Overexpression of an Arabidopsis thaliana galactinol synthase gene improves drought tolerance in transgenic rice and increased grain yield in the field

Michael Gomez Selvaraj, Takuma Ishizaki, Milton Valencia, Satoshi Ogawa, Beata Dedicova, Takuya Ogata, Kyouko Yoshiwara, Kyonoshin Maruyama, Miyako Kusano, Kazuki Saito, Fuminori Takahashi, Kazuo Shinozaki, Kazuo Nakashima and Manabu Ishitani

Introduction

Drought is a major abiotic stress condition critically limiting crop production and yield (Edmeades, 2008). Climate prediction models suggest that abiotic stresses will increase in the near future because of global climate change (Ahuja et al., 2010). The ever-rising world population and recurrent global climate change challenge the agricultural system to produce sufficient food to feed the world (Godfray et al., 2010). As the world's second-largest crop, rice plays a critical role in food security for more than half of the world's population (FAO, 2016: http://faostat3.fao.org/browse/Q/QC/E).

Rice accounts for about 27% of total cereal production, with a worldwide production of roughly 738.2 million tons (FAO, 2016). By 2035, a 26% increase in rice production will be required to feed the growing population (Cassman et al., 2003; Seck et al., 2012). Global water shortage is a major issue for cultivated rice, which needs large quantities of water (Manavalan et al., 2012). It was reported that the global reduction in rice production due to drought averages 18 million tons annually (O'Toole, 2004). Worldwide, drought affects approximately 23 million ha of rice production under rainfed conditions. Drought is particularly frequent in unbunded uplands, bunded uplands and shallow rainfed lowland fields in many parts of South and South-East Asia, sub-Saharan Africa and Latin America (Serraj et al., 2011). To resolve those global problems, it is important to improve crop yields especially within staple food crops like rice (Oryza sativa L.) through breeding-improved stress tolerance.

Transgenic technologies are one of the numerous tools available to plant breeding programmes, which help to open new avenues for crop improvement by developing crop cultivars resistant to various biotic and abiotic stresses (Younis et al., 2014). Around 175.2 million hectares of biotech crops were grown globally and transgenic acreage grew 3% in 2013, representing 35% of the global seed market (Marshall, 2014). In rice, progress has been made in the generation and evaluation of transgenic rice events against drought tolerance (Todaka et al., 2015).

Abstract

Drought stress has often caused significant decreases in crop production which could be associated with global warming. Enhancing drought tolerance without a grain yield penalty has been a great challenge in crop improvement. Here, we report the Arabidopsis thaliana galactinol synthase 2 gene (AtGolS2) was able to confer drought tolerance and increase grain yield in two different rice (Oryza sativa) genotypes under dry field conditions. The developed transgenic lines expressing AtGolS2 under the control of the constitutive maize ubiquitin promoter (Ubi:AtGolS2) also had higher levels of galactinol than the non-transgenic control. The increased grain yield of the transgenic rice under drought conditions was related to a higher number of panicles, grain fertility and biomass. Extensive confined field trials using Ubi:AtGolS2 transgenic lines in Curinga, tropical japonica and NERICA4, interspecific hybrid across two different seasons and environments revealed the verified lines have the proven field drought tolerance of the Ubi:AtGolS2 transgenic rice. The amended drought tolerance was associated with higher relative water content of leaves, higher photosynthesis activity, lesser reduction in plant growth and faster recovering ability. Collectively, our results provide strong evidence that AtGolS2 is a useful biotechnological tool to reduce grain yield losses in rice beyond genetic differences under field drought stress.
Plants have evolved several mechanisms to accustom to abiotic stresses through changes at the physiological levels and molecular levels (Todaka et al., 2012; Yamaguchi-Shinozaki and Shinozaki, 2006). It is suggested that overexpression of stress-related genes could improve drought tolerance in rice (reviewed by Nakashima et al., 2014 and Todaka et al., 2015). Despite such efforts to develop drought-tolerant rice plants, very few have been shown to improve grain yields under the field environments (Gaudin et al., 2013). Encouraging results include transgenic rice plants expressing OsNAC5 (Jeong et al., 2013), OsNAC9/OSNAC1 (Redil-las et al., 2012) or OsNAC10 (Jeong et al., 2010), which was shown to improve grain yield under field drought conditions. Many genes that may play an important role under drought have been mostly tested on a single model rice genetic background (Nipponbare) under laboratory conditions, but very few have been tested vigorously in a natural target environment using different commercial rice genetic backgrounds. For improved rice to be accepted by consumers, it is necessary to consider both adaptation to the target environments and fulfilment of local grain quality and taste preferences. This is predominantly important in transgenic studies in which the recipient genetic background is often chosen according to its ability to be transformed rather than agronomic or cultural considerations (Gaudin et al., 2013).

The accumulations of metabolite or osmoprotectants are one of the key adaptive mechanisms for plants to handle with dehydration stress and cellular injury (Hare et al., 1998). Soluble sugars, including those in the sucrose, trehalose and raffinose families also known as oligosaccharides (RFOs), have been found to accumulate during drought stress in many plants (Collett et al., 2004; Farrant, 2007; Peters et al., 2007; Taji et al., 2002). Galactinol synthase (Gols), a key enzyme in the metabolic pathway leading to RFOs, synthesizes galactinol (from UDP-Gal and myoinositol), which serves as a galactosyl donor to form raffinose, stachyose and verbascose (Panikulangara et al., 2004). It has been reported that the production of enzymes involving the biosynthesis of RFOs, and the resulting accumulation of RFOs, plays critical roles in acquired tolerance of Arabidopsis thaliana to drought and heat stresses (Taji et al., 2002; Nishizawa et al., 2008; reviewed by Sengupta et al., 2015). In Arabidopsis, seven GolS genes and three putative GolS genes have been identified, and intricate induction patterns were reported (Nishizawa et al., 2008). AtGolS1 was inducible by drought, salinity (Taji et al., 2002) and temperature stresses (Panikulangara et al., 2004); AtGolS2 was induced only by drought and salinity stresses; and AtGolS3 induction was detected solely after cold stress (Taji et al., 2002). Overexpression of AtGolS2 caused the increase in galactinol and raffinose contents in leaves and exhibited enhanced drought tolerance of transgenic Arabidopsis (Taji et al., 2002).

Here, we describe the production of transgenic rice events that overexpress the AtGolS2 cDNA driven by the maize ubiquitin promoter (Ubi:AtGolS2) in the background of Curinga (a Brazilian local upland rice variety) and NERICA4 (a popular upland rice variety in African countries) and present the results of multiple confined field trials over two different environmental conditions. Our extensive field test over different seasons and environmental conditions using multiple rice genetic backgrounds clearly demonstrated that AtGolS2 overexpression consistently increased biomass and grain yield under drought stress conditions. These findings implied that the Ubi:AtGolS2 transgene played an important role in improving agronomic traits and yield characteristics of rice and that overexpression would be an efficient way to accelerate the rice breeding programme for drought tolerance.

Results

Generation and molecular analyses of rice events expressing Ubi:AtGolS2

Our goal was to produce and select best-performing Curinga and NERICA4 transgenic rice lines for drought tolerance by expressing AtGolS2 from the constitutive maize ubiquitin (Ubi) promoter. We accomplished this using Agrobacterium-mediated transformation (Ishizaki and Kumashiro, 2008; Zuniga-Soto et al., 2015). At least 20 independent transgenic events were produced from each variety. T3 or T4 seeds that possessed genetically fixed single copy of transgene were used for further analyses.

To link field performance of the transgenic lines to the transgene expression levels and metabolite accumulation levels. Therefore, quantitative PCR expression of Ubi:AtGolS2 transgenic Curinga and Ubi:AtGolS2 transgenic NERICA4 events was analysed by quanti-tative real-time PCR (RT-PCR). All of the transgenic Curinga lines expressed the transgene (Figure 1a). Galactinol synthase (Gols) catalyses the first committed step in the biosynthesis of raffinose family oligosaccharides (RFOs) including galactolin and raffinose and plays a key regulatory role in carbon partitioning between sucrose and RFOs (Saravitz et al., 1987; Taji et al., 2002). We measured galactinol content in the promising transgenic plants grown in glasshouse under unstressed conditions. The NT Curinga and NERICA4 were used as the control. Under normal growth conditions, each transgenic plant showed significantly higher accumulation of galactinol as compared with NT rice plants (Figure 1b). All of the transgenic Curinga lines expressed the transgene and the expression levels of the transgene in lines #3025 and #3214 were higher than in the other lines (#2580, #2590, #2783 and #3020). In case of NERICA4, the AtGolS2 gene was overexpressed and expression level of the gene and accumulation level of galactinol in line #1577 (NERICA4) were higher than in other lines tested (Figure 1a, b). We also analysed the expression of representative drought marker genes in the Ubi:AtGolS2 transgenic rice. Expression of genes for a transcription factor OsNAC6, isocitrate lyase (ICL) and late embryogenesis-abundant protein LEA3 (Maruyama et al., 2014; Nakashima et al., 2014) was not induced in the events for Ubi:AtGolS2 without drought stress (Figure S1). These results indicate that the accumulation of galactolin is not related to the expression of these drought-inducible genes.

Drought tolerance of Ubi:AtGolS2 Curinga lines at vegetative-stage stress

Drought tolerance during the seedling growth period was important for rice plant establishment in areas where early-season drought overlapped with the vegetative stage. We conducted vegetative-stage drought experiments using homozy-gous transgenic rice lines overexpressing Ubi:AtGolS2 in Curinga. The experiment was conducted in rainout shelter facility at CIAT, Colombia, and biomass was used as a main criterion to select promising lines.

To evaluate the growth performance of the Ubi:AtGolS2-overexpressing Curinga under drought conditions at the vegeta-tive stage, three-week-old transgenic and nontransgenic (NT) Curinga control plants were subjected to drought stress for up to 3 weeks in November-December 2011 (Figure 2a). Agronomic
data collected before stress treatment showed that there was no significant variation among the lines, which helps to explain the uniformity in the experiment.

During peak stress, the transgenic lines significantly maintained more plant height than NT Curinga (Figure 2e). The NT Curinga started to show visual symptoms of drought-induced leaf rolling at an earlier stage than the transgenic plants (Figure 2b). The transgenic lines showed low leaf rolling score, 2-3 compared with the NT Curinga plants with (6.5) during peak drought stress (Figure 2c). However, dry biomasses of the transgenic plants measured after rewatering were significantly higher than those of NT Curinga in all but one instance (Figure 2d).

Drought tolerance of the Ubi:AtGolS2 Curinga lines under Managed Drought Stress Environment (MDSE)

In order to confirm the drought tolerance of Curinga transgenic lines at the reproductive stage, three consecutive confined field trials were conducted under the removable rainout shelter facility at CIAT, Palmira. As mentioned above, we had conducted two drought stress trials in the year 2012 over two cultivating seasons (2012-rainy season-MDSE-Trial-1 and 2012-dry season-MDSE-Trial-2) and one in 2014 (2014-rainy season-MDSE-Trial) (Figure S2). Up to eight independent T4 single-copy homozygous transgenic and NT Curinga lines were used to conduct the dry-down experiments. In addition to the field drought experiments, one normal well-watered paddy field trial (WW-field trial) was also conducted during the dry season of 2012 (Table S1). In the WW-field trial, single plant grain yield of all the tested transgenic lines and NT Curinga was not significantly different (Table S1), which suggests no yield penalty in these transgenic lines. Based on these results, grain yield was also used as main parameter to compare yield in dry fields between these transgenic lines and NT Curinga.

Drought intensity varied among drought trials from mild to severe (Figure S2). Although similar levels of stress duration and environmental conditions occurred in the rainy season drought experiments, it was observed that average single plant yield of NT Curinga was sharply reduced compared to the other two rainy season experiments (Figure 3a, b and c). This suggests a dry season effect. Additionally, we found that soil moisture rapidly decreased within the 0- to 40-cm soil layer compared to other two rainy season experiments (Figure S2b). The level of drought stress imposed under upland rainout shelter conditions was equivalent to that which caused an average reduction of around 60%–70% in the single plant grain yield obtained in the NT Curinga under well-watered paddy field conditions (Table S1 and Figure 3a, b and c).

Statistical analysis of yield and yield-related parameters scored for three rainout shelter drought experiments revealed that the Ubi:AtGolS2 overexpression consistently produced grain yield compared to NT across the season. Interestingly, in the Ubi:AtGolS2 Curinga lines, the morphophysiological trait performance was significantly better than NT Curinga (Table S2 and Figure 3). In each trial, we found some transgenic lines that had significantly higher yield: three of eight transgenic

---

**Figure 1** Ectopic overexpression of AtGolS2 gene confers higher galactinol accumulation on transgenic Curinga and NERICA4 rice. (a) Expression of AtGolS2 in Ubi:AtGolS2 transgenic rice was analysed by quantitative real-time PCR. Expression of OsUbi1 was analysed as an internal control to normalize the expression of AtGolS2. (b) Accumulation of galactinol in Ubi:AtGolS2 transgenic rice was analysed using GC-TOF-MS. The highest average value of the samples was set as 100, and the relative values were shown in (a) and (b). Five plants were combined into one sample for each line, and the relative values are mean ± SD of three technical replicates. Nontransgenic (NT) Curinga and NERICA4 were used as the control.
lines in 2012-rainy-MDSE-Trial-1, four of six transgenic lines in 2012-dry-MDSE-Trial-2 and two of five transgenic lines in 2014-rainy-MDSE-Trial, respectively (Figure 3a, b and c). We also found two promising transgenic lines (#2580 and #2590), which consistently outperformed NT Curinga in terms of grain yield over the drought experiments (Figure 3). In these lines, the higher grain yield under severe drought stress (2012-dry-MDSE-Trial-2) was associated with a significantly higher number of panicles, higher accumulation of biomass, low leaf rolling and leaf drying score, faster recovering ability, early flowering (Table S2), panicle length and grain fertility. Furthermore, under moderate stress (drought experiments in rainy season), no significant difference in panicle number and biomass between transgenic and nontransgenic lines was observed. Altogether, these results demonstrate that the Ubi:AtGolS2 expression increased grain yield under drought stress conditions imposed at the reproductive stage through a mechanism that involves the maintenance of early flowering, increased vegetative biomass, higher numbers of panicles and enhanced grain fertility (Table S2).

Analysis of physiological parameters in Ubi:AtGolS2 Curinga lines

Based on the results of the rainout shelter experiments in 2012 (the 2012-MDSE-Trial-1 and 2), five promising T4 Ubi:AtGolS2 Curinga lines were selected for further analysis of physiological parameters and field gene expression analysis in the 2014-rainy-MDSE-Trial. During the dry-down experiment, the most uniform transgenic lines for physiological analysis were tagged and repeated measurements were taken during drought stress.

Relative water content (RWC) was measured before and after subjecting the transgenic lines and NT Curinga to the drought stress treatment. Before stress, there were no obvious differences in the leaf RWC between NT Curinga and transgenic lines, and the RWC was within the range of 92%–95% (Figure 4a). After the lines were subjected to water stress for one week, the RWC of the NT Curinga leaves reduced sharply with respect to their first reading (before stress) from 95% to 89%, whereas the RWC of most of the transgenic lines declined very slowly (#2580, 94%; #2590, 94%; #2783, 94%; #3020, 94%; and #3214, 93%). After three weeks of drought stress, the RWC of the transgenic lines had declined by just 18%–22% as compared to 30% in the NT Curinga. Lines #2580, #2590, #2783, #3020 and #3214 maintained RWC very well even three weeks after drought stress, with RWC percentages of 73, 77, 77, 72 and 76, respectively. The rapid decline of the RWC (average of 66%) was observed in the NT Curinga after three weeks of drought stress.

To further verify the mechanism of drought tolerance, we measured Fv/Fm values of the transgenic and NT Curinga during stress period using FluorPen-FP100, (Photon Systems Instruments, spol. s r.o., Czech Republic) (Figure 4b). The Fv/Fm values represent the maximum photochemical efficiency of photo
system (PS) II in a dark-adapted state, where \( F_v \) stands for variable fluorescence and \( F_m \) stands for maximum fluorescence. Initially under unstressed conditions, the \( F_v/F_m \) values of both NT and transgenic plants were similar, ranging from 0.70 to 0.74. After one week of drought stress, the \( F_v/F_m \) value of the NT Curinga slightly decreased (0.68), but we did not find any significant differences between NT Curinga and the \textit{Ubi:AtGolS2} transgenic lines. However, after three weeks of drought stress, the \( F_v/F_m \) value for the transgenic lines #3214, #2580, #2590, #2783 and #3020 was maintained at 0.59, 0.67, 0.65, 0.65 and 0.61, respectively, while the NT Curinga value significantly decreased to 0.56 (Figure 4b). The promising transgenic lines #2580 and #2590 showed significantly higher \( F_v/F_m \) values compared to NT Curinga even after three weeks of stress.

Chlorophyll content was measured using a SPAD-502 Chlorophyll Meter (Konica Minolta Inc., Tokyo, Japan). Transgenic and NT Curinga plants were measured for their chlorophyll content before and during peak stress (Figure 4c). Before stress, the SPAD values of the transgenic and NT Curinga plants were not significantly different ranging from 37 to 40. At peak stress (three weeks after stress), the chlorophyll values of the NT Curinga plants were reduced (average of 34) compared to the transgenic lines. After one week of drought stress, the chlorophyll content of the NT Curinga plants was maintained a similar chlorophyll content after three weeks of the stress development to confirm the expression of the \textit{Ubi:AtGolS2} transgene under field conditions (Figure 4d).

**Drought tolerance of \textit{Ubi:AtGolS2} Curinga in the different environments—Target Environment (TE) Trial**

Based on the initial vegetative and reproductive drought stress experiments, up to six potential transgenic Curinga lines along with the NT Curinga were chosen for upland rainfed field trials at CIAT Santa Rosa station, Villavicencio, Colombia. To test the hypothesis of gene x environment interactions of \textit{Ubi:AtGolS2}, the three consecutive TE field trials were carried out from 2012 to 2015. The field trial conditions, design and the plot size were well described in the experimental procedure section. The rainfall and temperature pattern of this site during trial period is shown in Figure 5. Ten years of rainfall data from this site revealed that natural rainfall failure events usually occur in the months of January–February, which coincides with the reproductive stage of the crop. For instance, trial years TE-2012-13 and TE-2013-14 were very dry with continuous rain-free days of 31 and 39, respectively (Figure 5a and b). However, trial year TE-2014-15 had rainfall on and off during the reproductive stage (Figure 5c).

In the first two rainfed trials (TE-2012-13 and TE-2013-14), Curinga transgenic lines reached 50% flowering significantly earlier than NT Curinga (4 and 5 days earlier), indicating that \textit{Ubi:AtGolS2} overexpression induced earliness in Curinga lines (Table S3). The continuous rain-free period during the reproductive stage caused a marked reduction in soil moisture in the 0-
To understand how the expressing Ubi:AtGolS2 drought tolerance in the different genetic background coincided the grain-filling stage (Figure 5c). Field vegetative stress was mild; there was about 19 days rain-free period that (Figure 6b and c). In the third rainfed trial (TE-2014-15), drought lines in the second (TE-2013-14) and third (TE-2014-15) field trials We also observed yield gains continued in promising transgenic Curinga (Figure 6a and Table S3) in the first trial (TE-2012-13). 49%, 18%, 34% and 17%, respectively, compared to NT showed significantly higher grain yield (GY) with relative gains of 40-cm soil layer (Figure S3). Under these severe stress conditions, lines #2580 and #2590 maintained higher panicle number and #2580 was much better than NT Curinga as shown in Figure 6d. Thus, the average grain yields of promising Ubi:AtGolS2 transgenic lines were significantly increased compared to NT Curinga in three field trials (Figure 6).

Drought tolerance in the different genetic background expressing Ubi:AtGolS2

To understand how the Ubi:AtGolS2 ubiquitously works in a rice genotype, we conducted drought tolerance experiments in homozygous transgenic lines overexpressing Ubi:AtGolS2 in the interspecific hybrid, NERICA4. We first tested seedling survival rate in a glasshouse pot experiment at Japan International Research Center for Agriculture Sciences (JIRCAS) in Japan. Seedling survival of NERICA4 transgenic lines was evaluated through a previously reported method (Ishizaki et al., 2013). Before drought stress treatment, no obvious phenotypic differences were observed between the NT NERICA4 plants and the Ubi:AtGolS2 transgenic NERICA4 lines. After nine days of drought treatment and subsequent recovery for seven days, the majority of NT NERICA4 never recovered and only 11.5% survived. By contrast, four of seven Ubi:AtGolS2 transgenic NERICA4 lines exhibited a significantly higher survival ratio, ranging from 26.2% to 34.5% (Table S4). These results demonstrate that Ubi:AtGolS2 can significantly improve seedling survival under drought in NERICA4.

The Ubi:AtGolS2-NERICA4 lines were also evaluated during the second (TE-2013-14) and third (TE-2014-15) rainfed reproductive trials in Colombia along with Curinga lines; NERICA4 lines were not included in the trial 1 (TE-2012-13) (Figure 7 and Table S5). AquaPro soil moisture profiles indicated that the conditions of NERICA4 plots were similar to those of Curinga plots in TE-2013-14-Trial and TE-2014-15-Trial (Figure S4a and b). Under severe drought stress conditions in the second trial (TE-2013-14), the Ubi:AtGolS2-NERICA4 lines #1577 and #2344 showed significantly higher GY with relative gains of 34% and 49%, respectively, compared to NT NERICA4 (Figure 7a). In the third trial (TE-2014-15), #1577, #2361 and #2362 showed significantly higher grain yield than NT NERICA4 (Figure 7b). Interestingly, line 1577 consistently performed well in both vegetative and reproductive experiments (Table S4 and Figure 7).

Correlation between accumulation level of galactinol and grain yield

To link field performance of the transgenic lines to the expression level of transgene AtGolS2 and the galactinol accumulation levels, we calculated Pearson’s coefficient of correlation between accumulation level of galactinol and mRNA level of AtGolS2, SPY and GY in Curinga and NERICA4 evaluated under field (Figure S5). The expression level of AtGolS2 correlated with the accumulation level of galactinol: Pearson’s coefficient of correlation between those factors was 0.72 in Curinga and 0.94 in NERICA4, revealing
that the expression of AtGolS2 certainly conferred the accumulation of galactinol in rice plant. However, SPY and GY did not always correlate with the accumulation level of galactinol: Pearson’s coefficient of correlation between those factors ranged from -0.05 to 0.65, suggesting the galactinol did not have dose effects on yield under field.

Discussion

Ubi:AtGolS2 is versatile: improving drought tolerance across stages of rice, genetic background, drought intensity and environments

While developing drought-tolerant crops, plant productivity should be taken into consideration. Plant productivity is widely affected by natural drought incidences under field conditions (Todaka et al., 2015). Droughts are random events and dry spells can occur at virtually any time during the rice growing period in drought-prone areas, leading to drought stress of varying intensity. Although rice is highly sensitive to drought stress during the reproductive stage (Venuprasad et al., 2007), drought at early vegetative stage of rice growth can considerably affect plant performance. Commonly, drought survival test during the vegetative stage is obtained under laboratory or glasshouse conditions and is therefore not perfectly comparable to interpretations made under real-field conditions. Extensive field trials are thus critical for the appropriate evaluation of stress-tolerant transgenic crops (Todaka et al., 2015). In this paper, we carried out field trials in CIAT, Colombia, and demonstrated that Ubi:AtGolS2 overexpression in rice was effective at conferring drought tolerance during both the vegetative and the reproductive stages (Figures 2 and 3). It is a very rare phenomenon when the results obtained from vegetative screening experiments concur with reproductive stage, indicating that the Ubi:AtGolS2 overexpression can be exploited for both early- and mid-season drought. This is very important in the perspective of targeting rice varieties to rainfed environments where rainfall uncertainty is expected.

In rice, several reports are available that examine field drought tolerance caused by overexpression of transgenes (Xiao et al., 2009; You et al., 2012; Yu et al., 2013). However, most of these studies were conducted on plants that were grown under glasshouse conditions. There have been instances where a transgene-mediated trait expressed in the glasshouse was unstable under field conditions (Brandle et al., 1995). As it was reported that the effect of transgene expression in wheat varied from year to year based on the climatic conditions of a particular growing season (Bahieldin et al., 2005), it was considered essential to explore the yield stability of the Ubi:AtGolS2

Figure 5 Rainfall and temperature pattern during crop period in upland confined rainfed field trial, CIAT, Santa Rosa upland station. (a) Climatic profile of TE-2012-13-Trial. (b) Climatic profile of TE-2013-14-Trial. (c) Climatic profile of TE-2014-15-Trial. Black bar shows amount of rainfall received during crop period, and dotted line graph shows the maximum daily temperature during trial period. Temperature data are daily averages and rainfall is daily total.
transgenic rice in different seasons, for example rainy and dry and under several environmental conditions such as rainfed and well-watered conditions.

In this study, higher GY in the Ubi:AtGolS2 transgenic lines was consistently observed over the season and environments (Figures 3, 6 and 7). The promising Ubi:AtGolS2 transgenic lines showed significantly enhanced drought tolerance in the field across different genetic backgrounds, Curinga and NERICA4, with a grain yield of 17%–100% higher than NT Curinga under mild to severe drought stress, whereas the transgenic lines displayed no significant differences under normal growth conditions (Figures 3, 6, 7 and Table S1). These improvements of grain

Figure 6 Ubi:AtGolS2 improves Curinga grain yield in Target Environment (TE)—Santa Rosa rainfed Trial, CIAT upland rainfed station, Villavicencio. (a) Grain yield performance of Curinga transgenic lines in TE-2012-13-Trial. (b) Grain yield performance of Curinga transgenic lines in TE-2013-14-Trial. (c) Grain yield performance of Curinga transgenic lines in TE-2014-15-Trial. (d) Field performance of NT Curinga and promising transgenic event 2580 at TE-2013-14-Trial. Photographs were taken during stress at the grain-filling stage. Estimated grain yield (kg/ha) was derived from plot yield from three replications, and value represents the mean ± SE (n = 3). Different letters in each column denote significant differences at P < 0.05 by Tukey–Kramer method.

Figure 7 Ubi:AtGolS2 improves NERICA4 grain yield in rainfed upland trials. (a) Grain yield performance of NERICA4 lines in Target Environment (TE)-2013-14-Trial at Santa Rosa rainfed trial. (b) Grain yield performance of NERICA4 lines in TE-2014-15-Trial at Santa Rosa rainfed trial. Estimated grain yield (kg/ha) derived from plot yield. Estimated grain yield (kg/ha) was derived from plot yield from three replications, and value represents the mean ± SE (n = 3). Different letters in each column denote significant differences at P < 0.05 by Tukey–Kramer method.
yields under drought can be considered greater than what has been reported for other transgenic rice lines expressing genes conferring field drought tolerance, which have often been challenged with milder stresses as demonstrated by the grain yield reduction under drought of the control checks (Oh et al., 2009). However, in this study, the drought intensity was mild because plants were irrigated to evade leaf rolling, which resulted in a yield loss of around 32% in the WT. In another study, rice plants overexpressing OsNAC10 showed enhanced drought tolerance during the flowering stage and increased grain yield by 25%–42% compared to WT, but again milder drought stress conditions were applied (Jeong et al., 2010).

A limited number of studies applied field drought conditions similar to our work (Todaka et al., 2015). Under severe stress, rice transgenic lines expressing OsCPR1 showed 2.5- to 3-fold greater FY over the control, for which yield dropped 90% (Huang et al., 2007). Likewise, plants overexpressing LOS5 and ZAT10 exhibited gains between 11% and 36% compared to their controls which suffered 82% yield reduction (Xiao et al., 2009).

Through this international collaborative project, we realized the importance of conducting the initial screening efforts in a farmer-adapted variety, because these are popular over large growing areas, locally adapted and because relatively quick introgression of the transgene into other megavarieties is possible (Gaudin et al., 2013). In our study, we attempted to improve two farmer-adapted varieties, one from Latin America (Curinga) and another one from Africa (NERICA4). The phenotype might be controlled by genes (G) including transgenes and genetic background (genotypes) in transgenic plants, and plant responses to drought are also influenced by environment (E) including intensity, duration and frequency of the stress as well as by diverse plant-soil-atmosphere interactions (Saint Pierre et al., 2012). It is always suggested to test G × G and G × E interactions/stability before recommending a potential transgene into the breeding pipeline. In this study, we have conducted drought experiments in two contrasting field seasons (rainy and dry). We found the response of the Ubi:AtGolS2 lines to be different than the NT control based on the season. However, regardless of the season, the promising lines #2580 and #2590 had significantly greater plant biomass and panicle numbers. A similar result was found within the target environment.

Our results provide strong evidence that overexpression of AtGolS2 is a useful biotechnological tool to reduce yield losses under field drought conditions under different environmental conditions (E) and in different rice genetic backgrounds (G), which suggests that AtGolS2 is an essential gene to improve drought tolerance in rice regardless of G × G and G × E interactions.

**Mechanism of drought tolerance offered by the Ubi: AtGolS2 transgene**

Even though many stress resistance genes have been identified in noncrop species such as Arabidopsis, evaluation of the effect of these genes on improving field drought tolerance in a given crop has seldom been reported (Xiao et al., 2009). GolS plays a key role in the accumulation of galactinol under abiotic stress conditions, conferring drought stress tolerance to plants, because galactinol may function as osmoprotectants and scavenger of hydroxyl radicals (Nishizawa et al., 2008; Taji et al., 2002). Based on our extensive evaluation of transgenic lines, the Ubi:AtGolS2 transgene improved grain yield of rice under drought conditions. Although high expression of AtGolS2 and accumulation of galactinol were confirmed in Ubi:AtGolS2 events, no significant change in the expression of drought marker genes was induced (Figure 1 and Figure S1). These results suggest that drought tolerance of Ubi:AtGolS2 transgenic lines with the accumulation of galactinol was not correlated with the expression of drought-responsive genes. This could be contributed through the following mechanisms. First, the transgenic lines are more tolerant to drought and gain yield over the NT because they are protected by elevated galactinol (RFOS) that can act as osmoprotectants and scavenger of hydroxyl radicals (Figure 1b). The increased transcription of GolS genes during drought has been reported in many plants and crops. In Cucumis melo, it was observed that GolS activates accumulation of RFO in plants submitted to drought stresses (Volk et al., 2003).

As second physiological perspective, the promising Curinga transgenic lines have a better maximum photochemical efficiency (Fv/Fm) and leaf chlorophyll content than NT under drought stress (Figure 4b). The decrease in Fv/Fm, and SPAD chlorophyll values under drought stress could be an indicator of oxidative stress and damage in PSII (Farooq et al., 2009). Under severe drought stress, we observed high SPAD chlorophyll and Fv/Fm values in the Ubi: AtGolS2 Curinga than in NT Curinga, and the Ubi:AtGolS2 leaves were greener than those of the NT, which confirmed normal photosynthesis in transgenic Ubi:AtGolS2 rice. In addition to photosynthetic-related traits, stress-related traits such as RWC, leaf rolling and drying of transgenic lines were significantly better than NT Curinga (Tables S2, S3 and S5). Maintenance of high plant water status, as expressed in high RWC of the Ubi:AtGolS2 rice, was an good indicator of drought tolerance (as shown by Babu et al., 2004), and capacity of transgenic lines maintained higher leaf RWC compared with NT Curinga under drought stress, which was consistent with their ability to postpone dehydration (as indicated by Castonguay and Markhart, 1992). In this study, we observed that the Ubi:AtGolS2 transgenic lines had higher RWC values than NT rice during peak drought stress and had a lower leaf rolling and leaf drying score (Figure 4a; Tables S2, S3 and S5). By contrast, we observed that NT rice quickly wilted and dried as compared to those transgenic lines with higher RWC values under drought stress.

Third, the AtGolS2 Curinga transgenic lines showed earlier flowering than NT Curinga (Table S3) under drought stress, but displayed no difference in growth under normal growth conditions (Table S1). In rice, early maturation is an escape mechanism to ensure production under conditions of stress (Gur et al., 2010). In this study, the Ubi:AtGolS2 Curinga lines showed the earliest flowering and exhibited higher grain fertility (82%) than NT (70%), which may be due to the drought escape mechanism of transgenic lines (data not shown). However, early flowering was not observed in the Ubi:AtGolS2 NERICA4 background (Table S5). As NERICA4 is a short-duration variety, early flowering of the Ubi: AtGolS2 lines may be profound in Curinga due to the long duration nature.

Transcription levels of the AtGolS2 gene of the transgenic lines correlated with the accumulation level of galactinol; however, the accumulation level of galactinol did not correspond with their field performance (Figures 1 and S5). These results suggest that good field performance might not always be associated with levels of gene expression and of accumulation of galactinol. The complexities of environments and other factors influencing performance of rice plants under drought may be reasons for no dosage effects of galactinol on grain yield under field.
Conclusions and prospects

Our study reported extensive field evaluation of transgenic rice plants expressing the Ubi:AtGolS2 transgene under drought stress environments under field conditions in Colombia. We clearly observed that the Ubi:AtGolS2 expression and the accumulation of galactinol significantly enhanced grain yield under drought field conditions, but did not affect either grain yield or plant growth under well-watered paddy field conditions. Improved grain yield under stress was associated with early flowering, higher biomass accumulation, higher number of panicles and lower panicle sterility.

In this study, the same gene construct, the Ubi:AtGolS2 transgene was tested on two different commercial genetic backgrounds, Curinga and NERICA4, and contrasting different seasons and different environments. We presented the results of extensive confined field testing of transgenic rice overexpressing AtGolS2 and the responses of these transgenic rice plants to contrasting environments. Notably, we evaluated the agronomic traits of these transgenic lines at all stages of plant growth in the field as a function of the environment and genetic background. As the Ubi:AtGolS2 transgene was tested in the commercial rice genetic backgrounds of Latin America (Curinga) and Africa (NERICA4), we think it is easy to pyramid the Ubi:AtGolS2 transgene into ongoing transgenic rice breeding programmes in Latin America and Africa. The promising NERICA4 transgenic lines selected from this study can be integrated into ongoing NEWEST—the NERICA4 (Nitrogen-use Efficient, Water-use Efficient and Salt Tolerant) rice project where extensive transgenic field trials are currently being implemented in Ghana, Uganda and Nigeria through USAID feed the future programme. Development of this drought-tolerant rice through the Ubi:AtGolS2 transgene should have significant economic and environmental benefits in low-input agricultural systems like Latin America and Africa.

Experimental procedures

Generation of Ubi:AtGolS2 Plants

To generate transgenic rice plants overexpressing AtGolS2 encoding galactinol synthase 2 of Arabidopsis thaliana (Taji et al., 2002), the pBIG-ubi vector was used (Becker, 1990; Ito et al., 2006). AtGolS2 cDNA was amplified using BamHI linker primers. The resulting DNA fragment carrying BamHI sites at the 5' and 3' termini was inserted into pBIG-ubi at the BamHI site. The construct was introduced into rice cv. Curinga and NERICA4 by Agrobacterium-mediated transformation as described previously (Ishizaki and Kumashiro, 2008; Zuniga-Soto et al., 2015). The molecular characterization of putative transgenic events involved PCR and Southern blot analysis. The primers used for this study are reported in Table S6.

Expression analysis of rice plants expressing Ubi:AtGolS2

The transgenic and nontransgenic (NT) rice plants (Curinga and NERICA4) were grown in soil-filled, open-bottomed 50-ml plastic tubes in the glasshouse. After the drought treatment, the leaves from five plants were collected, frozen in liquid nitrogen and stored at -80 °C. Total RNA was isolated from the leaf samples using RNAiso Plus reagent (Takara Bio, Shiga, Japan). Extracted RNA was subjected to a DNase treatment using a RQ1 DNase (Promega, WI), and complementary DNA was synthesized using a PrimeScript RT Master Mix (Takara Bio). Real-time quantitative RT-PCR was performed with the QuantStudio 7 Flex real-time PCR system (Thermo Fisher Scientific, MA) using SYBR Premix Ex Taq (Takara Bio). Primers used for qRT-PCR are listed in Table S6.

Sugar metabolite analysis of rice plants expressing Ubi: AtGolS2

The transgenic and control rice lines were grown in soil-filled, open-bottomed 50-ml plastic tubes in the glasshouse. After the drought treatment, the leaves from five plants were collected, frozen in liquid nitrogen and stored at -80 °C. Sugar metabolites were analysed using GC-TOF-MS as described previously by Maruyama et al. (2014).

Seedling survival test of NERICA4 transgenic events

The ability of NERICA4 transgenic events to survive under rapid drying was evaluated by the reported method (Ishizaki et al., 2013).

Vegetative drought stress experiment in confined field

To evaluate the drought tolerance of transgenic rice plants at vegetative stage, single-copy independent homozygous lines of Ubi:AtGolS2 Curinga transgenic lines, together with nontransgenic (NT) Curinga controls, were direct-seeded in confined field conditions under a rainout shelter at the International Center for Tropical Agriculture (CIAT), Palmira, Colombia, in the dry season, November–December 2011. A randomized block design was employed with three replicates with each event sown in two rows placed 16 cm apart in a rainout shelter where the depth of restructured soil was 85 cm. Each row was 1 m long and 40 plants were accommodated in each row with equal spacing (5 cm) between plants. Drought stress was imposed by withholding irrigation at initial tillering stage (21 days after direct sowing) and rewatered after 21 days (3 weeks) until severe wilting symptoms appeared in NT Curinga (Figure 2a). The intensity of drought was monitored through AquaPro soil moisture probes (AquaPro sensors Inc, California, USA). Plant height, the number of tillers and destructive plant dry biomass data were measured from uniform tagged plants at the before, during peak stress and at the end of the harvest after rewatering. Leaf rolling was determined at the time of peak drought stress.

Managed Drought Stress Environment (MDSE) Trial—Rainout Shelter (RS) reproductive Stage trial at CIAT, Palmira, Colombia

All rainout shelter reproductive stress experiments were carried out at our confined field facility at CIAT, Palmira, Colombia. For Curinga, three confined field drought trials (from 2012 to 2014) over two contrasting seasons were conducted under the movable semi-automatic rainout shelter facility. All three experiments were conducted using same protocol with respect to designs and field drought characterization. A randomized block design with three replications was followed to layout the experiment under the rainout shelter facility at CIAT. The seeds of T4 homozygous lines were sown in the dry soil of the experimental plots in rows (20 cm spacing between rows). Each event was sown in two rows placed 20 cm apart where the depth of restructured soil was 85 cm. Each row was 2 m long and had 20 plants with equal spacing. The recommended fertilizer application for upland rice was used. Drought was imposed by withholding irrigation when panicle initiation was around 10 mm long (60–66 days after sowing in the case of Curinga) for 3–4 weeks (or) until severe leaf rolling and drying appeared in the NT control. Then, the plants were rewatered to 90% field capacity until physiological maturity. The intensity of drought was monitored through AquaPro soil moisture probes.
moisture probes that were installed to measure moisture in the soil profile to a depth of 0.85 m.

Leaf rolling (LR), leaf drying (LD) and drought recovery scores were recorded on a 1-9 IRRI scale standardized for rice (IRRI, 2002). The following agronomic traits were measured based on the criteria established in the Standard Evaluation System for Rice (SES) (IRRI, 2002): flowering date, plant height (cm), single plant dry biomass (g), panicle length (cm), the number of tillers, the number of fully emerged panicles and grain fertility (%). Single plant yield (from five more uniform tagged plants from each block with three replications) and plot yield were also recorded.

The degree of relative chlorophyll content in the fully expanded flag leaf was determined while the plant was under stress, using a SPAD-502 Chlorophyll Meter (Konica Minolta Co., Tokyo, Japan). Chlorophyll a fluorescence parameters were also measured using a FluorPen FP100 chlorophyll (Photon Systems Instruments, spol. s r.o., Czech Republic). Relative water content was calculated using the protocol based on Schonfeld et al. (1988).

Well-watered experiment in confined field conditions

To evaluate the yield components of the transgenic Curinga lines under normal well-watered field conditions, selected independent T₄ homozygous lines of Ubi:AtGolS2 transgenic plants together with NT controls were transplanted to a rice paddy confined field at CIAT, Palmira (dry season, September–January 2013). A randomized design was employed with three replicates of two 2-m-long rows per plot. For each plot, 20 seedlings per line were randomly transplanted with a 20 x 10 cm spacing 25 days after sowing. The recommended fertilizer application for lowland rice was used. Yield parameters were scored for five tagged uniform plants per plot.

RT-PCR analysis of field samples—Rainout shelter trials

Total RNA was extracted from the flag leaves of tested promising transgenic lines at before stress, peak stress and after rewatering from the 2014-rainy-MDSE-rainout shelter trial using the Trizol reagent (Invitrogen). Reverse transcription was carried out using DNase (Promega) and SuperScript II (Invitrogen). Endpoint PCR was conducted with primers listed in Table S6, using standard protocols and an annealing temperature of 55 °C. PCR products were checked on 1% agarose gel with SYBR-safe stain.

Target Environment (TE) Trial—Confined Field Evaluation of Transgenic lines at Santa Rosa, Colombia

To evaluate yield components of transgenic plants under rainfed upland conditions with natural drought condition, the most promising independent T₄ homozygous lines of Curinga and NERICA4 from the previous drought experiments along with their NT controls were evaluated in a replicated plot trial with randomized block design from 2013 to 2015 (three consecutive field trials) at CIAT Santa Rosa rainfed upland station, Colombia. Promising NERICA4 lines were selected based on the survival test results at Japan and rainout shelter trials. An upland field trial was laid out in a random complete block design with three replications. The transgenic events along with NT rice were sown in 2 x 2 m plots with 25 x 10 cm spacing. Seeds were sown directly by hand at the rate of 120 kg/ha when soil moisture was about 80% of field capacity. The recommended fertilizer application for upland rice was used. The plants were established in dry soil and irrigation was provided until 50 days after sowing (DAS) via sprinklers to establish the crop. After plant establishment, irrigation was stopped and plants were totally dependent on rainfall. The soil moisture was monitored throughout the cropping period by Aquapro soil moisture device. Plant growth and development of each of the transgenic events relative to NT rice was monitored regularly and plot yield (g) was recorded. Grain yield (kg/ha) was estimated from plot yield based on plant density.

Data analysis

Data were analysed by one-factor ANOVA at P < 0.05. When significant differences were found, multiple comparisons by the Tukey–Kramer method (P < 0.05) were made.

Acknowledgements

We are grateful for technical support provided by Emiko Kishi and Mihoko Kishimoto of JIRCAS. We also thank Dr. Jagadish Ranne, AL.Chavez, Cesar Zuluaga, Santiago Jaramillo and Maria Recko of CIAT for the technical assistance and Dr. Joe Tohme, Director of Agrobiodiversity Research Area of CIAT, for critical discussion on field phenotyping. We also thank Richard Bruton, Texas A&M, and Angela Fernando, CIAT, for their critical English edition and suggestions of the manuscript. This work was supported by the grants from the Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan for a project: Development of Drought-Tolerant Crops for Developing Countries. The authors declare no conflict of interest.

Author contributions

K.S., K.N. and M.I designed the total experiments. M. G. S., S.O. and M.O.V. planned, conducted and analysed drought field experiments. B.D. and M.I. conducted transformation experiments at CIAT. T.I. conducted transformation experiments and glasshouse experiments at JIRCAS. T.O. and K.N. conducted gene expression experiments. K.Y., K. M., M. K. and K. S. conducted sugar analysis. F.T. made the construct for transformation. M.G.S. wrote the manuscript, and all the authors checked it.

Ethical standards

The authors declare that the transgenic experiments comply with the current biosafety laws of the country in which they were performed.

References

Ahuja, I., de Vos, R.C., Bones, A.M. and Hall, R.D. (2010) Plant molecular stress responses face climate change. Trends Plant Sci. 15, 664–674.
Babu, R.C., Zhang, J., Blum, A., Ho, T.-H.D., Wu, R. and Nguyen, H. (2004) HvA1, a LEA gene from barley confers dehydration tolerance in transgenic rice (Oryza sativa L.) via cell membrane protection. Plant Sci. 166, 855–862.
Beahdilin, A., Mahlouf, H.T., Eissa, H.F., Saleh, O.M., Ramadan, A.M., Ahmed, I.A., Dyer, W.E. et al. (2005) Field evaluation of transgenic wheat plants stably expressing the HvA1 gene for drought tolerance. Physiol. Plantarum, 123, 421–427.
Becker, D. (1990) Binary vectors which allow the exchange of plant selectable markers and reporter genes. Nucleic Acids Res. 18, 203.
Brandl, J., McHugh, S., James, L., Labbe, H. and Miki, B. (1995) Instability of transgene expression in field grown tobacco carrying the csr1-1 gene for sulfonylurea herbicide resistance. Nat. Biotechnol. 13, 994-998.
Cassman, K.G., Dobermann, A., Walters, D.T. and Yang, H. (2003) Meeting cereal demand while protecting natural resources and improving environmental quality. Annu. Rev. Environ. Resour. 28, 315–358.
Castonguay, Y. and Markhart, A.H. (1992) Leaf gas exchange in water-stressed common bean and tepary bean. Crop Sci. 32, 980–986.

Collett, H., Shen, A., Gardner, M., Farrant, J.M., Denby, K.J. and Illing, N. (2004) Towards transcript profiling of desiccation tolerance in Xerophyta humilis: construction of a normalized 11 k X humilis cDNA set and microarray expression analysis of 424 cDNAs in response to dehydration. Physiol. Plantarum. 122, 39–53.

Edmeades, G.O. (2008) Drought tolerance in maize: an emerging reality: a feature in Climate. 2008. Global Status of Commercialized Biotech/GM Crops: 2008. In: ISAAA Brief No. 39. (Greg O., Edmeades, ed), pp. 1–11. Ithaca, NY: ISAAA.

FAO (2016) Rice Market Monitor, April 2016. Volume XIX Issue No. 1 available at http://www.fao.org/fileadmin/templates/est/COMM_MARKETS MONITORING/Rice/Images/RMM/MMM_ARP16.pdf.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D. and Basra, S.M.A. (2009) Plant drought stress: effects, mechanisms and management. Agron. Sustain. Dev. 29, 185–212.

Farrant, J.M. (2007) Mechanisms of desiccation tolerance in angiosperm resurrection plants. In Plant Desiccation Tolerance (Jenks, M.A. and W.A.J., eds), pp. 51–90. Oxford, UK: Blackwell.

Gaudin, A.C., Henry, A., Sparks, A.H. and Slamet-Loedin, I.H. (2013) Taking transgenic rice drought screening to the field. J. Exp. Bot. 64, 109–117.

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J. et al. (2010) Food security: the challenge of feeding 9 billion people. Science, 327, 812–818.

Gur, A., Osorio, S., Fridman, E., Fernie, A.R. and Zamir, D. (2010) h2-1, A QTL which improves harvest index, earliness and alters metabolite accumulation of processing tomatoes. Theor. Appl. Genet. 121, 1587–1599.

Hare, P., Cress, W. and Van Staden, J. (1998) Dissecting the roles of osmolyte accumulation during stress. Plant. Cell Environ. 21, 535–553.

Huang, Y., Xiao, B. and Xiong, L. (2007) Characterization of a stress responsive proteinase inhibitor gene with positive effect in improving drought resistance in rice. Planta, 226, 73–85.

IRRI. (2002) Standard Evaluation System (SES), pp. 11–30. Manila, Philippines: International Rice Research Institute.

Ishizaki, T., Maruyama, K., Obara, M., Fukutani, A., Yamaguchi-Shinozaki, K., Ito, Y. and Kumashiro, T. (2013) Expression of Arabidopsis DREB1C improves survival, growth, and yield of upland New Rice for Africa (NERICA) under drought. Mol Breed. 31, 255–264.

Ito, H., Iwamoto, I., Morishita, R., Nozawa, Y., Asano, T. and Nagata, K.-I. (2006) Identification of a PDZ protein, PIST, as a binding partner for Rho effector Rethokin: biochemical and cell-biological characterization of Rethokin-PIST interaction. Biochem. J. 397, 389–398.

Jeong, J.S., Kim, Y.S., Baek, K.H., Jung, H., Ha, S.H., Do Choi, Y., Kim, M. et al. (2010) Root-specific expression of OsNAC9 improves drought tolerance and grain yield in rice under field drought conditions. Plant Physiol. 153, 185–197.

Jeong, J.S., Kim, Y.S., Redillas, M.C., Jang, G., Jung, H., Bang, S.W., Choi, Y.D., Ha, S.H. et al. (2012) The overexpression of OsNAC9 alters the root architecture of rice plants enhancing drought resistance and grain yield under field conditions. Plant Biotech. J. 10, 792–805.

Saint Pierre, C., Clossa, J.L., Bonnett, D., Yamaguchi-Shinozaki, K. and Reynolds, M.P. (2012) Phenotyping transgenic wheat for drought resistance. J. Exp. Bot. 63, 1799–1808.

Saravitz, D.M., Pharr, D.M. and Carter, T.E. (1987) Galactinol synthase activity in resurrection plants. In James, Clive. 2008. Global Status of Commercialized Biotech/GM Crops: ISAAA Brief No. 39 (Greg O., Edmeades, ed), pp. 1–79. Ithaca, NY: ISAAA.
Zuniga-Soto, E., Mullins, E. and Dedicova, B. (2015) Ensifer-mediated transformation: an efficient non-Agrobacterium protocol for the genetic modification of rice. SpringerPlus, 4, 600.

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1 Expression analysis of drought responsive genes in Ubi:AtGolS2 transgenic rice.

Figure S2 Aquapro soil moisture profile and work schedules of Curinga plots during rainout shelter managed drought stress environment (MDSE) trial, CIAT, Palmira.

Figure S3 Aquapro soil moisture profile and work schedules of Curinga plots during Target environment (TE) trial, CIAT, Santa Rosa upland rainfed station.

Figure S4 Aquapro soil moisture profile and work schedules of NERICA4 plots during Target Environment (TE) trial, CIAT, Santa Rosa upland rainfed station.

Figure S5 Pearson’s correlation coefficient between accumulation level of galactinol and mRNA level of AtGolS2, single plant yield (SPY), and grain yield (GY) in Curinga and NERICA4 evaluated under field.

Table S1 Agronomic data capture from 2012 well-watered paddy field trial at CIAT, Palmira, using Ubi:AtGolS2 Curinga lines.

Table S2 Agronomic data capture from rainout-shelter trial (2012-rainy-MDSE-Trial-1, 2012-dry-MDSE-Trial-2, and 2014-rainy-Trial) at CIAT, Palmira using Ubi:AtGolS2 Curinga transgenic lines.

Table S3 Agronomic data capture from Santa Rosa target Environment (TE) upland trial using Ubi:AtGolS2 Curinga lines.

Table S4 The survival rates of NT NERICA4, Ubi:AtGolS2 NERICA4 lines under drought stress.

Table S5 Agronomic data capture from Santa Rosa target environment (TE) trial using Ubi:AtGolS2 NERICA4 lines.

Table S6 Primer sequences used in this study.