Transcriptome analysis of wheat introgression of GPC-QTL from wild emmer wheat under nitrogen starvation at seedling stage

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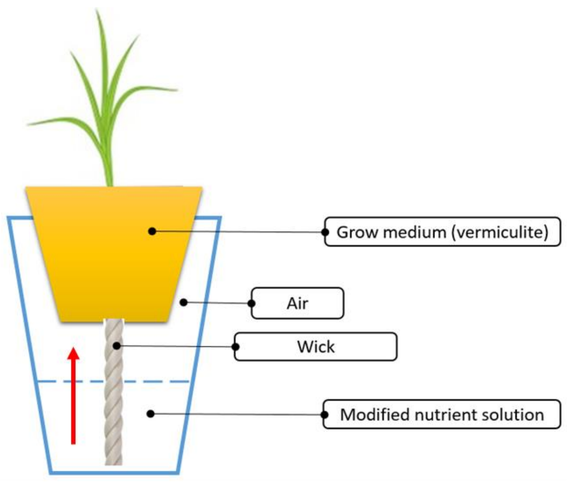
Increasing productivity and grain protein concentration (GPC) in wheat depend on nitrogen (N) availability. Globally, the application of N fertilizer tends to be excessive, and can negatively affect the environment since plants can utilize 30% to 40% of all N fertilizer applied. N use efficiency (NUE) in wheat is low, and it is a genetically controlled trait that varies greatly between wheat cultivars. Improving NUE and yield stability under low-N conditions is essential for the development of more sustainable agriculture.
For functional characterization of genes that may be associated high NUE we used RNAseq analysis of near-isogenic lines (NILs) under N stress (10% of normal application) vs. normal N, at the seedling stage. The use of NILs can reduce the noise of genetic background and help to understand the molecular mechanisms controlling NUE. We compared whole transcriptome response between a bread wheat cultivar Ruta, and its introgressions from wild emmer wheat: (a) IL99 (BC_3F_5) with the full GPC-QTL from Chr. 5B (*QGpc.huj.uh-5B.2*); (b) IL99-26 and IL99-46 (BC_4F_3), recombinants each containing partial segments of the QTL, generated by an additional backcross of IL99 with Ruta.



a

b

Figure 1. We used a semi-hydroponics growth system – The system contain two parts, inserted one on top of other, with a cotton wick connecting between parts. The nutrient solutions from lower container is transferred by capillary move through the cotton wick to upper part which is filled with vermiculite as growth support medium. Plants were grown for 14 days in a controlled growth chamber at 21-23°C. Growth parameters were measured, and leaves were harvested after 14 days for RNA extraction, transcriptome analysis was conducted at Novogene (Europe);
Nutrient solution – we used a modified Hogland solution, N was supplied as 1.0 mM KNO_3; 1.0 mM $(NH_4)_2SO_4$ was used for full N and 0.1 mM was used for the N stress treatment. The solutions were replaced every two days.

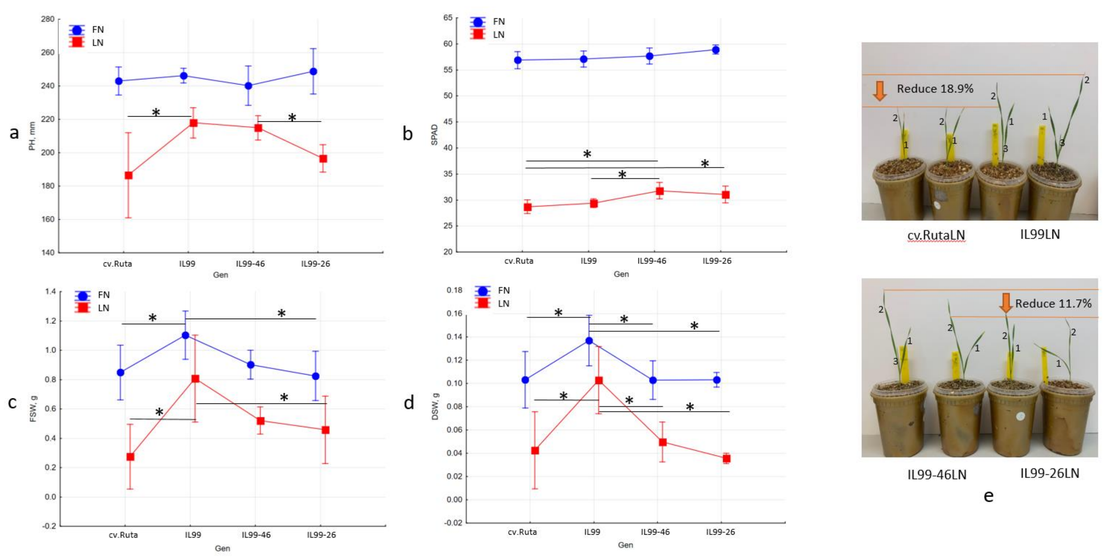


Figure 2. Growth parameters of tested wheat lines : Ruta, IL99, IL99-46, and IL99-26 under Full N (FN) and N stress (LN) conditions - (a) Plant height (PH; cm); (b) SPAD; (c) Fresh shoot weight (FSW; gr); (d) Dry shoot weight (DSW; gr); (e) – plant development at 14 days under N stress in IL99 vs. cv.Ruta, and IL99-46 vs. IL99-26.
All parameters were negatively affected by N stress; SFW and DSW were significant different between Ruta and IL99 both under FN and LN. PH of IL99 and IL99-46 were significantly higher (*p<0.05) by 18.9% and 11.7% on average than in Ruta and IL99-26, respectively. Development of the third leaf was faster in N stress in IL99 and IL99-46.

Transcriptome analysis

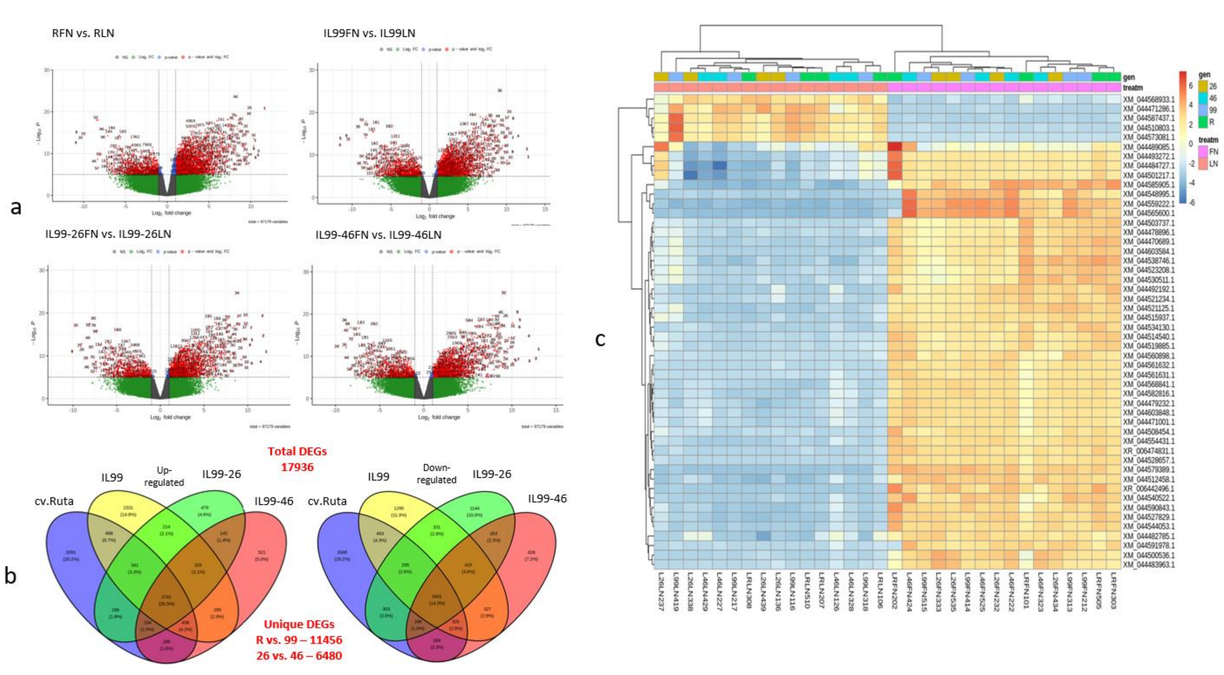


Figure 3. DEGs under N starvation. (a) – Volcano plots of significant differentially expressed genes (DEGs) between FN vs. LN. in Ruta vs. IL99, and IL99-46 vs. IL99-26 (log 2-fold change from P-log10 value). DEGs were considered as significant above the line representing the correct Bonferroni significance limit; (b) – Venn diagrams of up- and down DEGs obtained from pairwise comparisons; The overlapping portion of the Venn diagrams indicate the overall DEG between the compared combinations; (c) – Heat map of expression patterns of the highest 100 DEGs in the four tested genotypes cv.Ruta, IL99, IL99-46 and IL99-26.

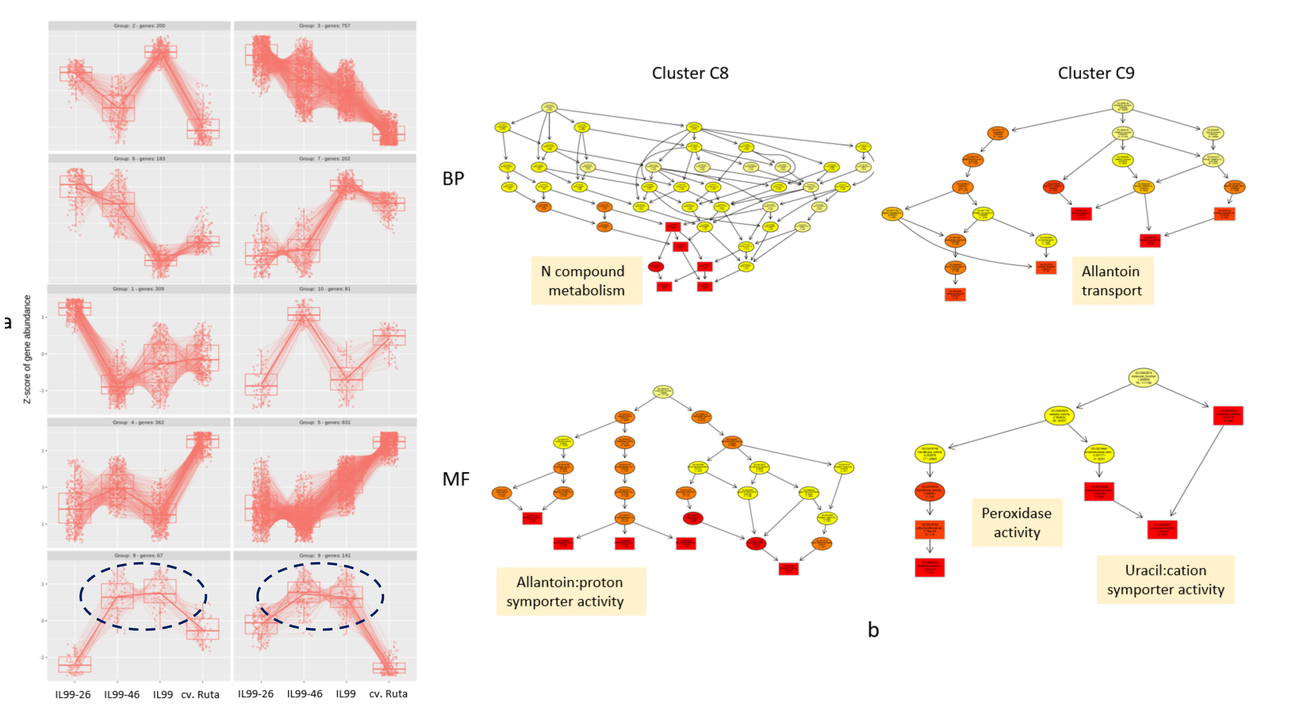


Figure 4. Patterns of regulation of common DEGs found by hierarchical clustering, and enrichment analysis of annotated DEGs included in clusters 8 and 9 (a) – Ruta, IL99, IL99-46, and IL99-26 are shown on the x-axis and the y-axis indicates the Z-score of genes abundance; (b) – Enrichment analysis of clusters C8 and C9 based on biological process (BP) and molecular function (MF).
Hierarchical clustering identified 3133 DEGs in 10 clusters, cluster 8 and 9 included a total of 208 DEGs (67 and 141 DEGs), sharing similar patterns of up-regulation in IL99 and IL99-46, and down-regulation in Ruta and IL99-26. These DEGs can be regarded as candidates for high NUE.

CONCLUSIONS

- **Transcriptome results show that N stress affected whole genome response in introgressions of 5B GPC-QTL from wild emmer wheat.**
- **Enrichment analysis shows that different pathways and candidate genes associated with NUE under N stress were activated in cv. Ruta and in the 3 introgressions from WEW.**
- **The full and partial introgression (IL99 and IL99-46), had an advantage over cv. Ruta and IL99-26 in growth parameters under N stress, at the seedling stage.**
- **IL99 and IL99-46 response to N stress included activation of N transporters ureide permease (UPs) that encode ureide permease typically found in legumes and are involved in the transport of the N compounds.**
- **IL99-26 and cv. Ruta activated (NRT, PODs, and ARf) that encode nitrate transporter, peroxidase activity, auxin response factor, and transcription factor (PHR).**
- **IL99 also showed up-regulation of transcription factors (ERF, bHLH, NLP, and MADS-box) on Chr. 1A, 1B, 1D, 2D, 3A, 4B, antioxidant enzyme catalase (CATs) on Chr. 6B.**

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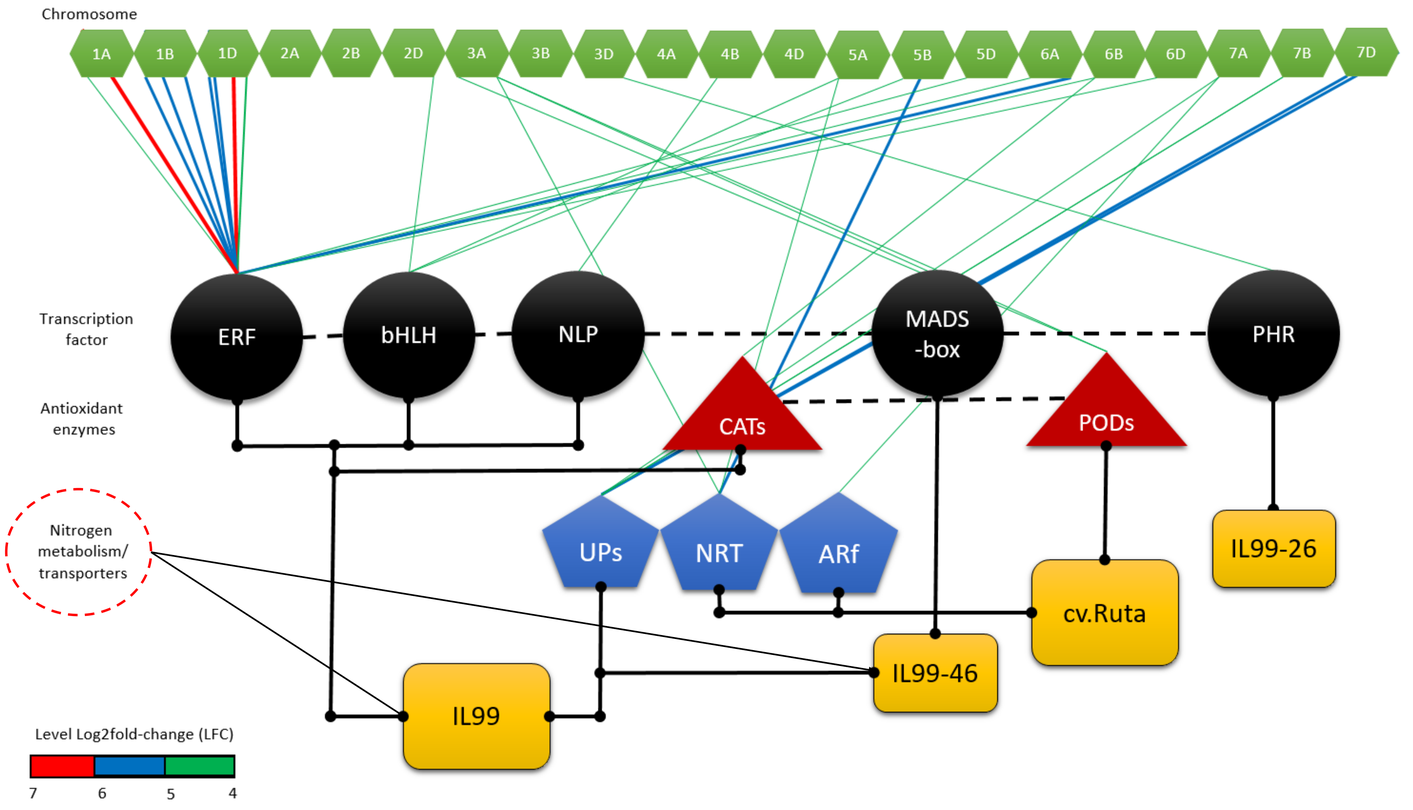

Figure 5. A model illustrated based on most activated DEGs under LN in Ruta vs. IL99 and two recombinant lines IL99-46 vs. IL99-26. The model demonstrates activation of transcription factors, antioxidant enzymes, genes involved in N transport and metabolism, and Auxin response factors; in 21 wheat chromosomes; identified in Ruta, IL99, IL99-46, and IL99-26. The red lines show LFC from 6.0 to 7.0, the blue lines LFC from 5.0 to 6.0, and the green lines LFC from 4.0 to 5.0. The black lines with nodes show associations of DEGs with the different four tested lines.