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Jeanette L. Balindong
Southern Cross University

Rachel M. Ward
NSW Department of Primary Industries

Lei Liu
Southern Cross University

Terry J. Rose
Southern Cross University

Laura A. Pallas
NSW Department of Primary Industries

See next page for additional authors

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Authors

Jeanette L. Balindong, Rachel M. Ward, Lei Liu, Terry J. Rose, Laura A. Pallas, Ben W. Oviden, Peter J. Snell, and Daniel LE Waters

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Jeanette L Balindong¹, Rachelle M Ward², Lei Liu¹, Terry J Rose¹, Laura A Pallas², Ben W
Ovenden², Peter J Snell², and Daniel LE Waters^{1,3*}

¹Southern Cross Plant Science, Southern Cross University, Lismore, NSW, 2480, Australia

²Yanco Agricultural Institute, NSW Department of Primary Industries, Yanco, NSW, 2703,
Australia

³ARC ITTC for Functional Grains, Charles Sturt University, Wagga Wagga, NSW, 2650,
Australia

Corresponding author

*Name: Daniel LE Waters; Email: dawaters@csu.edu.au; Telephone number: +61 (02) 6933
2161; Postal address: ARC ITTC for Functional Grains, Charles Sturt University, Locked
Bag 588, Wagga Wagga NSW 2678 Australia

Email addresses of co-authors

Jeanette L Balindong: j.balindong.10@student.scu.edu.au; Rachelle M Ward:

rachelle.ward@dpi.nsw.gov.au; Lei Liu: lliu@scu.edu.au; Terry J Rose:

terry.rose@scu.edu.au; Laura A Pallas: laura.pallas@dpi.nsw.gov.au; Ben W Ovenden:

ben.ovenden@dpi.nsw.gov.au and Peter J Snell: peter.snell@dpi.nsw.gov.au

1 **Abstract**

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

18

19 **Keywords:** *Oryza sativa*; grain quality; prolamins; texture

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

28 **1. Introduction**

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

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

41 Rice grains are composed of approximately 80-85% starch, 4-10% protein, 1% lipid and
42 10% moisture. Amylose content, amylopectin fine structure, and intra and/or inter-molecular
43 interactions of starch with other components such as proteins, lipids and non-starch
44 polysaccharides, are some of the factors involved in determining rice grain quality. Among
45 these, amylose content is perhaps the most important factor that determines cooking
46 behaviour. Low amylose cooked rice is generally soft with individual grains adhering closely
47 while high amylose rice is harder and less sticky (Rani and Bhattacharya, 1989).
48 Amylopectin chain length distribution also has an impact on rice grain quality through its
49 effect on starch gelatinisation temperature (GT) (Umemoto et al., 2004) which is associated
50 with cooking time.

51 The primary genetic determinants of amylose content and amylopectin chain length
52 distribution are known. The *Waxy/waxy* gene codes for granule-bound starch synthase I and is
53 the most important controller of amylose content (Wang et al., 1995), while *Alk/alk* codes for

54 starch synthase IIa and is responsible for the most important differences in amylopectin chain
55 length distribution (Umemoto et al., 2004). Although several alleles of the *Waxy/waxy* and
56 *Alk/alk* genes associated with different forms of starch have been identified (Waters et al.,
57 2006), other starch biosynthesis genes in addition to *Waxy/waxy* and *Alk/alk* affect rice
58 cooking and eating quality (Kharabian-Masouleh et al., 2013). However, starch structure does
59 not explain all variation in rice grain quality parameters in all rice germplasm (Kharabian-
60 Masouleh et al., 2013), and flour derived from cultivars with similar amylose content may
61 have different pasting and textural properties (Champagne et al., 1999). Furthermore,
62 viscosity differences between rice flour and rice starch (Singh et al., 2000) suggest
63 components other than starch affect these properties of rice.

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

75 In common with all cereal grain, rice grain storage proteins are composed of globulins,
76 glutelins and prolamins which are traditionally defined by their differences in solubility.
77 Globulins are salt-soluble, the glutelins dissolve in dilute acid or alkali solutions while the
78 prolamins, including those of rice which are poorly digested by monogastric animals

79 including humans (Resurreccion and Juliano, 1981; Kubota et al., 2010), are solubilised by
80 aqueous alcohol. Rice grain protein content differs between cultivars and within populations
81 derived from two parental cultivars (Zhang et al., 2008), however, the relationship between
82 rice grain protein composition and rice eating quality is not well understood. Here, we report
83 upon the relationship between rice grain protein composition and instrumental measures of
84 rice eating quality in a range of advanced rice breeding lines.

85

86 **2. Materials and methods**

87 ***2.1. Rice samples***

88 Flour from the milled grain of 80 medium grain and 80 long grain advanced rice
89 breeding lines within a narrow range of 17% to 20% apparent amylose content (AAC) from
90 the 2014 rice breeding and quality trials at Yanco, New South Wales, Australia (34° 36'S;
91 146° 23' E) was used in this study. The accumulated thermal time for the rice growing season
92 for years 2011 to 2014 appear in Supplementary Figure 1.

93 ***2.2. Rice grain grinding, RVA and TA analysis***

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

104 measured using a Perten TVT6700 texture analyser fitted with a 5 kg load cell and 20 mm
105 probe, and supported by TexCalc software.

106 **2.3. Rice grain protein extraction**

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

111 **2.4. Characterisation of protein composition**

112 Two high-performance liquid chromatography (HPLC) methods utilising a C8-5
113 column were used to separate the proteins. The HPLC gradient for prolamins and albumins
114 was as follows: 25% aqueous acetonitrile (ACN) at 0 min, 40% at 5 min, 45% at 15 min,
115 60% at 25 min, concluding at 95% at 26 and 27 min and returning to 25% at 28 to 33 min
116 (Balindong et al., 2016). For glutelins and globulins, the HPLC gradient commenced at 25%
117 ACN, increased to 35% at 5 min, 40% at 10 and 15 min, 50% at 25 min and reached a
118 maximum of 95% at 26 and 27 min before returning to 25% between 28 and 33 min
119 (Balindong et al., 2016).

120 The HPLC analysis was carried out using an Agilent 1260 HPLC System equipped with
121 a vacuum degasser, quaternary pump, auto-injector, and diode array detector. Column
122 temperature was set at 50 °C and absorbance was measured at 280 nm (Balindong et al.,
123 2016).

124 **2.5. Data analysis**

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

129

130 **3. Results**

131 Following HPLC analysis, two distinct prolamin HPLC profiles were revealed within
132 each grain type based on the pattern of peak retention times. Sixty seven of the 80 medium
133 grain rice samples were classified as medium grain profile 1 (MG-Profile1) while 13 samples
134 were classified as medium grain profile 2 (MG-Profile2) (Figure 1a and 1b). Similarly, the
135 long grain rice samples were divided into two profiles, long grain profile 1 (LG-Profile1)
136 which included 53 long grain rice samples, and long grain profile 2 (LG-Profile2) which
137 comprised 27 samples (Figure 1c and 1d). Only the major peaks which discriminated between
138 MG-Profile1/MG-Profile2 and LG-Profile1/LG-Profile2 are depicted in Figure 1a, 1b, 1c and
139 1d. Correlation analyses were undertaken to identify relationships between all parameters
140 followed by regression analyses of those parameters found to be correlated.

141 **3.1. Medium grain rice**

142 The 67 medium grain rice lines grouped as MG-Profile1 not only fell within a narrow
143 range of AAC (17 to 20%), but also a narrow range of total protein content (5.6% to 7.8%)
144 and pasting temperature (66°C to 69°C). There were 23 prolamin, five glutelin and 19
145 globulin peaks in MG-Profile1. In this profile, the RVA parameters peak viscosity ($r = -0.50$
146 to -0.81), trough ($r = -0.51$ to -0.81), breakdown ($r = -0.51$ to -0.73) and setback ($r = 0.50$ to
147 0.74) were more highly correlated with individual (Supplementary Table 1a) and total (Table
148 1) prolamin peaks than total protein content. Although TA peak force (g) was regarded as a
149 measure of hardness, it was -82 % (MG) and -64 % (LG) correlated with cohesiveness and
150 exhibited weak negative correlations with protein fractions (data not shown). Most MG-
151 Profile1 prolamin peaks (18 prolamin peaks) displayed strong associations with TA
152 parameter height which indicates swelling (Table 1 and Supplementary Table 1a).

153 Setback was of particular interest because it is a common RVA parameter used to
154 indicate cooked rice firmness. Total glutelin ($r = -0.47$), the predominant protein in rice
155 endosperm, and individual glutelin peaks ($r = 0$ to -0.46) were negatively correlated with
156 setback while AAC ($r = 0.01$), total globulin ($r = 0.16$) and its individual peaks ($r = -0.35$ to
157 0.39) exhibited very weak relationships with RVA parameters (Supplementary Table 1b).
158 MG-Profile1 total protein content explained very little of the variation in setback ($R^2 = 0.18$)
159 (Figure 2b.1), however, total prolamin content explained more of the variation in setback (R^2
160 $= 0.44$, data not shown) while the ratio of total prolamin:total prolamin + total glutelin
161 explained an even greater proportion of the variation in setback ($R^2 = 0.67$) (Figure 2c.1).
162 Mean peak areas and coefficient of variation (CVs) of those protein fractions (prolamins and
163 glutelins) associated with RVA or TA parameters were; prolamin mean peak area = 248, CV
164 = 28; glutelin mean peak area = 583, CV = 24 (Supplementary Table 2).

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

175

176 **3.2. Long grain rice**

177 LG-Profile1 was composed of 53 long grain rice lines with total protein content of
178 6.3% to 10.3% and pasting temperature of 68°C to 77°C. The total prolamin:total prolamin +
179 total glutelin ratio was the major contributor to LG-Profile1 variation in setback ($R^2 = 0.44$)
180 (Figure 3c.1) while AAC ($R^2 = 0.09$) while total protein content ($R^2 = 0.05$) made a very
181 small contribution (Figure 3a.1 and 3b.1). AAC ($r = 0.31$), total protein content ($r = 0.22$)
182 (Supplementary Table 4a), total glutelin content ($r = -0.24$) and total globulin content ($r = -$
183 0.05) (Supplementary Table 4b) in LG-Profile1 had weak correlations with setback.

184 In contrast to MG-Profile1, only a few individual protein peaks in LG-Profile1 were
185 correlated with RVA parameters, however, some prolamin, glutelin and globulin peaks were
186 correlated with TA parameters (Table 3 and Supplementary Table 4). Six prolamin peaks
187 were positively correlated with stringiness ($r = 0.59$ to 0.77), peak time ($r = 0.61$ to 0.79) and
188 adhesiveness ($r = 0.63$ to 0.76) (Supplementary Table 4a). These same six prolamin peaks
189 were negatively correlated with cohesiveness ($r = -0.63$ to -0.72) (Supplementary Table 4a).
190 Two glutelin peaks and some globulin peaks were negatively correlated with stringiness ($r = -$
191 0.50 to -0.60), peak time ($r = -0.53$ to -0.65) and adhesiveness ($r = -0.63$ to -0.64) and
192 cohesiveness (Supplementary Table 4b).

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

199

200

201 **4. Discussion**

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

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

222 Cultivated *Oryza sativa* is composed of two major subspecies *japonica* and *indica*. The
223 grains derived from *japonica* varieties including medium grains are stickier when cooked
224 than *indica* rice grains which are often firmer with discrete grains. These textural differences
225 are correlated with the amylose content; low amylose rice is generally soft and sticky while
226 high amylose rice is relatively firm (Rani and Bhattacharya, 1989). The data here

227 demonstrate variation in rice grain texture occurs in both medium and long grain cultivars
228 despite cultivars being similar in amylose content and GT, confirming reports that cultivars
229 with similar amylose content have differences in pasting and textural properties (Champagne
230 et al., 1999), and that factors other than starch such as protein composition may influence rice
231 grain texture.

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

239 Experiments which extracted and re-added each protein component, albumin, globulin,
240 glutelin and prolamin, individually and in combination found each type of protein has
241 different effects on RVA parameters (Baxter et al., 2004; 2010; 2014). In addition, the
242 viscosity properties of rice flour change as it ages and a significant proportion of these
243 changes can be attributed to changes in protein structure (Zhou et al., 2010). We have found
244 the prolamins and glutelins exhibited wide variation in content in rice breeding lines which
245 were grown in the one season and location, and the amount and ratio between these two
246 proteins influenced instrumental measures of rice eating quality in some sets of germplasm.
247 Each of these protein fractions display different levels of aqueous solubility and so it is
248 possible they affect rice grain quality by having an impact on the rates of starch hydration
249 during cooking. However, the observed effects may be due to molecular interactions either
250 between or within each of the different types of protein or between protein and starch or lipid
251 and this has a direct effect on rice grain physico-chemical properties.

252 Setback, an RVA parameter defined by the difference between final and peak viscosity,
253 is a predictor of the final texture of rice indicating the firmness of cooked rice (Gravois and
254 Webb, 1997). The greater the setback, the firmer the rice texture and this parameter is
255 therefore used in rice breeding programs as a selection tool for rice eating quality. Prolamins
256 and the prolamin: prolamin + glutelin ratio were most closely associated with this parameter
257 in this set of germplasm. RVA is not, however, highly correlated with all sensory panel
258 parameters and textural attributes of the cooked rice in all germplasm (Champagne et al.,
259 1999) and so it is possible measuring protein composition directly may be a more useful
260 predictor of some of the components of rice grain quality such as cooked rice firmness in
261 some sets of germplasm.

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

272 Rice grain protein can be an important contributor to total protein intake in some
273 communities and so development of high yielding, high protein rice cultivars for these
274 communities is a priority. Identification of Quantitative Trait Loci (QTL) and genes that
275 control total protein content is a means of assisting in the development of high yielding, high
276 protein cultivars (Zhang et al., 2008). However, the data here demonstrate the underlying

277 constituents of total protein, particularly glutelin and prolamin, can be very variable within a
278 narrow range of total protein content, highlighting the need to identify not only QTL that
279 control total protein content, but also the QTL which control albumin, globulin, glutelin and
280 prolamin content. The wide variation in prolamin and glutelin content also has direct
281 implications for human nutrition. Prolamins are poorly digested by humans (Resurreccion
282 and Juliano, 1981; Kubota et al., 2010), thus breeding for rice with low prolamin content may
283 not only generate rice with a softer texture, but also more nutritious rice.

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

298 In common with many traits, rice grain composition is controlled and or influenced by
299 genetics and environment. For example, high temperature stress during rice grain
300 development increases the occurrence of chalky grain in susceptible genotypes (Lisle et al.,
301 2000), alters starch and protein composition (Lin et al., 2010) and leads to higher rates of

302 grain breakage during milling (Lyman et al., 2013). There are more than 10, 000 known rice
303 varieties and temperature is but one of many environmental variables which may influence
304 rice grain composition in a genotype dependant manner. Soil type, water availability, relative
305 humidity, altitude, day length and crop nitrogen status are other factors which can have a
306 genotype dependant impact on rice plant development and grain composition. We have
307 shown there can be wide variation in rice grain protein composition and this variation
308 influences instrumental measures of rice grain quality, however, further research is required
309 to establish the dimensions of the genotype x environment matrix which govern rice grain
310 protein composition.

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

315

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321

322 **Appendix A: Supplementary material**

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

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

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

Parameter	Correlation distribution of individual glutelin peaks	Total glutelin correlation	Total protein correlation
TA Cohesiveness	3/5 of peaks 0.50 to 0.58	0.86	0.64
TA Adhesiveness	2/5 of peaks -0.77 to -0.84	-0.62	-0.12

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488 **Table 3.** Long grain Profile 1 (53 samples) correlations greater than 0.5 or less than -0.5
 489 between rapid visco/ texture analyser parameters and high-performance liquid
 490 chromatography separated protein components.

Parameter	Correlation distribution of individual glutenin peaks	Total glutelin correlation	Total protein correlation
TA Cohesiveness	2/5 of peaks 0.59 to 0.64	0.60	0.31
TA Stringiness	2/5 of peaks -0.50 to -0.60	-0.56	-0.29
TA Adhesiveness	2/5 of peaks -0.63 to -0.64	-0.63	-0.30
Parameter	Correlation distribution of individual prolamins peaks	Total prolamins correlation	Total protein correlation
RVA Breakdown	0/23 of peaks > 0.50	-0.50	-0.36
RVA Setback	0/23 of peaks > 0.50	0.44	0.22

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504 **Figure captions**

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

508
509 **Figure 2.** Scatter diagram and linear regression of (a.1; a.2) apparent amylose content; (b.1;
510 b.2) % protein; (c.1; c.2) total prolamin:sum of total prolamin and total glutelin
511 ratio vs setback of medium grain rice. Medium grain profile 1, MG-Profile1 (a.1;
512 b.1; c.1) and medium grain profile 2, MG-Profile2 (a.2; b.2; c.2).

513
514 **Figure 3.** Scatter diagram and linear regression of (a.1; a.2) apparent amylose content; (b.1;
515 b.2) % protein; (c.1; c.2) total prolamin:sum of total prolamin and total glutelin
516 ratio vs setback of long grain rice. Long grain profile 1, LG-Profile1 (a.1; b.1; c.1)
517 and long grain profile 2, LG-Profile2 (a.2; b.2; c.2).

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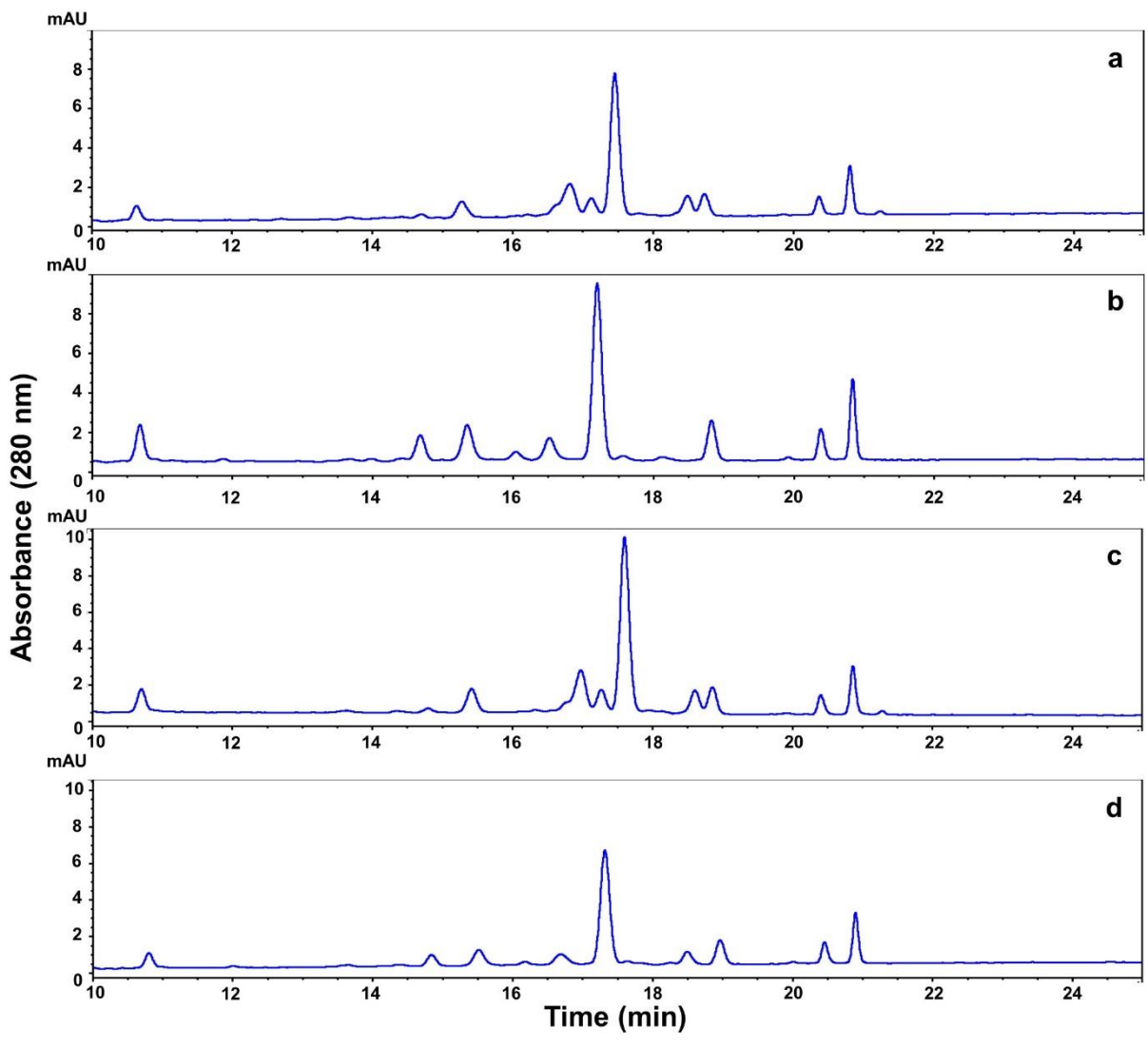


Figure 1.

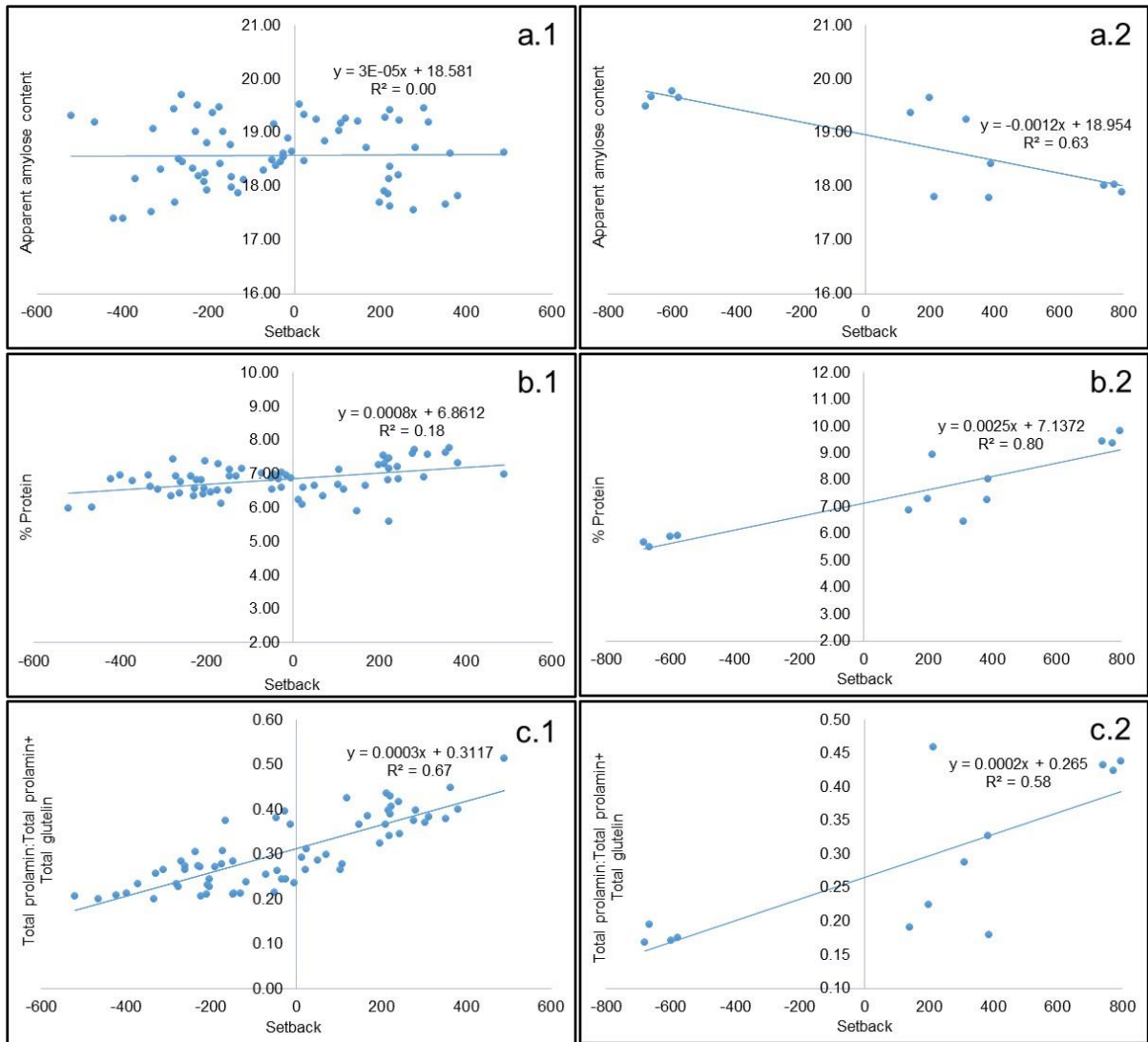


Figure 2.

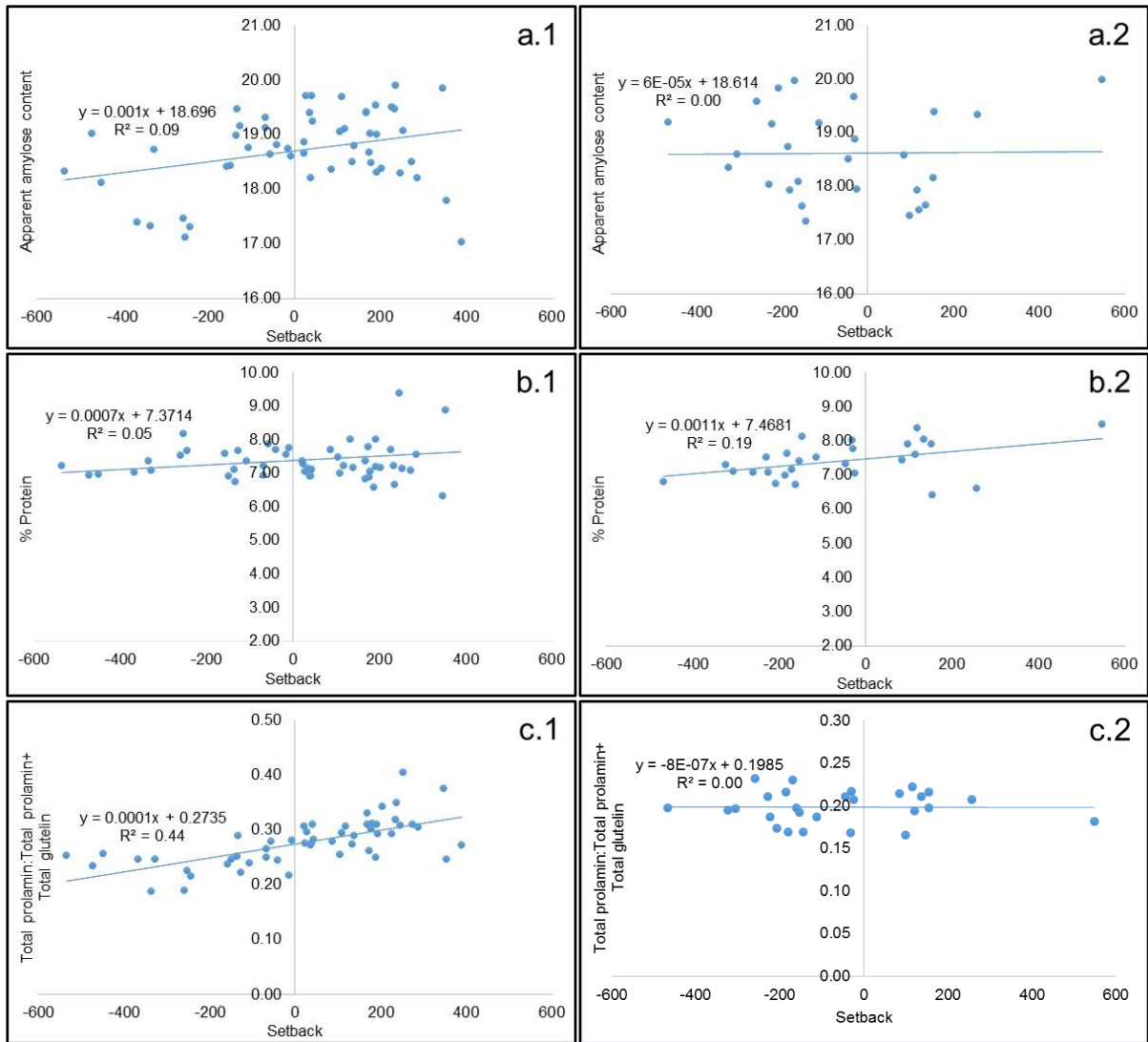


Figure 3.