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Rice grain protein composition influences instrumental measures of rice cooking and eating quality

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Abstract

Rice cultivar starch composition differences do not completely explain variation in rice cooking and eating quality. Rice grain storage proteins possess divergent solubility properties suggesting they may contribute to cultivar differences in rice grain quality. Application of high-performance liquid chromatography (HPLC) analysis to protein extracts derived from medium and long grain advanced rice breeding lines revealed rice grain protein composition differences which were associated with instrumental measures of grain quality. Globulin content displayed little variation in both grain types. The mean glutelin content was higher in long grain rice lines than medium grains. Although the mean content of prolamins in medium and long grain rice were similar, the prolamin content of medium grains was more variable. Individual medium grain prolamin HPLC peaks, total prolamin content and the prolamin:glutelin + prolamin ratio were positively correlated with several rapid visco analyser (RVA) parameters. The long grain HPLC data displayed a similar pattern of correlations with RVA parameters but of reduced magnitude. Protein composition was associated with instrumental measures of grain quality in this set of germplasm and although the textural properties of rice are complex, these data suggest consideration of rice grain protein composition could contribute to breeding high quality rice.

Keywords: Oryza sativa; grain quality; prolamin; texture

Abbreviations used: AAC, apparent amylose content; AACC, American Association of Cereal Chemists; ACN, acetonitrile; CV, coefficient of variation; GI, glycaemic index; GT, gelatinisation temperature; HPLC, high-performance liquid chromatography; LG, long grain; MG, medium grain; QTL, quantitative trait loci; RVA, rapid visco analyser; TA, texture analyser

1. Introduction
Rice (Oryza sativa L.) is the staple food of many countries, particularly in Asia, and is increasing in popularity in many other parts of the world. Consumers pay attention to rice grain quality, displaying a preference for rice with a particular visual appearance, texture, flavour and aroma (Fitzgerald et al., 2009). There is a strong cultural dimension to these preferences resulting in a wide array of different types of cultivated rice originating from around the globe.

The texture of cooked rice is considered by many consumers to be a quality trait of primary importance. Although texture has been defined as a multidimensional characteristic that only humans can perceive, describe and measure (Szczesniak, 1968), several instrumental tests are used to predict these sensory attributes or textural properties of rice. These tests involve assays that measure the physico-chemical properties of rice such as alkali spreading value, gel consistency, rapid visco analyser (RVA) and texture analyser (TA).

Rice grains are composed of approximately 80-85% starch, 4-10% protein, 1% lipid and 10% moisture. Amylose content, amylopectin fine structure, and intra and/or inter-molecular interactions of starch with other components such as proteins, lipids and non-starch polysaccharides, are some of the factors involved in determining rice grain quality. Among these, amylose content is perhaps the most important factor that determines cooking behaviour. Low amylose cooked rice is generally soft with individual grains adhering closely while high amylose rice is harder and less sticky (Rani and Bhattacharya, 1989).

Amylopectin chain length distribution also has an impact on rice grain quality through its effect on starch gelatinisation temperature (GT) (Umemoto et al., 2004) which is associated with cooking time.

The primary genetic determinants of amylose content and amylopectin chain length distribution are known. The Waxy/waxy gene codes for granule-bound starch synthase I and is the most important controller of amylose content (Wang et al., 1995), while Alk/alk codes for
starch synthase IIa and is responsible for the most important differences in amylopectin chain length distribution (Umemoto et al., 2004). Although several alleles of the Waxy/waxy and Alk/alk genes associated with different forms of starch have been identified (Waters et al., 2006), other starch biosynthesis genes in addition to Waxy/waxy and Alk/alk affect rice cooking and eating quality (Kharabian-Masouleh et al., 2013). However, starch structure does not explain all variation in rice grain quality parameters in all rice germplasm (Kharabian-Masouleh et al., 2013), and flour derived from cultivars with similar amylose content may have different pasting and textural properties (Champagne et al., 1999). Furthermore, viscosity differences between rice flour and rice starch (Singh et al., 2000) suggest components other than starch affect these properties of rice.

Rice grain protein is the second most abundant component of milled rice grain and has been studied extensively in the context of its important role as a nutrient. The net protein utilisation of rice is the highest among the cereal grains, despite rice having the lowest protein content (Juliano, 1992). In the context of eating quality, several studies have reported total protein content is related to cooked rice texture (Xie et al., 2008) with high protein content associated with harder cooked rice (Lyon et al., 1999). Martin and Fitzgerald (2002) demonstrated total protein content had a significant influence on rice eating quality by digesting the rice flour proteins of six cultivars with protease and observing the RVA profiles differed between samples of similar amylose content. Experiments which have removed and replaced each of the protein fractions also suggest protein composition may influence rice eating quality (Baxter et al., 2004; 2010; 2014).

In common with all cereal grain, rice grain storage proteins are composed of globulins, glutelins and prolamins which are traditionally defined by their differences in solubility. Globulins are salt-soluble, the glutelins dissolve in dilute acid or alkali solutions while the prolamins, including those of rice which are poorly digested by monogastric animals
including humans (Resurreccion and Juliano, 1981; Kubota et al., 2010), are solubilised by aqueous alcohol. Rice grain protein content differs between cultivars and within populations derived from two parental cultivars (Zhang et al., 2008), however, the relationship between rice grain protein composition and rice eating quality is not well understood. Here, we report upon the relationship between rice grain protein composition and instrumental measures of rice eating quality in a range of advanced rice breeding lines.

2. Materials and methods

2.1. Rice samples

Flour from the milled grain of 80 medium grain and 80 long grain advanced rice breeding lines within a narrow range of 17% to 20% apparent amylose content (AAC) from the 2014 rice breeding and quality trials at Yanco, New South Wales, Australia (34°36’S; 146°23’ E) was used in this study. The accumulated thermal time for the rice growing season for years 2011 to 2014 appear in Supplementary Figure 1.

2.2. Rice grain grinding, RVA and TA analysis

Milled rice grains (10 g) were ground to pass through a 0.5 mm screen (Cyclotec 1093 sample mill, Tecator, Hoganas, Sweden). Amylose content was measured using a modification of American Association of Cereal Chemists (AACC) Method 61-03 and UV5 spectrophotometer (Mettler-Toledo). Gelatinisation temperature was measured using differential scanning calorimetry (DSC) (Mettler-Toledo) (Waters et al., 2006) while the viscosity was measured by RVA (TecMaster and 4500 model) supported by Thermocline software following AACC Method 61-02. Peak viscosity, trough viscosity, final viscosity, breakdown viscosity, setback, peak time and pasting temperature were measured by a RVA to evaluate rheological properties of starch structure (Perten RVA 4500, Segeltorp, Sweden) according to the manufacturer’s instructions. After RVA analysis, texture parameters were
measured using a Perten TVT6700 texture analyser fitted with a 5 kg load cell and 20 mm probe, and supported by TexCalc software.

### 2.3. Rice grain protein extraction

Duplicate 250 mg sub-samples of rice flour were transferred to 2 mL microfuge tubes. Prolamins and albumins were extracted with 60% n-propanol while 5 M acetic acid was used to isolate glutelins and globulins. Each extraction was done independently following the modified protocol of Balindong et al. (2016).

### 2.4. Characterisation of protein composition

Two high-performance liquid chromatography (HPLC) methods utilising a C8-5 column were used to separate the proteins. The HPLC gradient for prolamsins and albumins was as follows: 25% aqueous acetonitrile (ACN) at 0 min, 40% at 5 min, 45% at 15 min, 60% at 25 min, concluding at 95% at 26 and 27 min and returning to 25% at 28 to 33 min (Balindong et al., 2016). For glutelins and globulins, the HPLC gradient commenced at 25% ACN, increased to 35% at 5 min, 40% at 10 and 15 min, 50% at 25 min and reached a maximum of 95% at 26 and 27 min before returning to 25% between 28 and 33 min (Balindong et al., 2016).

The HPLC analysis was carried out using an Agilent 1260 HPLC System equipped with a vacuum degasser, quaternary pump, auto-injector, and diode array detector. Column temperature was set at 50 °C and absorbance was measured at 280 nm (Balindong et al., 2016).

### 2.5. Data analysis

Each protein peak was analysed using the ChemStation software B.04.03. Storage proteins (prolamins, glutelins and globulins) were selected for quantification and association with RVA and TA parameters. Data were compiled in Microsoft Excel 2013 and imported into GenStat 64-bit Release 17.1 for statistical analysis.
3. Results

Following HPLC analysis, two distinct prolamin HPLC profiles were revealed within each grain type based on the pattern of peak retention times. Sixty seven of the 80 medium grain rice samples were classified as medium grain profile 1 (MG-Profile1) while 13 samples were classified as medium grain profile 2 (MG-Profile2) (Figure 1a and 1b). Similarly, the long grain rice samples were divided into two profiles, long grain profile 1 (LG-Profile1) which included 53 long grain rice samples, and long grain profile 2 (LG-Profile2) which comprised 27 samples (Figure 1c and 1d). Only the major peaks which discriminated between MG-Profile1/MG-Profile2 and LG-Profile1/LG-Profile2 are depicted in Figure 1a, 1b, 1c and 1d. Correlation analyses were undertaken to identify relationships between all parameters followed by regression analyses of those parameters found to be correlated.

3.1. Medium grain rice

The 67 medium grain rice lines grouped as MG-Profile1 not only fell within a narrow range of AAC (17 to 20%), but also a narrow range of total protein content (5.6% to 7.8%) and pasting temperature (66°C to 69°C). There were 23 prolamin, five glutelin and 19 globulin peaks in MG-Profile1. In this profile, the RVA parameters peak viscosity ($r = -0.50$ to -0.81), trough ($r = -0.51$ to -0.81), breakdown ($r = -0.51$ to -0.73) and setback ($r = 0.50$ to 0.74) were more highly correlated with individual (Supplementary Table 1a) and total (Table 1) prolamin peaks than total protein content. Although TA peak force (g) was regarded as a measure of hardness, it was -82 % (MG) and -64 % (LG) correlated with cohesiveness and exhibited weak negative correlations with protein fractions (data not shown). Most MG-Profile1 prolamin peaks (18 prolamin peaks) displayed strong associations with TA parameter height which indicates swelling (Table 1 and Supplementary Table 1a).
Setback was of particular interest because it is a common RVA parameter used to indicate cooked rice firmness. Total glutelin \((r = -0.47)\), the predominant protein in rice endosperm, and individual glutelin peaks \((r = 0 \text{ to } -0.46)\) were negatively correlated with setback while AAC \((r = 0.01)\), total globulin \((r = 0.16)\) and its individual peaks \((r = -0.35 \text{ to } 0.39)\) exhibited very weak relationships with RVA parameters (Supplementary Table 1b).

MG-Profile1 total protein content explained very little of the variation in setback \((R^2 = 0.18)\) (Figure 2b.1), however, total prolamin content explained more of the variation in setback \((R^2 = 0.44, \text{ data not shown})\) while the ratio of total prolamin:total prolamin + total glutelin explained an even greater proportion of the variation in setback \((R^2 = 0.67)\) (Figure 2c.1).

Mean peak areas and coefficient of variation \((CVs)\) of those protein fractions (prolamins and glutelins) associated with RVA or TA parameters were; prolamin mean peak area = 248, CV = 28; glutelin mean peak area = 583, CV = 24 (Supplementary Table 2).

Although the remaining 13 medium grain rice samples comprising MG-Profile2 were also selected on the basis of being within a range of 17 to 20% AAC, they exhibited a wider range of total protein content \((5.5\% \text{ to } 9.8\%)\) and pasting temperature \((66^\circ C \text{ to } 76^\circ C)\). The associations observed between the MG-Profile2 proteins and RVA or TA parameters differed from those obtained from MG-Profile1. Taking the example of RVA setback, although many protein peaks (Supplementary Table 3) and the total prolamin:total prolamin + total glutelin \((R^2 = 0.58)\) (Figure 2c.2) were positively correlated with setback, total protein was more highly correlated \((r = 0.89)\) (Supplementary Table 3) in all cases, a pattern followed by all RVA parameters (Supplementary Table 3). Total glutelin was more highly correlated than total protein in TA parameter cohesiveness \((r = 0.86)\) and adhesiveness \((r = -0.62)\) (Table 2).

### 3.2. Long grain rice
LG-Profile1 was composed of 53 long grain rice lines with total protein content of 6.3% to 10.3% and pasting temperature of 68°C to 77°C. The total prolamin:total prolamin + total glutelin ratio was the major contributor to LG-Profile1 variation in setback ($R^2 = 0.44$) (Figure 3c.1) while AAC ($R^2 = 0.09$) while total protein content ($R^2 = 0.05$) made a very small contribution (Figure 3a.1 and 3b.1). AAC ($r = 0.31$), total protein content ($r = 0.22$) (Supplementary Table 4a), total glutelin content ($r = -0.24$) and total globulin content ($r = -0.05$) (Supplementary Table 4b) in LG-Profile1 had weak correlations with setback.

In contrast to MG-Profile1, only a few individual protein peaks in LG-Profile1 were correlated with RVA parameters, however, some prolamin, glutelin and globulin peaks were correlated with TA parameters (Table 3 and Supplementary Table 4). Six prolamin peaks were positively correlated with stringiness ($r = 0.59$ to 0.77), peak time ($r = 0.61$ to 0.79) and adhesiveness ($r = 0.63$ to 0.76) (Supplementary Table 4a). These same six prolamin peaks were negatively correlated with cohesiveness ($r = -0.63$ to -0.72) (Supplementary Table 4a). Two glutelin peaks and some globulin peaks were negatively correlated with stringiness ($r = -0.50$ to -0.60), peak time ($r = -0.53$ to -0.65) and adhesiveness ($r = -0.63$ to -0.64) and cohesiveness (Supplementary Table 4b).

LG-Profile2 comprised 27 long grain rice lines of AAC 17% to 20%, total protein content of 6.4% to 8.5% and pasting temperature of 67°C to 78°C. Total protein content explained more of the variation in setback ($r=0.44$) than total prolamin content ($r=0.08$), total glutelin content ($r=0.07$) and total globulin content ($r=0.15$) (Supplementary Table 5). No single protein peak nor AAC exhibited significant associations with any RVA or TA parameter (Figure 3a.2, 3c.2 and Supplementary Table 5).

4. Discussion
Starch comprises the largest portion of the milled rice grain and is integral in determining the texture and defining the cooking and eating qualities of rice. Although many studies have investigated the relationship between starch and its structure in the context of rice eating quality, starch does not explain all variation in rice grain quality across all rice germplasm (Kharabian-Masouleh et al., 2013). Protein and its constituent components also play a role (Martin and Fitzgerald, 2002; Zhou et al., 2002), however, the extent to which natural variation in rice grain protein composition influences rice grain textural properties is not well understood. This study analysed rice grain protein composition in a set of medium amylose content rice breeding lines in order to identify the extent to which rice grain textural properties are influenced by rice grain protein composition.

Total protein content and protein composition differed between medium and long grain cultivars which primarily arose from the higher glutelin content of long grain rice. The other feature of note was although the mean prolamin content of medium and long grain cultivars were similar, the prolamin content within medium grain rice was more variable than that of long grain rice. The relatively low level of variation in long grain protein composition observed here may in part explain why the role of protein composition in influencing long grain rice texture it is not as prominent as it is in medium grain rice. The domestication history of rice has an influence on rice traits (Kovach et al., 2007) and the preferential selection by humans for eating qualities influenced by protein composition may explain why long and medium grain rice differ in protein composition.

Cultivated *Oryza sativa* is composed of two major subspecies *japonica* and *indica*. The grains derived from *japonica* varieties including medium grains are stickier when cooked than *indica* rice grains which are often firmer with discrete grains. These textural differences are correlated with the amylose content; low amylose rice is generally soft and sticky while high amylose rice is relatively firm (Rani and Bhattacharya, 1989). The data here
demonstrate variation in rice grain texture occurs in both medium and long grain cultivars despite cultivars being similar in amylose content and GT, confirming reports that cultivars with similar amylose content have differences in pasting and textural properties (Champagne et al., 1999), and that factors other than starch such as protein composition may influence rice grain texture.

The globulins displayed very low levels of variation within and between the different types of rice and this was reflected in their little to no association with RVA or TA parameters. Low levels of variation suggest globulins play an important functional role which does not allow divergence from a level necessary to maintain that function. The globulins are synthesised early in rice grain development (Yamagata et al., 1982) and may play a role as structural proteins that maintain protein body integrity (Shewry, 2003), and this may explain their low level of variation.

Experiments which extracted and re-added each protein component, albumin, globulin, glutelin and prolamain, individually and in combination found each type of protein has different effects on RVA parameters (Baxter et al., 2004; 2010; 2014). In addition, the viscosity properties of rice flour change as it ages and a significant proportion of these changes can be attributed to changes in protein structure (Zhou et al., 2010). We have found the prolamins and glutelins exhibited wide variation in content in rice breeding lines which were grown in the one season and location, and the amount and ratio between these two proteins influenced instrumental measures of rice eating quality in some sets of germplasm.

Each of these protein fractions display different levels of aqueous solubility and so it is possible they affect rice grain quality by having an impact on the rates of starch hydration during cooking. However, the observed effects may be due to molecular interactions either between or within each of the different types of protein or between protein and starch or lipid and this has a direct effect on rice grain physico-chemical properties.
Setback, an RVA parameter defined by the difference between final and peak viscosity, is a predictor of the final texture of rice indicating the firmness of cooked rice (Gravois and Webb, 1997). The greater the setback, the firmer the rice texture and this parameter is therefore used in rice breeding programs as a selection tool for rice eating quality. Prolamins and the prolamin: prolamin + glutelin ratio were most closely associated with this parameter in this set of germplasm. RVA is not, however, highly correlated with all sensory panel parameters and textural attributes of the cooked rice in all germplasm (Champagne et al., 1999) and so it is possible measuring protein composition directly may be a more useful predictor of some of the components of rice grain quality such as cooked rice firmness in some sets of germplasm.

Texture analysers repeatedly compress a sample simulating the masticating action of the human mouth and generating a force-time curve (Manickavasagan et al., 2012). The textural attributes measured by TA include hardness which is defined as the force applied to compress a food; adhesiveness, which is the ability of food to adhere to teeth; springiness, which measures how well a deformed material springs back to its undeformed condition; and cohesiveness, which describes how well a product withstands a second deformation relative to its first deformation (Paula and Conti-Silva, 2014). Glutelin in long and medium grain samples was more highly correlated with adhesiveness, cohesiveness and stringiness than total protein content, further illustrating that each discrete component of rice grain protein has a unique impact on rice grain texture.

Rice grain protein can be an important contributor to total protein intake in some communities and so development of high yielding, high protein rice cultivars for these communities is a priority. Identification of Quantitative Trait Loci (QTL) and genes that control total protein content is a means of assisting in the development of high yielding, high protein cultivars (Zhang et al., 2008). However, the data here demonstrate the underlying
constituents of total protein, particularly glutelin and prolamin, can be very variable within a narrow range of total protein content, highlighting the need to identify not only QTL that control total protein content, but also the QTL which control albumin, globulin, glutelin and prolamin content. The wide variation in prolamin and glutelin content also has direct implications for human nutrition. Prolamins are poorly digested by humans (Resurreccion and Juliano, 1981; Kubota et al., 2010), thus breeding for rice with low prolamin content may not only generate rice with a softer texture, but also more nutritious rice.

Amylose content is not only the primary controller of rice grain physico-chemical properties but also rice starch digestibility or glycaemic index (GI) (Fitzgerald et al., 2009). However, for any set of rice cultivars with the same AAC, the GI can vary by up 20 GI units as estimated by in vitro analysis; variation which is not explained by differences in GT (Fitzgerald et al., 2009), which is perhaps the second most important rice starch property after AAC. Although the impact of rice protein composition on rice starch digestibility is not known, addition of native rice protein to wheat reduces wheat starch digestibility in vitro (López-Barón et al., 2017), suggesting rice grain storage proteins may have a role in modulating rice starch digestibility. This is not without precedent in the cereals given the proteins of sorghum (Rooney and Pflugfelder, 1986) and wheat (Bhattacharai et al., 2016) modulate starch digestibility, perhaps through interacting with digestive enzymes in vivo (Bhattacharai et al., 2016). Given the differing properties of rice grain storage proteins, it is conceivable their interactions with starch, or in vivo digestive enzymes, could would also differ, affecting rice starch digestibility.

In common with many traits, rice grain composition is controlled and or influenced by genetics and environment. For example, high temperature stress during rice grain development increases the occurrence of chalky grain in susceptible genotypes (Lisle et al., 2000), alters starch and protein composition (Lin et al., 2010) and leads to higher rates of
grain breakage during milling (Lyman et al., 2013). There are more than 10,000 known rice varieties and temperature is but one of many environmental variables which may influence rice grain composition in a genotype dependant manner. Soil type, water availability, relative humidity, altitude, day length and crop nitrogen status are other factors which can have a genotype dependant impact on rice plant development and grain composition. We have shown there can be wide variation in rice grain protein composition and this variation influences instrumental measures of rice grain quality, however, further research is required to establish the dimensions of the genotype x environment matrix which govern rice grain protein composition.

In conclusion, rice grain protein composition was associated with instrumental measures of grain quality in this set of germplasm. Although the textural properties of rice are complex, these data suggest that consideration of rice grain protein composition in rice breeding programs will contribute to the generation of rice cultivars with high grain quality.

5. Acknowledgements

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Appendix A: Supplementary material
6. References


Table 1. Medium grain Profile 1 (67 samples) correlations greater than 0.5 or less than -0.5 between rapid visco/texture analyser parameters and high-performance liquid chromatography separated protein components.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Correlation distribution of individual prolamin peaks</th>
<th>Total prolamin correlation</th>
<th>Total protein correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>RVA Peak viscosity</td>
<td>20/23 of peaks -0.50 to -0.79</td>
<td>-0.81</td>
<td>-0.52</td>
</tr>
<tr>
<td>RVA Trough</td>
<td>14/23 of peaks -0.51 to -0.81</td>
<td>-0.67</td>
<td>-0.31</td>
</tr>
<tr>
<td>RVA Breakdown</td>
<td>17/23 of peaks -0.51 to -0.73</td>
<td>-0.72</td>
<td>-0.54</td>
</tr>
<tr>
<td>RVA Setback</td>
<td>16/23 of peaks 0.50 to 0.74</td>
<td>0.66</td>
<td>0.42</td>
</tr>
<tr>
<td>TA Height</td>
<td>18/23 of peaks 0.55 to 0.85</td>
<td>0.83</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Table 2. Medium grain Profile 2 (13 samples) correlations greater than 0.5 or less than -0.5 between rapid visco/texture analyser parameters and high-performance liquid chromatography separated protein components.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Correlation distribution of individual glutelin peaks</th>
<th>Total glutelin correlation</th>
<th>Total protein correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA Cohesiveness</td>
<td>3/5 of peaks 0.50 to 0.58</td>
<td>0.86</td>
<td>0.64</td>
</tr>
<tr>
<td>TA Adhesiveness</td>
<td>2/5 of peaks -0.77 to -0.84</td>
<td>-0.62</td>
<td>-0.12</td>
</tr>
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</table>
Table 3. Long grain Profile 1 (53 samples) correlations greater than 0.5 or less than -0.5 between rapid visco/texture analyser parameters and high-performance liquid chromatography separated protein components.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Correlation distribution of individual protein peaks</th>
<th>Total glutelin correlation</th>
<th>Total protein correlation</th>
</tr>
</thead>
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<tr>
<td>TA Cohesiveness</td>
<td>2/5 of peaks</td>
<td>0.60</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>0.59 to 0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA Stringiness</td>
<td>2/5 of peaks</td>
<td>-0.56</td>
<td>-0.29</td>
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<td></td>
<td>-0.50 to -0.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA Adhesiveness</td>
<td>2/5 of peaks</td>
<td>-0.63</td>
<td>-0.30</td>
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<tr>
<td></td>
<td>-0.63 to -0.64</td>
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<table>
<thead>
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<th>Parameter</th>
<th>Correlation distribution of individual prolamin peaks</th>
<th>Total prolamin correlation</th>
<th>Total protein correlation</th>
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<tr>
<td>RVA Breakdown</td>
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**Figure captions**

**Figure 1.** Prolamin HPLC profiles of (a) medium grain profile 1 (MG-Profile1); (b) medium grain profile 2 (MG-Profile2); (c) long grain profile 1 (LG-Profile1); (d) long grain profile 2 (LG-Profile2).

**Figure 2.** Scatter diagram and linear regression of (a.1; a.2) apparent amylose content; (b.1; b.2) % protein; (c.1; c.2) total prolamin:sum of total prolamin and total glutelin ratio vs setback of medium grain rice. Medium grain profile 1, MG-Profile1 (a.1; b.1; c.1) and medium grain profile 2, MG-Profile2 (a.2; b.2; c.2).

**Figure 3.** Scatter diagram and linear regression of (a.1; a.2) apparent amylose content; (b.1; b.2) % protein; (c.1; c.2) total prolamin:sum of total prolamin and total glutelin ratio vs setback of long grain rice. Long grain profile 1, LG-Profile1 (a.1; b.1; c.1) and long grain profile 2, LG-Profile2 (a.2; b.2; c.2).
Figure 1.
Figure 2.
Figure 3.