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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

Table 1
Proximate compositions and other nutritional components of Xa21 rice and non-transgenic IR72 rice seeds.

	Xa21 rice	IR72	Ref. range[a]
Proximate compositions			
Moisture (%)	13.56 ± 0.25	13.58 ± 0.27	14.0
Ash (%)	1.43 ± 0.01	1.41 ± 0.01	1.0–1.5
Lipid (%)	2.03 ± 0.01	2.02 ± 0.01	1.6–2.8
Carbohydrate (%)	75.84 ± 1.08	73.66 ± 0.33	72.9–75.9
Protein (%)	8.21 ± 0.06	8.06 ± 0.12	7.1–8.3
Minerals (mg/100 g)			
Sodium	4.3 ± 0.32	3.63 ± 0.23	2–40
Potassium	254.25 ± 13.78	252.75 ± 2.53	70–320
Copper	0.35 ± 0.02	0.25 ± 0.01	0.1–0.7
Magnesium	116.9 ± 1.47	105.71 ± 1.45	20–170
Manganese	1.36 ± 0.11	1.34 ± 0.12	0.2–4.2
Calcium	0.72 ± 0.09	0.84 ± 0.10	1–6
Iron	1.04 ± 0.17	0.88 ± 0.17	0.2–6.0
Zinc	1.81 ± 0.06	1.50 ± 0.35	0.7–3.3
Vitamins			
Thiamine (mg/100 g)	0.45 ± 0.01	0.45 ± 0.01	0.29–0.61
Niacin (mg/100 g)	6.64 ± 0.36	6.60 ± 0.12	3.5–5.3
Anti-nutrient			
Phytic acid (g/100 g)	1.15 ± 0.03	1.13 ± 0.09	0.72–1.20

Values are mean ± SE; $n = 3$.
[a] Source: OECD (2004).

Table 2
Amino acids composition of Xa21 rice and non-transgenic IR72 rice seeds.

Components (%)	Xa21 rice	IR72	Ref. range[a]
Alanine	5.13 ± 0.32	5.52 ± 0.04	5.3–9.3
Arginine	6.81 ± 0.48	7.25 ± 0.08	8.0–13.0
Asparagine	9.68 ± 0.21	10.09 ± 0.05	8.7–14.9
Cysteine	1.03 ± 0.01	0.92 ± 0.09	1.3–2.2
Glutamic acid	23.40 ± 5.87	19.38 ± 0.01	12.7–24.2
Glycine	4.76 ± 0.27	4.59 ± 0.21	4.4–6.9
Histidine	2.37 ± 0.58	2.15 ± 0.01	2.7–4.4
Isoleucine	4.55 ± 0.47	4.71 ± 0.01	2.7–4.2
Leucine	7.55 ± 0.43	8.19 ± 0.01	6.7–11.5
Lysine	2.90 ± 0.33	2.91 ± 0.01	3.1–5.4
Methionine	1.55 ± 0.04	2.01 ± 0.09	1.3–2.1
Phenylalanine	5.19 ± 0.59	5.42 ± 0.01	5.1–7.8
Proline	3.99 ± 0.31	4.28 ± 0.08	3.8–6.0
Serine	4.20 ± 0.31	4.29 ± 0.06	4.3–7.7
Threonine	3.29 ± 0.40	3.40 ± 0.01	3.3–5.8
Tyrosine	3.76 ± 0.47	3.93 ± 0.03	3.3–5.2
Valine	9.58 ± 0.65	10.16 ± 0.01	4.2–6.9

Values are mean ± SE; $n = 3$.
[a] Source: Wang et al. (2012).

known protein marker. Student's *t*-test was performed for statistical analysis and protein spots above 2.0-fold for up-regulation and below 0.5-fold for down-regulation were considered as differentially expressed proteins.

2.8. Protein digestion and MALDI TOF MS/MS analysis

After PDQuest analysis, protein spots were excised manually from the gels, washed three times with ultra-pure water. The spots were digested with trypsin using in vitro trypsin digestion kit (Pierce, USA) following instruction manual (Pierce). The lyophilized proteins were dissolved in 5 μl of 0.1% TFA and 50% acetonitrile solution and used for peptide identification following MALDI-TOF-MS/MS analyzer (Bruker Daltonics, Germany). The instrument was calibrated with the Bruker peptide standard Mixture. Spectra were collected with the Flex Control software and data analysis was carried out using the software Flex Analysis 3.4.

The Protein search was carried out using the MASCOT program (Matrix Science, London, England) and identified by NCBI nr protein sequence database (National Center for Biotechnology Information, Bethesda, MD, USA) using a MOWSE algorithm as implemented in the MASCOT search engine version 3.5 (Matrix science: http://www.matrixscience.com).

The following parameters were used for database searches: taxonomy: *O. sativa* (25805290 sequences); cleavage specificity: trypsin with one missed cleavages allowed; mass tolerance of 100 ppm for precursor ions and a tolerance of 0.7 Da for the fragment ions; allowed modifications: carbamidomethyl (fixed), oxidation of methionine (variable); cleavage by trypsin: cuts C-term side of KR unless the next residue is P. According to MASCOT probability analysis, only significant hits ($P < 0.05$) were considered.

2.9. Statistical analysis

All statistical analysis was performed using the Graph Pad Prism 5 software and data was expressed as mean ± SEM. Statistical significance of the data was analyzed by Bonferroni Post-tests.

3. Results and discussion

According to the OECD, the nutritional quality of a GMO should be as substantial as the corresponding non-transgenic material, and emerging studies assessing nutrient composition and quality in various transgenic crops have increasingly revealed the importance of such criteria in establishing biosafety. Hence, in order to

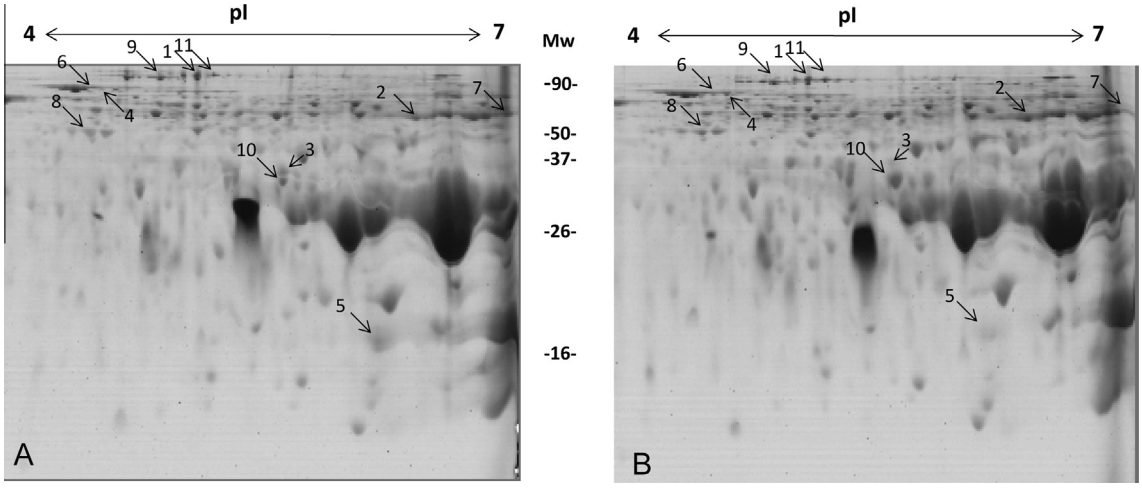

Fig. 1. Two-dimensional gel electrophoresis of total rice seed protein (A) transgenic Xa21 and (B) control IR72.

conduct a comprehensive biosafety evaluation, the nutritional components of transgenic Xa21 rice seed were compared with the non-transgenic IR72 rice seed, as similarly reported in earlier transgenic crop studies (Gayen et al., 2013; Junhua et al., 2005; Li et al., 2007; Oberdoerfer, Shillito, de Beuckeleer, & Mitten, 2005; Wang et al., 2012).

3.1. Nutritional composition analysis

3.1.1. Proximate analysis

When assessing the practical and nutritional aspects of a genetically modified organism, proximate analysis is one of the most important tools and takes into account factors such as moisture, ash, crude fat, carbohydrate, and protein content (Table 1). Grain moisture content in particular is an important indicator of shelf life, and grain moisture content above a certain threshold level can lead to deterioration of the nutritional quality of rice seeds. In this study the moisture content of both transgenic and non-transgenic rice varieties was maintained around 14%, while the ash content of both rice seeds was also comparable and within an appropriate reference range as determined by the OECD.

Rice is an excellent source of carbohydrates, fat, and protein, all of which are all essential nutrients in human health. For the people of developing countries who often suffer from protein deficiency, rice can serve as an inexpensive source of this crucial dietary nutrient. For these reasons, it was important to examine any alterations in these macronutrients upon IR72 modification. However, no statistically significant difference in protein content was found between the transgenic and non-transgenic rice seed, similar to a recent study on transgenic ferritin rice seeds (Gayen et al., 2013).

The lipid content of the transgenic rice seeds (2.03%) was also almost indistinguishable from that of the non-transgenic rice seeds (2.02%). The carbohydrate content of transgenic and non-transgenic rice seeds was 75.84% and 73.66%, respectively, both of these values lying within the suitable reference range reported by OECD (2004). All together, these results revealed that the proximate compositions and nutritional quality of the Xa21 rice seeds are essentially equivalent with the non-transgenic control, as also shown in the previous study (Gayen et al., 2013).

3.1.2. Mineral content

In addition to the nutrients above, rice can also provide a number of important minerals that are essential for human physiological functions (Bhullar & Gruissem, 2013). Therefore, quantifying the mineral content of the transgenic rice seed was another crucial step in evaluating its performance relative to the unmodified rice. As shown in Table 1, atomic absorption spectroscopy afforded a precise handle on the mineral content of both the transgenic and unmodified rice seed, revealing only small variations in the Na, Fe and Zn compositions within each seed type. All data were again found to be within the desired reference range as reported by the OECD and any observed variations between seeds were statistically insignificant, as also shown recently in other crops (Rayan & Abbott, 2015).

3.1.3. Vitamin and anti-nutrient content

In addition to the nutrients discussed above, rice contains certain essential vitamins that have been shown to have significant health benefits. In this study, niacin and thiamine were considered for a substantial equivalence analysis (Table 1). It was found that niacin (6.64 and 6.60 mg/100 g) and thiamine (0.45 and 0.45 mg/100 g) content in transgenic and control rice respectively. This study also support by Gayen et al. (2013), where it was found that the vitamin content of transgenic ferritin rice was statistically indistinguishable from non-transgenic IR68144 rice. We also evaluated the phytic acid content of transgenic Xa21 (1.15%) and non-

Table 3
Identification of differentially expressed protein in transgenic Xa21 and non-transgenic IR72 rice seeds.

Spot No	Accession No	Score	Number of peptides	Sequence coverage (%)	Theoretical pI/MW	Experimental pI/MW	Protein name	Cellular component	Function	Fold change
Energy/carbohydrate metabolism										
1	gi|262345485	831	36	41	5.58/102517.2	5.50/112710.0	Pullulanase	Outer membrane	Glycogen catabolic process	3.4↑
2	gi|253992891	603	21	37	7.9/66569.21	6.02/70240	Granule-bound starch synthase	Periplasmic	Starch biosynthesis	0.41↓
Stress responsive										
3	gi|158513197	110	11	40	6.01/35661.9	5.7/35660	Late embryogenesis abundant protein 1	Periplasmic; cytoplasmic	Starch biosynthesis	4.72↑
4	gi|108707472	291	20	38	5.29/71634.1	5.29/80670	Heat shock cognate 70 kDa protein	Cytoplasmic	Stress responsive	0.41↓
Seed storage protein										
5	gi|115464709	297	12	38	7.48/21054.7	5.76/16540	α-Globulin (19 kDa)	Extracellular; periplasmic	Nutrient reservoir activity	20.0↑
Transcription factor										
6	gi|2267006	673	26	40	5.30/73540.4	5.1/80000	Endosperm lumenal binding protein	Cytoplasmic	ATP binding	0.38↓
Hypothetical										
7	gi|218190412	584	22	37	9.3/54062.5	6.98/67820	Hypothetical protein Osl_06572	Outer membrane	Nutrient reservoir activity	6.31↑
8	gi|125533732	660	20	36	4.97/56840.5	4.56/62760	Hypothetical protein Osl_35452	Endoplasmic reticulum	Protein disulfide isomerase activity	0.41↓
9	gi|218196777	579	30	39	5.37/93643.4	5.44/107260	Hypothetical protein Osl_19920	Cytoplasmic	Pyruvate metabolic process	0.28↓
10	gi|125550788	331	16	46	6.02/32281.6	5.70/32280	Hypothetical protein Osl_18399	Cytoplasmic	Oxidoreductase activity	0.22↓
11	gi|222628355	1140	38	42	5.58/99959.1	5.64/111800	Hypothetical protein Osj_13773	Outer membrane	Carbohydrate metabolism process	0.31↓

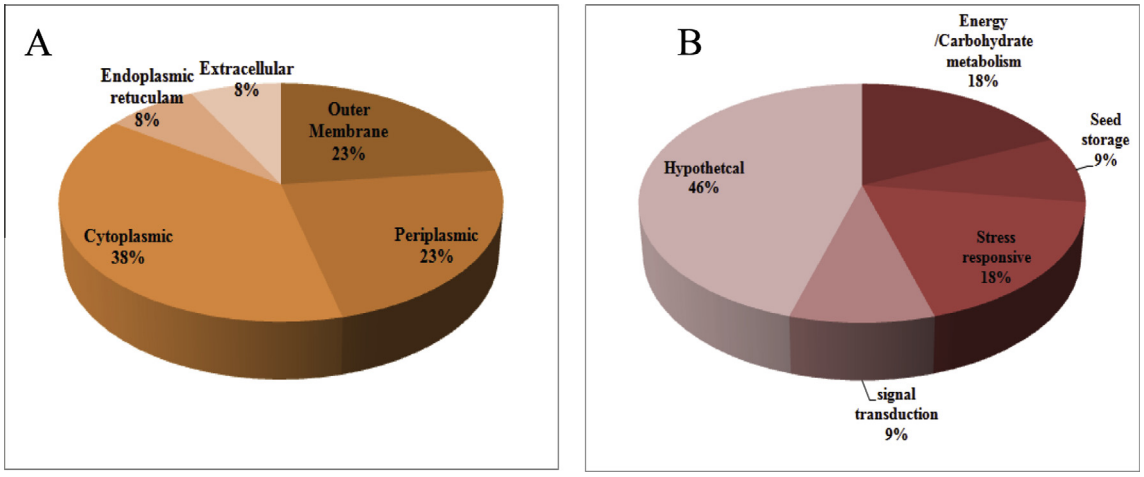

Fig. 2. Classification of the identified differentially expressed proteins (DEPs) based on (A) sub cellular localization (B) physiological function analysis.

transgenic (1.13%) rice seeds (Table 1) and found no statistically significant difference.

3.1.4. Amino acid content

For people in developing countries who may suffer from protein deficiency, rice can serve as an inexpensive source of this crucial dietary nutrient. In this study, we used HPLC to evaluate 17 essential and non-essential amino acids and found that the transgenic Xa21 and IR72 rice seeds exhibited nearly identical amino acid profiles (Table 2). No statistically significant differences were observed between the rice samples with the exception of glutamic acid, which was slightly higher in the transgenic seeds (23.40%) than the non-transgenic seeds (19.38%). However, all the amino acid values were within the previously determined reference range reported by Wang et al. (2012), and thus even this difference for glutamic acid is not expected to be biologically significant. Thus the amino acid analysis clearly demonstrated that the transgenic cultivar is nutritionally competitive with the control cultivar.

3.2. Analysis of protein profile of transgenic and non-transgenic rice seed

As discussed above, the objective of the study was to make an initial biosafety assessment of the Xa21 rice through the substantial equivalents analysis, an evaluation which has also been carried out in other transgenic crops (Lepping, Herman, & Potts, 2013; Rayan & Abbott, 2015). Proteomics can also provide important information regarding biological safety (Gong, Li, Yu, Wang, & Wang, 2012) and has already been carried out for a wide variety of GMOs (Albo et al., 2007; Brandão, Barbosa, & Arruda, 2010; Gong et al., 2012; Ren et al., 2009; Wang et al., 2015). We thus carried out a similar whole proteome analysis of the transgenic Xa21 rice seeds in order to investigate any alteration of proteome levels upon integration of the *Xa21* gene into the genome of the IR72 rice plant. The proteins expressed in the Xa21 rice seeds were separated by two-dimensional gel electrophoresis (Fig. 1). We considered differentially accumulated protein spots >2.0-fold and <0.5-fold to be differentially expressed proteins (DEPs). Based on comparison of the wild-type and transgenic lines, 11 DEPs were selected for subsequent MALDI analysis for mass determination. Among this smaller group, four proteins were found upregulated and seven were down-regulated (Table 3). Interestingly, the Xa21 protein was not detected in 2DE gel, likely due to the low abundance of protein, with a similar result was also observed by Wang et al. (2015).

The 11 identified proteins were classified into five categories based on Gene ontology annotation and physiological function: energy & carbohydrate metabolism (18.0%), stress responsive (18.0%), signal transduction (9.0%), hypothetical (46.0%), and seed storage (9.0%) (Fig. 2). The proteins were found to be predominantly localized in cytoplasm (38.0%), followed by the periplasm and outer membrane (23.0%), and finally the endoplasmic reticulum and extra cellular components (8%).

Some variations were observed in the proteome profile of the transgenic seeds compared with non-transgenic seeds. The proteomics study revealed that pullulanase protein was found to be up accumulated by 3-fold in transgenic seeds over the non-transgenic seeds. The pullulanase enzyme plays a key role for the physical property of starch (Yamasaki, Nakashima, & Konno, 2008). Therefore, the increased expression of this enzyme of transgenic rice seed will lead to alter structure of starch component in the rice endosperm. Gong et al. (2012) found a similar enhancement in pullulanase activity in the Ming Hui rice variety compared to D68 and ZH10 controls.

We also found that expression of the late embryogenesis abundant (LEA) protein was enhanced by 4-fold in the Xa21 rice seeds compared to control rice seeds. This particular protein functions as a general "spacer" molecule (molecular shield) that can prevent protein aggregation during water loss. Alternatively, this protein can also act as a specific protector of individual target molecules (Goyal, Walton, & Tunnacliffe, 2005).

The proteomics study showed that nutrient reservoir activity of transgenic rice seeds was upregulated. It has been found that nutrient reservoir activity of transgenic rice seed was enhanced greatly (20-fold) over the IR72 rice seeds, similar to the result found by Wang et al. (2012) in their study of transgenic BT rice expressing the *cry1ab/ac* gene. This protein plays a significant role in the storage of nutritious substrates and therefore its upregulation is anticipated to lead to enhanced nutritional storage capacity in the Xa21 rice. The down-regulation was also observed in the case of some proteins within the Xa21 rice, with most of these proteins involved in the carbohydrate metabolism pathway.

3.3. Agronomic study of transgenic rice plant

Agronomic performance of the transgenic and WT plants was evaluated to determine the phenotypic alteration due to genetic manipulation. In this study, we considered rice plant height, panicle length, seed length and weight of 1000 grains of rice for a comprehensive assessment. All the transgenic and WT plants showed similar morphological nature. Moreover, no statistically significant

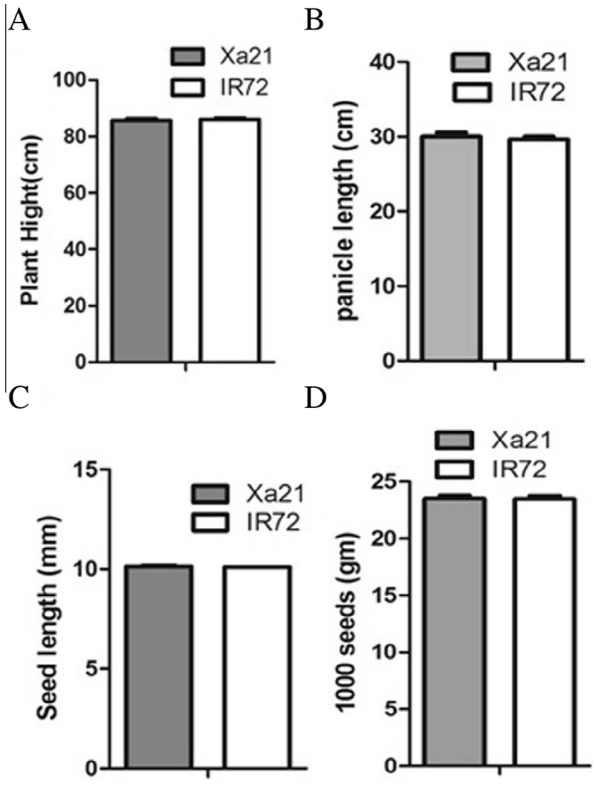

Fig. 3. Agronomic performances of transgenic Xa21 plant with respect to non-transgenic control IR72 (A) plant height (B) panicle length (C) seed length (D) 1000 seeds weight.

differences were found between transgenic and non-transgenic plants (Fig. 3).

4. Conclusions

Through nutritional quality assessment and 2D gel electrophoresis, the substantial equivalence study was performed by comparing transgenic Xa21 rice with the non-transgenic IR72 rice in order to characterize the changes resulting upon insertion of *Xa21* gene in the genome of IR72 rice. The nutritional quality assessment, which included comparison of amino acid, mineral, and vitamin content, revealed no statistically significant differences between the Xa21 and IR72 varieties, with all differences lying within the reference ranges provided by the OECD (Gayen et al., 2013). While the proteomics study did reveal some level of alteration in the protein profile of the Xa21, none of the altered proteins were found to be toxic, allergenic, or detrimental to the growth capabilities of the transgenic rice. Notably, the proteomics analysis revealed that a majority of the differentially expressed, up-regulated proteins of the Xa21 rice are involved in plant defense mechanisms, starch biosynthesis pathways, and nutritional component storage, thus contributing some possible advantages to this transgenic variety over the IR72 rice. The result of this entire study of nutritional components and proteome profile of transgenic Xa21 rice seeds revealed that due to integration of foreign gene, no detrimental changes were observed in the transgenic seeds.

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