

Exogenous GA₃ Enhances Nitrogen Uptake and Metabolism under Low Nitrate Conditions in 'Duli' (*Pyrus betulifolia* Bunge) Seedlings

Weilong Zhang, Xiaohua Cheng, Zhaotian Jing, Ying Cao, Shuai Yuan, Haixia Zhang and Yuxing Zhang *

College of Horticulture, Hebei Agricultural University, Baoding 071001, China; 18322711826@163.com (W.Z.); chengxiaoahua2016@126.com (X.C.); 13409298238@163.com (S.Y.); zhx2323a@163.com (H.Z.)

* Correspondence: zhyx@hebau.edu.cn

Supplementary Data

Table S1 Effects of GA₃ and PAC on the growth, chlorophyll content and root length of 'Duli'. Data are presented as means \pm SD (n = 3). Values not followed by the same letter denote significant differences based on Tukey's multiple-range tests (P < 0.05).

| Concentration (mM) | Plant length | Leaf number | Leaf area per plant | Chlorophyll content | Main root length |
|------------------------|------------------|-------------------|---------------------|---------------------|--------------------|
| CK | 8.19 \pm 0.52c | 7.40 \pm 0.52a | 2.38 \pm 0.08b | 2.21 \pm 0.04e | 17.85 \pm 0.38cd |
| 0 | 7.68 \pm 0.33d | 6.60 \pm 0.52bc | 1.910 \pm 0.07e | 1.97 \pm 0.07g | 18.21 \pm 0.45cd |
| GA ₃ (0.01) | 7.82 \pm 0.34d | 6.40 \pm 0.52bc | 1.96 \pm 0.05e | 1.94 \pm 0.02g | 18.38 \pm 0.34bc |
| GA ₃ (0.05) | 8.28 \pm 0.25c | 6.80 \pm 0.42b | 2.16 \pm 0.09d | 1.98 \pm 0.05fg | 18.91 \pm 0.49b |
| GA ₃ (0.1) | 9.44 \pm 0.34a | 7.40 \pm 0.84a | 2.61 \pm 0.13a | 2.04 \pm 0.05fg | 19.82 \pm 0.70a |
| GA ₃ (0.15) | 8.54 \pm 0.30b | 7.60 \pm 0.52a | 2.27 \pm 0.13c | 2.10 \pm 0.05ef | 18.92 \pm 0.53b |
| PAC (0.005) | 7.70 \pm 0.09d | 6.20 \pm 0.42cd | 1.90 \pm 0.06e | 2.73 \pm 0.09c | 18.11 \pm 1.08cd |
| PAC (0.01) | 7.31 \pm 0.10e | 5.80 \pm 0.42de | 1.55 \pm 0.04f | 3.17 \pm 0.05a | 17.66 \pm 0.85de |
| PAC (0.02) | 7.06 \pm 0.14e | 5.60 \pm 0.52e | 1.30 \pm 0.08 | 3.00 \pm 0.09b | 17.16 \pm 0.40e |
| PAC (0.04) | 6.73 \pm 0.26f | 5.40 \pm 0.52e | 0.94 \pm 0.07h | 2.92 \pm 0.06b | 14.76 \pm 0.41f |
| PAC (0.1) | 6.49 \pm 0.22f | 5.30 \pm 0.48e | 0.59 \pm 0.05i | 2.48 \pm 0.10d | 14.64 \pm 0.27f |

Table S2 The membership function values and comprehensive evaluation of different GA₃/PAC concentration.

| Concentration (mM) | Plant length | Leaf number | Leaf area per plant | | | Chlorophyll content | Main root length | Comprehensive assessment | Ranking |
|------------------------|--------------|-------------|---------------------|-----------|-------------|---------------------|------------------|--------------------------|---------|
| | | | area | per plant | chlorophyll | | | | |
| CK | 0.58 | 0.91 | 0.89 | 0.22 | 0.62 | 0.64 | 0.62 | 0.64 | 3 |
| 0 | 0.45 | 0.48 | 0.74 | 0.00 | 0.72 | 0.48 | 0.58 | 0.48 | 8 |
| GA ₃ (0.01) | 0.45 | 0.48 | 0.74 | 0.00 | 0.72 | 0.48 | 0.58 | 0.48 | 8 |
| GA ₃ (0.05) | 0.61 | 0.65 | 0.83 | 0.03 | 0.82 | 0.59 | 0.82 | 0.59 | 6 |
| GA ₃ (0.1) | 1.00 | 0.91 | 1.00 | 0.08 | 1.00 | 0.80 | 1.00 | 0.80 | 1 |
| GA ₃ (0.15) | 0.69 | 1.00 | 0.85 | 0.13 | 0.83 | 0.70 | 0.83 | 0.70 | 2 |
| PAC (0.005) | 0.59 | 0.39 | 0.71 | 0.64 | 0.67 | 0.60 | 0.67 | 0.60 | 5 |
| PAC (0.01) | 0.72 | 0.22 | 0.57 | 1.00 | 0.58 | 0.62 | 0.58 | 0.62 | 4 |
| PAC (0.02) | 0.81 | 0.13 | 0.47 | 0.86 | 0.49 | 0.55 | 0.49 | 0.55 | 7 |
| PAC (0.04) | 0.92 | 0.04 | 0.35 | 0.80 | 0.02 | 0.43 | 0.02 | 0.43 | 10 |

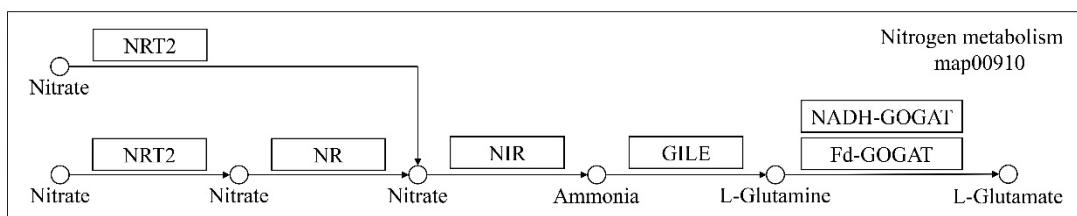


Figure S1. Map of key gene pathways involved in N uptake and metabolism.

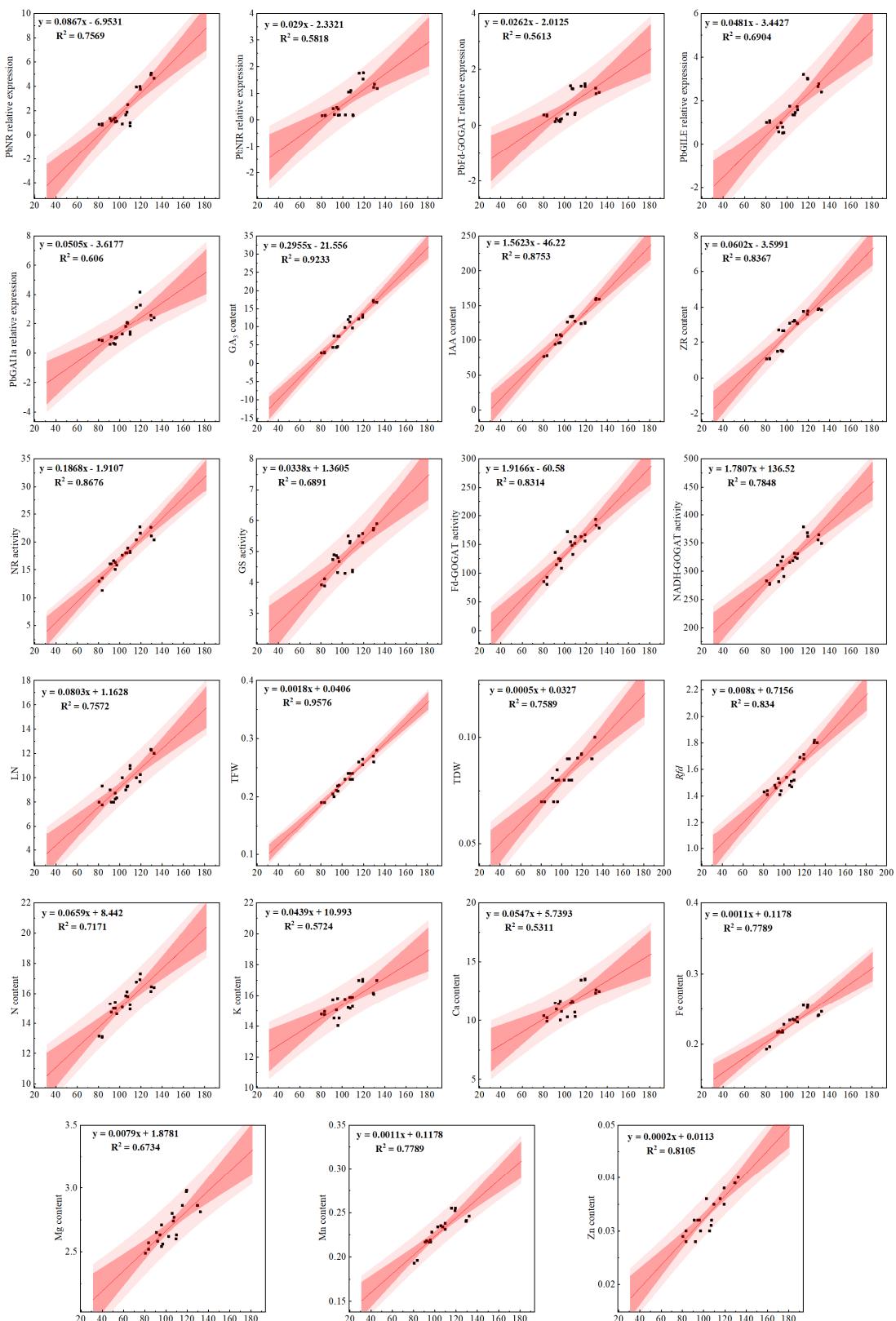


Figure S2. Relationships between the aboveground index and plant length (Pearson's correlation analysis). Only the aboveground indices that are significantly correlated with plant length are shown in the figure.

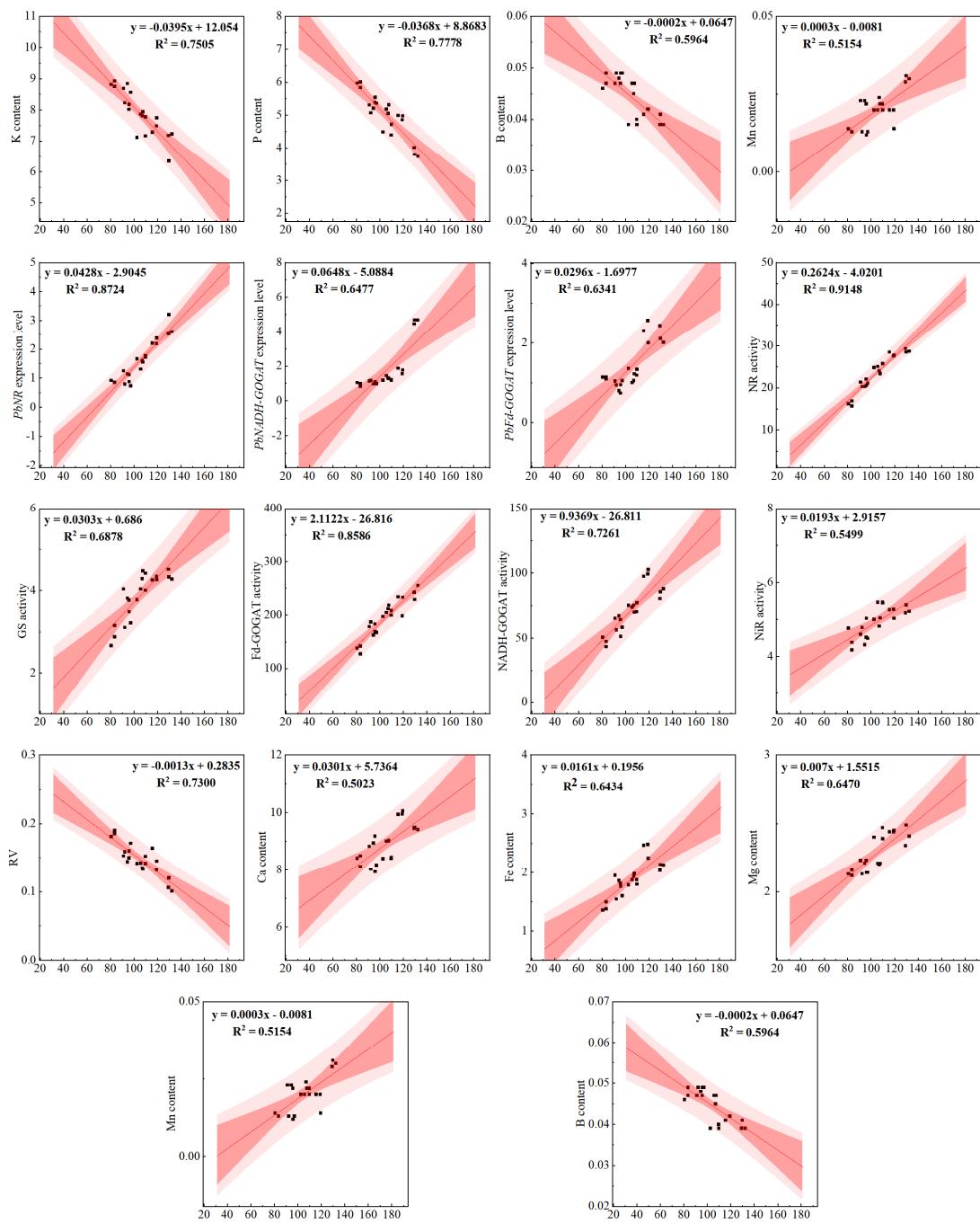


Figure S3. Relationships between the root index and plant length (Pearson's correlation analysis). Only the root indices that are significantly correlated with plant length are shown in the figure.

Table S3 Comprehensive score of exogenous GA₃ in 'Duli' under NO₃⁻ deficiency. SCK, 0.5 mM NO₃⁻ solution; SGT, 0.5 mM NO₃⁻ solution with 0.1 mM GA₃; SPT, 0.5 mM NO₃⁻ solution with 0.01 mM PAC; DCK, 8 mM NO₃⁻ solution; DGT, 8 mM NO₃⁻ solution with 0.1 mM GA₃; DPT, 8 mM NO₃⁻ solution with 0.01 mM PAC; CK, 16 mM NO₃⁻ solution.

| Treatment | PC1 | PC2 | PC3 | PC4 | PC5 | Comprehensive score | Ranking |
|-----------|-------|-------|-------|-------|-------|---------------------|---------|
| SCK | -0.19 | -0.34 | -0.07 | 0.02 | 0.02 | -0.55 | 6 |
| SGT | 0.10 | -0.35 | 0.04 | -0.02 | -0.04 | -0.27 | 5 |
| SPT | -0.98 | 0.04 | 0.01 | -0.06 | 0.02 | -0.96 | 7 |
| DCK | 0.31 | 0.05 | -0.03 | 0.08 | 0.01 | 0.43 | 3 |
| DGT | 0.50 | 0.09 | 0.17 | -0.01 | 0.01 | 0.77 | 2 |
| DPT | -0.57 | 0.34 | -0.02 | 0.04 | -0.03 | -0.25 | 4 |
| CK | 0.81 | 0.17 | -0.11 | -0.06 | 0.00 | 0.82 | 1 |

Table S4 The concentrations of nutrition solution and allogenic material of each treatment. SCK, 0.5 mM NO₃⁻ solution; SGT, 0.5 mM NO₃⁻ solution with 0.1 mM GA₃; SPT, 0.5 mM NO₃⁻ solution with 0.01 mM PAC; DCK, 8 mM NO₃⁻ solution; DGT, 8 mM NO₃⁻ solution with 0.1 mM GA₃; DPT, 8 mM NO₃⁻ solution with 0.01 mM PAC; CK, 16 mM NO₃⁻ solution.

| Composition | | Purity (≥%) | SCK | SGT | SPT | DCK | DGT | DPT | CK |
|---------------------------|--------------------------------------|----------------|-------|-------|-------|-------|-------|-------|-------|
| Macroelement (mM) | KH ₂ PO ₄ | 99.50 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | MgSO ₄ ·7H ₂ O | 99.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| | KCl | 99.50 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| | Ca(NO ₃) ₂ | 98.50 | 0.50 | 0.50 | 0.50 | 8.00 | 8.00 | 8.00 | 16.00 |
| Microelement (mg/L) | CaCl ₂ | 96.00 | 7.50 | 7.50 | 7.50 | 8.00 | 8.00 | 8.00 | 0.00 |
| | KI | 99.00 | 0.83 | 0.83 | 0.83 | 0.83 | 0.83 | 0.83 | 0.83 |
| | H ₃ BO ₃ | 99.50 | 6.20 | 6.20 | 6.20 | 6.20 | 6.20 | 6.20 | 6.20 |
| | ZnSO ₄ | 99.50 | 8.60 | 8.60 | 8.60 | 8.60 | 8.60 | 8.60 | 8.60 |
| | Na ₂ MoO ₄ | 99.00 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| | CuSO ₄ | 99.00 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| Iron salt (mM) | CoCl ₂ | 99.00 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| | MnSO ₄ | 99.00 | 22.30 | 22.30 | 22.30 | 22.30 | 22.30 | 22.30 | 22.30 |
| Allogenic material(mM) | EDTA-Fe | 99.50 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| | PAC | 95.00 | 0 | 0 | 0.01 | 0 | 0 | 0.01 | 0 |
| | GA ₃ | 99.00 | 0 | 0.10 | 0 | 0 | 0.10 | 0 | 0 |

Table S5 Sequences of primers used in qRT-PCR.

| Gene | Primer sequence (5'-3') |
|------------------|-------------------------|
| <i>PbACTIN F</i> | TTGGTATGGGTCAAGAGG |
| <i>PbACTIN R</i> | CTGTGAGCAGAACTGGGTG |
| <i>PbNRT2 F</i> | AAGATCATCGTGGTATGTTGC |
| <i>PbNRT2 R</i> | GAAGGTGGAGAAGAAGTGGAG |

| | |
|-----------------------|----------------------|
| <i>PbNR</i> F | CCCACTCTGCGACTTGCTTA |
| <i>PbNR</i> R | AACCACTTCACCATCCGACC |
| <i>PbNiR</i> F | GCATTGACCCCCTCGAGATT |
| <i>PbNiR</i> R | TGAATCCGAACCTGCCATCC |
| <i>PbNADH-GOGAT</i> F | TAGAGGTTGGCGCTGCATAG |
| <i>PbNADH-GOGAT</i> R | CCACTTGTGTGCGTTCAAGG |
| <i>PbFd-GOGAT</i> F | CGGACTCTCGTCGTCGATT |
| <i>PbFd-GOGAT</i> R | AGTGGCAATTCTGGAGACG |
| <i>PbGILE</i> F | AGCAAAGCAAGGACTCTGCC |
| <i>PbGILE</i> R | AGCCCTCTTGTGGTTGGAA |
| <i>PbGAI1a</i> F | TCCCTCCGAACTCCTCTT |
| <i>PbGAI1a</i> R | GTAGCCGATCTGGGTATC |
