In Vitro Identification of Low Glycemic Index (GI) White Rice using Nutriscan GI Analyzer

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ABSTRACT

The Glycemic Index (GI) of food is a measure of glucose releases rate upon consumption of a specified quantity and plays vital role in diabetes management. The GI and digestibility of rice starch depends upon its amylose and amylopectin content and structure. Looking to the availability of diverse rice landraces and genetic variability in IGKV, Raipur, an effort was made to screen the selected rice genotypes for their GI values. A simple in vitro method for predicted Glycemic Index (GI) determination using “Nutriscan Glycemic Index Analyser” was standardized. Four white and one brown grains of rice (Oryza sativa) varieties were used to determine GI together with influencing traits. Glycemic response of selected rice varieties varied from 55 to 68 units which categories them as low to medium GI rice type. Carbohydrate, apparent amylose content and crude fibre content varied from 83.3% to 89%, 23.7% to 25.2% and 0.39% to 2.11% respectively. The genetic determinants and allelic variations of marker RM190 for the waxy gene have also shown variations for tested rice genotypes. The allele in low pGI genotype, Chhattisgarh Madhuraj Dhan-55 (CGMD-55) is intermediate type with ~110-115 bp compare to that of 105-110 bp for Swarna and ~122-126bp for IR64. The GI value of white grain of one of the tested rice landrace of Chhattisgarh named as CGMD-55 belonging to Gurmatiya group was lowest i.e. 55 units. The local landrace from Chhattisgarh, portrayed lower rate of starch digestibility compare to other improved popular rice varieties in consumption and help in diabetes management.

Key words Amylose, Carbohydrate, Diabetes, Glycemic Index, Rice

Food sources and their postprandial blood glucose responses. The characterization of various food stuff based on their ability to raise blood glucose level is termed as ‘Glycemic Index’. Jenkins et al. defined GI as the incremental area under blood glucose response curve of a 50 g carbohydrate with a reference food like white bread or glucose taken by the same subject on a specified period of time(5). GI categorises foods as low (GI value d<55), medium (GI value 56-69) or high GI foods (e>70) (Fitzgerald, et al., 2011; Srinivasa, et al., 2013; Odenigbo et al., 2014).

Foods with a high GI score contain rapidly digested carbohydrate, which produces a large rapid rise and fall in the level of blood glucose. In contrast, foods with a low GI score contain slowly digested carbohydrate, which produces a gradual, relatively low rise in the level of blood glucose (Fitzgerald, et al., 2011; Jain et al., 2012; http://www.glycemicindex.com/testing_research.php: Glycemic Index Testing & Research). Studies have shown the potential of lower GI foods to help to improve the glycemic control in diabetics, which also positively effect in preventing the prevalence of cardiovascular diseases (CVD).

Rice the major stable food serve majorly to satisfy hunger and nutritional requirements of more than half the world’s population. It is the major source of carbohydrate and even protein. Rice accounts for daily supply of 27% energy, 20% protein and 3% fat in most developing countries (Srinivasa et al., 2013; Odenigbo et al., 2014; Jain et al., 2012; Tian et al., 2009 and Biselli et al., 2014).

Approximately, 90% of milled rice comprised of starch and the digestibility as well as eating/cooking quality of rice is influenced by the starch characteristics, which include amylose content (AC), gelatinization temperatures (GT) etc. Strong association of starch structure with physical behaviour and functionality have been established. Studies have demonstrated the influence of various nutritional components on starch digestibility which include; protein, moisture, fibre etc. Fibres are a form of complex carbohydrate found in many plant cell walls which cannot be digested by the human digestive tract and passed to the colon for bacterial degradation. It affects glucose assimilation, reduces blood cholesterol level and increasing fibre in the diet is also associated with a reduction of insulin resistance (Anderson et al., 2009; Vuksan et al., 2009; Jozinovic et al., 2012 and Fatema et al., 2010). Other factors that influence GI include geographical location, varieties, preparation of food before consumption etc. So it is important to measure the quality and starch digestibility of rice as it’s of particular interest and vital for consumers as health point of view (Fitzgerald et al., 2011; Odenigbo et al., 2013).
al., 2014; Jain et al., 2012 and Odenigbo et al., 2013).

Although prevention of type II diabetes through more judicious food choices may be the frontline strategy, but behavioural change is difficult to achieve in practice, especially in the short term. Improving the carbohydrate quality and lowering the GI of staple foods such as rice offers potential as a dietary strategy for preventing and managing type II diabetes, and its co-morbidities. Selecting low GI rice genotypes of high nutritional value from existing germplasm collection may be an efficient and reliable way to deliver benefits to farmers and society (Fitgerald et al., 2011 and Jain et al., 2012).

Research activities for determining nutritional quality and in some cases GI have been documented on rice varieties. Despite these studies, there is a lack of information on GI of rice varieties that needs to be generated. This study therefore aimed to analyse the rate of in vitro starch digestion to evaluate the predicted glycemic index (GI) of five non-parboiled milled rice varieties grown in India and also to develop a simple, relatively inexpensive procedure for predicting GI as an useful alternative to in vivo method. Accordingly the objective of this study is to characterise rice genotypes mainly for differences in predicted GI values and other related parameters by the in vitro means and to support the food or food product development based on these characteristics. The appropriate characterizations will be very useful for maintaining a good public health which is the ultimate purpose of the study.

MATERIALS AND METHODS

Experimental material

Grains of five different rice (Oryza sativa) genotypes (Table 1) were used for the estimation of the predicted glycemic index (GI) value, carbohydrate (CHO), apparent amylose content (AAC), gelatinization temperature (GT) and crude fibre content. These rice genotypes belongs to Indica subtype rice varieties which includes two popular rice varieties (Swarna, HMT), a commercial brown rice from market (termed as BR check), a local landrace selected from a group of various Gurmatiya types of Chhattisgarh region of India named CGMD-55 (Figure 1) and one high zinc rice variety of IGKV Raipur (CGZR1). In addition to these varieties, an international rice variety IR64 (developed at IRRI, Philippines) was also used for genotyping purpose at Waxy gene locus as a reference. All the varieties were grown at the Research cum Instructional Farm, IGKV, Raipur (C.G.) India.

Processing of rice grains

Before analyzing the GI and other traits seeds of all the varieties except BR check rice (which is a commercial brown rice) were subjected to dehusking and polishing (Figure 2). After the crop reached physiological maturity, rice seeds were carefully harvested and dried under clean shade to reduce post harvest grain moisture concentration up to ~12%. Seeds of each rice genotype were carefully dehusked using a polyurethane coated dehusker. The grains of each variety were polished up to commercial level whiteness (reflectance ~40-50). These grain samples were then kept at 35-40°C for the analysis in a clean, contaminant-free oven. Up to 5g whole polished grain of each rice variety was ground into fine powder and also kept in oven for the analysis. Utmost care was taken at every step to avoid dust and any other contamination. The whole white (polished) grain or powdered samples of all the varieties above prepared were used for the analysis as per the protocol.

Estimation of carbohydrate, apparent amylose content, crude fibre, gelatinization temperature and predicted glycemic index

Total carbohydrate levels for all rice varieties were estimated by phenol sulfuric acid method described by DuBois et al., (1956). The apparent amylose content was determined by iodine-colorimetric approach as per the procedure described by Juliano (1971). Alkali digestibility test is an estimate of gelatinization temperature Little et al., (1958). If the alkali spreading value (ASV) is low, the GT will be high (>74°C), if the ASV is intermediate, the GT will be intermediate (70-74°C) and if the ASV is high then the GT

<table>
<thead>
<tr>
<th>S No</th>
<th>Rice Variety/Genotype Name</th>
<th>Grain Type (L, W)</th>
<th>Importance</th>
<th>Species</th>
<th>Subtype</th>
<th>Pedigree/Parant age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Swarna</td>
<td>Medium</td>
<td>High yielding, Check</td>
<td>Oryza sativa</td>
<td>Indica</td>
<td>Vaisishtha x Mahsuri</td>
</tr>
<tr>
<td>2</td>
<td>CGMD-55</td>
<td>Bold</td>
<td>Heat Tolerant, late</td>
<td>Oryza sativa</td>
<td>Indica</td>
<td>Landrace</td>
</tr>
<tr>
<td>3</td>
<td>HMT</td>
<td>Medium Thin, Small</td>
<td>Popular commercial variety</td>
<td>Oryza sativa</td>
<td>Indica</td>
<td>Selection from ‘Patel 3’</td>
</tr>
<tr>
<td>4</td>
<td>CGZR1</td>
<td>Long, Bold</td>
<td>High zinc &gt;22 ppm white grain</td>
<td>Oryza sativa</td>
<td>Indica</td>
<td>Poomima x Ananda</td>
</tr>
<tr>
<td>5</td>
<td>BR Check (Brown Rice)a</td>
<td>Medium Thin, Small</td>
<td>Commercial Low GI rice</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>IR 64b</td>
<td>Long, Slender</td>
<td>First IR variety with intermediate amylose and intermediate gelatinization temperature, International Check</td>
<td>Oryza sativa</td>
<td>Indica</td>
<td>IR 5657-35-2-1 x IR 2061465-1-5-5</td>
</tr>
</tbody>
</table>

a – used only for phenotypic characterization; b – used only for RM190 based genotypic characterization as a reference.
will be low (55-69°C) (Bhonsle et al., 2010). GT of white rice grains was estimated by the alkali spreading value as described by the Cruz and Khush (2000). Crude fibre content of the rice grain samples were determined by the filtration method by using the Fibra Plus system (Pelican Instruments made) according to ISO 6865:2000 method with slight modifications (ISO 6865:2000).

The in vitro predicted Glycemic Index (GI) values of rice varieties was determined using “Nutriscan Glycemic Index Analyser (NutriScan GI20)” an automated instrument for predicting the GI in vitro: developed by the CSIRO, Division of Food and Animal Nutrition, Adelaide, South Australia. The NutriScan GI20 is a 20 sample capacity analyser that simulates the digestion of a sample of food as it passes through the human gut. All the reagents/chemicals and enzymes required for the analysis are specific and supplied from the manufacturer of the instrument as kit.

The grains of each variety was first, cooked and then used for estimation of GI (Figure 3). About 100 mg (± 0.5) polished rice grain samples were weighed and cooked with 2ml (sufficient enough for cooking; excess water may leads to loss of the sample) water in a glass tube by using water bath for 15-20 min. Time required for cooking defers among the genotypes because of their complex chemical structure and composition which can be estimated by analysing gelatinization temperature in addition to this other factors like nature of seed i.e. polished/unpolished also affect the cooking time and should be consider for proper cooking for sample preparation. After cooking rice samples were allowed to cool for few minutes and directly transferred i.e. without draining (including whatever water it have) into the sample cups of the NutriScan GI20 machine that contains a stirrer bar and kept in a heating block to maintain the temperature to 37°C. Three enzyme mixtures (not disclosed by the company) were added to each sample cup in a specific sequence along with buffers. Samples were digested as occurs inside the human system and the GI20 measures the amount of glucose released from these digested samples at successive intervals (30, 60, 120, 240, and 300 minutes) during the digestion process. Digestion of food is a long, continuous process and depends upon the type of food. During the digestion there is a state at which release of glucose stabilizes at this point of time reading of glucose should be consider. For rice sample we observed that time as 300 min (5 hours) and taken the reading after 300 min. The glucose is measured using a Glucose Analyser based on the following reaction:

\[
\beta-D-glucose + O_2 + H_2O \rightarrow \text{Gluconic Acid} + H_2O_2
\]

(Gluconic Acid = D-glucono-1, 5-lactone; GOD = Glucose Oxidase)

An oxygen electrode placed in the reaction cell measures the consumption of O2, which is proportional to the amount of glucose that is oxidised by the glucose oxidase reagent. The glucose concentrations of the digests are plotted over 5 hours. The total amount of glucose released is then used to predict the GI of the original sample. The predicted GLs of the rice were calculated as a percentage of available CHO converted to glucose over the duration of the incubation. The Glycemic Index is calculated from the following equation:

\[
\text{Glycemic Index} = \frac{\text{Glycemic Response}}{\text{Available carbohydrates in the Sample}}
\]

**Allelic diversity for Waxy gene**

Simple sequence repeat (SSR) marker RM 190 (CT,) located within the ‘Waxy (Wx)’ locus a major gene influencing the rice grain amylose content was used for analysing the presence of genetic variability among the selected rice genotypes with IR64 rice as reference for RM190 loci. Genomic DNA was extracted from leaf by the MiniPrep method (Doyle and Doyle, 1987). Polymerase chain reaction (PCR) amplification was performed in a total volume of 20 il and the reaction mixture contained 1X PCR buffer, 0.2 mM dNTP mix, 0.5 iM forward and reverse primers, ~50 ng of template DNA and 0.075 unit Taq polymerase in Applied Biosystems thermal cycler. After an initial denaturation step of 94°C for 5 minutes, the amplification was carried out for 35 cycles comprising 30 seconds each of 94°C, 52°C and 72°C. The final extension step was performed for 10 minutes at 72°C followed by storage at 4°C. After the PCR reaction was completed, 5 μl of 6 X loading dye was added to PCR product and total 8 il of was loaded on 5% poly acrylamide gel (PAGE) in a mini vertical electrophoresis system (CBS scientific, model MGV-202-33). Additionally 100bp DNA ladder was also loaded as standard to estimate the product size. DNA fragments were then stained with ethidium bromide, visualized and documented with a Gel Documentation system (Bio-rad).

**Statistical analysis**

Standard statistical tools were used to calculate mean of all traits. Pearson’s correlation coefficient (r) was used to determine the existence of relation among the trait analysed.

**RESULTS AND DISCUSSION**

**Grain Quality characteristics**

Variation for grain amylose content, gelatinization quality and amount of fibre content are responsible for making rice highly palatable and digestive food. Initially, ACC, GT and fibre contents of all five rice genotypes were recorded (table 2). The Grain ACC levels of five tested rice genotypes were intermediate to high amylose types, which ranged from 25.2% for selected Gurmatiya genotype named CGMD-55and Swarna to 23.70% for HMT. Similarly, the grain GT values for three rice genotypes fall into Low GT (55-69°C) and two rice genotypes had intermediate GT levels (70-74°C). This clearly indicated that all tested rice genotypes bear’s good grain quality especially in term of palatability and digestibility.

The grain fibre is another important character considered to affect the starch digestibility intern the Glycemic index value. Therefore, the crude fibre contents of rice genotypes were estimated, which varied from 0.39% for HMT to 2.11% for BR check.

**Predicted Glycemic Index (GI) value**

Variations from 83.3% to 89% (mean value) for total carbohydrate content in four white and one brown grain (BR check) among the 5 different rice varieties under study
were observed (table 3). Result showed the highest carbohydrate content i.e. 89% for Swarna and lowest carbohydrate content i.e. 83.3% for BR check rice with an average of 85.7%.

The predicted Glycemic Index (\(\gamma\)GI) values and carbohydrate value for five selected rice varieties under study were determined (Table 3 and Figure 4) by the in vitro method described above. A noticeable variation for predicted GI values ranged from 55% to 68% was observed with carbohydrate value ranged from 83.3% to 89% among the tested rice varieties. The average \(\gamma\)GI value recorded was 64%. Out of five varieties tested, four (Swarna, HMT, CGZR1 and BR check) have shown medium \(\gamma\)GI value (mean value), and only one variety, CGMD-55 shown the \(\gamma\)GI value of 55% which categorises it as low GI type rice variety (high GI above 69, moderate GI between 56-69 and low GI below 56).

Table 2. Grain quality characteristics of rice genotypes

<table>
<thead>
<tr>
<th>Rice Variety/Genotypes</th>
<th>AAC (%)</th>
<th>Fibre (%)</th>
<th>ASV</th>
<th>Alkali Spreading Class</th>
<th>GT (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swarna</td>
<td>25.2</td>
<td>0.52</td>
<td>6</td>
<td>High</td>
<td>Low (55-69)</td>
</tr>
<tr>
<td>CGMD-55</td>
<td>25.2</td>
<td>0.68</td>
<td>5</td>
<td>Intermediate</td>
<td>Intermediate (70-74)</td>
</tr>
<tr>
<td>HMT</td>
<td>23.7</td>
<td>0.39</td>
<td>6</td>
<td>High</td>
<td>Low (55-69)</td>
</tr>
<tr>
<td>CGZR1</td>
<td>24.6</td>
<td>0.66</td>
<td>7</td>
<td>High</td>
<td>Low (55-69)</td>
</tr>
<tr>
<td>BR Check (Brown)</td>
<td>24.3</td>
<td>2.11</td>
<td>5</td>
<td>Intermediate</td>
<td>Intermediate (70-74)</td>
</tr>
<tr>
<td>Average</td>
<td>24.6</td>
<td>0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(\gamma\)GI values and carbohydrate were significant (at 5% level) other than fibre content was observed. The correlation between AAC and carbohydrate content was also detected, whereas fibre content showed negative correlation to the carbohydrate content. AAC and carbohydrate was significant (at 5% level) other than this no trait showed significant relation.

Table 3. Mean performance of rice genotypes for carbohydrate and predicted glycemic index (\(\gamma\)GI) values

<table>
<thead>
<tr>
<th>Rice Variety/Genotypes</th>
<th>Whitness (%)</th>
<th>CHO (%)</th>
<th>(\gamma)GI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swarna</td>
<td>50.5</td>
<td>89.0</td>
<td>67</td>
</tr>
<tr>
<td>CGMD-55</td>
<td>57.8</td>
<td>87.6</td>
<td>55</td>
</tr>
<tr>
<td>HMT</td>
<td>57.0</td>
<td>83.4</td>
<td>68</td>
</tr>
<tr>
<td>CGZR1</td>
<td>63.3</td>
<td>85.5</td>
<td>68</td>
</tr>
<tr>
<td>BR Check (Brown)</td>
<td>14.5</td>
<td>83.3</td>
<td>61</td>
</tr>
<tr>
<td>Average</td>
<td>58.5</td>
<td>85.7</td>
<td>64</td>
</tr>
</tbody>
</table>

Alleric variation affecting grain amylose content

Waxy gene (known to have major effect on amylose content) specific simple sequence repeat (SSR) marker RM 190 (CT,) located within the ‘waxy’ locus was used to assess the presence of genetic variability among the selected rice genotypes. Rice variety IR64 used earlier by other researchers known to have 17-CT repeats (Juliano et al., 2012) at the RM190 locus was used as reference allele to characterize the rice varieties under study except BR check rice (commercial rice purchased from market) due to unavailability of DNA. The PCR product generated via RM190 marker was analysed on the 5% poly acrylamied gel, banding pattern of the above samples was manually analysed to predict size of products generated by referring 100bp DNA ladder. Total three types of bands were observed (Figure 5) among the five genotypes including IR64. Swarna showed the smallest band and rest three test varieties i.e. CGMD-55, HMT and CGZR1 gave the intermediate band whereas band of the reference variety IR64 was of largest size among the five rice genotypes. Based on the pattern of ladder the approximate product size for Swarna was estimated between 105-110bp, 110-115bp for CGMD-55, HMT and CGZR1 whereas for IR64 the size was close to 122-126 bp which is also supported by the gel image mentioned on the gramene marker page (http://archive.gramene.org/markers/) for RM190. Accordingly the estimated difference between the alleles of Swarna and IR64 was expected from 14 to 16 bp that indicated towards the presence of allele with 9 or 10 CT repeat type. Similarly the difference between three rice varieties i.e. CGMD-55, HMT, CGZR1 and IR64 was estimated from 12 to 14bp which correspond towards the presence of allele having 10 or 11 CT repeat type.

Correlation among \(\gamma\)GI and grain quality traits

Pearson’s correlation coefficient (r) value for grain carbohydrate, \(\gamma\)GI, AAC, fibre content and ASV was calculated by using mean values of the above traits for five rice varieties and presented in table 4. Negative correlation between \(\gamma\)GI and carbohydrate, AAC, fibre content were observed, whereas ASV showed the high positive correlation to the \(\gamma\)GI. High positive correlation between AAC and carbohydrate content was observed additionally very slight positive correlation between ASV and carbohydrate was also detected, whereas fibre content showed negative correlation to the carbohydrate content. AAC content was negatively correlated to the ASV and fibre content also a negative correlation between ASV and fibre content was observed. The correlation between AAC and carbohydrate was significant (at 5% level) other than this no trait showed significant relation.

The problem of lifestyle-related diseases like obesity, cardiovascular complications and type II diabetes are the major health concern rapidly increasing throughout the world (Post et al., 2012).The quality of staple food like rice influence human health through its nutritional value and digestibility. Among various factors starch quality and quantity of food are key functional property, primarily influencing blood glucose level and diabetes related disorders. The incomplete digestion-absorption of starch in the small intestine leads to non-digestible starch fractions...
which have physiological functions similar to dietary fibre with beneficial impacts. Study showed that dietary glycemic index is positively associated with diabetes risk and the choice of carbohydrates, particularly those with low GI or slowly digestible carbohydrates is able to assist in the management and prevention of type II diabetes (Masouleh et al., 2012 and Mir et al., 2013).

Rice is among the traditional staple food and primary dietary source of carbohydrates for a major part of the world’s population. Dietary strategy with improved carbohydrate quality rice would be of great importance for the management of type II diabetes and related disorders. Development of potential rice varieties for reducing the incidence and severity of type II diabetes has not been done because unavailable information for the variability of GI in rice and the genetics behind it. Studies in this area have also been hampered by the low throughput, poor precision and expensive in vivo method of GI detection (Fitzgerald et al., 2011).

Present investigation categorises the rice varieties as low and medium GI type varieties based on their $\text{GI}$ value. This indicates that the variability for GI content is present in the rice genotypes and there are such rice type exist which can be utilised for managing diabetes. Out of five varieties tested, only one CGMD-55 have shown $\sim 55\%$ $\text{GI}$ value and characterised as low GI rice type. This finding is also supported by the traditional believe of slow digestion of this variety and its stay in stomach for longer period of time. Additionally, the identified low $\text{GI}$ variety is already in consumption pattern of local peoples and provides an option for rice lovers who force to change their food habit due to diabetes and related disorders. The identified low GI variety can be further validated by in vivo method and can be recommended for the diabetes. The low $\text{GI}$ value of the traditional landrace and higher $\text{GI}$ of popular commercial varieties also indicates towards the selection bias of various breeding programmes. Based on an in vivo study Fatema et al., (2010) reported 3 commonly consumed Bangladeshi rice varieties (BR-14, BR-29 and BR-44) as low GI rice (Fatema et al., 2010). Indrasari et al., (2010) evaluated the GI value of 9 rice varieties and reported low, medium and high GI ranging from 34 to 79 among the nine rice varieties. Fitzgerald et al. reported large variability in GI, ranging from 48-92 (low, intermediate and high GI), with an average of 64 using a diverse set of 235 rice varieties both in vivo and in vitro method and reported the usefulness of the in vitro approach for predicting the GI of rice samples (Fitzgerald et al., 2011). Trinidad et al., (2014) analysed the GI of brown and milled rice of four rice varieties representing waxy, low-AC, intermediate-AC and high-AC rice types by in vivo approach and reported the GI of 94, 85, 69, 59 for milled rice and 77, 69, 61, 57 for brown rice samples respectively (Trinidad et al., 2014).

The high average total carbohydrate content

![Fig. 1. Plant and grain architecture of CGMD-55](image)

### Table 4. Correlation Coefficient ($r$) between Predicted Glycemic Index ($\text{GI}$) values with other grain quality characters studied

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean CHO (%)</th>
<th>$\text{GI}$ (%)</th>
<th>Mean AAC (%)</th>
<th>Mean Fibre (%)</th>
<th>ASV</th>
<th>P-value (at 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean CHO (%)</td>
<td>1</td>
<td>-0.1701</td>
<td>0.9188*</td>
<td>-0.4717</td>
<td>0.0485</td>
<td>0.878</td>
</tr>
<tr>
<td>$\text{GI}$</td>
<td></td>
<td></td>
<td>-0.4454</td>
<td>-0.3638</td>
<td>0.8256</td>
<td></td>
</tr>
<tr>
<td>Mean AAC%</td>
<td></td>
<td></td>
<td></td>
<td>-0.1312</td>
<td>-0.1738</td>
<td></td>
</tr>
<tr>
<td>Mean Fibre (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.5380</td>
<td></td>
</tr>
<tr>
<td>ASV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

* Significant at 5%
obtained describes the energy rich nature of rice and the range indicates towards the existence of variability for grain carbohydrate content. Odenigbo et al. estimated total carbohydrate content of milled grains of 9 different rice varieties and reported the carbohydrate content between 77.90 to 82.30 % (Odenigbo et al., 2014). In a study with five rice varieties Oko and Ugwu also reported carbohydrate content ranges from 76.92 to 86.03 % (Oko and Ugwa, 2011). Similarly Olembo et al. find the carbohydrate mean values of 82% in milled rice sample (Olembo et al., 2010).

Apparent amylose content (AAC) observed in the study reflects the presence of intermediate and high amylose containing genotypes only. These is probably because of the less number of genotypes and the fact that all the rice varieties tested belongs to Indica type which normally have higher amylose content than the Japonica type varieties (Biselli et al., 2014 and Cheng et al., 2012) and majorly comes under the intermediate to high amylose classes. The AAC obtained for CGMD-55 is closer to the popular commercial rice varieties which indicates that, it’s cooking and eating quality are similar to the varieties which are already in the market and largely consumed. Beside this a vast variability for AAC content among the various rice genotypes ranging from 0% (waxy) to more than 25% (high) has been reported by many researchers (Fasahat et al., 2012 and Jeevetha et al., 2014).

Result of gelatinization temperature (GT) analysis based on the alkali spreading score categorise the five tested rice varieties as low (55-69°C) and intermediate (70-74°C) GT type this indicates that temperature required for normal cooking time for these varieties is 55-74°C. In a study Bhonsle and Sellappan reported low, intermediated and high GT rice classes among the 25 tested rice varieties (Bhonsle and Sellappan, 2010). Similar to this Odenigbo et al. also reported variation on GT from 63.42 to 78.34°C among 10 rice genotypes tested and showed significant variation among samples for gelatinization parameters (Odenigbo et al., 2013).

Although a small amount of fibre content has been observed and was higher for the brown rice grain in comparison to the polished rice. The study gave encouraging results and indicates towards the existence of some variability for grain fibre content in rice. Similar to this result, Fatema et al. reported the fibre content from 1 to 1.1% among the three common Bangladeshi rice varieties (Fatema et al., 2010).

Various factors such as amylose, fat, dietary fibre, protein, resistant starch and starch properties viz granule size, architecture, crystalline pattern, degree of crystallinity, surface pores or channels, degree of polymerisation including nutritional composition of food, processing, food osmotic power, as well as antinutritional substances known to affects the GI of food (Rimbawan and Siagian, 2014 and Widowati et al., 2006).

According to Widowati et al. high amylose polished rice in general has low GI values whereas low amylose varieties show high GI values (Widowati et al., 2007a). Fitzgerald et al. reported strong negative correlations between amylose content, Waxy locus and GI among 235 rice varieties and indicated amylose as the major grain constituent affecting GI (Fitzergerald et al., 2011). They also indicated the existence of outliers among classes of amylose. Negative correlation between GI and AAC observed in the present study validate the existing inverse association among the GI and AC. In their study involving eight rice varieties Trinidad et al. reported the significant correlation of GI with AC, ASV, total and available carbohydrates (Trinida et al., 2014). The significant positive correlation observed between AAC and carbohydrate is indicative of the quality of the starch of rice varieties and

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**Fig. 2.** Showing appearance of grains of selected rice genotypes at different stages (i.e. undehusked, brown and polished)
Sample Preparation for GI estimation

Seeds of each rice variety were dehulled, polished (up to commercial level whiteness i.e., -60-50) using Chinese dehuller and polisher. Then 100 mg of polished rice grains of each variety was cooked in boiling tubes with 2 ml of distilled water for 15-20 minutes and used for estimation of predicted GI in nutiscan GI analyser.

Initialization and calibration of instrument

For initialization switch on GI machine and perform self-check. Fill glucose reagent and all the required solutions/buffers in their corresponding vials/bottles and check all the tubing, then press the buttons and switches. Now open the GI test software from computer and connect the computer to instrument.

Loading and analysis of GI of Cooked rice samples

- Transfer complete cooked rice samples in the digestion cup and placed it in the Nutriscan GI analyser.
- Pump the buffers in to enzyme A, B and C bottles and allowed to max for 10 minutes.
- Dispense 2 ml of Enzyme A into each digestion cup.
- Dispense the 5 ml of Enzyme B into each digestion cup.
- Incubate the digests at 37°C for 30 minutes under gentle agitation.
- Add 1 ml of buffer 4 and 23 ml of buffer 5 to the digests to neutralise the solution.
- Add 1 ml of enzyme C in each digestion cup and keep at 37°C with gentle agitation.
- After 300 minutes of incubation pump 1 ml of digest of each sample and inject into the GM9 Glucose Analyzer for analysis.
- For each digested sample, glucose concentration will be captured and stored.

Based on the banding pattern RM 190 marker (CT) revealed three different allelic forms of Waxy gene among the five genotypes including IR64. The allele of the reference variety IR64 was of largest size (122-126bp and 17-CT) whereas smallest allele was observed for Swarna (105-110bp) and expected to have 9 or 10 CT repeat types which gave 25.2% AAC. Other than Swarna rest three test varieties i.e. CGMD-55, HMT and CGZR1 gave the intermediate allele (110-115bp) with expected CT repeat of 10 or 11 types which gave 25.2%, 23.7% and 24.6% AAC respectively for the three varieties.

Genetic basis of rice grain quality is mainly affected by three physicochemical properties: AC, gel consistency (GC) and GT, but so far only one major gene, the Waxy gene has been found to affect AC, GC and GT (Tian et al., 2009; Biselli et al., 2014). Fitzgerald et al. also reported Waxy locus as the gene major affecting GI and indicated towards the variable glycemic responses generated by different Waxy gene alleles and suggests the presence of other loci or modifiers influencing the Waxy gene (Fitzgerald et al., 2011). In addition Fasahat et al. also reported Waxy gene as the principal gene controlling amylose content (Fasahat et al., 2012) but different researchers has identified various different QTLs which indicates influence of multiple genes on AC.

Fig. 3. Summary of estimation of predicted Glycemic Index value of rice grains using Nutriscan GI Analyser

probably the reason behind the inverse relation between GI and carbohydrate content. The negative correlation obtained among GI and fibre content is also in the agreement of the high fibre-low GI concept. Negative correlation of fibre with carbohydrate and AAC is an indicative of the increase in the proportion of other grain constituents with decrease in carbohydrate quantity.

Negative correlation of AAC and fibre to ASV reflects their positive relation with GT that is an indicative of the role of amount and structures of grain constituents like AC and fibre which when present in higher amount normally increases the firmness of the grain. In a study by Trinidad et al. ASV showed intermediate GT for intermediate amylose and low GT for waxy and low amylose rice varieties (Trinidad et al., 2014). Fitzgerald et al reported the firm and soft texture of rice after cooking due to differences in the amount and structure of amylose within the grain (Fitzgerald et al., 2011). High positive correlation between ASV and GI is suggestive of inverse relation among GT and GI which indicates that soft textured rice varieties normally have higher GI values than the firm rice varieties and suggests towards the influence of the amount and structure of the starch granules on the GI. Positive correlation of ASV to carbohydrate is suggestive of increase in amount carbohydrate and so the amylpectin which may leads to softer grain texture. Fitzgerald et al. analased the effect of textures of rice on GI but no clear relation was observed (Fitzgerald et al., 2011). Although they have mentioned the role of structure of grain constituents and so its texture on GI but it is suggested that the amount is more important than the structure.

Based on the banding pattern RM 190 marker (CT) revealed three different allelic forms of Waxy gene among the five genotypes including IR64. The allele of the reference variety IR64 was of largest size (122-126bp and 17-CT) whereas smallest allele was observed for Swarna (105-110bp) and expected to have 9 or 10 CT repeat types which gave 25.2% AAC. Other than Swarna rest three test varieties i.e. CGMD-55, HMT and CGZR1 gave the intermediate allele (110-115bp) with expected CT repeat of 10 or 11 types which gave 25.2%, 23.7% and 24.6% AAC respectively for the three varieties.

Wan et al. analysed the allelic variation for the Wx gene in 50 non-glutinous rice varieties and reported six homozygous namely CT20, CT19, CT18, CT17, CT16, CT14, CT11, CT10, and a heterozygous genotype CT11/CT18, of
Fig. 4. Chart showing the variation in predicted Glycemic Index (GI) level and their CHO value among the five rice genotypes

which CT11 and CT18 were predominant (Wan et al., 2007). According to the study conducted among the 47 rice varieties by Roferos et al. the major alleles of the Waxy gene were CT10, CT11, CT17 and CT20 (Roferos et al., 2008).

According to Cheng et al. total 16 Wx microsatellite alleles, ranging from CT4 to CT22, are known and correlated to amylose content (Cheng et al., 2012). In their study they reported five microsatellite alleles for the Wx gene viz CT10, CT11, CT16, CT17, and CT18 among the 15 rice varieties of which CT11 and CT17 were most frequent. It is also reported that varieties with high amylose content (above 24%) were associated with shorter repeat alleles, namely CT10 and CT11, while varieties with low and intermediate amylose contents (below 24%) were carried longer repeat alleles, including CT16, CT17 and CT18.

By using the germplasm collection of 127 rice accessions Biselli et al. identified ten different alleles which have CT-9, 10, 11, 13, 14, 17, 18, 19, 20, 21 for the RM190 with CT18 as the most frequent type (S). Among these CT9, CT10 and CT14 were present only in genotypes with 23–24.85% AAC, while CT11 and CT20 were only in AAC higher than 25%. Allele CT17, CT18 and CT19 represents a wide range of AAC (14.92-23.27%).

According to the Biselli et al. RM190 microsatellite (CTn) marker, is responsible for 74.9% of the AAC variation and different alleles of this marker are known which discriminate low, intermediate and high AAC genotypes (Biselli et al., 2014). Due to lack of specific correlation between the number of CT repeats and an AAC group it can be predicted that RM190 is only a closely linked marker and not the actual cause of variation for AAC (Cheng et al., 2012) and which indicate towards the existence of other variation like SNPs and or modifier genes influencing amylose content.

Based on the present study among the tested varieties a local landrace selection of Chhattisgarh region of India CGMD-55 was identified as low GI rice (GI-55) together with good CHO (87.6%), high AAC (25.2%), higher fibre (0.68%), and intermediate GT (70-74) type. The slow sugar release nature of CGMD-55s also supported by the traditional knowledge and belief of local people which provides strengths towards the usefulness of this variety for sugar patients. Since this variety is already in consumption of these people it also fulfills the test or other cooking/eating quality requirements and makes the identified low GI landrace CGMD-55s a healthy choice to the type-II diabetes patients. In addition to the normal rice consumption this variety is an excellent raw material for various rice based food products like poha etc normally consumed by various people and can be helpful in improving the public health without changing their diet. At the same time the less time consuming in vitro glycemic index prediction method is a useful alternative of the in vivo method of measuring glycemic responses of food. Results from the work provided initial basic information for in vitro GI estimation work required for large scale screening of various available germplasm and further research in this area. The methodology and database represent a unique resource that will be utilized to accelerate the development of low GI rice varieties.

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