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Agro-Morphological Characters and PCR Based Markers for NEP NGU at Binh Dinh, Vietnam

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Abstract— *Nep Ngu rice is a valuable genetic of glutinous rice resource and cultural heritage with a long history of cultivation and utilization in Binh Dinh .A total of 102 traditional Nep Ngu varieties selectd at Binh Dinh province Vietnam, were used to explore this diversity using SSR markers and quantitative morphological characters. The study aims to evaluate the genetic diversity of Nep Ngu (Binh Dinh) varieties and involves molecular diversity analysis using 62 polymorphic SSR markers revealed among the 102 varieties. The Nep Ngu (Binh Dinh) varieties generated three clusters at 0.63 similarity coefficient. Some varieties with similar names were grouped into different clusters as molecular analysis showed that they were actually genetically different. The 102 Nep Ngu (Binh Dinh) varieties collected were evaluated phenotypically. In the analysis of quantitative traits, the range of coefficients of variability was high. It varied from 130.74-93.05 % (filled grain) to 30.38–18.16% (unfilled grain). This shows that these traits can be considered most stable as exemplified by their coefficients of variability. The highest values seen in unfilled grain indicate that this character is more affected by the environment and farmers' cultural management practices. The mean values of quantitative trait measurements were higher (137.87–155.70 cm). The highest values noted in yield (36.33–56.52 g).Looking at agro-morphology, ANOVA showed highly significant differences among the 102 traditional rice varieties. The standardized Shannon-Weaver diversity indices for the quantitative morphological characters ranged from 0.68 to 0.89 with a mean of H' = 0.79. Cluster analysis using UPGMA grouped the 102 traditional varieties into 3 major clusters. Varieties collected with two lines good for aroma with Line 81 and line 52. Sequence of Nep Ngu 52; Nep Ngu 81 were submitted to GenBank with accession number OR880900 and OR880901 respectively.*

Keywords— ***Coefficients of variability, molecular analysis, DNA dequencing, quantitative morphological characters, NepNgu varieties.***

I. INTRODUCTION

Nep Ngu selected at Binh Dinh called landraces (glutinous rice)or local varieties or farmers, form the foundation for building better rice crops. Landraces are often considered a rich source of genetic variation. Moreover, local varieties offer farmers alternatives in areas where modern rice varieties are not well adapted and contribute to diversity at the field level. However, the number of traditional varieties grown has declined, with a number of relatively uniform high-yielding and high-yielding varieties dominating the rice landscape. Genetic diversity is fundamental to the survival of a species. Recombinant processes and gene mutations provide continuous input to new variants, as well as processes of environmental adaptation and random drift shape the distribution of genetic diversity in time and space (Brown et al., 1989).

The easiest and most common tool for assessing genetic diversity is to measure differences in morphological traits or phenotypes. Farmers use certain phenotypic features of plants for selection and identification. Thus, morphological traits are associated with genetic diversity, and the naming of these varieties suggests that farmers have some understanding of the genetic diversity of crops grown in their fields (Jarvis et al., 2000). Recent advances in molecular biology, primarily the development of polymerase chain reaction (PCR) for DNA amplification, DNA sequencing, and data analysis have led to powerful techniques that can be used to screen, characterize, and assess genetic diversity.

Characterization and evaluation of diversity among traditional varieties will provide plant breeders with the information needed in determining the starting materials for breeding to produce varieties with improved yield and quality. This article is: assessing the genetic diversity of traditional rice varieties in the gene bank of Binh Dinh glutinous, VietNam using morphological characteristics and microscopic markers; To study correlations between letters for application in plant breeding, and to relate results between morphological features and molecular markers.

With molecular marker techniques, powerful tools have been developed so that gene sources can be accurately assessed and characterized. Several types of molecular markers are available to assess the degree of genetic variation in rice (Ni et al., 2002). These include limited segment length polymorphism (RFLP) (Botstein et al., 1980), randomly amplified polymorphic DNA (RAPD) (Williams et al., 1990), amplified piece-length polymorphism (AFLP) (Vos et al., 1995), Many researchers evaluated genetic diversity of Indian rice germplasm using other SSRs, but SSRs from UCGM have not been used for studying diversity previously (Yadav et al. 2013). Studies were also conducted globally on classifying rice genotypes based on their genetic diversity and population structure using molecular markers (Shinada et al. 2014). and microsatellites or simple sequence repeaters (SSR) (Lang et al., 2009, Lang et al 2014). SSR markers, also called DNA microsatellites, are regions of DNA (often forming part of the non-coding regions) where sequences of one to five nucleotides are repeated, and they are uniformly distributed in the genomes of most eukaryotes. The SSR sequences found in plants are frequently made up of AT and GA nucleotide repeats(Jae-Ryoung et al.,2019).The InDel organelle markers identified 70% of the purple rice landraces in this study as tropical *japonica*, 8% as temperate *japonica*, and 22% as *indica* (Suksan et al.,2021) Characterization and evaluation of diversity among traditional varieties will provide plant breeders with the information needed in determining the starting materials for breeding to produce varieties with improved yield and quality. This article is: assessing the genetic diversity of traditional glutinous rice varieties in the gene bank of Binh Dinh glutinous using morphological characteristics and microsatellite markers; correlation research for traits . This information is expected to be useful in the germplasm management and enable breeders selected promising accessions for glutinous rice improvement efforts by plant breeding program.

II. MATERIALS AND METHODS

Plant Materials

A total of 102 accessions of traditional varieties collected from Binh Dinh, Vietnam, conserved in genebank of HATRI in Vietnam were used. Passport information of these accessions is presented in Table 1.

Agro-morphology- Based Diversity Analysis

One hundred traditional varieties were planted in the field at the HATRI Vietnam during the wet season 2022-2023. Seeds were sown in raised seed beds and 18-21day old seedlings were transplanted at one seedling per hill. Rice transplantation were established at distances of 15 x 20 cm. The standard cultural management practices for rice were followed (Bui, 1986).

Data Collection

Data were collected for quantitative traits following the Descriptors for Rice *Oryza sativa* L. (IBPGR–IRRI Advisory Committee, 1980). The following is the list of morphological and agronomic traits and the number of samples that were measured to assess diversity and relationships of the different rice accessions.

Table 1. Passport information of the 102 traditional varieties used in the study.

NO	ACCESSION	NAME OF VARIETY	PASSPORT INFORMATION	NO	ACCESSION	NAME OF VARIETY	PASSPORT INFORMATION
1	1	Nếp Ngự (01)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229	13	13	Nếp Ngự (13)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of

							108°59'30.57"E or 14.443229, saline soil
2	2	Nếp Ngự (02)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229	14	14	Nếp Ngự (14)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229 00 N, 100 00 E
3	3	Nếp Ngự (03)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229	15	15	Nếp Ngự (15)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229
4	4	Nếp Ngự (04)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229	16	16	Nếp Ngự (16)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229
5	5	Nếp Ngự (05)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229	17	17	Nếp Ngự (17)	Wetland rice, Thailand, 15 00 N, 100 00 E
6	6	Nếp Ngự (06)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229	18	18	Nếp Ngự (18)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229 alluvial soil
7	7	Nếp Ngự (07)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229	19	19	Nếp Ngự (19)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229 00 N, 100 00 E
8	8	Nếp Ngự (08)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229	20	21	Nếp Ngự (20)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229 ', alluvial soil
9	9	Nếp Ngự (09)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229	21	22	Nếp Ngự (21)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229 alluvial soil
10	375	Nếp Ngự (10)	Ap Binh Dinh city of country Vietnam lies on the geographical coordinates of 10° 10' 0" N, 106° 1' 58" E. Latitude and Longitude of the Ap Binh Dinh city.	22	23	Nếp Ngự (22)	Lua nuoc troi, Longan, Vietnam, $105^0$30' 30"-$106^0$47' 02" longitude and $10^0$23'40"-$11^0$02' 00" latitude, alluvial soil
11	11	Nếp Ngự (11)	Ap Binh Dinh city of country Vietnam lies on the geographical coordinates of 10° 10' 0" N, 106° 1' 58" E. Latitude and Longitude of the Ap Binh Dinh city.	23	26	Nếp Ngự (23)	Ap Binh Dinh city of country Vietnam lies on the geographical coordinates of 10° 10' 0" N, 106° 1' 58" E. Latitude and Longitude of the Ap Binh Dinh city.
12	12	Nếp Ngự (12)	Ap Binh Dinh city of country Vietnam lies on the geographical coordinates of 10° 10' 0" N, 106° 1' 58"	24	27	Nếp Ngự (24)	Ap Binh Dinh city of country Vietnam lies on the geographical coordinates of 10° 10' 0" N, 106° 1' 58"

			E. Latitude and Longitude of the Ap Binh Dinh city.				E. Latitude and Longitude of the Ap Binh Dinh city.

Table 2. Continued…

NO	ACCESSION	NAME OF VARIETY	PASSPORT INFORMATION	NO	ACCESSION	NAME OF VARIETY	PASSPORT INFORMATION
25	25	Nếp Ngự (25)	Huyện Hoài Nhơn. Latitude, 14.50535000. Longitude, 109.02315000.	38	38	Nếp Ngự (38)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229
26	26	Nếp Ngự (26)	Huyện Hoài Nhơn. Latitude, 14.50535000. Longitude, 109.02315000.	39	39	Nếp Ngự (39)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229
27	27	Nếp Ngự (27)	Huyện Hoài Nhơn. Latitude, 14.50535000. Longitude, 109.02315000.	40	40	Nếp Ngự (40)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229
28	28	Nếp Ngự (28)	Huyện Hoài Nhơn. Latitude, 14.50535000. Longitude, 109.02315000.	41	41	Nếp Ngự (41)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229
29	29	Nếp Ngự (29)	Huyện Hoài Nhơn. Latitude, 14.50535000. Longitude, 109.02315000.	42	42	Nếp Ngự (42)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229
30	30	Nếp Ngự (30)	Huyện Hoài Nhơn. Latitude, 14.50535000. Longitude, 109.02315000.	43	43	Nếp Ngự (43)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229, 100 00 E
31	37	Nếp Ngự (11)	Huyện Hoài Nhơn. Latitude, 14.50535000. Longitude, 109.02315000.	44	376	Nếp Ngự (44)	Hoài Nhơn District, has a latitude of 14°26'35.62"N and a longitude of 108°59'30.57"E or 14.443229 parallels of northern latitude
32	32	Nếp Ngự (32)	Hoài Ân is a district (huyện) of Bình Định Province in the South Central Coast region of Vietnam. Latitude: 14° 25' 1.20" N Longitude: 108° 49' 58.80" E.	45	45	Nếp Ngự (45)	Hoài Ân is a district (huyện) of Bình Định Province in the South Central Coast region of Vietnam. Latitude: 14° 25' 1.20" N Longitude: 108° 49' 58.80" E. soil
33	33	Nếp Ngự (33)	Hoài Ân is a district (huyện) of Bình Định Province in the South Central Coast region of Vietnam. Latitude: 14° 25' 1.20" N Longitude: 108° 49' 58.80" E.	46	46	Nếp Ngự (46)	Hoài Ân is a district (huyện) of Bình Định Province in the South Central Coast region of Vietnam. Latitude: 14° 25' 1.20" N Longitude: 108° 49' 58.80" E.” latitude
34	34	Nếp Ngự (34)	Hoài Ân is a district (huyện) of Bình Định Province in the South Central Coast region of Vietnam. Latitude: 14° 25'	47	47	Nếp Ngự (47)	Hoài Ân is a district (huyện) of Bình Định Province in the South Central Coast region of Vietnam. Latitude: 14° 25'

			1.20" N Longitude: 108° 49' 58.80" E.				1.20" N Longitude: 108° 49' 58.80" E. soil
35	35	Nếp Ngự (35)	Hoài Ân is a district (huyện) of Bình Định Province in the South Central Coast region of Vietnam. Latitude: 14° 25' 1.20" N Longitude: 108° 49' 58.80" E.	48	48	Nếp Ngự (48)	Hoài Ân is a district (huyện) of Bình Định Province in the South Central Coast region of Vietnam. Latitude: 14° 25' 1.20" N Longitude: 108° 49' 58.80" E. soil
36	36	Nếp Ngự (36)	Hoài Ân is a district (huyện) of Bình Định Province in the South Central Coast region of Vietnam. Latitude: 14° 25' 1.20" N Longitude: 108° 49' 58.80" E	49	49	Nếp Ngự (49)	Hoài Ân is a district (huyện) of Bình Định Province in the South Central Coast region of Vietnam. Latitude: 14° 25' 1.20" N Longitude: 108° 49' 58.80" E
37	37	Nếp Ngự (37)	Hoài Ân is a district (huyện) of Bình Định Province in the South Central Coast region of Vietnam. Latitude: 14° 25' 1.20" N Longitude: 108° 49' 58.80" E	50	50	Nếp Ngự (50)	Hoài Ân is a district (huyện) of Bình Định Province in the South Central Coast region of Vietnam. Latitude: 14° 25' 1.20" N Longitude: 108° 49' 58.80" E latitude

Table 3. Continued…

NO	ACCESSION	NAME OF VARIETY	PASSPORT INFORMATION	NO	ACCESSION	NAME OF VARIETY	PASSPORT INFORMATION
51	378	Nếp Ngự (51)	Hoài Ân is a district (huyện) of Bình Định Province in the South Central Coast region of Vietnam. Latitude: 14° 25' 1.20" N Longitude: 108° 49' 58.80" E.	63	299	Nếp Ngự (63)	Hoài Ân is a district (huyện) of Bình Định Province in the South Central Coast region of Vietnam. Latitude: 14° 25' 1.20" N Longitude: 108° 49' 58.80" E.
52	52	Nếp Ngự (52)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60").	64	64	Nếp Ngự (64)	Hoài Ân is a district (huyện) of Bình Định Province in the South Central Coast region of Vietnam. Latitude: 14° 25' 1.20" N Longitude: 108° 49' 58.80" E. longitude
53	53	Nếp Ngự (53)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")	65	65	Nếp Ngự (65)	Hoài Ân is a district (huyện) of Bình Định Province in the South Central Coast region of Vietnam. Latitude: 14° 25' 1.20" N Longitude: 108° 49' 58.80" E.
54	54	Ngếp Ngự (54)	Latitude and Longitude of Vietnam; Binh Dinh / An Nhon, 13°55'N · 109°07'E;	66	66	Nếp Ngự (66)	Hoài Ân is a district (huyện) of Bình Định Province in the South Central Coast region of Vietnam. Latitude: 14° 25' 1.20" N Longitude: 108° 49' 58.80" E.
55	55	Nếp Ngự (55)	Latitude and Longitude of Vietnam; Binh Dinh / An Nhon, 13°55'N · 109°07'E; latitude, alluvial soil	67	67	Nếp Ngự (67)	Hoài Ân is a district (huyện) of Bình Định Province in the South Central Coast region of Vietnam. Latitude: 14° 25' 1.20" N Longitude: 108° 49' 58.80" E.

56	56	Nếp Ngự (56)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")	68	68	Nếp Ngự (68)	Hoài Ân is a district (huyện) of Bình Định Province in the South Central Coast region of Vietnam. Latitude: 14° 25' 1.20" N Longitude: 108° 49' 58.80" E. latitude
57	57	Nếp Ngự (57)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")	69	69	Nếp Ngự (69)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60") latitude
58	58	Nếp Ngự (58)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")	70	70	Nếp Ngự (70)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")
59	59	Nếp Ngự (59)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")	71	71	Nếp Ngự (71)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")
60	60	Nếp Ngự (60)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")	72	72	Nếp Ngự (72)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")
61	61	Nếp Ngự (61)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")	73	73	Nếp Ngự (73)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")
62	62	Nếp Ngự (62)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")	74	74	Nếp Ngự (74)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")

Table 4. Continued…

NO	ACCESSION	NAME OF VARIETY	PASSPORT INFORMATION	NO	ACCESSION	NAME OF VARIETY	PASSPORT INFORMATION
75	75	Nếp Ngự (75)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")	83	83	Nếp Ngự (83)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")
76	76	Nếp Ngự (76)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")	84	84	Nếp Ngự (84)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")
77	77	Nếp Ngự (77)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")	85	85	Nếp Ngự (85)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")
78	78	Nếp Ngự (78)	Latitude: 14° 9' 59.5152". Longitude: 108° 54' 9.6588". Latitude: N 14° 9.9919'. Longitude: E 108° 54.161'. Latitude: 14.166532°. Longitude	86	86	Nếp Ngự (86)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")
79	79	Nếp Ngự (79)	Latitude: 14° 9' 59.5152". Longitude: 108° 54' 9.6588".	87	87	Nếp Ngự (87)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60") latitude

80	80	Nếp Ngự (80)	Latitude: 14° 9' 59.5152". Longitude: 108° 54' 9.6588".	88	88	Nếp Ngự (88)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")
81	81	Nếp Ngự (81)	Latitude: 14° 9' 59.5152". Longitude: 108° 54' 9.6588".	89	89	Nếp Ngự (89)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")
82	82	Nếp Ngự (82)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")	90	90	Nếp Ngự (90)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60")

Table 5. Continued....

NO	ACCESSION	NAME OF VARIETY	PASSPORT INFORMATION	NO	ACCESSION	NAME OF VARIETY	PASSPORT INFORMATION
91	91	Nếp Ngự (91)	Latitude: 14° 9' 59.5152". Longitude: 108° 54' 9.6588".	97	97	Nếp Ngự (97)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60"), saline soil
92	92	Nếp Ngự (92)	Latitude: 14° 9' 59.5152". Longitude: 108° 54' 9.6588". Longitude: E 108° 54.161'. Latitude: 14.166532°. Longitude	98	98	Nếp Ngự (98)	Hoài Nhơn District, Bình Định Province, Vietnam (N 14° 26' 24", E 109° 5' 60") 00 N, 100 00 E
93	93	Nếp Ngự (93)	Latitude and longitude coordinates: 109.04532, 14.4666386	99	99	Nếp Ngự (99)	Latitude: 14° 9' 59.5152". Longitude: 108° 54' 9.6588". Latitude: N 14° 9.9919'. Longitude: E 108° 54.161'. Latitude: 14.166532°. Longitude
94	94	Nếp Ngự (94)	Lua nuoc troi, Latitude and longitude coordinates: 109.04532, 14.4666386	100	100	100 (Nếp Ngự Quảng Ngãi)	District on the north. Latitude: 15° 06' 60.00" N Longitude: 108° 47'
95	95	Nếp Ngự (95)	Lua nuoc troi, Latitude and longitude coordinates: 109.04532, 14.4666386	101	101	IR 29	IRRI
96	96	Nếp Ngự (96)	Latitude and longitude coordinates: 109.04532, 14.4666386	102	102	HATRI 04 nếp	HATRI
97	97	Nếp Ngự (97)	Latitude and longitude coordinates: 109.04532, 14.4666386				

Quantitative traits

1. Panicle length (cm)-length of panicle at maturity measured from the base to the tip of the panicle (from 10 randomly selected primary panicles per accession per replication).
2. Panicles per plant (number)-total number of panicles per plant (from 10 randomly selected primary panicles per accession per replication).
3. 1000-grain weight (gram)-weight of 1000 well-developed grains at 14% MC (from 5 randomly selected primary panicles per accession per replication).
4. Days to maturity-days from seeding when 80% of the grains are fully ripened on a per replication basis.
5. Filled grains (number)-obtained from counts of total number of filled grains per panicle (from 5 randomly selected primary panicles per accession per replication).
6. Unfilled grains (number) - obtained from counts of total number of unfilled grains per panicle (from 5

randomly selected primary panicles per accession per replication).

7. Yield-obtained from the harvested plants in each replication. Harvested grains were threshed, cleaned, dried, and weighed for each accession per replication. Moisture content (MC) per plot was determined immediately after weighing using a moisture meter.

   Yield = wt. of harvest (g)/ no. of hills harvest x no. of possible hills x MF

   where: $MF = \frac{100 - MC}{100}$ of the harvest grains

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

Data Analysis

Analysis of variance. The agro-morphological data collected were initially analyzed through analysis of variance to verify genetic variation in the traits measured. The few traits with insignificant genetic variation, based on the F-test, were not considered for further analyses.

Shannon-Weaver diversity Index. Diversity indices for the various traits were computed using the following formula:

$$H' = \frac{-\sum pi * \log_2(pi)}{\log_2 n}$$

where: *n* is the number of phenotypic classes for a character and

pi is the portion of the total number of entires belonging to the i class. The Shannon -Weaver diversity index was standardized by dividing H' by the $\log_2$ of the total number of phenotypic classes. To have an estimate of the phenotypic diversity of the varieties, H' was computed in the MS-Excel for each of the morpho-agronomic descriptors. The mean phenotypic diversity index was computed for the pooled diversity estimates per descriptor. The standardized value ranged from 0 to 1, with 1 indicating maximum diversity.

Correlation analysis. Correlation coefficient (*r*) is a measure of the association between two or more variables. It is a measure of symmetrical association between variables and does not measure the dependence of one variable over other. Correlation among agro-morphological traits was calculated by using SAS program.

Distance matrix. Distance matrix was calculated by means of Euclidean Distance Coefficient (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.

Cluster analysis. Cluster analysis was carried out for agro-morphology–based genetic distance matrix using UPGMA clustering method in the NTSYS program. The results of the UPGMA were used to draw the dendrogram of the 102 traditional varieties.

Principal component analysis. The main function of PCA was to explain variance with the linear combination of the variables. Principal component analysis was done using NTSYS and SAS programs.

Molecular-based characterization and analysis using SSR DNA extraction. The 102 varieties were grown in pots. Maximum protection was employed to ensure healthy and disease-free seedlings. The leaves were collected 2-3 weeks after planting for DNA extraction. Standard molecular grade chemicals and general techniques for preparing stock solutions, buffers, reagents, and equipment were followed according to Lang 2002. Molecular work was conducted at the Genetics and Plant Breeding Department of the Hight Aricultural technology Insitute, Cantho, Vietnam (HATRI). DNA suitable for PCR analysis was prepared using a simplified procedure (McCouch et al., 1988). A piece of a young rice leaf (2 cm) was collected and placed in a labeled 1.5 ml centrifuge tube in ice. The leaf was ground using a polished glass rod in a well of a spot test plate (Thomas Scientific) after adding 400 µl of extraction buffer. Grinding was done until the buffer turned green, an indication of cell breakage and release of chloroplasts and cell contents. Another 400 µl of extraction buffer was added into the well by pipetting. Around 400 µl of the lysate was transferred to the original tube of the leaf sample. The lysate was deproteinized using 400 µl of chloroform. The aqueous supernatant was transferred to a new 1.5 ml tube and DNA was precipitated using absolute ethanol. DNA was air-dried and resuspended in 50 µl of TE buffer (Lang., 2002). DNA quality checks used 1% agarose by melting 3 g of agarose in 300 ml of TAE buffer. The mixture was heated in a microwave for 5-6 min and then cooled to around 55-60 0 C. This was then poured on a previously prepared electrophoresis box with combs. Gels were prepared and the combs removed after about 45 min. Seven microliters of DNA sample plus 3 µl of loading buffer (Tris 1 M pH = 8.0, glycerol, EDTA 0.5 M pH = 8.0, xylene cyanol 0.2%, bromphenol blue 0.2%, and distilled

water) was run at 70-80 v, 60 mA for 45 min or until the loading buffer dye moved far away from the wells. The gel was then taken out and stained with ethidium bromide, after which it was observed under UV light.

Microsatellite analysis :the whole microsatellite analysis included PCR assay, polyacrylamide gel electrophoresis, and band detection and scoring. PCR assay Microsatellite primers were used to survey polymorphism on the samples. These were randomly selected from the 135 microsatellite primer pairs currently available for rice (Temnykh et al., 2000).

The PCR reaction was as follows: Reactions were overlaid with mineral oil and processed in a programmable thermal controller set for 35 cycles of 1 min at 94 0 C, 1 min at 55 0 C, and 2 min at 72 0 C, with a final extension at 75 0 C for 5 min. After amplification, 10 µl of stop solution was added to the PCR product, which was then denatured at 94 0 C for 2 min. Eight microliters of each reaction were run on polyacrylamide gel. Band detection and scoring Plates were separated using a plastic wedge and were removed from the tank. The acrylamide gel was soaked in ethidium bromide staining solution for 15 to 20 min. Bands in the ethidium bromide-stained gels were detected and photographed under UV light. Allelic bands were scored as 1 (present) or 0 (absent), respectively. 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. These coefficients were calculated using Nei and Li's distance measure (Nei and Li, 1979) in the NTSYS–PC Numerical Taxonomy and Multivariate Analysis System (Rohlf, 1990). The lines were clustered on the basis of similarity coefficients using the unweighted pair group method-arithmetic average (UPGMA) clustering algorithm.

III. RESULTS AND DISCUSSION

Polymorphism of microsatellite markers

To overcome this, an assessment of genetic diversity of initial material sources is necessary. PCR amplification was performed with DNA samples extracted from 102 traditional Nep Ngu rice varieties. Several representative DNA samples were used as template in the PCR amplification reaction using SSR markers as 135 primers on 12 chromosome , but only 62 primers were polymorphic. Amplified PCR products were electrophoresed on 3% agarose gel with 1X TBE buffer solution, stained with ethidium bromide, then observed under UV-transilluminator. In the amplification of genomic DNA of the 102 rice genotypes using 135 primers, 62 were found to be polymorphic. The number of amplified fragments ranged from 2 to 4. All of the primer pairs used in this study generated polymorphic bands among the genotypes. A total of 62 loci were assigned to the 62 microsatellite primer pairs. A total of 178 alleles were detected among the 102 rice genotypes with an average of 1.26 alleles per locus (Table 3). The number of alleles per locus ranged from 2 to 5 (in RM11125). The total alleles identified in the 102 genotypes were classified into 3 categories: The PIC values for the microsatellite loci ranged from 0.16 to 0.87 with an average of 0.57 (Table 2). The low PIC values were observed among the primers of RM228 (0.16) RM26212(0.22); the PIC value high such as primers S11049 (0.74), RM115 (0.87). High PIC values indicate that the selected microsatellite markers are efficient at evaluating a large number of genetic resources. In this study, the mean PIC value of the microsatellite markers used to assess the diversity of rice genetic resources was 0.57, which was much lower than the PIC values of microsatellite markers used in other studies (Surapaneni et al .,2016; Jae-Ryoung et al.,2019).

A dendrogram based on cluster analysis using UPGMA with the module of SAHN in the NTSYS-pc package was created. Cluster analysis showed significant genetic variation among the landrace rice varieties studied, with genetic distance ranging from 0 to 0.84 (Figure 1). With a genetic distance of 0.63, the cluster revealed 3 major groups, A, B, and C, in the Binh Dinh Nep Ngu rice varieties. Group A was divided into sub-clusters A1 and A2 (27%); Group B and Group C (70.58%); and Group C consisted of 3 traditional varieties (2.94%) such as Line 52 , Line 81 and line 44.

Table 2: Primers and Chromosome, PIC values for survival 102 varieties from Binh Dinh VietNam.

STT	Primer	Chromosome location	No. of allele	Size (bp)	PIC VALUES
1	RM228	**9**	**2**	**240-250**	**0.16**
2	**RM26212**	**4**	**2**	**200-220**	**0.22**
3	RM243	**1**	**2**	**190-210**	**0.41**
4	RM10649	**1**	**2**	**180-210**	**0.45**
5	RM24	**1**	**3**	**200-205**	**0.63**
6	RM7643	**1**	**3**	**205-220**	**0.66**
7	RM472	**1**	**3**	**210-242**	**0.64**

8	RM11125	**1**	**5**	**160-200**	**0.79**
9	RM10843	**1**	**4**	**180-200**	**0.73**
10	RM3412b	**1**	**3**	**190-200**	**0.64**
11	RM10793	**1**	**3**	**210-220**	**0.63**
12	Salt 1	**1**	**4**	**200-220**	**0.74**
13	Salt 2	**1**	**2**	**210-220**	**0.45**
14	RM 152	**8**	**3**	**175-200**	**0.63**
15	RM5806	**10**	**3**	**210-230**	**0.66**
16	RM110	**11**	**2**	**230-210**	**0.67**
17	RM211	**2**	**3**	**200-250**	**0.65**
18	RM17	**12**	**5**	**160-190**	**0.79**
19	RM310	**8**	**4**	**200-210**	**0.72**
20	RM27877	**12**	**3**	**215-240**	**0.63**
21	RM221	**2**	**3**	**220-230**	**0.66**
22	RM28746	**12**	**3**	**200-210**	**0.63**
23	RM5436	**7**	**4**	**200-210**	**0.73**
24	RM3867	**3**	**4**	**210-230**	**0.74**
25	RM6329	**3**	**3**	**220-230**	**0.61**
26	RM249	**5**	**3**	**210-230**	**0.56**
27	RM5626	**3**	**5**	**200-210**	**0.78**
28	RM18	**7**	**3**	**190-200**	**0.64**
29	RM21	**11**	**5**	**210-220**	**0.27**
30	RM163	**5**	2	**255-260**	**0.45**
31	S11049	**11**	4	**200-210**	**0.74**
32	RM140	**1**	3	**190-200**	**0.61**
33	RM169	**5**	4	**240-250**	**0.73**
34	RM9	**1**	2	**230-240**	**0.49**
35	RM10852	**1**	3	**220-230**	**0.64**
36	RM10890	**1**	3	**205-210**	**0.66**
37	RM10927	**1**	2	**240-245**	**0.40**
38	RM154	**2**	2	**160-180**	**0.45**
39	RM231	**3**	3	**200-210**	**0.67**
40	RM21539	**7**	2	**205-210**	**0.45**
41	RM122	**5**	3	**205-230**	**0.64**
42	RM510	**6**	2	**220-230**	**0.42**
43	RM547	**8**	2	**200-210**	**0.49**
44	RM23662	**9**	3	**210-220**	**0.64**
45	RM219	**9**	3	**200-215**	**0.65**
46	RM24013	**9**	2	**215-220**	**0.42**
47	RM3	**6**	2	**220-225**	**0.50**
48	RM223	**8**	2	**200-210**	**0.46**
35	RM10852	**1**	3	**220-230**	**0.64**
36	RM10890	**1**	3	**205-210**	**0.66**

37	RM10927	**1**	2	**240-245**	**0.40**
38	RM154	**2**	2	**160-180**	**0.45**
39	RM231	**3**	3	**200-210**	**0.67**
40	RM21539	**7**	2	**205-210**	**0.45**
41	RM122	**5**	3	**205-230**	**0.64**
42	RM510	**6**	2	**220-230**	**0.42**
43	RM547	**8**	2	**200-210**	**0.49**
44	RM23662	**9**	3	**210-220**	**0.64**
45	RM219	**9**	3	**200-215**	**0.65**
46	RM24013	**9**	2	**215-220**	**0.42**
47	RM3	**6**	2	**220-225**	**0.50**
48	RM223	**8**	2	**200-210**	**0.46**
49	RM315	**1**	2	**210-230**	**0.49**
50	RM13	**5**	**3**	**190-210**	**0.63**
51	RM166	**2**	**3**	**190-200**	**0.65**
52	RM140	**1**	**3**	**200-210**	**0.63**
53	RM220	**1**	**3**	**210-220**	**0.64**
54	RM227	**3**	**3**	**200-220**	**0.65**
55	RM148	**3**	**2**	**190-210**	**0.43**
56	**FMU1-2**	**8**	**3**	190-210bp	**0.52**
57	**RM115**	**6**	**2**	**210-300**	**0.87**
58	**Indel 5**	**7**	**2**	**213-250**	**0.23**
59	**RM106**	**2**	**2**	**300-350**	**0.56**
60	**RM244**	**10**	**3**	**250-230**	**0.31**
61	**RM105**	**9**	**2**	**210-215**	**0.27**
62	RM10115	**1**	**2**	**240-250**	**0.72**
	Total		**178**		**0.57**

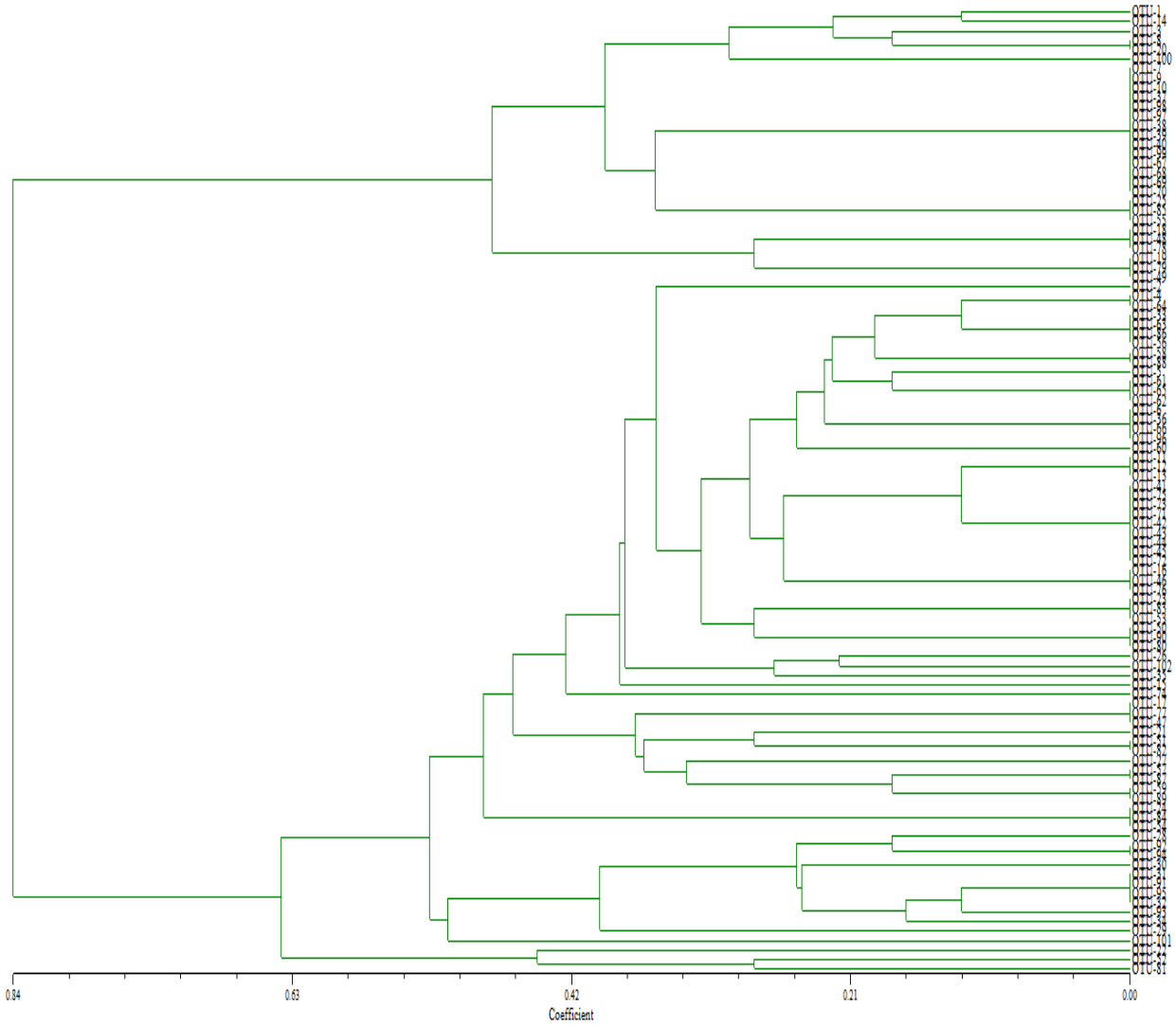

Fig 1. Classification of rice varieties based on genetic distance calculated from 62 microsatellite markers of 102 rice varieties

Table 3: Mean number of alleles based on microsatellite markers on different rice chromosomes

Group	Sub group	Mean of allele No. per SSR markers												**Mean**
		Chromosome												
		1	2	3	4	5	6	7	8	9	10	11	12	
A	1	1.23	1.56	1.42	0	1.42	0.92	1.74	1.17	1.24	1.53	1.82	1.48	1.29
	2	1.35	1.72	1.39	0	1.56	0.83	1.65	1.03	1.3	1.83	1.83	1.62	1.34
	Mean	1.29	1.64	1.4	0	1.49	0.87	1.69	1.1	1.27	1.68	1.83	1.55	1.31
B	1	1.52	1.68	1.65	0	1.74	1.12	1.78	1.46	1.75	1	2.92	1.64	1.52
	2	1.27	1.77	1.53	0	1.52	0.89	1.08	1.17	1.65	1.28	2.19	1.78	1.34
	3	1.33	1.25	1.58	0	1.65	0.1	1.55	1.32	1.42	1.79	2.36	1.98	1.36
	Mean	*1.37*	*1.56*	*1.58*	*0*	*1.63*	*0.7*	*1.47*	*1.31*	*1.6*	*1.36*	*2.49*	*1.8*	*1.4*

C	1	1.74	1.56	1.65	1.2	1.76	1.11	1,63	1.47	1.12	1.58	2.8	1.56	1.59
	Mean	1.74	1.56	1.65	1.2	1.76	1.11	1,63	1.47	1.12	1.58	2.8	1.56	1.59
Total	*Mean*	1.46	1.58	1.54	1.2	1.62	0.89	1.58	1.29	1.33	1.54	2.37	1.63	1.43

Mean of allele number per locus and each chromosome reveal much lower in the 1.43 (Table 3). The mean of allele number per locus group A is 1.34. The mean number of alleles per locus observed was group B is 1.40 similar with sub group C is 1.59.

Table 4. Mean and range of different quantitative traits used in measuring genetic distances among 102 landrace varieties.

	Max	**Min**	**Mean**	**CV**	**P**	**h^2**
Plant height (cm)	155.70	120.05	137.87	0.75	**	0.74
No. of panicle/ hill	30.22	5.69	15.89	3.07	**	0.68
Panicle length (cm)	28.06	17.19	22.62	3.17	**	0.81
Percentage of fertile grain (%)	130.74	55.35	93,05	0.56	**	0.71
Percentage of unfertile grain (%)	30.38	5.94	18.16	0.73	**	0.89
1000grain Weight (gram)	30.77	20.47	25.62	2.00	**	0.84
Duration (days)	155.78	120.00	139.39	0.52	**	0.84
Biomas(gram)	100.00	16.00	58.33	1.23	**	0.87
Yield (gram/hill)	56.52	16.15	36.33	1.40	**	0.89

Morphological table diversity analysis, using the analysis of agromorphological features of variance. For each of the 10 quantitative characteristics, the mean, range (maximum and minimum), standard deviation, coefficient of variation (CV), mean standard error, and F-value were calculated (Table 4).

Frequency distribution of the varieties with respect to maturity, panicles per plant, number of filled grains, number of unfilled grains, 1000-g weight, yield, biomass harvest index and survival day affters stress NaCl showed the diversity of traditional varieties. These quantitative characters were found to be significant at 1% and all measurements were not too far from normal distribution (Figures 2a-h).

Hight plant showed normal distributions (figure 2a). Distribution of varieties for the number of filled grains was slightly skewed to the right with only a few varieties near the maximum value (Figure 2d) while distribution of varieties for the number of unfilled grains was slightly skewed to the left with only a few varieties near the maximum value Figure figure 2e. For traits like 1000 grain weight, yield and panicles per plant, unimodal distributions were observed with most varieties skewed to the left of the curve. Such distribution is favorable particularly with respect to number of unfilled grains because lower number of unfilled grains would mean higher yield. This is an important objective for most plant breeders in improving present day varieties.

Yield showed near normal distributions, was slightly skewed to the right with only a few varieties near the maximum value (Figures 2h). With regards to maturity, almost half of the varieties investigated exhibited long maturity duration. Analysis of variance (ANOVA) showed high variability among the varieties in terms of number of unfilled grains, yield, number of filled grains, The results showed that most quantitative characteristics vary widely. For maturity, the earliest maturity genotype matures after 130 days while the maximum number of days to maturity is 140 days. The maximum value obtained in terms of yield (56.52g / hill).

With regard to maturation, most varieties mature in 130-140 days. The challenge still exists for breeders to develop shorter duration varieties without sacrificing yields. In general, morphological characteristics show that most traditional varieties have a higher number of filled seeds, a lower number of unfilled seeds, late maturity, tall with wider leaves, a higher weight of 1000 grain, and late maturity. The variation in agricultural morphological features discussed above can be explained by genetic variability between the tested varieties. This change can be used as a raw material for plant breeders to improve rice for better crop grade, better grain quality, and higher photosynthesis efficiency(Lang et al 2014).

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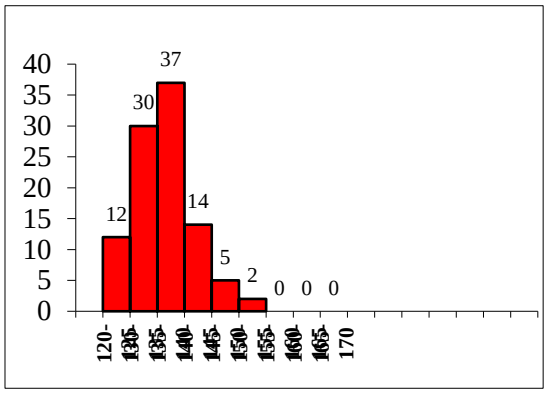
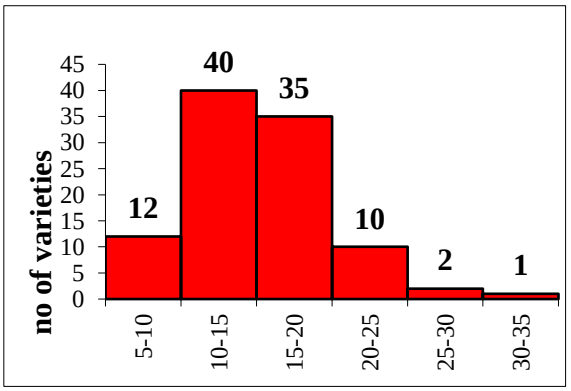
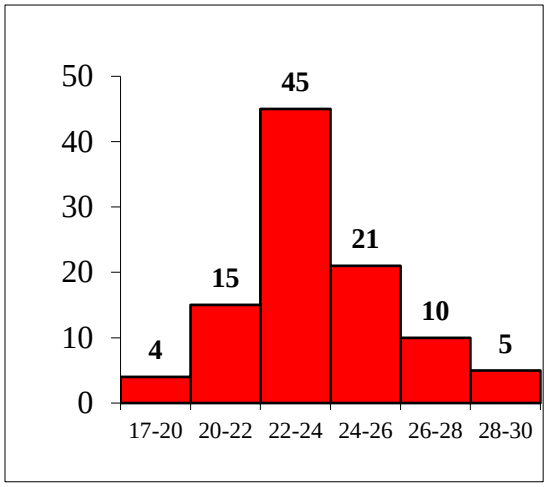


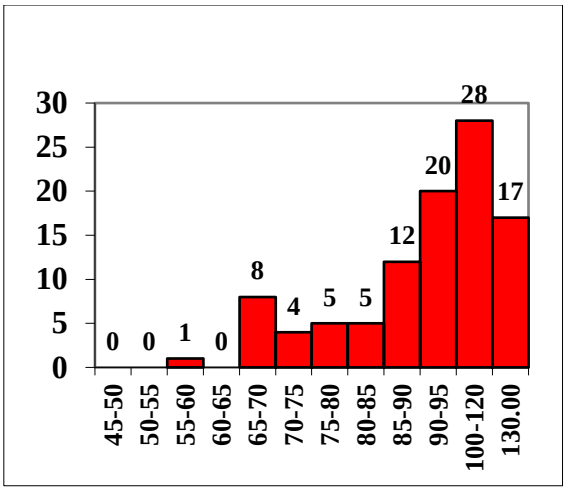
a. Plant height



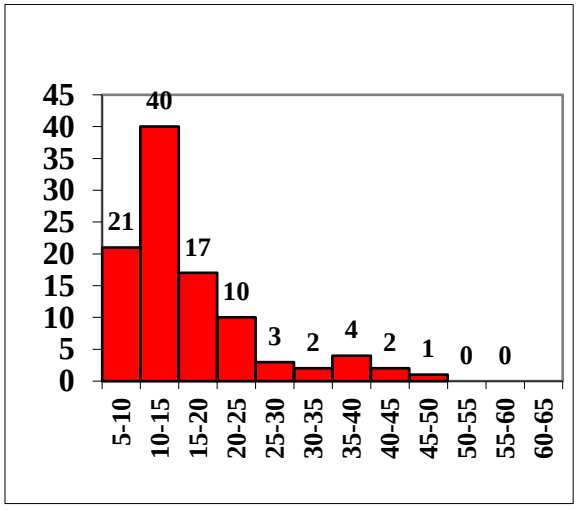
b. Panicle/ Hill



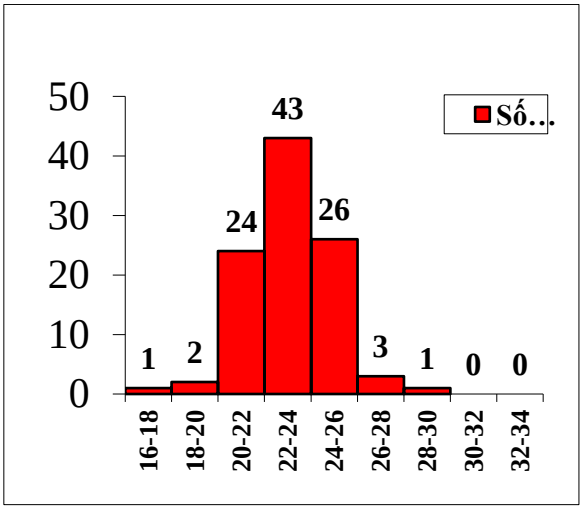
c. Length Panicle



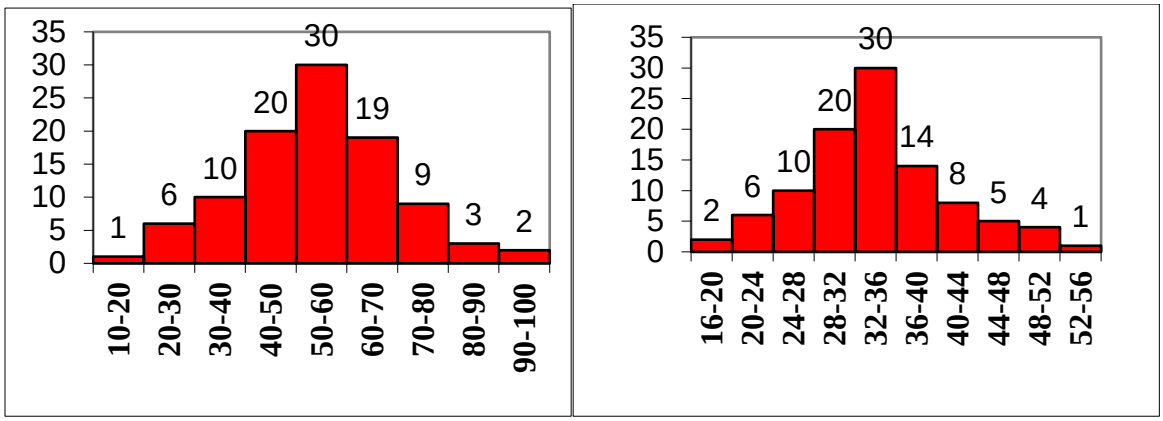
d. Filling distribution



e. unfilling distribution



f. 1000 grain weight



g.Biomass distribution h.Frequency distribution on yield

Fig.2: Frequency distribution of the varieties base on a/hight plant duration, b/ panicles per plant, c/Length Panicle, d/number of filled grains, e/ number of unfilledgrains, f/1000-g weight, f/biomas and g/yield, showed the diversity of landrace Nep Ngu varieties of Binh Dinh.

Correlation Among Agro-morphological Traits

Correlation between agricultural morphological features. The correlation coefficients between the measured traits are shown in Table 5. The number of strong filling per plant was significantly correlated with yield (r = 0.623**) and biomass (r = 0.725**) suggesting that varieties with more also had higher yields. Significant correlations were also found between panicle length plant height (r = 0.425*), duration time (r = 0.474*) which can be explained by the principle of morphological compatibility in rice architecture. Other characteristics are strongly correlated with the length of panicle. Weight 1000 g, closely related to yield (r = 0.795**) and biomass (r = 0.715). Yield correlates greatly with biomass (r = 0.856**) but moderately correlated with duration (r = 0.017ns). Other traits that were found to be poorly correlated with other agro-morphological traits. It exhibited negative correlation with panicle length and filling grain (-0.025), Weight gran 1000 (0.135ns). Some late maturing varieties had negative with yield (r=-0.043). The result the differnce with Lang et al 2014 expland for landrace varieties with Nep Ngu at Binh Dinh.

Table 5: Correlation coefficients among 9 agro-morphological traits of 102 Nep Ngu rice varieties

	Plant height	Duration	Length of panicle	Filling grain	Unfilled grain	Weight 1000	Yield	Biomass
Plant height	**1.000**							
Duration	0.223ns	**1.000**						
Panicle length	0.435*	0.474*	**1.000**					
Filling grain	-0.064ns	-0.117ns	-0.025ns	**1.000**				
Unfilled grain	0.275ns	0.176	0.198ns	-0.064ns	**1.000**			
Weight grain 1000	0.125ns	-0.167ns	0.135ns	0.084ns	-0.015ns	**1.000**		
Yield	0.457*	0.355	0.188	**0.623**	**0.751**	**0.795**	**1.000**	
Biomass	0.073ns	0.017	0.060	0.725	-0.033	0.715	**0.856**	**1.000**

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IV. CONCLUSIONS

Agro-morphological characters and PCR based markers have provided valuable information about genetic diversity of rice collection in Binh Dinh, VietNam. In molecular-based analysis, results showed that SSR markers were very useful and effective in characterizing and estimating the extent and distribution of genetic variation in the 102 rice landraces. Clustering of the varieties based on genetic distance (0.63) allowed grouping of the 102 varieties into three clusters

In general, both morphological and SSR markers were able to group the varieties into ecotypes, rainfed and landrade rice.

Quantitative agro-morphological characters and molecular markers of 102 accessions were analyzed using clustering, correlation coefficient, principal component analysis and analysis of variance. Diversity of the collection was analyzed using Shannon-Weaver diversity index. The objective of the study was to determine the extent of diversity using agro-morphological and molecular markers (SSRs).

Using quantitative agro-morphological characters, ANOVA showed highly significant differences among the traits of the 102 rice landraces except panicles per plant and yield. Correlation coefficients showed that all the traits were significantly correlated with each other except yield, which was only slightly correlated with other traits. The diversity indices for quantitative descriptors were high ranging from H' = 0.68 to 0.79. Mean diversity index for all traits among the 102 traditional varieties was high (H' = 0.7).

From these results, the following recommendations are presented:

1. Diversity analysis based on agro-morphological traits of rice landraces need to be continued to further confirm relationships among them.

2. Extensive molecular marker analysis may be conducted by considering more primers for its relevant application and efficient attainment of breeding objectives in rice improvement.

3. Continue analysis for the rest of the traits from difference traits such as grain quality and tolerance with biotic and biotic stress to Identification of novel resistance gene in rice germplasm for 102 lines Nep Ngu at Binh Dinh.

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