



Xuan Hanh Tran¹, Thanh Son Tran², Tan Ho², Thi Hue Truong², Dinh Thi Nguyen¹, Thanh Liem Nguyen^{2*}

¹ University of Agriculture and Forestry, Hue University, Hue, Vietnam
² Faculty of Natural Sciences, Quynhon University, Quynhon 590000, Binhdinh, Vietnam
*Corresponding author's email: <u>nguyenthanhliem@qnu.edu.vn</u>

Received: 03 Jan 2025; Received in revised form: 05 Feb 2025; Accepted: 11 Feb 2025; Available online: 17 Feb 2025 ©2025 The Author(s). Published by Infogain Publication. This is an open-access article under the CC BY license (<u>https://creativecommons.org/licenses/by/4.0/</u>).

Abstract— The analysis of genetic relationship among traditional glutinous rice varieties is crucial for genetic management, conservation, and the development of new varieties. The experiment was conducted from 2023 to 2024 in Duc Pho district, Quang Ngai province, Vietnam to assess the agro-morphological characteristics and genetic diversity of 106 traditional glutinous rice varieties. The study used 48 polymorphic SSR markers to calculate the polymorphic information content (PIC). Cluster analysis of the rice collection was then conducted using a similarity matrix and the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA). The results revealed a total of 193 alleles, with an average of 4.02 alleles per locus. PIC values ranged from 0.33 to 0.87, with an average of 0.61. The genetic similarity coefficients of the 106 traditional glutinous rice varieties ranged from 0.41 to 0.98. Based on the UPGMA analysis of molecular data using NTSYS-pc 2.11a software, the 106 traditional glutinous rice varieties were grouped into two major clusters, indicating genetic differences. In addition, the varieties exhibited relatively wide range of growth time with most belonging to the short-day and medium-day groups. They displayed medium-to-high plant heights, medium-to-long panicle lengths, and 1000-grain weights ranging from high to extra-high. The correlation coefficient analysis showed that all the traits were correlated with each other, with the yield being strongly correlated with the 1000-grain weight. Therefore, the research findings not only provide an essential starting material for the development of high-quality, high-yield rice varieties but also contribute to the conservation and promotion of the Ngu glutinous rice genotype, which is closely associated with the Sa Huynh cultural heritage.



Keywords— Quantitative morphological traits, alleles, genetic diversity, genetic resources, Sa Huỳnh's Ngự glutinous rice.

I. INTRODUCTION

Glutinous rice is one of the specialty rice varieties widely grown in Vietnam and has a long-standing history. Currently, more than 250 glutinous rice varieties are cultivated across the country, grown in almost all localities, both in upland fields and lowland paddies, and are closely associated with traditional culture and national cuisine. Glutinous rice accounts for about 10% of the

ISSN: 2456-1878 (Int. J. Environ. Agric. Biotech.) https://dx.doi.org/10.22161/ijeab.101.11 country's total rice production and commands a higher price compared to regular rice. Annually, it contributes approximately 20% of the total rice export volume. Indigenous rice varieties have evolved over generations, adapting to local cultivation conditions, and are known by various indigenous names (Rana *et al.*, 2009). However, the number and cultivation area of traditional rice varieties, particularly specialty glutinous rice varieties, have significantly declined, replaced by hybrid and highyielding improved rice varieties (Schiller et al., 2006). Laos is a major producer of glutinous rice, but in the 2000s, many indigenous glutinous rice varieties were no longer cultivated or were lost in the central and southern regions (Appa Rao et al., 2006). Therefore, it is crucial to collect, evaluate and conserve indigenous varieties before Vietnam's genetic diversity is further eroded. Genetic diversity forms the foundation for species survival. Processes such as genetic recombination and mutation provide a continuous source of new variations, while environmental adaptation and random genetic drift shape the distribution of genetic diversity over time and space (Brown et al., 1989; 1990). Thus, studying the genetic diversity of glutinous rice resources is not only crucial for conserving specialty rice varieties but also contributes significantly to the breeding of high-quality rice varieties, which supports sustainable agriculture and ensures food security (Begum et al., 2018; Acevedo-Siaca et al., 2020).

In the past, the assessment of genetic diversity was often based on phenotypes (agro-morphological traits). with farmers using certain morphological characteristics of crops for selection and identification. As a result, morphological traits are closely linked to genetic diversity, and the naming of these varieties reflects farmers' understanding of the genetic diversity present in their fields (Jarvis et al., 2000). Thus, agro-morphological characterization remains one of the simplest and most standardized methods for measuring crop genetic diversity (Watson & Eyzaguirre, 2002; Guru et al., 2017). Nowadays, with the advent of molecular marker techniques, powerful tools have been developed to accurately assess and describe genetic resources. Modern rice breeders are particularly interested in diversity at the molecular level (Becerra et al., 2015; Singh et al., 2016; Bhujel et al., 2018; Suvi et al., 2019; Park et al., 2019; Ab Razak et al., 2020). Various types of molecular markers are available for evaluating genetic variation in rice (Ni et al., 2002), including Restriction Fragment Length Polymorphism (RFLP) (Botstein et al., 1980), Random Amplified Polymorphic DNA (RAPD) (Williams et al., 1990), Amplified Fragment Length Polymorphism (AFLP) (Vos et al., 1995), and Simple Sequence Repeat (SSR) markers. SSRs are DNA regions (often part of non-coding regions) where one to five nucleotide sequences are repeated, evenly distributed across the genomes of most eukaryotic organisms. In plants, SSR sequences typically consist of AT and GA nucleotide repeats (Jae-Ryoung et

ISSN: 2456-1878 (Int. J. Environ. Agric. Biotech.) https://dx.doi.org/10.22161/ijeab.101.11 al., 2019). The use of SSR molecular markers is a powerful tool for assessing genetic variation, elucidating genetic relationships within and between species, and offering advantages such as rapid, accurate, highly polymorphic, and stable analysis (Virk et al., 2000). Characterizing and evaluating diversity among traditional varieties provides breeders with essential information for identifying initial breeding materials to develop varieties with improved yield and quality. Many studies have utilized SSR markers to investigate genetic diversity and population structure in rice (Rana et al., 2009; Salgotra et al., 2015; Vilayheuang et al., 2016; Wunna et al., 2016; Prabha et al., 2017; Verma et al., 2017; Aljumaili et al., 2018; Muto et al., 2019; Nilthong et al., 2020). In Vietnam, genetic diversity among local indigenous rice varieties has been reported (Lang et al., 2009; Nguyen et al., 2012; Lang et al., 2014; Doan et al., 2016; Tran et al., 2021; Lang et al., 2024). However, the agronomic traits and genetic diversity of indigenous glutinous rice varieties remain largely unknown, particularly those of the Sa Huỳnh's Ngự glutinous rice.

In this study, we assessed the genetic diversity of traditional glutinous rice varieties known as Ngự Sa Huỳnh, also referred to as King's glutinous rice. This variety is closely associated with Sa Huynh region, a significant archaeological site of the ancient Sa Huỳnh culture-a nationally recognized cultural heritage site dating back approximately 3,000 years. The evaluation was conducted using agro-morphological traits and SSR markers. The findings of this study will contribute to conservation efforts, provide valuable genetic resource information, and support the exploitation and development of Sa Huỳnh's Ngự glutinous rice brand, renowned for its high quality. Additionally, the results will offer plant breeders essential information for identifying initial breeding materials to develop rice varieties with higher yield and improved quality.

II. MATERIALS AND METHODS

Plant Materials

A total of 106 Ngr glutinous rice samples were collected, including 100 samples from Quang Ngai, 4 samples from Binh Dinh, and 2 samples from the Plant Resources Center (PRC). The origins of these glutinous rice samples are presented in Table 1.

No.	Accession number	Name of variety	Locations	No.	Accession number	Name of variety	Locations
1	NSH-1	Sa Huỳnh's Ngự glutinous rice -NSH1	Pho Chau ward, Duc Pho District, has a latitude of 14°38'34.8"N and alongitude of 109°03'19.9" E	54	NSH-54	Sa Huỳnh's Ngự glutinous rice -NSH54	Pho Chau ward, Duc Pho District, has a latitude of 14°38'39.0"N and alongitude of 109°03'13.0" E
2	NSH-2	Sa Huỳnh's Ngự glutinous rice -NSH2	Pho Chau ward, Duc Pho District (14°38'39.0"N and 109°03'37.4" E)	55	NSH-55	Sa Huỳnh's Ngự glutinous rice -NSH55	Sa Huynh, Duc Pho District, has a latitude of 14°38'31.9"N and alongitude of 109°03'46.2" E
3	NSH-3	Sa Huỳnh's Ngự glutinous rice -NSH3	Pho Chau ward, Duc Pho District, has a latitude of 14°38'31.9"N and alongitude of 109°03'41.1" E	56	NSH-56	Sa Huỳnh's Ngự glutinous rice -NSH56	Pho Chau ward, Duc Pho District, Quang Ngai province (14°38'34.8"N, E 109°03'19.9")
4	NSH-4	Sa Huỳnh's Ngự glutinous rice -NSH4	Pho Chau ward, Duc Pho District, has a latitude of 14°38'26.8"N and alongitude of 109°03'25.4" E	57	NSH-57	Sa Huỳnh's Ngự glutinous rice -NSH57	Pho Chau ward, Duc Pho District, has a latitude of 14°38'34.8"N and alongitude of 109°03'19.9" E
5	NSH-5	Sa Huỳnh's Ngự glutinous rice -NSH5	Pho Chau ward, Duc Pho District, has a latitude of 14°38'23.5"N and alongitude of 109°03'33.7" E	58	NSH-58	Sa Huỳnh's Ngự glutinous rice -NSH58	Pho Chauward, Duc Pho District, has a latitude of 14°38'34.8"N and alongitude of 109°03'19.9" E
6	NSH-6	Sa Huỳnh's Ngự glutinous rice -NSH6	Pho Chau ward, Duc Pho District, has a latitude of 14°38'31.9"N and alongitude of 109°03'41.1" E	59	NSH-59	Sa Huỳnh's Ngự glutinous rice -NSH59	Pho Chau ward, Duc Pho District, has a latitude of 14°38'31.9"N and alongitude of 109°03'41.1" E
7	NSH-7	Sa Huỳnh's Ngự glutinous rice -NSH7	Pho Chau Pho District, has a latitude of 14°38'38.8"N and alongitude of 109°03'41.1" E	60	NSH-60	Sa Huỳnh's Ngự glutinous rice -NSH60	Sa Huynh, Duc Pho District, has a latitude of 14°38'31.9"N and alongitude of 109°03'46.2" E
8	NSH-8	Sa Huỳnh's Ngự glutinous rice -NSH8	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'37.7"N and alongitude of 109°03'33.7" E	61	NSH-61	Sa Huỳnh's Ngự glutinous rice -NSH61	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'31.9"N and alongitude of 109°03'41.1" E
9	NSH-9	Sa Huỳnh's Ngự glutinous rice -NSH9	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'26.8"N and alongitude of 109°03'25.4" E	62	NSH-62	Sa Huỳnh's Ngự glutinous rice -NSH62	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'32.0"N and alongitude of 109°03'22.0" E
10	NSH-10	Sa Huỳnh's Ngự glutinous rice -NSH10	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'34.8"N and alongitude of 109°03'19.9" E	63	NSH-63	Sa Huỳnh's Ngự glutinous rice -NSH63	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'26.9"N and alongitude of 109°03'19.9" E

Table 1. Accession numbers and locations of the 106 traditional glutinous rice varieties used in the study

No.	Accession number	Name of variety	Locations	No.	Accession number	Name of variety	Locations
11	NSH-11	Sa Huỳnh's Ngự glutinous rice -NSH11	Pho Thanh ward, Duc Pho District, Vietnam (14°38'26.9"N, E 109°03'19.9")	64	NSH-64	Sa Huỳnh's Ngự glutinous rice -NSH64	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'34.8"N and alongitude of 109°03'19.9" E
12	NSH-12	Sa Huỳnh's Ngự glutinous rice -NSH12	Sa Huynh, Duc Pho District, Quang Ngai province, Vietnam (14°38'31.9"N and 109°03'46.2" E)	65	NSH-65	Sa Huỳnh's Ngự glutinous rice -NSH65	Pho Chau ward, Duc Pho District, has a latitude of 14°38'34.1"N and alongitude of 109°03'48.0"E
13	NSH-13	Sa Huỳnh's Ngự glutinous rice -NSH13	Pho Thanh ward, Duc Pho District, has a latitude of 14°39'29.2"N and alongitude of 109°03'41.1" E	66	NSH-66	Sa Huỳnh's Ngự glutinous rice -NSH66	Pho Chau ward, Duc Pho District, has a latitude of 14°38'34.1"N and alongitude of 109°03'48.0"E
14	NSH-14	Sa Huỳnh's Ngự glutinous rice -NSH14	Pho Chau ward, Duc Pho District, has a latitude of 14°38'23.5"N and alongitude of 109°03'33.7" E	67	NSH-67	Sa Huỳnh's Ngự glutinous rice -NSH67	Pho Chauward, Duc Pho District, has a latitude of 14°38'34.8"N and alongitude of 109°03'19.9" E
15	NSH-15	Sa Huỳnh's Ngự glutinous rice -NSH15	Pho Chau ward, Duc Pho District, has a latitude of 14°38'31.9"N and alongitude of 109°03'41.1" E	68	NSH-68	Sa Huỳnh's Ngự glutinous rice -NSH68	Pho Chau ward, Duc Pho District, has a latitude of 14°38'31.9"N and alongitude of 109°03'41.1" E
16	NSH-16	Sa Huỳnh's Ngự glutinous rice -NSH16	Pho Chau Pho District, has a latitude of 14°38'38.8"N and alongitude of 109°03'41.1" E	69	NSH-69	Sa Huỳnh's Ngự glutinous rice -NSH69	Sa Huynh, Duc Pho District, has a latitude of 14°38'31.9"N and alongitude of 109°03'46.2" E
17	NSH-17	Sa Huỳnh's Ngự glutinous rice -NSH17	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'37.7"N and alongitude of 109°03'33.7" E	70	NSH-70	Sa Huỳnh's Ngự glutinous rice -NSH70	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'31.9"N and alongitude of 109°03'41.1" E
18	NSH-18	Sa Huỳnh's Ngự glutinous rice -NSH18	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'26.8"N and alongitude of 109°03'25.4" E	71	NSH-71	Sa Huỳnh's Ngự glutinous rice -NSH71	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'32.0"N and alongitude of 109°03'22.0" E
19	NSH-19	Sa Huỳnh's Ngự glutinous rice -NSH19	Sa Huynh, Duc Pho District, has a latitude of 14°39'33.3"N and alongitude of 109°03'48.8"E	72	NSH-72	Sa Huỳnh's Ngự glutinous rice -NSH72	Pho Chauward, Duc Pho District, has a latitude of 14°38'26.9"N and alongitude of 109°03'56.2"E
20	NSH-20	Sa Huỳnh's Ngự glutinous rice -NSH20	Pho Chauward, Duc Pho District, has a latitude of 14°38'34.8"N and alongitude of 109°03'19.9" E	73	NSH-73	Sa Huỳnh's Ngự glutinous rice -NSH73	Pho Thanh ward, Duc Pho District, has a latitude of 14°40'37.8"N and alongitude of 109°03'30.0"E
21	NSH-21	Sa Huỳnh's Ngự glutinous rice -NSH21	Pho Thanh ward, Duc Pho District, Vietnam (14°38'34.8"N, E	74	NSH-74	Sa Huỳnh's Ngự glutinous rice -NSH74	Pho Thanh ward, Duc PhoDistrict,Vietnam14°38'26.9"N,E

No.	Accession number	Name of variety	Locations	No.	Accession number	Name of variety	Locations
			109°03'19.9")				109°03'19.9")
22	NSH-22	Sa Huỳnh's Ngự glutinous rice -NSH22	Pho Thanh ward, Duc Pho District, Vietnam (14°38'31.9"N, 109°03'41.1" E)	75	NSH-75	Sa Huỳnh's Ngự glutinous rice -NSH75	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'34.8"N and alongitude of 109°03'19.9" E
23	NSH-23	Sa Huỳnh's Ngự glutinous rice -NSH23	Sa Huynh, Duc Pho District, has a latitude of 14°38'31.9"N and alongitude of 109°03'46.2" E	76	NSH-76	Sa Huỳnh's Ngự glutinous rice -NSH76	Pho Chau ward, Duc Pho District (14°38'34.1"N, 109°03'48.0"E)
24	NSH-24	Sa Huỳnh's Ngự glutinous rice -NSH24	Pho Thanh ward, Duc Pho District (14°39'29.2"N, 109°03'41.1" E)	77	NSH-77	Sa Huỳnh's Ngự glutinous rice -NSH77	Pho Chau ward, Duc Pho District, has a latitude of 14°38'34.1"N and alongitude of 109°03'48.0"E
25	NSH-25	Sa Huỳnh's Ngự glutinous rice -NSH25	s 14°38'23.5"N and alongitude of 109°03'33.7" E		NSH-78	Sa Huỳnh's Ngự glutinous rice -NSH78	Pho Chauward, Duc Pho District (14°38'34.8"N, 109°03'19.9" E)
26	NSH-26	Sa Huỳnh's Ngự glutinous rice -NSH26	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'26.8"N and alongitude of 109°03'25.4" E	79	NSH-79	Sa Huỳnh's Ngự glutinous rice -NSH79	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'32.0"N and alongitude of 109°03'22.0" E
27	NSH-27	Sa Huỳnh's Ngự glutinous rice -NSH27	Pho Thanh ward, Duc Pho District, Vietnam (14°38'31.9"N, 109°03'41.1" E)	80	NSH-80	Sa Huỳnh's Ngự glutinous rice -NSH80	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'34.8"N and alongitude of 109°03'19.9" E
28	NSH-28	Sa Huỳnh's Ngự glutinous rice -NSH28	Sa Huynh, Duc Pho District, has a latitude of 14°38'31.9"N and alongitude of 109°03'46.2" E	81	NSH-81	Sa Huỳnh's Ngự glutinous rice -NSH81	Pho Chau ward, Duc Pho District (14°38'34.1"N, 109°03'48.0"E)
29	NSH-29	Sa Huỳnh's Ngự glutinous rice -NSH29	Pho Chau ward, Duc Pho District, has a latitude of 14°38'23.5"N and alongitude of 109°03'33.7" E	82	NSH-82	Sa Huỳnh's Ngự glutinous rice -NSH82	Pho Chauward, Duc Pho District, has a latitude of 14°38'34.8"N and alongitude of 109°03'19.9" E
30	NSH-30	Sa Huỳnh's Ngự glutinous rice -NSH30	Pho Chau ward, Duc Pho District, has a latitude of 14°38'31.9"N and alongitude of 109°03'41.1" E	83	NSH-83	Sa Huỳnh's Ngự glutinous rice -NSH83	Pho Chau ward, Duc Pho District, has a latitude of 14°38'31.9"N and alongitude of 109°03'41.1" E
31	NSH-31	Sa Huỳnh's Ngự glutinous rice -NSH31	Pho Chau ward, Duc Pho District, Quang Ngai province (14°38'34.8"N, E 109°03'19.9")	84	NSH-84	Sa Huỳnh's Ngự glutinous rice -NSH84	Sa Huynh, Duc Pho District, Quang Ngai province, Vietnam (14°38'31.9"N and 109°03'46.2" E)

No.	Accession number	Name of variety	Locations	No.	Accession number	Name of variety	Locations
32	NSH-32	Sa Huỳnh's Ngự glutinous rice -NSH32	Pho Chau ward, Duc Pho District, has a latitude of 14°38'34.1"N and alongitude of 109°03'48.0"E	85	NSH-85	Sa Huỳnh's Ngự glutinous rice -NSH85	Pho Thanh ward, Duc Pho District (14°39'29.2"N, 109°03'41.1" E)
33	NSH-33	Sa Huỳnh's Ngự glutinous rice -NSH33	Pho Thanh ward, Duc Pho District, Vietnam (14°40'33.1"N - 109°03'25.1"E)	86	NSH-86	Sa Huỳnh's Ngự glutinous rice -NSH86	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'34.8"N and alongitude of 109°03'19.9" E
34	NSH-34	Sa Huỳnh's Ngự glutinous rice -NSH34	Sa Huynh, Duc Pho District, Quang Ngai province, Vietnam (14°39'34.6"N - 109°03'48.0"E)	87	NSH-87	Sa Huỳnh's Ngự glutinous rice -NSH87	Pho Chau ward, Duc Pho District (14°38'26.9"N and 109°03'56.2"E)
35	NSH-35	Sa Huỳnh's Ngự glutinous rice -NSH35	Pho Thanh ward, Duc Pho District, Duc Pho province (14°39'29.2"N and 109°03'41.1" E)	88	NSH-88	Sa Huỳnh's Ngự glutinous rice -NSH88	Pho Chau ward, Duc Pho District, has a latitude of 14°38'34.1"N and alongitude of 109°03'48.0"E
36	NSH-36	Sa Huỳnh's Ngự glutinous rice -NSH36	Pho Chau ward, Duc Pho District, has a latitude of 14°38'23.5"N and alongitude of 109°03'33.7" E	89	NSH-89	Sa Huỳnh's Ngự glutinous rice -NSH89	Pho Chauward, Duc Pho District (14°38'24.7"N- 109°03'36.6"E)
37	NSH-37	Sa Huỳnh's Ngự glutinous rice -NSH37	Pho Chau ward, Duc Pho District, has a latitude of 14°38'31.9"N and alongitude of 109°03'41.1" E	90	NSH-90	Sa Huỳnh's Ngự glutinous rice -NSH90	Pho Chau ward, Duc Pho District, has a latitude of 14°38'31.9"N and alongitude of 109°03'41.1" E
38	NSH-38	Sa Huỳnh's Ngự glutinous rice -NSH38	Pho Chau Pho District (14°38'38.8"N and 109°03'41.1" E)	91	NSH-91	Sa Huỳnh's Ngự glutinous rice -NSH91	Sa Huynh, Duc Pho District, has a latitude of 14°38'31.9"N and alongitude of 109°03'46.2" E
39	NSH-39	Sa Huỳnh's Ngự glutinous rice -NSH39	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'37.7"N and alongitude of 109°03'33.7" E	92	NSH-92	Sa Huỳnh's Ngự glutinous rice -NSH92	Pho Thanh ward, Duc Pho District (14°38'31.9"N and 109°03'41.1" E)
40	NSH-40	Sa Huỳnh's Ngự glutinous rice -NSH40	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'26.8"N and alongitude of 109°03'25.4" E	93	NSH-93	Sa Huỳnh's Ngự glutinous rice -NSH93	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'32.0"N and alongitude of 109°03'22.0" E
41	NSH-41	Sa Huỳnh's Ngự glutinous rice -NSH41	Sa Huynh, Duc Pho District, Quang Ngai province, Vietnam (14°39'33.3"N and 109°03'48.8"E)	94	NSH-94	Sa Huỳnh's Ngự glutinous rice -NSH94	Pho Chauward, Duc Pho District, has a latitude of 14°38'26.9"N and alongitude of 109°03'56.2"E
42	NSH-42	Sa Huỳnh's Ngự glutinous rice -NSH42	Pho Chau ward, Duc Pho District, has a latitude of 14°38'34.8"N and alongitude of 109°03'19.9"	95	NSH-95	Sa Huỳnh's Ngự glutinous rice -NSH95	Pho Thanh ward, Duc Pho District (14°40'37.8"N and 109°03'30.0"E)

No.	Accession number	Name of variety	Locations	No.	Accession number	Name of variety	Locations
			Е				
43	NSH-43	Sa Huỳnh's Ngự glutinous rice -NSH43	Pho Thanh ward, Duc Pho District, Vietnam (14°38'34.8"N, E 109°03'19.9")	96	NSH-96	Sa Huỳnh's Ngự glutinous rice -NSH96	Pho Thanh ward, Duc Pho District, Vietnam (14°38'26.9"N, E 109°03'19.9")
44	NSH-44	Sa Huỳnh's Ngự glutinous rice -NSH44	Pho Thanh ward, Duc Pho District, Vietnam (14°38'31.9"N, 109°03'41.1" E)	97	NSH-97	Sa Huỳnh's Ngự glutinous rice -NSH97	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'34.8"N and alongitude of 109°03'19.9" E
45	NSH-45	Sa Huỳnh's Ngự glutinous rice -NSH45	Sa Huynh, Duc Pho District, has a latitude of 14°38'31.9"N and alongitude of 109°03'46.2" E	98	NSH-98	Sa Huỳnh's Ngự glutinous rice -NSH98	Pho Chau ward, Duc Pho District (14°38'34.1"N, 109°03'48.0"E)
46	NSH-46	Sa Huỳnh's Ngự glutinous rice -NSH46	Pho Thanh ward, Duc Pho District (14°39'29.2"N, 109°03'41.1" E)	99	NSH-99	Sa Huỳnh's Ngự glutinous rice -NSH99	Pho Chau ward, Duc Pho District, has a latitude of 14°38'34.1"N and alongitude of 109°03'48.0"E
47	NSH-47	Sa Huỳnh's Ngự glutinous rice -NSH47	Pho Chau ward, Duc Pho District, has a latitude of 14°38'23.5"N and alongitude of 109°03'33.7" E	100	NSH-100	Sa Huỳnh's Ngự glutinous rice -NSH100	Pho Chauward, Duc Pho District (14°38'34.8"N, 109°03'19.9" E)
48	NSH-48	Sa Huỳnh's Ngự glutinous rice -NSH48	Pho Thanh ward, Duc Pho District, has a latitude of 14°38'26.8"N and alongitude of 109°03'25.4" E	101	NBD-101	Hoai Nhon, Binh Dinh glutinous rice -NBD101	Hoai Nhon District, Binh Dinh province has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229
49	NSH-49	Sa Huỳnh's Ngự glutinous rice -NSH49	Pho Thanh ward, Duc Pho District, Vietnam (14°38'31.9"N, 109°03'41.1" E)	102	NBD-102	Hoai Nhon, Binh Dinh glutinous rice - NBD102	Hoai Nhon District, Binh Dinh province has a latitude of 14°26'35.62" and a longitude of 108°59'30.57"E
50	NSH-50	Sa Huỳnh's Ngự glutinous rice -NSH50	Sa Huynh, Duc Pho District, has a latitude of 14°38'31.9"N and alongitude of 109°03'46.2" E	103	NBD-103	Hoai Nhon, Binh Dinh glutinous rice - NBD103	Hoai Nhon District, Binh Dinh province (N14° 26' 24", E 109° 5' 60")
51	NSH-51	Sa Huỳnh's Ngự glutinous rice -NSH51	Pho Chau ward, Duc Pho District, has a latitude of 14°38'23.5"N and alongitude of 109°03'33.7" E	104	NBD-104	Hoai Nhon, Binh Dinh glutinous rice - NBD104	Hoai Nhon District, Binh Dinh province (N14° 26' 24", E 109° 5' 60")
52	NSH-52	Sa Huỳnh's Ngự glutinous rice -NSH52	Pho Chau ward, Duc Pho District (14°38'21.5"N - 109°03'41.8"E)	105	NPCR- 105	Quang Ngai glutinous rice -NSH105	The Plant Resources Center (PRC)

Tran et al. Agro-Morphological Characteristics and Genetic Diversity of Traditional Glutinous Rice Varieties in Quang Ngai Province, Vietnam

No.	Accession number	Name of variety	Locations	No.	Accession number	Name of variety	Locations
53	NSH-53	Sa Huỳnh's Ngự glutinous rice -NSH53	Pho Chau ward, Duc Pho District, Quang Ngai province (14°38'34.8"N, E 109°03'19.9")	106	NPCR- 106	Quang Ngai glutinous rice -NSH106	The Plant Resources Center (PRC)

Plant cultivation and care

A total of 106 traditional rice varieties were cultivated in the specialized Sa Huỳnh's Ngự glutinous rice fields in Pho Chau commune, Duc Pho district, Quang Ngaiprovince, Vietnam, during the 2023-2024 winterspring crop. The seeds were sown on raised seedbeds, and 12-15-day-old seedlings were transplanted, with one seedling per hill. The experiment was conducted following the genetic resource evaluation method of the International Plant Genetic Resources Institute (IPGRI, Experimental farming methods and care 2001). techniques were implemented in accordance with the prevailing rice cultivation practices in the region. The process was overseen by the Departments of Agriculture and Rural Development of Quang Ngai Province and Duc Pho District to ensure compliance with local standards and best practices.

Indicators and measures of quantitative traits

The monitoring criteria in the field were assessed according to the rice genetic resource evaluation criteria of the International Rice Research Institute (IRRI, 2014).

Growth time (days): The number of days from sowing to seed maturity, defined as the stage when 85% of the seeds on the mature panicle are fully matured.

Plant height (cm): Measured from the soil surface to the tip of the tallest panicle (excluding awns). This measurement was taken from 10 randomly selected plants per accession per replication.

Panicle length (cm): Measured from the panicle neck to the tip of the main panicle. Thismeasurement was taken from 10 randomly selected primary panicles per accession per replication.

Number of panicles/m²: Determined by randomly sampling three representative one-square-meter regions from each plot.

Number of filled spikelets/panicle: The total number of filled spikelets per panicle was counted from 10 randomly selected clusters for each experimental plot.

Empty spikelet ratio (%): The percentage of unfilled spikelets per panicle.

ISSN: 2456-1878 (Int. J. Environ. Agric. Biotech.) https://dx.doi.org/10.22161/ijeab.101.11 1000-grain weight (g): The weight (in grams) of 1000 well-developed whole grains at 14% moisture content, taken from five randomly selected primary panicles per accession per replication. Yield (g/hill): Determined using the following formula:

$$Yield (g/hill) = \frac{Weight of harvest grain (g)}{No. of hills x No. of possible hills x MF}$$

Where:

$$MF = \frac{100 - MC}{86}$$

The moisture content (MC) of the harvested grains per plot was measured immediately after weighing, using a moisture meter.

Biomass-weight of 10 plants harvested from each accession per replication. Harvested plants were dried before weighing.

Harvest index (%): The harvest index was determined by the following formula:

Harvest index (%) =
$$\frac{Economic yield}{Biological yield} \times 100$$

Where: Economic yield was defined as the total grain weight harvested from 10 plants per accession per replication. Biological yield encompassed the total grain weight and biomass from the same 10 plants per accession per replication.

DNA Extraction and PCR Analysis

DNA was extracted from freshly frozen leaves of individuals using an improved CTAB method (Oliveira *et al.*, 2015). A 2-3 cm section of traditional glutinous rice leaf was collected and placed in a labeled 1.5 mL centrifuge tube on ice after being ground into a fine powder. The tissue powder was mixed into an emulsion with a glass rod after adding 10 mL of extraction buffer (containing 2% CTAB, 0.7 M NaCl, 50 mM Tris-HCl pH 8.0, and 10 mM EDTA pH 8.0). The mixture was then incubated at 65°C for 1 hour. Then, a 10 mL mixture of chloroform and isoamyl alcohol (v:v/24:1) was added,

and the solution was vigorously shaken to form an emulsion. The mixture was centrifuged at 14,000 rpm for 15 minutes at 20°C. The resulting supernatant was transferred to a fresh tube, and 10 μ L of RNase (10 mg/mL) was added. The mixture was incubated at room temperature for 10 minutes. DNA was precipitated by gently shaking the mixture after adding 0.6 volume of isopropanol. The precipitated DNA was carefully collected and transferred into 70% ethanol for washing. The DNA was air-dried and subsequently resuspended in 50 μ L of TE buffer. The extracted DNA was stored in ultra-pure water at -20°C in a refrigeratorfor future use.

DNA quality was assessed using 1% agarose gel electrophoresis. To prepare the gel, 3 g of agarose was dissolved in 300 ml of TAE buffer. The mixture was heated in a microwave for 5–6 minutes until fully melted and then allowed to cool to approximately 55–60°C. The cooled agarose solution was poured into a previously prepared electrophoresis box fitted with combs to create wells. The gel was allowed to set for approximately 45 minutes before the combs were carefully removed. A10 µl mixture containing 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM MgCl₂, 50 mM each of dNTPs, 0.2 mM primer pairs, 0.5 U Taq polymerase (Merck KGaA, Darmstadt, Germany), and 20 ng DNA template was loaded into the wells. Electrophoresis was performed at 70–80 V and 60 mA for 45-48 minutes.

A total of 108 simple-sequence-repeat (SSR) markers used in the experiment were located on the12 chromosomes (Chr) of the rice genome (Table 2). The genomic sequences and SSR markers were obtained from the public database GRAMENE (http://archive.gramene.org/markers/index.html).

The polymerase chain reaction (PCR) for SSR markers was carried out in 10 μ l mixture. The PCR cycle began with an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, followed by extension at 72°C for 30 seconds, and 72°C for 10 minutes using a Mastercycler Gradient PCR system (Eppendorf). 5 μ l of each reaction mixture were loaded onto a polyacrylamide gel for electrophoresis. After the run, the plates were separated using a plastic wedge and removed from the electrophoresis tank. The acrylamide gel was then soaked in an ethidium bromide staining solution for 15-20 minutes. Stained bands were visualized and photographed under UV light.

Data analysis

Analysis of variance: The agro-morphological data collected were initially analyzed using analysis of

variance (ANOVA) to verify genetic variation in the traits measured. Traits with insignificant genetic variation, based on the F-test, were not considered for further analyses.

Correlation analysis: The correlation coefficient (r) measured the degree of association between two or more variables. It provided a symmetrical measure of association and did not indicate dependency between variables. Correlation among agro-morphological traits was calculated using Statistix 10.0 software.

Distance matrix: The distance matrix was calculated using the Euclidean distance coefficient, as described by Sneath and Sokal (1973).

$$Eij = [\sum_{k} (X_{ki} - X_{kj})^2]^{1/2}$$

Where: Eij = 0 to ∞ , the larger the value, the more distant the degree of relationship; Xi and Xj are the standardized values for the ith and jth characters in kth varieties.

Genetic diversity of Sa Huỳnh's Ngự glutinous rice varieties studied based on genotype: Molecular data were collected by scoring 1 for presence of band and 0 for absence of band. The data were entered directly into an Excel spreadsheet.

Data analysis :Pairwise comparisons of lines, based on the presence of unique and shared polymorphic products, were used to calculate the genetic similarity coefficients. Polymorphic Information Content (PIC) was calculated using the method of Weir (1996) with the following equation:

$PIC(i) = 1 - \sum Pij^2$

Where: Pij is the frequency of jth allele with at the ith locus. The collected data were analyzed using NTSYS-pc 2.11a software. A cluster analysis was performed based on the similarity matrix, using the unweighted pair-group method with arithmetic average (UPGMA) clustering algorithm.

Cluster analysis: Cluster analysis was carried out for agro-morphology–based genetic distance matrix using UPGMA clustering method in the NTSYS-pc 2.11a software. The results of the UPGMA were used to draw the dendrogram of the 106 traditional glutinous rice varieties.

Statistical analysis: Mean, range and standard error were calculated using Statistix 10.0 software. Based on the LSD test (P < 0.01 and P < 0.05), statistical analyses were performed using one-way ANOVA to

determine the significant difference between experiment means.

III. RESULTS AND DISCUSSION

Polymorphism of simple-sequence-repeat markers

PCR amplification was conducted using DNA samples extracted from 106 traditional glutinous rice varieties. The amplified PCR products were electrophoresed on a 3% agarose gel prepared with 1X TBE buffer, stained with ethidium bromide, and visualized under a UV transilluminator. A total of 108 SSR markers distributed throughout the 12 rice chromosomes were applied to analyze polymorphisms, among which only 48 (44. 44 %) SSR markers were found to be polymorphic. In total, 193 alleles were detected, with the number of amplified fragments ranging from 2 to 8 alleles per locus, and an average of 4.02 alleles per locus. Two primers, RM6314 (chromosome 4) and RM316 (chromosome 9), produced the highest number of alleles (8). The PIC values of the 48 SSR markers used in this study ranged from 0.33 (RM333) to 0.87 (RM6314), with an average PIC value of 0.61. This result is slightly lower than that of some previous studies on quality rice varieties. For example, Victoria (2007) reported an average PIC value of 0.68 when assessing the genetic diversity of quality rice varieties in the Philippines. Other studies, such as those by Shaptadvipa et al. (2009), Borba et al. (2009), and Upadhyay et al. (2011), reported higher PIC values of 0.923, 0.75, and 0.78, respectively. Khanin Pathak et al. (2016) used 65 polymorphic SSR markers to evaluate the genetic diversity of 100 glutinous rice samples, obtaining PIC values ranging from 0.613 to 0.995. More recently, Hoque M.I. et al. (2021) assessed the genetic diversity of 83 native rice varieties (both glutinous and non-glutinous) in Bangladesh using 9 SSR markers, reporting PIC values ranging from 0.766 to 0.951. In contrast, the PIC values observed in this experiment were higher than those reported in studies on specialty rice varieties. For example, Ravi et al. (2003) and Jain et al. (2004) reported average PIC values of 0.48 and 0.51, respectively. Similarly, studies on purple rice varieties by Ngo Thi Hong Tuoi et al. (2014) and local glutinous rice by Doan Thanh Quynh et al. (2016) showed average PIC values of 0.46 and 0.5, respectively. Lei et al. (2021), in their study on Kam glutinous rice varieties, reported an average PIC value of 0.5576 (Table 2).

Table 2. Results of polymorphic analysis of 106 traditional glutinous rice varieties based on SSR markers

	51 5 1		0		
No.	Primer SSR	Chr	No.of alleles	Size (bp)	PIC value
1	RM140	1	3	190-200	0.63
2	RM10843	1	5	180-200	0.73
3	RM10890	1	3	205-210	0.66
4	RM10115	1	4	240-250	0.72
5	RM284	1	3	147-155	0.57
6	RM221	2	3	220-230	0.66
7	RM106	2	2	300-350	0.56
8	RM154	2	2	165-199	0.45
9	RM71	2	3	210-230	0.56
10	RM347	3	4	207-240	0.48
11	RM3867	3	4	210-230	0.74
12	RM148	3	6	190-210	0.43
13	RM231	3	6	200-210	0.67
14	RM 514	3	7	240-280	0.86
15	RM317	4	2	146-166	0.46
16	RM1155	4	3	200-245	0.40
17	RM551	4	3	168-214	0.75
18	RM6314	4	8	142-169	0.87

Total			193	-	-	
Maxi			8	-	0.87	
Avera	-		4.02	-	0.61	
Minir			2	-	0.33	-
48	RM12	12	3	162-184	0.74	
47	RM5364	12	3	150-160	0.66	
46	RM 270	12	5	110-140	0.77	
45	RM463	12	4	210-225	0.52	
44	RM277	12	3	115-125	0.54	
43	RM224	11	2	120-157	0.41	
42	RM 536	11	7	230-268	0.71	
41	RM11049	11	4	200-210	0.74	
40	RM333	10	3	164-215	0.33	
39	RM484	10	3	291-299	0.48	
38	RM 171	10	7	321-353	0.73	
37	RM 316	9	8	195-220	0.84	
36	RM23662	9	3	210-220	0.64	
35	RM24013	9	6	215-220	0.42	
34	RM433	8	5	222-232	0.66	
33	RM284	8	4	141-149	0.48	
32	RM152	8	3	175-200	0.63	
31	RM547	8	5	200-210	0.49	
30	RM346	7	5	140-160	0.68	
29	RM418	7	4	200-215	0.56	
28	RM 455	7	3	130-140	0.58	
27	RM5436	7	3	200-210	0.73	
26	RM21539	7	2	205-210	0.45	
25	RM510	6	4	220-230	0.67	
24	RM190	6	3	104-124	0.65	
23	RM454	6	3	267-290	0.40	
22	RM334	5	4	146-197	0.58	
21	RM5948	5	4	138-145	0.49	
20	RM13	5	5	190-210	0.63	
19 20	RM122	5	4	205-230	0.64	
10	D) (100	-		207.220	0.54	

Note: Chr: Chromosome; PIC: Polymorphic Information Content

. In addition, a dendrogram was constructed using cluster analysis based on the UPGMA method with the SAHN module in NTSYS-pc 2.11a, and it was generated and showed significant genetic variation among the traditional glutinous rice varieties studied in Figure 1. At genetic distance of 0.41, the 106 traditional glutinous rice varieties were separated into 2 major clusters namely, A and B. At a genetic distance of 0.55, cluster A was

divided into sub-clusters A1 (11.32%), A2 (12.26%) and A3 (21.7%). Cluster B was divided into sub-clusters B1

(16.04%) and B2 (38.68%).

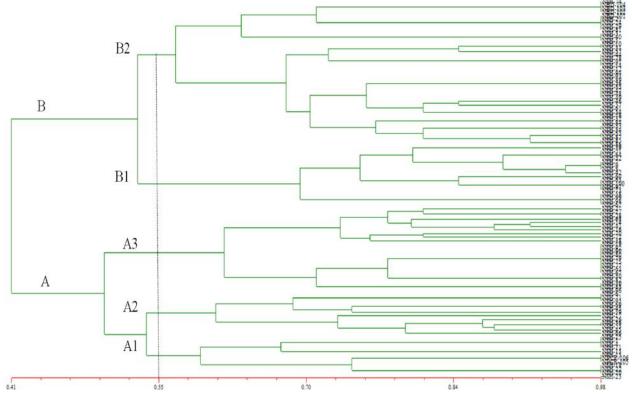


Fig.1. Classification of 106 traditional glutinous rice varieties based on genetic distance calculated from 48 SSR markers

Variance in agro-morphological characteristics

Morphological diversity analysis was conducted using the variance of agro-morphological features. For each of the 11 quantitative traits, key statistical parameters were calculated, including the mean, range (maximum and minimum), standard deviation, coefficient of variation (CV), mean standard error, and F-value. The results indicate that most of the quantitative traits exhibited high variability and all measurements followed a normal distribution (Table 3, Figures 2 to 4).

Table 3. Descriptive statistics of quantitative traits among 106 traditional glutinous rice varieties

Traits	Max	Min	Mean	CV (%)	Р
Growing time (days)	132.00	106.00	115.28	0.42	< 0.05
Plant height (cm)	154.90	115.00	140.31	0.55	< 0.05
Effective tiller number/plant (no.)	4.00	1.00	1.82	4.80	< 0.05
Panicle length (cm)	30.30	21.00	25.83	0.75	< 0.05
Number of panicle/m ² (no.)	387.00	146.00	260.45	1.94	< 0.05
Number of filled spikelet/panicle (no.)	147.00	30.00	87.83	2.91	< 0.05
Empty spikelet ratio (%)	40.30	1.12	9.65	6.55	< 0.05
1000-grain weight (g)	41.02	31.08	34.61	0.52	< 0.05
Yield (g/hill)	66.90	24.00	41.24	1.72	< 0.05
Biomass (g)	83.98	33.75	62.29	1.31	< 0.05
Harvest index (%)	0.72	0.12	0.50	2.72	< 0.05

Note: CV: *Coefficient of Variation; Significantly different at* p < 0.05

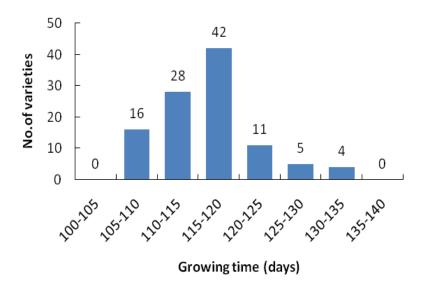


Fig.2. Frequency distribution of 106 traditional glutinous rice varieties based on GT

Table 4. The groups of 106 traditional glutinous rice varieties based on GT

Trait group	The number of varieties	Ratio (%)
Extra short-day (< 100 days)	0	0
Short-day (100-115 days)	44	41.51
Medium-day (116-130 days)	58	54.72
Long-day (> 130 days)	4	3.77

The growing time (GT)

The GT of rice varieties is a genetic trait that is affected by environmental factors, the earliest maturing genotype matured in 106 days, the latest maturing one took 132 days (Table 3). There are 44 short-day traditional glutinous rice varieties (41.51%), 58 mediumday varieties (54.72%) and 4 long-day varieties (3.77%), and no extra short-day varieties (Table 4 and Figure 2). Thus, the GT of the studied rice varieties was relatively diverse, with the majority of them falling into the shortand medium-day groups. The results of grouping according to GT are similar to the sub-group of commonly grown rice varieties in the northwest (short and medium-day varieties) in Doan Thi Thuy Linh'sprevious report (2013). Similarly, Van Quang Tran et al. (2021) also reported comparable findings regarding the agro-morphological characteristics and genetic diversity of glutinous rice landraces in northern Vietnam. GT is a valuable trait for selecting and developing new rice varieties; however, breeders still face the challenge of without developing varieties with shorter GT compromising yield.

Agronomic Characteristics

The plant height of traditional glutinous rice varieties ranged from 115 cm to 154.9 cm. Among them, 104 varieties (98.11%) had a plant height greater than 120 cm, while only 2 varieties (1.89%) fell into the mediumheight category, with a plant height ranging between 100 and 120 cm.No semi-dwarf varieties were observed. The semi-dwarf stature contributed most to production gains during the green revolution due to associated improvements in harvest index and lodging under heavy fertilizer doses (Hargrove et al., 1980). Thus, breeders need to improve height of traditional glutinous rice to increase lodging resistance. Traditional glutinous rice varieties, in particular, and the traditional rice landraces, in general, produced alow number of tillers per plant (Rabara et al., 2014). In this study, traditional glutinous rice varieties exhibited low tiller numbers, with the effective tiller number per plant ranging from 1 to 4. Of these, 103 varieties (97.17%) had a low panicle level, and 3 varieties (2.83%) had a medium panicle level (Table 5 and Figure 3).

Table 5. Groups of 106 traditional glutinous rice varieties based on plant height and effective tiller number per plant

ISSN: 2456-1878 (Int. J. Environ. Agric. Biotech.) https://dx.doi.org/10.22161/ijeab.101.11

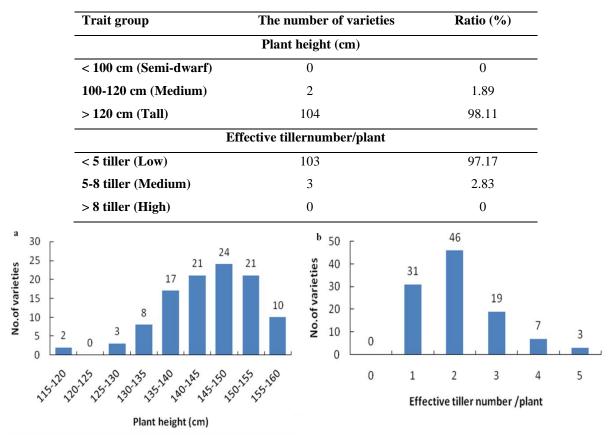


Fig.3. Frequency distribution of 106 traditional glutinous rice varieties based on plant height (a) and effective tiller number per plant (b).

The panicle length ranged from 21 cm to 30.3 cm. There were 22 varieties (20.75%) with medium panicles (20-25 cm), 77 varieties (72.64%) with long panicles (26-30 cm), and 7 varieties (6.61%) with extra-long panicles (>30 cm). The number of filled spikelets per panicle ranged from 30 to 147, and the number of panicles per square meter ranged from 146 to 387, showing high variation among varieties. The empty spikelet ratio ranged from 1.12% to 40.3%. The weight of 1000 grains varied among varieties, ranging from 31.08 g to 41.02 g. There are 42 varieties (39.62%) with high grain weight (30-35 g) and 64 varieties (60.38%) with extra-high grain weight (> 35 g). Since this trait is one of the most important yield components, the traditional glutinous rice varieties

identified can serve as an important source of starting material for breeding rice varieties with higher yield. The individual yield ranged from 24 to 66.9 g per hill,with an average of 41.24 g per hill (Table 3, Figure 4a-f). This result is similar to the individual yield of rice landraces varieties in Vietnam (41.92 g/hill) reported in Nguyen Thi Lang's study (2014), but higher than the yield reported for Nep Ngu rice (36.33 g per hill) in Binh Dinh (2023). The biomass ranged from 33.75 to 83.98 g, with a mean of 62.29 g, and the harvest index ranged from 0.12% to 0.72%, with a mean of 0.5% (Table 3, Figure 4g-h). The average biomass values identified in this experiment were higher than the result reported for Nep Ngu rice (58.33 g) in Binh Dinh (2023).

Tran et al. Agro-Morphological Characteristics and Genetic Diversity of Traditional Glutinous Rice Varieties in Quang Ngai Province, Vietnam

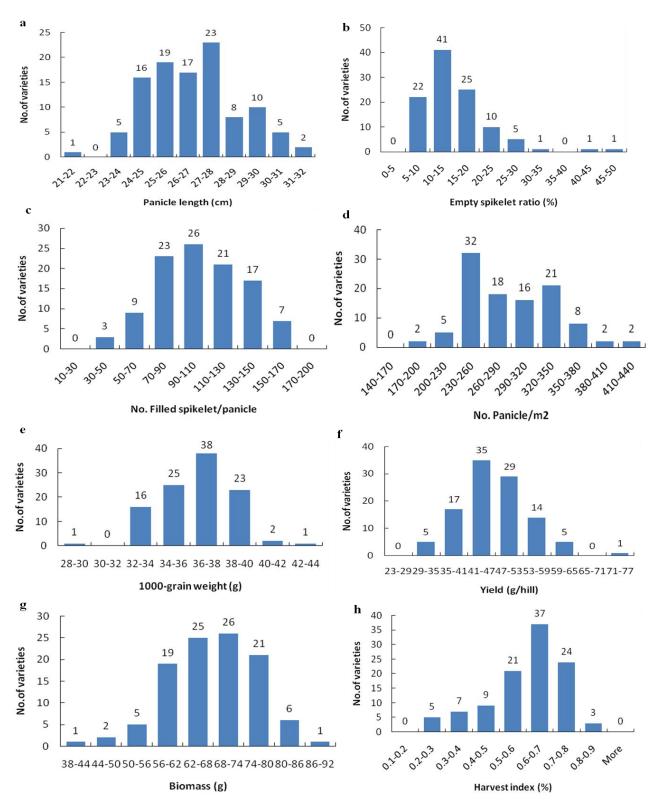


Fig.4. Frequency distribution of 106 traditional glutinous rice varieties based on panicle length (a), empty spikelet ratio (b), number of filled spikelet per panicle (c), number of panicle per m^2 (d), 1000-grain weight (e), yield (f), biomass (g) and harvest index (h).

Morphological characteristics show that most the traditional rice varieties, in general, and the traditional glutinous rice varieties, in particular, have late maturity, tall plant, and a higher number of filled seeds, a lower empty spikelet ratio, and a higher weight of 1000 grains. The variation in agro-morphological traits mentioned

ISSN: 2456-1878 (Int. J. Environ. Agric. Biotech.) https://dx.doi.org/10.22161/ijeab.101.11 above can be attributed to genetic variability among the tested varieties. This diversity serves as a valuable resource for plant breeders to develop rice varieties with improved crop yields, superior grain quality, and greater photosynthetic efficiency.

Correlation among agro-morphological traits

The correlation coefficients among the agromorphological traits measured are shown in Table 6. Panicle length was significantly correlated with plant height ($r = 0.375^*$), which can be explained by the principle of morphological compatibility in plant architecture among 106 traditional glutinous rice varieties. This result is similar to Nguyen Thi Lang'sprevious report (2014). Other characteristics were strongly correlated with the panicle length. A significant correlation was also found between growing time and biomass ($r = 0.301^*$). There was a strong negative correlation between the number of filled spikelet per panicle and the empty spikelet ratio ($r = -0.485^{**}$), indicating that as the number of filled spikelet per panicle increases, the empty spikelet ratio decreases, and vice versa. The weight of 1000 grain was significantly correlated with the number of filled spikelets per panicle $(r = 0.454^{**})$ and individual yield $(r = 0.41^{**})$. There was significantly correlated between individual yield and biomass (r =0.492**). Although the correlation coefficients did not show significant differences, individual yield and biomass were positively proportional to the panicle length and the number of panicles per square meter, while they were negatively proportional to plant height. The results differ from those of Lang et al. (2014), which can be explained by the unique characteristics of landrace varieties. The results indicate that higher yield is correlated with reproductive traits, growing time, panicle length, empty spikelet ratio, number of filled spikelet per panicle, number of panicle per square meter and especially the weight of 1000 grains. The information obtained from this study is critical for breeding and can guide further research on the relationship between 1000-grain weight and yield. Other traits were found to have weak correlations with agromorphological traits.

Traits	GT (days)	PH (cm)	ET/P (no.)	PL (cm)	NP/m ² (no.)	NFS/P (no.)	ESR (%)	1000W (g)	Y (g/hill)	B (g)	HI (%)
GT (days)	1										
PH (cm)	-0.009	1									
ET/P (no.)	0.058	0.091	1								
PL (cm)	-0.028	0.373*	0.133	1							
NP/m² (no.)	0.081	-0.080	-0.030	-0.080	1						
NFS/P (no.)	0.075	0.011	0.084	0.027	0.085	1					
ESR (%)	-0.112	0.071	-0.050	0.033	0.028	-0.485**	1				
1000W (g)	-0.107	0.156	-0.018	0.066	-0.231	0.454*	0.059	1			
Y (g/hill)	0.176	-0.100	0.055	0.136	0.127	0.049	0.116	0.410**	1		
B (g)	0.301*	-0.202	0.028	0.211	0.110	0.057	-0.050	-0.079	0.492**	1	
HI (%)	0.021	0.004	0.014	0.041	0.143	-0.135	0.028	0.079	0.017	-0.046	1

Table 6. Correlation coefficients among 11 agro-morphological traits of 106 traditional glutinous rice varieties

Note: GT: Growing time;PH: Plant height; ET/P: Effective tiller number /plant (no.); PL: Panicle length; NP/m²: No. Panicle/m² (no.); NFS/P: No. Filled spikelet/panicle (no.); ESR: Empty spikelet ratio (%); 1000W:1000-grain weight (g); Y: yield (g/hill); B: Biomass (g); HI: Harvest index (%). Means with * and ** are significantly different at p < 0.05 and 0.01, respectively.

IV. CONCLUSIONS

This study assessed the agro-morphological characteristics and genetic diversity of 106 traditional *ISSN: 2456-1878 (Int. J. Environ. Agric. Biotech.)* https://dx.doi.org/10.22161/ijeab.101.11

glutinous rice varieties. Agro-morphological traits and PCR-based markers provided valuable insights into the genetic diversity of Sa Huỳnh's Ngự glutinous rice varieties in Quang Ngai province, Vietnam. Molecular analysis revealed that SSR markers were highly effective in characterizing and assessing the extent and distribution of genetic variation among the 106 traditional glutinous rice varieties. Using UPGMA for molecular data analysis with NTSYS pc 2.11a software, the 106 traditional glutinous rice varieties were divided into two major clusters based on genetic differences. The data collected in this study provide valuable insights for breeding glutinous rice landraces using both traditional and molecular methods.

Using quantitative agro-morphological characters, ANOVA revealed highly significant differences among the traits of the106 traditional glutinous rice varieties. The traditional glutinous rice varieties exhibit relatively diverse growing times, with the majority falling into short-day and medium-day groups, they have medium-tohigh plant heights, medium-to-long panicle lengths, and 1000-grain weights ranging from high to extra-high. Correlation coefficients showed that all the traits were correlated with each other, especially the yield, which was strongly correlated with 1000-grain weight but only slightly correlated with other traits.

ACKNOWLEDGEMENTS

This paper presents findings from "Investigation, collection, evaluation, and conservation of genetic recourses of Sa Huỳnh's Ngự glutinous rice and local Cút glutinous rice in Quang Ngai province" project. We thank the Quang Ngai People's Committees and Department of Science and Technology for supporting this project under grant number QGT35.DT.01/2022.

REFERENCES

- Ab Razak, S., Nor Azman, N. H. E., Kamaruzaman, R., Saidon, S. A., Mohd Yusof, M. F., Ismail, S. N., Jaafar, M. A. and Abdullah, N. (2020). Genetic diversity of released Malaysian rice varieties based on single nucleotide polymorphism markers. Czech J. *Genet. Plant Breed.* 56: 62-70.
- [2] Acevedo-Siaca, L. G., Coe, R., Wang, Yu., Kromdijk, J., Quick, W. P. and Long, S. P. (2020). Variation in photosynthetic induction between rice accessions and its potential for improving productivity. *New Phytol.* 227:1097-08.
- [3] Aljumaili, S. J., Rafii, M. Y., Latif, M. A., Sakimin, S. Z., Arolu, I. W. and Miah, G. (2018). Genetic diversity of aromatic rice germplasm revealed by SSR markers. *Bio. Med. Res. Int.* Article No. 7658032.
- [4] Appa Rao, S., Schiller, J. M., Bounphanousay, C. and Jackson, M. T. (2006). Diversity within the traditional rice

ISSN: 2456-1878 (Int. J. Environ. Agric. Biotech.) https://dx.doi.org/10.22161/ijeab.101.11 varieties of Laos. In: Rice in Laos. (Schiller J. M., Chanphengxay, M. B., Linquist, B., Appa Rao, S. Eds.). International Rice Research Institute, Los Banos, pp.123-159.

- [5] Becerra, V., Paredes, M., Gutierrez, E. and Rojo, C. (2015). Genetic diversity, identification, and certification of Chilean rice varieties using molecular markers. *Chil. J. Agric. Res.* 75:267-75.
- [6] Begum, M., Rashid, Md. M., Arifuzzaman, Md., Hasan, M. S. and Hasan, M. A. A. Z. (2018). Genetic variability and crossing among blast resistant and susceptible fine aromatic rice (*Oryza sativa* L) land races. *Res. Crops* 19:450-57.
- [7] Bhujel, J., Sharma, S., Shrestha, J. and Bhattarai, A. (2018). Correlation and path coefficient analysis in normal irrigated rice (*Oryza sativa* L.). *Farm. Manage*. 3:19-22.
- [8] Borba T. C. O., BrondaniR. P., Rangel P. H., Brondani C., (2009). Microsatellite marker-mediated analysis of the EMBRAPA rice core collection genetic diversity. *Genetica* 137(3): 293-304.
- [9] Botstein D, White RL, Skolnick M, Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet* 32: 314–331.
- [10] Brown, A.H.D., O.H. Frankel, D.R. Marshall & J.T. Williams. (1989). The Use of Plant Genetic Resources (1st ed.). Cambridge University Press.
- [11] Brown, H.D., M.T. Clegg, A.L. Kahler & B.S. Weir. (1990). Plant Population Genetics, Breeding, and Genetic Resources (1st ed.). Sinauer Assoc. Inc., Sunderland, Massachusetts.
- [12] Guru, T., Padma, V., Reddy, D. V. V., Rao, P. R., Rao, D. S., Ramesh, T. and Radhakrishna, K. V. (2017). Genetic diversity analysis for yield attributing traits in rice genotypes. *Res. Crops* 18:311-15.
- [13] Hargrove, T.R., Coffman, W.R. and Cabanilla, V.L. (1980). Ancestry of improved cultivars of Asian Rice. *Crop Science* 20:721-727.
- [14] Hoque M.I., M.M. Islam, S.N. Begum, F. Yasmine, M.S.R. Khanom and M. M. Islam, (2021). Genetic diversity analysis of rice (*Oryzae sativa* L.) landraces using SSR markers in Bangladesh. *SAARC J. Agric.* 19(2):13-25.
- [15] IRRI (2001). Design and analysis of evaluation trails of genetic resources collections, IPGRRI Via dei Tre Denari 472/a 00057 Maccarese, Rome Italy, ISSN 92-9043-505-4.
- [16] IRRI (2014). Standard Evaluation System for Rice (5th ed.). Los Banos, International Rice Research Institute. Philippines.
- [17] Jae-Ryoung Park, Won-Tae Yang, Yong-Sham Kwon, Hyeon-Nam Kim, Kyung-Min Kim, and Doh-Hoon Kim (2019). Assessment of the Genetic Diversity of Rice Germplasms Characterized by Black-Purple and Red Pericarp Color Using Simple Sequence Repeat Markers. *Plants* 8(11): 471.
- [18] Jain S., Jain R. K., McCouch S. R., (2004). Genetic analysis of Indian aromatic and quality rice (*Oryza sativa* L.) germplasm using panels of fluorescently-labeled

microsatellite markers. *Theor. Appl. Genet.* 109(5): 965-977.

- [19] Jarvis, D.I., L. Myer, H. Klemick, L. Guarino, M. Smale, A.H.D. Brown, M. Sadiki, B. Sthapit And T. Hodgkin. (2000). A training guide for in situ conservation on farm (1st ed.). International Plant Genetic Resources Institute, Rome, Italy.
- [20] Khanin Pathak; Sunayana Rathi; R N Sarma and Samindra Baishya (2016). Assessment of Genetic Diversity of Indigenous Glutinous Rice Genotypes of Assam using SSR Marker. *Indian Journal of Agricultural Biochemistry* 29(1):54-61.
- [21] Lang (NT), Pham Thi Be Tu, Nguyen Chi Thanh, Bui Chi Buu and Ismail A (2009). Genetic diversity of salt-tolerant rice landraces in Vietnam. J. Plant Breed. Crop Sci. 1(5): 230-243.
- [22] Lang Thi Nguyen, Bui Phuoc Tam, Nguyen Van Hieu, Chau Thanh Nha, Abdelbagi Ismail, Russell Reinke and Bui Chi Buu (2014). Evaluation of Rice Landraces In Vietnam Using Ssr Markers And Morphological Characters. Sabrao Journal of Breeding and Genetics 46(1):1-20.
- [23] Lang Thi Nguyen, Tran Khanh Thi Nguyen, Hieu Chi Bui, Khoa Anh Bien, Buu Chi Bui (2023). Agro-Morphological Characters and PCR Based Markers for NEP NGU at Binh Dinh, Vietnam.*Int. J. Environ. Agric. Biotech.* 8(6):139-155.
- [24] Lei, Q.-Y., Zhou, J.-J., Xiong, Y., Zhang, W.-H., Luo, J., & Long, C.-L. (2021). Genetic Diversity Evaluation and Conservation of Kam Fragrant Glutinous Rice (*Oryza* sativa L.) Germplasm in Southeast Guizhou, China. *Plants* 10(9):1898.
- [25] Linh Thi Thuy Doan, Nguyen Van Khoa (2013). Genetic diversity of native rice (Oryza sativa L.) in northwest basedon morphological characteristics. The 5th National Scientific Conference on Ecology and Biological Resources, 1132-1139.
- [26] Muto, C., Ebana, K., Kawano, K., Bounphanousay, V., Bounphanousay, C., Kanyavong, K., Inthapanya, P., Boualaphanh, C., Sato, T., Ishikawa, R., Sato, Yo-Ichiro., Yanagihara, S. and Fukuta, Y. (2019). Genetic variation in rice (*Oryza sativa* L.) germplasm from northern Laos. *Breed. Sci.* 69:272-78.
- [27] Nguyen, T. T. T., Nguyen, N. M. T., Hoang, L. H., Furuya, Naruto and Tsuchiya, K. (2012). Genetic diversity in Vietnamese upland rice germplasm revealed by SSR markers. *Fac. Agric. Kyushu Univ.* 57:383-91.
- [28] Ni J, Colowit PM and MacKill DJ (2002). Evaluation of genetic diversity in rice subspecies using microsatellite markers. *Crop Sci.* 42: 601- 607.
- [29] Nilthong, S., Chukeatirote, E. and Nilthong, R. (2020). Assessment of genetic diversity in Thai upland rice varieties using SSR markers. *Aust. J. Crop Sci.* 14:597-04.
- [30] Oliveira, R.R., A.J. Viana, A.C. Reátegui and M.G. Vincentz., (2015). Short Communication An efficient method for simultaneous extraction of high-quality RNA

and DNA from various plant tissues. Gen. Mol. Res. 14:18828.

- [31] Park, J. R., Yang, W. T., Kwon, Y. S., Kim, H. N., Kim, K. M. and Kim, D. H. (2019). Assessment of the genetic diversity of rice germplasms characterized by black-purple and red pericarp color using simple sequence repeat markers. *Plants (Basel)* 8:471.
- [32] Prabha, S., Yada, A., Yadav, H. K., Kumar, S. and Kumar, R. (2017). Importance of molecular marker in linseed (*Linum usitatissimum*) genome analysis-A review. *Crop Res.* 52:61-66.
- [33] Rabara, R. C., Ferrer, M. C., Diaz, C. L., Newingham, M. C. V. and Romero, G. O. (2014). Phenotypic diversity of farmers' traditional rice varieties in the Philippines. *Agron* 4:217-41.
- [34] Rana, J. C., Negi, K. S., Wani, S. A., Saxena, S., Pradheep, K., Kak, A., Pareek, S. K. and Sofi, P. A. (2009). Genetic resources of rice in the Western Himalayan region of India: Current status. Genet. Resour. *Crop Evol*. 56:963-73.
- [35] Ravi M., Geethanjali S., Sameeyafarheen F., Maheswaran M (2003). Molecular Marker based Genetic Diversity Analysis in Rice (*Oryzasativa* L.) using RAPD and SSR markers. *Euphytica* 133:243-252.
- [36] Schiller, J. M., Hatsadong and Doungsila, K. (2006). A history of rice in Laos. In: Rice in Laos. (Schiller, J. M., Chanphengxay, M. B., Linquist, B. A., Appa Rao, S. Eds.). International Rice Research Institute, Los Banos, Philippines, pp.9-28.
- [37] Shaptadvipa B., Sarma N. R., (2009). Study on Apparent Amylose Content in Context of Polymorphism Information Content along with Indices of Genetic Relationship Derived through SSR Markers in Birain, Bora and Chokuwa Groups of Traditional Glutinous Rice (*Oryza* sativa L.) of Assam. Asian J. Biochem. 4:45-54.
- [38] Singh, N., Choudhry D. R., Tiwwari, G., Singh A. K., Kumar, S., Srinivasan, K., Tyagi R. K., Sharma, A. D., Singh, N. K. and Singh, R. (2016). Genetic diversity trend in Indian rice varieties: an analysis using SSR markers. *BMC Genet*. 17:127.
- [39] Sneath PA and Sokal RR (1973). Numerical taxonomy.W.H. Freeman Co, San Francisco, USA, pp. 179-241
- [40] Suksan Tonapha, Pusadee, Chanakan Prom-u-thai, Benjavan Rerkasem, and Sansanee Jamjod. (2021). Diversity of Purple Rice (Oryza sativa L.) Landraces in Northern Thailand. Agronomy 11(10): 2029.
- [41] Suvi, W. T., Shimelis, H., Laing, M., Mathew, I. and Shayanowako, A. I. T. (2019). Assessment of the genetic diversity and population structure of rice genotypes using SSR markers. *Acta Agric. Scand. B Soil Plant Sci.* 70:76-86.
- [42] Thanh Quynh Doan, Nguyen Thi Hao, Vu Thi Thu Hien, Tran Van Quang (2016). Analysis of Genetic Diversity in Local Sticky Rice Based on Morphological Characteristics and SSR Markers. *Vietnam J. Agri. Sci.* (14)4:527-538.
- [43] Tuoi Thi Hong Ngo, Pham Van Cuong, Nguyen Van Hoan (2014). Analysis of Genetic Diversity in Black Rice by SSR Markers. J. Sci. & Devel. 12(4):485-494.

ISSN: 2456-1878 (Int. J. Environ. Agric. Biotech.) https://dx.doi.org/10.22161/ijeab.101.11

- [44] Upadhyay P., Singh V. K., Neeraja C. N. (2011). Identification of genotype specific alleles and molecular diversity assessment of popular rice (*Oryza sativa* L.) varieties of India. *Int. J. Plant Breed. Genet.* 5(2):130-140.
- [45] Van Quang Tran, Thanh Quynh Doan, Thi Thu Hien Vu, Thi Huyen Tran and Thanh Tuan Nguyen (2021). Agromorphological characteristics and genetic diversity of glutinous rice (*Oryza sativa*) landraces in north Vietnam. *Res. on Crops* 22(1):1-9.
- [46] Verma, R. K., Chetia, S. K. Rahman, A., Dey, P. C., Sen, P. and Modi, M. K. (2017). Study on genetic diversity and population structure of upland rice accessions using SSR markers associated with grain yield under drought. *Crop Res.* 52:180-87.
- [47] Victoria C.L, Darshan S. Bar, Toshinori Abe, Edilberto D. Redona (2007).Ssessment of Genetic Diversity of Philippine Rice Cultivars Carring Good Quality trait using SSR marker. *Breeding Science* 57:263-270.
- [48] Vilayheuang, K., Machida-Hirano, R., Bounphanousay, C. and Watanabe, K. N. (2016). Genetic diversity and population structure of 'Khao Kai Noi", a Lao rice (*Oryza* sativa L.) landrace, revealed by microsatellite DNA markers. *Breed. Sci.* 66:204-12.
- [49] Virk, P. S., Newbury, J. H., Bryan, G. J., Jackson, M. T. and Ford-Lloyd, B. V. (2000). Are mapped or anonymous markers more useful for assessing genetic diversity. *Theor. Appl. Genet.* 100: 607-13.
- [50] Weir, B. S. (1996). Genetic data analysis II.. Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts, Sinauer Associates, pp-445.
- [51] Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. (1990). DNA polymorphism amplified by arbitrary primers is useful as genetic markers. *Nucleic Acids Res.* 18:6531-6535.
- [52] Wunna, W. K. N., Ohsawa, R., Obara, M., Yanagihara, S., Aung, P. P. and Fukuta, Y. (2016). Genetic variation of rice (*Oryza sativa* L.) germplasm in Myanmar based on genomic compositions of DNA markers. *Breed. Sci.* 66:762-67.