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Single nucleotide polymorphisms in *GBBSI* and *SSIIa* genes in relation to starch physicochemical properties in selected rice (*Oryza sativa* L.) varieties

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ABSTRACT

Starch quality is one of the most important agronomic traits in rice (*Oryza sativa* L). In this study, we identified single nucleotide polymorphisms (SNPs) in the *Waxy* and *Alk* genes of eight rice varieties and their associations with starch physicochemical properties. i.e. amylose content (AC) and gelatinization temperature (GT). Seven Sri Lankan rice varieties, *Pachchaperumal, Herathbanda, At* 354*, Bg* 352*, Balasuriya, H* 6 and *Bw* 295-5 were detected as high amylose varieties while *Nipponbare* exhibited low amylose content. *In silico* analysis of the *Waxy* gene revealed that all tested Sri Lankan varieties possessed 'G' (Wx^a allele) instead of 'T' in the first intron which could explain varieties with high and intermediate amylose content. All Sri Lankan varieties had 'A' instead of 'C' in exon 6 of the *Waxy* gene and this fact was tally with the varieties showing high amylose content. Therefore, possessing the Wx^a allele in the first intron and 'A' in exon 6 could be used as a molecular marker for the selection of high amylose varieties as validated using several Sri Lankan varieties. All Sri Lankan varieties except, *Bw* 295-5 exhibited the intermediate type of GT which could not be explained using the so far reported allelic differences in the *Alk* gene. However, *Bw* 295-5 which is a low GT variety had two nucleotide polymorphisms in the last exon of the *Alk* gene, i.e. 'G' and 'TT' that represent low GT class. Therefore, it can be concluded that sequence variations of *Waxy* and *Alk* genes reported in this study are useful in breeding local rice varieties with preferential amylose content and GT class.

Key word: *Alk* gene, amylose content, single nucleotide polymorphism, *Waxy* gene

INTRODUCTION: Rice *(Oryza sativa* L.) is one of the leading food crops of the world. More than half of the world's population relies on rice as the major daily source of calories and protein (Sartaj and Suraweera, 2005). After grain yield, quality is the most important aspect of rice breeding. Grain size and shape largely determine the market acceptability of rice, while cooking quality is influenced by the properties of starch.

In rice grains starch is the major component that primarily controls rice quality. Starch consists of two forms of glucose polymers, relatively unbranched amylose and a highly branched amylopectin. Starch-synthesizing genes may contribute to variation in starch physicochemical properties because they affect the amount and structure of amylose and amylopectin in rice grain (Kharabian-Masouleh *et al.*, 2012).

Amylose content (AC), gelatinization temperature (GT) and gel consistency (GC) is the three most important determinants of eating and cooking quality. Amylose content is the ratio of amylose amount present in endosperm to total starch content. Rice varieties are grouped based on their amylose content into *waxy* (0-2%), very low (3-9%), low (10-19%), intermediate (20-25%), and high (> 25%) (Kongseree and Juliano, 1972). The most widely used method for amylose determination is a colorimetric assay where iodine binds with amylose to produce a blue-purple color, which is measured spectrophotometrically at a single wavelength (620nm). Low amylose content is usually associated with tender, cohesive and glossy cooked rice; while, high amylose content is associated with firm, fluffy and separate grains of cooked rice.

The *Waxy (Wx)* gene, which encodes granule-bound starch synthase I (*GBSSI*), is the major gene controlling AC in rice (Nakamura, 2002). The *Waxy* gene is located on chromosome six and various single nucleotide polymorphisms (SNPs) of *Wx* were found, including a 'G' to 'T' SNP of the first intron, 'A' to 'C' SNP of the sixth exon and 'C' to 'T' SNP of the tenth exon (Larkin and Park, 2003). The 'AG**G**TATA' sequence at the 5'splice-junction coincides with the presence of the Wx^a allele, while the 'AG**T**TATA' sequence coincides with the presence of the Wx^b allele. Therefore, all intermediate and high amylose cultivars had 'G' nucleotide while low amylose cultivars had 'T' nucleotide at the putative leader intron 5′ splice site.

The cytosine and thymidine (CT) dinucleotide repeats in the 5'-untranslated region (UTR) of the *Waxy* gene were reported to be a factor associated with AC. However, the relationship between these polymorphisms and amylose contents is not clear.

Amylopectin chain length distribution plays a very important role to determine GT in cooked rice. The time required for cooking is determined by the gelatinization temperature of starch. It is important because it affects the texture of cooked rice and it is related to the cooking time of rice. The gelatinization temperature is estimated by the alkali digestibility test. It is measured by the alkali spreading value (ASV). The degree of spreading value of individual milled rice kernels in a weak alkali solution (1.7% KOH) is very closely correlated with gelatinized temperature. According to the ASV, rice varieties may be classified as low (55 to 69˚C), intermediate (70 to 74˚C) and high (> 74˚C) GT classes. In a breeding program ASV is extensively used to estimate the gelatinization

temperature.

The synthesis of amylopectin is more complex than that of amylose. Polymorphisms in the *starch synthase IIa* (*SSIIa*) gene which is recognized as the *Alk* gene are responsible for the differences in GT in rice (Umemoto and Aoki, 2005; Waters *et al.*, 2006). Two single nucleotide polymorphisms (SNPs) in the last exon of the *Alk* gene are responsible for the differences in GT in rice. The biochemical analysis clearly showed that the function of the amino acids caused by these two SNPs is essential for *SSIIa* enzyme activity (Nakamura *et al.*, 2005) and those are 'G'/'A' SNP at 4424 bp position and 'GC'/'TT' SNPs at 4533/4534 bp position with reference to *Nipponbare* rice genomic sequence. Based on the SNPs, Low *SSIIa* enzyme activity results in S-type amylopectin, which is enriched in short chains whereas high *SSIIa* enzyme activity produces L-type amylopectin (Umemoto *et al.*, 2004). Therefore, the combination of 'G' at SNP3 and 'GC' at SNP4 is required to produce L-type rice starch and this has a higher GT relative to S-type starch. GC is a standard assay that is used in rice improvement programs to determine the texture of softness and firmness in high amylose rice cultivars. Intermediate and low amylose rice usually has soft gel consistency. Sequence variation in exon 10 of the *Waxy* gene associates with GC (Tran *et al.*, 2011).

OBJECTIVES: The objectives of this study were to detect polymorphisms in major starch synthesizing genes among several rice cultivars as models and to determine the relationship between their SNP variations and starch physicochemical properties. Also, we analyzed major starch synthesizing gene sequences of several Sri Lankan rice varieties in silico aiming at utilizing this information in rice breeding programs.

MATERIALS AND METHODS: Plant materials: Seeds of eight *Oryza sativa* L. accessions were obtained from the Rice Research and Development Institute (RRDI), Bathalagoda, Sri Lanka and Gene Bank of Plant Genetic Resource Center (PGRC), Gannoruwa.

Characterization of grain physical parameters: Grain length and width were determined using a vernier caliper. Ten grains from each sample were collected randomly and measured to obtain the average length and width of the milled rice. The average length and width were recorded as their length and width. Based on the length and width of the grains, the milled rice grains were classified into four classes (table 1) according to the method accepted by RRDI Bathalagoda, Sri Lanka.

Length (mm)	Grain size	Common category name	L/W ratio	Grain shape	Category
6-7	Long	Kora	>3	Slender	L/S
6-7	Long	Kora	2.40-3	Medium	L/M
4.5-5.99	Intermediate	Nadu	2-2.39	Bold	I/B
<4.5	Short	Samba	<2.00	Round	S/R

Table 1: The classification of milled rice grain.

According to the scale L/S – Long Slender, L/M – Long Medium, I/B – Intermediate Bold and S/R –Short Round

Analysis of amylose content: Initially, rice samples were dehusked and polished prior to milling. Ten whole – milled rice kernels of eight rice samples were ground separately by using mortar and pestle. Amylose content per 100 mg was determined by measuring the blue value of rice varieties as described by Juliano (1971). About 100mg rice sample was shifted into a 100 mL volumetric flask and 1mL of 95% ethanol was added. Then 9mL of 1N NaOH was added and the content was boiled for 20min. at boiling temperature to gelatinize the starch. After cooling the content, the volume was made up to 100mL and 5mL of starch solution was pipetted out into a 100mL volumetric flask. The blue color was developed by adding 1mL of 1N acetic acid and 2 mL of iodine solution (0.2g iodine and 2.0g potassium iodine in 10 mL aqueous solution). Then volume was made up to 100mL with distilled water and the solution was kept for 20min. after shaking. Finally, the absorbance of the solution was measured at 620nm using Spectrophotometer T80 (PG Instruments Limited) as described by Juliano (1971).

The standard curve was prepared using 40mg of potato-amylose to calculate the amylose content of rice varieties through absorbance values. Forty mg of potato amylose was put into a 100 mL of volumetric flask and 1ml of 95% ethanol and 9mL of NaOH were added and content was heated for 20min at boiling temperature. After cooling the content volume of the solution was made up to 100mL using distilled water. Then 1mL, 2mL, 3mL, 4mL and 5mL of amylose solution were pipetted out into 100mL flasks. Then 0.2mL, 0.4mL, 0.6mL, 0.8mL and 1mL of 1N acetic acid were added to the flasks respectively. Finally, 2mL of iodine solution was added to each flask and volume was made up to 100mL with distilled water. Solutions were stood up for 20min. after shaking and absorbance values were measured at 620nm. Measured absorbance values were plotted at 620nm against the concentration of anhydrous amylose (mg).

Analysis of gelatinization temperature: GT was indirectly measured on rice by the alkali spreading value. Husked and polished seeds per accession were used for the analysis. Selected duplicate sets of six milled grains without cracks of each sample were put into Petri dishes. About 10mL of 1.7% KOH was added and grains were spread in the petri dish to provide enough space. The constant temperature at 30°C was maintained to ensure better reproducibility. After 23hrs, the degree of disintegration was quantified by a standard protocol with a numerical scale of 1–7 (table 2) as reported by Cruz and Khush (2000). As reported by Juliano (2003), GT of rice was determined using the alkaline spreading scale, where 1.0-2.5: High (74-80 °C), 2.6-3.4: High-intermediate (70-74 °C), 3.5-5.4: Intermediate (70-74 °C) and 5.5-7.0 Low: (55-70 °C).

Bioinformatics and statistical analysis: The available literature was used to identify the most likely candidate genes associated with rice starch quality and their SNPs of each gene (Hirose *et al.*, 2006; Waters and Henry, 2007; Tran *et al.*, 2011). In all the tested varieties except *Bg* 352 and *At* 354, the DNA sequence of each gene was retrieved from the Rice SNP Seek database (http://snp-seek.irri.org/). The gene sequences of *At* 354 and *Bg* 352 were obtained from the National Research Council 16-016 project, Wayamba University of Sri Lanka. Multiple sequence alignment was conducted for the DNA sequence using Clustal Omega software (https://www.ebi.ac.uk/Tools/msa/clustalo/).

Starch physiochemical data obtained were subjected to a one-way analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test (DNMRT) to determine the statistical differences among varieties at the significance level of $p \leq 0.05$.

Statistical analysis was done using SAS version 9.1 (SAS, 2004).

RESULTS AND DISCUSSION: Physical properties of rice grains: Physical properties such as length, width, size, shape and pericarp color of rice grains obtained from eight different rice varieties are given in table 3. Classification of rice grains was carried out, according to their sizes and shapes based on Juliano (1985). The size of the rice grains was determined as per grain length while grain shape was determined by means of length and width ratio of the rice kernel. In the local market, rice is classified as *Samba* (short grain), *Nadu* (intermediate grain) and *Kora* (long/medium) based on the size of the grain (Pathiraje *et al.*, 2010). Lengths of rice kernels were varied from 5.58 to 6.725 mm for all varieties. The highest grain length and width were given by *At* 354 and *Pachchaperumal* respectively. The varieties, *Bw* 295-5 and *H* 6 showed a length: width ratio over 3 which is considered as slender in grain shape. *Bw* 295-5, *H* 6, *At* 354, *Bg* 352 and *Nipponbare* possessed white pericarp and others possessed red pericarp.

Relationship between amylose content and SNPs variation of *waxy* loci in selected varieties: Amylose content was measured in seven Sri Lankan rice varieties and one exotic rice variety. Amylose content of the evaluated varieties varied significantly with $p \leq 0.05$ with the lowest of 15.11% and highest of 28.63% which were found in *Nipponbare* and *Bw* 295-5, respectively (table 4). The majority of the evaluated varieties fell into the high AC category (between 25-28%). Only *Nipponbare* could be clearly categorized under the low amylose group (table 4).

Description	Score
Grain not affected	1
Grain swollen	2
Grain swollen, collar incomplete or narrow	3
Grain swollen. Collar complete and wide	4
Grain split or segmented, collar complete and wide	5
Grain dispersed, merging with collar	6
Grain completely dispersed and disintegrated	7

Table 2: Description of alkali spreading value for milled rice, after soaking in 1.7% KOH for 23 hours

Rice Variety	Length (L Mm)	Grain size	Width (W Mm)	L/W ratio	Grain shape	Pericarp color
*Pachchaperumal**	6.720 ± 0.000^{A}	Medium	2.315 ± 0.007^{A}	2.9	Medium	Red
Balasuriya (Ac.No.004896)	5.615 ± 0.007^{E}	Intermediate	2.300 ± 0.028^{A}	2.4	Medium	Red
Bw 295-5 (Ac.No.004895)	6.190 ± 0.028^{C}	Medium	1.575 ± 0.007^{E}	3.9	Slender	White
H 6 (Ac.No.003113)	6.360 ± 0.000^{B}	Medium	1.955 ± 0.007^{D}	3.3	Slender	White
*Herathbanda**	5.615 ± 0.063^{E}	Intermediate	2.280 ± 0.000^{BC}	2.5	Medium	Red
At 354*	6.725 ± 0.007^{A}	Medium	2.300 ± 0.014^{A}	2.9	Medium	White
Bg 352*	5.810 ± 0.014^{D}	Intermediate	2.275 ± 0.007^{BC}	2.6	Medium	White
*Nipponbare**	5.580 ± 0.000^{E}	Intermediate	2.280 ± 0.014^{BC}	2.4	Medium	White

Table 3: Variation of physical properties of selected rice varieties.

* Seeds obtained from RRDI, Bathalagoda, Sri Lanka.

Means with the same letter are not significantly different from each other at $P \leq 0.05$

Variety	Amylose %	Amylose class	In 1/ Ex 1	Exon 6	Exon 10
Pachchaperumal	25.432 ± 0.566	High	G	A	C
Herathbanda	26.734 ± 0.566	High	G	A	C
At 354	28.485 ± 0.212	High	G	A	C
H 6	27.334 ± 0.000	High	G	A	T
Bg 352	28.286 ± 0.637	High	G	A	T
Balasuriya	26.383 ± 0.212	High	G	A	C
Nipponbare	15.119 ± 0.141	Low	T	A	C
Bw 295-5	28.636 ± 0.141	High	K	A	T

Table 4: Mean amylose content for different haplotypes of intron 1/exon 1 junction site 'G'/'T'. exon 6 'A'/'C' and exon 10 'C'/'T' polymorphisms of *Wx*.

The amylose content of *Bg* 352, *Pachchaperumal* and *Herathbanda* have already been determined by early studies of Rebeira *et al.* (2014) and Fernando *et al.* (2015). Most of the data obtained in the present experiment has agreed with the results of previous studies.

Major genes such as *Waxy* and their functional SNPs have a major influence on amylose in rice (Nakamura *et al.*, 2005). Accordingly, single nucleotide polymorphism, 'G'/'T', at the 5' leader intron splice site of the *GBSSI* has explained the variation in amylose content of varieties. Accordingly, high and intermediate amylose varieties have 'AGGTATA' while low amylose varieties have the sequence 'AGTTATA', which might lead to a decrease in the splicing efficiency. Therefore, the *GBSSI* activity of *Nipponbare* might be considerably weak and resulted in starch with low amylose content. Hence, producing 'G'/'T' polymorphism clearly differentiates low amylose rice varieties, as reported by Nakamura *et al.* (2005).

In *GBSSI,* Larkin and Park (2003) identified an 'A'/'C' polymorphism in exon 6 and a 'C'/'T' polymorphism in exon 10 which resulted in non- synonymous amino acid change. Chen *et*

al. (2008) reported that the non-synonymous 'A'/'C' SNP at exon 6 had the highest possible impact on *GBSSI*. Accordingly, the 'A'/'C' polymorphism in exon 6 causes a tyrosine/serine amino acid substitution while the 'C'/'T' polymorphism in exon 10 causes a serine/proline amino acid substitution. In view of this information, there is a relationship between the polymorphism detected by *in silico* analysis and amylose content obtained from our experiment.

Out of the eight tested rice varieties, only one variety, *Nipponbare* was categorized as low amylose variety (10-19%) and it exhibited 'T' nucleotide at the intron splice site (table 4; figure 1).

Figure 1: Clustal Omega alignments of 'G'/'T', 'A'/'C' and 'C'/'T' SNPs in intron 1/exon 1 junction site, exon 6 and exon 10 respectively of *GBSSI*. The SNPs are marked.

Varieties such as *Pachchaperumal*, *Balasuriya*, *Bw* 295-5, *H* 6, *Herathbanda*, *At* 354 and *Bg* 352 which contained high amylose (> 25%), had 'G' and 'A' nucleotides at intron splice site and exon 6 respectively (table 4; figure 1). The predominant allelic pattern of intron splice site and exon 6 are different in varieties containing intermediate amylose content (20-25%) which showed 'G' and 'C' nucleotides respectively. Of these selected rice varieties, none of the intermediate type amylose variety was found.

Relationship between gel consistency and SNPs variation in *Waxy* loci: In this study, GC data of *Herathbanda*, *Hondarawalu*, *Kuruluthuda*, *Pachchaperumal* and *Bg* 352 were obtained from Fernando *et al.* (2015). The results of Tran *et al.* (2011) showed that the exon 10 'C'/'T' SNP of *Wx* has mainly affected GC. Accordingly, rice with a 'C' at exon 10 had soft and viscous gels once cooked. However, a sample with a 'T' had short and firm gels. In this study, *Herathbanda*, *Hondarawalu*, *Kuruluthuda* and *Pachchaperumal* had 'C' nucleotide and *Bg* 352 had 'T' nucleotide in exon 10 (table 5; figure 2). However, 'C'/'T' substitution analysis could not be used to explain the GC of tested varieties.

Rice variety	Gel consistency*	Exon 10
Pachchaperumal	Hard	C
Herathbanda	Soft	C
Hondarawalu	Soft	C
Kuruluthuda	Soft	C
Bg 352	Intermediate	T

Table 5: Comparison of the gel consistency with SNP of the *Waxy* locus among five rice cultivars.
*Data obtained by Fernando *et al.* (2015).

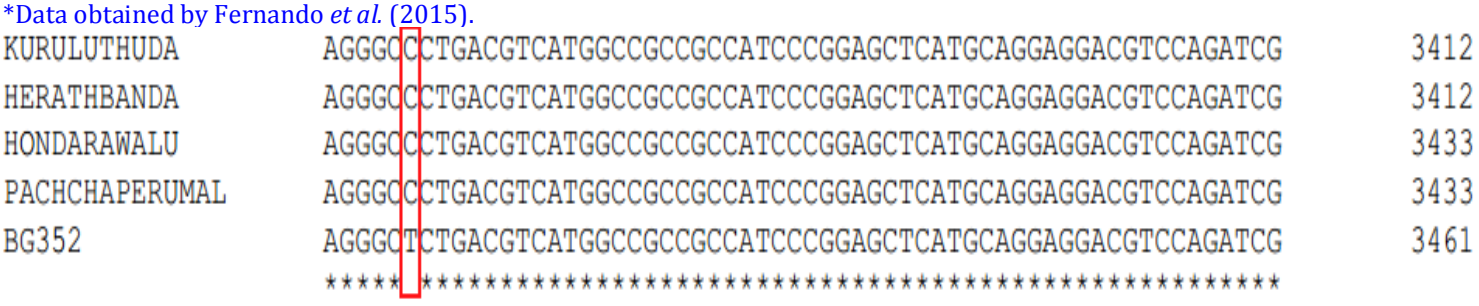

Figure 2: Clustal Omega alignment of 'C'/'T' polymorphism in exon 10 of *GBSSI* gene. SNP is marked.

Relationship between gelatinized temperature and SNPs variation of *Alk* loci in selected rice varieties: Although there were differences in the scores, the degree of disintegration of all samples was saturated at 23 hrs. Most of the selected rice

varieties showed the intermediate disintegration score. Varieties, *Pachchaperumal, Balasuriya, H 6, Herathbanda, At* 354 and *Bg* 352 were categorized into intermediate GT class (70–74˚C) as indicated by an alkali spreading (AS) value of 5 (table 6; figure 3). *Nipponbare* and *Bw* 295-5 showed the highest disintegration score indicating the dispersion of all grains. Hence these varieties were categorized into low GT class (55-69˚C) as indicated by an AS value of 6 (table 6; figure 3). However, high GT class rice varieties (> 74˚C) were not found in the tested samples.

Rice variety	AS value	Alkali digestion	GT classification	Haplotypes		
				SNP2	SNP3	SNP4
Bg 352	5	Intermediate	Intermediate	A	G	GC
At 354	5	Intermediate	Intermediate	G	G	GC
Pachchaperumal	5	Intermediate	Intermediate	G	G	GC
Balasuriya	5	Intermediate	Intermediate	G	G	GC
Herathbanda	5	Intermediate	Intermediate	G	G	GC
H 6	5	Intermediate	Intermediate	A	G	GC
Bw 295-5	6	High	Low	A	G	TT
Nipponbare	6	High	Low	A	A	GC

Table 6: Gelatinization temperature for different haplotypes of exon 8 'G'/'A', 'G'/'A' and 'GC'/'TT' polymorphisms of *Alk* AS - alkali spreading, GT - gelatinization temperature.

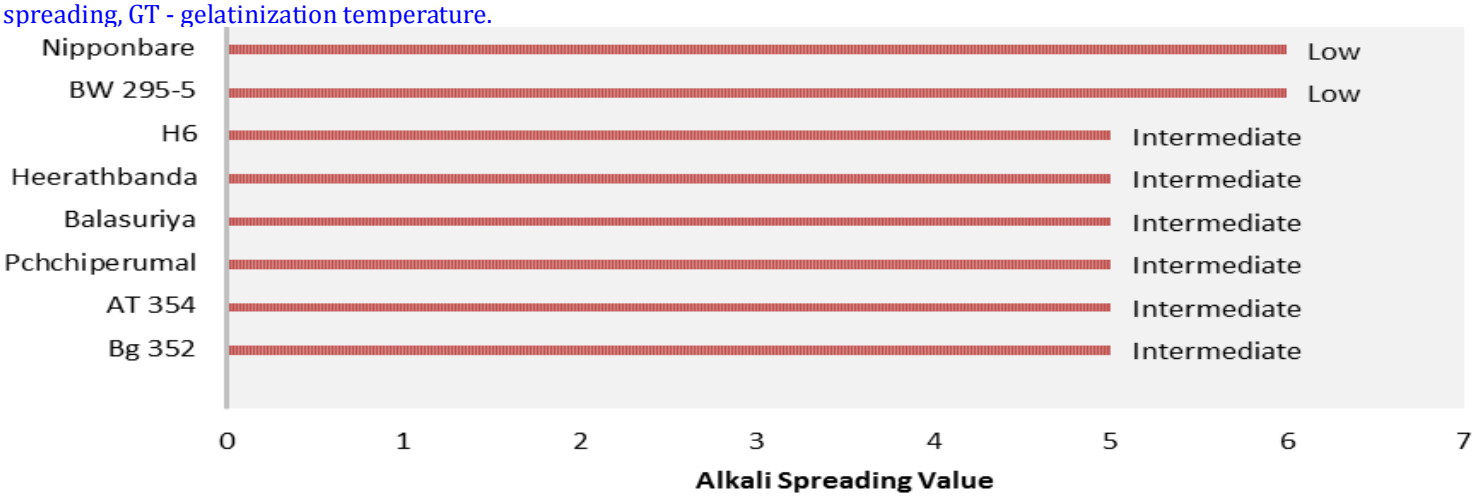

Figure 3: Alkali spreading value of rice in 1.7% KOH and their corresponding GT classification.

Chromosomal mutation within the *Alk* gene has led to a number of single nucleotide polymorphisms (SNPs). Umemoto *et al.* (2004) identified four SNPs in *Alk* gene. Thus, SNP3 and SNP4 may be important genetic polymorphisms that are associated with GT class. According to the SNP3 and SNP4, eight rice varieties could be classified into either high GT or low GT types. If there is 'A' instead of 'G' at 4424 bp position of *Alk* gene with reference to *Nipponbare* rice genomic sequence, it codes methionine instead of valine amino acid residue in *SSIIa*, whilst two adjacent SNPs at bases 4533 and 4534 code for either leucine ('GC') or phenylalanine ('TT').

Rice varieties with high GT starch had a combination of valine and leucine at these residues. Rice varieties with low GT starch had a combination of either methionine and leucine or valine and phenylalanine at these same residues. *Nipponbar*e carried the 'A' and 'GC' nucleotides, while *Bw* 295-5 carried the 'G' and 'TT' nucleotides. Hence these varieties were classified into low GT class. Varieties such as *Pachchaperumal, Balasuriya, H* 6, *Herathbanda, At* 354 and *Bg* 352 carried 'G' and 'GC' nucleotides and these varieties were classified into high GT rice varieties. However, intermediate GT status could not be determined by SNP3 and SNP4 mutation of *Alk* gene (table 6; figure 4).

***In silico* analysis of the polymorphisms in *GBSSI* gene and *Alk* genes of rice varieties retrieved from Rice-SNP-database:** In this study, *GBSSI* gene and *Alk* gene were compared with the sequences retrieved from the Rice-SNP-Seek database to validate the SNPs further. As previously reported by Ayres *et al.* (1997), all low amylose varieties had the sequence 'AGTTATA' in exon 1. In agreement with preliminary work done by Larkin and Park (2003), all of the intermediate amylose varieties have the allelic pattern of GCC. All of the high amylose varieties have either the GAC or GAT allele of *GBSSI*. Among 42 rice accessions with the Sri Lankan pedigree, four allelic patterns were found; TAC, GCC, GAC and GAT (table 7). In this allelic pattern, the first letter corresponds to the 'G'/'T' polymorphism in 5' leader intron splice-junction, the second letter corresponds to the 'A'/'C' polymorphism in exon 6 and the third letter corresponds to the 'C'/'T' polymorphism in exon10 of *Waxy* gene. Analysis of the 'G'/'T' polymorphism in the Wx locus showed that 41 rice cultivars shared the same 'AGGTATA' sequence at the 5' leader intron splice-junction. But only 1 rice cultivar, *Puttu nellu* was found with 'T' nucleotide in intron1/exon1 junction site, which could be categorized as a low amylose variety (table 7). As discussed above, varieties with an intermediate level of apparent amylose could be reliably distinguished from those with higher apparent amylose based on a SNP in exon 6. Hence, only three rice varieties *Nalumoolai Karuppan*, *Pannithi* and *Godawel* with 'C' nucleotide in exon 6 exhibited the possibility of containing intermediate amylose content (table 7). High activity of *GBSSI* produces high amylose content leading to a non-waxy, non-sticky or non-glutinous phenotype. Therefore, according to the *in silico*

genotypic results, rest of the 38 rice varieties may produce high amylose content in the endosperm (table 7). Proving this phenomenon. Abeysekera *et al.* (2017) has reported that usually, most of Sri Lankan rice varieties contain high amylose content.

Targeted sequence analysis of exon 8 of the *Alk* gene in 42 different rice cultivars were found with three SNP polymorphisms that resulted in a changed amino acid sequence and, of these three SNPs, two SNPs were reported to be correlated with possible GT differences. Accordingly, *Puttu nellu* and 3210 rice varieties carried the 'G' and 'TT' nucleotides in SNP3 and SNP4 respectively (table 7). Hence these varieties can be classified into low GT class and except these two; other rice varieties carried the 'G' and 'GC' nucleotides in SNP3 and SNP4 respectively. Therefore, those varieties can possibly be classified into high GT rice varieties (table 7). However, further experiments are necessary to check the phenotypic variations for grain amylose content and GT class of *in silico* analyzed rice varieties.

	Allele of *Waxy* SNPs				Allele of *Alk* SNPs	
Variety	**In1/ Ex1 junction**	**Exon 6**	**Exon 10**	**SNP2**	**SNP3**	**SNP4**
Matholuwa	G	A	C	G	G	GC
Moddai karuppan	G	A	C	A	G	GC
Mudaliga wee	G	A	C	G	G	GC
Murunga	G	A	C	G	G	GC
Muttu samba	G	A	C	G	G	GC
Nalumoolai karuppan	G	C	C	G	G	GC
Pannithi	G	C	C	A	G	GC
Periya vellai	G	A	C	G	G	GC
Podi heenati	G	A	C	G	G	GC
Podiwee	G	A	C	A	G	GC
Pokkali	G	A	C	G	G	GC
Puttu nellu	T	A	C	A	G	TT
Race perumal	G	A	C	G	G	GC
Rangoon samba	G	A	T	G	G	GC
Ranruwan	G	A	C	G	G	GC
Samba	G	A	C	G	G	GC
Sayam	G	A	C	G	G	GC
Sigadis	G	A	T	A	G	GC
Sinna sitira kali	G	A	Y	A	G	GC
Sithaiyan kottaisamba	G	A	T	G	G	GC
Sudu karayal	G	A	C	G	G	GC
105	G	A	C	G	G	GC
3210	G	A	T	A	G	TT
A 69-1	G	A	C	G	G	GC
Alagusamba	G	A	C	A	G	GC
Chandina	G	A	C	G	G	GC
Galawaka handeran	G	A	C	G	G	GC
Godawel	G	C	C	A	G	GC
Halsudu heenati	G	A	C	G	G	GC
Heendik wee	G	A	C	A	G	GC
Hoderawala	G	A	T	G	G	GC
Hodarawala	G	A	C	G	G	GC
Kahatawee	G	A	C	G	G	GC
Kalu Ilankalayan	G	A	C	G	G	GC
Karutha seenati	G	A	C	G	G	GC
Kotteyaran	G	A	C	G	G	GC
Kur karuppan	G	A	C	G	G	GC
Kalu karuppan	G	A	C	G	G	GC
Kurulu wee	G	A	C	G	G	GC
Kurulutuda	G	A	C	G	G	GC
Vellai kolamban	G	A	C	G	G	GC
Wir1391	G	A	C	G	G	GC

Table 7: Single nucleotide polymorphisms (SNPs) genotypes in *Waxy* and *Alk* genes in 42 Sri Lankan rice varieties.

Figure 4: Clustal Omega alignment of 'A'/'G', 'A'/'G' and 'TT'/'GC' SNPs in *SSIIa* gene. SNPs are marked.

CONCLUSION: Present results revealed the relationship between SNPs variation at *Waxy* loci and the amylose content of selected rice varieties. Accordingly, *Pachchaperumal, At* 354, *Bg* 352, *Herathbanda, H* 6, *Balasuriya* and *Bw* 295-5 with high amylose content had 'G' instead of 'T' in the first intron exhibiting the presence of Wx^a allele with reference to *Nipponbare* which had low amylose content. Also all tested varieties had 'A' in exon 6 of the *Waxy* gene. Thus present findings i.e. presence of Wx^a allele and SNP 'A' in exon 6 could be used as a potential molecular marker for the selection of high amylose varieties. In addition, *Bw* 295-5 which is a low GT variety, had two SNPs variations in the last exon of the *Alk* gene i.e. 'G' and 'TT' which is likely to be used to represent low GT class. Accordingly, sequence variations identified in *Waxy* and *Alk* genes could be utilized in the future rice breeding programs for the development of varieties with preferential amylose content and GT class.

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