

New Insights into the Transcriptional Regulation of Genes Involved in the Nitrogen Use Efficiency under Potassium Chlorate in Rice (*Oryza sativa* L.)

Nkulu Rolly Kabange ¹, So-Yeon Park ¹, Ji-Yun Lee ¹, Dongjin Shin ¹, So-Myeong Lee ¹, Youngho Kwon ¹, Jin-Kyung Cha ¹, Jun-Hyeon Cho ¹, Dang Van Duyen ², Jong-Min Ko ¹ and Jong-Hee Lee ¹.
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¹ Department of Southern Area Crop Science, National Institute of Crop Science, RDA, 50424, Miryang, Korea; N.R.K., rollykabange@korea.kr; S.-Y.P., f55261788@korea.kr; J.-Y.L., minitia@korea.kr; D.-J. S., jacob1223@korea.kr; S.M.L., olivetti90@korea.kr; Y.H.K., kwon6344@korea.kr; J.K.C., jknzz5@korea.kr; J.M.K., kojmin@korea.kr
² Molecular Biology Department, Agricultural Genetic Institute, Hanoi, Vietnam; dangvandung79@gmail.com

* Correspondence: J.H.L, ccrljh@korea.kr; Tel.: +82-53-350-1168, Fax: +82-55-352-3059

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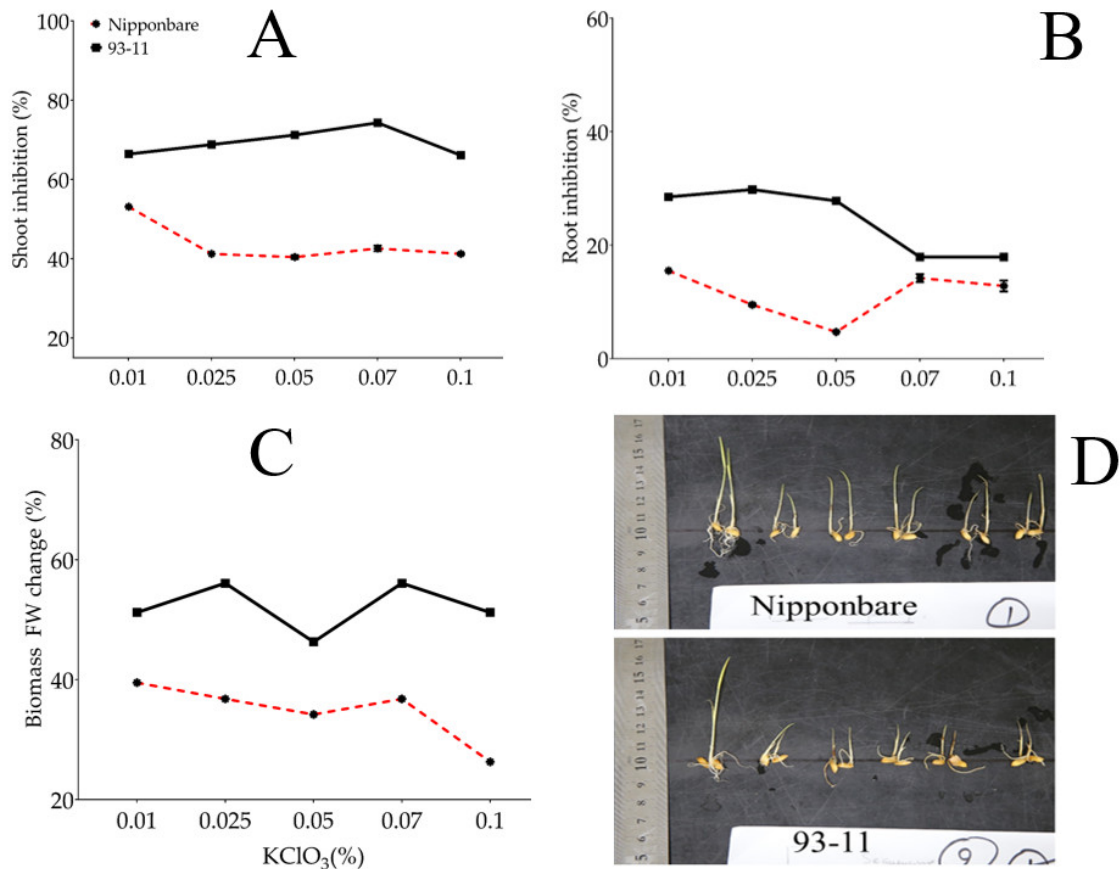


Figure S1. Optimization of potassium chlorate (KClO₃) concentration using Nipponbare and 93-11 rice cultivars. (A) The pattern of shoot inhibition percentages of Nipponbare (typical *japonica* ssp.) and cv.93-11 (typical *indica* ssp.) in response to gradients KClO₃ concentrations (0.01, 0.025, 0.05, 0.07, and 0.1%). (B) The roots inhibition pattern under gradient KClO₃ concentrations. (C) Changes of biomass fresh weight under the same conditions. (D) Phenotypes of seedlings exposed to gradients KClO₃ concentrations 7 days after treatment.

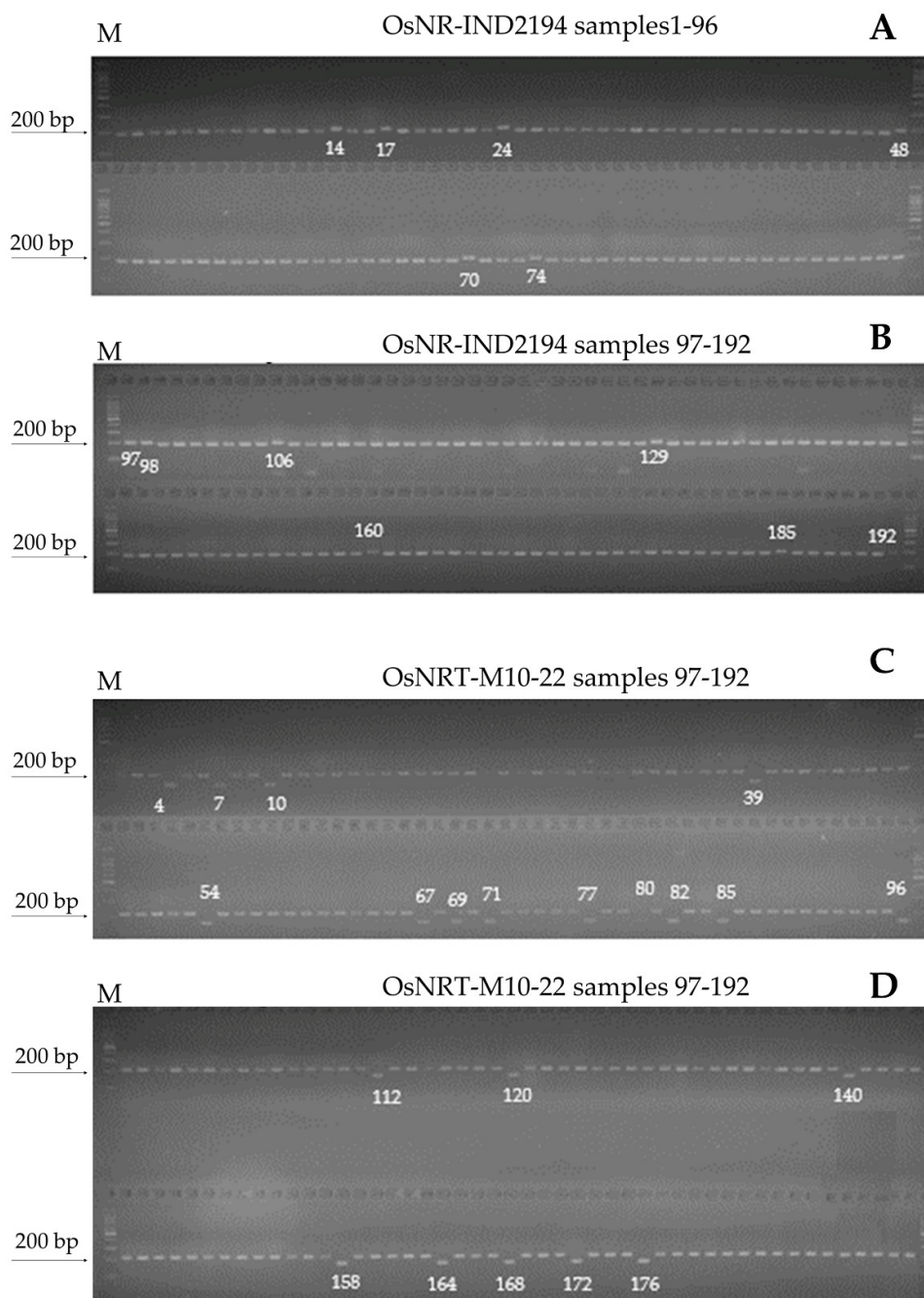


Figure S2. Identified nitrate reductase (NR) and nitrate transporter (NRT) introgression lines. This figure shows the genotyping results of representative rice lines pulled from 420 BC2F7 rice lines derived from a cross between Saeilmi × Milyang23, *japonica* and *indica* subspecies, respectively, using insertion/deletion (InDel) markers. (A–B) NR introgression lines amplifying the *indica* allele of nitrate reductase (200 bp), and (C–D) NRT introgression rice lines amplifying the *indica* allele of nitrate transporter (165 bp).

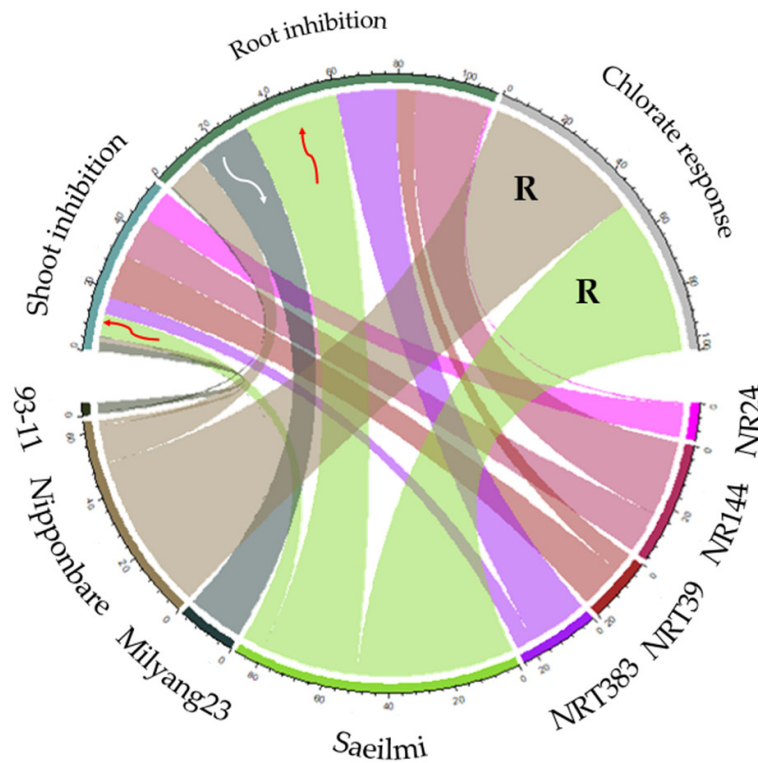


Figure S3. Chord diagram showing the shoot and roots growth patterns of Saeilmi (P1) and Milyang23 (P2), and four BC2F7 introgression lines. This figure shows the proportion of the shoot growth and roots growth, and the potassium chlorate response of two Nitrate reductase (NR) and nitrate transporter (NRT) introgression lines, the parental lines (Saeilmi, P1 *japonica*; Milyang23, P2 *indica*), as well as that of Nipponbare and 93-11, typical *japonica* and *indica* cultivars used as reference for the phenotypic evaluation under KClO_3 treatment. The bottom side of the circular Chord diagram comprise the rice lines evaluated, and the upper side is the traits. Bands or links show the linkage between rice lines and traits. The wider the band width the larger the proportion (increase or decrease). The arrow directed downward indicates a reduction in that specific parameter in the parental line, Milyang23. The R indicates resistant. No indication means reduction of that specific trait in the NR or NRT introgression lines under potassium chlorate (KClO_3) treatment.

Table S1. List of primer sequences for gene expression used in the study

| Marker/gene names | Locus | Forward primer (5'→3') | Reverse primer (5'→3') | Length (F/R) | Tm °C (F/R) | GC contents (F/R) | Amplicon size (bp) |
|---|------------------------------|------------------------|------------------------|--------------|-------------|-------------------|--|
| Genotyping InDel marker primers | | | | | | | |
| OsNR-IND2194 | LOC_Os02g53130 /BGIOGA005531 | GTGCTGACCTCACGTCCATC | GTAGCCCGAGCTTCTGGTC | 20/ 19 | 59.5/ 59.2 | 60/63.2 | 200 (<i>indica</i>)/ 188 (<i>japonica</i>) |
| OsNRT-M10-22 | LOC_Os10g40600/ BGIOGA031434 | TCGCGTGACAAATATGACAT | CCACTGCAAGATCCAAGTCT | 20/ 20 | 51.3/ 55.4 | 50/45 | 165 (<i>indica</i>)/ 213 (<i>japonica</i>) |
| High affinity nitrate transporters encoding gene | | | | | | | |
| <i>OsNRT1.1B</i> | LOC_Os10g40600 | GGCTCGACTACTTCTACTGGC | CGAGGCGCTTCTCCTTGTAG | 21/20 | 59.8/59.5 | 57.1 / 60 | 102 |
| High affinity ammonium transporters encoding genes | | | | | | | |
| <i>OsAMT1.3</i> | LOC_Os02g40710 | GCGCGCTCTTCTACTACCTC | GTAGTCGTACCCTGTCTGCG | 20/ 20 | 59.5/ 59.5 | 60/ 60 | 120 |
| <i>OsAMT2.3</i> | LOC_Os01g61550 | CGGATGAACATCAAGGCGTG | TATCCGCCGGAGTAGTCGAT | 20/ 20 | 57.5/ 57.5 | 55.0/ 55 | 116 |
| Glutamate synthase involved in the initial step of nitrogen assimilation | | | | | | | |
| <i>GLU1</i> | LOC_Os01g48960 | TGTTGCTGTCAGTTCGCTCT | AAACCCAAACAAGGGTGCAGA | 20/20 | 55.4/ 55.4 | 50.0/ 50.0 | 140 |
| <i>GLU2</i> | LOC_Os05g48200 | AATGCTCTCCCAACCTGG | CTGCTGTTAATCCGTGCTGC | 20/20 | 57.5/ 57.5 | 55.0/ 55.0 | 110 |
| Nitrate reductase catalyzing the conversion of nitrate (NO ₃) to nitrite (NO ₂) | | | | | | | |
| <i>OsNR2</i> | LOC_Os02g53130 | TCCTCGCTACATGCAGAAC | ATGCGCTTGAGCCATTCAC | 20/20 | 57.5/ 55.4 | 55.0/ 50.0 | 112 |
| Nitric oxide biosynthetic genes | | | | | | | |
| <i>OsNIA1</i> | LOC_Os08g36480 | TCGGCAAGCACATCTTCGT | ACTTGGGGTGCTGTTCTTG | 19/ 20 | 54.9/ 57.5 | 52.6/ 55.0 | 138 |
| <i>OsNIA2</i> | LOC_Os08g36500 | TGTACCAGGTCATCCAGTCG | CGATGACGTACCACACCTTG | 20 /20 | 57.5/ 57.5 | 55.0/ 55.0 | 162 |
| Housekeeping gene | | | | | | | |
| <i>OsActin1</i> | LOC_Os05g36290 | CTAGCGGTGCAACAACCTGGT | ACCGGAGGATAGCATGAGGA | 20/ 20 | 57.5/ 57.5 | 55.0/ 55.0 | 102 |