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Comparative nutritional compositions and proteomics analysis of transgenic Xa21 rice seeds compared to conventional rice

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ABSTRACT

Transgenic rice expressing the *Xa21* gene have enhanced resistant to most devastating bacterial blight diseases caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). However, identification of unintended modifications, owing to the genetic modification, is an important aspect of transgenic crop safety assessment. In this study, the nutritional compositions of seeds from transgenic rice plants expressing the *Xa21* gene were compared against non-transgenic rice seeds. In addition, to detect any changes in protein translation levels as a result of *Xa21* gene expression, rice seed proteome analyses were also performed by two-dimensional gel electrophoresis. No significant differences were found in the nutritional compositions (proximate components, amino acids, minerals, vitamins and anti-nutrients) of the transgenic and non-transgenic rice seeds. Although gel electrophoresis identified 11 proteins that were differentially expressed between the transgenic and non-transgenic seed, only one of these (with a 20-fold up-regulation in the transgenic seed) shows nutrient reservoir activity. No new toxins or allergens were detected in the transgenic seeds.

1. Introduction

Owing to its high nutritional content, rice (*Oryza Sativa* L.) is one of the most important food crops, providing 21% of the dietary energy and 15% of the protein for the developing world (Bhullar & Gruissem, 2013). Currently, the world population is about 7.3 billion, and with projected growth to 8.0 billion by 2020 (Datta, 2004) rice production will need to increase by 25–40% over the next five years to match current daily consumption levels. Rice production has increased over the last decade in response to the demands of the growing world population, though a huge amount of rice crop is also being lost due to different abiotic and biotic stresses. Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most devastating diseases afflicting rice and can reduce crops in tropical and temperate regions by as much as 80% of total initial production (Kumar et al., 2013). In pursuit of a rice variety that is resistant to bacterial blight, the *Xa21* gene was previously inserted into the *indica* rice variety IR72 through particle bombardment (Tu et al., 1998).

Genetic modification in conjunction with a conventional breeding program may play a major role in the development of improved varieties of rice exhibiting better nutrition and biotic and abiotic stress tolerance. With the development of such improved traits, however, unintended modification in the genome may change the gene expression profile, which can modulate biochemical pathways in plants (Ioset et al., 2007). These unforseen changes, arising from the integration of a modified gene or the interaction between gene products and the endogenous genome of the genetically modified organism (GMO), can in turn be analyzed by examination of transgene integration sites, transgene function, proteomics, and transgene-related metabolic pathways. Transgene integration may cause unintended alterations of the genome by deletions, insertions, or rearrangement, which are responsible for the pleiotropic effects (Cellini et al., 2004; García-Cañas, Simó, León, Ibáñez, & Cifuentes, 2010; Kuiper, Kleter, Noteborn, & Kok, 2001). Therefore, evaluation of the safety of genetically modified crops is of vital importance for eventual commercialization of transgenic crops (Herman, 2011; Rayan & Abbott, 2015; Wang et al., 2012) and a systematic comparative analysis can provide important revelation of unforeseen effects (Cellini et al., 2004; García-Cañas et al., 2010). The wide implementation of such studies and assessment of overall biosafety of GMOs is of increasing importance as their worldwide commercialization becomes more widespread (Agapito-Tenfen, Guerra, Wikmark, & Nodari, 2013).

The OECD (Organization for Economic Cooperation and Development), World Health Organization, Food and Agriculture Organization of the United Nations, and Codex Alimentarius Commission

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have all played significant roles internationally in setting standards for and assessing the safety of genetically modified food products (Kitta, 2013). In particular, the substantial equivalent study has been formulated by the OECD for extensive comparative studies of essential macro- and micronutrients and anti-nutrients in GMO crops and their corresponding controls (OECD, 2004). Within just the last decade, many substantial equivalence studies have been performed to assess the safety of GMOs with respect to conventional counterparts (Xue, Yang, Liu, & Xue, 2012). Targeted analysis of known compounds of high nutritional quality have also been analyzed for biosafety comparison with genetically modified crops. More recently, non-targeted proteomics profiling has become a promising tool to comprehend the changes on a translation level due to integration of a particular gene. Newly expressed proteins can serve many important roles in trait improvement, while at the same time they may also act as toxins, anti-nutrient factors, or allergens and thus have detrimental effects on human or animal health. Therefore, comparative proteomics is another important strategy in the comprehensive assessment of genetically modified organisms (Xue et al., 2012). Here, we present the results of a comprehensive proteomic profile and nutritional quality assessment of genetically modified bacterial blight-resistant rice plant, Xa21, and the non-transgenic parent IR72 rice, and discuss the relevance of proteome changes in the overall nutritional efficacy of Xa21.

2. Materials and methods

2.1. Rice sample

Homozygous transgenic bacterial blight (BB) registrant rice line used in this study was developed by integration of *Xa21* gene into the genome of elite *indica* rice cultivar IR72 (Tu et al., 1998). The transgenic Xa21 and control IR72 rice was grown under the greenhouse condition for substantial equivalence analysis. After harvest, rice seeds were dried to obtain final moisture content around 14%.

2.2. Proximate analysis

The entire proximate component such as total protein, ash, carbohydrate, crude fat and energy content of transgenic Xa21 rice seeds was analyzed following the protocol as described in previous study (Gayen, Sarkar, Datta, & Datta, 2013). The moisture content was measured by gravimetric analysis by drying at 105 °C (AOAC, 1990). Crude protein content was measured by total nitrogen content using the kjeldahl method (AOAC, 2000). Ash content was measured by gravimetric method after ignition of rice sample in a muffle furnace at a 600 °C temperature for 12 h (AOAC, 1990). Crude fat content was measured by Soxhlet apparatus using *n*-hexane (AOAC, 1990). Carbohydrate was measured following the method reported by Gayen et al. (2013).

2.3. Amino acid analysis

Amino acid analysis was performed using AccQ-Tag method (Gayen et al., 2014). About 20 mg of rice power was digested with 2 ml of 6 N HCl containing 0.1% phenol at 110 °C temperature for 16 h in the closed glass vial. The digested sample was filtered through 0.22 μm syringe filter. 100 μl of the clear extract was neutralised with 100 μl of 6 N NaOH. 10 μl of digested rice sample was taken in 1.5 ml microcentrifuge tube and 70 μl of AccQ Fluor reagent was added into the same tube. 20 μl of the AccQ Fluor derivative agent was added for derivatization at 55 °C for 10 min. The AccQ-Fluor amino acid derivatives were separated on a Waters 2695 Separations Module HPLC System attached to a Waters 2996 fluorescence detector. 10 μl samples were injected into a Waters AccQ-Taq Column (150 mm × 3.9 mm). The AccQ-Tag Eluent A diluted (1:10) was used as eluent A (WAT052890) and 60% acetonitrile as eluent B in a separation gradient according to manufacture protocol.

2.4. Mineral analysis

Minerals of rice seed were analyzed by Atomic Absorption Spectroscopy (AAS) using a modified method of Jiang, Wu, Feng, Yang, and Shi (2007). About 2.0 g of brown seed was taken in a crucible and ignited in a muffle furnace at 550–600 °C for 10 h. The ash of the rice sample was dissolved in 0.2 N HCl and filtered through whatman-42 filter paper. The filtrate was used for AAS analysis with respective hollow-cathode lamp (HCl).

2.5. Vitamin and anti-nutrient factor estimation

The niacin and thiamine content of rice seed were estimated by a spectrofluorometric method (Sadasivam & Manikam, 1991). Phytic acid was extracted with 2.4% HCl and estimated by spectrophotometer (Bhandari & Kawabata, 2006).

2.6. Two-dimension polyacrylamide gel electrophoresis (2-DE)

Total protein was isolated from rice seed (2.0 g) by phenol extraction method with some modification (Paul, Gayen, Datta, & Datta, 2015). The dehusked rice seeds were ground to a fine powder with liquid nitrogen using chilled mortar and pestle. The seed powder was suspended with 10.0 ml extraction buffer containing 0.5 M Tris–HCl (pH-7.5), 30% sucrose, 50 mM Na-EDTA, 2% SDS, 2% β-ME, 2% PVP and 2% PMSF, 2% DTT in 50 ml tube. The equal volume of Tris saturated phenol (pH-8.0) was added and incubated at 4 °C for 30 min, followed by centrifugation at 5000*g* for 30 min. After collecting the aqueous phase, equal volume of extraction buffer was added and incubated for 30 min at 4 °C. The upper aqueous phase was recollected by centrifugation and five volume of methanol containing 0.1 M ammonium acetate was added. The tube was stored at −20 °C for overnight. The isolated protein was precipitated by centrifugation at 5000*g* for 30 min and protein pellet was washed with cold methanol and acetone. The protein was air dried in laminar flow and resuspended in 2-DE sample buffer consisting of 7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 20 mM DTT and 1% (v/v) Bio-Lyte pH 3-10 (Bio-Rad, Hercules, CA, USA) and protein concentration was measured by the Bradford method (Bradford, 1976). The protein (700 μg) was diluted to a final volume of 300 μl and loaded into immobilized pH gradient (IPG) strip holder containing 17 cm strips, pH gradient 4–7 (Bio-Rad, Hercules, CA, USA). Isoelectric focusing (IEF) was carried out with the IEF Cell (Bio-Rad, USA) using following condition: 250 V linear for 30 min, 10,000 V linear for 4 h, 10,000 V for 43,000 Vh, 1000 V for 5 min. The strips were equilibrated twice in equilibration buffer I and equilibrium buffer II (Bio-Rad, USA) respectively, for 15 min each. The 2DE was carried out in 12% SDS–PAGE using PROTEAN Tetra Cell (Bio-Rad, USA). After electrophoresis, protein spots were stained by colloidal Coomassie Brilliant blue R 350 solutions and the gel was scanned by Calibrated Imaging Densitometer (Bio-Rad, GS-800).

2.7. Image analysis

The gel image was analyzed by PDQuest Software, version 8.0 (Bio-Rad, USA). Statistical analysis (*t*-test) was performed to determine the significant differences between the two groups (WT and transgenic). The apparent molecular weight (Mr) and isoelectric point (pI) of each spot were determined by comparison with