



Drought stress-induced compositional changes in tolerant transgenic rice and its wild type



Kyong-Hee Nam^a, Do-Young Kim^a, Hee Jae Shin^a, Ki Jung Nam^a, Joo Hee An^a, In-Soon Pack^a, Jung-Ho Park^a, Soon-Chun Jeong^a, Ho Bang Kim^b, Chang-Gi Kim^{a,*}

^aBio-Evaluation Center, KRIBB, 30 Yeongudanji-ro, Ochang-eup, Cheongwon, 363-883, Republic of Korea

^bLife Sciences Research Institute, Biomedic Co., Ltd., Bucheon 420-020, Republic of Korea

ARTICLE INFO

Article history:

Received 27 June 2013

Received in revised form 11 November 2013

Accepted 10 December 2013

Available online 16 December 2013

Keywords:

Chemical composition

Drought tolerance

Food safety assessment

Oryza sativa L.

Rainout shelter

Transgenic crop

ABSTRACT

Comparing well-watered versus deficit conditions, we evaluated the chemical composition of grains harvested from wild-type (WT) and drought-tolerant, transgenic rice (*Oryza sativa* L.). The latter had been developed by inserting *AtCYP78A7*, which encodes a cytochrome P450 protein. Two transgenic lines, '10B-5' and '18A-4', and the 'Hwayoung' WT were grown under a rainout shelter. After the harvested grains were polished, their levels of key components, including proximates, amino acids, fatty acids, minerals and vitamins were analysed to determine the effect of watering system and genotype. Drought treatment significantly influenced the levels of some nutritional components in both transgenic and WT grains. In particular, the amounts of lignoceric acid and copper in the WT decreased by 12.6% and 39.5%, respectively, by drought stress, whereas those of copper and potassium in the transgenics rose by 88.1–113.3% and 10.4–11.9%, respectively, under water-deficit conditions.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Diverse abiotic stresses can influence plants throughout their life cycles. Drought is one of the greatest constraints on growth and development. In response to a water deficit, plants decrease their photochemical efficiency and activity by ribulose-1,5-bisphosphate carboxylase/oxygenase, while increasing the levels of stress metabolites such as proline, poly-sugars, glycine betaine, glutathione, carotenoids and abscisic acid (ABA) (Guha, Sengupta, Rasineni, & Reddy, 2010; Reddy, Chaitanya, & Vivekanandan, 2004). Drought is also associated with alterations in lipid metabolism, including the inhibition of lipid biosynthesis and stimulation of antioxidant activity (Basu, Roychoudhury, Saha, & Sengupta, 2010; Monteiro de Paula et al., 1993). Understanding these biochemical changes in response to such stress can assist researchers in the development of drought-tolerant transgenic plants.

AtNCED3 encodes 9-*cis*-epoxycarotenoid dioxygenase, a key enzyme in ABA biosynthesis. Overexpression of this gene elevates tolerance to drought in transformed *Arabidopsis* (Iuchi et al., 2001). Transgenic plants over-expressing the galactinol synthase gene

accumulate endogenous galactinol and raffinose and have improved drought tolerance (Taji et al., 2002). Moreover, the accumulation of proline in transgenic plants over-expressing proline dehydrogenase, *ProDH*, is more resistant to the effects of osmotic stress (Nanjo et al., 1999).

Rice (*Oryza sativa* L.) is one of the most important crops worldwide, serving as the staple food for more than half of the world's population. Rain-fed environments comprise a large percentage of the global region for its cultivation, and drought stress greatly deteriorates both grain quantity and quality. For rice, transcription factors, including family members for the dehydration responsive element, ABA responsive element, basic leucine zipper protein and NAC (NAM, ATAF and CUC) have been employed to generate a drought-tolerant trait in this crop (Kang, Choi, Im, & Kim, 2002; Lu, Gao, Zheng, & Han, 2009; Oh et al., 2005; Zheng, Chen, Lu, & Han, 2009). However, none of these transgenic lines have yet been commercialised (James, 2011). Approval of transgenic crops as a food source requires an extensive and systematic safety assessment. In particular, because the overexpression of genes regulating signal transduction and plant metabolism may have unintended consequences, more careful considerations are essential for ensuring the food safety of drought-tolerant crops.

To our knowledge, research has not yet been reported on the metabolic responses of drought-tolerant transgenic rice under practical water-limited conditions. Most studies related to drought have

Abbreviations: ABA, abscisic acid; AOAC, Association of Official Analytical Chemists; MFDS, Ministry of Food and Drug Safety; WT, wild type.

* Corresponding author. Tel.: +82 43 240 6543; fax: +82 43 240 6549.

E-mail address: cgkim@kribb.re.kr (C.-G. Kim).

focused on vegetative organs, such as leaves and roots, rather than on edible parts. Realizing the influence of drought on the overall chemical composition of the grain is important not only in terms of food-safety assessments, but also for nutritional quality.

Previously, we analysed the key nutrients in drought-tolerant rice, which was developed by inserting *AtCYP78A* to encode a cytochrome P450 protein. By growing the plants in a paddy field under conventional water management practices, we were able to confirm substantial equivalence between transgenic and non-transgenic rice (Nam et al., 2013). As a continuation of that earlier work, we now describe the compositional differences between drought-tolerant rice and its wild type (WT). This current evaluation was conducted for both well-watered and water-deficit systems, using a rainout shelter under field conditions.

2. Materials and methods

2.1. Establishment of the rainout shelter

The rainout shelter was built in an experimental field at the Korea Research Institute of Bioscience and Biotechnology (KRIBB), Cheongwon-gun, Republic of Korea (36°43'N, 127°26'E; elevation 35 m). It was divided into two systems: well-watered and water-deficit. The container within each system was 21 m long, 6 m wide and 1.1 m deep. Four valves were installed in holes on the left- and right-hand walls, at 15 cm from both the top and bottom of the container, so that water could either be added or drained. Each container had a 15-cm-deep gravel layer on the bottom to facilitate water drainage, which was then topped with an 80-cm-deep layer of field soil. To exclude rainfall from designated plots in the containers, the shelter was equipped with a moisture sensor so that the polyvinyl roof and sides of the system could close automatically during rain events but re-open under dry conditions.

Water was supplied via sub-surface drip irrigation. The driplines were installed at the soil surface, with emitters spaced at 0.2 m and each having a flow rate of 1.49 l h⁻¹. In each container, three EasyAG soil moisture sensors (Sentek, Australia) were installed at depths of 10, 20, 30, 40, and 50 cm, and changes in water content were monitored at 30-min intervals.

2.2. Plant materials

Drought-tolerant transgenic rice was developed by inserting *AtCYP78A7*, a gene derived from *Arabidopsis thaliana* that encodes a cytochrome P450 protein. For overexpression, the PCR product containing this gene was cloned between the *CaMV* 35S promoter and *ocs3'* of pART7. The transgenic cassette was sub-cloned into the pCAMBIA1301 binary vector. The binary vector construct was then transformed, via electroporation, into rice cv. Hwayoung by *Agrobacterium tumefaciens* strain AGL1. Finally, we used Southern blot hybridisation and inverse polymerase chain reactions to verify that a single copy of *AtCYP78A7* had been inserted into transgenic lines '10B-5' and '18A-4'.

2.3. Growing conditions

Seeds of the 'Hwayoung' WT and Lines '10B-5' and '18A-4' were sterilised for 24 h with prochloraz and fludioxonil solutions, then pre-germinated at room temperature for 72 h. The seeds were then sown in a seedbed filled with rice seedling culture soil (Chamgrow Co., Hongseong, Korea) and allowed to grow in a greenhouse maintained with day/night temperatures of 28 ± 3 °C/22 ± 3 °C and long-day (16 h light/8 h dark) conditions. When 5 weeks old, the seedlings were transplanted into soil in the rainout shelter. Both well-watered and water-deficit systems were established in three

replicated plots in a randomised complete block design. In each plot, 114 seedlings were placed on 30 cm × 15 cm spacings. Before the drought treatments were applied, all seedlings in both systems were irrigated according to conventional practices to strengthen the rooting ability. As a basal treatment, fertiliser was applied before transplanting to provide the equivalent of 4.6, 5.4 and 5.2 kg per 10 a of N, P, and K, respectively.

2.4. Drought treatments

Drought stress was imposed as the tillering stage began (17 d after transplanting). For plots in the water-deficit system, irrigation was stopped for 2 weeks, then resumed for another week before being halted again until harvesting. In the well-watered system, plants received 30 min of daily irrigation, which continued for 13 weeks after transplanting. Samples for analysing chemical compositions were collected at 18 weeks post-transplanting.

2.5. Compositional analysis of brown rice

Whole grains collected from each plot were hulled by a rice-polishing machine (SY88-TH; Ssang Yung Machine Industry Co., Ltd, Incheon, Korea) to obtain brown rice. The samples were then ground to a fine powder for compositional analysis. Contents of moisture, crude protein, crude fat, crude ash, and crude fibre were measured by oven-drying, the Kjeldahl method, the Soxhlet extraction method, dry-ashing and Henneberg–Stohman protocols, respectively, according to the AOAC official requirements (AOAC, 2000, 2005, 2006). Carbohydrate levels were calculated by the following formula: % carbohydrates = 100% – (% crude protein + % crude fat + % crude ash). The concentrations of all amino acids, except for tryptophan, were determined with an automatic amino acid analyser (Biochrom 30 Amino Acid Analyzer; Biochrom, Cambridge, UK) after hydrolysis with hydrochloric acid, according to the procedure stipulated by the Korean Food Code (MFDS, 2011). For tryptophan, a sodium hydroxide solution was added before hydrolysis, and analysis was carried out with HPLC system (1100 Series; Agilent, Santa Clara, CA, USA). The fatty acid profile for samples extracted with hydrochloric acid and diethyl ether was evaluated on a gas chromatograph (7890 GC; Agilent) in accordance with the AOAC method (AOAC, 2005). Mineral elements were monitored after nitric acid treatment, using an inductively coupled plasma-optical emission spectrometer (Optima 8300; PerkinElmer, Waltham, MA, USA) and a UV/VIS spectrophotometer (Optizen 2120 UV Plus; Mecasys, Daejeon, Korea), according to the method in the Korean Food Code (MFDS, 2011). Concentrations of vitamin B₁ and B₂ were analysed by LC–MS/MS (API 4000; AB Sciex, Framingham, MA, USA) based on the procedure described by Martins-Junior et al. (2008). Vitamin E levels were evaluated by HPLC (1100 Series; Agilent) according to the AOAC method (AOAC, 2005).

2.6. Statistical analysis

All statistical analyses were conducted with STATISTICA (Version 8.0; StatSoft Inc., Tulsa, OK, USA). The effects of watering system and genotype on the level of each grain component were assessed by two-way ANOVA. Statistically significant differences between means were identified by a Tukey's HSD test ($p < 0.05$).

3. Results

3.1. Changes in soil moisture contents between well-watered and water-deficit systems

In the water-deficit system, the soil moisture content declined during the first 2 weeks of drought treatment (Fig. 1). Afterward,

Download English Version:

<https://daneshyari.com/en/article/7598312>

Download Persian Version:

<https://daneshyari.com/article/7598312>

[Daneshyari.com](https://daneshyari.com)