

Figure S1 Root morphology of two *Brassica napus* genotypes (D4-15, D2-1) in response to N availability in the hydroponic environments. Fifteen-day-old plants were transferred to a nutrient solution lacking N. After a further 15 days, N was re-supplied to the solution. The number of root tips (A), root surface area (B), root volume (C) and average root diameter (D) were measured after 0, 1, 3, 5, 7, 9 and 15 days of N starvation and 1, 3 and 5 days after N resupply (R timepoints). Bars indicate means \pm SE (n = 3). The effects of genotype, days and the interaction between genotype and days were analyzed using two-way ANOVA with the Tukey HSD post hoc test (# P < 0.05; NS, not significant). Statistical comparisons among different time points were made using one-way ANOVA with the Tukey HSD post hoc test (* P < 0.05).



Figure S2 Overview of the polymorphic genes in the whole genome of D4-15 and D2-1 compared to that of Damor*bzh.* Venn diagram showed the number of polymorphic genes identified between D4-15 and Damor*bzh* (red), and between D2-1 and Damor*bzh* (blue). Chromosomal distributions (B) of the polymorphic genes were displayed. The density of polymorphic genes per 100 kb between D2-1 and Damor*bzh* was showed in blue color, and the density of polymorphic genes per 100 kb between D4-15 and Damor*bzh* was showed in red color.



KEGG pathways enrichment

Figure S3 Chromosomal distribution and annotation of the polymorphic genes in the whole genome of D4-15 and D2-1. A, chromosomal distribution of the unique and common polymorphic genes. B, the gene ontology (GO) and KEGG pathway analysis of the genes with sequence variation in the genomic level between D4-15 and D2-1.

Biological Process

Molecular Function

Α



Figure S4 Comparative gene ontology (GO) enrichment analysis of differentially expressed genes (DEGs) in D4-15 and D2-1. Cellular component, molecular function and biological process of the DEGs in shoots (A) and roots (B) of D4-15 and D2-1 were displayed based on the pairwise comparisons of $D2-1_{-N}/D2-1_{+N}$, $D4-15_{-N}/D4-15_{+N}$, respectively. Colored panels indicated the *P* values of each GO term.



Figure S5 KEGG pathway analysis of differentially expressed genes (DEGs) in D4-15 and D2-1. The top 20 enriched pathways of DEGs in D4-15 in comparison with D2-1 in shoots (A) and roots (B) under N starvation conditions were shown. The X-axis indicated the enrichment factor. The dot color and size indicated the Q value and gene number as shown on the right.



Figure S6 Venn diagrams of genetic variants and differentially expressed genes (DEGs) identified in shoots (A) and roots (B) of the two *Brassica napus* genotypes (D4-15 and D2-1) seven days after N starvation. The number of genes were showed. Different colors indicated the genetic variants identified in related to N efficiency in the genomic level (red), DEGs between D4-15 and D2-1 (blue), DEGs in D2-1 between -N (0 mM N) and +N (6 mM N) conditions (yellow), and DEGs in D4-15 between -N (0 mM N) and +N (6 mM N) treatments (green).



Figure S7 Transporter genes identified using RNA-seq in roots of D4-15 and D2-1 under +N (6 mM N) and -N (0 mM N) treatments. (A) Heat maps of all the transporter genes. (B) FPKM values of the four selected genes.