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Article Addendum

Rice transcription factor *AP37* involved in grain yield increase under drought stress

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Drought is a serious threat to the sustainability of rice yields in rainfed agriculture. In particular, exposure to drought conditions during the stage of panicle development of a rice plant results in a delayed flowering time, reduced number of spikelets and poor grain filling. In our recent report, we functionally characterized the rice *AP37* gene for drought tolerance during the vegetative and reproductive growth. Transgenic overexpression of the *AP37* with the *OsCc1* promoter in rice increased the tolerance to drought, high salinity and low temperature at the vegetative stage. The transgenic plants *OsCc1:AP37* also showed significantly enhanced drought tolerance at the reproductive stage, as evidenced by the increase in grain yield by 16–57% over controls under severe field drought conditions. Thus, our results suggest that the *AP37* gene has the potential to improve drought tolerance without causing undesirable growth phenotypes.

Introduction

Water deficit and global warming can cause serious problem of crop productivity in arable land. Drought stress is among the most serious challenges to crop production worldwide. Upon exposure of plants to drought conditions, many stress-related genes are induced and their products are thought to function as cellular protectants of stress-induced damage.[1,2] APETALA2 (AP2) factors appear to be widespread in plants with the genomes of rice and Arabidopsis predicted to contain 139 and 122 *AP2* genes, respectively.[3] Members of the AP2 family have been implicated in diverse functions in cellular processes involving flower development, spikelet meristem determinacy, plant growth and stress tolerance.[4-8]

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It is important to evaluate the transgenic plants under drought condition, and to understand the physiological effect of the transgene in the natural conditions to overcome abiotic stresses. To date, a number of studies have suggested that overexpression of stress related genes could improve drought tolerance in rice to some extent.[9-15] Despite such efforts to develop drought-tolerant rice plants, very few of these have been shown to improve grain yields under field conditions. Examples of positive effects include transgenic rice plants expressing *SNAC1*,[13] and *OsLEA3*,[16] which was shown to improve grain yield under field drought conditions. In our current study, we examined in-field performance of *AP37* transgenic rice plants under field drought conditions.[17]

Stress Tolerance of *OsCc1:AP37* Plants at the Vegetative Stage

A full-length cDNA of AP37 gene was isolated from rice, linked to the *OsCc1* promoter for constitutive expression, and transformed into rice. Three independent T_{4-5} homozygous lines of *OsCc1:AP37* plants were selected and transcript levels of AP37 were enhanced in the transgenic plants as compared to those in the nontransgenic (NT) controls. To investigate whether the overexpression of *AP37* correlated with stress tolerance in rice, four-week-old transgenic plants and NT controls were exposed to drought stress. The NT plants started to show visual symptoms of drought-induced damage, such as leaf rolling and wilting with a concomitant loss of chlorophylls, at an earlier stage than the *OsCc1:AP37* plants. The transgenic plants also recovered faster than the NT plants upon re-watering. Consequently, the NT plants remained severely affected by the time at which all of the transgenic lines had fully recovered. To further verify the stress-tolerance phenotype, we measured the Fv/Fm values of the transgenic and NT control plants, all at the vegetative stage. The Fv/Fm values represent the maximum photochemical efficiency of PS II in a dark-adapted state. The Fv/Fm levels were about 15–30% higher in the *OsCc1:AP37* plants than in the NT plants under drought, high salinity and low temperature conditions, indicating tolerance of the plants to high salinity and low temperature as well as to drought at the vegetative stage.

Stress Tolerance of *OsCc1:AP37* Plants at the Reproductive Stage

Grain yield from rice plants is severely affected when they are exposed to drought stress at the reproductive stage. To evaluate whether any improvements in grain yield had occurred in our transgenic rice under drought conditions, we transplanted T_5 homozygous lines of *OsCc1:AP37* plants to the field in 2008. The plants were exposed to drought stress at the panicle heading stage from 10-d prior to heading to 20-d after heading in field conditions. The *OsCc1:AP37* plants showed significantly enhanced drought tolerance in the field, with a grain yield of 16–57% higher than the controls under severe drought conditions. Thus, we found that the overexpression of *AP37* in rice was effective against drought stress at the reproductive stage as well as at vegetative stage. In addition, the overexpression of *AP37* does not seem to affect the development of reproductive organs whilst conferring stress tolerance in transgenic plants. Development of the panicle and/or spikelet meristem is repressed in rice under drought conditions, resulting in a reduction in the number of panicles and/or spikelets.[18,19] The lower decreases in the filling rate and in the number of spikelets of *OsCc1:AP37* plants under drought conditions implied that the developmental processes for panicles and spikelets had been protected from drought stress, indicating drought tolerance of *OsCc1:AP37* plants at the reproductive stage. To date, the potential impact of homeotic genes like the AP2 factors upon grain yield have received relatively little attention, because of their negative effects on fertility, plant growth and development. It is thus important to evaluate agronomic traits in transgenic crops throughout the entire stages of plant growth to address the advantages of using such homeotic genes for improving stress tolerance.

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