Supplemental Information

The Supplemental Material for this article can be found online at:

**Supplemental Table S1****.** Predicted miR159 target genes in soybean

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **miRNA** | **Target gene** | | | |
| miR159a | *Glyma.06g134200* | *Glyma.05g169200* | *Glyma.18g014400* | *Glyma.09g053400* |
|  | *Glyma.02g205700* | *Glyma.17g094800* | *Glyma.03g045700* | *Glyma.14g220600* |
|  | *Glyma.17g259500* | *Glyma.15g231300* | *Glyma.15g231100* | *Glyma.15g230900* |
|  | *Glyma.15g233100* | *Glyma.20g248200* | *Glyma.18g265900* | *Glyma.15g233400* |
| miR159b | *Glyma.09g202900* | *Glyma.18g062700* | *Glyma.02g220200* | *Glyma.14g187600* |
|  | *Glyma.11g200800* |  |  |  |
| miR159c | *Glyma.20g047600* | *Glyma.13g073400* | *Glyma.15g225300* | *Glyma.13g187500* |
|  | *Glyma.19g206100* | *Glyma.06g312900* | *Glyma.04g125700* | *Glyma.16g029800* |
|  | *Glyma.19g121000* | *Glyma.16g144200* | *Glyma.13g031500* | *Glyma.14g154200* |
|  | *Glyma.03g099000* | *Glyma.03g005000* | *Glyma.04g161400* | *Glyma.05g022800* |
|  | *Glyma.06g204300* | *Glyma.06g096000* | *Glyma.07g061200* | *Glyma.03g187400* |
|  | *Glyma.08g032100* | *Glyma.15g181500* | *Glyma.16g145300* | *Glyma.07g061100* |
| miR159d | *Glyma.02g086800* | *Glyma.16g214900* | *Glyma.U035700* | *Glyma.16g214600* |
|  | *Glyma.16g215000* |  |  |  |
| miR159e-3p | *Glyma.12g032600* | *Glyma.06g312900* | *Glyma.04g125700* | *Glyma.03g115400* |
|  | *Glyma.04g100900* | *Glyma.20g047600* | *Glyma.13g073400* | *Glyma.15g079800* |
|  | *Glyma.15g225300* | *Glyma.13g187500* | *Glyma.13g271900* | *Glyma.12g228100* |
|  | *Glyma.14g107600* | *Glyma.12g217800* | *Glyma.13g282800* | *Glyma.20g211100* |
|  | *Glyma.02g061200* | *Glyma.20g149200* | *Glyma.18g277700* | *Glyma.08g240200* |
|  | *Glyma.03g222300* | *Glyma.19g219500* | *Glyma.11g107600* | *Glyma.14g125900* |
|  | *Glyma.14g125600* | *Glyma.15g271900* | *Glyma.08g030100* | *Glyma.08g152500* |
|  | *Glyma.20g211800* |  |  |  |
| miR159e-5p | *Glyma.06g134200* | *Glyma.18g014400* | *Glyma.09g053400* | *Glyma.05g169200* |
|  | *Glyma.03g045700* | *Glyma.14g220600* | *Glyma.17g259500* | *Glyma.15g231300* |
|  | *Glyma.15g231100* | *Glyma.15g230900* | *Glyma.15g233400* | *Glyma.18g265900* |
|  | *Glyma.20g248200* | *Glyma.02g205700* | *Glyma.17g094800* |  |
| miR159f | *Glyma.09g202900* | *Glyma.18g062700* | *Glyma.02g220200* | *Glyma.14g187600* |
|  | *Glyma.11g200800* |  |  |  |

Note: Target genes were predicted using the psRNA Target website (http://psrna target.org). Genes with a score of ≤3.0 were selected.

A picture containing text, diagram, screenshot, line

Description automatically generated

**Supplemental Figure S1.** Effects of *MIR159e* over expression on growth and development. **WT**, wild type (YC03-3); ***OE-2***, *miR159eOE-2*; ***OE-4***, *miR159eOE-4*; **HP**, plants treated with a high phosphorus concentration of 250 μM; **LP**, plants treated with a low-P concentration of 5 μM; treatment time was 40 days. Results are means ± SE from three independent experiments. Student’s t-test was used to compare the differences between Col-0 and transgenic *MIR159eOE* plants(\*P < 0.05).

A picture containing text, diagram, screenshot, line

Description automatically generated

**Supplemental Figure S2.** Total phosphorus, nitrogen, and soluble phosphorus contents in soybean overexpressing *MIR159e* under low-nitrogen conditions. **WT**, wild type (YC03-3); **HPLN**, high phosphorus and low nitrogen; treatment was performed for 40 days. Results are means ± SE from three independent experiments. Student’s t-test was used to compare the differences between Col-0 and the transgenic line *MIR159eOE* in the same plant part sampled at the same time point (\*P < 0.05).

**Supplemental Table S2.** List of primer pairs used in this study.

| **Name of primer** | **Primer sequence（5’-3’）** | **Application** |
| --- | --- | --- |
| Gma-miR159a.qF | AATTAAAGGGGATTATGAAGTGGA | qRT-PCR |
| Gma-miR159a.qR | AGAAAAGAAGAGAAGGGTGTAGAG | qRT-PCR |
| Gma-miR159b.qF | TGCTAGTTCATGGATACCTCTG | qRT-PCR |
| Gma-miR159b.qR | TCCCTTCACTCCAATACCAAA | qRT-PCR |
| Gma-miR159c.qF | ACCCAAGTTGGAGCTCTCT | qRT-PCR |
| Gma-miR159c.qR | AAGGCCTAATTCGGAGCTC | qRT-PCR |
| Gma-miR159d.qF | GGGTGAATTGAGCTGCTTAG | qRT-PCR |
| Gma-miR159d.qR | AGGCAAATGAAGCTCCCC | qRT-PCR |
| Gma-miR159e.qF | CAAAGGGGGTTATGGAGTGG | qRT-PCR |
| Gma-miR159e.qR | ACAAAAGGGGAGAAGGGTG | qRT-PCR |
| Gma-miR159f.qR | ACCCTCTGGAGCTCCCTT | qRT-PCR |
| GmEF-1α.qF | TGCAAAGGAGGCTGCTAACT | qRT-PCR |
| GmEF-1α.qR | CAGCATCACCGTTCTTCAAA | qRT-PCR |
| pro-miR159e.qF | AAgaattcAAGCCGGTTCCCGAGACATA | Promoter activity analysis |
| pro-miR159e.qR | AAggatccTTTGTGATTCCCTTTGACCT | Promoter activity analysis |
| Gma-miR159e.oxF | AAcccgggTAGCAAGGGTTTAGGTGGTG | Overexpression |
| Gma-miR159e.oxR | AAtctagaAGAGCAAGAACGAGATTATGG | Overexpression |

Note: Lowercase letters indicate different *MIR159* gene family members.