x Environment x Management Interactions to Improve Yield, Water Use Efficiency and Grain Protein in Wheat. In J. H. J. Spiertz, P. C. Struik, & H. H. V. Laar (Eds.), Scale and Complexity in Plant Systems Research - Gene-Plant-Crop Relations (91-101).

Aufhammer W, Kaul H, Herz P, Nalborczyk E, Dalbiak A, & Gontarczyk M., 1995. Grain yield formation and nitrogen uptake of amaranth. European journal of agronomy. 4(3): 379-386

Baggs EM, Rees RM, Smith KA, Vinten AJA., 2000. Nitrous oxide emission from soils after incorporating crop residues. Soil Use Management, 16(2): 82–87.

Bancroft I, Morgan C, Fraser F, Higgins J, Wells R, Clissold L., 2011. Dissecting the genome of the polyploid crop oilseed rape by transcriptome sequencing. Nature Biotechnology. 29: 762–766.

Bänziger M, Setimela PS, Hodson D, Vivek B., 2006. Breeding for improved abiotic stress tolerance in maize adapted to southern Africa. Agriculture Water Management, 80: 212–224.

Barbottin A., 2004. Utilisation d'un modèle de culture pour évaluer le comportement des génotypes: Pertinence de l'utilisation d'Azodyn pour analyser la variabilité du rendement et de la teneur en protéines du blé tendre. Thèse de Doctorat, INA P-G, Paris

Barton L, Murphy DV, Kiese R, Butterbach-Bahl K., 2010. Soil nitrous oxide and methane fluxes are low from a bioenergy crop (canola) grown in a semi-arid climate. Global Change Biology Bioenergy, 2, 1–15

Basunanda P, Radoev M, Ecke W, Friedt W, Becker HC, Snowdon RJ., 2010. Comparative mapping of quantitative trait loci involved in heterosis for seedling and yield traits in oilseed rape (Brassica napus L.). Theoretical Applied Genetics. 120: 271–281.

Bateman EJ, Baggs EM., 2005. Contributions of nitrification and denitrification to N_2O emissions from soils at different water-filled pore space. Biology and Fertility of Soils. 41: 379-388.

Benjamini Y, Hochberg Y.,1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Society Statistical Society. 57: 289–300.

Berry PM, Spink JH., 2006. A physiological analysis of oilseed rape yields: Past and future. Journal of Agricultural Science. 144: 381-392.

Berry PM, Teakle G, Foulkes MJ, White PJ, Spink J., 2008. Breeding for Improved Nitrogen Use Efficiency in Oilseed Rape. Acta Horticulturae. 867: 97-101.

Berry PM, Spink JH., 2009. “Canopy Management” and late nitrogen applications to improve yield of oilseed rape. Home-Grown Cereals Authority. Project Report No447 HGCA.

Berry PM, Spink JH, Foulkes MJ, White PJ., 2010 The physiological basis of genotypic differences in nitrogen use efficiency in oilseed rape (Brassica napus L.). Field Crops Research. 119: 365-373.

Berry PM, Roques S, Clarke S., 2011a. Predicting the optimum rate and timing of nitrogen fertiliser for winter oilseed rape. 13th International Rapeseed Congress. Prague, 5-9 June 2011.

Berry PM, Foulkes MJ, Carvalho P, Teakle G, White PJ, White C, Roques S., 2011b. Breeding oilseed rape with a low requirement for nitrogen fertiliser. HGCA Report 479 and Sustainable Arable LINK Project LK0979.

Bloom AJ., 2015. Photorespiration and nitrate assimilation: a major intersection between plant carbon and nitrogen. Photosynthesis Research. 123(2):117-28.

Blümel M, Dally N, Jung C., 2014. Flowering time regulation in crops—What did we learn from Arabidopsis? Current opinion in biotechnology. 32: 121-129.

Bogard M, Jourdan M, Allard V, Martre P, Perretant MR, Ravel C, Heumez E, Orford S, Snape J, Griffiths S, Gaju O, Foulkes J, Le Gouis J., 2011. Anthesis date mainly explained correlations between post-anthesis leaf senescence, grain yield, and grain protein concentration in a winter wheat population segregating for flowering time QTLs. Journal of Experimental Botany. 62(10): 3621-36

Boote KJ, Jones JW, Batchelor WD, Nafziger ED, Myers O., 2003. Genetic coefficients in the CROPGRO-Soybean model: links to field performance and genomics, Agronomy Journal. 95: 32–51.

Böttcher U, Rampin E, Hartmann K, Zanetti F, Flenet F, Morison MV, Kage H., 2016. A phenological model of winter oilseed rape according to the BBCH scale. Crop and Pasture Science. 67: 345 - 358.

Böttcher U, Wiebke W, Jeroen WM, Pullens J, Olesen E, Henning K., 2020. Development and evaluation of HUME-OSR: A dynamic crop growth model for winter oilseed rape. Field crop research. 246.

Bouchet AS, Nesi N, Bissuel C et al., 2014. Genetic control of yield and yield components in winter oilseed rape (Brassica napus L.) grown under nitrogen limitation. 2014. Euphytica. 199: 183-205.

Bouchet AS, Laperche A, Bissuel-Belaygue C et al., 2016. Nitrogen use efficiency in rapeseed. A review. Agronomy for Sustainable Development. 36:36

Bowles D, Lim EK, Poppenberger B, Vaistij FE., 2006. Glycosyltransferases of lipophilic small molecules. Annual Review of Plant Biology. 57: 567–597.

Bradbury NJ, Whitmore AP, Hart PBS, Jenkinson DS., 1993. Modelling the fate of Nitrogen in crop and soil in the years following application of N-15-labeled fertiliser to winter-wheat. Journal of Agricultural Science. 121: 363-379.

Bruce DM, Farrent JW, Morgan CL, Child RD., 2002. PA—Precision Agriculture: Determining the Oilseed Rape Pod Strength Needed to Reduce Seed Loss due to Pod Shatter. Biosystems Engineering. 81(2): 179-184,

Burney JA, Davis SJ, and Lobell DB., 2010. Greenhouse gas mitigation by agricultural intensification. Proceedings of the National Academy of Sciences. U.S.A. 107: 12052–12057.

Bus A, Körber N, Snowdon R, Stich B., 2011. Patterns of molecular variation in a species-wide germplasm set of Brassica napus. Theoretical and Applied Genetics. 123: 1413–1423.

Bus A, Körber N, Parkin IAP, Samans B, Snowdon RJ, Li J., 2014. Species- and genome-wide dissection of the shoot ionome in Brassica napus and its relationship to seedling development. Frontier Plant Science. 5:485.

Cai HM, Xiao JH, Zhang QF, Lian, XM., 2010. Co-suppressed glutamine synthetase2 gene modifies nitrogen metabolism and plant growth in rice. Chinese Science Bulletin. 55:823–33.

Casadebaig P, Guilioni L, Lecoeur J, et al., SUNFLO, a model to simulate genotype-specific performance of the sunflower crop in contrasting environments Agricultural and Forest meteorology. 151 (2):163-178

Castro Marin I, Loef I, Bartetzko L, Searle I, Coupland G, Stitt M, et al., 2011. Nitrate regulates floral induction in Arabidopsis, acting independently of light, gibberellin and autonomous pathways. Planta. 233: 539–552.

Chen W, Zhang Y, Liu X, et al., 2007. Detection of QTL for six yield-related traits in oilseed rape (Brassica napus) using DH and immortalized F2 populations. Theoretical and Applied Genetics. 115: 849–858

Chiu C, Lin CS, Hsia AP, Su RC, Lin HL, Tsay YF., 2004. Mutation of a nitrate transporter, AtNRT1:4, results in a reduced petiole nitrate content and altered leaf development. Plant and Cell Physiology. 45. 1139–1148.

Chopin F, Orsel M, Dorbe MF, Chardon F, Truong HN, Miller AJ, Krapp A, Daniel-Vedele F., 2007. The Arabidopsis ATNRT2.7 nitrate transporter controls nitrate content in seeds. Plant Cell 19: 1590-1602

Colneme C, Meynard JM, Reau R, Justes E, Merrien A., 1998. Determination of a critical dilution curve for winter oilseed rape. Annals of Botany. 81: 311–317.

Crawford NM., 1995. Nitrate: Nutrient and signal for plant growth. Plant Cell. 7: 859–868.

Czolpinska M, Rurek M., 2018. Plant Glycine-Rich Proteins in Stress Response: An Emerging, Still Prospective Story. Frontiers Plant Science. 9: 302.

Daniel-Vedele F, Filleur S, Caboche M., 1998. Nitrate transport: a key step in nitrate assimilation. Current Opinion Plant Biology. 1: 235–239.

David C, Jeuffroy MH, Recous S, Dorsainville F., 2004. Adaptation and assessment of the Azodyn model for managing the nitrogen fertilization of organic winter wheat, European Journal of Agronomy. 21: 249–266.

David C, Jeuffroy MH, Laurent F, Mangin M, Meynard JM., 2005. The assessment of a decision-making tool for managing nitrogen fertilization of organic winter wheat, European Journal of Agronomy. 23: 225–242.

De Koeijer TJ, Wossink GAA, Van Ittersum MK, Struik PC, Renkema JA., 1999. A conceptual model for analysing input-output coefficients in arable farming systems: from diagnosis towards design. Agricultural Systems. 61: 33–44.

Deery D, Jimenez-Berni J, Jones H, Sirault X, Furbank R., 2014. Proximal remote sensing buggies and potential applications for field-based phenotyping. Agronomy. 4: 349–379.

DEFRA., 2016. Annual statistics about agriculture in the United Kingdom to 2016.

DEFRA., 2017. British survey of fertiliser practice 2017 - Annual report

DEFRA., 2018. Annual statistics about agriculture in the United Kingdom to 2018.

DEFRA., 2019. The Future Farming and Environment Evidence Compendium.

Dejoux JF, Recous S, Meynard JM, Trinsoutrot I, Leterme P., 2000. The fate of nitrogen from winter-frozen rapeseed leaves: mineralization, fluxes to the environment and uptake by rapeseed crop in spring. Plant and Soil 218: 257–272.

Delourme R, Falentin C, Huteau V, Clouet V, Horvais, R, Gandon B, Specel S, Hanneton L, Dheu J, Deschamps M., 2006. Genetic control of oil content in oilseed rape (Brassica napus L.). Theoretical and Applied Genetics. 113: 1331–45.

Derkx AP, Orford S, Griffiths S, Foulkes MJ, Hawkesford MJ., 2012. Identification of differentially senescing mutants of wheat and impacts on yield, biomass and nitrogen partitioning. Journal of Integrative Plant Biology. 54: 555–566.

Desclos M, Etienne P, Coquet L, et al., 2009. A combined 15N tracing/proteomics study in Brassica napus reveals the chronology of proteomics events associated with N remobilisation during leaf senescence induced by nitrate limitation or starvation. Proteomics. 9(13): 3580-3608.

Diepenbrock W., 2000. Yield analysis of winter oilseed rape (Brassica napus L.): a review. Field Crops Research. 67: 35-49.

Ding G, Zhao Z, Liao Y, Hu Y, Shi L, Long Y, Xu Fe., 2012. Quantitative trait loci for seed yield and yield-related traits, and their responses to reduced phosphorus supply in Brassica napus. Annals of Botany 109: 747–759.

Dise NB, Ashmore MR, Belyazid S, Sutton M., 2011. Nitrogen as a threat to european 474 terrestrial biodiversity. In: The european nitrogen assessment: Sources, effects and policy 475 perspectives. Cambridge University Press.

Dobbie KE, Smith, KA, 2003. Impact of different forms of N fertilizer on N_2O emissions from intensive grassland. Nutrient Cycling in Agroecosystems, 67: 37-46.

Don A, Schumacher J, Freibauer A., 2011. Impact of tropical land-use change on soil carbon stocks—a meta-analysis. Global Change Biology. 17:1658–1670

Dresboll DB, Thorup-Kristensen K., 2014. Will breeding for nitrogen use efficient crops lead to nitrogen use efficient cropping systems? : a simulation study of GxExM interactions. Euphytica. 199: 97-117.

Drewer J, Finch JW, LLoyd CR, Baggs E, Skiba U., 2011. 'How do soil emissions of N_2O, CH4 and CO2 from perennial bioenergy crops differ from arable annual crops?'. Global Change Biology Bioenergy. 4: 408-419.

Dun X, Tao Z, Wang J, Wang X, Liu G, Wang H., 2016. Comparative transcriptome analysis of primary roots of Brassica napus seedlings with extremely different primary root lengths using RNA sequencing. Frontier Plant Science. 7:1238.

Dunn OJ., 1961. Multiple comparisons among means. Journal of the American Statistical Association. 56: 52–64.

Dupre C, Stevens CJ, Ranke T, et al., 2010. Changes in species richness and composition in european 487 acidic grasslands over the past 70 years: The contribution of cumulative atmospheric nitrogen 488 deposition. Global Change Biology. 16: 344-357.

Ecke W, Clemens R, Honsdorf N, Becker HC., 2010. Extent and structure of linkage disequilibrium in canola quality winter rapeseed (Brassica napus L.). Theoretical and Applied Genetics. 120: 921–931.

Erley GSA, Behrens T, Ulas A., Wiesler F,. Horst WJ., 2011. Agronomic traits contributing to nitrogen efficiency of winter oilseed rape cultivars. Field Crops Research. 124: 114-123.

Evans T., 2015. Considerations for the use of transcriptomics in identifying the ‘genes that matter’ for environmental adaptation. Journal of Experimental Biology 2015. 218: 1925-1935;

Fan SC, Lin CS, Hsu PK, Lin SH, Tsay YF., 2009. The Arabidopsis nitrate transporter NRT1.7, expressed in phloem, is responsible for source-to-sink remobilization of nitrate. Plant Cell. 21(9): 2750–2761

Feder ME, Walser JC., 2005. The biological limitations of transcriptomics in elucidating stress and stress responses. Journal of Evolutionary Biology. 18: 901-910.

Gabrielle B, Denoroy P, Gosse G, Justes E, Andersen MN., 1998a. A model of leaf area development and senescence for winter oilseed rape. Field Crops Research. 57: 209-222.

Gabrielle B, Denoroy P, Gosse G, Justes E, Andersen MN., 1998b. Development and evaluation of a CERES-type model for winter oilseed rape. Field Crops Research. 57: 95-111.

Gaju O, Allard V, Martre P, Gouis JL, Moreau D, Bogard M, Hubbart S, Foulkes J., 2014. Nitrogen partitioning and remobilization in relation to leaf senescence, grain yield and grain nitrogen concentration in wheat cultivars. Field Crops Research. 155: 213-223.

Gansel X, Muños S, Pascal T, Gojon A., 2001. Differential regulation of the NO^{-}_{3} and NH_4^+ transporter genes AtNRT2.1 and AtAMT1.1 in Arabidopsis: relation with long-distance and local controls by N status of the plant. The Plant journal : for cell and molecular biology. 26: 143-55.

George BJ., 1984. Design and interpretation of nitrogen response experiments. In: The nitrogen requirements of cereals. MAFF book: 133-149.

Girondé A, Etienne P, Trouverie J, Bouchereau A, Cahérec F, Leport L, Orsel M, Niogret MF, Nesi N, Carole D, et al., 2015. The contrasting N management of two oilseed rape genotypes reveals the mechanisms of proteolysis associated with leaf N remobilization and the respective contributions of leaves and stems to N storage and remobilization during seed filling. BMC Plant Biology. 15: 59

Gombert J, Le Dily F, Lothier J, Etienne P, Rossato L, Allirand JM, Jullien A, Savin A, Ourry A., 2010. Effect of nitrogen fertilization on nitrogen dynamics in oilseed rape using ^{15}N-labeling field experiment. Z. Pflanzenernähr Bodenk. 173: 875-884.

Good AG, Shrawat AK, Muench DG., 2004. Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? Trends in Plant Science. 9(12): 597–605.

Gracey A., 2007. Interpreting physiological responses to environmental change through gene expression profiling. Journal of Experimental Biology. 210: 1584-1592

Greenbaum D, Colangelo C, Williams K. et al., 2003. Comparing protein abundance and mRNA expression levels on a genomic scale. Genome Biology. 4: 117

Gül MK., 2002. QTL mapping and analysis of QTL x nitrogen interactions for some yield components in Brassica napus L. Turkish Journal of Agriculture and Forestry. 27:71–76

Guo J, Jia Y, Chen H, Zhang L, Yang J, Zhang J, Hu X, Li Y, Zhou Y., 2019. Growth, photosynthesis, and nutrient uptake in wheat are affected by differences in nitrogen levels and forms and potassium supply. Scientific Report, 9:1248.

Habekotté B., 1993. Quantitative analysis of pod formation, seed set and seed filling in winter oilseed rape (Brassica napus L.) under field conditions. Field Crops Research. 35 (1): 21-33,

Habekotte B., 1997a. A model of the phenological development of winter oilseed rape (Brassica napus L.). Field Crops Research. 54: 127-136.

Habekotte B., 1997b. Evaluation of seed yield determining factors of winter oilseed rape (Brassica napus L.) by means of crop growth modelling. Field Crops Research. 54: 137-151.

Hammer G, Jordan DR., 2007. An integrated systems approach to crop improvement. 45-61.

Han YL, Song HX, Liao Q., 2016. Nitrogen Use Efficiency Is Mediated by Vacuolar Nitrate Sequestration Capacity in Roots of Brassica napus. Plant Physiology; 170(3):1684–1698.

Harper FR, Berkenkamp B., 1975. Revised growth-stage key for Brassica campestris and B. napus. Canadia Journal of Plant Science. 55: 657-658.

Harper AL, Trick M, Higgins J, Fraser F, Clissold L, Wells R., 2012. Associative transcriptomics of traits in the polyploid crop species Brassica napus. Nature Biotechnology. 30: 798–802.

Harper AL, McKinney LV, Nielsen LR, et al., 2016. Molecular markers for tolerance of European ash (Fraxinus excelsior) to dieback disease identified using Associative Transcriptomics. Science Reports -UK, 6: 19335

Havlickova L, He Z, Wang L, Langer S, Harper AL, Kaur H, et al., 2017. Validation of an updated Associative Transcriptomics platform for the polyploid crop species Brassica napus by dissection of the genetic architecture of erucic acid and tocopherol isoform variation in seeds. The Plant Journal. 10:1

Havlin JL, Tisdale SL, Beaton JD, Nelson WL., 2005. Soil Fertility and Fertilizers. Upper Saddle River, NY: Pearson Education, Inc.

Hawkesford MJ., 2014. Reducing the reliance on nitrogen fertilizer for wheat production. Journal of Cereal Science. 59 (3): 276-283.

He Z, Cheng F, Li Y, Wang X, Parkin IA, Chalhoub B, et al., 2015. Construction of Brassica A and C genome-based ordered pan-transcriptomes for use in rapeseed genomic research. Data Brief, 4357–362.

He Z, Wang L, Harper AL, Havlickova L, Pradhan AK, Parkin IAP, et al., 2016. Extensive homoeologous genome exchanges in allopolyploid crops revealed by mRNAseq-based visualization. Plant Biotechnology. J. 15: 594–604.

He H, Yang R, Li Y, et al., 2017a. Genotypic Variation in Nitrogen Utilization Efficiency of Oilseed Rape (Brassica napus) Under Contrasting N Supply in Pot and Field Experiments. Frontiers in Plant Science. 8: 1825.

He Y, Wu D, Wei D, et al., 2017b. GWAS, QTL mapping and gene expression analyses in Brassica napus reveal genetic control of branching morphogenesis. Scientific Reports. 7(1): 15971.

Henke J, Breustedt G, Sieling K, Kage H., 2007. Impact of uncertainty on the optimum nitrogen fertilization rate and agronomic, ecological and economic factors in an oilseed rape based crop rotation. Journal of Agricultural Science. 145: 455-468.

HGCA, 2013. Oilseed rape growth guide

Hirel B, Lea P., 2001. Ammonia Assimilation. In: Lea, P.J. and Morot-Gaudry, J.F., Eds., Plant Nitrogen, Springer-Verlag, Berlin, 79-100.

Hirel B, Gouis J, Ney B, Gallais A., 2007. The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches, Journal of Experimental Botany, 58 (9): 2369–2387.

Hochman Z, Keating B., 2014. APSIM - Evolution towards a new generation of agricultural systems simulation. Environmental Modelling and Software. 62: 327-350.

Hoffmann MP, Jacobs A, Whitebread AM., 2015. Crop modelling based analysis of site-specific production limitations of winter oilseed rape in northern Germany. Field Crops Research. 178: 49-62.

Hohmann M, Stahl A, Rudloff J, Wittkop B, Snowdon RJ., 2016. Not a load of rubbish: simulated field trials in large-scale containers. Plant, Cell and Environment. 39(9): 2064-73

Holzworth DP, Huth NI, deVoil PG, Zurcher EJ, Herrmann NI, McLean G, Chenu K, Oosterom EJ, Snow V, Murphy C, Moore AD, Brown H, Whish JPM, Verrall S, Fainges J, Bell LW, Peake AS, Poulton PL, Hochman Z, Thorburn PJ, Gaydon DS, Dalgliesh NP, Rodriguez D, Cox H, Chapman S, Doherty A, Teixeira E, Sharp J, Cichota R, Vogeler I, Wang FY, Hammer GL, Robertson MJ, Dimes JP, Whitbread AM, Hunt J, Rees H, McClelland T, Carberry PS, Hargreaves JNG, MacLeod N, McDonald C, Harsdorf J, Wedgwood S, Keating BA., 2014. APSIM - Evolution towards a new generation of agricultural systems simulation. Environmental Modelling and Software. 62: 327-350.

Honsdorf N, Becker H, Ecke W., 2010. Association mapping for phenological, morphological, and quality traits in canola quality winter rapeseed (Brassica napus L.) Genome / National Research Council Canada = Génome / Conseil national de recherches Canada. 53: 899-907.

Houles V, Mary B, Guérif M, Makowski D, Justes E., 2004. Evaluation of the ability of the crop model STICS to recommended nitrogen fertilisation rates according to agro-environmental criteria, Agronomy. 24: 339–349.

Huala E, Dickerman A, Garcia-Hernandez M, Weems D, Reiser L, LaFond F, et al., 2001. The Arabidopsis Information Resource (TAIR): a comprehensive database and web-based information retrieval, analysis, and visualization system for a model plant. Nucleic Acids Research. 29: 102–105.

Hunt ER, Hively WD, Fujikawa SJ, Linden DS, Daughtry CST, McCarty GW., 2010. Acquisition of NIR-Green-Blue Digital Photographs from Unmanned Aircraft for Crop Monitoring. Remote Sensing. 2: 290-305.

Ito S, Song YH, Josephson-Day AR, Miller RJ, Breton G, Olmstead RG, Imaizumi T., 2012. FLOWERING BHLH transcriptional activators control expression of the photoperiodic flowering regulator CONSTANS in Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America, 109 (9): 3582–3587.

Jack T., 2004. Molecular and genetic mechanisms of floral control. Plant Cell, 16: (1-17)

Jeuffroy MH, Ney B, Ourry A., 2002. Integrated physiological and agronomic modelling of N capture and use within the plant. Journal of Experimental Botany. 53: 809-823

Jeuffroy MH, Casadebaig P, Debaeke P. et al., 2014. Agronomic model uses to predict cultivar performance in various environments and cropping systems. A review. Agronomy of Sustainable Development. 34: 121–137

Jones JW, Hoogenboom G, Porter CH, Boote KJ, Batchelor WD, Hunt LA, Wilkens PW, Singh U, Gijsman AJ, Ritchie JT., 2003. The DSSAT cropping system model. The European Journal of Agronomy. 18: 235–265

Jullien A, Mathieu A, Allirand J-M, et al., 2011. Characterization of the interactions between architecture and source–sink relationships in winter oilseed rape (Brassica napus) using the GreenLab model. Annals of Botany. 107(5): 765-779.

Kaiser EA, Kohrs K, Kücke M, Schnug E, Heinemeyer O, Munch JC., 1998. Nitrous oxide release from arable soil: importance of N-fertilization, crops and temporal variation. Soil Biology and Biochemistry. 30(12): 1553–1563.

Kamh M, Wiesler F, Ulas A, Horst WJ., 2005. Root growth and N-uptake activity of oilseed rape (Brassica napus L.) cultivars differing in nitrogen efficiency. Journal of Plant Nutrition and Soil Science. 168: 130-137.

Keane BJ, Ineson P, Vallack HW, Blei E, Bentley M, Howarth S, Toet S., 2018. Greenhouse gas emissions from the energy crop oilseed rape (Brassica napus); the role of photosynthetically active radiation in diurnal N_2O flux variation. GCB Bioenergy. 10: 306– 319.

Kessel B, Schierholt A, Becker HC., 2012. Nitrogen Use Efficiency in a Genetically Diverse Set of Winter Oilseed Rape (Brassica napus L.). Crop Science. 52: 2546-2554.

Kindred D, Berry P, Burch O, Sylvester-Bradley R., 2008. Effect of nitrogen fertiliser use on greenhouse gas emissions and land use change. Aspects of Applied Biology. 88: 53–56.

Kjeldahl, J., 1883. New Method for the Determination of Nitrogen; Chem. News 1240: 101-102.

Koeslin-Findeklee F, Meyer A, Girke A, Beckmann K, Horst WJ., 2014. The superior nitrogen efficiency of winter oilseed rape (Brassica napus L.) hybrids is not related to delayed nitrogen starvation-induced leaf senescence. Plant and Soil. 384: 347-362.

Koeslin-Findeklee F, Safavi Rizi V, Becker MA, Parra-Londono S, Arif M, Balazadeh S, Mueller-Roeber M, Kunze R, Horst WJ. 2015. Transcriptomic analysis of nitrogen starvation- and cultivar-specific leaf senescence in winter oilseed rape (Brassica napus L.). Plant Science. 233: 174–185

Koprivova A, Harper A, Trick M, Bancroft I, Kopriva S., 2014. Dissection of the Control of Anion Homeostasis by Associative Transcriptomics in Brassica napus. Plant Physiology. 166: 442-450.

Körber N, Wittkop B, Bus A, Friedt W, Snowdon RJ, Stich B., 2012. Seedling development in a Brassica napus diversity set and its relationship to agronomic performance. Theoretical and Applied Genetics.125: 1275–1287.

Körber N, Bus A, Li J, Higgins J, Bancroft I, Higgins E., et al., 2015. Seedling development traits in Brassica napus examined by gene expression analysis and association mapping. BMC Plant Biology. 15: 136.

Körschens M., 2006. The importance of long-term field experiments for soil science and environmental research—a review. Plant Soil and Environment. 52: 1–8.

Krapp A, David LC, Chardin C, Girin T, Marmagne A, Leprince AS, Daniel-Vedele F., 2014. Nitrate transport and signalling in Arabidopsis. Journal of Experimental Botany. 65: 789–798.

Lainé P, Ourry A, Alain JH, Macduff H, Boucaud J, Salette J., 2006. Kinetic parameters of nitrate uptake by different catch crop species: Effects of low temperatures or previous nitrate starvation. Physiologia Plantarum. 88: 85-92.

Lamboni M, Makowski D, Monod H., 2008. Multivariate global sensitivity analysis for discrete-time models. Report number: Rapport technique 2008-3,

Larson MH, Gilbert LA, Wang X, Lim WA, Weissman J, Qi LS., 2013. CRISPR interference (CRISPRi) for sequence-specific control of gene expression. Nature Protocols. 8: 2180-2196.

Lassaletta L, Billen G, Grizzetti B, Anglade J, Garnier J., 2014. 50 year trends in nitrogen use efficiency of world cropping systems: The relationship between yield and nitrogen input to cropland. Environmental Research Letters. 105011.

Le Bail M, Makowski D., 2004. A model-based approach for optimizing segregation of soft wheat in country elevator, The European Journal of Agronomy. 21: 171–180.

Le Roy J, Huss B, Creach A, Hawkins S, Neutelings G., 2016. Glycosylation Is a Major Regulator of Phenylpropanoid Availability and Biological Activity in Plants. Frontier Plant Science. 7: 735.

Lecomte C, Prost L, Cerf M, Meynard JM., 2010. Basis for designing a tool to evaluate new cultivars. Agronomy of Sustainable Develpment. 30: 667–677.

Lehner B., 2008. Selection to minimise noise in living systems and its implications for the evolution of gene expression. Molecular systems biology. 4: 170.

Li X, Chen T, Li Y., 2019. ETR1/RDO3 Regulates Seed Dormancy by Relieving the Inhibitory Effect of the ERF12-TPL Complex on delay of germination1 Expression. Plant Cell. 31(4): 832-847.

Lipka AE, Tian F, Wang Q, Peiffer J, Li M, Bradbury PJ et al., 2012. GAPIT: genome association and prediction integrated tool. Bioinformatics. 28: 2397–2399.

Liu XJ, Mosier AR, Halvorson AD, Zhang FS., 2005. Tillage and nitrogen application effects on nitrous and nitric oxide emissions from irrigated corn fields. Plant and Soil. 276: 235–249.

Lu G, Harper AL, Trick M, Morgan C, Fraser F, O'Neill C., 2014. Associative transcriptomics study dissects the genetic architecture of seed glucosinolate content in Brassica napus. DNA Research. 21: 613–625.

Lunn GD, Spink JH, Stokes DT, Clare RW, Wade A, Scott RK., 2001. Canopy management in winter oilseed rape. Project Report No. OS 47 Home Grown Cereals Authority, London.

Luo X, Ma C, Yue Y, Hu K, Li Y, Duan Z, Wu M, Tu J, Shen J, Yi B., 2015. Unravelling the complex trait of harvest index in rapeseed (Brassica napus L.) with association mapping. BMC Genomics. 16: 379

Maathuis F., 2009. Physiological functions of mineral nutrients, Current Opinion in Plant Biology. 12: 250-258.

MacNeil L, Walhout A., 2011. Gene regulatory networks and the role of robustness and stochasticity in the control of gene expression. Genome research. 21(5): 645–657.

Mahmuti M, West JS, Watts J, Gladders P, Fitt BDL., 2009. Controlling crop disease contributes to both food security and climate change mitigation. International Journal of Agricultural Sustainability. 7: 189-202.

Makowski D, Naud C, Jeuffroy MH, Barbottin A, Monod H., 2006. Global sensitivity analysis for calculating the contribution of genetic parameters to the variance of crop model predictions. Reliability Engineering and System Safe. 91: 1142–1147.

Malagoli P, Laine P, Rossato L, Ourry A., 2005. Dynamics of nitrogen uptake and mobilization in field-grown winter oilseed rape (Brassica napus) from stem extension to harvest: I. Global N flows between vegetative and reproductive tissues in relation to leaf fall and their residual N. Annals of Botany. 95(5): 853–861.

Malagoli P, Meuriot F, Laine P, Le Deunff, E., 2009. Modelling Nitrate Uptake and Nitrogen Dynamics in Winter Oilseed Rape (Brassica napus L.). Quantifying and Understanding Plant Nitrogen Uptake for Systems Modelling. 47-69.

Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A., 2010. Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture, Annals of Botany. 105 (7): 1141–1157.

Maskell LC, Smart SM, Bullock JM., 2010. Nitrogen deposition causes widespread loss of species 514 richness in british habitats. Global Change Biology. 16: 671-679.

Medici A, Marshall-Colon A, Ronzier E, Szponarski W, Wang R, Gojon A., 2015. AtNIGT1/HRS1 integrates nitrate and phosphate signals at the Arabidopsis root tip. Nature Communication. 6: 6274

Mendham NJ, Shipway PA, Scott RK., 1981. The effects of delayed sowing and weather on growth, development, and yield of winter oil-seed rape (Brassica napus L.). Journal of Agricultural Science. 96: 389-416.

Miller AJ, Fan X, Orsel M, Smith SJ, Wells DM., 2007. Nitrate transport and signalling. Journal of Experimental Botany. 58: 2297–2306.

Miro B, 2010. Identification of traits for nitrogen use efficiency in oilseed rape (Brassica napus L.). School of Agriculture Food and Rural Development, Newcastle University, Newcastle

Moll RH, Kamprath EJ, Jackson WA., 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilisation. Agronomy Journal. 74: 562-564.

Muller J, Behrens T, Diepenbrock W, 2006. Use of a new sigmoid growth equation to estimate organ area indices from canopy area index in winter oilseed rape (Brassica napus L.). Field Crops Research. 96: 279-295.

Muller K, Bottcher U, Meyer-Schatz F, Kage H., 2008. Analysis of vegetation indices derived from hyperspectral reflection measurements foe estimation crop canopy parameters of oilseed rape (Brassica napus L.). Biosystems Engineering. 101: 172-182.

Nakamura Y, Teo NZW, Shui G, Chua CHL, Cheong W, Parameswaran S, Koizumi R, Ohta H, Wenk MR, Ito T., 2014. Transcriptomic and lipidomic profiles of glycerolipids during Arabidopsis flower development New Phytologist. 203 (1): 310-22.

Nelson MN, Rajasekaran R, Smith, A., 2014. Quantitative trait loci for thermal time to flowering and photoperiod responsiveness discovered in summer annual-type Brassica napus L. Plos one, 9.

Noguero M, Lacombe B., 2016. Transporters Involved in Root Nitrate Uptake and Sensing by Arabidopsis. Frontier Plant Science. 7: 1391.

Nyikako J, Schierholt A, Kessel B, Becker HC., 2014. Genetic variation in nitrogen uptake and utilization efficiency in a segregating DH population of winter oilseed rape. Euphytica. 199: 3-11.

Oaks A., 2011. Primary nitrogen assimilation in higher plants and its regulation. Canadian Journal of Botany. 72: 739-750.

Orsel M, Filleur S, Fraisier V, Daniel-Vedele F., 2002. Nitrate transport in plants: which gene and which control? Journal of Experimental Botany. 53 (370): 825-33.

Payne RJ, Dise B, Field CD, Dore AJ, Caporn, Simon JM, Stevens CJ., 2017. Nitrogen deposition and plant biodiversity: past, present, and future. Frontiers in Ecology and the Environment. 15 (8): 431-436.

Pérez AJ, López F, Benlloch JV, Christensen S., 2000. Colour and shape analysis techniques for weed detection in cereal fields. Computers and Electronics in Agriculture. 25: 197-212.

Poisson E, Mollier A, Trouverie J, Avice JC, Brunel-Muguet S., 2018. SuMoToRI model simulations for optimizing sulphur fertilization in oilseed rape in the context of increased spring temperatures. European Journal of Agronomy. 97(10): 1016

Popescu AA, Harper AL, Trick M, Bancroft I, Huber KT., 2014. A novel and fast approach for population structure inference using Kernel-PCA and optimisation (PSIKO). Genetics. 198: 1421–1431.

Qi LS, Larson MH, Gilbert LA, Doudna, JA, Weissman JS, Arkin AP, Lim WA., 2013. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell. 152: 1173-1183.

Quaggiotti S, Ruperti B, Borsa P, Destro T, Malagoli M., 2003. Expression of a putative high-affinity NO3– transporter and of an H+-ATPase in relation to whole plant nitrate transport physiology in two maize genotypes differently responsive to low nitrogen availability. Journal of Experimental Botany. 54: 1023-31.

R Core Team., 2015. R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing.

Rasmusson DC., 1991. A plant breeder's experience with ideotype breeding. Field Crops Research, 26 (2): 191-200,

Rathke GW, Behrens T, Diepenbrock W., 2006. Integrated nitrogen management strategies to improve seed yield, oil content and nitrogen efficiency of winter oilseed rape (Brassica napus L.): a review. Agriculture, Ecosystems & Environment. 117(2–3): 80–108.

Razi H, Howell EC, Newbury HJ, Kearsey MJ., 2008. Does sequence polymorphism of FLC paralogues underlie flowering time QTL in Brassica oleracea. Theoretical and Applied Genetics. 116: 179–192.

Robertson MJ, Holland JF, Kirkegaard JA, Smith CJ., 1999. Simulating growth and development of canola in Australia. In ‘Proceedings 10th International Rapeseed Congress’. 26–29 Sept. Canberra, ACT.

Robertson MJ, Lilley JM., 2016. Simulation of growth, development and yield of canola (Brassica napus) in APSIM. Crop & Pasture Science. 67: 332-344.

Rossato L, Laine P, Ourry A., 2001. Nitrogen storage and remobilization in Brassica napus L. during the growth cycle: nitrogen fluxes within the plant and changes in soluble protein patterns. Journal of Experimental Botany. 5: 1655-1663.

Ruffel S, Krouk G, Ristova D, Shasha D, Birnbaum KD, Coruzzi GM., 2011. Nitrogen economics of root foraging: transitive closure of the nitrate-cytokinin relay and distinct systemic signaling for N supply vs. demand. Proceedings of the National Academy of Science. U.S.A. 108: 18524–18529.

Ruffel S, Poitout A, Krouk G, Coruzzi GM, Lacombe B., 2016. Long-distance nitrate signaling displays cytokinin dependent and independent branches. Journal of Integrative Plant Biology. 58: 226–229.

Schiessl SV, Huettel B, Kuehn D, Reinhardt R, Snowdon RJ., 2017. Flowering Time Gene Variation in Brassica Species Shows Evolutionary Principles. Frontier Plant Science. 8: 1742.

Schjoerring JK, Bock JGH, Gammelvind, L et al., 1995. Nitrogen incorporation and remobilization in different shoot components of field-grown winter oilseed rape (Brassica napus L.) as affected by rate of nitrogen application and irrigation. Plant Soil. 177**:** 255–264

Schranz ME, Quijada P, Sung SB, Lukens L, Amasino R, Osborn TC., 2002. Characterization and effects of the replicated flowering time gene FLC in Brassica rapa. Genetics. 162: 1457–1468.

Schröder JJ., 2014. The position of mineral nitrogen fertilizer in efficient use of nitrogen and land: a review. The Natural Resources Journal. 5(15): 936–948.

Schulte auf'm Erley G, Wijaya KA, Ulas A, Becker H, Wiesler F, Horst WJ., 2007. Leaf senescence and N uptake parameters as selection traits for nitrogen efficiency of oilseed rape cultivars. Physiologia Plantarum. 130: 519-531

Seiffert B, Zhou ZW, Wallbraun M, Lohaus G, Möllers C., 2004. Expression of a bacterial asparagines synthetase gene in oilseed rape (Brassica napus L.) and its effect on traits related to nitrogen efficiency. Plant Physiology.121: 656-665.

Shi J, Li R, Qiu D, Jiang C, Long Y, Morgan C, Bancroft I, Zhao J, Meng J., 2009. Unraveling the complex trait of crop yield with quantitative trait loci mapping in Brassica napus. Genetics. 182: 851–861.

Shishi L, Lantao L, Wenhan G, Yukun Z, Yinuo L, Shanqin W, Jianwei L., 2018. Diagnosis of nitrogen status in winter oilseed rape (Brassica napus L .) using in-situ hyperspectral data and unmanned aerial vehicle (UAV) multispectral images. Computers and Electronics in Agriculture. 151: 185-195.

Shurpali N, Rannik Ü, Jokinen, S. et al., 2016. Neglecting diurnal variations leads to uncertainties in terrestrial nitrous oxide emissions. 6: 25739.

Sibley KJ, Astatkie T, Brewster G, Struik PC, Adsett JF, Pruski K., 2009. Field-scale validation of an automated soil nitrate extraction and measurement system. Precision Agriculture. 10: 162–174.

Sieling K, Kage H., 2010. Efficient N management using winter oilseed rape. A review. Agronomy for Sustainable Development. 30: 271-279.

Smirnoff N, Stewart G., 1985. Nitrate assimilation and translocation by higher plants: Comparative physiology and ecological consequences. Plant Physiology. 64: 133–140

Snowdon RJ, Iniguez Luy FL., 2012. Potential to improve oilseed rape and canola breeding in the genomics era. Plant Breeding. 131: 351-360.

Soto-Cerda BJ, and Cloutier S., 2012. Association Mapping in Plant Genomes. In: Caliskan, M., Ed., Genetic Diversity in Plants, InTech.

Srikanth A, Schmid M., 2011. Regulation of flowering time: all roads lead to Rome. The Cellular Molecular Life Sciences. 68**:** 2013–2037

Stafford JA., 1996. The effects of prochloraz on the growth and yield of oilseed rape. PhD Thesis, Nottingham University.

Stahl A, Friedt W, Wittkop B, Snowdon RJ., 2016. Complementary diversity for nitrogen uptake and utilisation efficiency reveals broad potential for increased sustainability of oilseed rape production. Plant and Soil. 400: 245-262.

Stahl A, Pfeifer M, Frisch M, Wittkop B, Snowdon RJ., 2017. Recent Genetic Gains in Nitrogen Use Efficiency in Oilseed Rape. Frontier Plant Science. 8: 963

Stockle C, Donatelli M, Roger N. 2003., CropSyst, a cropping systems simulation model. European Journal of Agronomy. 289-307.

Storer K, Berry P, Kindred D, Sylvester-Bradley R., 2018. Identifying oilseed rape varieties with high yield and low nitrogen fertiliser requirement. Field Crops Research. 225: 104-116.

Sylvester-Bradley R, Makepeace RJ., 1984. A code for stages of development in oilseed rape (Brassic napus L.). Aspects of Applied Biology. 6: 399-419.

Sylvester-Bradley R, Kindred DR., 2009. Analysing nitrogen responses of cereals to prioritize routes to the improvement of nitrogen use efficiency. Journal of Experimental Botany. 60: 1939-1951.

Tang Y, Sun X, Hu C, Tan Q, Zhao X., 2013. Genotypic differences in nitrate uptake, translocation and assimilation of two Chinese cabbage cultivars [Brassica campestris L. ssp. Chinensis (L.)]. Brassica campestris L. ssp. Chinesnsis. L. Plant Physiology Biochemistry. 70: 14–20.

Tang W, Xin H, Lunwen Q, Feng W, Zhenhua Z, Chao S, Liangbin L, Chunyun G., 2019. Comparative Transcriptome Analysis in Oilseed Rape (Brassica napus) Reveals Distinct Gene Expression Details between Nitrate and Ammonium Nutrition. Science Reports. 9: 1248

Taylor M, Tornqvist CE, Zhao X, Grabowski P, Doerge R, Ma J, Jiang Y., 2018. Genome-Wide Association Study in Pseudo-F_2 Populations of Switchgrass Identifies Genetic Loci Affecting Heading and Anthesis Dates. Frontiers in Plant Science. 9: 1250.

Thomas C, Graham N, Hayden R, Meacham M, Neugebauer K, Nightingale Mark, Dupuy L, Hammond J, White P, Broadley M., 2016. High-throughput phenotyping (HTP) identifies seedling root traits linked to variation in seed yield and nutrient capture in field-grown oilseed rape (Brassica napus L.). Annals of Botany. 118: 655-665

Thorup-Kristensen K, Kirkegaard J., 2016. Root system-based limits to agricultural productivity and efficiency: the farming systems context. Annals of Botany. 118(4): 573-592.

Tian Q, Stepaniants SB, Mao M, Weng L, Feetham MC, Doyle MJ, Yi EC, Dai H, Thorsson V, Eng J., 2004. Integrated genomic and proteomic analyses of gene expression in mammalian cells. Molecular & Cellular Proteomics. 3: 960-969.

Trick M, Long Y, Meng J, Bancroft I., 2009. Single nucleotide polymorphism (SNP) discovery in the polyploid Brassica napus using Solexa transcriptome sequencing. Plant Biotechnology. J. 7: 334–346.

Ulas A, Erley GSA, Kamh M, Wiesler F, Horst WJ., 2012. Root-growth characteristics contributing to genotypic variation in nitrogen efficiency of oilseed rape. Journal of Plant Nutrition and Soil Science. 175: 489-498.

Ulas A, Behrens T, Wiesler F, Horst WJ, Erley GSA., 2013. Does genotypic variation in nitrogen remobilisation efficiency contribute to nitrogen efficiency of winter oilseed-rape (Brassica napus L.) cultivars? Plant and Soil. 371: 463-471.

United Nations., 2013. Food and Agriculture Organization of the United Nations, Rome

Vaclav S., 2004. Enriching the Earth: Fritz Haber, Carl Bosch, and the Transformation of World Food Production (1st ed.). Cambridge, MA: MIT.

Venterea RT, Halvorson AD, Kitchen N, Liebig MA, Cavigelli MA, Del Grosso SJ, Collins H., 2012. Challenges and opportunities for mitigating nitrous oxide emissions from fertilized cropping systems. Frontiers in Ecology and the Environment. 10: 562-570.

Vinten A, Vivian B, Wright F, Howard S., 1994. A comparative study of nitrate leaching from soils of differing textures under similar climatic and cropping conditions. Journal of Hydrology. 159(1–4): 197-213

Wang Z, Gerstein M, Snyder M., 2009. RNA-Seq: A revolutionary tool for transcriptomics. Nature Reviews Genetics. 10: 57–63.

Wei D, Mei J, Fu Y, Disi JO, Li J, Qian W., 2014. Quantitative trait loci analyses for resistance to Sclerotinia sclerotiorum and flowering time in Brassica napus, Molecular Breeding. 34: 1797–804.

White PJ, George TS, Gregory PJ, Bengough AG, Hallett PD, McKenzie BM., 2013. Matching roots to their environment. Annals of Botany. 112: 207-222.

White C, Roques S, Berry P., 2013. Effects of foliar-applied nitrogen fertilizer on oilseed rape (Brassica napus). The Journal of Agricultural Science. 153: 42-55.

White C, Sylvester-Bradley R, Berry P., 2015. Root length densities of UK wheat and oilseed rape crops with implications for water capture and yield, Journal of Experimental Botany. 66(8); 2293–2303

Wiesler F, Behrens T, Horst WJ., 2001. The role of nitrogen-efficient cultivars in sustainable agriculture. The Scientific World. 1(S2): 61–69.

Xu L, Hu K, Zhang Z, Guan C, Chen S, Hua W, Li J, Wen J, Yi B, Shen J, Ma C, Tu J, Fu T., 2017. Genome-wide association study reveals the genetic architecture of flowering time in rapeseed (Brassica napus L.). Frontiers in Plant Science, 8:1246

Yang G, Liu J, Zhao C, et al., 2017. Unmanned Aerial Vehicle Remote Sensing for Field-Based Crop Phenotyping: Current Status and Perspectives. Frontiers in Plant Science. 8:1111.

Yin X et al., 2017 Multi-model uncertainty analysis in predicting grain N for crop rotations in Europe. European Journal of Agronomy. 84: 152–65

Yong HY, Zou Z, Kok EP, Kwan BH, Chow K, Nasu S, Nanzyo M, Kitashiba H, Nishio T., 2014. Comparative transcriptome analysis of leaves and roots in response to sudden increase in salinity in Brassica napus by RNA-seq. Biomed Research International. 19. 2014:467395

Zaman-Allah M, Vergara O, Araus JL, Tarekegne A. Magorokosho C, Zarco-Tejada PJ., Cairns J., 2015. Unmanned aerial platform-based multi-spectral imaging for field phenotyping of maize. Plant Methods. 11: 35.

Zhang XY, Loyce C, Meynard JM, Savary S., 2006. Characterization of multiple disease systems and cultivar susceptibilities for the analysis of yield losses in winter wheat. Crop Protection. 25:1013–1023

Zhang Z, Ersoz E, Lai C, Todhunter RJ, Tiwari HK, Gore MA., 2010. Mixed linear model approach adapted for genome-wide association studies. Nature Genetics. 42: 355–360.

Zhang X, Lee X, Griffis TJ., 2014. The influence of plants on atmospheric methane in an agriculture-dominated landscape. The International Journal of Biometeorology. 58: 819–833

Zhang XM, Meng L, Liu B, Hu YY, Cheng F, Liang JL, Aarts MGM, Wang XW, Wu JA., 2015. Transposon insertion in FLOWERING LOCUS T is associated with delayed flowering in Brassica rapa. Plant Science. 241: 211–220.

Zhang Y, Mahowald N, Scanza R, Journet E, Desboeufs K, Albani S, Kok J, Zhuang, Chen, Y, Cohen D, Patey M, Achterberg, E, Engelbrecht, J, Fomba K., 2015. Modeling the global emission, transport and deposition of trace elements associated with mineral dust. Biogeosciences. 12: 19

Zhang Z, Xiong S, Wei Y, Meng X, Wang X, Ma X., 2017. The role of glutamine synthetase isozymes in enhancing nitrogen use efficiency of N-efficient winter wheat. Scientific Reports. 7(1): 1000.

Zhao FJ, Evans EJ, Bilsborrow PE, Syers JK., 1993. Influence of sulphur and nitrogen on seed yield and quality of low glucosinolate oilseed rape (Brassica-Napus L). Journal of the Science of Food and Agriculture. 63: 29-37.

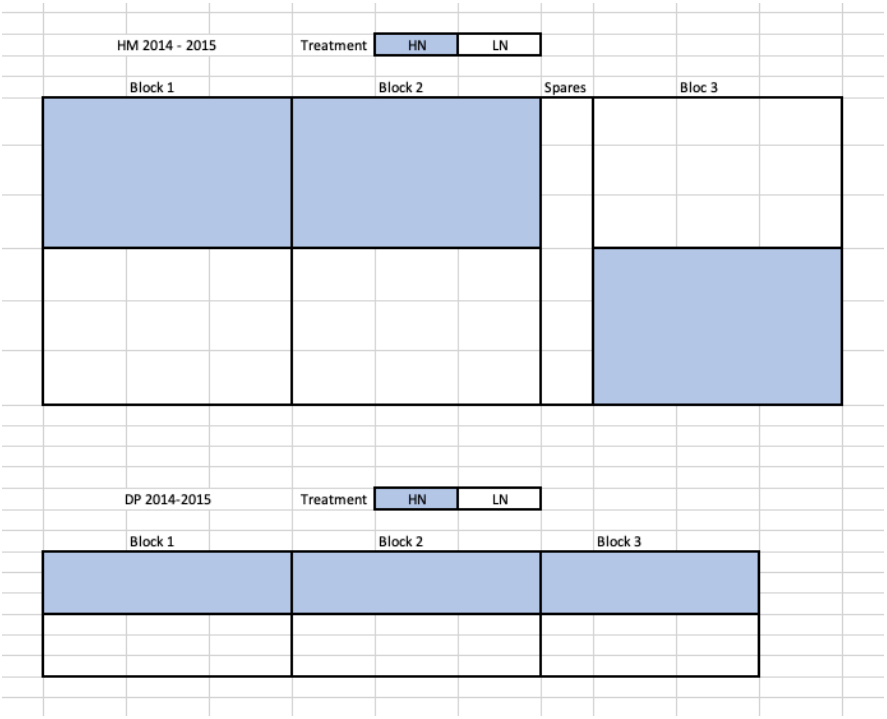
Zhao J, Becker HC, Zhang D, Zhang Y, Ecke W., 2006. Conditional QTL mapping of oil content in rapeseed with respect to protein content and traits related to plant development and grain yield. Theoretical and Applied Genetics.113: 33–8.

Zhou L, Yan T, Chen X, Li Z, Wu D, Hua S, Jiang L., 2018. Effect of high night temperature on storage lipids and transcriptome changes in developing seeds of oilseed rape. Journal of Experimental Botany. 69 (7): 1721–1733

Zou J, Jiang C, Cao Z, Li R, Long Y, Chen S, Meng J., 2010. Association mapping of seed oil content in Brassica napus and comparison with quantitative trait loci identified from linkage mapping. Genome. 53: 908–16.

Appendices

Appendix, Figure 1. Trial design for the HM 2014-2015 and DP 2014-2015 trial site. Experiment were split into two treatments, HN and LN, with plots randomised within.



Appendix table 1. Brassica genotypes used in field trials during 2014/2015 and 2015/16. Trial key column records differences in genotypes between trials; o = genotype not in Deeping 2014-2015 trial, * = genotype not in Ompton 2015-2016 trial and x = additional genotype in Ompton 2015-2016 trial only.

ASSYST line number	Founder accession	Type	Origin	Trial key
BnASSYST-001	Alesi	Modern WOSR	France	*
BnASSYST-002	Remy	Modern WOSR		x
BnASSYST-005	Pirola	Modern WOSR	France	*
BnASSYST-009	Agalon	Modern WOSR	UK	
BnASSYST-014	Rodeo	Modern WOSR	Germany	
BnASSYST-015	Rapid	Modern WOSR	France	
BnASSYST-016	Boston	Modern WOSR	France	
BnASSYST-017	Escort	Modern WOSR		x

BnASSYST-018	Montego	Modern WOSR		x
BnASSYST-020	Pacific	Modern WOSR	France	
BnASSYST-021	Savannah	Modern WOSR	France	
BnASSYST-024	Ladoga	Modern WOSR		x
BnASSYST-025	Atlantic	Modern WOSR		x
BnASSYST-028	Capitol	Modern WOSR		x
BnASSYST-029	Idol	Modern WOSR	France	
BnASSYST-031	Bristol	Modern WOSR		x
BnASSYST-033	Lisabeth	Modern WOSR	Germany	*
BnASSYST-034	Lipid	Modern WOSR	Germany	*
BnASSYST-035	Lipton	Modern WOSR	Germany	
BnASSYST-039	OASE	Modern WOSR	Germany	
BnASSYST-041	Recital	Modern WOSR		x
BnASSYST-044	Laser	Modern WOSR	France	
BnASSYST-045	Fortis	Modern WOSR	Unknown	
BnASSYST-046	Smart	Modern WOSR	France	
BnASSYST-047	Roxet	Modern WOSR	France	
BnASSYST-048	NK Bravour	Modern WOSR		x
BnASSYST-049	NK Fair	Modern WOSR		x
BnASSYST-050	Aviso	Modern WOSR		x
BnASSYST-051	Sansibar	Modern WOSR	Unknown	*
BnASSYST-052	SWGospel	Modern WOSR	Sweden	
BnASSYST-053	Verona	Modern WOSR		x
BnASSYST-054	Tenor	Modern WOSR		x
BnASSYST-055	Expert	Modern WOSR		x
BnASSYST-056	Musette	Modern WOSR	Unknown	
BnASSYST-057	Kvintett	Modern WOSR	Unknown	
BnASSYST-058	Falstaff	Modern WOSR		
BnASSYST-060	Viking	Modern WOSR	Germany	
BnASSYST-061	Aragon	Modern WOSR	Germany	
BnASSYST-062	Aurum	Modern WOSR		x
BnASSYST-065	Rasmus	Modern WOSR		x
BnASSYST-067	Nugget	Modern WOSR	Canada	
BnASSYST-068	Zephir	Modern WOSR	Germany	
BnASSYST-069	SLM 0413	Modern WOSR	Germany	

BnASSYST-070	SLM 0512	Modern WOSR	Germany	
BnASSYST-071	LSF 0519	Modern WOSR	Germany	
BnASSYST-072	Beluga	Modern WOSR	Germany	
BnASSYST-073	Amor	Modern WOSR	Germany	
BnASSYST-075	Campari	Modern WOSR		x
BnASSYST-076	Caramba	Modern WOSR		x
BnASSYST-078	Express 617	Modern WOSR		x
BnASSYST-080	Orlando	Modern WOSR	Former GDR	
BnASSYST-081	Pollen	Modern WOSR		x
BnASSYST-082	Prince	Modern WOSR	Germany	
BnASSYST-083	Wotan	Modern WOSR		x
BnASSYST-084	NK Nemax	Modern WOSR		x
BnASSYST-085	NK Passion	Modern WOSR		x
BnASSYST-087	Barrel	Modern WOSR		x
BnASSYST-088	KW1519	Modern WOSR		x
BnASSYST-093	Canberra x Courage DH	WOSR		x
BnASSYST-096	Hansen X Gaspard DH	WOSR	France	
BnASSYST-097	Madrigal x Recital DH	WOSR	UK	
BnASSYST-098	Rafal DH1	WOSR		
BnASSYST-099	Tapidor DH	WOSR		x
BnASSYST-102	Lesira	WOSR		x
BnASSYST-103	Primor	WOSR		x
BnASSYST-107	Janetzki Schlesischer	WOSR		x
BnASSYST-109	Norin	WOSR	Japan	
BnASSYST-110	Olimpiade	WOSR		x
BnASSYST-112	Mansholt	WOSR	Germany	
BnASSYST-113	Samourai	WOSR	Germany	x
BnASSYST-115	Akela	WOSR		
BnASSYST-117	Maplus	WOSR		
BnASSYST-118	Askari	WOSR	Germany	
BnASSYST-119	Lirabon	WOSR	Germany	*
BnASSYST-120	Darmor	WOSR		x
BnASSYST-122	Cobra	WOSR	Germany	*
BnASSYST-124	Mohican	WOSR	France	*
BnASSYST-125	Flip	WOSR	unknown	*

BnASSYST-126	Zenith	WOSR	France	*
BnASSYST-127	Phil	WOSR	unknown	*
BnASSYST-128	Leopard	WOSR	Germany	
BnASSYST-129	RESYN-H048	WOSR		
BnASSYST-133	Baltia	WOSR	France	*
BnASSYST-138	Diamant	WOSR	Germany	*
BnASSYST-140	Doral	WOSR		*
BnASSYST-142	Erra	WOSR		x
BnASSYST-149	Krapphauser	WOSR	Germany	*
BnASSYST-156	Lirakotta	WOSR	Germany	*
BnASSYST-157	Madora	WOSR	Former GDR	
BnASSYST-163	Mytnickij	WOSR	Russia	*
BnASSYST-165	Norde	WOSR	Sweden	*
BnASSYST-173	Sobotkowski	WOSR		x
BnASSYST-179	Wolynski	WOSR		x
BnASSYST-180	V8	WOSR		x
BnASSYST-182	25629-3	WOSR		x
BnASSYST-184	R53	WOSR		x
BnASSYST-186	Moana rape	Winter fodder		x
BnASSYST-187	Winfred	Winter fodder	Netherlands	
BnASSYST-188	Emerald	Winter fodder		
BnASSYST-190	Aphid Resistant Rape	Winter fodder		x
BnASSYST-193	Dwarf Essex	Winter fodder		x
BnASSYST-194	English Giant	Winter fodder		x
BnASSYST-195	Liragrün	Winter fodder	Germany	
BnASSYST-198	Palu	Winter fodder		x
BnASSYST-199	Parapluie	Winter fodder		x
BnASSYST-201	Silona	Winter fodder		x
BnASSYST-202	Chuosenshu	Winter vegetable		
BnASSYST-203	Taisetsu	Winter vegetable		x
BnASSYST-207	Chembere Dzagumhana	Unspecified		x
BnASSYST-208	Couve Nabica	Cauve nabica		x
BnASSYST-212	Slapska, Slapy	Unspecified		x
BnASSYST-213	Abukuma Natane	Unspecified	Japan	*

BnASSYST-217	Evvin	var. napus		x
BnASSYST-218	Groene Groninger Snijmoes	unspecified		x
BnASSYST-219	Gruner Schnittkohl	Siberian kale		x
BnASSYST-224	Wild Accession	Wild accession	Algeria	
BnASSYST-229		Semiwinter		x
BnASSYST-231	SWU Chinese 3	Semiwinter	China	*
BnASSYST-237	SWU Chinese 5	Semiwinter	China	*
BnASSYST-403	Fortin Family	Swede		x
BnASSYST-413	Pike	Swede		x
ELITE	Extrovert	Modern WOSR		*o
ELITE	Popular	Modern WOSR		* 0
ELITE	PT 211	Modern WOSR		*
ELITE	Charger	Modern WOSR		
ELITE	DIMENSION	Modern WOSR		O
ELITE	Harper	Modern WOSR		
ELITE	INCENTIVE	Modern WOSR		O
ELITE	Sesame	Modern WOSR		
ELITE	Trinity	Modern WOSR		
ELITE	Troy	Modern WOSR		
ELITE	DK Carbernet	Modern WOSR	Germany	
ELITE	Ex calibur	Modern WOSR	Germany	
ELITE	Expower	Modern WOSR		
ELITE	Castile	Modern WOSR	France	
ELITE	Elgar	Modern WOSR	Germany	
ELITE	PR46W21	Modern WOSR		
ELITE	Compass	Modern WOSR		
ELITE	Quartz	Modern WOSR		
ELITE	Temple	Modern WOSR	UK	
ELITE	V316OL	Modern WOSR		x
ELITE	SY Harnas	Modern WOSR		x
ELITE	Picto	Modern WOSR		x
ELITE	Wembley	Modern WOSR		x

Appendix table 2. Trait mean, min, max at low N for all trials; DP14, DP15, HM14, OM15. Traits include; Plant counts, phenology scores, Mid-flowering tissue data and Maturity tissue data.

		HM14	DP14	OM15	DP15
Plant count	Mean	44.61	29.56	34.09	30.96
	Min	17.07	10.00	22.93	16.00

	Max	59.11	39.00	44.71	41.33
Flower score	Mean	6.36	4.58	4.77	4.48
	Min	4.00	2.50	1.33	1.33
	Max	9.00	8.00	8.00	8.00
Mid-flowering dry weight leaves (t ha^{-1})	Mean	0.86	1.45	0.85	1.70
	Min	0.40	0.35	0.43	0.59
	Max	1.64	3.12	3.25	3.43
Mid-flowering dry weight stem (t ha^{-1})	Mean	5.67	4.53	3.45	4.09
	Min	2.49	1.24	1.70	1.47
	Max	8.34	8.51	5.81	6.94
Mid-flowering leaf (%N)	Mean	2.48	1.63	2.15	1.41
	Min	1.04	0.78	0.96	1.05
	Max	4.15	2.47	3.13	2.09
Mid-flowering stem (%N)	Mean	1.10	0.92	0.57	1.01
	Min	0.60	0.57	0.29	0.62
	Max	2.05	1.63	0.93	1.68
Mid-flowering leaf total N (t ha^{-1})	Mean	21.51	23.52	18.25	23.89
	Min	8.75	5.67	7.46	9.61
	Max	42.81	54.95	55.68	56.30
Mid-flowering stem total N (t ha^{-1})	Mean	59.44	41.44	19.82	40.89
	Min	20.70	15.43	3.87	17.23
	Max	108.33	132.58	46.49	80.90
Mid-flowering plant total N (t ha^{-1})	Mean	79.89	65.20	34.94	64.04
	Min	35.16	21.12	35.25	23.72
	Max	126.74	162.17	150.17	120.77
Maturity	Mean	6.22	7.46	7.33	6.68
	Min	3.00	5.00	1.00	2.33
	Max	9.00	8.50	8.33	9.00
Maturity dry weight stem (t ha^{-1})	Mean	3.27	1.89	1.21	2.10
	Min	1.11	0.63	0.31	0.89
	Max	5.12	4.17	3.63	4.65
	Mean	2.39	1.01	1.04	0.70

Maturity dry weight chaff (t ha^{-1})	Min	0.86	0.25	0.30	0.24
	Max	3.92	2.25	3.73	1.95
Maturity stem (%N)	Mean	0.25	0.39	0.45	0.86
	Min	0.14	0.09	0.18	0.48
	Max	0.41	0.74	1.24	1.26
Maturity chaff (%N)	Mean	0.37	0.51	0.57	1.04
	Min	0.16	0.30	0.21	0.48
	Max	0.62	0.97	1.10	1.56
Maturity seed (%N)	Mean	3.14	2.56	2.77	2.91
	Min	1.92	1.92	2.14	2.11
	Max	3.86	3.22	3.37	3.61
Maturity stem total N (t ha^{-1})	Mean	7.95	7.35	5.30	17.82
	Min	3.25	1.67	0.78	6.60
	Max	16.55	18.24	17.66	52.01
Maturity chaff total N (t ha^{-1})	Mean	8.69	5.13	6.11	7.22
	Min	2.85	1.32	1.55	1.26
	Max	18.40	11.66	23.06	25.87
Maturity seed total N (t ha^{-1})	Mean	86.14	59.20	22.99	29.26
	Min	43.13	7.76	4.25	7.84
	Max	140.57	86.46	43.00	53.12
Maturity plant total N (t ha^{-1})	Mean	102.03	71.29	33.89	51.99
	Min	42.34	22.24	7.75	25.44
	Max	162.70	100.53	77.71	92.68
N-uptake post flowering (kg/ha)	Mean	21.39	9.78	-3.08	-0.44
	Min	-26.84	-84.38	-56.51	-91.21
	Max	83.01	55.93	39.74	86.65
Stem N remobilisation (kg/ha)	Mean	51.59	24.53	13.51	21.21
	Min	12.22	1.00	-8.94	-28.37
	Max	99.27	71.99	32.15	63.27
NUE	Mean	41.07	15.97	11.39	36.46
	Min	17.99	2.72	2.90	10.62
	Max	63.23	24.17	21.31	58.50

N utilisation seed	Mean	29.71	32.89	25.34	20.27
	Min	23.84	18.97	14.18	5.68
	Max	36.37	41.55	36.18	31.75
N utilisation biomass	Mean	84.08	74.19	87.99	71.14
	Min	68.94	50.04	68.02	47.94
	Max	100.79	128.08	134.40	108.92

Appendix table 3. Mean values of morphological traits for ASSYST and ELITE genotypes for LN plots at; DP14, DP15, HM14 and OM15. P values for t-tests.

	HM14			**DP14**			**OM15**			**DP15**		
	ASSYST	**ELITE**	**T test P value**	**ASSYST**	**ELITE**	**T test P value**	**ASSYST**	**ELITE**	**T test P value**	**ASSYST**	**ELITE**	**T test P value**
NUE	38.15	51.44	0.00	15.02	20.88	0.00	34.33	44.87	0.00	10.43	15.73	0.00
YIELD	2.82	3.81	0.00	2.18	3.03	0.00	0.96	1.26	0.00	0.76	1.15	0.00
MTNSD	80.77	105.23	0.00	56.55	72.85	0.00	27.65	35.60	0.00	21.22	30.86	0.00
MTNP	95.75	124.38	0.00	68.51	85.59	0.00	50.06	59.68	0.00	31.70	43.55	0.01
MDWP	8.23	10.28	0.00	4.80	6.17	0.00	3.63	4.38	0.06	2.98	3.51	0.01
NUTLSD	29.36	30.93	0.02	32.28	36.05	0.00	19.93	21.66	0.00	24.73	28.07	0.26
MDWS	3.11	3.82	0.00	1.84	2.15	0.08	2.02	2.40	0.23	1.18	1.36	0.05
N uptake post	15.14	43.63	0.00	7.26	22.79	0.08	-2.45	7.48	0.05	-4.50	3.29	0.26

flowering												
Flower score	6.60	5.50	0.00	4.63	4.27	0.33	4.65	3.80	0.01	4.99	4.14	0.08
Establishment score	43.37	49.02	0.00	7.47	8.31	0.01	3.93	4.08	0.32	2.21	2.29	0.46
MFDWP	6.41	7.15	0.04	5.92	6.22	0.62	5.62	6.36	0.09	4.23	4.65	0.08
MNSD	3.16	3.04	0.12	2.59	2.41	0.04	2.91	2.93	0.02	2.79	2.68	0.78
MFDWS	5.57	6.03	0.15	4.50	4.72	0.64	3.99	4.47	0.04	3.38	3.80	0.14
MFNL	2.54	2.28	0.09	1.64	1.59	0.53	1.42	1.37	0.05	2.11	2.30	0.32
MNS	0.25	0.24	0.32	0.40	0.35	0.05	0.85	0.88	0.00	0.47	0.36	0.62
Maturity score	6.25	6.12	0.70	7.43	7.58	0.49	6.49	7.35	0.00	3.59	4.41	0.19
HEIGHT	134.22	138.84	0.07	126.12	126.35	0.91	119.26	122.65	0.05	109.72	114.51	0.43
MNC	0.38	0.36	0.42	0.52	0.48	0.34	1.02	1.14	0.70	0.57	0.59	0.06
MFTNL	21.07	23.05	0.36	23.72	22.49	0.71	23.42	25.75	0.39	17.96	19.53	0.34
MFDWL	0.81	1.03	0.00	1.44	1.50	0.73	1.65	1.91	0.99	0.85	0.85	0.11
MDWC	2.30	2.69	0.00	0.99	1.09	0.37	0.69	0.72	0.77	1.04	1.00	0.76
NUTLBIO	84.22	83.58	0.73	74.34	73.39	0.83	71.85	68.29	0.09	88.93	83.82	0.27
MTNS	7.69	8.86	0.12	7.30	7.61	0.73	17.08	20.65	1.00	5.30	5.30	0.15
MFTNP	79.58	81.01	0.79	65.31	64.64	0.94	62.58	69.80	0.19	34.55	38.34	0.16

SNR	51.76	50.98	0.87	24.29	25.77	0.75	22.58	16.07	0.36	13.19	14.94	0.17
MFTNS	59.33	59.80	0.92	41.31	42.14	0.89	39.94	44.62	0.34	19.49	21.25	0.17
MTNC	8.33	9.95	0.03	5.13	5.12	0.99	7.14	7.51	0.68	6.03	6.47	0.74
MFNS	1.12	1.04	0.26	0.92	0.93	0.86	1.01	1.02	0.57	0.57	0.56	0.79