Analysis of Physicochemical Characteristics Diversity of 46 Accessions of Indonesian Local Rice Germplasms

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Abstract. Physicochemical characterization of rice germplasms is an important step of the rice breeding program. The physicochemical characteristics are important for consumers' acceptance. The objective of this study was to analyze physicochemical characteristics information to obtain suitable accessions for breeding programs and to cluster accessions according to their main characteristics. A total of 46 accessions of ICRR & ICABIOGRD collection were evaluated for 24 physicochemical traits. Principal component Analysis (PCA) was carried out to analyze the variation and the contribution of each trait to the total variance. This study showed that physicochemical variation might be divided into 5 principal component: Principal component 1 (PC1) was dominated by grain roundness, PC2 by low amylose content, PC3 by high milling yield, PC4 by long and slender grain, and PC5 by premium quality. Clustering analysis yielded 4 clusters. The 1st cluster was dominated by round grain, 2nd cluster by low milling yield, 3rd by high broken grain, and the last cluster by high milling yield and premium quality. This work showed that there were 7 potential accessions to be included in the rice breeding program of high milling yield and premium quality rice, i.e. accession no. 10479 (Umbul-umbul), 10486 (Babad Cianjur), 10491 (Marus), 10499 (619), 10515 (Siregi), 10551 (Logawa), and 10562 (Umbul-umbul). Those varieties can be used by rice breeders to develop new varieties to answer the global challenge of food security.

Keywords: Rice germplasms, physicochemical characteristics, Principal component analysis, Clustering analysis

1. Introduction

Indonesia has a diversified rice germplasms [1]. Their conservation is needed since rice is staple food of the country. Nowadays, rice germplasms' conservation is carried out by Indonesian Center for Rice Research (ICRR) and Indonesian Centre for Agricultural Biotechnology and Genetic Resources Research and Development (ICABGRRD). The conservation program is then followed by characterization process of the characteristics of the rice plants to obtain information about their advantages. The characterization process is also one of the activities to conserve the rice germplasms as the genetic sources in rice breeding program [2].

Rice quality is an important aspect for those who produce, process, and consume them. In the end, the quality of rice will affect its economic value or selling price [3]. The important quality components of rice are physical characteristics including appearance and milling quality and physicochemical characteristics such as cooking quality [4].

Rice appearance is mainly determined by its shape; such as length, width, length-width ratio, and the endosperm translucency [5]. The shape of rice is an important quality component that has huge impact to the selling value and also to the yield [6]. It is determined by genetic factor from the parents. There are several genes that affect rice shape, such as GS2, GS3, GS5, GS6, qGL3, GW2, GW5/qSW5/GSE5, GW7/GL7/SLG7, dan GLW7 [7].

Rice milling quality is one of the main factor of rice quality. It includes several components, such as milling yield, head rice percentage, broken rice percentage, and the milling degree [8]. Head rice yield is the main requirement on the determination of the unhusked rice quality because it will determine the weight of the produced rice and, finally, the economic value. The head rice yield is varied and affected by several factors, i.e. variety, grain category, chalky grain, crop management, environmental factors, post-harvest handling including harvesting, threshing, storing, and milling [9]. On the other hand, the main parameter of chemical characteristic determining the rice quality is the amylose content. Several studies showed that the amylose content is correlated with the texture of the cooked rice [10,11]. The higher the amylose content, the harder of the cooked rice texture; and vice versa [12].

All the quality characteristics mentioned before will affect the consumer acceptance. The decree of Ministry of Agricultre (Kepmentan) no. 1091 year 2018 regarding standard operational procedure of the variety assessment in order to release food plant variety includes quality prerequisites, such as brown rice yield, milled rice yield, head rice percentage, amylose content, rice shape, rice length, chalky grain, and rice texture. It means, the information regarding rice characteristics is needed to support rice breeders in order to breed and release new kind of rice varieties that will be accepted by consumer and have high yield. In this study, 46 rice germplasm accessions were characterized their physicochemical characteristics to obtain several accessions that will be suitable as parents on the breeding program. Futhermore, accessions will be categorized according to the dominant characteristics that will help rice breeders to understand the characters of each accessions.

2. Methods

Materials

Forty four rice accessions obtained from ICRR and ICABGRRD germplasm collection. (Table 1). *Materials Preparation*

Planting was done on the experiment farm at Sukamandi in the dry season of 2017. Cropping technique was adopted from the recommended integrated crop management [13] with modification on the varieties and planting spacing. Planting spacing of 25 cm x 25 cm and spacing between plots of 50 cm was used. A kilogram of dry unhusked rice was sampled from each varieties to be characterized. Unhusked rice was dehusked using rice husker THU 35A (Satake). The brown rice was then polished using TM-05 (Satake). The milled rice is then analyzed to obtain its physicochemical characteristics.

Identification of the Unhusked Rice Physical Quality

The determination of water content (WCG) of the unhusked rice was carried out using air oven method as described in SNI 01-0224-1987 [14]. The percentage of empty grain and adulterants (EG), the calky green grain (CGG), the damaged yellow grain (DYG), the red grain (RG) were counted manually. The density of the grain (DG) and the one thousand grain's weight (W1000) were measured using scale.

The Measurement of the Milled Rice Physicochemical Quality Rice shape

Rice shape was determined by measuring length (L) and width (W) of the rice grain using a caliper. From those value, length/width ratio (LWR) was obtained.

Water content

Water content of milled rice (WCR) was measured using air oven method according to SNI 6128:2015 [15].

Physical quality of the milled rice

Determination of head rice percentage (HRP), broken rice (BRP), menir/small broken rice (SBRP), chalky rice (CR), and damaged yellow rice (DYRP) was done by experts of ICRR according to SNI 6128:2015 [15]. Whiteness degree (WD) and milling degree (MD) was measured by milling meter.

Amylose Content (AC)

Amylose content was determined using spectrophotometry method. A hundred gram of rice flour was prepared and then put inside a 100 ml volumetric flask. A mililiter etanol 95% dan 9 mL NaOH 1N were added and then the solution was kept for a night. The solution was diluted by adding aquades until the volume was 100 ml. As much as 5 ml of the solution was pippeted into 100 ml volumetric flask and then 2 ml Iod solution and 1 ml asetic acid 0,5N were added. The solution then was diluted again using aquadest. Its absorbance was measured using spectrophotometer at 620 nm wave length. The same procedure was carried out for the standard (potato amylose) that had been prepared to have different amylose content. The amylose content of the rice was calculated by comparing the absorbance of the sample with the standard, multiplied by dilution factor.

From the amylose content, a new variable was derived, ie softness deviation (SD), and calculated using the following formula:

 $SD_i = |23 - AC_i|$

with *AC* was the amylose content. The variable showed how big the deviation against the amylose content of Ciherang. It will be important for rice breders to obtain varieties that have the texture commonly liked by the people. Ciherang variety has amylose content of 23% with soft texture [16].

Gel consistency (GC)

A hundred milligram of rice flour was put into reaction flask. Alcohol 95% (contains 0.025% thymol blue) and KOH 0.2N were added as much as 0.2 ml and 2 ml, accordingly. The solution was stirred using vortex mixer. The flask then was heated on the water bath at 90°C for 15 minutes, then removed and cooled down. The cooling process was speed up using icy water. After it was done, the flask was put down horizontally on top of a millimeter paper for an hour. The gel length inside the flask was measured.

Gelatinization temperature (GT)

As many as 6 rice grains were submerged in an alkali solution (KOH 1.7%) for 23 hours at room temperature. Gelatinization temperature was determined on the swelling and cracking value of the grains.

Data Analysis

Principal Component Analysis (PCA) was used as statistical test, followed by Hierarchical Clustering on Principal Components (HCPC). PCA was carried out to reduce a great number of variables into a new variables that were smaller in size. PCA and HCPC tests both were done using FactoMineR library [17,18,19].

The data was standardized first before PCA was carried out to ensure all characters had same weight to be compared [20]. It was done using the following formula:

$$
x_{standard,i} = \frac{x_i - mean(x)}{sd(x)}
$$

With *mean(x)* was the average value of x and $sd(x)$ was its standard deviation.

How many numbers of components to be included on the analysis was determined using eigenvalue. It showed the percentage of variability that can be explained by each principal components [21]. The higher of its eigenvalue, the higher the principal component can explain the variability on the data. The principal components that had eigenvalue greater than 1 were chosen, as [22] did. The characters that were chosen were the ones that have absolute value of loading more than 0.4. It indicated that the character was a good indicator [23]. Value of loadings of accessions that were greater than 1 on each principal components were used as main tool to choose accession that had particular dominant characters [22].

Results and discussion

Germplasm accessions had variability on each characters. Table 2 shows the minimum, maximum, mean, and coefficient of variation of each characters. There were several characters that had high coefficient of variation, such as empty grain and adulterant, chalky green grain, damaged yellow grain, red grain, broken rice, small broken rice, chalky rice, damaged yellow rice, and softness deviation.

PCA was carried out on the 24 physicochemical characters of unhusked and milled rice of 46 rice germplasms from ICRR and ICABGRRD and resulted in 25 new variables (principal components) that were independent from each other and could explain all the variation within the data. From those 25 principal components, 8 components were chosen that had eigenvalue more than 1. With those 8 components, as much as 84.676% variation could be explained (Table 3). The first principal component (PC1) had eigenvalue of 4.512 with the percentage of variance was 18.049%. PC8 had eigenvalue of 1.074 and the percentage of variance of 4.188%.

Graphical representation of variance percentage can be seen on the Figure 1. It can be shown that the first three of principal components have higher percentageof variance than the remaining components. On the other hand, PC4 and PC5 both have high enough variance to be included as indicators to determine important physicochemical characteristics.

Characters	Minimum value	Maximum value	Mean	Coefficient of variation $(\%)$
Unhusked rice water content $(\%)$	11.60	13.90	12.72	4.47
Empty grain and adulterants (%)	0.26	5.60	2.03 532.4	61.38
Grain density (g/l)	352.50	606.50	2	6.90
1000 grains weight (g)	14.96	28.63	23.10	13.54
Calky green grain $(\%)$	0.04	5.15	1.18	104.79
Damaged yellow grain (%)	0.08	3.68	1.34	65.41
Red grain $(\%)$	0.00	100.00	7.34	255.82
Milled rice water content $(\%)$	10.15	12.20	10.97	4.60
Brown rice yield (%)	74.95	81.24	78.82	1.57
Milled rice yield (%)	66.38	73.39	70.52	2.31
Head rice $(\%)$	73.16	99.57	94.94	5.08
Broken rice $(\%)$	0.39	26.63	4.94	97.13
Small broken rice (%)	0.02	0.36	0.12	63.84
Calky rice (%	0.00	3.26	0.92	91.66
Damaged yellow rice (%)	0.05	2.74	0.66	82.18
Length (mm)	3.16	7.60	6.37	12.15
Width (mm)	2.06	3.23	2.45	9.45
L/W ratio	0.98	3.52	2.63	18.07
Whiteness $(\%)$	34.85	59.50	46.78	13.64
Translucency (%)	0.59	2.39	1.55 120.8	31.04
Milling degree $(\%)$	66.00	179.50	2	24.73
Amylose content $(\%)$	3.32	26.48	18.50	25.66
Gelatinization temperature*	1.00	3.00	2.39	35.79
Gel consistency (mm)	0.00	91.50	59.43	28.18
Softness deviation (%)	0.07	19.68	4.88	89.19

Table 2. The minimum, maximum, mean, and coefficient of variation of physicochemical characters of the 46 rice accessions

***)** 1: gelatinization temperature 55-69°C, 2: gelatinization temperature 70-74°C, 3: gelatinization temperature > 74°C

Principal component matrix

Rice germplasms were analyzed using PCA on their 24 physicochemical characters to obtain loading value on each characters. Those value could be used as indicators to choose important characters on each principal components. The value of each accession on each principal components, finally, could be used to show which accessions that have potential to be used in breeding program. Further analysis were using only 5 principle components since those 5 components were enough to conclude the correlation between several important quality characters. The PCA value of each characters is shown on the Table 4.

Tabel 3. *Eigenvalue*, % of variance, % of cumulative variance of physicochemical characters of the 46 rice accessions

Components	Eigenvalue	% of Variance	% of Cumulative Variance
PC1	4.512	18.049	18.049
PC ₂	4.220	16.880	34.930
PC3	3.677	14.707	49.637
PC4	2.745	10.981	60.618
PC5	1.872	7.489	68.107
PC ₆	1.714	6.857	74.964
PC7	1.381	5.524	80.488
PC8	1.047	4.188	84.676
PC9	0.755	3.019	87.696
PC10	0.641	2.563	90.259
PC11	0.499	1.995	92.254
PC12	0.410	1.641	93.895
PC13	0.346	1.383	95.279
PC14	0.281	1.125	96.403
PC15	0.252	1.009	97.413
PC16	0.181	0.725	98.138
PC17	0.131	0.523	98.661
PC18	0.109	0.436	99.097
PC19	0.093	0.373	99.471
PC20	0.053	0.212	99.683
PC21	0.036	0.143	99.826
PC22	0.026	0.102	99.928
PC ₂₃	0.014	0.056	99.984
PC24	0.004	0.016	100.000
PC ₂₅	4.11E-07	1.64E-06	100.000

Explanation: PC1 was the first principal component, PC2 was the second principal component, etc. Principal components were new reduced variables that were not correlated between each other in order to help to take a conclusion.

Figure 1. Percentage of variance scree plot of physicochemical characters of the 46 rice accessions.

1 apel 4. Loading value of each characteristics					
Karakter	Components				
	PC1	PC ₂	PC3	PC4	PC5
Unhusked rice water content	0.337	-0.310	-0.682	0.051	0.081
Empty grain and adulterants	-0.166	-0.356	0.125	-0.337	0.093
Grain density	0.428	-0.355	0.581	-0.226	0.015
1000 grains weight	-0.385	0.646	0.452	-0.034	0.143
Calky green grain	0.283	0.469	0.237	0.437	-0.039
Damaged yellow grain	-0.102	0.703	0.032	0.134	0.131
Red grain	-0.170	0.000	0.040	0.565	-0.514
Milled rice water content	0.141	-0.175	-0.655	0.066	0.099
Brown rice yield	-0.112	0.064	0.705	0.108	0.423
Milled rice yield	-0.457	-0.272	0.592	0.028	0.335
Head rice	0.007	-0.521	-0.345	0.300	0.616
Broken rice	-0.013	0.515	0.349	-0.306	-0.617
Small broken rice	0.334	0.541	-0.182	0.251	-0.164
Calky rice	0.388	0.467	-0.037	0.426	0.045
Damaged yellow rice	-0.454	0.322	0.094	-0.312	0.228
Length	-0.795	0.186	0.173	0.447	0.044
Width	0.600	0.372	0.099	-0.483	0.026
L/W ratio	-0.786	-0.076	0.028	0.507	0.025
Whiteness	0.501	0.617	-0.164	0.041	0.355
Translucency	0.165	-0.094	0.491	-0.467	0.207
Milling degree	0.530	0.587	-0.098	0.030	0.385
Amylose content	0.607	-0.404	0.511	0.272	-0.102
Gelatinization temperature	0.347	0.149	0.275	0.538	0.243
Gel consistency	-0.518	0.449	-0.265	-0.400	0.096
Softness deviation	-0.532	0.426	-0.557	-0.265	0.113

Tabel 4. Loading value of each characteristics

Information: \vert loading $\vert \ge 0.4$ was bolden. Chosen parameter was boldened and underlined. Minus (-) sign indicates negative correlation.

PC1 shows important positive correlation (loading>0.4) on grain density (DG), width (W), whiteness (WD), milling degree (MD), and amylose content (AC) and negative correlation (loading<-0.4) on milled rice yield (MRY), damaged yellow rice (DYRP), length (L), L/W ratio (LWR), gel consistency (GC), and softness deviation (SD). Thus, PC1 can be used to select accessions that have round grain, high amylose content with hard texture, and white colored. On the other hand, PC1 is negatively correlated with milled rice yield which is undesireable. Graphical representation of PC1 is shown on Figure 2.

 Figure 2. Diagram of the principal component matrix of physicochemical characters on PC1.

PC2 shows positive correlation (loading>0.4) on weight of 1000 grains (W1000), calky green grain (CGG), damaged yellow grain (DYG), broken rice percentage (BRP), small broken rice (SBRP), calky rice (CRP), whiteness (WD), milling degree (MD), gel consistency (GC), and softness deviation (SD). On the other hand, it is negatively correlated (loading<-0.4) with head rice percentage (HRP) and amylose content (AC). This indicates bad quality characteristics with low head rice, but high broken grain. Nevertheless, PC2 still can be utilized to select which accessions having low amylose content (sticky rice). Graphical representation of the PC is shown on Figure.

Figure 3. Diagram of the principal component matrix of physicochemical characters on PC2.

PC3 indicates important positive correlation (loading > 0.4) on grain density (GD), weight of 1000 grains (W1000), brown rice yield (BRY), milled rice yield (MRY), translucency (T), and amylose content (AC) . On the other hand, it negatively correlates $(loading<0.4)$ with softness deviation (SD). This component has good correlation with high yield character with high density and weight. It also indicates low deviation on softness. That means this

component can be used to select accession with high yield and likeable texture. PC3's graph

Figure 4. Diagram of the principal component matrix of physicochemical characters on PC3.

PC4 shows important positive correlation (loading>0.5) on calky green grain (CGG), red grain (RG), calky rice (CRP), length (L), length/width ratio (LWR), and gelatinization temperature (GT). It was negatively correlated $\text{(loading} < -0.4)$ with width (W), transluecency (T), and gel consistency (GC). This component is suitable as indicator for long and slender grain. On the other hand, it has several undesirable characteristics, such as higher percentage of green, red, and

calky grain. It also has more opaque appearance. Graph of the PC4 is shown on the Figure 5.

Figure 5. Diagram of the principal component matrix of physicochemical characters on PC4.

PC5 positively correlates (loading>0.4) with brown rice yield (BRY) and head rice percentage (HRP); and negatively correlates (loading<-0.4) with red grain (RG) and broken rice percentage (BRP). This component is suitable to be an indicator for accessions that are potential for developing premium rice. Graphical representation of PC5 is shown on Figure 6.

Figure 6. Diagram of the principal component matrix of physicochemical characters on PC5.

Table 5. Loading score of each accessions

Information: Loading > 1 was boldened. Accessions that have loading > 1 on both PC3 and PC5 were boldened and shaded.

Accession selection according to the components' score

Table 5 shows each accession's score on each principal components. On PC1, the loading is from -3.978 (accession 10480) to 7.177 (accession 10500). On PC2, the loading is from - 4.644 (accession 10497) to 6.163 (accession 10489). On PC3, the loading is from -7.721 (accession 10489) to 3.445 (accession 10515). On PC4, the loading is from -3.781 (accession 10599) to 4.640 (accession 10510). On PC5, the loading is from -3.655 (accession 10510) to 1.929 (accession 10527). The loading ranging from negative to positive on all five principal components means the characteristics of the accession have high degree of variation.

According to Gour et al. (2017), the value of cutoff used should be 1. It means, accessions to be considered will have loading score >1. Accessions that have the score >1 on PC1 were correlated with roundness shape and high amylose content with low milling yield (13 accessions); on PC2, they were correlated with low amylose content, ie sticky rice (9 accessions); on PC3, they were correlated with high yield rice and likeable texture (16 accessions); on PC4, they were correlated with long and slender grain (10 accessions); and finally, on PC5, they were correlated with premium rice (11 accessions). The grouping is shown on Table 6.

PC1	\circ PC2	PC ₃	PC4	PC ₅
10500(7,167)	10489(6,163)	10515(3,445)	10510(4,64)	10527(1,929)
10592(3,752)	10474(5,218)	10543(2,907)	10470(3,234)	10479(1,809)
10477(3,562)	10535(4,329)	10535(2,818)	10474(2,127)	10562(1,759)
10496(2,672)	10592(2,032)	10475(2,476)	10513(1,892)	10515(1,737)
10515(2,594)	10496(1,73)	10499(1,865)	10592(1,878)	10551(1,736)
10577(2,407)	10515(1,716)	10539(1,507)	10515(1,729)	10499(1,627)
10482(2,317)	10476(1,696)	10560(1,424)	10496(1,61)	10545(1,503)
10572(2,002)	10572(1,447)	10486(1,422)	10491(1,472)	10513(1,33)
10506(1,699)	10560(1,236)	10551(1,407)	10471(1,191)	10486(1,268)
10548(1,653)		10514(1,387)	10499(1,104)	10564(1,227)
10591(1,58)		10471(1,341)		10491(1,103)
10474(1,396)		10562(1,29)		
10550(1,146)		10479(1,288)		
		10485(1,195)		
		10491(1,16)		
		10541(1,089)		

Table 6. Chosen accession having loading >1 on each components

Information: Accessions that have both loading >1 on PC3 and PC5 were boldened

Suitable accessions for round-shaped rice development are 10500, 10592, 10477, 10496, 10515, 10577, 10482, 10572, 10506, 10548, 10591, 10474, dan 10550. For developing low amylose content or sticky rice, accession 10489, 10474, 10535, 10592, 10496, 10515, 10476, 10572, dan 10560 are considered. For long and slender grain, accession 10510, 10470, 10474, 10513, 10592, 10515, 10496, 10491, 10471, dan 10499 can be used as parents in the breeding program.

PC3 and PC5 can be used in tandem as indicator for accession with good milling quality, yield, and well liked by consumers. There are 7 potential accessions (Table 7) as genetic materials for premium rice development, i.e. 10479 (Umbul-umbul), 10486 (Babad Cianjur), 10491 (Marus), 10499 (619), 10515 (Siregi), 10551 (Logawa), dan 10562 (Umbul-umbul).

Table 7. Potential accessions having loading > 1 on both PC3 and PC5

The grouping of accessions

The grouping of accessions from PCA result using HCPC showed that the accessions could be

grouped into 4 according to their physicochemical characters. Group I included 11 accessions, group II was 3 accessions, group III was 8 accessions, and group IV had 24 accessions.

By reviewing their score on each components, it was shown that group I had mainly positive score on PC1 and PC3, group II had negative score on PC1 and PC3, group III had negative score on PC2, PC3, and PC5, finally, group IV has positive score on PC3 and PC5. It was not surprising that 6 of 7 potential accessions for premium rice were members of group IV (Figure 7).

Figure 7. Dendogram of accessions' group with score on each component: a) dendogram Dendogram pengelompokan aksesi serta nilai pada masing-masing komponen: a) dendogram resulted from HCPC analysis. Number I to IV indicates group's number. Potential accessions are shaded in grey. b) accessions' loading score on each components

Conclusion

Potential accessions to be used as parents on the breeding program for high yield and premium quality are 10479 (Umbul-umbul), 10486 (Babad Cianjur), 10491 (Marus), 10499 (619), 10515 (Siregi), 10551 (Logawa), dan 10562 (Umbul-umbul). The grouping of accessions resulted in 4 main group with the group IV is dominated by high yield and premium quality characters.

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