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Research article

Straw digestibility of Thai rice accessions

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Abstract

Rice straw is a major source of agricultural biomass in Southeast Asia. However, it has not been fully utilized due to its recalcitrance to breakdown by an enzymatic process. Recent work demonstrated a distribution of straw digestibility among rice varieties, but this trait has not been detailed for the rice population in Thailand. Identifying rice varieties with highly digestible straw would provide information for biofuel, biorefinery and animal feed use. The current study assessed the digestibility of straw from 49 Thai rice accessions using the enzymatic-based high-throughput platform. Straw saccharification released sugars in the range 50.53–114.52 nmol/mg/hr. The highest and lowest saccharification potentials were from a glutinous variety "Muey Nawng 62 M" and an upland variety "Dawk Pa-Yawm", respectively. Rice accessions with high digestibility for potential use were identified. These data provide a preliminary assessment of rice straw from the Thai population and should be useful for studying the genes responsible for the digestibility trait and for breeding programs for both rice grain production and straw utilization.

Introduction

Rice straw is a major agricultural biomass waste produced in Southeast Asia and particularly in Thailand, and yet most of this biomass is left unutilized and is often burned in the field causing severe environmental problems at both local and global levels (Oanh, et al., 2011; Junpen et al., 2018). It is estimated that over 20 million t of rice straw are produced in Thailand every year (Gadde et al., 2009) and that during the peak seasons of rice harvesting, straw is burned on 90% of rice paddies to get rid of it (Tipayarom and Oanh, 2007). Open rice straw burning results in incomplete combustion giving rise to high levels of particulate black carbon, carbon monoxide and ozone, which cause respiratory problems and premature deaths among the human population, crop yield loss and atmospheric warming (United Nations Environment Programme and World Meteorological Organization, 2011). As a substantial waste burden, rice straw is posing a critical threat by burning, but also provides a great opportunity to benefit humankind by converting the waste into biomass-based products (Satlewal et al., 2018).

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Rice straw is rich in polysaccharides (approximately 70% dry weight), and therefore, the large volume of straw produced annually could provide valuable lignocellulose feedstock for producing biofuels, chemicals and animal feed (Chen and Dixon, 2007; Harris and DeBolt, 2010). Unfortunately, the compositional characteristics of rice straw currently hinder its applications as it is inherently difficult to digest using enzymes, making it costly for biofuel and chemical production through fermentation. Furthermore, rice straw has notably high levels of silica (up to 15% dry weight), which makes it particularly unpalatable to animals and consequently poor quality as animal feed (Virk et al., 2019). Nonetheless, recent work has shown that there is large natural variation in all of these parameters among commercial rice varieties, suggesting that there is substantial scope for improving the characteristics of rice straw without having negative impacts on plant performance and grain yield (Marriott et al., 2016; Garrido et al., 2018). Furthermore, recent work using Genome-Wide Association Studies (GWAS) have identified some regions in genomes that are responsible for variation in rice straw digestibility (Liu et al., 2016).

The current study investigated rice straw saccharification analysis of 49 Thai rice accessions (taken from both commercial and landrace varieties) using enzymatic digestion based on a platform developed by Gomez et al. (2010). The central aim of this work was to lay the groundwork to drive widespread use of rice straw in animal feed, biofuel and bioenergy applications in Thailand. Selection of specific varieties with high straw saccharification potential will pave ways for the development of rice breeds with high straw and husk values for further exploitation.

Materials and Methods

Plant materials

Seeds of 49 Thai rice accessions were obtained from the Pathum-Thani Rice Research Centre, Thailand (Table 1). For straw sample collection, rice was grown in a pot (30 cm diameter and 22.5 cm depth) using one germinating seed per pot and five pot replicates per variety. The rice pots were arrayed in a completely randomized design on a plain field in Chiang Mai province, Thailand (18°52’47.5”N, 99°02’43.2”E) during January–April 2016. Rice cultivation was performed using a submerged irrigation method with regular water supply for 4 mth or until flowering after which the crop was left to dry for up to 2 wk. Stems from the main tillers were collected and straw samples were taken from the third and fourth nodes. The straw samples were further dried in an incubator at 45°C for 2 d before being cut into approximately 4 mm lengths and pulverized to a fine powder using a robotic grinding platform described by Gomez et al. (2010).

Enzymatic saccharification analysis

Rice straw saccharification was performed based on 96-well-plate formats using Celluclast (cellulose from *Trichoderma reesei*) and Novozyme 188 (Novozymes A/S; Bagavaerd, Denmark) at a ratio of 4:1 at an enzyme loading of 22.5 filter paper units (FPUs) per gram of material and a robotic platform (Tecan Evo200; Tecan Group Ltd.; Mannedorf, Switzerland), as described by Gomez et al. (2010). Briefly, with the equipped robotic tools, 4 mg of straw powder were loaded into 96-deep-well plates, pre-treated with 350 µL of 0.5 M NaOH solution at 90°C for 30 min, washed five times with 500 uL of 25 mM sodium acetate buffer (pH 4.5) and then incubated with the enzyme cocktail at 50°C for 8 hr. It should be noted that this 8 hr digestion was programmed for verifying the saccharification potential or digestibility of straw among rice accessions and not to determine the total saccharification values of straw biomass. Biomass hydrolysates were aliquoted by the robotic platform into 96-well-plates and analyzed for released reducing sugar using a modified MBTH method (Gomez et al., 2010; 2011). The pre-treatment using 0.5 M NaOH was required to remove the majority of hemicellulose from the straw materials before enzymatic hydrolysis that resulted in the saccharified products consisting mainly of glucose from cellulose (Moradi et al., 2013; Wood et al., 2016). The experiment was performed using three biological replicates, each with four technical replicates. Optical density reads were converted to amounts of reducing sugars released in nanomoles using glucose standards, as previously described.

Statistical analysis

Data analysis was performed in the R environment (R Core Team, 2016) using Agricolae (de Mendiburu, 2014) for statistical analysis. The dataset was assessed for significant differences among rice accessions using analysis of variance and means were compared using the Tukey method with significance tested at $p < 0.05$.

Results and Discussion

The saccharification potential was explored based on straw samples collected from 49 Thai rice varieties using an automated enzymatic hydrolysis platform developed by Gomez et al. (2010). The mean values of the sugar released from the straw biomass from these 49 varieties was in the range 50.53–114.52 nmol/mg/hr (Fig. 1). The highest and lowest saccharification potentials were from R30 (a glutinous variety “Muey Nawng 62 M”) and R13 (an upland variety “Dawk Pa-Yawm”), respectively (Table 1). A normal distribution was observed for the sugar released values of the 49 accessions (Fig. 2). Because of data continuity, statistical analysis was unable to clearly distinguish between accessions regarding the saccharification potential. Nonetheless, some accessions could be grouped based on the Tukey analysis using high saccharification potential, for example, R30, R47, R31, R02, R43, R34, R37, R44, R40 and R03. No correlation was observed between the saccharification values and rice groups based on grain type and high or low land accessions.

Table 1 Mean and SD of saccharification (mmol/mg/hr) of 49 Thai rice accessions

	Identifier	Accession	Grain type	Mean	SD
1	R01	KDML 105	NG	77.92^{jklmnopqr}	11.7
2	R02	RD15	NG	103.36^{abcd}	10.7
3	R03	Leb Meu Nahng 111	NG	97.28^{abcdefghi}	9.2
4	R04	RD51	NG	74.92^{klmnopqr}	8.2
5	R05	Goo Meuang Luang	NG	86.72^{defghijklmn}	10.2
6	R06	Khao Tah Haeng 17	NG	88.40^{defghijklm}	7.6
7	R07	Nahng Mon S-4	NG	78.54^{ijklmnopqr}	22.1
8	R08	Leuang Pratew 123	NG	72.65^{lmnopqrs}	8.0
9	R09	Prachin Buri 2	NG	80.95^{ghijklmnopq}	10.8
10	R10	Pin Gaew 56	NG	90.32^{cdefghijkl}	8.2
11	R11	Plai Ngahm Prachin Buri	NG	76.96^{jklmnopqr}	6.9
12	R12	Sao Hai	NG	83.92^{efghijklmno}	7.5
13	R13	Dawk Pa-Yawm	NG	50.53^{t}	5.7
14	R14	Sinlek	NG	69.08^{nopqrs}	4.7
15	R15	Sang Yod Phattalung	NG	76.58^{jklmnopqr}	10.7
16	R16	Hommali Daeng	NG	83.00^{fghijklmno}	10.1
17	R17	Gaen Jan	NG	84.91^{efghijklmno}	11.0
18	R18	Khem Tawng Phatthalung	NG	88.68^{defghijklm}	6.5
19	R19	Hantra 60	NG	89.58^{cdefghijklm}	5.2
20	R20	Ta-pow Gaew 161	NG	88.36^{defghijklm}	7.7
21	R21	Leuang no.1	NG	62.90^{qrst}	6.1
22	R22	RD19	NG	62.16^{rst}	9.8
23	R23	RD6	G	77.15^{jklmnopqr}	12.9
24	R24	Leum Pua	G	56.10st	13.7
25	R25	Hahng Yi 71	G	77.79^{jklmnopqr}	7.7
26	R26	Niaw Kiaw Ngoo	G	69.25^{nopqrs}	8.8
27	R27	Nahng Cha-lawng	G	71.21^{mnopqrs}	14.2
28	R29	Sew Mae Jan	G	79.70^{hijklmnopqr}	19.1
29	R30	Muey Nawng 62M	G	114.52^{a}	18.9
30	R31	RD16	G	106.92^{abc}	15.1
31	R32	Pathum Thani 1	NG	82.43^{fghijklmnop}	10.7
32	R33	Suphan Buri 1	NG	75.72^{jklmnopqr}	10.4
33	R34	Phitsanulok 2	NG	101.63^{abcde}	7.5
34	R35	Chai Nat 1	NG	67.19^{opqrst}	11.0
35	R36	Chai Nat 80 (RD29)	NG	84.56^{efghijklmno}	7.0
36	R37	RD39	NG	100.13^{abcdef}	13.9
37	R38	RD43	NG	78.86^{ijklmnopqr}	10.8
38	R39	RD49	NG	69.80^{nopqrs}	16.3
39	R40	RD21	NG	97.42^{abcdefgh}	13.5
40	R41	Pathum Thani 80 (RD31)	NG	68.33^{opqrst}	18.4
41	R42	Hawm Suphan	NG	84.97^{efghijklmno}	9.7
42	R43	RD33	NG	103.12^{abcd}	13.0
43	R44	RD41	NG	99.33^{abcdefg}	8.3
44	R45	RD47	NG	92.65^{bcdefghijk}	9.6
45	R46	Homnil	NG	93.03^{bcdefghij}	5.2
46	R47	Riceberry	NG	110.37^{ab}	8.9
47	R48	San-pah-tawng 1	G	77.72^{jklmnopqr}	9.4
48	R49	RD10	G	64.74^{pqrst}	9.4
49	R50	RD14	G	83.88^{efghijklmno}	9.1

NG = non-glutinous variety; G = glutinous variety

Means superscripted with different lowercase letters denote significant difference (p<0.05).

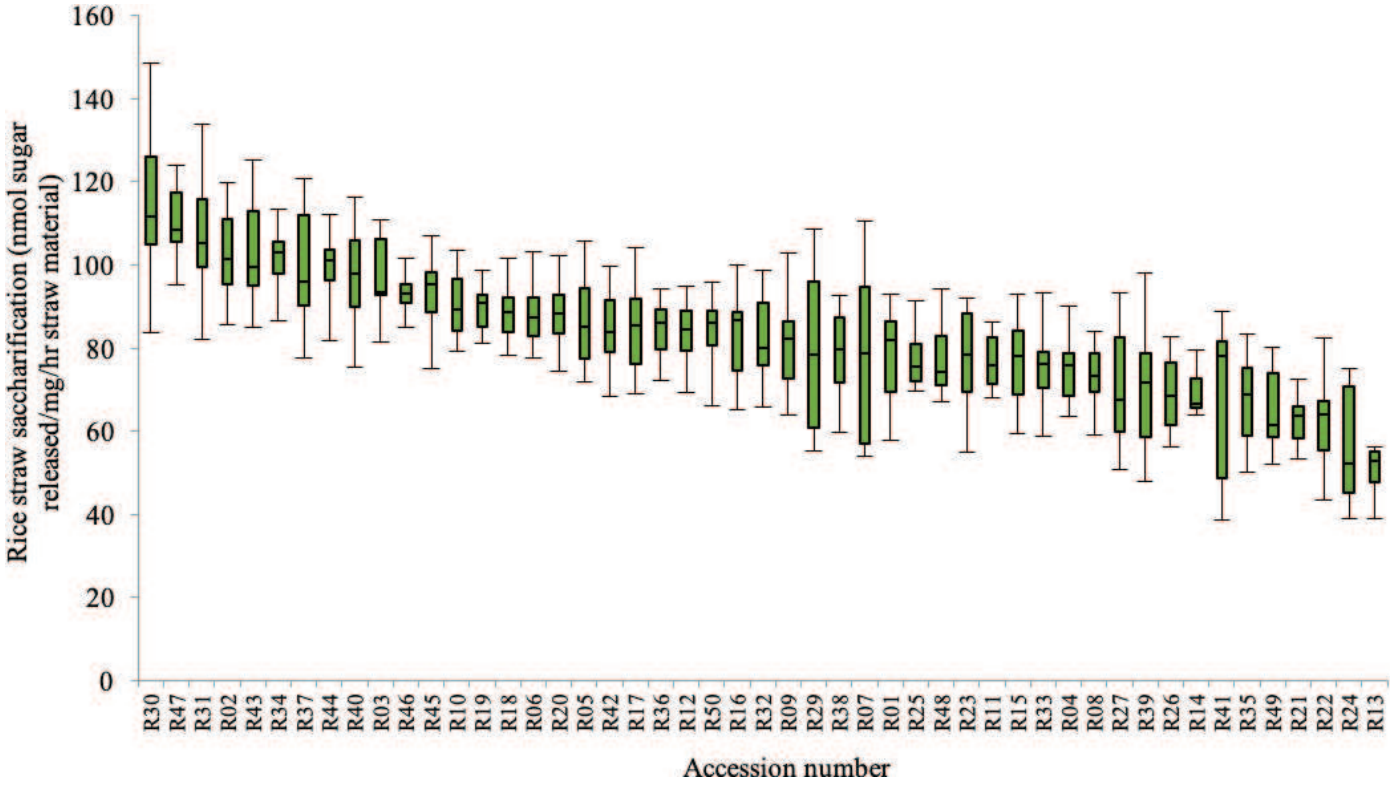

Fig. 1 Rice straw saccharification of 49 Thai rice accessions, where Box plots represent distribution of reducing sugars released by enzymatic hydrolysis of pre-treated rice straw (three biological replicates and each with four technical replicates)

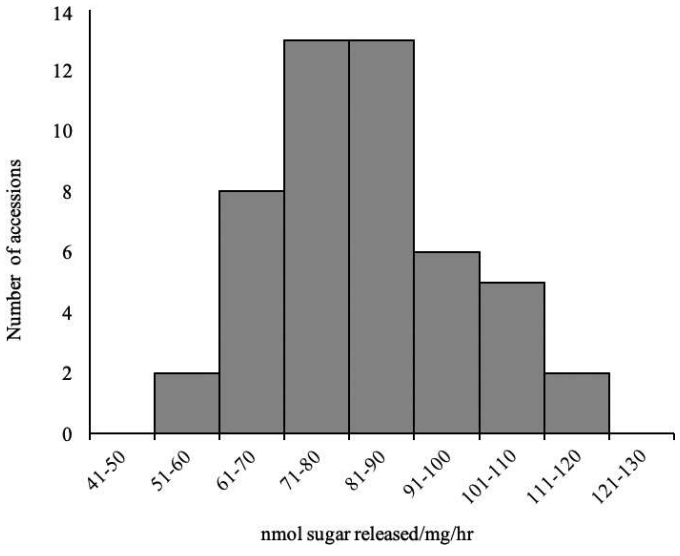

Fig. 2 Distribution of straw saccharification potential among 49 rice accessions

Previously, results of rice straw saccharification using enzymatic hydrolysis indicated various ranges in the sugar released from different rice populations, including those from China (59–116 nmol/mg/hr; Liu et al., 2016) and Vietnam (20–134 nmol/mg/hr; Nguyen, 2016). The current analysis among selected Thai rice accessions showed a similar distribution and range of saccharification potential, demonstrating that the broad trait diversity is presented within the Thai population. As these analyses were performed using the same platform and experimental conditions, a comparison can be made of the saccharification potential. Furthermore, the highest saccharification value from the current study of 114 nmol/mg/hr suggested that the Thai accessions were comparable with the populations from China and Vietnam. However, most of the top Thai saccharification values were from accessions with restricted propagation, local uses or low production, thereby limiting a direct utilization as a large-scale biomass resource. Thus, these accessions are more likely to be useful for introgression breeding for high digestibility traits. A recent analysis of biomass digestibility and productivity traits showed that both traits are not correlated to one another, which indicated that these crops could be bred for both high productivity and high digestibility traits (Garrido et al., 2018).

Various studies of biomass saccharification have revealed some insight into the plant cell wall recalcitrance, which results from a multitude of contributing pathways, particularly the cell wall polysaccharide components and especially cellulose and

hemicelluloses (Demartini et al., 2013; Marriott et al., 2014). Follow-up studies of biomass production from grasses and rice demonstrated that lignins also play key roles in the recalcitrance (Fu et al., 2011; Bartley et al., 2013; Bouvier d'Yvoire et al., 2013). Interestingly, silica appeared to be one of the key factors in the recalcitrance of rice straw and husk (Zhang et al., 2015), acting as a rice defense to pathogens and even deterring foraging animals (Agbagla-Dohnani et al., 2003). Changes in the complexity of the biomass in many aspects including composition, structure and molecular arrangement could affect the saccharification potential (Gomez et al., 2010; Marriott et al., 2014; Whitehead et al., 2018). Though not cell wall related, starch remained in rice straw biomass also provides an add-on to final saccharification products (Teramura et al., 2013). Other aspects of plant cell walls responsible for biomass recalcitrance are now being discovered, and much recent progress is owed to the work of genetic variation and genome analysis, including GWAS and quantitative trait loci analysis. Newly identified genes will be beneficial both for marker-assisted selection for selecting breeding lines with low recalcitrance and providing further insight into the genetics underlying straw digestibility. When the genomic data of the rice accessions used here become available, the related saccharification data would be useful for identifying loci responsible for the straw recalcitrance.

Quantitative traits are largely affected by the environment; indeed, saccharification is also prone to this effect. For example, Nguyen (2016) reported straw saccharification results differed greatly between two consecutive years (20–134 nmol/mg/hr and 23–72 nmol/mg/hr from 2013 and 2014, respectively) under well-planned paddy field experiments in Vietnam. Thus, it is difficult to directly compare biomass saccharification results among different experiments. Although the current analysis was based on straw materials collected from rice grown in pots, which may pose a different situation from those grown in the paddy fields, the current results are useful for uniformly comparing the saccharification potential among the tested accessions. Pot-cultivation with a controlled environment including well-mixed compost, watering and fertilizer feeding should minimize the environmental effects usually encountered in samples collected from the field. However, it is crucial in the future to perform the analysis using straw samples from rice cultivated in paddy fields for accessing biomass yields in the field and their saccharified products.

In conclusion, the enzymatic straw saccharification of Thai rice accessions presented here can serve as preliminary data to support the utilization of rice straw biomass in Thailand. The rice accessions with high saccharification potential identified here could be beneficial for both direct biomass production and for breeding programs. In addition, these data should prompt saccharification screening on a more extensive collection of Thai rice accessions. A more detailed study using plot experimental designs, DNA sequence data and genetic analysis tools such as GWAS would allow this straw waste to be utilized more effectively. Diverting rice straw from burning will not only reduce the harmful environmental consequences but also provide a new source of animal feed, biofuels and bioenergy with additional environmental benefits arising from reducing the land required to produce forage and the use of fossil fuels.

Ethics Statements

Approvals were not required regarding biosafety or human or animal ethical issues for this work.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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