Regulation of grain yield in rice under well-watered and drought stress conditions by GUDK

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Increasing the grain yield of cereals, which is stable under unfavorable environmental stress, is a major objective to sustain production and feed the growing world population. Recently, we functionally characterized a receptor-like cytoplasmic kinase, named GROWTH UNDER DROUGHT KINASE (GUDK), revealing its role in regulating grain yield under well-watered and drought stress conditions by transphosphorylating the OsAP37 transcription factor. GUDK is induced under several stresses and its loss-of-function increased the sensitivity of rice seedlings to salinity, osmotic stress, and abscisic acid treatment. In addition to reduced tolerance of gudk mutant plants to drought stress at vegetative stage, a significant reduction in grain yield was observed under well-watered and drought stress conditions at reproductive stage. Gene co-expression analysis supports the role of GUDK in regulating important biological processes both under control and stress conditions. Thus, our results suggest that GUDK has the potential to regulate grain yield both under favorable and unfavorable conditions.

Rice, feeds more than half the world’s population, and consumes about 30% of all the fresh water used in agriculture.1 Water scarcity, caused by the needs of the rapidly increasing global population threatens sustainable production of rice. Therefore, improvement of yield and maintaining yield stability under optimal as well as water-limited conditions is essential for food security of the growing global population. In recent years, significant examples of achieving drought tolerance have been reported using transgenic-based methods by overexpression of transcription factors and other upstream regulatory factors such as kinases and phosphatases.2,3 However, most of these studies using Arabidopsis for increasing drought tolerance at the vegetative stage, have poor translation of results to increase yield of crops under drought stress.4-14 A limited number of studies have shown the relevance of regulatory genes upstream of transcription factors in regulating grain yield under drought.15-19 In addition, little importance has been given for simultaneous improvement of rice yield under well-watered and drought conditions. In this context, it has been recently proposed that yield potential should be selected under favorable environmental conditions,20 as there is a positive correlation between yield potential under normal conditions and drought stress conditions.20 In this study, we show that the rice receptor-like cytoplasmic kinase, GROWTH UNDER DROUGHT KINASE (GUDK), is induced under multiple stresses and support its role in determining grain yield under well-watered and drought stress conditions by regulating the OsAP37 transcription factor.

GUDK is Induced under Multiple Stresses

In our previous report we have shown that GUDK (LOC_Os03g08170) is a drought stress inducible receptor-like cytoplasmic kinase. GUDK encodes a protein of 425 amino acids containing only an intracellular kinase domain and no transmembrane or extracellular domains.21 Quantitative polymerase chain reaction (qPCR) analyses showed that GUDK was induced by dehydration, salinity (200 mM NaCl), heat (45°C), and cold (4°C) within 1 h of exposure (Fig. 1A). GUDK expression was induced or maintained throughout the stress treatments and also under recovery. Under treatments of abscisic acid (ABA, 100 μM), methyl jasmonate (MeJA, 100 μM), salicylic acid (SA, 100 μM), hydrogen peroxide (H2O2, 10 mM) and wounding, the expression of GUDK was induced within 1 h; except for SA and H2O2 treatments, wherein the expression was down-regulated at the early periods but induced later (Fig. 1B). The late induction of GUDK under SA treatment is probably a secondary response mediated by reactive oxygen species accumulation.22

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Among all the stresses, high induction was observed under dehydration, salinity, heat and MeJA treatments. Together, results indicate the possible involvement of GUDK in regulating multiple stress responses.

GUDK Regulates Stress Response at both Seedling and Vegetative Stage

To test the role of GUDK in regulating abiotic stress response, 2 independent T-DNA insertion lines (gudk-1 and gadk-2, termed as mutant lines) were identified. Loss-of-function of GUDK was confirmed by qPCR analysis with no expression of GUDK in both mutant lines compared to wild-type plants.21 To investigate the role of GUDK in regulating salinity, osmotic stress and ABA response, pre-germinated seedlings were treated with NaCl, PEG and ABA. The mutant lines showed a significant reduction in root length, shoot length, and biomass under all stresses. Further, to investigate whether the loss-of-function of GUDK alters drought stress response of rice, mutant lines were exposed to severe drought by dry-down and controlled drought by gravimetric method. During dry-down, the gadk mutant lines showed drought-induced changes such as leaf rolling and wilting
at an early stage when compared to wild-type plants. The mutant plants also recovered later than the wild-type plants upon re-watering with concomitant 43%–55% reduction in survival rate compared to wild-type. Under controlled drought stress, wherein the plants were maintained at 40% field capacity for 10 days, measurement of physiological parameters revealed substantial reduction in drought tolerance of mutant plants. Mutant plants showed reduction in relative water content, photosynthesis rate, instantaneous water use efficiency, photochemical efficiency of PSI in a light-adapted state and biomass suggesting the role of GUDK in drought tolerance at vegetative stage.

**GUDK Regulates Grain Yield under Well-watered and Drought Stress Conditions**

Rice is very sensitive to drought stress at reproductive stage, with moderate drought stress severely affecting grain yield. The *gudk* mutant plants were exposed to drought stress at pre-anthesis stage, and showed significant (29%–35%) reduction in grain yield compared to wild-type plants. Interestingly, mutant plants also showed a 25%–28% reduced grain yield under well-watered condition compared to wild-type plants. Reduced yield under well-watered and drought stress conditions is mainly contributed by increased spikelet sterility.

To determine which biological processes are regulated by GUDK, we evaluated the association of all kinases in the rice genome with biological process terms in the gene ontology (GO) database. Two separate genome wide kinase regulatory networks, representing state of kinase interactions under normal tissue/developmental (control) conditions and environmental perturbation (stress) conditions were derived from a collection of publicly available microarray expression profiles using ‘specific’ correlation scores as a measure of co-expression between every kinase-gene pair. From these global networks, we explored the association of GUDK targets with gene-sets in the biological processes category of GO database using a parametric analysis model. The results showed that GUDK is associated in expression with several biological processes such as photosynthesis and primary metabolic processes that are common to both control and stress (Fig. 2), therefore supporting the role of GUDK gene both under well-watered and drought stress conditions. Furthermore, phosphoproteome analysis and *in vitro* assays identified OsAP37 as a phosphorylation target of GUDK. This rice transcription factor OsAP37 has been previously shown to increase grain yield in rice under drought through regulation of several stress responsive genes. In planta transactivation assays showed the requirement of GUDK function for the regulation of stress genes by OsAP37, suggesting that GUDK regulates yield by phosphorylating the OsAP37 transcription factor.

Regulation of yield under drought and normal conditions has also been shown for 3 NAC family transcription factors. Field evaluations of rice plants overexpressing OsNAC5, OsNAC9 and OsNAC10 transcription factors revealed grain yield increase of 9%–26%, 13%–32% and 5%–14% under well-watered conditions, respectively. However, most of these results have been overlooked with more emphasis on yield under drought stress. Our results show the potential role of GUDK in regulating grain yield under both well-watered and drought stress conditions acting upstream of transcription factors. GUDK is therefore a primary regulator of grain yield in rice and offers the opportunity to improve and stabilize rice grain yield under normal and drought stress conditions.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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