Genome-Wide Association Study Reveals Candidate Genes Related to Low Temperature Tolerance in Rice (*Oryza sativa*) during Germination

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Abstract

Direct-seeding has become increasingly popular and an inevitable trend in rice growing region due to its labor-saving and lower production costs. However, weak seedling establishment, slow and poor seedling emergence will cause yield loss, so that restrict the technique's application and popularity. In rice, mesocotyl as a primary organ for seedling emergence can push the shoot tip toward the soil surface. Therefore, it is necessary to introduce mesocotyl elongation related genes into elite varieties and screen genetic resources of mesocotyl elongation to enhance seedling emergence.

Methodology and Theoretical Orientation:

Mesocotyl length was measured in various rice accessions under agar condition and carried out association analysis to identify candidate genes related to mesocotyl elongation using a natural population comprising 137 rice cultivars and inbred lines selected from the Korean rice core set. Genome-wide association study (GWAS) using ~1.44 million high-quality SNPs, which were identified by re-sequencing all rice collections.

Findings:

55 candidate genes on chromosome 1, 3 and 4 in the high peak SNP sites of associated loci for mesocotyl length were revealed. By detecting highly associated variations located inside genic regions and performing functional annotation of the genes, we ultimately identified 22 genes that may be responsible for mesocotyl length. In addition, the haplotype and sequence analysis of the candidate gene (Os01g0272600), which was revealed the correlation between sequences of functional variations and phenotypic. Several novel mesocotyl length related candidate genes previously were known for the function during rice germination and uncovered their substantial natural variations.

Conclusion and Significance:

Our research identified some mesocotyl elongation related novel candidate genes and their substantial natural variations in rice seedling emergence stage. The revealed candidate genes of mesocotyl elongation would provide valuable resources for molecular breeding and genetic improvement of the seedling emergence after direct seeding.

Image:

Image:

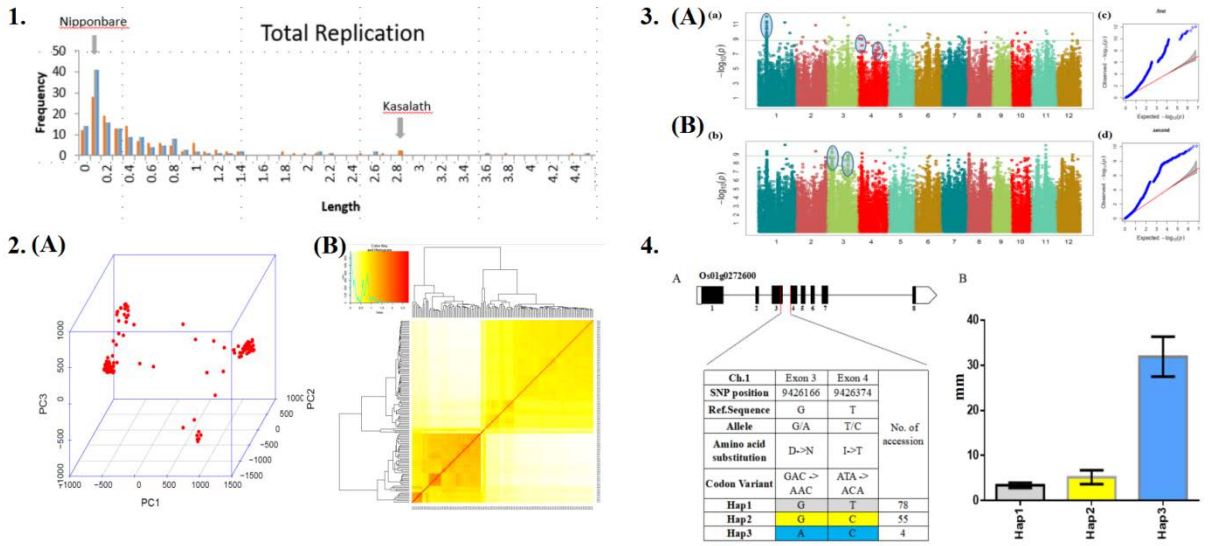


Table 1

Chr.	Name	Description	Position
1	Os01g0270700	catalytic domain containing protein. (Os01t0270700-00)	chr01:9,348,658..9,350,721 (+ strand)
1	Os01g0273300	BSD domain containing protein. (Os01t0273300-01)	chr01:9,454,976..9,456,973 (+ strand)
1	Os01g0271200	Similar to Glutamyl-tRNA synthetase. (Os01t0271200-01)	chr01:9,373,756..9,378,200 (- strand)
1	Os01g0273500	Heat shock protein (Os01t0273500-01)	chr01:9,470,130..9,475,187 (+ strand)
1	Os01g0272600	Similar to Root determined nodulation 1. (Os01t0272600-01)	chr01:9,424,556..9,429,189 (+ strand)
1	Os01g0269900	Actin/actin-like family protein. (Os01t0269900-01)Actin/actin-like family protein. (Os01t0269900-01)	chr01:9,319,015..9,323,339 (-strand)
1	Os01g0270300	Similar to Cationic peroxidase isozyme 40K precursor. (Os01t0270300-01)	chr01:9,339,815..9,342,240 (+ strand)
1	Os01g0271400	ClpP family protein. (Os01t0271400-00)	chr01:9,379,123..9,383,262 (-strand)
1	Os01g0271500	Ubiquitin activating enzyme E1-like protein. (Os01t0271500-01)	chr01:9,386,202..9,390,616(+ strand)
3	Os03g0213900	Similar to ROUGH SHEATH2-interacting KH-domain protein. (Os03t0213900-02)	chr03:5,967,215..5,973,703 (+ strand)
3	Os03g0640400	KxDL motif-containing protein(Os03t0640400-01)	chr03:24,638,186..24,641,449 (- strand)
3	Os03g0124000	Homeodomain-related domain containing protein. (Os03t0124000-01)	chr03:1,394,429..1,440,133 (+ strand)
3	Os03g0156000	Alpha-expansin OsEXPA19. (Os03t0156000-00)	chr03:3,026,522..3,027,521 (+ strand)
3	Os03g0209500	Zinc finger%2C DHHC-type domain containing protein. (Os03t0209500-01)	chr03:5,710,240..5,715,009 (- strand)
3	Os03g0640300	Similar to phosphatidylinositol N-acetylglucosaminyltransferase subunit P. (Os03t0640300-01)	chr03:24,630,180..24,634,746 (- strand)
3	Os03g0640800	Class III homeodomainLeu zipper (HD-Zip III) family member (Os03t0640800-01)	chr03:24,651,848..24,657,650 (- strand)
3	Os03g2103300	Phosphopantetheine attachment site domain containing protein. (Os03t0213300-01)	chr03:5,907,703..5,907,030 (+ strand)
4	OS04g0161100	Similar to OSIGBa0102O13.5 protein. (Os04t0161100-00)	chr04:4,216,591..4,218,547 (+ strand)
4	Os04g0623800	Glycine cleavage system T protein (GCVT). (Os04t0623800-01)BSimilar to H0215F08.10 protein. (Os04t0623800-02)	chr04:31,700,976..31,703,589 (+ strand)
4	Os04g0166400	Similar to OSIGBa0136O08-OSIGBa0153H12.2 protein. (Os04t0166400-01)	chr04:4,509,613..4,513,848 (- strand)
4	Os04g0463800	Pentatricopeptide repeat domain containing protein. (Os04t0463800-01)	chr04:23,153,453..23,158,544 (- strand)
4	Os04g0452500	Similar to H0505F09.1 protein. (Os04t0452500-00)	chr04:22,582,841..22,592,982 (+ strand)

Figure 1. Verification of replications in mesocotyl phenotype by histogram table of accessions.
Figure 2. Genetic relatedness in the diversity panel evaluated on the SNP data used for GWAS. (A) Samples' relationship in the three-dimensional space of the first three principal components derived from a PCA. Red dots represent samples. (B) Kinship analysis on the panel. Top left, the distribution of estimated kinship values. In the main panel, the pairwise kinship values depicted in increasing tones of red. Outside the matrix, the resulting clustering tree.

Figure 3. Manhattan plots and Q-Q plots of rice mesocotyl elongation. A and B: Manhattan plots of mesocotyl elongation two replication; a and b: Manhattan plots of mesocotyl elongation; c and d: Q-Q plots of mesocotyl elongation.

Figure 4. Haplotyping and sequence analysis of Os01g0272600, which was correlated with a phenotypic difference. A: gene structure of Os01g0272600 and results of haplotype analysis. Black boxes represent coding regions, solid lines represent introns and red line represent the location of amino acids. B: Average mesocotyl length of each group by results of haplotype analysis.

Table 1. List of annotated candidate genes associated with mesocotyl elongation on chromosome 1, 3, 4.

Recent Publications

1. B Kim, S Woo, MJ Kim, **SW Kwon**, ... & HJ Koh. (2018) Identification and quantification of flavonoids in yellow grain mutant of rice (Oryza sativa L.). **Food Chemistry** 241:154-162.
2. H Wang, SY Park, ... & **SW Kwon**. (2018) Next-generation sequencing yields the complete chloroplast genome of *C. goeringii* acc. smg222 and phylogenetic analysis. ***Mitochondrial DNA Part B,*** *3*(1), 215-216.
3. H Wang, AR Lee, ... & **SW Kwon**. (2018) Genome-wide association study reveals candidate genes related to low temperature tolerance in rice (*Oryza sativa*) during germination. ***3 Biotech*.**
4. **SW Kwon**, M Kim, H Kim, & J Lee **(2016)**. Shotgun quantitative proteomic analysis of proteins responding to drought stress in *Brassica rapa* L. (Inbred Line (Chiifu)). **International Journal of Genomics**
5. TS Kim, Q He, …, **SW Kwon,** ... & YJ Park **(2016)**. Genome-wide resequencing of KRICE_CORE reveals their potential for future breeding, as well as functional and evolutionary studies in the post-genomic era. **BMC Genomics** doi: 10.1186/s12864-016-2734-y
6. **SW Kwon**, YC Cho, ... & YG Kim **(2011).** Identification of quantitative trait loci associated with rice eating quality traits using a population of recombinant inbred lines derived from a cross between two temperate japonica cultivars. **Molecules and Cells** 31(5):437-445



Biography (150-word limit)

Soon-Wook Kwon has been an associate professor in Pusan National University since 2013. He has been studied in Rice Breeding with a focus on eating quality and abiotic stress and studied in Molecular Breeding in post-genomic era. He received a B.A, M.S, and Ph. D from Seoul National University in 1999, 2002, and 2008, respectively. He also had involved in IRRI (International Rice Research Institute) as a Collaborator and Kongju National Univ. as a Post Doc.

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