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Grain Amylose Content and its Stability over Seasons in a Selected Set of Rice Varieties Grown in Sri Lanka

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ABSTRACT

Grain amylose content, one of the key parameters determining cooking and eating quality of rice, varies among varieties. Identification of varieties with different grain amylose contents that are stable over seasons is important to improve the quality of rice. Present study evaluated 39 varieties of Sri Lanka for grain amylose content and its stability over seasons. Twenty six traditional and 12 improved rice varieties and 1 introduced rice variety were evaluated over 2006 Yala and 2006-07 Maha seasons. Experiment in each season was conducted in a Randomized Complete Block Design at the Regional Rice Research and Development Center, Bombuwela. Amylose content was determined by standard iodine colorimetric method. Grain amylose content significantly varied from 23 to 31% among varieties. Irrespective of traditional or improved, all the varieties except Suduru Samba and Basmathi 370 (intermediate amylose contents: 20-24%), had high amylose contents (≥25%) in both seasons. In both traditional and improved varieties, varietal differences for stability of grain amylose content over seasons were found. Higher proportion of improved varieties showed stable amylose content over seasons than that of traditional varieties. Interestingly, both the varieties with intermediate amylose showed stability over seasons. Varieties with high and intermediate amylose contents that are stable over seasons are valuable germplasm in terms of grain amylose content. Therefore, such varieties can be used in rice grain quality improvement program in Sri Lanka.

Keywords: *Grain amylose content, rice, stability over seasons, traditional and improved varieties, Sri Lanka*

INTRODUCTION

Rice mainly consists of starch (Juliano, 2003) and there are two different types of storage starches namely amylose and amylopectin (Juliano, 1985; Juliano, 2003; Fitzgerald *et al*., 2009a; Chen *et al*., 2012). Amylose content of rice is considered as the most important predictor influencing the sensory quality of rice (Fitzgerald *et al*., 2009a). It is commonly used as an objective index for cooked rice texture (Webb, 1991; Asghar *et al*., 2012). Low amylose levels are associated with cohesive, tender, and glossy cooked rice (Juliano, 1971). Conversely, high levels of amylose cause rice to absorb more water and consequently expand comparatively more during cooking and the grains tend to cook dry, fluffy, and separate (Juliano, 1971; Fitzgerald *et al*., 2009a; Patindol *et al*., 2010).

Countries around the world have different preferences for rice with varying amylose contents (Juliano, 1985; Juliano, 2003; Calingacion *et al*., 2014). Consumer preference for rice around the world, when eaten an intact grain is largely dependent on a desire for its cooked texture to be either firm and non-sticky or soft and sticky (Chen *et al*., 2007). Amylose content is the major factor related to cooked rice texture (Fitzgerald *et al*., 2009b). Therefore,

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rice breeders invariably are concerned with and routinely select for desired amylose content in new lines (Fitzgerald *et al*., 2009b; Calingacion *et al*., 2014).

The grain amylose content can vary among varieties due to genetic and environmental factors (Chen *et al*., 2007; Chen *et al*., 2012). The environmental conditions such as the temperature and day length and genotype × environment interaction also affect the amylose content of different varieties of rice (Lin *et al*., 2005). Several other studies have shown that the interaction between genotype and ambient temperature also has an effect on amylose content in rice grains (Nkori Kibanda and Luzi-kihupi, 2007; Patindol *et al*., 2015). Thus, amylose content varies over seasons in the same site of cultivation even with the same variety (Inatsu, 1979; Asaoka *et al*., 1984). Grain amylose content of rice is influenced by ambient temperature (Chen *et al*., 2007; Ahmed *et al*., 2015) and it decreases with the increasing environmental temperatures (Ahmed *et al*., 2015). Environmental temperature during seed development affects the amylose synthesis in rice with the Wxb allele. The level of the Wx protein increase in lower temperatures, resulting in higher amylose contents in mature seeds (Inatsu, 1979; Asaoka *et al*., 1984). Chen *et al*., (2007) reported that higher air temperature during grain development associated with a decrease in amylose content of rice varieties having low and intermediate amylose contents, but with an increase in rice varieties with high amylose content. Heritability that shows the proportion of genetic variability relative to total phenotypic variability for grain amylose content in rice has also been studied. Heritability values from low to high have been reported. Rafii *et al*., (2014) reported very low (31.74%) while Nirmaladevi *et al*., (2015) reported very high (93%) broad sense heritability estimates. Asfaliza *et al*., (2012) reported low broad sense (45.45%) as well as narrow sense (39.26%) heritability values.

Studies on how starch characteristics vary in response to seasonal differences and development of rice varieties with desirable amylose contents that do not change over seasons are important. The current trend of rice breeding research is also for the development of rice varieties having desirable grain quality traits with less sensitivity particularly to seasonal differences. Therefore, the objective of the present study was to evaluate the variety and variety × season interaction effects of the grain amylose content of rice using a set of rice genotypes in Sri Lanka.

MATERIALS AND METHODS

Materials

A total of twenty six traditional and twelve improved local rice varieties and one introduced '*Basmathi*' rice variety were used in the study (Table 01).

Experimental design

The experiment was conducted at the Regional Rice Research and Development Center (RRRDC), Bombuwala, over 2006 *Yala* and 2006/07 *Maha* seasons. Rice varieties were planted in a Randomized Complete Block Design (RCBD) with two replications in each season. Plot size of the experiment was 1.9 × 1.2 m and rice seedlings (18 days old) were transplanted in the experimental plots at a spacing of 15 × 20 cm within and between rows with one plant per hill.

Amylose Content

Varieties were harvested at physiological maturity. Rice seeds were stored at 8° C prior to analysis of grain amylose. Rice seed samples were dehulled using a dehuller machine (Satake THU 35B, Japan) and stored in airtight containers at 8 °C in a cold room. The milled rice grains were used for the determination of amylose content using the standard Iodine Colorimetric Method (Juliano, 1985). One mL of 95% ethanol and 9 mL of 1 N sodium hydroxide were added to 100 mg of flour sample. The sample was heated for 10 min in a boiling water bath to gelatinize the starch. Sample was allowed to cool and volume was made up to 100 mL. Five milliliters of solution, 1 mL of 1 N acetic acid and 2 mL of 0.2 % iodine solution were transferred to 100 mL volumetric flack and volume was made up to 100 mL. Flask was shaken and allowed to stand for 20 min at 30 °C. Absorbance was measured at 620 nm using a UV/visible spectrophotometer (GBC-911A, Scientific Equipment, Australia). Total amylose content was determined from a standard potato amylose curve (Potato, Sigma Type III). Analysis was performed in quadruplicate for each and every variety in each season. Based on the amylose content, rice varieties were then classified into low amylose (12-20%), intermediate amylose (20-25%) and high amylose (25-33%) groups (Frei *et al*., 2003).

Statistical Analysis

Data on grain amylose content of 39 rice varieties were analyzed using the method proposed in Abeysiriwardena *et al*., (1991) using SAS statistical software. Deviation (d_{ijk}) of the grain amylose content of each variety in each plot within a season from the season mean (plot deviation) was computed as follows;

$$d_{ijk} = y_{ijk} - \bar{y}_j$$

Where, d_{ijk} is the deviation of the grain amylose content of the i^{th} variety in the k^{th} block in the j^{th} season from j^{th} seasonal mean (average over all varieties and blocks). Sum over variety deviations for each season was zero so that the effect of season became zero. The mean of variety deviations across seasons for each variety (D_i) estimated the average effect of that variety across seasons and it was calculated as follows;

$$D_1 = \sum_{j=1}^{n} \bar{d}_{ij} \Big/ n$$

Where, “n” is the number of seasons.

Combined analysis of variance (ANOVA) over seasons on the plot deviations for amylose content was performed. When interaction effect of variety × season was found to be significant, it was further analyzed to derive stability parameters (S^2) one corresponding to each variety (S_i^2) as follows;

$$S_i^2 = \left[\sum_{j=1}^{n} d_{ij}^2 - \frac{\left(\sum_{j=1}^{n} d_{ij}\right)^2}{n} \right] \Big/ q^{(n-1)}$$

Each variety’s S_i^2 could be tested against the pooled error by an “F” test to see whether it was different from zero or significant. Non-significant S_i^2 of a variety indicates that the variety does not interact with the environment in an unpredictable manner so that the variety is stable over seasons with respect to the amylose content. Duncan New Multiple Range Test (DNMRT) was performed to separate variety means. Thus, this method provides the opportunity to select stable varieties over seasons at a desired content of amylose.

RESULTS AND DISCUSSION

The selected Sri Lankan traditional rice varieties are the ones that are still cultivated and available among farmers. Most of the improved Sri Lankan rice varieties used in the study are the most popular and widely grown and consumed varieties in the country. *Yala* is the season with comparatively higher rainfall starting from March and ending in August and *Maha* is the season with comparatively lower rainfall starting from September and ending in February of the following year in the Low Country Wet Zone of Sri Lanka. Maximum and minimum temperatures of *Yala* and *Maha* experimental seasons were 29.1 - 31.8 °C and 23.1 - 26.9 °C and 29.5 - 31.7 °C and 22.1 - 25.1°C, respectively. Although *Yala* season is reported to have a higher ambient temperature than in *Maha* season only the minimum temperature in *Yala* was about 1°C more than that of *Maha* in the present study.

Amylose content of rice varieties

The results from the combined analysis of variance performed on plot deviations of grain amylose content from seasonal means indicated that both variety and the variety × season interaction effects were highly significant. Hence, the mean deviation of the amylose content over seasons or the 'D' value and the stability parameter (S^2) over seasons corresponding to each variety were estimated and tested against the pooled error. Variability of 'D' values exactly represents the variability of actual average amylose contents of varieties over seasons so that mean separation of 'D' values is as same as the mean separation of actual average amylose contents of varieties over seasons (Abeysiriwardena *et al*., 1991). Therefore, instead of reporting 'D' values, actual average mean amylose contents of varieties over seasons are reported with the same mean separation. Amylose content and its stability over seasons of 26 traditional and 12 improved rice varieties and one introduced '*Basmati*' variety grown in 2006 *Yala* and 2006/07 *Maha* seasons in the LCWZ of Sri Lanka are presented in Table 01.

In the present study grouping of rice varieties was based on overall average amylose content of varieties and variety stability estimates in amylose content over seasons derived by analyzing the variety × season interaction effect. All the rice varieties except *Suduru Samba* and *Basmati 370* were high (> 25%) in grain amylose content. Amylose content of *Suduru Samba* and *Basmati 370* was intermediate (20 - 25%). Out of 25 high amylose traditional varieties, 17 recorded higher but only 8 recorded lower grain amylose content than the overall average amylose content across seasons (27.86%), respectively. Similarly, out of 12 high amylose improved varieties, 6 recorded higher and 6 recorded lower grain amylose content than the overall average amylose content across seasons, respectively. Genetics play a major role in the amylose content of rice. The waxy gene, located in rice chromosome 6, encodes the enzyme granule bound starch synthase (GBSS) which plays a key role in amylose synthesis (Smith *et al*., 1997; Chen *et al*., 2007). Jeng *et al*., (2003) and Nishi *et al*., (2001) reported that the activity of GBSS at the grain filling stage was positively correlated with the amylose content in rice grains. Two waxy gene alleles, Wxa and Wxb, have been associated with the contents of GBSS and amylose in rice endosperm. Rice strains with the Wxb allele contain 15-20% amylose in contrast to rice strains with the Wxa allele with ≥ 20-25% amylose content in the rice grain (Sano, 1984).

Out of 25 high amylose traditional varieties, only 11 were found to be stable over seasons. Similarly out of 12 high amylose improved varieties, only 7 were found to be stable over seasons. Thus, higher proportion of improved varieties (58%) than that of traditional varieties (44%) showed stable amylose content over seasons in the Low Country Wet Zone of Sri Lanka. Interestingly, both varieties, *Suduru Samba* and *Basmathi 370*, with intermediate amylose content were also found to be stable over seasons.

Table 01: Grain amylose contents and their stability parameters (S^2) of 39 rice varieties grown in Maha and Yala seasons in the Low Country Wet Zone of Sri Lanka

	Amylose content %			
Variety†	2006/07 Maha	2006 Yala	Mean over seasons ±	Stability parameter (S^2)
Rathal	29.27	30.05	29.66 a	0.689*
Murungakayan	30.10	28.55	29.32ab	2.25**
Kahata Wee	30.94	27.60	29.27ab	10.824***
Kalu Heeneti	29.15	29.20	29.18abc	0.010
Dik Wee	28.28	30.07	29.17abc	3.386**
Rath Suwandel	29.10	29.20	29.16abc	0.023
Hondarawala	29.20	28.69	28.94bcd	0.212
Sulai	28.79	28.64	28.72cde	0.009
KaluBala Wee	29.03	28.23	28.63def	0.562*
Madathawalu	28.60	28.18	28.39efg	0.130
Batapolal	28.64	28.11	28.38efg	0.235
Kottayar	27.63	28.96	28.29efg	1.904**
Masuran	27.82	28.60	28.21efgh	0.681*
Gonabaru	30.19	27.24	28.18fghi	15.761***
Goda Heeneti	27.31	29.01	28.16fghi	3.028**
Molligoda	28.79	27.24	28.01ghij	2.25**
Herath Banda	27.51	28.50	28.00ghij	1.082*
Devaraddiri	27.38	28.01	27.70hijk	0.462*
Wannidahanala	28.52	26.83	27.67hijk	2.722**
Pachaperumal	27.65	27.65	27.65ijkl	0.003
Dahanala	27.14	27.99	27.57jkl	0.801*
Beheth Heeneti	28.11	26.78	27.44 kl	1.651**
Sudu Heeneti	27.53	27.17	27.35 kl	0.096
Kattamanjal	26.22	28.43	27.32 kl	5.085**
Rathu Heeneti	26.59	26.42	26.50 m	0.013
Suduru Samba	24.70	24.21	24.45 p	0.292
Bg 352	28.98	29.90	29.44ab	0.951*
Bg 359	29.30	29.15	29.25ab	0.021
At 354	28.23	29.95	29.10bcd	3.133**
Bw 267-3	28.28	29.08	28.68cdef	0.7225*
At 353	28.47	27.63	28.05ghij	0.632*
Bg 300	28.26	27.82	28.04ghij	0.148
Bg 379-2	27.77	27.60	27.69hijk	0.014
Bw 361	27.46	27.22	27.34 kl	0.034
Bw 272-6b	26.68	27.58	27.13 l	0.893*
Bg 360	26.39	26.22	26.31 m	0.014
At 306	25.40	25.76	25.58 h	0.172
Bg 406	25.16	25.01	25.08 o	0.010
Basmati 370	23.44	23.34	23.39 p	0.002

† Varieties with names are traditional and varieties with numbers are improved except 'Basmathi 370' which is introduced
± Values with the same letter are not significantly different at 0.05 probability level
*, **, *** Significant at 0.05, 0.01 and 0.001 probability levels, respectively

Rice with high amylose content is preferred in South Asia including Sri Lanka. Sri Lankans traditionally eat and tend to favor flaky, somewhat hard non cohesive rice. Thus, it is quite clear that high amylose rice as a breeding objective is justified. Brekenridge (1980) also reported that high grain amylose content close to 25% was taken as a breeding objective in the Sri Lanka rice improvement program in the past. Either traditional or improved, almost all the Sri Lankan rice varieties recorded high grain amylose contents. This is in agreement with Brekenridge (1980) who reported that all Sri Lankan rice, both traditional and improved belonged to the high amylose group with grain amylose contents ranging from 25 - 29%. However, only some of the varieties included in the present study, including both traditional (*Kalu Heeneti*, *Rath Suwandel*, *Hondarawala*, *Sulai*, *Madathawalu*, *Batapolal*, *Pachaperumal*, *Sudu Heeneti* and *Rathu Heeneti*) and improved (Bg 359, Bg 300, Bg 379-2, Bw 361, Bg 360 or *Keera Samba*, Bg 406 and At 306), showed stability of grain amylose content over seasons in the LCWZ and those are valuable germplasm that can be used in rice improvement programs for high and stable grain amylose content over seasons.

Unfortunately, the variety Bw 272-6b which is high in grain Fe, Zn and protein contents (unpublished data) and the variety Bw 267-3 which is reported to be tolerant to Fe toxicity (Bentota and Weerasinghe, 2005) were not stable in grain amylose content over seasons in the LCWZ of Sri Lanka. Among all traditional and improved varieties, two traditional varieties namely *Kahata Wee* and *Gonabaru* appeared highly unstable in grain amylose content over seasons. They have recorded higher amylose in *Maha* season than that of in *Yala* season and this difference cannot be attributed to temperature as *Maha* and *Yala* recorded almost the same temperature during the course of the study. However, this seasonal difference in grain amylose content may be attributed to day length (Lin *et al*., 2005) as day length in *Maha* season is shorter than that of *Yala* season and grain amylose content of these two traditional varieties may be sensitive to day length. In addition, identification of one traditional variety (*Suduru Samba*) and one introduced variety (*Basmathi 370*) having intermediate grain amylose content with stability over seasons is also immense importance as valuable germplasm.

CONCLUSIONS

Significant variation in grain amylose content ranging from high (30.07) to intermediate (23.34) among the selected Sri Lankan traditional and improved rice varieties was observed. Varietal differences for stability in grain amylose content over seasons in both traditional and improved varieties were also observed. Varieties with stable grain amylose contents over seasons can be utilized in rice variety improvement programs for grain quality improvement in the LCWZ of Sri Lanka.

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