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Article in *Analytical Biochemistry* · July 2016

DOI: 10.4172/2161-1009.1000285.

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Physicochemical Characterization of Selected Rice (*Oryza Sativa* L.) Genotypes Based on Gel Consistency and Alkali Digestion

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Rec date: May 26, 2016; Acc date: Jul 19, 2016; Pub date: Jul 22, 2016.

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Abstract

Quality assessment of rice genotypes involves physicochemical and sensory test. A physicochemical test relies on the rice chemical composition, cooking quality, gelatinization temperature and physical properties of cooked rice. The aim of this study was to determine the physicochemical characteristics of selected Kenyan and Tanzanian genotypes based on gel consistency and alkali digestion. Diverse classes of rice exist with different expression levels of gel consistency and alkali digestion. As these traits are major contributors to the eating and cooking quality traits, unscrupulous traders mix both low and high-grade rice hence making enormous profits from their sales. Minitab 17.0 software package was used to determine the means and the standard error of means of the gel consistency physicochemical test results while the alkali digestion values were determined based on the standard evaluation system by International Rice Research Institute. Majority of the genotypes had high alkali digestion. Based on the gel consistency test, the average GC values ranged from 31.50 mm in ITA 310 to 99.5 mm in IR 2793. Genotype BS 217 showed significant difference from Kilombero, IR 64, Kahogo, Saro 5, ITA 310, IR 54, Wahiwahi and BW 196. These results show that physicochemical characterization using the available test procedures can be effectively utilized in analysis of diversity in rice germplasm

Keywords: Rice; Gel consistency; Alkali digestion; Physicochemical characterization

Introduction

Rice belongs to the genus *Oryza*, which is, divided into four species complexes which include; *O. sativa*, *O. officialis*, *O. ridelyi* and *O. granulata*. The genus contains 25 recognized species where 23 are wild species and two are cultivated species namely; *O. Sativa* and *O. glaberrima* [1]. The 23 wild species represents the 10 genome types, which are distributed throughout the tropics, and subtropics whereby Africa has five of these genome types. In Kenya and Tanzania, there are many rice varieties, which are either glutinous (waxy) or non-waxy (non-glutinous) with different levels of the amylose content [2].

Rice grain quality traits encompass the totality of all characteristics and features of rice or the rice products that meets the consumer demands and preference [3]. Grain quality features the physical, physiological and its biochemical features [4]. The physical properties include the grain shape, degree of milling, grain appearance, milling recovery, kernel shape. The physicochemical properties comprises of gel consistency (GC), alkali digestion, amylose content (AC) [4-6]. Different rice genotypes have diverse physicochemical and physical properties, which influences the grain quality properties.

These ECQ traits are influenced by complex genetic control of both minor and major quantitative traits loci (QTL), environmental factors and soil's nitrogen levels. QTL analysis indicates that the AC, GC, paste viscosity parameters and gel texture are controlled by the waxy locus (Wx) gene and other minor QTLs. The Wx is located on chromosome six and it encodes granule-bound starch synthase-I (GBSSI), which determines the ratio between amylose and amylopectin in the

endosperm starch. Gel consistency measures the cold paste viscosity of cooked milled rice flour an index used in distinguishing cooked rice texture of high amylose genotypes. Gel consistency varies from soft to hard [7]. Genotypes are grouped into arbitrarily set classes based on the length of the gel: hard (length of gel <40 mm), medium (length of gel 41 – 60 mm), and soft (length of gel > 61 mm) [8]. Association of starch polymers in the aqueous phase determines weak and rigid gels. , rice with soft gel consistency has a higher preference amongst the consumers. Therefore, breeders tend to develop rice genotypes with soft gel consistency [9].

Alkali digestion quality trait is located on chromosome six and the QTL that controls it is in the alk locus. The alky gene encodes the starch synthase IIa (SSIIa) that determines the gelatinization temperature of cooked rice [10]. Disintegration of rice starch granules in 1.7% KOH alkali solution and gelatinization temperature of milled rice have a significant correlation. Alkali digestion values are dependent on the nature of the amylopectin molecules. Amylopectin is a polysaccharide found in the rice starch endosperm. It has a large molecular weight and highly branched compared to amylose. Amylopectin is composed of α -1-4-glycosidic linkages that link the D-glucose units while the branched chains are linked by the α -1-6-glycosidic linkages [11].

The starch branching enzymes (SBE) plays a major role in the synthesis of amylopectin since it is the only enzyme that introduces the α -1-6-glycosidic linkages into the α -polyglucans in the endosperm starch, the amylopectin side chains play a major role in the disintegration of rice starch granules in alkali solution [12,13]. The degree of polymerization (DP) characterises the amylopectin into four categories [14]. The $DP \leq 12$ forms the A short chains while $13 \leq DP \leq 24$ forms the B1 short chains. Moreover, $25 \leq DP \leq 36$ is attributed to

the long B2 chains while DP ≥ 37 forms the B3 long chains. Rice starch granules with amylopectin enriched with the shorter chains easily disintegrates in alkali solution than starch granules that have longer chains of amylopectin [15].

The SSIIa plays a vital role in the elongation of the short amylopectin chains which directly influences the degree of disintegration of the starch molecules in alkali solution [13]. The degree of alkali digestion is inversely proportional to the gelatinization temperature, that is, when the alkali digestion is low, the gelatinization temperature is high [16]. Rice with low GT disintegrates completely in 1.7% KOH solution, whereas rice with intermediate GT shows partial disintegration while high GT remains largely unaffected in alkali solution.

Materials and Methods

Sample collection and preparation

A total of 500g of the rice grains of twelve selected Kenyan and Tanzanian rice genotypes were collected from two repositories; Mwea Irrigation and Agricultural Development (MIAD) and Kilimanjaro Agricultural Training Centre (KATC) in Moshi, Tanzania. The rice grains were brought to Kenyatta University Plant Transformation Laboratory (PTL) and stored under dry conditions with similar moisture content to facilitate further determination physicochemical traits. Rice grains were dehusked to facilitate the alkali digestion test and an electric mill was used to mill dehusked rice into rice flour for gel consistency test. Genotype IR 64 was selected as model genotype since it's a superior variety with soft gel consistency and intermediate alkali digestion (Table 1).

Genotype	Source	Attribute
IR 2793	Kenya	Improved genotype
BS 217	Kenya	Improved genotype
BS 370	Kenya	Improved genotype
BW 196	Kenya	Improved genotype
ITA 310	Kenya	Improved genotype
Red Afaa	Tanzania	Landrace genotype
IR 54	Philippines	Improved genotype
Kilombero	Tanzania	Landrace genotype
IR 64	Philippines	Improved genotype
Kahogo	Tanzania	Landrace genotype
Saro 5	Tanzania	Improved genotype
Wahiwahi	Tanzania	Landrace genotype

Table 1: Rice genotypes, origin and attributes of rice genotypes used in the study.

Gel consistency

Gel consistency in rice genotypes was determined using protocol described by [4,7] with slight modifications. Approximately 500 mg rice flour from each rice genotype was weighed in triplicates and

placed in a 13 mm × 100 mm test tube. A total volume of 0.026 ml of 95% ethanol containing 0.025% thymol blue was added to each tube and mixed so as to prevent clumping of the rice flour. The mixture was vortexed gently, and then 2 ml of 0.2 N KOH was added and vortexed again. Each tube was covered with glass marbles to prevent steam loss and reflux of the samples and placed over a boiling water bath at 92oC for 6 minutes. They were then kept at room temperature for 5 minutes and finally transferred to an ice bath for 15 minutes. The tubes were placed horizontally on a graph paper on a flat laboratory bench for 30 minutes, and the blue gel length was measured from the bottom of the tube to the end of the gel in millimetres (Figure 1). This test was performed in triplicates to ensure accuracy and validity of the results.

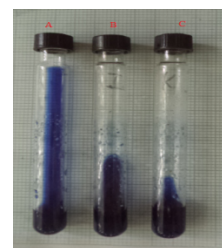


Figure 1: Gel consistency of rice genotypes under study.

Alkali digestion

Alkali digestion was determined using methodology described by [17] with slight modifications. Disintegration was determined by placing 10 polished rice grains in a Petri plate containing 10 ml of freshly prepared 1.7% (w/v) KOH solution. Seeds were arranged with the provision of space between the grains for spreading. The Petri plates were then covered and placed in a 30oC incubator for 23 hours. This test was performed in triplicates to facilitate the accuracy and validity of the test results. The degree of disintegration of each of the grains was rated visually according to standard evaluation system for rice by International Rice Research Institute [18] (Table 2).

Degree of digestion	Alkali digestion classification	Alkali digestion value
Grain not affected	Low	1
Grain swollen	Low	2
Grain swollen, collar incomplete and narrow	Low	3
Grain swollen, collar complete and wide	Intermediate	4
Grain split or segmented, collar complete and wide	Intermediate	5
Grain dispersed, merging with collar	High	6
Grain completely dispersed and intermingled	High	7

Table 2: The seven-point scale used in assigning of the alkali digestion values.

Data Analysis

Data on gel consistency values were transferred to a spreadsheet. The data was subjected to descriptive statistics and expressed as Mean \pm SEM. It was analysed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for pair-wise separation and comparison of means. Statistical significance was set at 95% confidence interval ($P \leq 0.05$). Minitab 17.0 software package (State College, Pennsylvania) was used for statistical analysis. Alkali digestion results were descriptive hence; classifications were done visually based on the IRR standard evaluation system.

Results

Alkali digestion

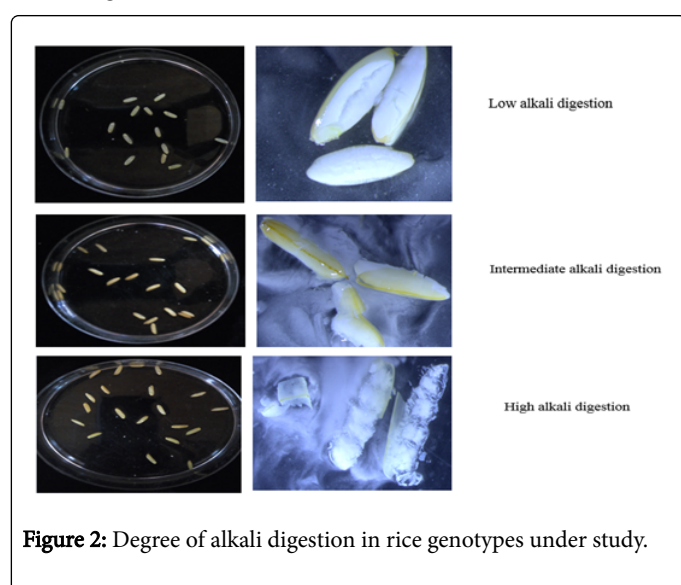


Figure 2: Degree of alkali digestion in rice genotypes under study.

Genotype	Degree of degradation	Alkali digestion value [ADV]
IR 2793	Intermediate	4.0
BS 217	Intermediate	5.0
BS 370	Intermediate	5.0
BW 196	High	6.0
ITA 310	High	7.0
Saro 5	High	6.0
IR 64	Intermediate	4.0
Kilombero	High	7.0
Red Afaa	Low	1.0
Kahogo	High	7.0
IR 54	High	7.0
Wahiwahi	High	7.0

Table 3: Degree of degradation, alkali digestion values of the 12 genotypes.

Based on the degree of alkali digestion observed (Figure 2), the rice genotypes were classified into three groups; low, intermediate and high alkali digestion values. Genotype with low degree of alkali digestion value was Red Afaa while four genotypes had intermediate alkali digestion namely; IR 2793, BS 217, BS 370 and IR 64. High alkali digestion was observed in 7 genotypes; Saro 5 and BW 196 had an alkali digestion value of 6.0 while Wahiwahi, ITA 310, IR 54, Kahogo and Kilombero had an alkali digestion value of 7.0 (Table 3).

Gel consistency (GC)

Based on GC values shown in Table 4, the average GC values ranged from 31.50 mm in ITA 310 to 99.5 mm in IR 2793. Genotype BS 217 had the second highest GC values after IR 2793 with no significant differences amongst their mean values. There was no significant difference between the GC values of BS 217, Red Afaa and BS 370 ($P \geq 0.05$; Table 4). Gel consistency values of BS 370, an improved Kenya genotype, revealed no significant difference from Kahogo, BW 196, Saro 5, IR 64, Kilombero and Red Afaa ($P \geq 0.05$; Table 4). Genotypes BS 370, BW 196, Saro 5, IR 64, Kilombero, Kahogo, IR 54 and Wahiwahi had no significant difference in gel consistency values. Improved genotypes, ITA 310 and IR 54, had gel consistency values insignificantly different from Wahiwahi, a landrace genotype from Tanzania ($P \geq 0.05$; Table 4).

Gel consistency value of genotype BS 217, an improved Kenyan rice genotype was significantly different from Kilombero, IR 64, Kahogo, Saro 5, ITA 310, IR 54, Wahiwahi and BW 196 ($P < 0.05$; Table 4). Genotype Red Afaa showed significant difference in the mean gel consistency measurements from genotypes ITA 310, Wahiwahi and IR 54 values ($P < 0.05$; Table 4). Improved genotype ITA 310 with mean GC value of 31.50 mm showed significant difference from nine rice genotypes except Wahiwahi and IR 54 ($P < 0.05$; Table 4). The GC value of improved Kenyan genotype IR 2793, was significantly different from the gel consistency values of genotypes ITA 310, Wahiwahi, IR 54, Saro 5, BW 196, Kahogo, IR 64, Kilombero and BS 370 ($P < 0.05$; Table 4).

Genotype	Gel consistency values (mm)
IR 2793	99.50 \pm 0.50 a
BS 217	97.00 \pm 1.29 ab
BS 370	75.25 \pm 3.90 bcd
BW 196	67.00 \pm 3.39 cd
ITA 310	31.50 \pm 4.48 e
Saro 5	66.50 \pm 3.48 cd
IR 64	67.75 \pm 3.42 cd
Kilombero	69.50 \pm 3.07 cd
Red Afaa	78.50 \pm 9.54 abc
Kahogo	67.50 \pm 5.50 cd
IR 54	53.25 \pm 7.09 de
Wahiwahi	54.25 \pm 3.33 de

Table 4: Analysis of variance of the gel consistency values of the 12 rice genotypes.

Discussion

Evaluation of the rice grain quality traits is achieved through the determination of the physicochemical properties of the rice starch, which provides an indirect index on the eating and cooking quality trait of each genotype. Determination of the ECQ traits is imperative given that it influences the multiple uses of rice at both industrial and domestic scale [19]. Genetic background and environmental interactions cause a variation in ECQs of different germplasms [20].

Alkali digestion is one of the important indicators of the eating, cooking and processing quality of rice starch [12]. In this study, determination of the alkali digestion classified the rice genotypes into three groups namely; low, intermediate and high alkali digestion. Similar classifications have been reported in Thai rice cultivars [21]. Genotypes that were least affected by the alkali solution had a low ADV, which could be attributed to the presence of more long amylopectin chains (B2 and B3) than the short (A and B1) amylopectin chains [15]. The activity of the SSIIa is highest in the low alkali digestion cluster than in other classification. This is due to the SSIIa gene increasing the proportion of the short amylopectin chains [13]. Given that there is an inversely proportional relationship between alkali digestion value and the gelatinization temperature, the genotypes with low alkali digestion have a high gelatinization temperature above [22, 23].

The intermediate alkali digestion group consisted of grains that were either swollen or segmented with complete and wide collars. Similar observations have been reported by in Myanmar local rice cultivars and in Thai rice cultivars [21,24]. This observation could be attributed to the presence of intermediate number of both the long and the short chains. The SSIIa plays a vital role in the elongation of the short amylopectin chains which directly influences the degree of disintegration of the starch molecules in alkali solution. Hence, the SSIIa activity is lower than the low ADV classification and higher than the SSIIa activity of the high alkali digestion cluster [13]. The intermediate ADV corresponds to an intermediate gelatinization temperature, which ranges from 70 to 74 The intermediate alkali digestion genotypes are the most preferred worldwide given their good cooking qualities such as water absorption, moistness, volume expansion and softness upon cooling [25].

The rice grains that were highly affected by alkali solution had a high ADV. This observation could be attributed to the presence of amylopectin with a high number of short chains (A and B1) with minimal number of long amylopectin chains. The SSIIa activity is maximal amongst these genotypes since this gene plays a vital role in the elongation of the short amylopectin chains [13,26]. The high alkali disintegration corresponds to gelatinization temperature below. The low gelatinization temperature ascribed to these genotypes is an economically important indicator of quality given that consideration of the shorter cooking duration results in significant savings of the fuel costs. Consumer preference of the low GT rice is minimal due to the negative outcome on the linear kernel elongation, water absorption and volume expansion of the rice genotypes [27]. The diverse ADV classification of IR 54 and IR 64, despite both genotypes being improved genotypes from Philippines, is attributed to crop improvement strategies that have been undertaken on both crops. The IR64 rice genotype is a superior rice genotype released by IRRRI after undergoing crop improvement process to confer good quality traits [28] as well as resistance to biotic stress such as *Xanthomonas oryzae* and abiotic stresses, such as flooding [29].

Gel consistency test was developed as an indirect method used in screening cooked rice for its hardness especially in rice with high amylose content [30]. This physicochemical test is used in rice improvement programs to ascertain if high amylose genotypes are soft or hard textured when cooked [31]. In this study, genotypes were classified into the soft, intermediate and hard GC based on the gel consistency values [8]. The three classifications could be as a result of different expression levels of the waxy gene and the variations in the *Wx* gene locus [32].

Hard gel consistency observed in this study was due to formation of rigid rice gels, which occur as a result association of starch polymers in the aqueous phase. Amylose polymers leach when the starch granules are heated and they subsequently form networks once the gel cools [33-35]. Physicochemical characterization of double haploid rice population also reported on the same classification [36]. The intermediate gel consistency observed in this study could be due to effects of minor genes, for instance, gene interaction between waxy and pullulanase or waxy and BEIII genes [36]. Indian cultivars Sharbati and HBC-19 expressed intermediate GC values of 54, 58 and 53 mm respectively [37].

Genotypes with soft GC had a gel length that is more than 61 mm. This trait was observed in most of the rice genotypes under study. The model variety IR 64 had an average GC of 67.75 mm, which is a soft gel consistency genotype [28]. The IR 64 classification in this study was contrary to earlier studies, which classified IR 64 under the hard gel consistency category [38]. Traditional aromatic Indian BS 370 rice genotype had an intermediate GC, which was different from findings of this study where Kenyan BS 370 had a GC value of 75.25 mm [39]. The different classification of IR 64 and BS 370 could be attributed to different environmental conditions when these studies were done. The soft and hard GC values are accounted for by the biallelic variability at the waxy locus [32]. The wide diversification of the physicochemical test results was due to the wide genetic background of the rice genotypes under study, that is, the rice composed of landraces and improved rice genotypes from different geographical regions. Similar classification of rice into three GC groups has been reported in 63 non-waxy rice varieties [40,41].

Conclusion

From this study, it can be concluded that physicochemical classification showed considerable variation based on gel consistency and alkali digestion. Most of the rice genotypes under study had high alkali digestion values implying that it saves on fuel cost. In addition, these verities compromises on the linear kernel elongation, water absorption and volume expansion hence tend to have poor eating and cooking quality traits. Soft gel consistency was also the most observed trait, which is a favorable trait amongst rice since they remain soft after cooling.

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