**Supplementary Table S1.** Number of up- and down-regulated DEGs responsible for amino acid, carbon and nitrogen metabolism in the roots of contrasting nitrogen efficient cotton genotypes in response to N starvation and resupply.

|   | XLZ-30 CCI |      | CRI-69 |      |
|---|------------|------|--------|------|
| Amino acid metabolism                       | Up         | Down | Up     | Down |
| Alanine, aspartate and glutamate metabolism | 2          | 4    | 3      | 5    |
| Arginine biosynthesis                       | 2          | 10   | 2      | 10   |
| Cysteine and methionine metabolism          | 1          | 6    | 2      | 6    |
| Glutathione metabolism                      | 0          | 1    | 0      | 1    |
| Glycine, serine and threonine metabolism    | 0          | 5    | 2      | 5    |
| Histidine metabolism                        | 0          | 2    | 0      | 2    |
| Lysine degradation                          | 2          | 0    | 2      | 0    |
| Monobactam biosynthesis                     | 0          | 1    | 0      | 1    |
| Pyrimidine metabolism                       | 0          | 1    | 0      | 1    |
| Valine, leucine and isoleucine biosynthesis | 0          | 3    | 0      | 3    |
| Carbon metabolism                           |            |      |        |      |
| Amino sugar and nucleotide sugar metabolism | 1          | 1    | 1      | 0    |
| Carbon fixation                             | 0          | 1    | 2      | 0    |
| Glyoxylate and dicarboxylate metabolism     | 0          | 2    | 6      | 0    |
| Starch and sucrose metabolism               | 0          | 5    | 0      | 4    |
| Glycolysis / Gluconeogenesis                | 1          | 15   | 4      | 12   |
| Pentose phosphate pathway                   | 0          | 12   | 0      | 11   |
| Fructose and mannose metabolism             | 2          | 1    | 2      | 1    |
| Galactose metabolism                        | 2          | 1    | 2      | 1    |
| Fatty acid elongation                       | 1          | 0    | 1      | 0    |
| Pentose and glucuronate interconversions    | 1          | 4    | 2      | 4    |
| Phenylpropanoid biosynthesis                | 1          | 0    | 1      | 1    |
| Nitrogen metabolism                         |            |      |        |      |
| Nitrate reductase                           | 0          | 4    | 0      | 4    |
| Glutamine synthetase                        | 2          | 6    | 2      | 4    |
| Nitrite reductase                           | 0          | 2    | 0      | 2    |
| Glutamate synthase                          | 0          | 1    | 0      | 1    |
| Glutamate dehydrogenase                     | 0          | 1    | 0      | 1    |
| Asparagine synthetase                       | 0          | 3    | 0      | 4    |
| cytochrome b5                               | 1          | 2    | 1      | 1    |

**Supplementary Table S2.** Number of up- and down-regulated DEGs responsible for amino acid, carbon and nitrogen metabolism in the shoots of contrasting nitrogen efficient cotton genotypes in response to N starvation and resupply.

|   | ,  | (LZ-30 | С  | CRI-69 |
|---|----|--------|----|--------|
| Amino acid metabolism                               | Up | Down   | Up | Down   |
| Alanine, aspartate and glutamate metabolism         | 4  | 6      | 8  | 3      |
| Arginine and proline metabolism                     | 4  | 1      | 2  | 2      |
| Arginine biosynthesis                               | 5  | 2      | 6  | 2      |
| Cysteine and methionine metabolism                  | 2  | 7      | 8  | 1      |
| Glycine, serine and threonine metabolism            | 1  | 3      | 7  | 0      |
| Histidine metabolism                                | 0  | 0      | 1  | 0      |
| Lysine biosynthesis                                 | 0  | 1      | 2  | 0      |
| Lysine degradation                                  | 2  | 0      | 1  | 0      |
| Monobactam biosynthesis                             | 0  | 0      | 2  | 0      |
| Phenylalanine metabolism                            | 0  | 0      | 2  | 0      |
| Phenylalanine, tyrosine and tryptophan biosynthesis | 0  | 1      | 8  | 0      |
| Pyrimidine metabolism                               | 0  | 0      | 1  | 0      |
| Purine metabolism                                   | 1  | 0      | 0  | 1      |
| Synthesis and degradation of ketone bodies          | 0  | 0      | 2  | 0      |
| Taurine and hypotaurine metabolism                  | 0  | 1      | 1  | 0      |
| Valine, leucine and isoleucine biosynthesis         | 2  | 1      | 5  | 0      |
| Amino sugar and nucleotide sugar metabolism         | 0  | 0      | 0  | 1      |
| Carbon metabolism                                   |    |        |    |        |
| Carbon fixation                                     | 1  | 2      | 2  | 1      |
| Cutin, suberine and wax biosynthesis                | 0  | 2      | 1  | 0      |
| Cyanoamino acid metabolism                          | 0  | 1      | 1  | 2      |
| Fructose and mannose metabolism                     | 0  | 2      | 2  | 1      |
| Galactose metabolism                                | 3  | 2      | 5  | 2      |
| Glycolysis / Gluconeogenesis                        | 5  | 5      | 12 | 5      |
| N-Glycan biosynthesis                               | 0  | 0      | 1  | 0      |
| Oxidative phosphorylation                           | 0  | 0      | 0  | 1      |
| Pentose and glucuronate interconversions            | 1  | 5      | 4  | 12     |
| Pentose phosphate pathway                           | 0  | 2      | 4  | 0      |
| Phenylpropanoid biosynthesis                        | 1  | 2      | 3  | 1      |
| Starch and sucrose metabolism                       | 8  | 11     | 19 | 9      |
| Nitrogen metabolism                                 |    |        |    |        |
| Nitrate reductase                                   | 0  | 4      | 2  | 1      |
| Glutamine synthetase                                | 2  | 1      | 2  | 1      |
| Nitrite reductase                                   |    | 2      | 0  | 0      |
| Glutamate synthase                                  | 0  | 2      | 0  | 0      |
| Glutamate dehydrogenase                             | 0  | 0      | 1  | 0      |
| Asparagine synthetase                               | 2  | 2      | 3  | 0      |
| cytochrome b5                                       | 0  | 0      | 2  | 1      |

**Supplementary Table S3.** Hub genes up and down-regulated in the root of contrasting N-efficient cotton genotypes in response to N starvation and resupply.

|            |                 |               | <u>-</u>      |
|------------|-----------------|---------------|---------------|
| Category   | Gene ID         | CCRI-69       | XLZ-30        |
| Amino acid | Ghir_D13G024390 | Downregulated | Downregulated |
| Amino acid | Ghir_A13G023660 | Downregulated | Downregulated |
| Amino acid | Ghir_A09G009700 | Downregulated | Downregulated |
| Amino acid | Ghir_A13G023660 | Downregulated | Downregulated |
| Amino acid | Ghir_D02G003120 | Downregulated | Downregulated |
| Amino acid | Ghir_A06G006700 | Downregulated | Downregulated |
| Amino acid | Ghir_A13G005930 | Upregulated   | Upregulated   |
| Amino acid | Ghir_D09G020360 | Downregulated | Downregulated |
| Amino acid | Ghir_A04G016360 | Downregulated | Downregulated |
| Amino acid | Ghir_D05G014390 | Downregulated | Downregulated |
| Amino acid | Ghir_A02G002720 | Downregulated | Downregulated |
| Amino acid | Ghir_A13G005930 | Upregulated   | Upregulated   |
| Amino acid | Ghir_D09G020360 | Downregulated | Downregulated |
| Amino acid | Ghir_A05G038060 | Downregulated | Downregulated |
| Amino acid | Ghir_D04G004990 | Downregulated | Downregulated |
| Amino acid | Ghir_D09G021650 | Downregulated | Downregulated |
| Amino acid | Ghir_A07G022270 | Downregulated | Downregulated |
| Amino acid | Ghir_D07G022350 | Upregulated   | Upregulated   |
| Amino acid | Ghir_D08G025080 | Downregulated | Downregulated |
| Amino acid | Ghir_A08G024180 | Downregulated | Downregulated |
| Amino acid | Ghir_D06G014880 | Downregulated | Downregulated |
| Amino acid | Ghir_A10G012370 | Downregulated | Downregulated |
| Amino acid | Ghir_A06G008720 | Upregulated   | Upregulated   |
| Amino acid | Ghir_D02G003060 | Downregulated | Downregulated |
| Amino acid | Ghir_D06G008920 | Upregulated   | Upregulated   |
| Nitrogen   | Ghir_D12G026390 | Downregulated | Downregulated |
| Nitrogen   | Ghir_D02G003120 | Downregulated | Downregulated |
| Nitrogen   | Ghir_A02G002720 | Downregulated | Downregulated |
| Nitrogen   | Ghir_A13G005930 | Upregulated   | Upregulated   |
| Nitrogen   | Ghir_D09G020360 | Downregulated | Downregulated |
| Nitrogen   | Ghir_A13G023660 | Downregulated | Downregulated |
| Nitrogen   | Ghir_A09G009700 | Downregulated | Downregulated |
| Nitrogen   | Ghir_D13G024390 | Downregulated | Downregulated |
| Carbon     | Ghir_D11G017390 | Upregulated   | Upregulated   |
| Carbon     | Ghir_A11G017320 | Upregulated   | Upregulated   |
| Carbon     | Ghir_D04G005980 | Upregulated   | Upregulated   |
| Carbon     | Ghir_D06G004020 | Downregulated | Downregulated |
| Carbon     | Ghir_A06G003910 | Downregulated | Downregulated |
| Carbon     | Ghir_A02G009250 | Downregulated | Downregulated |
| Carbon     | Ghir_D02G015800 | Downregulated | Downregulated |
|            |                 |               |               |

Carbon Ghir\_A12G024710 Downregulated Downregulated

**Supplementary Table S4.** Hub genes up and down-regulated in the shoot of contrasting N-efficient cotton genotypes in response to N starvation and resupply.

| Category   | Gene ID         | CCRI-69       | XLZ-30        |
|------------|-----------------|---------------|---------------|
| Amino acid | Ghir_A09G021540 | Upregulated   | Downregulated |
| Amino acid | Ghir_A13G017290 | Upregulated   | Downregulated |
| Amino acid | Ghir_D13G017980 | Upregulated   | Downregulated |
| Amino acid | Ghir_D12G002180 | Upregulated   | Downregulated |
| Amino acid | Ghir_A13G023000 | Downregulated | Upregulated   |
| Amino acid | Ghir_A06G009280 | Upregulated   | Downregulated |
| Amino acid | Ghir_D06G009760 | Upregulated   | Downregulated |
| Amino acid | Ghir_A06G006700 | Upregulated   | Downregulated |
| Amino acid | Ghir_A12G011030 | Upregulated   | Downregulated |
| Amino acid | Ghir_D05G003480 | Upregulated   | Downregulated |
| Amino acid | Ghir_A12G028500 | Upregulated   | Downregulated |
| Amino acid | Ghir_A13G014560 | Upregulated   | Downregulated |
| Amino acid | Ghir_A09G009680 | Upregulated   | No change     |
| Amino acid | Ghir_D09G020360 | Downregulated | Upregulated   |
| Amino acid | Ghir_A07G018740 | Upregulated   | Downregulated |
| Amino acid | Ghir_D12G028670 | Upregulated   | Downregulated |
| Amino acid | Ghir_A07G018790 | Upregulated   | Downregulated |
| Amino acid | Ghir_A07G012910 | Upregulated   | Downregulated |
| Amino acid | Ghir_A06G014110 | Upregulated   | Downregulated |
| Amino acid | Ghir_A01G011530 | Upregulated   | Downregulated |
| Amino acid | Ghir_D07G017980 | Upregulated   | Upregulated   |
| Amino acid | Ghir_A09G020960 | Downregulated | Upregulated   |
| Amino acid | Ghir_A12G016440 | Downregulated | No change     |
| Amino acid | Ghir_D13G023640 | Downregulated | Upregulated   |
| Amino acid | Ghir_D06G007420 | Upregulated   | Downregulated |
| Amino acid | Ghir_D10G022890 | Upregulated   | Downregulated |
| Amino acid | Ghir_D01G022210 | Upregulated   | Upregulated   |
| Amino acid | Ghir_D04G012180 | Upregulated   | No change     |
| Amino acid | Ghir_A05G022930 | Upregulated   | Downregulated |
| Amino acid | Ghir_A07G002780 | Upregulated   | No change     |
| Amino acid | Ghir_D07G005800 | Upregulated   | Downregulated |
| Amino acid | Ghir_D12G011120 | Upregulated   | Downregulated |
| Amino acid | Ghir_A07G017570 | Upregulated   | Downregulated |
| Amino acid | Ghir_A04G014400 | Upregulated   | Downregulated |
| Amino acid | Ghir_D05G006020 | Upregulated   | No change     |
| Amino acid | Ghir_D13G024390 | Upregulated   | Downregulated |
| Amino acid | Ghir_A03G010810 | Upregulated   | Downregulated |
| Amino acid | Ghir_A09G022390 | Upregulated   | Downregulated |
|            |                 | 4             |               |

| Amino acid | Ghir_D09G021650 | Upregulated   | Downregulated |
|------------|-----------------|---------------|---------------|
| carbon     | Ghir_D10G008680 | Downregulated | Upregulated   |
| carbon     | Ghir_A01G011760 | Upregulated   | Downregulated |
| carbon     | Ghir_A08G009680 | Upregulated   | Downregulated |
| carbon     | Ghir_D04G005320 | Downregulated | No change     |
| carbon     | Ghir_A03G008550 | Downregulated | Downregulated |
| carbon     | Ghir_D13G008490 | Downregulated | No change     |
| carbon     | Ghir_D01G013270 | Upregulated   | Downregulated |
| carbon     | Ghir_D03G011050 | Upregulated   | Downregulated |
| carbon     | Ghir_A01G020200 | Upregulated   | Downregulated |
| carbon     | Ghir_D09G014570 | Upregulated   | Downregulated |
| carbon     | Ghir_A02G011710 | Downregulated | Upregulated   |
| carbon     | Ghir_A12G023160 | Downregulated | Upregulated   |
| carbon     | Ghir_D11G006370 | Upregulated   | Downregulated |
| carbon     | Ghir_D11G017960 | Upregulated   | Downregulated |
| carbon     | Ghir_A13G023760 | Downregulated | Upregulated   |
| carbon     | Ghir_A05G013940 | No change     | No change     |
| carbon     | Ghir_A13G020910 | Upregulated   | Downregulated |
| carbon     | Ghir_D13G001770 | Upregulated   | Downregulated |
| carbon     | Ghir_A11G017920 | Upregulated   | Downregulated |
| carbon     | Ghir_D03G006990 | Downregulated | Upregulated   |
| carbon     | Ghir_A03G013490 | Downregulated | No change     |
| carbon     | Ghir_D12G020110 | Downregulated | Upregulated   |
| carbon     | Ghir_A12G019870 | Downregulated | No change     |
| carbon     | Ghir_A07G019010 | Upregulated   | No change     |
| carbon     | Ghir_D13G021730 | Upregulated   | Downregulated |
| carbon     | Ghir_D07G019350 | Upregulated   | Downregulated |
| carbon     | Ghir_A07G001840 | Downregulated | No change     |
| carbon     | Ghir_A11G022120 | Upregulated   | No change     |
| carbon     | Ghir_A11G015470 | Downregulated | Upregulated   |
| carbon     | Ghir_D09G001040 | Upregulated   | Downregulated |
| Nitrogen   | Ghir_D09G020360 | Downregulated | Upregulated   |
| Nitrogen   | Ghir_A09G009680 | Upregulated   | No change     |
| Nitrogen   | Ghir_A13G023660 | Upregulated   | Downregulated |
| Nitrogen   | Ghir_A07G018790 | Upregulated   | Downregulated |
| Nitrogen   | Ghir_A09G020960 | Downregulated | Upregulated   |
| Nitrogen   | Ghir_D13G024390 | Upregulated   | Downregulated |

## **Supplementary Table S5.** Gene specific primers used for RT-qPCR.

| Gene ID         | Gene name | Specific primer        |
|-----------------|-----------|------------------------|
| Ghir_A01G018110 | NR-1F     | TGGTATGGTATTCGAGCACCCA |
| Ghir_A01G018110 | NR-1R     | GTCGTAGATATGACCATGGAC  |
| Ghir_A02G007870 | NR-2F     | TCGAAGGAGCTGAGCATTTG   |

| Ghir A02G007870 | NR-2R      | TTCGTTGTAACAATGATTCGT    |
|-----------------|------------|--------------------------|
| Ghir D02G003120 | GS-1F      | GTGGAGTTCTCTGGTACTGA     |
| Ghir D02G003120 | GS-1R      | AACGTATTCAGCGATGATCT     |
| Ghir_A04G011580 | GS-2F      | ATCGATGAGAAATGATGGTGG    |
| Ghir_A04G011580 | GS-2R      | GGCAGTCTCGTGAAGACCT      |
| Ghir_A13G003840 | NiR-1F     | GGATTGAAGTGTTCAGATCAGAG  |
| Ghir_A13G003840 | NiR-1R     | GTTCGTCCATGTCGTCAGCTTGG  |
| Ghir_D13G004040 | NiR-2F     | GTATTGAAGTGTTCAGATCAGAAG |
| Ghir_D13G004040 | NiR-2R     | GTTCGTCCATGTCGTCGGCTTG   |
| Ghir_A12G026360 | GOGAT-1F   | ATGAGCGTTATGGGTTGTAA     |
| Ghir_A12G026360 | GOGAT-1R   | ACATCACCAACGAGATGCAA     |
| Ghir_D06G018750 | GOGAT-2F   | GTGAAGCCCAGTAACACGGA     |
| Ghir_D06G018750 | GOGAT-2R   | CTGAGCCATTTGATAGCTGATG   |
| Ghir_A03G007160 | GDH-1F     | TCAAGGTTTCATGTGGGATGAAG  |
| Ghir_A03G007160 | GDH-1R     | GTTCACCCCAAGTGTAAATGCGC  |
| Ghir_D10G006710 | GDH-2F     | TCTTGCAGAGTTGAATAAAGA    |
| Ghir_D10G006710 | GDH-2R     | CCATCCTCGTCAACCAAATACC   |
| Ghir_D09G009410 | ASN-1F     | GACCGAAAGACTGATGACTGA    |
| Ghir_D09G009410 | ASN-1R     | TAGTCTGCAACCTCCCTTGC     |
| Ghir_D13G024390 | ASN-2F     | TTGTTATTTGGCTCATCAACGC   |
| Ghir_D13G024390 | ASN-2R     | GTACTCCTCATACAAATGTGC    |
| Ghir_A02G008900 | Cytb5-1F   | GTGACAAAGTTCATGGAAGA     |
| Ghir_A02G008900 | Cytb5-1R   | GGAGCAAATTCTGAGCTGAC     |
| Ghir_D02G009090 | Cytb5-2F   | GAACGTAACAAAGTTCCTGGAA   |
| Ghir_D02G009090 | Cytb5-2R   | GAGCAAATTCTGAGCCGACTT    |
| AT5G09810       | Gh H 3.3-F | CCTTGTGGGTCTTTTTGAA      |
| AT5G09810       | Gh H 3.3-R | AACTGGATGTCCTTGGGC       |