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Evaluation of agro-morphological and molecular characters of 22 rice landraces of East Timor

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Abstract. *Branco LM, Palupi ER, Ilyas S, Purwoko BS, Purwito A. 2023. Evaluation of agro-morphological and molecular characters of 22 rice landraces of East Timor. Biodiversitas 24: 2536-2546.* Landraces play an important role in the local food security and sustainable development of agriculture as a source of genes that control important traits adaptive to environmental conditions. The study aimed to characterize the agro-morphological and molecular characteristics of 22 rice landraces of East Timor (Timor Leste). The research was conducted in April-August 2018 in Caibada-Baucau-East Timor. Soil analysis was conducted at the Soil Laboratory of the Agronomy and Horticulture Department, IPB University. Materials used were 22 rice landraces seeds and one check variety. The seeds were germinated in seedbed seedlings with a basin (30 cm x 25 cm). The seedlings were planted in the field 16 days after sowing in a plot of 1.5 m x 7.0 m and a spacing of 25 cm x 25 cm, with an isolation distance between plots of ±3 m. Data collection was based on nine growth phases of rice. The cluster analysis based on agro-morphological characters resulted in 6 groups with a similarity coefficient of 0.17 or 17%, showing a high level of diversity. The lowest yield was Ale Kukefuhu (290 g/m^2), and the highest was Hare Nona Portu (979 g/m^2). Using 10 RAPD markers, the 22 rice landraces samples produced 77 DNA fragment bands with sizes between 300 bp-1475 bp, averaging 3.35 alleles per landrace; polymorphic rates of 8.1. OPA8, OPH1, and OPH7 markers were the highest (100.00%), and OPF5 was the lowest (66.67%) polymorphic percentage. Based on the molecular marker, the coefficient of similarity of the landraces ranged from 0.12 to 0.51, and they were grouped into five clusters.

Keywords: Cluster analysis, coefficient of similarity, genetic diversity, RAPD

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food for most of the people of East Timor (Timor Leste) (93.97%) to meet their daily food needs (Dos and Utomo 2010). However, rice production is inadequate to meet the demand; hence the availability of rice becomes a huge challenge for the government. The national rice demand reaches 150 000 tons per year, with consumption per capita/year reaching 90 kg/person/year. Domestic rice production only reaches 30-40% of the total demand; therefore, the government has to import rice to meet the demand. The major constraints to rice production are the lack of quality seeds and inappropriate cultivation technology. The local rice planted by farmers is not of superior variety, resulting in a productivity of only 1.5-2.0 t ha^{-1} (Young 2013).

Landraces play a vital role in local food security and the sustainable development of agriculture (Walter 2018; Eka et al. 2019). The major objective of the rice breeding program is to maintain the desirable traits with an increase in the yield potential of landraces. Rice breeding to obtain high-yielding variety (HYV) has been a key approach to improving agricultural productivity and reducing global hunger and poverty (Rahman and Connor 2022). New improved rice varieties are expected to have high yield potential and be more adaptive to the local biotic and abiotic stresses (Verma et al. 2021). Landraces are potential germplasm, function as a source of genes that control important traits adaptive to environmental conditions in East Timor, and can be used for cross-breeding with other cultivars to obtain new high-yielding varieties. Rice landraces have a high level of genetic diversity that will be very important input for future improvement and sustainability of the rice development system. Improvement in the yield potential of rice is the major strategy to increase rice production. Increasing rice yield potential strategies include ideotype breeding, conventional hybridization, heterosis breeding, male sterility, wide hybridization, genetic engineering, and molecular marker-assisted breeding (Khan et al. 2015).

Exploration, conservation, and characterization efforts must be carried out to avoid the extinction of potential landraces (Rabara et al. 2015; Bigyan et al. 2021; Surje et al. 2022). Through characterization, the important traits of economic value, either qualitative and quantitative or the characteristic of the variety in inquiries, are identified (Sandra et al. 2015). Observable characters can be morphological, agronomic, physiological, and molecular characters. The landraces of different genotypes need to be conserved and used for breeding purposes (Pathak 2018). Based on their higher economic characteristics, local landraces can be utilized for breeding new high-yielding varieties (Sinha et al. 2015; Saidah et al. 2015; Ahmed et al. 2016). The superior properties of the germplasms are identified through characterization to obtain potential landraces for further development (Babu et al. 2020). Agro-morphological and molecular characterization to identify the potential and the genetic diversity between landraces will provide the necessary information for rice crop development programs (Ziyi et al. 2022).

The use of the RAPD (Random Amplified Polymorphic DNA) technique has been widely practiced to identify the genetic diversity of rice plants. The advantages of using the technique are that it is simple, fast, cost-effective, requires a small amount of DNA, and effectively detects polymorphism (Karande et al. 2017). In addition, the molecular marker is very useful, especially in selecting genotypes for making crosses, thus minimizing the number of individuals needed with a range of diversity and not affected by the environment (Maulan et al. 2014; Chauhan et al. 2015; Bhuiyan et al. 2019).

The diversity of landraces or local rice is a valuable basic capital in rice plants to support the national food security program (Mohidem et al. 2022). East Timor has a wide variety of landraces, but the available data and information on their whereabouts are limited. Therefore, this study aimed to obtain information on the agro-morphological and molecular characters of 22 East Timor landraces and to determine the genetic diversity of the landraces based on the agro-morphological and molecular characters.

MATERIALS AND METHODS

This research was conducted in April-September 2018 in the rice fields of Caibada Village, Baucau District, East Timor. Soil samples were analyzed at Soil Laboratory, and molecular analysis was carried out at the Bio-Molecular Laboratory of Agronomy and Horticulture Department, Institut Pertanian Bogor (IPB). The seeds of 22 landraces from different areas of different topography and elevation (Table 1) and one check variety were used for this study.

A descriptive method was used in analyzing the 22 landraces and a check variety as control. First, the seeds were soaked in water for 12 h and wrapped with leaves. After the seeds germinated, they were sown in a 30 cm x 25 cm basin containing the planting medium (soil:sand:compost=2:1:1). The seedlings were transplanted into a paddy field with a plot size of 1.5 m x 7 m, with a spacing of 25 cm x 25 cm at 16 days after sowing. The isolation distance between landraces was ≥3 meters.

Agro-morphological analysis

Data on agro-morphological characters of the landraces were collected based on nine phases of rice plant growth (Table 2). Five sample plants per plot of each landrace were selected randomly for data collection.

Molecular analysis

DNA extraction

The DNA for RAPD analysis was extracted from young, vigorously growing fresh leaves collected from 16 days old seedlings of each landrace. DNA extraction used 0.1 g leaf samples, previously rinsed with 70% alcohol, using the acetyl trimethyl ammonium bromide (CTAB) mini-prep method. CTAB extraction buffer of approximately 700 µL was added and ground until evenly distributed. The sample was transferred into 2 mL of microtubes. The microtube was soaked in a water bath (65℃) for 30 minutes. Approximately 700 µL of Chloroform isoamyl alcohol (CIA) was added to the sample and vortexed to mix. The sample was centrifuged (10000-15000 rpm) at 4℃ for 10-15 minutes. The supernatant (400-600 µL) was transferred to a new microtube and added with absolute ethanol twice as much as the sample volume or added with isopropanol as much as the sample volume, then flipped slowly. Samples were incubated at room temperature for 10 minutes or in the freezer overnight. The sample was centrifuged again (4000-7000 rpm) at 4℃ for 10-15 minutes, and the liquid was discharged. DNA deposits (pellets) were washed with 70% ethanol and dried at room temperature for 15-20 minutes or using a vacuum. DNA was then dissolved in 50-200 µL double distilled water (ddH_2O) and was stored at -20℃ (Alam et al. 2014).

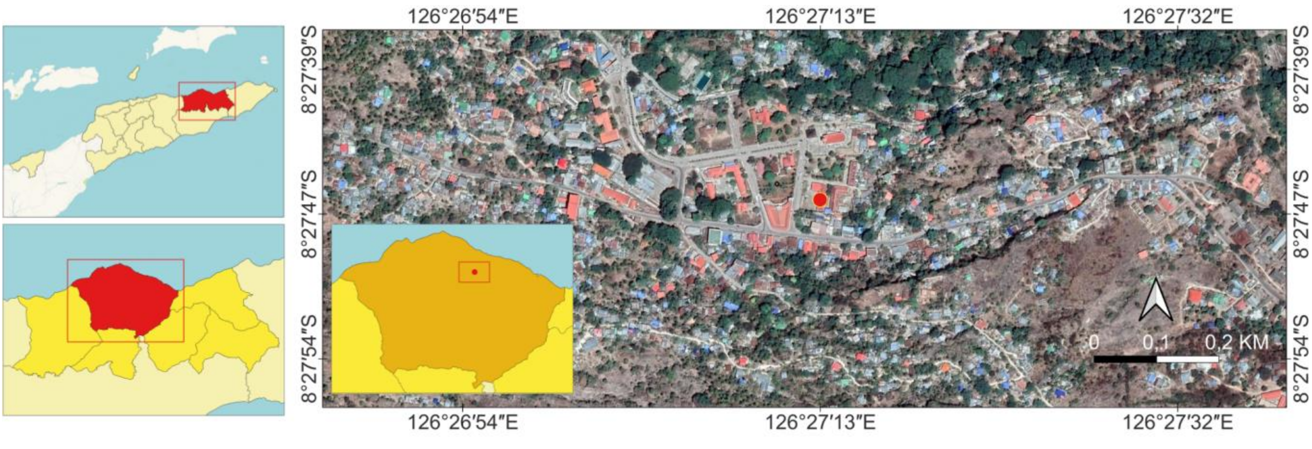

Figure 1. Research location in Caibada Village, Baucau, East Timor

Table 1. Topography and elevation of the place origin of the 22 rice landraces and the check variety

Landrace name	Place of origin	Topography	Elevation (m asl.)
Hare metan	Bobonaro-Cailaco	Upland	543
Ale kukefuhu	Lautem-Luro	Upland	410
Hare metama	Bobonaro- Cailaco	Upland	543
Hare R-oitu	Viqueque-Bikari	Low land	10
R-sinco	Viqueque-Bikari	Low land	10
Mean morin	Viqueque-Vila	Low land	47
Fos mean	Viqueque-Vila	Low land	39
Hare metan 8	Baucau-Venilale	Upland	830
Ale hendik	Lautem-Luro	Upland	881
Ale kalaha asan	Lautem-Luro	Upland	899
Hare mean 11	Maliana-Ritabou	Middle land	244
Ale mamea ula lesa	Lautem-Luro	Upland	881
Hare cla'an	Bobonaro-Maliana	Middle land	244
Hare mean 14	Maliana- Ritabou	Middle land	244
Ale isu puti	Lautem-Luro	Upland	881
Hare nona portu	Bobonaro-Maliana	Middle land	244
Hare belit	Viqueque-Vila	Low land	47
Resa silaun	Baucau-Vemasse	Low land	10
Ale mamea	Lautem-Luro	Upland	410
Ale kalaha dikar	Lautem-Luro	Upland	410
Hare mean 21	Baucau-Venilale	Upland	830
Hare mean 22	Maliana-Ritabou	Middle land	244
Hare nacroma*	Baucau-Venilale	Upland	830

Note: *Check variety; asl: above sea level

Table 2. Growth stages of rice plants subject to observation (IRRI 2013)

Growth phase	Observed characters
Seedling	Plant height
Tillering	Leaf ligule color
Stem elongation	Leaf-sheath color and ligule color
Booting	Leaf blade color
Heading	Leaf shape, flag leaf angle
Flowering	Stigma color, the day of flowering
Milk ripe grain	Leaf length, leaf width, number of productive tillers
Half ripened grain	Culm color, culm angle, culm segment color, awn, awn length
Full mature grain	Panicle length, panicle type, secondary panicle branch, day to harvest, number of seeds/panicle, grain length, grain width, grains color, rice husk color, 1000 seeds weight, seed weight/panicle, seed weight/hill, and seed weight /m^2

Polymerase Chain Reaction (PCR)

DNA (2.5 µL) was inserted into several microtubes and was diluted with ddH_2O. PCR reactions were performed in a ten µl reaction mix containing one µL Ampli Taq polymerase buffer (10X), 2.5 µL Primer (10 µM), one µL dNTPs (250 µM), 0.2 µL Ampli Taq DNA polymerase and 3.3 µL sterile deionized water. Into the reaction mix, two µL DNA was added, making the total volume 10 µL. DNA amplification was performed in an oil-free thermal cycler. The reaction mix was preheated at 94℃ for 3 minutes, followed by 40 cycles of 1 min denaturation at 94℃, 1 min annealing at 54℃, and elongation or extension at 72℃ for 2 minutes. After the last cycle, a final step for 7 min at 72℃ to allow complete extension of all amplified fragments. After completion of the cycling program, reactions were held at 40℃. Electrophoresis was carried out in 0.5 x TBE buffer on a 1.5% agarose gel, and amplified fragments were visualized by staining with ethidium bromide (Alam et al. 2014).

Gel electrophoresis

Agarose gel was prepared by adding 20 mL of TAE buffer 1x to 0.2 g of agarose powder in a goblet glass and heating on a hot plate until homogeneous. The solution was poured into a mold, and a comb was installed until it solidified. The PCR result DNA with a loading dye was loaded into the wells, and the gel was run into the electrophoration chamber containing a 1 x TAE buffer (the top of the gel that was a gel well in the negative cathode/pole position). Electrophoresis was run (100V) for 20 minutes. Then, the gel was soaked in CTAB for 30 minutes, and the DNA band was observed using UV light (Haquarsum et al. 2015).

Data analysis

The characterization of the traits was based on descriptors for rice (*Oryza sativa* L.) procedure from the IBPGR-IRRI rice advisory committee and descriptors for wild and cultivated rice (*Oryza* spp.) (IRRI 2013). Agro-morphological data were cluster analyzed using the SAS software. Cluster analysis grouped the landraces based on the similarity of characters and formed a dendrogram. Molecular data were collected. Molecular characterization was based on the presence or absence of DNA bands in binary data. The creation of matrix and dendrogram data was carried out using The Unweighted Pair-Group Method Arithmetic (UPGMA) with SAS software.

RESULTS AND DISCUSSION

Up to now, East Timor has one national rice variety (Hare nacroma) that was introduced from Indonesia in 2002. However, farmers cultivate many landraces for various reasons, such as traditional festivities, preferences, etc., in which the farmers produce their seeds. The landraces for this study were taken from 5 rice production centers in East Timor, i.e., Bobonaro, Maliana, Viqueque, Baucau, and Lautem (Figure 1).

Agro-morphological characterization

The agro-morphological characteristics of the landraces varied considerably, with the lowest coefficient variation being 5.59% for days to harvest and the highest being 50.72% for the culm angle. The qualitative and quantitative characteristics of the landraces showed differences in leaf shape, length, width, culm angle, leaf sheath color, and plant height (Table 3). Most landraces (14 of 22) have hairy leaves, and nine are hairless (Table 3). All the landraces have longer leaves (34.50-69.00 cm) than the check variety of 27.83 cm, while for the leaf width, only one landrace (Resa silaun) has narrower leaves than the check variety.

Based on the culm angle, most landraces (16 of 22) have an erect growth habit, three are open, and four have a medium growth habit. All landraces have green leaf sheath color, except two landraces (Hare R-oitu and Resa silaun) have purple color. In terms of plant height, only one landrace (Ale kalaha asan) is taller than the check variety and represents the tallest (188.75 cm), while the other landraces are shorter than the check variety, with Fos mean is the shortest (76.25 cm) (Table 3). The variation in the agro-morphological traits is likely due to genetic factors as they have adapted to the local environment.

The culm and leaf sheaths form the basal of the rice plant, which is composed of the mechanical tissue layers, and vascular bundles that play dominant roles in the lodging resistance of rice plants (Liu et al. 2022). Based on the data 4 landraces (Ale hendik, Hare nona portu, Hare mean 21, and Hare mean 22) are expected to have weak lodging resistance, 5 landraces (Hare metan, Ale kukefuhu, Hare R-oitu, Hare metan 8, and Ale isu puti) medium lodging resistance, and the other 14 landraces (Hare metama, Hare R-sinco, Mean morin, Fos mean, Ale hendik, Hare mean 11, Ale mamea ula lesa, Hare claán, Hare mean 14, Hare belit, Resa silaun, Ale mamea, Ale kalaha dikar, and Hare nacroma) strong lodging resistance. The stronger the lodging resistance, the higher the potential to produce high yield and quality seed. More detailed research is needed to clarify the lodging resistance of these landraces.

Table 3. Diversity of some agro-morphological characters of 22 landraces of East Timor rice at the vegetative phase

Name of landrace	Leaf hairiness	Leaf length (cm)	Leaf width (cm)	Culm angle	Leaf-sheaths color	Plant height (cm)
Hare metan	Hairless	44.67	1.33	Open	Green	144.75
Ale kukefuhu	Hairless	44.67	1.53	Medium	Green	134.75
Hare metama	Hairy	39.37	1.28	Erect	Green	87.50
Hare R-oitu	Hairy	45.50	1.33	Erect	Purple	105.50
Hare R-sinco	Hairless	40.20	1.20	Erect	Green	89.67
Mean morin	Hairy	34.50	1.18	Erect	Green	85.75
Fos mean	Hairy	39.47	1.13	Erect	Green	76.25
Hare metan 8	Hairless	53.47	1.00	Erect	Green	122.75
Ale hendik	Hairy	37.5	1.25	Open	Green	106.25
Ale kalaha asan	Hairy	53.47	1.18	Erect	Green	188.75
Hare mean 11	Hairy	38.97	1.20	Erect	Green	95.25
Ale mamea ula lesa	Hairy	69.00	1.00	Medium	Green	98.75
Hare cla'an	Hairy	43.53	1.25	Erect	Green	94.25
Hare mean 14	Hairy	48.00	1.10	Erect	Green	127.00
Ale isu puti	Hairless	38.50	1.00	Erect	Green	98.50
Hare nona portu	Hairless	40.83	1.18	Open	Green	92.50
Hare belit	Hairless	51.23	1.10	Erect	Green	91.00
Resa silaun	Hairy	42.70	0.93	Erect	Purple	96.75
Ale mamea	Hairy	43.63	1.05	Medium	Green	121.25
Ale kalaha dikar	Hairy	35.70	1.05	Medium	Green	83.00
Hare mean 21	Hairy	58.70	1.13	Erect	Green	147.50
Hare mean 22	Hairless	45.700	1.33	Erect	Green	146.00
Hare nacroma*	Hairless	27.83	0.97	Erect	Green	149.50
CV (%)	31.02	31.10	12.42	50.72	18.42	11.23

Note: *Check variety

The number of productive tillers varied considerably among landraces (CV=45.24%); nine landraces produced productive tillers higher than the check variety, with Fos mean having the highest productive tillers of 53.5, while Hare metan has the lowest (8.3) (Table 4). Chaniago (2017) classified rice plants based on the number of productive rice tillers into three categories, i.e., ≤10 tillers are classified as low, 11-20 tillers are moderate, and ≥20 are high. Therefore, one landrace (Hare metan) under study is classified as having low productive tillers, 9 with moderate productive tillers, and 13 with high productive tillers. The number of productive tillers per hill is one of the most important yield components; the higher the number of productive tillers, the higher the yield (Anwari et al. 2019).

The day of flowering, which indicates 50% of plants flower, is often used as a counting guide for crossing in the breeding of varieties. Four landraces (Hare metan, Ale kukefuhu, Hare metama and Hare metan 8) were early-flowering landraces (83-87 DAS), and six landraces (Ale mamea, Hare nona portu, Hare belit, Hare mean 21, Hare mean 22 and Hare R-oitu) were late-flowering (113-119 DAS) (Table 4). However, the early flowering landraces were not necessarily had early harvest time. The early-flowering landraces of Hare metan, Ale kukefuhu, and Hare metama were harvested at 116 (DAS), while Hare metan 8 was harvested at 123 (DAS) (Table 5).

The flag leaf position of the landraces varied considerably (CV=46.47%), 3 landraces have erect flag leaves, 6 landraces medium, and 14 landraces descending (Table 4). The position of the flag leaf is important for maximum penetration of sunlight. Moreover, the morphology of the flag leaf affects the yield potential and grain quality, as flag leaf is a major source of assimilate for grain filling (Rahman et al. 2013; Sinha and Misrha 2013; Pillai and Surya 2021).

The panicle type varied considerably (CV=40.12%), and 13 landraces were categorized as having a compact type, nine landraces as medium type, and one landrace of medium-open type (Table 4). In addition, many genes are reported to control the panicle type and the formation and arrangement of branches and spikelets in a panicle (Li et al. 2021).

Based on panicle length, rice varieties are grouped into three categories, i.e., short (≤ 20 cm), medium (20-30 cm), and long (>30 cm). Using this classification, four landraces of this experiment are categorized as having short panicles, and the others (23.5-27.5 cm) are having medium-length panicles, including the check variety (Table 5). Panicle length is an important trait for improving panicle architecture and grain yield. Panicle length affects the number of grains; the longer the panicle, the higher the number of grains. Planting density also affects panicle length; low planting density results in longer panicles than a high planting density.

Table 4. Diversity of some agro-morphological characters of 22 landraces of East Timor rice at the reproductive phase

Name of landrace	Culm lodging resistance	Productive tillers	Days of flowering (50%) (DAS)	Flag leaf position	Panicle type
Hare metan	Medium	8.25	86	Erect	Compact
Ale kukefuhu	Medium	11.25	87	Descending	Compact
Hare metama	Strong	21.00	84	Medium	Compact
Hare R-oitu	Medium	21.75	113	Descending	Compact
R-sinco	Strong	19.75	104	Descending	Compact
Mean morin	Strong	16.75	106	Descending	Compact
Fos mean	Strong	53.50	108	Descending	Compact
Hare metan 8	Medium	11.75	83	Medium	Medium
Ale hendik	Weak	10.75	99	Medium	Medium
Ale kalaha asan	Strong	16.75	95	Descending	Compact
Hare mean 11	Strong	36.00	93	Descending	Compact
Ale mamea ula lesa	Strong	17.50	95	Descending	Medium
Hare cla'an	Strong	17.75	100	Medium	Med. Open
Hare mean 14	Strong	23.75	95	Descending	Medium
Ale isu puti	Medium	23.50	109	Descending	Medium
Hare nona portu	Weak	35.25	114	Descending	Compact
Hare belit	Strong	27.25	114	Descending	Medium
Resa silaun	Strong	25.75	103	Descending	Compact
Ale mamea	Strong	35.75	119	Medium	Compact
Ale kalaha dikar	Strong	21.50	100	Descending	Medium
Hare mean 21	Weak	25.00	113	Erect	Medium
Hare mean 22	Weak	15.50	114	Medium	Medium
Hare nacroma*	Strong	21.75	96	Erect	Compact
CV (%)	32.35	45.24	10.53	46.47	40.12

Note: *Check variety

The awn length is one of the morphological characteristics to distinguish local rice from one another, 11 landraces, including check variety, have no awn, while 11 landraces have awn with varying lengths between landraces (Table 5). For example, Hare R-oitu has the shortest awn (1.1 mm), while Ale kalaha asan has the longest (23.4 mm). Based on time to harvest, Dewi et al. (2012) classified rice plants into three categories, i.e., <125 days after sowing (DAS) as early-ripening, 125-150 DAS as medium-ripening, and >150 DAS as late-ripening. Thus, of the 22 landraces, three (Hare metan, Ale kukefuhu and Hare metama) are early-ripening and the other 19 landraces and one check variety are medium-ripening.

Other factors affecting the number of tillers were planting distance, growing season, and the use of fertilizers. Wide planting, supported by a good environment, will increase the number of tillers (Asmamaw 2015; Huang et al. 2021). The grains' length ranged from 6.42-9.86 mm (CV=12.73%), and Ale kalaha dikar has the longest grain of 9.86 mm and Hare mean 14, the shortest of 6.42 mm. While grains width ranged from 1.98-3.70 mm (CV=18.39%), Hare R-oitu has the widest grain of 3.70 mm, and Hare nona portu and Hare belit have the narrowest grain (Table 5). Grain size is an important factor in determining yield in rice because the grain size, which comprises grain length and width, greatly contributes to grain yield and quality in rice (Wang et al. 2019; Zhan et al. 2022).

The grain color is one of the useful characteristics for identifying mixtures of other varieties (off-type). Most of the landraces under study have straw yellow grain color, including the check variety, except Ale kalaha asan and Ale kalaha dikar, which are red, and Hare cla'an is purple (Table 6). The shape of grain is classified into four groups, 7 landraces (Hare R-sinco, Ale hendik, Hare mean 11, Ale mamea ula lesa, Hare claán, Resa silaun, and Hare nacroma) are oval, 1 landrace (Hare metama) is slender, 8 landraces (Hare metan, Ale kukefuhu, Hare R-oitu, Mean morin, Fos mean, Hare metan 8, Ale isu puti, and Hare belit) are medium, and 7 landraces (Ale kalaha asan, Hare mean 14, Hare nona portu, Ale mamea, Ale kalaha dikar, Hare mean 21, and Hare mean) are round.

The number of grains per panicle is one of the characteristics used in selecting parental lines to obtain a new high-yielding variety since it directly determines grain yield (Deng et al. 2021; Lu et al. 2022). Hare cla'an produced 297 grains per panicle, the highest among landraces and the check variety (Table 6), while Ale kukefuhu, Hare metama, R-oitu, and R-sinco were of the low number of grains. The grain yield is calculated by the number of grains per panicle, the spikelet number per plant (determined by the number of productive tillers), and planting density (Zhao et al. 2015; Khan and Kifayatullah 2022).

Hare metan and Ale kukefuhu produced the highest 1000 grains weight of 35.30 g and 35.11 g, respectively, and Hare nacroma, the check variety, was the lowest. The 1000 grains' weight indicates the quality of the seeds and is a stable character of a landrace. The size of the grain is determined at the time of panicle exertion, so the size of the filled grain is based on the size of the grain. The characteristics of 1000 grains' weight, grain size, and panicle length are generally influenced by genetic factors rather than environmental factors (Fukushima 2019).

Table 5. Diversity of some agro-morphological characters of 22 landraces of East Timor rice at the ripening phase

Name of landrace	Panicle length (cm)	Awn length (mm)	Days to harvest (DAS)	Grain length (mm)	Grain width (mm)
Hare metan	27.50	4.75	116	9.81	3.42
Ale kukefuhu	27.50	3.35	116	9.70	3.51
Hare metama	27.30	2.27	116	9.72	3.45
Hare R-oitu	27.50	1.10	132	9.54	3.70
R-sinco	27.30	0.00	132	9.23	3.67
Mean morin	26.40	0.00	132	9.27	3.52
Fos mean	26.00	0.00	141	8.77	3.18
Hare metan 8	24.10	0.00	123	8.18	2.81
Ale hendik	23.80	0.00	132	8.16	2.55
Ale kalaha asan	23.50	23.40	132	7.97	2.56
Hare mean 11	23.50	4.36	123	7.99	2.70
Ale mamea ula lesa	24.10	6.67	129	8.22	2.90
Hare cla'an	23.60	8.70	126	8.47	2.95
Hare mean 14	18.10	6.49	123	6.42	2.21
Ale isu puti	18.20	4.34	136	6.64	2.15
Hare nona portu	18.30	2.04	134	6.83	1.98
Hare belit	18.30	0.00	134	7.09	1.98
Resa silaun	24.60	0.00	132	9.38	2.61
Ale mamea	24.90	0.00	145	9.52	2.65
Ale kalaha dikar	25.00	0.00	129	9.86	2.62
Hare mean 21	25.80	0.00	132	9.61	2.56
Hare mean 22	25.50	0.00	132	9.79	2.70
Hare nacroma*	25.50	0.00	130	9.28	2.73
CV (%)	12.82	17.75	5.69	12.73	18.39

Note: *****Check variety

Table 6. Diversity of some agro-morphological characters and yield component of 22 landraces of East Timor rice at the harvesting phase

Name of landrace	Grain color	Grains shape	Total grains per panicle	1,000 grains weight (g)	Grain weight per panicle (g)	Grain weight per m2 (g)
Hare metan	Straw yellow	Medium	159	35.30	5.99	402
Ale kukefuhu	Straw yellow	Medium	133	35.11	5.82	270
Hare metama	Straw yellow	Slender	129	28.70	4.16	324
Hare R-oitu	Straw yellow	Medium	130	28.64	4.46	855
R-sinco	Straw yellow	Oval	131	28.55	4.74	635
Mean morin	Straw yellow	Medium	152	27.91	5.64	569
Fos mean	Straw yellow	Medium	162	27.45	4.81	861
Hare metan 8	Straw yellow	Medium	162	26.58	4.45	635
Ale hendik	Straw yellow	Oval	170	26.22	5.32	673
Ale kalaha asan	Red	Round	188	26.05	7.69	865
Hare mean 11	Straw yellow	Oval	220	25.99	5.13	644
Ale mamea ula lesa	Straw yellow	Oval	262	25.92	5.22	753
Hare cla'an	Purple	Oval	297	25.60	2.24	434
Hare mean 14	Straw yellow	Round	237	25.48	7.07	774
Ale isu puti	Straw yellow	Medium	210	25.44	6.47	662
Hare nona portu	Straw yellow	Round	188	25.31	5.57	979
Hare belit	Straw yellow	Medium	145	25.23	4.89	801
Resa silaun	Straw yellow	Oval	190	24.99	2.92	295
Ale mamea	Straw yellow	Round	193	24.95	6.09	807
Ale kalaha dikar	Red	Round	178	23.40	2.36	301
Hare mean 21	Straw yellow	Round	180	22.39	6.31	314
Hare mean 22	Straw yellow	Round	191	21.84	6.56	847
Hare nacroma*	Straw yellow	Oval	182	21.85	5.44	642
CV (%)	41.83	43.13	23.26	5.59	34.8	34.80

Note: *Check variety

Grain weight per panicle is a quantitative character with the lowest coefficient of variation (5.59 %) ranging from 2.24-7.69 g. Ale kalaha asan produced the highest grain weight per panicle of 7.69 g, and Hare claán the lowest at 2.24 g (Table 6). The grain weight directly determines the yield of a population, determined by the number of panicles per hill, the number of grains per panicle, and the grain weight (Lu et al. 2022). Ale kukefuhu produced the lowest yield with a grain weight per m^2 of 290 g, and Hare nona portu produced the highest grain weight per m^2 of 979 g. Therefore, the grain production can be used to determine the yield potential of the landraces. In general, the potential highest yield is determined genetically and is a benchmark of the ability to produce grain (Ding et al. 2014; Kadidaa et al. 2017).

The cluster analysis based on the agro-morphological characters resulted in a dendrogram that clustered the 22 rice landraces into 6 groups with a similarity coefficient of 0.17 or 17% (Figure 2). Group 1 consists of 1 landrace, Hare claán. Group 2 consists of 1 landrace, Hare metan. Group-3 consists of 5 landraces, Hare mean 21, Resa silaun, Ale kalaha dikar, Ale kukefuhu, and Hare metama. Group 4 consists of 7 landraces, Mean morin, Hare nacroma, Hare mean 11, Ale isu puti, Ale Hare R-sinco, and Hare metan 8. Group 5 consists of 2 landraces, Ale mamea ula lesa, and Hare mean 14, and Group 6 consists of seven landraces, Hare belit, Ale mamea, Hare nona portu, Hare R-oitu, Fos mean, Ale kalaha asan and Hare mean 22. The cluster analysis showed that Hare claán has a very distant relationship with all existing landraces.

The similarity of agro-morphological characters indicates the relationship level or closeness, resulting in the highest phenotypic similarity. All the landraces have a very distant relationship. Landraces with the closest degree of similarity are Resa silaun and Ale kalaha dikar, while the lines with the most distant similarity are: Hare claán. The value of genetic distance in the cluster is ≤0.8, and then crosses within the cluster cannot be performed. On the other hand, if the genetic distance is >0.8 then the landraces in the same cluster are descendants (Maulan et al. 2014).

Genetic diversity analysis

The genetic diversity of 22 rice landraces and one check variety (Table 7) was based on the number of alleles of each RAPD marker. The analysis produced 77 DNA bands with a fragment length of 300bp-1475bp, an average of 3.35 alleles per landraces. The average number of polymorphic bands per marker is 8.1. OPA8, OPH1, and OPH7 markers resulted in the highest percentage of polymorphism (100%), while OPF5 resulted in a smaller percentage of polymorphism (66.70%). One marker (OPA8) did not produce any band on the Hare metan. It is suspected that the marker did not produce DNA bands because the markers were not homogeneous to the DNA template (Maulan et al. 2014). Based on the matrix of similarity of the dispersal proportions of the DNA fragment bands can form the degree of similarity of 23 rice samples using 10 random markers from the RAPD (Table 7). The 23 samples used in this study were collected from four different districts with different topographic types, namely lowlands, middle lands, and uplands based on altitude. The

10 RAPD markers were effective in detecting the genetic diversity of the 22 rice landraces and the one check variety.

Some of the rice landraces have the same name from the area of origin and local language, where the local name of the landrace is based on the color of the rice pericarps and awn at the end of the grain and grain husks. However, the results of the present study showed that the landraces do not have a close kinship, which means that even landraces that have the same color of pericarp are of different genetic traits with different levels of kinship. This is as reported by Alam et al. (2014) that the diversity of a plant is influenced by its genetic component and not influenced by the geographical spread. Based on the results mentioned above, of the 22 landraces of local rice and one check variety, there is a long degree of kinship. All landraces have potential to be used in rice plant development programs in the future.

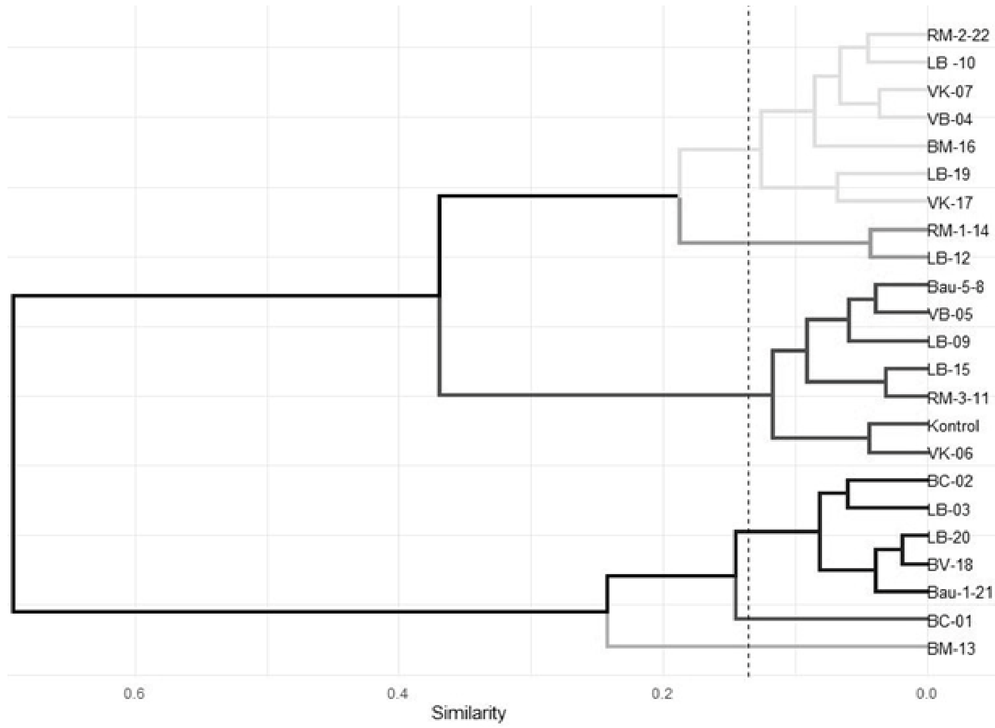

Figure 2. Dendrogram of 22 rice landraces and one check variety based on agro-morphological characters. BC-01: Hare Metan, BC-02: Hare Metama, LB-03: Ale Kukefuhu, VB04: Hare R-oitu, VB-05: Hare R-sinco, VK-06: Hare Mean Morin, VK-07:Fos Mean, Bau-5-8: Hare Metan 8, LB-09: Ale Hendik, RM-3-11: Hare Mean 11, LB-12: Ale Mamea Ula Lesa, BM-13: Hare Claán, RM-1-14: Hare Mean 14, LB-15: Ale Isu Puti, BM-16:Hare Nona Portu, VK-17: Hare Belit, BV-18:Resa Silaun, LB-19: Ale Mamea, LB-20: Ale Kalaha Dikar, Bau 1-21: Hare Mean 21, RM-2-22: Hare Mean 22, LB10: Ale kalaha asan, Kontrol: Hare Nacroma*

Table 7. Primer base arrangement and numbers of DNA bands amplified from 22 rice landraces and one check variety

Primer	Sequence primer 5'-3'	Fragment DNA	Polymorphism band	Monomorphism band	Total	Polymorphism (%)	Monomorphism(%)	PIC
OPA 8	GTGACGTAGG	325-1475	11	0	11	100.00	0.00	0.47
OPB18	CCACAGCAGT	525-1180	7	1	8	87.50	12.50	0.38
OPF4	GGTGATCAGG	325-1185	7	0	7	100.00	0.00	0.44
OPF5	CCGAATTCCC	350-700	4	2	6	66.70	33.33	0.52
OPH1	GGTCGGAGAA	300-1050	10	0	10	100.00	0.00	0.48
OPH7	CTGCATCGTG	325-1500	9	0	9	100.00	0.00	0.51
OPX1	CTGGGCACGA	350-1000	8	1	9	88.90	12.99	0.41
OPX2	TTCCGCCACC	400-950	6	2	8	75.00	25.00	0.44
OPX8	CAGGGGTGGA	300-950	8	1	9	88.90	11.11	0.32
OPX17	GACACGGACC	325-750	7	0	7	100.00	0.00	0.35
	Average		7.7	0.7	8.27	90.70	9.49	0.534
	Total		77	7	82.7	907.00	94.93	5.34

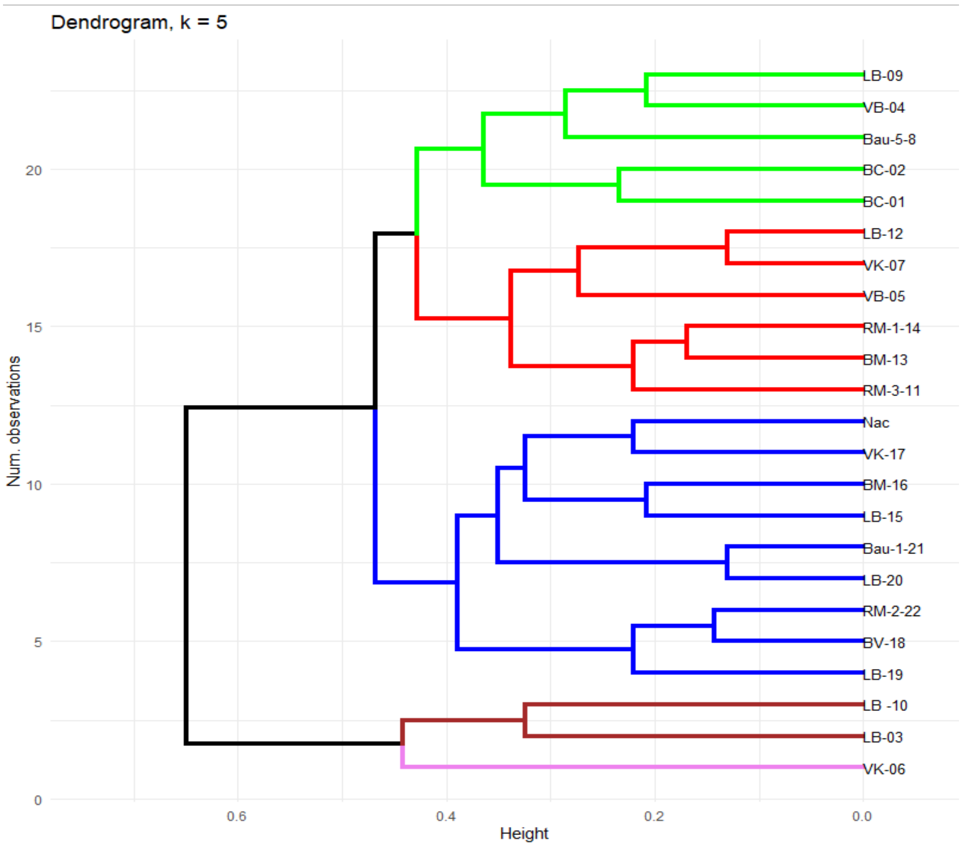

Figure 3. Dendrogram of 22 rice landraces and 1 check variety of UPGM analysis with 10 RAPD markers. BC-01: Hare Metan, BC-02: Hare Metama, LB-03: Ale Kukefuhu, VB04: Hare R-oitu, VB-05: Hare R-sinco, VK-06: Hare Mean Morin, VK-07:Fos Mean, Bau-5-8: Hare Metan 8, LB-09: Ale Hendik, RM-3-11: Hare Mean 11, LB-12: Ale Mamea Ula Lesa, BM-13: Hare Claán, RM-1-14: Hare Mean 14, LB-15: Ale Isu Puti, BM-16:Hare Nona Portu, VK-17: Hare Belit, BV-18:Resa Silaun, LB-19: Ale Mamea, LB-20: Ale Kalaha Dikar, Bau 1-21: Hare Mean 21, RM-2-22: Hare Mean 22, LB10: Ale kalaha asan, Nac: Hare Nacroma*

The polymorphic value in each individual or PIC serves to measure the diversity of alleles at each locus so that the degree of polymorphism at a particular locus can be known. In this study, the highest PIC value was generated by the OPF5 marker (0.52) and the lowest by the OPX8 marker (0.32). The PIC value largely determines the degree of efficiency of the molecular marker. The greater the PIC value, the locus is the best marker to be used as a marker. In addition, if the PIC value ≥0.50 then the marker is informative.

Kinship analysis based on specific DNA bands generated by the RAPD produces a dendrogram with five distinct groups (Figure 3). The dendrograms of similarity using UPGMA (Unweighted Pair-Group Method Arithmetic) grouped 23 samples into five groups with a similarity coefficient of 0.375, consisting of Group-1: Mean morin. Group-2: Ale kukefuhu and Ale kalaha asan. Group-3: Ale mamea, Resa silaun, Hare mean 22, Ale kalaha dikar, Hare mean 21, Ale isu puti, Hare nona portu, Hare belit and Hare nacroma*, Hare metan 8, Hare R-oitu, and Ale hendik. Group-4: Hare mean11, Hare claán, Hare mean 14, Hare R-sinco, Fos mean, Ale mamea ula lesa. Group-5: Hare metan, Hare metama, Hare metan 8, Hare R-oitu, Ale hendik. The dendrogram analysis data above shows that landrace Mean morin has a very far distance diversity from other landraces. Landraces Ale kalaha dikar and Hare mean 21, Fos mean, and Ale mamesa ula lesa have the closest distance similarities and formed separate individual clusters. There is a significant difference in landrace clustering between the use of morphological characters and molecular markers because morphological characters are strongly affected by environmental conditions, while molecular characters are only based on certain DNA sequences which are amplified using certain primers.

In conclusion, the results of the similarity analysis indicated phenotypic similarity coefficient of 0.17 or 17%. All the 22 landraces and one check variety used in this study clustered into six groups. The lowest grain yield is 290 g per m^2 (Ale kukefuhu) and the highest is 979 g per m^2 (Hare nona portu). The 10 RAPD markers used in this study produced 77 DNA fragment bands with a distance between 300bp-1475 bp, with an average of 3.3 alleles per landrace, and polymorphic bands per marker 8.1. The OPA8, OPH1, and OPH7 markers produced the highest polymorphic (100.00%). While the OPF5 marker results in a smaller polymorphic percentage of 66.67%. The coefficient of similarity of all landraces used in this study ranged from 0.12 to 0.51 and clustered 23 rice samples into five clusters.

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