Supplementary Material

**RNA CATABOLITES CONTRIBUTE TO THE NITROGEN POOL AND SUPPORT GROWTH RECOVERY OF WHEAT**

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**Supplementary Table 1. Primer sequences and amplicon length for target bread wheat genes**

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| --- | --- | --- | --- |
| Gene name | Forward primer sequence (5’-3’) | Reverse primer sequence (5’-3’) | Amplicon length (bp) |
| *TaADK* | CAACTGCCTCTGGCTTCTGG | GTCCTCGATGCTCTTCCCTTG | 207 |
| *TaENT1* | TACATGCAGGCTGTCGTCGC | TACAGGATTGCGCTTTGCC | 122 |
| *TaENT3* | TACAAAGGCAGCTTTCGAGA | CGGTAGTACTTCACGATGGG | 143 |
| *TaRNS2* | TATCCTGGTCTCCAACGGTA | GGACAATCTCCAGTGTCGAA | 268 |
| *TaCyc* | CAAGCCGCTGCACTACAAGG | AGGGGACGGTGCAGATGAA | 227 |
| *TaEFA* | CAGATTGGCAACGGCTACG | CGGACAGCAAAACGACCAAG | 227 |

**Supplementary Table 2. Shoot fresh weight, total nitrogen (N) and N metabolites measured in experiment 1**



**Supplementary Table 3. Identification of target bread wheat genes and orthologs**

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| --- | --- | --- | --- | --- | --- |
| GOI | Arabidopsis# | *Brachypodium distachyon* (brachypodium) | Oryza sativa (rice)\* | Triticum aestivum (bread wheat)\*\* | References for orthologs |
| Adenosine kinas (*ADK*) | AT3g09820 (ADK1)  AT5g03300 (ADK2) | Bradi3g49230 | LOC\_Os02g41590 | TGACv1\_scaffold\_473321\_6AL  TGACv1\_scaffold\_499360\_6BL  TGACv1\_scaffold\_526952\_6DL | (Moffatt et al., 2000) |
| Equilibrative nucleoside transporter (*ENT1*) | At1g70330 | Bradi3g17700 | LOC\_Os08g10450 | TGACv1\_scaffold\_472259\_6AL  TGACv1\_scaffold\_502717\_6BL  TGACv1\_scaffold\_526370\_6DL | (Li and Wang, 2000;Hirose et al., 2005) |
| Equilibrative nucleoside transporter (*ENT3*) | At4g05120 | Bradi1g24960 | LOC\_Os07g37100 | TGACv1\_scaffold\_16965\_2AS  TGACv1\_scaffold\_3010\_2BS  TGACv1\_scaffold\_15731\_2DS | (Hirose et al., 2005) |
| Ribonuclease T2 (*RNS2*) | At2g39780 | Bradi2g57608 | LOC\_Os01g67180 | TGACv1\_scaffold\_196569\_3AL  TGACv1\_scaffold\_224141\_3B  TGACv1\_scaffold\_252500\_3DL | (Taylor et al., 1993) |

#NCBI; \*Phytozome; \*\*sequences identified in the present study

**Supplementary Table 4. Amino acid identity (% and grey shading) of the target bread wheat genes compared with the orthologous genes**

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| --- | --- | --- | --- | --- | --- | --- |
| **A** |  |  |  |  |  |  |
|  | **TaRNS2\_3AL** | **TaRNS2\_3B** | **TaRNS2\_3DL** | **BdRNS2** | **OsRNS2** | **AtRNS2** |
| **TaRNS2\_3AL** |  | 97.8 | 96.1 | 78.1 | 72.9 | 44.7 |
| **TaRNS2\_3B** | 97.8 |  | 95.3 | 78.8 | 73.9 | 45.1 |
| **TaRNS2\_3DL** | 96.1 | 95.3 |  | 77.7 | 71.8 | 44.3 |
| **BdRNS2** | 78.1 | 78.8 | 77.7 |  | 74.5 | 47.7 |
| **OsRNS2** | 72.9 | 73.9 | 71.8 | 74.5 |  | 44.8 |
| **AtRNS2** | 44.7 | 45.1 | 44.3 | 47.7 | 44.8 |  |

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| --- | --- | --- | --- | --- | --- | --- |
| **B** |  |  |  |  |  |  |
|  | **TaENT1\_6AL** | **TaENT1\_6BL** | **TaENT1\_6DL** | **BdENT1** | **OsENT1** | **AtENT1** |
| **TaENT1\_6AL** |  | 99.0 | 98.8 | 92.2 | 81.6 | 62.6 |
| **TaENT1\_6BL** | 99.0 |  | 98.8 | 92.6 | 82.3 | 63.1 |
| **TaENT1\_6DL** | 98.8 | 98.8 |  | 92.4 | 82.3 | 62.6 |
| **BdENT1** | 92.2 | 92.6 | 92.4 |  | 84.2 | 62.3 |
| **OsENT1** | 81.6 | 82.3 | 82.3 | 84.2 |  | 63.3 |
| **AtENT1** | 62.6 | 63.1 | 62.6 | 62.3 | 63.3 |  |

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| **C** |  |  |  |  |  |  |
|  | **TaENT3\_2AS** | **TaENT3\_2BS** | **TaENT\_2DS** | **BdENT3** | **OsENT3** | **AtENT3** |
| **TaENT3\_2AS** |  | 99.5 | 99.5 | 88.8 | 82.1 | 64.2 |
| **TaENT3\_2BS** | 99.5 |  | 100.0 | 89.0 | 81.9 | 64.2 |
| **TaENT\_2DS** | 99.5 | 100.0 |  | 89.0 | 81.9 | 64.2 |
| **BdENT3** | 88.8 | 89.0 | 89.0 |  | 83.5 | 64.2 |
| **OsENT3** | 82.1 | 81.9 | 81.9 | 83.5 |  | 65.9 |
| **AtENT3** | 64.2 | 64.2 | 64.2 | 64.2 | 65.9 |  |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **D** |  |  |  |  |  |  |  |
|  | **TaADK\_6AL** | **TaADK\_6BL** | **TaADK\_6DL** | **BdADK** | **OsADK** | **AtADK1** | **AtADK2** |
| **TaADK\_6AL** |  | 98.3 | 99.1 | 94.8 | 93.0 | 82.8 | 82.9 |
| **TaADK\_6BL** | 98.3 |  | 98.8 | 95.4 | 93.3 | 82.9 | 83.2 |
| **TaADK\_6DL** | 99.1 | 98.8 |  | 95.1 | 93.3 | 83.1 | 83.2 |
| **BdADK** | 94.8 | 95.4 | 95.1 |  | 94.5 | 82.6 | 83.5 |
| **OsADK** | 93.0 | 93.3 | 93.3 | 94.5 |  | 81.1 | 82.0 |
| **AtADK1** | 82.8 | 82.9 | 83.1 | 82.6 | 81.1 |  | 92.5 |
| **AtADK2** | 82.9 | 83.2 | 83.2 | 83.5 | 82.0 | 92.5 |  |

**Supplementary Figure 1.** **The effect of N starvation on tissue accumulation of ammonium and total basic amino acid.** Ammonium and 20 standard free amino acids were identified by HPLC and authenticated with standards of known concentrations. Data was normalised to an internal standard (norvaline) and to the sample dry weight (DW) and expressed as per 1 g DW-1. Ammonium and the sum of 20 standard amino acids is presented.

