EVALUATION OF GENETIC DIVERSITY OF THE BLACK GLUTINOUS RICE BASED ON AGRO-MORPHOLOGICAL CHARACTERS

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Abstract – *The study assessed the variations in nine agro-morphological characters among and within the black glutinous rice (Oryza sativa) population from Chau Thanh District, Tra Vinh Province. The nine quantitative agro-morphological characters that were measured include culm length, leaf length, leaf width, number of panicles, panicle length, grain length, grain width, number of firm grain, and number of grain per panicle. The unweighted pair group method with arithmetic mean method and principal coordinate analysis by the NTSYS program were applied to classify the nine agro-morphological characters. In addition, to compare the variations in quantitative characters between O. sativa populations, one-way analysis of variance (ANOVA) was used. The results showed significant differences between the black glutinous rice populations for all quantitative agro-morphological characters. Moreover, some agro-morphological characters showed positive correlations to each other. The dendrogram generated from the analysis process of the agro-morphological data divided the O. sativa populations into two groups with unfamiliar features. However, the O. sativa populations assessed exhibited a wide range of variations in morphological characteristics, both within the same population and among other populations with the same strains.*

Keywords: agro-morphological characters, black glutinous rice, genetic diversity, Oryza sativa.

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I. INTRODUCTION

Rice, a member of Poaceae (Gramineae) belonging to the genus Oryza, is the world's most important staple food crop that feeds over half of the global population and estimated areas have grown approximately 163,000,000 hectares in over 100 countries [1]. Rice is also an indispensable source of energy, iron, calcium, vitamins, and protein in the diet of the Asian population [2]. There are 22 species in the genus, divided into two main cultivars: O. glaberrima which was domesticated in Africa, and O. sativa was in the humid tropics of South and Southeast to East Asia [3]. There are thousands of rice varieties throughout the world, and glutinous rice (Oryza sativa var. glutinosa) is one of the most popular varieties. It is commonly known as sticky rice, waxy rice, or sweet rice, and is considered one of the common ingredients in many kinds of traditional Asian desserts such as Japanese rice balls and rice cakes [4].

Black rice found in many Asian countries has the darkest color compared to other black rice strains in the world. Black rice is mostly glutinous rice and exists in both Indica and Japonica backgrounds. Black rice (O. sativa) is highly nutritious and contains various proteins, vitamins, and mineral levels that are higher than others kinds of rice [5]. Previous studies indicated that pigmented rice is rich in vitamins, minerals, and fiber, especially phenolic compounds–the main active component of antioxidants [6]. In numerous compounds of pigmented rice, anthocyanin is known to have free radical scavenging and antioxidant capacities, and many other benefits for our health. For its nutritious value, the worldwide consumers' demand for pigmented rice has been increasing. In the study investigating the impact of black rice anthocyanins against cancer cells in 2006, the authors indicated that liver cancer cells can be prevented by anthocyanin pigment if people take pigmented rice every day [7]. Chinese people have used pigmented rice to improve blood circulation, kidney function, and eye function [8]. Besides excellent nutritional value, anthocyanin-rich rice grains have been known as natural and safe sources of food colorants [9]. In addition, compared to white rice, black rice is relatively richer in the mineral contents such as Fe, Zn, Mn, and P and variability in mineral content depending on the varieties and soil types of the planting area [10].

The origin of black glutinous rice is not well understood, but it is believed that it derived from Asia countries such as China [11], India [12], Japan [13], the Philippines [14], and Vietnam [15]. Black glutinous rice is one of the colored rice varieties and its color is due to morphological characters and anthocyanin content. According to Nezu [16], morphology is also considered the simplest characteristic to distinguish different rice varieties. Local farmers distinguish different rice varieties based on morphological characteristics such as shape, size, and pigmentation of plant parts [17]. Previous studies reported that the seed of local rice varieties maintained by farmers are genetically diverse [18]. Because the farmers don't specifically know the production potential or origin of the rice, they are less able to follow unstable trends in the market and grow more profitable genetic strains.

There are several methods for researching genetic diversity, some of them based on genotype characters, or phenotype characters. The genetic relationship between two individuals or among populations can be measured based on the similarity of the characteristics by assuming that the different characteristics could be caused by the difference in genetic structure [19]. As the study of genetic diversity of morphological traits directly provide beneficial information for farmers or breeders, especially when the resource population is large, using morphological features to study genetic diversity is considered an uncomplicated and economical approach. Therefore, this classical method continues to be extensively employed in the evaluation of germplasm resources [20, 21]. This study aimed to explore the genetic relationships of 20 black glutinous rice grains from Chau Thanh District, Tra Vinh Province, by evaluating nine morphological characters.

II. MATERIALS AND METHODS

A. *Plant materials*

Twenty black sticky rice strains in Chau Thanh District in Tra Vinh Province were selected for the study. Each selected sample line was measured in morphological characteristics and analysis. To have a thorough evaluation of each rice line, the study used nine agro-morphological characteristics including plant height (PH), leaf length (LL), leaf width (LW), number of panicles (NP), panicle length (PL), grain length (GL), grain width (GW), number of firm grain (FG) and number of grain per panicle (GP). The purpose of measuring these nine agro-morphological characteristics was to offer a comprehensive assessments and overview of different black sticky rice strains to readers and researchers in this field. The assessment was carried out and adapted from the standard evaluation system for rice [2].

B. *Morphological characterization*

The nine morphological characters were observed and recorded from vegetative growth stages. Most measurements were conducted using either a tape measure or a ruler, except for grain number per panicle and the number of primary branches per panicle. The plant height (PH) was measured using a tape measure from the ground level or base of the plant to the base of the panicle and panicle length (PL) was measured from the base of the panicle to its tip. The leaf width (LW) was measured at the widest portion of the leaf. Grain length (GL) was measured as the distance from the base of the lowermost glume to the tip of the lemma or palea. The grain width (GW) was measured as the distance across the lemma and palea at the widest point. Grain number per panicle (GP) and the number of primary branches per panicle were determined by counting the respective parts.

C. Data analysis

The morphological data were analyzed using Statgraphic18-X64 software. One-way analysis of variance (ANOVA) was used to compare the variations in morphological characteristics among the populations for all the collected quantitative data. A correlation analysis was performed to determine the interrelationships among the assessed morphological characters. The unweighted pair group method with arithmetic mean (UPGMA) dendrogram was also built to assess the clustering of O. sativa of individuals from the four populations based on their genetic distance.

III. RESULTS AND DISCUSSION

A. Morphological characterization

Plant height is one of the most important bio-agronomic characteristics and is closely related to photosynthetic capacity, collapse resistance, and fertilization response. When the plant height is too short, the plant produces less dry matter; whereas, if it is too high, it is vulnerable to lodging and less responsive to N fertilization [22]. The increase in plant height is usually due to the elongation of stem internodes. Rice strains having longer internodes produce taller plants [23]. Besides the genetic makeup of the cultivar, plant height is governed by environmental factors [24].

The height of black glutinous rice was measured in the reproductive stage of flowering because the plant was not continuing growth after this stage. Plant height remained between 90 and 142 cm. The data analysis of the study illustrated the differences in height growth between rice strains. Particularly, the shortest height was found in NTCT41, while NTCT11 had the highest height from 140 to 142 cm. NTCT23 and NTCT37 had an average height of between 120 and 126 cm (Table 1).

The morphological characters of 20 black glutinous rice are presented in Table 1. The leaf plays a vital role in panicle size, grain size, and weight. The leaf width and length and panicle length are considered the basic characteristics which affect growth, development, photosynthetic rate, and productivity of the plant and are often used to select the best phenotype to increase the yield and plant resistance. The larger the leaf areas are, the more photosynthetic activities occur [23]. However, the leaf areas not only depended on genotype, and environmental condition, but also depended on plant population density and plant height [25].

Table 1 shows that the leaf length of the 20 black glutinous rice accessions fluctuated between 30 and 62 cm. NTCT24, NTCT29, NTCT37, NTCT57, NTCT63, and NTCT94 had the shortest leaves (30 to 38 cm) and were classified into the short-leaf group. Six black glutinous rice strains belonged to the intermediate leaf group (from 40 to 50 cm). The remaining 40% of samples belonged to the long-leaf group with leaf lengths of more than 50 cm, including NTCT11, NTCT12, NTCT13, NTCT15, NTCT27, NTCT33, NTCT44, and NTCT58.

The leaf width ranged from 1.0 to 1.8 cm. Panicle length has indirectly contributed to increments in rice grain yield by increasing the number of panicles per unit area. Furthermore, panicle length has a significant quadratic relationship with grain yield [26]. The longer the panicle length, the higher number of grain total. Panicle lengths of the black rice accessions in this study varied from average to short lengths (16.4 to 23.2 cm). Seventy-five percent of 20 selected glutinous black rice had a short panicle length (20.2 to 23.2 cm) and twenty-five percent had very short panicle lengths of below 20 cm. The results indicated that the panicle length of black rice strains in Chau Thanh, Tra Vinh, is shorter than the ones in the research of Phan [27].

As shown in Table 2 and Figure 1, grain length (GL) and grain width (GW) of 20 black glutinous rice were varied among different rice samples. The grain length ranged from 7.8 to 8.8 mm with an average of 8.5 mm. The NTCT11 was the shortest grain at 7.8 mm, while NTCT58 had an 8.8 mm grain length and was the longest grain. The values of grain width varied from 2.0 to 2.6 mm with an average value of 2.3 mm. According to Wang [28], the grain size and shape can be effectively used to improve yield and grain quality and seem like the first quality characteristics considered in developing new commercial varieties [29]. Therefore, breeding for specific grain weight together with other

Table 1: The growth of 20 black glutinous rice accessions in 2020

Accession number	Plant height (PH) (cm)	Leaf length (LL) (cm)	Leaf width (LW) (cm)	Number of panicle (NP)	Panicle length (PL) (cm)
NTCT11	142	59	1.4	6	21.9 ± 2.0^{abc}
NTCT12	134	61	1.2	3	20.6 ± 1.9^{abc}
NTCT13	140	57	1.4	23	19.2 ± 2.0^{abc}
NTCT15	133	51	1.5	2	16.4 ± 7.5^{c}
NTCT17	110	46	1.2	11	20.4 ± 4.0^{abc}
NTCT23	126	45	1.2	2	21.5 ± 2.3^{abc}
NTCT24	100	30	1.0	1	20.2 ± 6.7^{abc}
NTCT27	140	51	1.8	16	19.0 ± 3.5^{abc}
NTCT29	119	30	1.0	4	20.3 ± 3.6^{abc}
NTCT33	138	55	1.2	3	22.5 ± 2.2^{ab}
NTCT36	134	40	1.2	3	16.4 ± 3.5^{bc}
NTCT37	120	35	1.2	4	22.3 ± 0.9^{abc}
NTCT41	90	50	1.0	3	19.8 ± 2.0^{abc}
NTCT43	137	45	1.2	25	22.8 ± 3.9^{a}
NTCT44	137	51	1.4	6	$19.\ 7 \pm 2.3^{abc}$
NTCT57	104	32	1.0	6	21.5 ± 5.7^{abc}
NTCT58	140	62	1.1	8	17.8 ± 5.2^{abc}
NTCT63	110	31	1.2	10	22.1 ± 0.4^{abc}
NTCT93	112	48	1.2	7	21.3 ± 3.3^{abc}
NTCT94	120	38	1.0	5	23.2 ± 1.0^{a}

Note: Mean marked with the same letter not statistically significantly different at (p=0.05) according to ANOVA analysis. $\pm$ *standard deviation*

beneficial traits should be carefully considered. Thus, a variety range of grain sizes plays a vital role in the breeding program as a source of genetic materials. Furthermore, grain size diversity promotes the breeders' initiative in the breeding process, which may help breeders and farmers approach and satisfy consumer demand for specific requirements such as grain size and shapes in the near future.

B. Correlation among the agro-morphological characters in O. sativa cultivar

The degree of correlation between the nine agro-morphological characters is a key factor when analyzing black glutinous rice strains, especially complex traits related to economics such as yield. Correlation analysis is therefore necessary to determine the direction of selection and the number of characteristics that need to be considered in improving grain yield [30]. Table 3 shows the correlation coefficients for pairs of the nine traits that were used in characterizing the 20 black glutinous rice accessions.

The result of correlation analysis in Table 3 reveals that the number of firm grains exhibits the highest significantly positive correlation with the number of grains per panicle (r = 0.806**). In addition, there were significant positive correlations between the number of grains per panicle (GP) and quantitative morphological characteristics of plant height (PH), leaf length (LL), leaf width (LW), number of panicles (NP), panicle length (PL), grain length (GL), grain width (GW), and number of firm grain (FG). However, there were negative correlations between PL and PH, LL and LW; between GL and PH, LW and PL; between GW and PH, LL, LW and NP; as well as between GL and FG. Among the characters, LL gives the high correlation coefficient value because it is related to the site of photosynthesis for supplying carbon to grains [31]. The findings showed that all the traits should be considered in simultaneous selection on enhancing the yield for the next population. In addition, one of the crucial traits that need to be highlighted is the positive correlation of the number of firm grains per number of panicles [32].

Table 2: The morphological characters of 20 black glutinous rice accessions in 2020

Accession number	Grain length (GL) (mm)	Grain width (GW) (mm)	Firm grain number (FG)	Grain per panicle number (GP)
NTCT11	7.8±0.4[d]	2.2±0.3[abc]	63.7±73.7[cde]	82.3±67.3[cde]
NTCT12	8.6±0.8[abc]	2.4±0.2[abc]	34.0±42.3[de]	127.7±46.8[abc]
NTCT13	8.6±0.4[abc]	1.9±0.3[c]	47.7±42.1[cde]	89.3±2.9[cde]
NTCT15	8.5±0.4[abc]	2.4±0.2[abc]	84.7±31.0[bcd]	106.7±28.7[bcde]
NTCT17	8.9±0.7[a]	2.3±0.3[abc]	100±70.3[ab]	128±81.2[abc]
NTCT23	8.3±0.3[abcd]	2.2±0.8[abc]	105±34.1[a]	142.3±30.6[ab]
NTCT24	8.1±0.3[cd]	2.2±0.2[abc]	20.0±34.6[e]	63.3±17.2[de]
NTCT27	8.7±0.5[abcd]	2.3±0.3[abc]	101.3±30.3[ab]	122. 7±27.0[bcd]
NTCT29	8.6±0.4[abc]	2.3±0.3[abc]	62.7±42.9[cde]	111. 3±28.0[bcde]
NTCT33	8.2±0.4[bcd]	2.4±0.2[abc]	85.3±2.5[bcd]	105. 7±10.6[bcde]
NTCT36	8.6±0.2[abc]	2.2±0.5[abc]	51.0±46.2[cde]	84±26.6[cde]
NTCT37	8.3±0.7[abcd]	2.3±0.2[abc]	99.5±54.4[abc]	125.7±42.5[abc]
NTCT41	8.7±0.3[abc]	2.3±0.4[abc]	76.7±47.4[bcd]	97.3±50.5[bcde]
NTCT43	8.6±0.4[abc]	2.2±0.4[abc]	108.3±42.2[a]	154.3±23.1[a]
NTCT44	8.4±0.3[abcd]	2.1±0.2[abc]	70.0±57.6[cde]	117.3±28.9[bcd]
NTCT57	8.5±0.5[abc]	2.0±0.4[bc]	3.0±2.6[f]	71±24.6[de]
NTCT58	8.8±0.2[ab]	2.2±0.5[abc]	30.3±19.8[de]	56.3±42.7[e]
NTCT63	8.7±0.4[abc]	2.5±0.2[ab]	65.3±6.5[cde]	89±16.5[cde]
NTCT93	8.4±0.4[abcd]	2.6±0.5[a]	42.3±37.0[cde]	86.7±16.0[cde]
NTCT94	8.3±0.3[abcd]	2.3±0.2[abc]	108.3±27.4[a]	132±23.6[ab]

Note: Mean marked with the same letter not statistically significantly different at (p=0.05) according to ANOVA analysis. ± standard deviation.

Fig. 1: Morphological characters of 20 black glutinous rice accessions

Table 3: Correlation coefficients of 9 traits used in characterizing 20 black glutinous rice accessions

	PH	LL	LW	NP	PL	GL	GW	FG	GP
PH	1								
LL	0.618**	1							
LW	0.625**	0.466*	1						
NP	0.332	0.191	0.374	1					
PL	-0.218	-0.263	-0.327	0.101	1				
GL	-0.202	0.006	-0.222	0.225	-0.348	1			
GW	-0.206	-0.048	-0.014	-0.330	0.128	0.065	1		
FG	0.195	0.026	0.315	0.201	0.270	-0.085	0.229	1	
GP	0.202	0.013	0.204	0.228	0.369	0.033	0.115	0.806**	1

*Note: **. Correlation is significant at the 0.01 level*
**. Correlation is significant at the 0.05 level*
PH = plant height (cm), LL = leaf length (cm), LW = leaf width (cm), NP = number of panicle, PL = panicle length (cm), GL = grain length (mm), GW = grain width (mm), FG = number of firm grain, GP = number of grain per panicle.

C. Clustering of the O. sativa individuals from the twenty populations based on quantitative morphological data

As shown in Figure 2, the UPGMA dendrogram generated from the quantitative morphological data based on genetic distance grouped the two O. sativa individuals into two groups (A and B) based on plant height and several sub-clusters with the cophenetic correlation of cultivars in the grouping. Group A was divided into two subgroups, consisting of A1 (NTCT11, NTCT44 and NTCT13) and A2 (NTCT27 and NTCT58), which have plant heights from 137 to 140 cm. The plant height of group B was lower than 137 cm and was also divided into two subgroups comprised of B1 (NTCT12, NTCT36, NTCT33, NTCT17, NTCT63, NTCT23, NTCT37, NTCT43 and NTCT93) and group B2 (NTCT15, NTCT57, NTCT24, NTCT29, NTCT94, and NTCT41).

IV. CONCLUSION

This study mainly focused on the agro-morphological characteristics of black glutinous rice by using appropriate measurements to collect data. The research results indicate that agro-morphological traits play a vital role in classifying the plant, a tool to understand the adaptation of plants to the environment as well as in assessing genetic variability among phenotypically identifiable rice accessions. These findings provide valuable information to plant breeders in the selection of morphology and distinct individuals for their breeding programs. In addition, 20 black glutinous rice accessions were divided into two main groups using the UPGMA dendrogram. The results indicate both detailed characteristics and the average value of each black glutinous rice strain. In particular, 124.3 cm is the average height of black glutinous rice, 45.9 cm for leaf length, 1.2 cm for leaf width, 20.4 cm for panicle length, 68.0 grains for the number of firm grains, and 104.7 grains for the rate of grain per panicle.

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