**Species-wide variation in shoot nitrate concentration, and genetic loci controlling nitrate, phosphorus and potassium accumulation in *Brassica napus* L.**

**Running title: Nitrate, phosphorus, potassium accumulation targets**

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**Abstract**

Large nitrogen, phosphorus and potassium fertiliser inputs are used in many crop systems. Identifying genetic loci controlling nutrient accumulation may be useful in crop breeding strategies to increase fertiliser use efficiency and reduce financial and environmental costs. Here, variation in leaf nitrate concentration across a diversity population of 383 genotypes of *Brassica napus* was characterised. Genetic loci controlling variation leaf nitrate, phosphorus and potassium concentrations, were identified through Associative Transcriptomics using single nucleotide polymorphism (SNP) markers and gene expression markers (GEMs). Leaf nitrate concentration varied over 8-fold across the diversity population. A total of 455 SNP markers were associated with leaf nitrate concentration after false-discovery-rate (FDR) correction. In linkage disequilibrium of highly associated markers are a number of known nitrate transporters and sensors, including a gene thought to mediate expression of the major nitrate transporter NRT1.1. Several genes influencing root and root-hair development co-localise with chromosomal regions associated with leaf P concentration. Orthologues of three ABC-transporters involved in suberin synthesis in roots also co-localise with association peaks for both leaf nitrate and phosphorus. Allelic variation at nearby, highly associated SNPs confers large variation in leaf nitrate and phosphorus concentration. A total of five GEMs associate with leaf K concentration after FDR correction including a GEM that corresponds to an auxin-response family protein. Candidate loci, genes and favourable alleles identified here may prove useful in marker-assisted selection strategies to improve fertiliser use efficiency in *B. napus*.

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**1 Introduction**

The plant macronutrients nitrogen (N), phosphorus (P) and potassium (K) are required in large amounts by higher plants, which typically contain approximately 1.5 % N, 0.2 % P, and 1 % K on a dry weight (DW) basis (Hawkesford et al., 2012). Most N in soil is found in organic matter, which is largely unavailable to plants directly (Dechorgnat et al., 2011). There is evidence to suggest that plants can acquire organic N through root uptake of amino acids (Näsholm et al., 2009). However, the major source of N to plants in aerobic soils is thought to be nitrate (NO3-) which represents less than 2 % of soil N (Dechorgnat et al., 2011). Similarly, although total soil P content is generally considered to be relatively high, bioavailable P is present at much lower concentrations (White and Hammond, 2008). This is largely due to the ability of P to form insoluble complexes with cations including calcium (Ca), magnesium (Mg), iron (Fe) and aluminium (Al; Hinsinger, 2001). The availability of P in soils is also thought to be declining due to soil degradation, which is estimated to have affected over half of global agricultural land, and the majority of agricultural land in Africa (Lynch, 2011). The concentration of K in soils can vary greatly depending on soil type, moisture, and chemical composition (Maathuis and Sanders, 1996). Much of the K available to crops is present in the soil solution, which represents only 0.1 – 0.2 % of total soil K (White, 2013). Whilst further sources of K from exchangeable and non-exchangeable fractions of the soil are generally available to replenish soil solution K, typically 90 – 98 % of total soil K remains effectively inaccessible to crop plants.

Due to the highly intensive nature of modern agriculture, bioavailable supplies of N, P and K in arable soils can quickly become depleted (Jones et al., 2013). This can lead to the manifestation of symptoms including stunting, narrow leaves, and chlorosis under N insufficiency, inhibited shoot growth under P insufficiency, and retarded growth and shoot necrosis under K insufficiency (Hawkesford et al., 2012). In order to prevent such symptoms, huge quantities of fertiliser are applied to soils every year. In 2016, worldwide N fertiliser demand was 112 million tonnes, and demand is estimated to be increasing by 1.5 % annually (FAO, 2017). Global demand for P and K fertiliser in the same year were around 42 and 33 million tonnes, with demand expected to increase by 2.2 and 2.4 % per year, respectively (FAO, 2017). Fertiliser use is expensive, driving up costs to both the farmer and the consumer. It can also be harmful to the environment by contributing to greenhouse gas emissions, of which agricultural fertiliser use is the main source of atmospheric N2O release (Snyder et al., 2009). There is also a need to reduce excessive anthropogenic nutrient inputs to aquatic ecosystems through agricultural runoffs in order to reduce eutrophication and protect drinking water supplies (Conley et al., 2009). For example, in order to return to within Earth’s critical boundary set for excessive P in waterways, it is estimated that daily food supply per capita would have to drop below one tenth of the present supply under current agricultural practices and diets (Kahiluoto et al., 2013). Thus, a significant challenge in modern agriculture is the generation of new varieties better able to uptake and utilise available nutrients, in order to improve yields, whilst reducing fertiliser inputs.

*Brassica napus* L. is a crop species of global importance, encompassing oilseed varieties, commonly known as oilseed rape or canola, as well as vegetable crop types including swede or rutabaga, and leafy types including fodder and kale (Allender and King, 2010). Among major oilseeds, production of oilseed rape is the second largest behind soybean, with an estimated production of 74.28 million tonnes between April 2017 and April 2018 (USDA, 2018). It is also the third largest source of vegetable oil globally behind palm and soybean, with world supply predicted to be 28.84 million tonnes for the same period. Of this, approximately 10 million tonnes is estimated to be produced by the European Union, in which oilseed rape is the primary source of vegetable oil (European Commission, 2018). Only following intensive breeding programmes to reduce concentrations of toxic erucic acid and bitter glucosinolates from seed oil over approximately the last 50 years has *B. napus* reached global importance as a source of high quality vegetable oil (Allender and King, 2010). Hence, it is likely that there is still extensive scope for improvements in nutrient use efficiency in *B. napus*. For instance, N use efficiency in oilseed rape was estimated to be less than half that of barley and winter wheat (Berry et al., 2010).

There is significant potential for such improvements to be made through conventional breeding, as reflected by the variation in nutrient concentration traits previously observed (Ding et al., 2010; Bus et al., 2014; Koprivova et al., 2014; Thomas et al., 2016). For instance, in the study of Koprivova et al. (2014), leaf nitrate and phosphate concentrations varied 83- and 5.0-fold respectively across a population of 99 field-grown genotypes of *B. napus*. Approximately 2-fold variation was also observed in both leaf P and K concentration traits in a larger population of *B. napus* grown in compost (Thomas et al., 2016). However, efforts to breed for increased nutrient use efficiency could also benefit from the identification of associated molecular markers, which would allow a more targeted approach to crop improvement. A genome-wide association study (GWAS) performed with leaf anion concentration data from 84 genotypes in the study of Koprivova et al. (2014) led to the discovery of a number of significantly associated markers, indicating a genetic component of these traits. An updated, diversity population of 383 genotypes of *B. napus* has since been compiled and RNA-sequenced (Thomas et al., 2016; Havlickova et al., 2017). This forms an excellent foundation for Associative Transcriptomics (Harper et al., 2012) analyses to dissect genetic loci controlling nutrient accumulation in *B. napus* as has been recently shown for leaf Ca and Mg accumulation (Alcock et al., 2017). Multiple genetic loci were highly associated with leaf Ca and Mg concentration, and *A. thaliana* plants mutated in nearby candidate genes were shown to have perturbed nutrient concentration traits, thus indicating the efficacy of this approach.

In this study, variation in leaf NO3- concentration across a diversity population of 383 genotypes of *B. napus* is characterised. This is likely to reflect most of the species-wide variation in this trait. As the major source of N to crops grown in aerobic soils, NO3- ­is also a suitable measure of N uptake and use efficiency in *B. napus*. This data, along with leaf P and K concentration data previously described in the same population (Thomas et al., 2016), is then utilised in Associative Transcriptomics analyses to identify single nucleotide polymorphisms (SNPs) and gene expression markers (GEMs) associated with these traits, as well as allelic variants which differ in NO3-, P and K accumulation, and genes in linkage disequilibrium (LD) likely to be responsible for their control.

**2 Methods**

**2.1 Growth of plant material**

This study makes use of the Renewable Industrial Products from Rapeseed (RIPR) diversity population of inbred lines of *Brassica napus* genotypes (Thomas et al., 2016). A subset of 383 genotypes were selected, comprising 169 winter-, 123 spring-, and 11 semiwinter-oilseed rape (OSR), 27 swede, six fodder, three kale and 44 of unspecified growth types. All plant growth took place at the Sutton Bonington Campus of the University of Nottingham (52°49'58.9"N, 1°14'59.2"W) in compost in a designed experiment in a polytunnel as described previously (Thomas et al., 2016). Briefly, seeds were all sown into a fine-grade compost in propagation trays and allowed to grow in a glasshouse for approximately three months. They were then transferred to 5 L pots containing Levington C2 compost (Scotts Professional, Ipswich, UK) and placed into single skinned polytunnels. Pots were arranged in a randomised block design of five replicate blocks, each split into 12 sub-blocks with 36 pots randomly allocated within. Sixteen reference genotypes were included within each replicate block to allow more accurate normalisation. Pots were watered by automatic irrigation three times daily.

**2.2 Characterisation of leaf nitrate, phosphorus and potassium concentrations**

Leaves were sampled from all plants at the rosette stage (typically 6-8 true leaves showing) approximately four months after sowing as described previously (Thomas et al., 2016). Typically three leaves were taken from each plant and freeze dried for 48-60 hours (CHRIST Alpha 2-4 LD freeze dryer; Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany). Leaves were then homogenised in liquid N2 using a pestle and mortar and stored at -80°C prior to analyses. Leaf NO3- concentration was measured by ion chromatography (IC). For each dried, homogenised sample, 20 mg was weighed and transferred to a 1.5 mL Eppendorf tube (Eppendorf AG, Hamburg, Germany), to which 1.5 mL of Milli-Q water (18.2 MΩ cm; Fisher Scientific UK Ltd, Loughborough, UK) containing 20 mg of insoluble polyvinylpolypyrrolidone was added. This was incubated at 4°C for 60 minutes with frequent mixing, then at 90°C for 15 minutes in a water bath, prior to centrifugation at 5,000 RPM for 15 minutes at 4°C in an Eppendorf 5180 centrifuge (Eppendorf). The resulting clear supernatant was transferred into a 0.5 mL anion sample tube (PolyVials; Thermo Fisher Scientific). This was then analysed using a Dionex™ ICS-1100 Ion Chromatography System. Eluents used were Na2CO3 (Fisher) and NaHCO3 (Fisher) at 2.7 mM and 1.8 mM respectively prepared in 2 L deionised water. Standards used were sodium fluoride, sodium chloride, potassium nitrate, potassium di-hydrogen orthophosphate, potassium sulphate, and di-sodium DL-malate prepared to 1000 ppm then diluted between 10 and 150 ppm across six standard samples. Flow rate was 1.4 mL / minute and temperature was 30°C. The instrument was primed for 20 minutes prior to analysis. Leaf P and K concentrations in all genotypes were previously characterised by inductively coupled plasma-mass spectrometry (ICP-MS; Thomas et al., 2016). Briefly, a further 200 mg of dry, homogenous sample was digested in 2 mL 70% Trace Analysis Grade HNO3, 1 mL Milli-Q water, and 1 mL H2O2 in a microwave system (Multiwave 3000; Anton Paar GmbH, Graz, Austria). Leaf digestates were diluted 1-in-5 using Milli-Q water, and then analysed by ICP-MS with operational modes including (i) a helium collision-cell (He-cell) with kinetic energy discrimination to remove polyatomic interferences, (ii) standard mode (STD) in which the collision cell was evacuated, and (iii) a hydrogen collision-cell (H2-cell). The relative contribution of genotypic and non-genotypic variance components underlying variation in leaf NO3-, P and K concentration was calculated using a REML procedure in GenStat (17th Edition, VSN International Ltd, Hemel Hempstead, UK). For leaf NO3- concentration, genotype and experimental sources of variation were classed as random factors according to the model [IC\_analysis\_run\_number + (genotype\*replicate)]. For leaf P and K concentration traits, a further model was used [replicate + (replicate/sub-block) + genotype + (replicate/genotype)]. For each analysis, genotype was subsequently added as a fixed factor to estimate normalised genotype-means. Standard deviations between genotypes within each of six defined crop habits (spring-, winter- and semiwinter-OSR, fodder, kale and swede) as well as pairwise, Pearson correlation coefficients between leaf NO3-, P and K concentration data and two-sided tests of correlations against zero were calculated in GenStat.

**2.3 Transcriptome sequencing, SNP identification and quantification of transcript abundance**

Plant growth conditions, sampling techniques, RNA extraction, quality checking and Illumina transcriptome sequencing were described previously (He et al., 2017). For each genotype, RNA-seq data was mapped onto ordered *Brassica* A and C genome-based pan-transcriptomes developed by He et al. (2015) using methods described by Bancroft et al.(2011) and Higgins et al. (2012). SNP positions with a read depth below 10, base call quality below Q20, or missing data above 0.25, were excluded from alignment, as were SNP positions for which greater than three alleles were called. Across the 383 genotype panel, 46,307 single SNPs and 309,229 hemi-SNPs were detected and scored. Transcript abundance was quantified and normalised as reads per kb per million aligned reads (RPKM) for each genotype. Of the 116,098 coding DNA sequence (CDS) models in the pan-transcriptome, significant expression (mean >0.4 RPKM) was detected for 53,889. Transcriptome sequences are deposited within the Sequence Read Archive (Leinonen et al., 2011) under accession number PRJNA309367. Clustering of genotypes based on SNP data was previously carried out, and a dendogram visualisation can be found in Havlickova et al. (2017).

**2.4 Associative Transcriptomics**

Association analyses using SNPs and GEMs were carried out in R 3.2.0 (R Core Team, 2015) as previously described by Harper et al.(2012)with the modifications recently described by Havlickova et al. (2017). Inference of population structure by Q-matrix was obtained by Population Structure Inference using Kernel-PCA and Optimization (PSIKO; Popescu et al., 2014; highest likelihood subpopulation *k = 2*). SNP-based analyses were performed using a compressed mixed linear model approach (Zhang et al., 2010) implemented in the GAPIT R package (Lipka et al., 2012). GEM-based analyses were performed using fixed-effect linear modelling in R with RPKM values and PSIKO-inferred Q-matrix data as the explanatory variables and trait score the response variable. Coefficients of determination (R2), constants and significance values were output for each regression. Genomic control (Devlin and Roeder, 1999) was applied to the GEM analysis when the genomic inflation factor was observed to be greater than 1 to correct for spurious associations. Manhattan plots reporting –log10*p* values for each marker for each trait were generated using graph functions in R. SNPs with low second allele frequency (< 0.01) were filtered from the dataset prior to plot generation. In total 256,397 SNPs and 53,889 GEMs were plotted. SNP markers for which genetic mapping to the appropriate genome could not be confirmed due to sequence similarity are plotted in grey, and appear in both *Brassica* A and C sub-genomes. False Discovery Rate (FDR; Benjamini and Hochberg, 1995) and Bonferroni (Dunn, 1961) corrections were used to set significance thresholds at p < 0.05. QQ plots for all association analyses are included as supplementary material (supplementary figures 2-7).

**2.5 Candidate gene selection**

Genome browsers comprising sequences of *B. rapa* (A genome, Chiifu-401-41; Wang et al., 2011) and *B. oleracea* (C genome, TO1000DH3; Parkin et al., 2014) at Ensembl Plants (Kersey et al., 2016) as well as ordered *Brassica* pan-transcriptome data (He et al., 2015) were used to determine genes that fell within visually-determined association peaks and were within estimated LD decay (~ 1-2 cM on average; Ecke et al., 2010) of SNPs associated with measured traits. All genes that fulfilled these requirements were considered in candidate gene selection. Predicted gene function, inferred using orthologue annotations from the closely related *Arabidopsis thaliana* held by The Arabidopsis Information Resource (TAIR; Huala et al., 2001), gene expression data at The Bio-Analytic Resource for Plant Biology (Waese and Provart, 2017) and ionomic data at the Purdue Ionomics Information Management System (PIIMS; Baxter et al., 2007) to select for genes most likely contributing to the control of leaf NO3-, P and/or K concentration. For candidate genes derived from GEM analyses, only genes corresponding to GEMs directly associated with the measured traits were considered.

**2.6 Lead-marker allelic effects on leaf nitrate, phosphorus and potassium concentration**

For SNP-based association analyses, the allelic effects of lead-markers on leaf NO3- and P concentration traits were determined. Lead-markers were defined as the most highly associated SNP markers within visually-determined association peaks for a given trait that had a second allele frequency above 0.01 and had been correctly mapped to the appropriate *B. napus* sub-genome. For each lead-marker, leaf NO3- or P concentration data were plotted separately for each allele at the SNP locus. Box plots representing allelic effects were created in SigmaPlot (13th Edition, Systat Software Inc., San Jose, CA, USA). For leaf K concentration, scatter plots representing marker expression effects on the trait were plotted for all markers with –log10*p* values greater than the FDR corrected significance threshold (p = 0.05) as well as markers corresponding to described candidate genes (table 1). Scatter plots and coinciding linear regression lines were created out in SigmaPlot. R2 values and respective significance values were calculated in GenStat.

**3 Results**

**3.1 Oilseed rape varieties have the highest mean leaf concentrations of nitrate, phosphorus and potassium**

Mean leaf NO3- concentration varied 8.34-fold between genotypes, ranging from 368 to 3070 mg kg-1 dry weight (DW; figure 1). When compared between crop types, the highest average leaf NO3- concentrations were observed in oilseed rape (OSR) varieties, particularly semiwinter and spring OSR types. Ranked highest to lowest in terms of leaf NO3- concentration, the crop-types fall in the order of semiwinter OSR, spring OSR, winter OSR, fodder, swede, and kale, with average leaf NO3- concentrations of 1775, 1654, 1129, 1090, 890, and 637 mg kg-1 DW respectively. Standard deviations within these groups were 255, 556, 363, 282, 377 and 182, respectively. Large amounts of variation were observed within both winter and spring OSR types; 6.34- and 4.92-fold variation respectively (figure 1). Leaf P and K concentration data were reported previously (Thomas et al., 2016). Briefly, leaf P and K concentrations varied 1.98- and 2.07-fold from 3290 to 6526 mg kg-1 DW and 26228 to 54329 mg kg-1 DW respectively between all 383 genotypes. Mean leaf P concentration was highest in spring OSR types, followed by fodder, semiwinter OSR, winter OSR, kale, and finally swede types. Mean leaf K concentration was highest in semiwinter OSR, followed by spring OSR, winter OSR, swede, fodder, and finally kale types. Pearson correlation coefficient between leaf NO3- and K was 0.45; between leaf NO3- and P was 0.32; and between leaf P and leaf K was 0.25 (all *p* values < 0.001). The range of concentrations of each nutrient followed approximately a normal distribution across the diversity population (supplementary figure 1).

**3.2 Genes controlling root suberin synthesis and nitrate and ammonium transport are within LD decay of markers associated with leaf nitrate concentration**

A total of 455 and 10 SNP markers were significantly associated with leaf NO3- concentration after FDR and Bonferroni correction respectively (*p* = 0.05). This was reduced to 415 and 9 SNP markers respectively after filtering out markers with low second allele frequency (SAF; < 0.01). The majority of associated markers fell within visually-determined association peaks. The two most defined association peaks are located on chromosomes A1 and A3 and co-localise with one and four of the 10 SNPs above the Bonferroni corrected significance threshold respectively (*p* = 0.05). Further, notable association peaks are located on chromosomes A4, A9, A10, C1, C4, C8 and C9 (figure 2A). There were no GEMs that fell above FDR or Bonferroni corrected significance thresholds. However, less distinct association peaks were visually-determined on chromosomes A2, C2 and C9 (figure 2B). By searching genetic loci within linkage disequilibrium (LD) decay (~ 1-2 cM on average; Ecke et al., 2010) of SNP-based association peaks associated with leaf NO3- concentration, 15 candidate genes were identified (table 1). These were orthologous to nine unique *Arabidopsis thaliana* genes, with multiple, paralogous copies of some candidate genes within LD decay of associated markers. Within LD of the association peak on chromosome A1 lies Cab024292.1, an orthologue of *A. thaliana* AT4G24020.1. This gene encodes NIN (nodule inception)-LIKE PROTEIN 7 (NLP7), a putative transcription factor thought to be responsible for sensing and responding to exogenous nitrate concentrations (Castaings et al., 2009). A further orthologue of *NLP7* was found within the syntenous region of chromosome C1. Within the association peak on chromosome A3 lies Cab002472.4, an orthologue of *A. thaliana* AT5G10140.4. This encodes FLOWERING LOCUS C (*FLC*), a transcription factor responsible for repressing flowering prior to sufficient cold treatment (Sheldon et al., 2000). The most highly associated GEM on chromosome A3 also corresponds to Cab002472.4. Further candidate genes include seven members of the NITRATE TRANSPORTER 2 (*NRT2*) gene family, three genes encoding ABC transporters thought to be required for suberin synthesis in roots, a gene encoding a high-affinity ammonium transporter, and a chloride channel which is thought to be responsible for NO3- accumulation in vacuoles (table 1). For lead-markers within selected association peaks, genotype-specific allelic variants in the RIPR diversity population were identified and leaf nitrate concentration between variant and reference alleles were compared. The allelic variant was defined as the second most frequent allele in the population after the reference base call. Lead-markers for each association peak investigated are summarised in table 1. For lead-markers within association peaks on chromosomes A1, A3, A4, A9 (labelled A9.1 as two association peaks referred to in this chromosome in table 1), A10, C1, C8 and C9, the allelic variant was associated with a 1.86-, 1.93-, 2.06-, 1.84-, 1.24-, 1.85-, 1.92-, and 1.73-fold increase in leaf NO3- concentration respectively (figure 3; all *p* values < 0.001).

**3.3 Genes controlling root-hair development among candidates for leaf phosphorus concentration**

There were no SNPs or GEMs that associated with leaf P concentration above the level of Bonferroni or FDR corrected significance thresholds. However, a number of SNP markers had notably higher –log10*p* values than the majority of markers, and many of these formed visually-determinable association peaks (figure 2C). The most distinct of these are present on chromosomes A1, A5, C8 and C9. Further, less prominent association peaks were detected on chromosome A6, C2 and C4. A total of 19 candidate genes were identified within LD decay of associated markers at these loci (table 1). Most notable candidate genes are four genes involved in root or root hair development on chromosome C8. These are orthologous to *A. thaliana* AT1G12240.1 (*BFRUCT4*), AT1G12360.1 (*KEULE*), AT1G12560.1 (*EXPANSIN* *A7*) and AT1G12950.1 (*ROOT HAIR SPECIFIC 2*). An additional outstanding candidate is a Bo9g166650, an orthologue of *A. thaliana* AT5G13580.1. This encodes an ABC transporter of the same family as those within LD of markers associated with leaf NO3- concentration and this member is also thought to be required for root suberin synthesis. Further candidates include three genes encoding SCARECROW-LIKE proteins (3, 5 and 13) which are transcription factors involved in the control of developmental processes during the plant life cycle, as well as three genes encoding flowering time regulators, six transporter-protein encoding genes, a gene involved in response to low phosphate, and a transcription factor which may have a role in lignin synthesis (table 1). It is also worthy of note that two of the GEMs most highly associated with leaf P concentration (chromosomes A10 and A3, ranked 1st and 11threspectively; figure 2D) correspond to distinct, orthologous copies of *A. thaliana* AT5G10140.4 (*FLC*). The latter of these is identical to the gene within LD of the association peak identified for leaf NO3- concentration described above. For all eight loci within which candidate genes were identified, box plots were generated to visualise the effect of allelic variant at lead-marker position on leaf P concentration (figure 4). For lead-markers within association peaks on chromosomes A5, A6, C2, C4, and the former peak on C9 (C9.1), the allelic variant was associated with a 1.13-, 1.06-, 1.15-, 1.09-, and 1.14-fold increase in leaf P concentration compared to the reference allele respectively (*p* < 0.001, *p* = 0.010, *p* < 0.001, *p* < 0.001, *p* < 0.001 respectively). For lead-markers within association peaks on chromosomes A1, C8, and the latter peak on chromosome C9 (C9.2), the allelic variant was associated with a 1.09-, 1.18-, and 1.15-fold decrease in leaf P concentration compared to the reference allele (all *p* values < 0.001).

**3.4 The most highly associated GEM for leaf potassium concentration corresponds to an auxin responsive family protein**

No SNPs, but a total of 1 and 5 GEMs were associated with leaf K concentration above the level of Bonferroni and FDR corrected significance thresholds respectively after genomic control. The GEM above both the significance thresholds was located on chromosome A3 and corresponds to Cab001225.1, an orthologue of *A. thaliana* AT2G04850.1 which encodes an auxin-responsive family protein. Of the remaining four GEMs over the FDR corrected significance threshold, a further marker falls within chromosome A3, one within chromosome A10, and two within chromosome C9 (figure 2F). Unlike results from SNP-based analyses, for which any gene within LD decay of associated markers could be the cause of the variation observed in the trait, genes underlying GEMs are more likely to be directly involved in trait control. For this reason, genes under all five of the aforementioned GEMs were considered as likely candidate genes (table 1). However, two visually-determined association peaks were located towards the beginning of chromosomes A9 and C9, and hence genes within LD of these association peaks were also considered. Within LD decay of the more highly associated markers within these loci are the genes Cab037725.3 and Bo9g001030.1, both orthologous to *A. thaliana* AT1G05940.1, a gene that encodes a cationic amino acid transporter. Two further candidate genes with relatively high –log10*p* values in the corresponding GEM were considered; Cab024257.1 and Bo9g171810.1. The former of these is orthologous to *A. thaliana* AT4G23640.1 which encodes a potassium transporter family protein. The latter is paralogous to the gene underlying the GEM over the FDR corrected significance threshold on chromosome A10. The effects of expression levels of the GEMs underlying each of these candidate genes is modelled in figure 5. For GEMs underlying genes Cab024257.1, Cab001225.1, Cab002494.2 and Cab037664.1, an increase in expression was associated with an overall increase in genotype-specific mean leaf K concentration (r2 = 0.059, 0.098, 0.075 and 0.068 respectively, all *p* values < 0.001. For GEMs underlying genes Cab007712.1, Bo9g003730.1, Bo9g002280.1 and Bo9g171810.1, an increase in expression was associated with an overall decrease in genotype-specific mean leaf K concentration (r2 = 0.067, 0.075, 0.059 and 0.064 respectively, all *p* values < 0.001; figure 5).

**4 Discussion**

**4.1 Species-wide variation in leaf nitrateconcentration indicates scope for breeding for increased N uptake and use efficiency via traditional means**

Leaf NO3- concentration varied over 8-fold among the 383 genotypes in the RIPR diversity population, indicating large amounts of species-wide variation. The variation observed here is lower than the 83-fold variation shown previously in a population of 84 similar genotypes of *B. napus* grown under field conditions (Koprivova et al., 2014). After converting FW measurements to DW for comparison, assuming a 90 % leaf water content, genotypes in the study of Koprivova et al. (2014) had estimated minimum and maximum leaf NO3- concentrations of 192 and 15935 mg kg-1 DW respectively. Hence, values from the present study occupy similar orders of magnitude to those measured previously. The lower variance between genotypes observed here is likely linked to the age of the plants, which were sampled after approximately 4 months of growth. In the earlier study of Koprivova et al., (2014), they were sampled after only 8 weeks. It could also be linked the growth conditions, which in the present study comprised compost-filled pots under controlled irrigation placed in polytunnels, unlike the field conditions used in the earlier study. Despite these differences, it is clear that large amounts of species-wide variation in shoot NO3- concentration exist in *B. napus*, irrespective of growth condition or plant age. This knowledge could be applied in traditional breeding strategies for increasing N uptake and use efficiency, perhaps under low exogenous NO3- conditions. It is worth bearing in mind that a low leaf NO3- concentration may be indicative of a high N use efficiency. There are multiple definitions of N use efficiency including yield per unit of N input, amount of N in plant per unit of N input, and shoot weight per unit of shoot N (Good et al., 2004). If a crop has relatively low shoot concentrations of NO3- but still yields highly, it may possess traits that allow it to prosper under lower exogenous NO3- supply. Hence it is essential that genotype-specific yield be considered as well as leaf NO3- concentration in selecting suitable varieties for breeding.

**4.2 Identification of genes encoding nitrate and ammonium transporters within LD of leaf nitrate association peaks likely linked to altered expression between genotypes in the diversity population**

A total of eight genes encoding putative NO3- transporters and one gene encoding a putative ammonium (NH4+) transporter were identified within LD decay of markers highly associated with leaf NO3- concentration. Seven of the eight genes encoding NO3- transporters were members of the NITRATE TRANSPORTER 2 (*NRT2*) family and fall within three distinct loci on chromosomes A9 (two orthologues of *Arabidopsis thaliana NRT2.1*), A10 (orthologues of *A. thaliana NRT2.3* and *NRT2.4*) and C9 (further orthologues of *A. thaliana NRT2.3* and *NRT2.4*; table 1). The latter two loci are in regions of known shared sequence homology (Chalhoub et al., 2014), indicating that copies of these genes on both *B. napus* sub-genomes are likely to be important for leaf NO3- accumulation. Each of these genes encode high-affinity NO3- transporters, transcript abundance of which are high under low exogenous NO3- concentrations (Orsel et al., 2002). The eighth putative nitrate transporter gene encodes CHLORIDE CHANNEL-A (*CLCA*). Not only was this gene found to mediate nitrate accumulation in plant vacuoles in *A. thaliana* (De Angeli et al., 2006), but it was also identified as a contributor of the control of leaf NO3- homeostasis in a previous association analysis in *B. napus* (Koprivova et al., 2014). Thus, this is a very convincing candidate gene for controlling leaf NO3- concentration. The gene encoding an ammonium transporter, specifically AMMONIUM TRANSPORTER 2 (*AM2*; table 1), was identified on chromosome A4, close to a SNP at which locus allelic variation confers 2.1-fold variation in leaf NO3- concentration (figure 3). The encoded protein is thought to be localised to the plasma membrane, and experiments in *A. thaliana* indicate that it is a high-affinity transporter, expression of which is repressed by higher exogenous ammonium nitrate concentrations (Sohlenkamp et al., 2002). Whilst it seems clear that NH4+, and not NO3- is the substrate for this protein, it is likely that changes in NH4+ uptake efficiency will impact on leaf NO3- concentration and hence this association is perhaps unsurprising.

A further, highly interesting candidate for leaf NO3- concentration is NIN (nodule inception)-LIKE PROTEIN 7 (*NLP7*). Allelic variation in the nearby lead-marker within the strong association peak on chromosome A1 confers 1.9-fold variation in leaf NO3- concentration (figure 3). *NLP7* has recently been shown to modulate expression of the major NO3- sensor and transporter *NRT1.1* (Zhao et al., 2018). As a NO3- sensor, NRT1.1 appears to enable detection of NO3--rich soil patches, leading to subsequent proliferation of roots in these areas (Bouguyon et al., 2016). This protein can also act as a dual-affinity transporter, depending on the phosphorylation status of a threonine residue within the protein, and is active at a range of exogenous NO3- concentrations (Parker and Newstead, 2014). The association of SNP markers within LD decay of *NLP7* provides further evidence of its downstream role in NO3- uptake in *B. napus*. This gene provides an excellent candidate for further study, allelic variants in which may provide a suitable route towards improvement of NO3- uptake and N fertiliser use efficiency in *B. napus*.

The association of SNP markers close to the genes described here is indicative of variation in expression of these genes across the diversity population, which may subsequently impact on NO3- uptake and leaf accumulation. Whilst the corresponding GEMs were not associated with the trait, it is important to state that the GEMs used in this study are based on transcript abundance in young leaves. Hence, genotype-specific differences in the expression of genes in roots or at different growth stages are likely to be masked. Similarly, variation in the expression of genes that are specifically expressed in root tissue, and not expressed in leaves, will not be reflected here. Thus, further work will be required to confirm differences in expression of such genes between genotypes in the RIPR diversity population.

**4.3 Orthologues of three ABC transporters required for root suberin synthesis likely contribute to nitrate and phosphorus homeostasis**

Three and one gene(s) encoding putative ABC transporters were identified close to SNPs highly associated with leaf NO3- and P concentration respectively (table 1). These are orthologous to the three unique *A. thaliana* ABC-2 transporters G2 (AT2G37360), G6 (AT5G13580) and G20 (AT3G53510). All of these genes are root expressed and thought to be required for the transport of aliphatic polymer precursors of suberin or related structural components of the Casparian strip (Yadav et al., 2014). Root suberin is considered to be a major component of the Casparian strip which forms a chemical barrier restricting extracellular transport of water and dissolved nutrients to the vascular tissue (Baxter et al., 2009). This enables greater control of solute transport between plant tissues. *Arabidopsis thaliana* triple mutants in the aforementioned ABC transporter encoding genes were previously characterised and root systems were found to be more permeable to water as well as the solutes sodium chloride (NaCl) and potassium nitrate (KNO3; Yadav et al., 2014). The authors also deduced that whilst the mutant plants were able to make suberin, its structure was altered compared to wild-type plants. That all three of these genes were identified close to SNPs highly associated with leaf NO3- and P concentration in this study provides further evidence for the role of Casparian strip localised suberin in nutrient uptake in *B. napus*.

**4.4 Flowering time is an important marker for leaf nitrate and phosphorus concentration**

Within LD decay of highly associated markers for leaf NO3- and P concentration, were a number of genes thought to control flower development. These include orthologues of *A. thaliana* FLOWERING LOCUS C (*FLC*), a flowering time regulator, which were detected close to association peaks for leaf NO3- and P concentration on chromosomes A3 and C9 respectively, as well as orthologues of PISTILLATA (*PI*), and AGAMOUS-LIKE 29 (*AGL29*) close to association peaks for leaf P concentration on chromosomes C2 and C4 respectively (table 1). Allelic variation at the lead-marker close to *FLC* on chromosome A3 is associated with 1.9-fold variation in mean leaf NO3- concentration (figure 3). Allelic variation close to the *FLC* orthologue on chromosome C9 is also associated with 1.2-fold variation in leaf P concentration (figure 4). Interestingly, orthologues of *FLC* and other flowering time regulating genes were previously found within LD decay of association peaks for leaf Ca and Mg concentration (Alcock et al., 2017). Expression of *FLC* directly influences flowering time by suppressing activation of flowering until after sufficient cold treatment (vernalisation; Sheldon et al., 2000). It has previously been demonstrated that winter cultivars of *B. napus*, which have higher vernalisation requirements, have greater *FLC* transcript abundance than spring cultivars, which have lower vernalisation requirements (Tadege et al., 2001). In the present study, leaf NO3- concentration was found to vary between spring and winter crop types. Similarly, leaf P concentration data used for association analyses here was previously shown to vary between winter and spring crop types (Thomas et al., 2016). It is possible that the associations with flowering time regulator genes observed here and in previous studies are linked to spurious correlations between leaf nutrient concentration and flowering time between different crop types. However, it was previously shown that leaf N concentrations decline with leaf expansion during crop development (Liu et al., 2018). Hence, it is also possible that expression levels of *FLC* and other flowering time related genes could influence leaf nutrient concentrations indirectly by changing the duration of vegetative growth. Whilst further work will be required to elucidate the mechanisms controlling this association, the tendency of flowering time genes to associate with multiple nutrient traits indicates that they are suitable markers for leaf mineral concentration traits in *B. napus*.

**4.5 Root hair development genes are particularly important for leaf phosphorus accumulation**

No SNPs or GEMs were significantly associated with leaf P concentration above the FDR corrected significance threshold in this study. This indicates that unlike leaf NO3- concentration, leaf P concentration is under relatively low genetic control, or at the very least, that there are low levels of genetic variation that associate with variation in the trait. This is surprising, as variance components analysis in this population previously showed that 20 % of the variation in leaf P concentration was associated with genotype, indicating a moderate genetic component (Thomas et al., 2016). However, this result is consistent with a previous association study in a similar population of *B. napus*, in which although heritability of shoot P concentration was estimated at 0.43, no significant marker associations were detected for the trait (Bus et al., 2014). Despite this, a number of notable association peaks were detected in the Manhattan plot generated from SNP-based association data (figure 2C). Allelic variation in lead-marker positions within these loci were also associated with relatively high levels of variation in leaf P concentration (figure 4), hence indicating that genes within LD of these markers are likely to contribute to control of the trait.

The three phosphate transporter encoding genes PHOSPHATE TRANSPORTER (*PHT*) 1.4, 1.7 and 3.1 were located close to highly associated SNP markers on chromosomes C4, A6 and C9 respectively. The former two genes appear to encode high-affinity transporters for external phosphate uptake (Shin et al., 2004; *PHT1.7* function inferred by sequence similarity), and thus their proximity to markers associated with leaf P concentration is unsurprising. The latter transporter is expressed in mitochondria, and appears to modulate plant salt stress responses (Zhu et al., 2012). Hence, whilst it remains an interesting candidate to consider, it is less likely than the former transporters to be the causative gene within its respective association peak. A further candidate which may have a direct influence on phosphate uptake is PHOSPHATE 2, otherwise known as UBIQUITIN-CONJUGATING ENZYME 24 (Huang et al., 2013). This gene has been shown to be involved in the response to exogenous phosphate concentration through degradation of PHOSPHATE TRANSPORTER 1 (PHO1) under phosphate sufficiency (Liu et al., 2012). PHO1 is crucial for phosphate loading to the xylem and subsequent transport to the shoot (Hamburger et al., 2002). Hence, alterations in expression or protein structure of PHOSPHATE 2 may provide a suitable avenue for improving P-fertiliser use efficiency, through altering the plant’s response to exogenous phosphate supplies.

A number of candidate genes that may contribute indirectly to the accumulation of phosphorus were also identified, including genes within LD of SNPs highly associated with leaf P concentration on chromosome C8 that play a role in root system architecture. The genes, *EXPANSIN A7* and *ROOT HAIR SPECIFIC 2* are both crucial for root hair elongation, and *A. thaliana* mutants in these genes have shorter root hairs (Won et al., 2009; Lin et al., 2011). A further gene identified in close proximity, known as *KEULE*, plays a role in cytokinesis through proper vesicle tethering, which among other things is required for polar growth of root hairs (Wu et al., 2013). Root hairs have previously been linked to phosphorus acquisition, which increase in length and number under low exogenous supply or deficiency (Lynch, 2011; Wang et al., 2018). It has also recently been shown that the expression of auxin-inducible transcription factors is increased in root hairs and that auxin accumulates in root hair zones under low exogenous P conditions, hence promoting root hair elongation (Bhosale et al., 2018). It is clear that root hair traits can influence P accumulation by plants, and there is genetic variation in this trait that could be exploited to improve P acquisition and P fertiliser use efficiency (Wang et al., 2018).

**4.6 Allelic-variant analysis indicates scope for marker-assisted selection for improved nitrate and phosphorus use efficiency**

Investigation into the effects of allelic variation at lead-marker positions for leaf NO3- and P concentration indicated that huge differences in trait values between genotypes can be detected whilst only considering few, individual SNPs. For instance, a SNP in the lead-marker within an association peak on chromosome A4 was shown to confer over 2-fold mean variation in leaf NO3- concentration between genotypes (figure 3). The identification of trait values associated with these markers may prove useful in marker-assisted selection strategies to improve nutrient accumulation or use efficiency in *B. napus*. This could accelerate the development of suitable crop varieties for a changing world, which is likely to demand greater yield from crop plants whilst reducing fertiliser inputs.

**4.7 Shoot gene expression is likely to play a role in the control of potassium homeostasis**

As with leaf phosphorus concentration, no SNP markers were significantly associated with leaf K concentration in the RIPR diversity population. Similarly, no associations were identified for shoot K concentration in an association study in a different population of 509 *B. napus* genotypes (Bus et al., 2014). Thus, it is possible that leaf K concentration is under lower genetic control than leaf NO3- concentration. However, there were several GEMs that had –log10*p* values over the FDR corrected significant threshold. The GEM most highly associated with leaf K concentration is orthologous to *A. thaliana* At2g04850.Whilst relatively little is known about this gene, data from the SMART web-based tool (Schultz et al., 1998) indicates that it is membrane bound, and that is contains a Cytochrome b-561 / ferric reductase transmembrane domain. This gene is also thought to be auxin-responsive. Auxin is a plant hormone that is responsible for orchestrating multiple growth and development processes (Swarup and Bennett, 2003). Hence, it is possible that expression of this gene is related to development stage, which may correlate with leaf K concentration. Whilst the link between this gene and leaf K concentration is currently unclear, this was by far the most highly associated GEM, and hence is worthy of further consideration. A further GEM of interest underlies a gene orthologous to *A. thaliana*, *POTASSIUM TRANSPORTER 3* (*KT3*), also known as *TINY ROOT HAIR 1* (Daras et al., 2015). Whilst not associated with leaf K concentration above the FDR corrected significance threshold, the GEM corresponding to this gene is relatively highly associated with the trait, and shoot gene expression is positively correlated (figure 5). Experiments in *A. thaliana* showed that KT3 knockout lines, which have significantly smaller root hairs, have a reduced rate of K transport, although not enough for the plants to develop deficiency symptoms (Rigas et al., 2001). The presence and length of root hairs have also been correlated with increased K acquisition among plant species (White 2013, Hinsinger et al. 2017). Thus, genetic variation in this trait might be exploited to improve K acquisition and K fertiliser use efficiency. Within LD decay of co-localising GEM association peaks on chromosomes A9 and C9 are genes orthologous to *A. thaliana* AT1G05940.1, a gene that encodes a cationic amino acid transporter. This may play a role in K acquisition within *B. napus*. However, association peaks in the same loci were detected for seed glucosinolate content (Lu et al., 2014). Over approximately the last 50 years, intensive breeding programmes have been undertaken to reduce seed glucosinolate concentrations in *B. napus* (Allender and King, 2010). Due to such strong selection pressures, it is possible that a number of other traits were perturbed in the process, and hence the association peaks on chromosomes A9 and C9 detected here may be a result of unconscious co-selection.

It is clear from SNP and GEM association analyses that gene expression data is a better tool for elucidating genes controlling leaf K concentration. It is possible that examining the effects of root gene expression across the population would lead to the discovery of further genes controlling the trait. Root traits have previously been correlated with plant K acquisition (White 2013, Hinsinger et al. 2017), and genotype-specific gene expression within this tissue is likely to reflect variation in K uptake and consequently leaf K concentration. However, root gene expression data across a diversity population of *B. napus* is not available at this time. Despite the limitations of using shoot gene expression data for the determination of genes controlling leaf K concentration, a number of convincing candidate genes have been identified, expression of which could be modified to alter K accumulation and use efficiency.

**5 Conclusions and perspective**

This study has identified large amounts of variation in leaf NO3- concentrations across 383 genotypes of *B. napus* grown in controlled conditions. This likely reflects the majority of species-wide heterogeneity in the trait and demonstrates the scope for breeding for increased NO3--use efficiency through traditional techniques. This study has also determined genetic loci and allelic variants associated with leaf NO3-, P and K concentrations in *B. napus*. Several convincing candidate genes that may affect these traits either directly or indirectly have also been identified. These include genes which relate to suberin synthesis and root hair development, both of which are likely to be amenable traits for improving uptake- and use-efficiency of multiple nutrients (White et al., 2013). Characterising variation in traits related to suberin synthesis and root hair development across genotypes of the RIPR diversity population would likely add to the understanding of their function and how they can be applied in crop breeding strategies. Allelic variants associated with variation in leaf NO3-*,* P and K concentration identified here also provide an excellent resource for marker-assisted selection. Together, these results make significant progress in the understanding of nutrient homeostasis in *B. napus*, which may prove useful in the generation of more nutrient and fertiliser use efficient crop varieties.

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**Author contributions**

MB, IB, PW, TA, and NG conceived the project and contributed to experimental design. TA analysed leaf nitrate and Associative Transcriptomics data. LH and ZH prepared functional genotypes and performed Associative Transcriptomics. LW prepared leaf samples and carried out ion chromatography analyses. TA and NG wrote the manuscript. All authors contributed to and have read and approved the final version of the manuscript.

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**Data Availability Statement**

Leaf nitrate, phosphorus and potassium concentration data from the *Brassica napus* diversity population utilised in this study are included as supplementary information (supplementary table 1). Transcriptome sequences used in Associative Transcriptomics analyses are deposited within the Sequence Read Archive (Leinonen et al., 2011) under accession number PRJNA309367. Raw, Associative Transcriptomics outputs supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

**Conflict of interest statement**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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**Figure legends**

Figure 1: Leaf nitrate concentrations of *Brassica napus* plants grown in compost. Data are means of 339 genotypes for which crop growth type had been assigned including 169 winter-, 11 semiwinter-, and 123 spring-oilseed rape (OSR), 27 swede, six fodder and three kale types. Boxes represent the mid two quartiles with the median drawn; whiskers above and below the boxes indicate the 90th and 10th percentiles with any outliers shown.

Figure 2: –log10*p* values of SNPs and GEMs associated with leaf nitrate concentration (**A**, **B**, respectively), leaf phosphorus concentration (**C**, **D**, respectively) and leaf potassium concentration (**E**, **F**, respectively) in order of markers within the *Brassica napus* pan-transcriptome. Upper, gold, dashed line represents Bonferroni corrected significance threshold; lower, yellow, dashed line represents FDR corrected significance threshold (*p* = 0.05).

Figure 3: Effects of reference allele and most frequent allelic variant at lead-marker loci on leaf nitrate concentration. Each panel refers to a specific association peak; lead-markers at each peak are listed in table 1. Boxes represent the mid two quartiles with the median drawn; whiskers above and below the boxes indicate the 90th and 10th percentiles with any outliers shown. A, T, G and C alleles represent adenine, thymine, guanine and cytosine nucleotide calls respectively. S, R and K alleles represent Strong (C or G), purine (A or G) or Keto (C or T) nucleotide calls respectively, thus called due to unresolvable variation in closely related genes. Differences in leaf nitrate concentration between alleles at each loci significant at *p* < 0.001.

Figure 4: Effects of reference allele and most frequent allelic variant at lead-marker loci on leaf phosphorus concentration. Each panel refers to a specific association peak; lead-markers at each peak are listed in table 1. Boxes represent the mid two quartiles with the median drawn; whiskers above and below the boxes indicate the 90th and 10th percentiles with any outliers shown. A, T, G and C alleles represent adenine, thymine, guanine and cytosine nucleotide calls respectively. Differences in leaf phosphorus concentration between alleles at each loci significant at *p* < 0.001 except loci on chromosome A6; *p* = 0.010.

Figure 5: Effect of leaf transcript abundance of genes underlying candidate GEMs on leaf potassium concentration. Transcript abundance was quantified and normalised as reads per kb per million aligned reads (RPKM) for each genotype. R2 values and respective significance values calculated in GenStat are shown.

Table 1 – Candidate genes within estimated linkage disequilibrium decay of lead-markers associated with leaf nitrate, phosphorus, and/or potassium concentration. Putative functions obtained from annotation data at The Arabidopsis Information Resource (Huala et al., 2001).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Trait** | **Candidate gene** | ***Arabidopsis orthologue*** | **Putative function** | **Linkage group** | **Lead-marker ID** |
| Leaf NO3- concentration | Cab024292.1 | AT4G24020 | NIN-LIKE PROTEIN 7; involved in regulation of nitrate assimilation | A1 | Cab024343.4:2028:T |
| Leaf NO3- concentration | Cab002472.4 | AT5G10140 | FLOWERING LOCUS C; MADS-box transcription factor family protein | A3 | Cab003105.1:423:C |
| Leaf NO3- concentration | Cab035003.1 | AT2G37360 | ATP-BINDING CASSETTE G2; required for root suberin synthesis | A4 | Cab034977.1:588:G |
| Leaf NO3- concentration | Cab034940.1 | AT2G38290 | AMMONIUM TRANSPORTER 2 | A4 | Cab034977.1:588:G |
| Leaf NO3- concentration | Cab000535.1 | AT3G53510 | ATP-BINDING CASSETTE G20; required for root suberin synthesis | A9.1 | Cab000410.2:940:G |
| Leaf NO3- concentration | Cab014282.1 | AT1G08090 | NITRATE TRANSPORTER 2.1 | A9.2 | Cab014338.1:34:T |
| Leaf NO3- concentration | Cab014283.1 | AT1G08090 | NITRATE TRANSPORTER 2.1 | A9.2 | Cab014338.1:34:T |
| Leaf NO3- concentration | Cab017152.1 | AT5G60780 | NITRATE TRANSPORTER 2.3 | A10 | BnaA10g13370D:657:T |
| Leaf NO3- concentration | Cab017154.1 | AT5G60770 | NITRATE TRANSPORTER 2.4 | A10 | BnaA10g13370D:657:T |
| Leaf NO3- concentration | Bo1g037430.1 | AT4G24020 | NIN-LIKE PROTEIN 7; involved in regulation of nitrate assimilation | C1 | Bo1g036990.1:1395:C |
| Leaf NO3- concentration | Bo4g145580.1 | AT5G40890 | CHLORIDE CHANNEL A; involved in nitrate accumulation in vacuoles | C4 | Bo4g143900.1 |
| Leaf NO3- concentration | Bo8g082890.1 | AT3G53510 | ATP-BINDING CASSETTE G20; required for root suberin synthesis | C8 | Bo8g082080.1:192:A |
| Leaf NO3- concentration | Bo9g147020.1 | AT5G60780 | NITRATE TRANSPORTER 2.3 | C9 | Bo9g143680.1:1029:T |
| Leaf NO3- concentration | Bo9g146990.1 | AT5G60770 | NITRATE TRANSPORTER 2.4 | C9 | Bo9g143680.1:1029:T |
| Leaf NO3- concentration | Bo9g147000.1 | AT5G60770 | NITRATE TRANSPORTER 2.4 | C9 | Bo9g143680.1:1029:T |
| Leaf P concentration | Cab013031.3 | AT4G16480 | INOSITOL TRANSPORTER 4 | A1 | Cab013058.2:573:G |
| Leaf P concentration | Cab012953.1 | AT4G17230 | SCARECROW-LIKE 13; required for the regulation of hypocotyl elongation | A1 | Cab013058.2:573:G |
| Leaf P concentration | Cab023424.2 | AT3G08500 | MYB83; transcription factor required for activation of lignin synthesis | A5 | Cab023469.1:384:C |
| Leaf P concentration | Cab022857.1 | AT3G54700 | PHOSPHATE TRANSPORTER 1.7 | A6 | Cab022858.1:2706:G |
| Leaf P concentration | Cab022780.1 | AT1G50420 | SCARECROW-LIKE 3; involved in control of gibberellin-mediated plant growth | A6 | Cab022858.1:2706:G |
| Leaf P concentration | Cab022771.1 | AT1G50600 | SCARECROW-LIKE 5; transcription factor likely to be involved in plant growth | A6 | Cab022858.1:2706:G |
| Leaf P concentration | Bo2g020730.1 | AT5G20240 | PISTILLATA; MADS-box transcription factor involved in floral development | C2 | Bo2g017990.1:1221:G |
| Leaf P concentration | Bo2g018220.1 | AT5G19600 | SULFATE TRANSPORTER 3.5 | C2 | Bo2g017990.1:1221:G |
| Leaf P concentration | Bo4g182590.1 | AT2G34440 | AGAMOUS-LIKE 29; transcription factor likely involved in floral development | C4 | Bo4g182560.1:642:G |
| Leaf P concentration | Bo4g182170.1 | AT2G33770 | PHOSPHATE 2 / UBC24; involved in phosphate starvation response | C4 | Bo4g182560.1:642:G |
| Leaf P concentration | Bo4g185840.1 | AT2G38940 | PHOSPHATE TRANSPORTER 1.4 | C4 | Bo4g182560.1:642:G |
| Leaf P concentration | Bo4g184590.1 | AT2G35740 | INOSITOL TRANSPORTER 3 | C4 | Bo4g182560.1:642:G |
| Leaf P concentration | Bo8g108330.1 | AT1G12240 | BFRUCT4; plays a role in mobilising sucrose to sink organs and in root elongation | C8 | Bo8g108620.1:687:A |
| Leaf P concentration | Bo8g108240.1 | AT1G12360 | KEULE; regulates cytokinesis related vesicle trafficking and root hair formation | C8 | Bo8g108620.1:687:A |
| Leaf P concentration | Bo8g108070.1 | AT1G12560 | EXPANSIN A7; expressed in root hair cells and involved in elongation | C8 | Bo8g108620.1:687:A |
| Leaf P concentration | Bo8g107860.1 | AT1G12950 | ROOT HAIR SPECIFIC 2; positively mediates root hair elongation | C8 | Bo8g108620.1:687:A |
| Leaf P concentration | Bo9g166650.1 | AT5G13580 | ATP-BINDING CASSETTE G6; required for root suberin synthesis | C9.1 | Bo9g166760.1:437:A |
| Leaf P concentration | Bo9g166210.1 | AT5G14040 | PHOSPHATE TRANSPORTER 3.1 | C9.1 | Bo9g166760.1:437:A |
| Leaf P concentration | Bo9g173370.1 | AT5G10140 | FLOWERING LOCUS C; MADS-box transcription factor family protein | C9.2 | Bo9g173750.1:963:A |
| Leaf K concentration | Cab024257.1 | AT4G23640 | POTASSIUM TRANSPORTER 3; also required for tip growth of root hairs | A1 | GEM\_Cab024257.1 |
| Leaf K concentration | Cab001225.1 | AT2G04850 | Auxin-responsive family protein | A3 | GEM\_Cab001225.1 |
| Leaf K concentration | Cab002494.2 | AT2G30320 | Pseudouridine synthase family protein | A3 | GEM\_Cab002494.2 |
| Leaf K concentration | Cab037725.3 | AT1G05940 | CATIONIC AMINO ACID TRANSPORTER 9 | A9 | GEM\_Cab037664.1 |
| Leaf K concentration | Cab007712.1 | AT5G10740 | Protein phosphatase 2C family protein | A10 | GEM\_Cab007712.1 |
| Leaf K concentration | Bo9g003730.1 | AT4G01080 | TRICHOME BIREFRINGENCE-LIKE 26 | C9 | GEM\_Bo9g003730.1 |
| Leaf K concentration | Bo9g002280.1 | AT4G00360 | CYTOCHROME P450, FAMILY 86, SUBFAMILY A, POLYPEPTIDE 2 | C9 | GEM\_Bo9g002280.1 |
| Leaf K concentration | Bo9g001030.1 | AT1G05940 | CATIONIC AMINO ACID TRANSPORTER 9 | C9 | GEM\_Bo9g002280.1 |
| Leaf K concentration | Bo9g171810.1 | AT5G10740 | Protein phosphatase 2C family protein | C9 | GEM\_Bo9g171810.1 |