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Genetic diversity of red and black upland rice accessions from East Nusa Tenggara, Indonesia as revealed by agro-morphological characters

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Abstract. *Mau YS, Markus JER, Shirly, Oematan S, Ndiwa ASS, Handoko DD, Nasution A, Makbul K. 2017. Genetic diversity of red and black upland rice accessions from East Nusa Tenggara, Indonesia as revealed by agro-morphological characters. Biodiversitas 18: 197-211.* A number of upland red and black rice accessions were collected from various locations of East Nusa Tenggara Province, Indonesia. This germplasm collection is invaluable genetic resource that can be utilized to generate new improved varieties. The objective of the present study was to elucidate genetic diversity of the rice germplasm based on agro-morphological characters. The study was carried out in the Glasshouse involving 40 upland red and black rice accessions. Observed variables included qualitative and quantitative agro-morphological characters. A total of 26 qualitative and 16 quantitative characters were observed. Qualitative characters were descriptively analyzed while quantitative characters were subjected to analysis of variance. Both data were also subjected to cluster analysis. Research results revealed a significant difference among rice accessions in both qualitative and quantitative characters. The tested rice accessions exhibited substantial differences in most of the observed qualitative and quantitative variables. Cluster analysis employing qualitative variables classified the rice accessions into 4 clusters and 15 sub-clusters. The same analysis using quantitative characters placed the 40 rice accessions into 5 clusters and 8 sub-clusters. Evaluation of agro-morphological characters demonstrated that the rice germplasm under the present study possessed a high genetic diversity.

Keywords: upland rice, agro-morphology, characters, diversity

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important food crop in Indonesia since rice is the main staple food for most Indonesian people. The current improvement in the community welfare and prosperity has caused a shift in the community lifestyle, which nowadays is beginning to put more concern on health by adjusting their diet. This is done by consuming foods according to their nutritional values. Red and black rices are staple food that many consumers are looking for today due to its great health-promoting benefits (Kushwaha 2016). Various studies indicated that red and black rices possess better efficacy compared to the common white rice. In addition to carbohydrate, protein, vitamins, and minerals, red and black rices are also sources of anthocyanins that are beneficial to health of antioxidants (Satue-Gracia et al. 1997; Nam et al. 2006; Chutipaijit et al. 2011; Huang and Lai 2016), anti inflammation (Tsuda et al . 2002) and anti-cancer (Shao et al. 2004; Hyun et al. 2004) properties. High anthocyanin content is found in both red rice and black rices. The anthocyanin content of dark-red rice and black rices had been proved to be higher because the reddish dark to the blackish brown color is partly caused by differences in the anthocyanin content (Abdel-Aal 2006; Shao et al. 2011; Suliartini 2011). Red and black rices have also long been known to be very useful for preventing and curing diseases caused by deficiencies in vitamin A and vitamin B (Kristamtini and Purwaningsih 2009). Thus, the red and black rices are not only staple foods of carbohydrate sources but also health-promoting functional foods.

The red and black rices are now becoming more popular and the demand for this rice is expected to rise in the future along with the increased level of community welfare and awareness on the importance of healthy food. However, the current availability of red and black rices on the market is limited due to lack of improved varieties at the farmer level. Most farmers are producing red and black rices using local varieties that are generally low yielding, long duration, susceptible to logding and lower eating quality such as ‘pera’ taste, etc. (Santika and Rozakurniati 2010). Number of existing superior red rice varieties is very limited while the black rice superior variety is not available yet. Until 2012, there were only five Indonesian released red rice varieties, i.e. Mandel and Segreng Handayani (Ministry of Agriculture 2009a,b), Bahbutong and Aek Sibundong (Santika and Rozakurniati 2010) and Inpari 24 (IAARD 2012). These red rice varieties are all lowland rice varieties, while improved upland red rice varieties were only three varieties, i.e. Inpago 7, Inpago Unram 1, and Inpago Unsoed 1 (IAARD 2012).

The limited number of high-yielding varieties of red and black upland rices needs to be addressed to anticipate the future increasing demand. One way to address this gap is to select local germplasm, which can be proposed as a candidate variety or used as parental sources for assembling new superior varieties. A number of local red and black upland rice accessions have been collected from various locations in East Nusa Tenggara (ENT) Province, Indonesia (Mau et al. 2013). This collection is an invaluable genetic resource that can be used for varietal improvement. An important step to handle this germplasm is to determine its level of genetic diversity. The existence of high level genetic diversity is a valuable asset for varietal improvement. Genetic diversity of red and black upland rice accessions can be determined based on the agro-morphological traits. Information about these traits is important for selection and further employment for production of new superior varieties.

The aims of this study were: (i) to identify agro-morphological characters, (ii) to determine genetic diversity of red and black upland rice accessions from East Nusa Tenggara Province, Indonesia.

MATERIALS AND METHODS

Research location and design

The present experiment was carried in the Glasshouse of Faculty of Agriculture, Universitas Nusa Cendana, Kupang, East Nusa Tenggara (ENT), Indonesia from April to October 2015. The experiment was laid out in a Completely Randomized Design employing 40 rice accessions as treatments, each was four 4 replicates. The treatment consisted of 35 local red and black upland rice accessions from ENT Province and four nationally released varieties as the checks (Table 1). In total, 40 experimental units were included in the study.

Research pprocedures

Seeds of the rice accessions were directly sown in plastic pots containing 10 kg of soil and manure in a 4:1 ratio (8 kg Vertisols and two kg manure). All seeds were grown in perforated pots to prevent water lodging to suit the water-unsaturated nature of upland rice growing condition. Three to four seeds were seeded per pot but only two plants were retained in each growing pot until harvest. The rice plants were cultivated based on the standard rice cultivation technique. The plants were harvested when about 95% of the grain had matured and turned to straw or reddish/purple straw color, the panicles dropped down due to the heavy grain, and the grain fell off easily when squeezed.

Agro-morphological trait observation

Observed variables included both qualitative and quantitative agro-morphological characters of leaf, culm and grain. Observed qualitative characters included 26 traits (leaf blade pubescence, leaf blade surface texture, leaf blade attitude, flag leaf attitude, leaf blade color, leaf sheath color, ligule color, ligule shape, collar color, auricle color, culm strength at vegetative stage, culm strength at generative stage, culm node color, culm internote color, culm angle, panicle type, panicle base, apiculus color, stigma color, awning, awn color, lemma and pallea pubescence, sterile lemma color, grain shape, grain tip color) while the observed quantitative characters included 17 traits (leaf length, leaf width, ligule length, sterile lemma length, basal culm internode diameter, plant height at flowering, plant height at harvesting, flowering date, harvesting date, number of tiller at vegetative stage, number of productive tiller, panicle length, number of grain per panicle, grain length, grain width, 100-seed weight, grain weight per-plant. The observed qualitative traits were scored based on Standard Evaluation System for rice issued by IRRI (2014) (Table 2) prior to data analysis.

Data analysis

The observed qualitative data were subjected to descriptive analysis while the quantitative data were subjected to analysis of variance (ANOVA) to determine the treatment effect. Observed variables with significant treatment effect were further subjected to DMRT (5%) post hoc test to determine the difference among treatment means. Both the qualitative data that varied among the treatments and the quantitative data that were significantly affected by treatment were subjected to cluster analysis to further classify the tested accessions based on their genetic diversity. Analysis of variance was carried out using Genstat Version 12 (VSNi 2009) while cluster analysis was carried out using PAST (Hammer et al. 2001).

RESULTS AND DISCUSSIONS

Observed data presented in Table 3 demonstrate a substantial qualitative trait variation among the tested upland rice accessions. Most of the evaluated rice accessions (60%) exhibited pubescent leaf blade while the rest of the accessions exhibited glabrous (17.5%) and intermediate (22.5%) leaf blade pubescence. We also observed in the present study that approximately 27.5% of the evaluated rice accessions exhibited a smooth leaf surface while the remaining accessions (72.5%) exhibited a rough leaf surface. Data in Table 3 also demonstrate variation in flag leaf attitude where the percentage of rice accessions with erect, intermediate and descending flag leaf are, respectively, 32.5%, 7.5% and 60%.

Results of the present study in Table 3 also revealed that most of the tested rice accessions possessed light green leaf blade color (85%), green leaf sheath color (90%), white ligule color (92.5%), acute to acuminate ligule shape (97.5%), light green auricle color (90%), green culm node color (90%), green internode color (87.5%), erect culm attitude (95%), straw awn color (80%), moderate culm strength at vegetative stage (77.5%), white stigma color (67.5%), yellow sterile lemma color (62.5%) slender grain shape (62.5%), and straw green tip color (75.5%). The present study results also demonstrate that all tested rice accessions (100%) shared an erect leaf blade attitude and a light green collar color. Meanwhile, the evaluated rice accessions distributed almost evenly on the remaining observed traits, which ranged between 2.5-45% and 12.5 - 35%, respectively, for lemma and pallea color and for culm strength at generative stage. This implies that the red and black upland rice accessions evaluated in the present study exhibited a high variability in most of the observed qualitative traits. Samples of vegetative performance of the tested rice accessions are presented in Figure 1.

Quantitative trait characterization

Observed quantitative agro-morphological traits of the evaluated upland rice accessions are presented in Tables 4 and 5. As with qualitative traits, we also found significant quantitative trait variation among the 40 rice accessions evaluated under the present study. The variability we observed in the quantitative characters was much higher than that of the qualitative characters. This was shown by significant ($P<0.05$) or highly significant ($P<0.01$) genotypic effect on the observed variables (Anova results are not shown).

Data on Table 4 demonstrate that leaf blade width of the rice accessions ranged between 1.2 cm (min.) and 2.3 cm (max.) while leaf blade length ranged from 41.8 cm to 81.0 cm. Furthermore, ligule length of the rice accessions ranged from 15 mm to 39 mm. Only two classes of sterile lemma length were observed in this study, i.e. short (no longer than 1.5 mm) and medium (1.6 -2.5 mm). Twelve accessions exhibited short sterile lemma while the rest 28 accessions had medium sterile lemma length. We also found significant variation on basal culm internode diameter, which ranged from 0.4 cm to 1.7 cm. Remarkable variation in this trait had also been reported by other workers in red and black upland rice germplasm (do Nascimento et al. 2011; Shinta, et al. 2014; Nurhasanah et al. 2016).

The minimum plant height at flowering stage was 85 cm while the maximum was 153.3 cm (Table 4). Results of the present study also revealed that most of the evaluated rice accessions (90%) were above 100 cm high at flowering, and only four accessions (10%), i.e. IR-20, SBD-03, SBD-05, and Aek Sibundong were below 100 cm at the flowering stage. We also observed substantial plant height differences among rice accessions at harvest (Figure 2.A), which ranged from 85 cm (min.) to 153.5 cm (max.). It is interesting to observe in the present study that rice accession with the highest plant height at flowering stage was different from that at harvesting stage (Table 4). This might have occurred due to the difference in the way of measuring plant height at flowering and harvesting stages. Plant height at flowering stage was measured from the culm base to the tip of the longest leaf while that at harvesting stage was measured from the culm base to the tip of the highest/longest panicle. Additionally, we also found that only 6 (15%) rice accessions, i.e. IR-20, CBL-01, SBD-03, MGP-01, SLT-01, and Aek Sibundong were below 100 cm high at harvest. This number is higher than that reported in the previous study by Shinta, et al. (2014) who found none of the six local pigmented rice collection from various locations in Indonesia, except the check variety Aek Sibundong that exhibited <100 cm plant height. Nurhasanah et al. (2016) had also characterized 71 local rice cultivars (40 cultivars were upland rice) from East Kalimantan but the authors found no cultivars to be lower than 100 cm high. Interestingly, seven local accessions (17.5%) evaluated in the current study exhibited optimum plant height between 85 -100 cm, which suit the breeder's preference as donors for production of new rice variety in the breeding program (Rabara et al., 2014). Almost the same percentage of upland colored rices with this optimum plant height had also been reported by Ahmad et al. (2015).

A B

Figure 1. Performance of several tested rice accessions at vegetative stage: A. Observed ligules of SBD-02, B. Vegetative growth performances of SBD-05, TL-04 and IR-20.

Table 1. Name, origin, usage, and seed coat color of rice accessions evaluated in the present study

Accession name	Vernacular name	Origin	Seed coat color	Usage
ADN-03	Waha Miten	East Adonara Sub-District, East Flores District	Black	Staple food, special food for pregnant and newly-giving birth mothers
ADN-04	Waha Me’a	East Adonara Sub-District, East Flores District	Red	Staple food, special food for pregnant and newly-giving birth mothers
ADN-05	Waha Mare	East Adonara Sub-District, East Flores District	Purple	Staple food, special food for pregnant and newly-giving birth mothers
ALR-01	Eba	Northeast Alor Sub-District, Alor District	Red	Food for special occasion and traditional ceremony
BLA-01	Auhu Tajan	Tabundung Sub-District, East Sumba District	Reddish Purple	Food for special occasion and traditional ceremony
CBL-01	Laka	Cibal Sub-District, Manggarai District	Black	Food for special occasion and traditional ceremony
ISN-02	Aen Nuti	Insana Sub-District, TTU District	Red	Food for special occasion, to cure diarrhea disease
ISN-03	Aen Metan	Insana Sub-District, TTU District	Reddish Purple	Food for special occasion, to cure diarrhea disease
KMD-01	Laka	Komodo Sub-District, West Manggarai District	Dark Purple	Staple food, mixed with white rice, special ceremony
MANU-04	Aen Nuti	Biboki Anleu Sub-District, TTU District	Red	Staple food, mixed with white rice, special ceremony
MGP-01	Pare Mite	Magepanda Sub-District, Sikka District	Black	Staple food, mixed with white rice, special ceremony
MGR-012	Ari Molas	Elar Sub-District, East Manggarai District	Red	Staple food, mixed with white rice, special ceremony
MGR-04	Ari Molas	Elar Sub-District, East Maggarai District	Red	Staple food, mixed with white rice, special ceremony
MK-01	Pari Mite	North Kodi Sub-District, Southwest Sumba District	Black	Staple food, mixed with white rice, special ceremony
NGR-011	Pari Mete	Nangaroro Sub-District, Nagekeo District	Red	Food for special occasion and traditional ceremony
NGR-012	Pare Mite	Nangaroro Sub-District, Nagekeo District	Reddish Purple	Food for special occasion and traditional ceremony
NGR-021	Pare Mite	Nangaroro Sub-District, Nagekeo District	Reddish Purple	Food for special occasion and traditional ceremony
NGR-022	Pare Mite	Nangaroro Sub-District, Nagekeo District	Black	Food for special occasion and traditional ceremony
PAU-01	Pare Mete	Paubekok Sub-District, Sikka District	Red	Food for special occasion and traditional ceremony
PJ-01	Pari Joki	Kodi Sub-District, Southwest Sumba District	Red	Food for special occasion and traditional ceremony
PK-01	Pari Kadico	North Kodi Sub-District, Southwest Sumba District	Light Red	Food for special occasion and traditional ceremony
PKP-01	Pari Kadico	North Kodi Sub-District, Southwest Sumba District	Red	Food for special occasion and traditional ceremony
PM-01	Pari Mete	North Kodi Sub-District, Southwest Sumba District	Reddish Black	Food for special occasion and traditional ceremony
PMK-01	Pari Mete Kadico	North Kodi Sub-District, Southwest Sumba District	Red	Food for special occasion and traditional ceremony
PRA-01	Auhu Hengu	Tabundung Sub-District, East Sumba District	Red	Food for special occasion and traditional ceremony
SBD-01	Pari Mite	Wanakaka Sub-District, West Sumba District	Black	Food for special occasion and traditional ceremony
SBD-02	Pari Mite	West Wewewa Sub-District, Southwest Sumba District	Reddish Black	Food for special occasion and traditional ceremony
SBD-03	Pari Mite	Wewewa Sub-District, Southwest Sumba District	Black	Food for special occasion and traditional ceremony
SBD-04	Pari Mete	West Wewewa Sub-District, Southwest Sumba District	Red	Food for special occasion and traditional ceremony
SBD-05	Pari Mete	West Wewewa Sub-District, Southwest Sumba District	Red	Food for special occasion and traditional ceremony
SBR-01	Laka	Sambirampas Sub-District, East Manggarai District	Dark Purple	Food for special occasion and traditional ceremony
SLR-07	Tahan Topo	West Solor Sub-District, East Flores District	Black	Food for special occasion and traditional ceremony
SLT-01	Tahan Topo	East Solor Sub-District, East Flores District	Dark Purple	Food for special occasion and traditional ceremony
TLB-04	Pare Mete	Talibura Sub-District, East Flores District	Red	Food for special occasion and traditional ceremony
TLB-05	Pare Mite	Talibura Sub-District, East Flores District	Black	Food for special occasion and traditional ceremony
Aek Sibundong	Beras merah	Check Variety, Rice Research Institute, Sukamandi, West Java	Red	Staple food
Asahan	Beras putih	Check Variety, Rice Research Institute, Sukamandi, West Java	White	Staple food
Gajah Mungkur	Beras putih	Check Variety, Rice Research Institute, Sukamandi, West Java	White	Staple food
IR-20	Beras putih	Check Variety, Rice Research Institute, Sukamandi, West Java	White	Staple food

Table 2. Characterization of qualitative traits on red and black rices according to descriptors issued by IRRI (2014)

No.	Variable	Character and score
1.	Leaf blade pubescence	Glabrous (1), intermediate (2), pubescent (3)
2.	Leaf blade surface	Smooth (1), rough (2)
3.	Leaf blade attitude	Erect (1), horizontal (5), descending (9)
4.	Flag leaf attitude	Erect (1), intermediate (3), horizontal (5), descending (7)
5.	Leaf blade color	Light green (1), green (2), dark green (3), purple tips (4), purple margins (5), purple blotch (purple mixed with green) (6), purple (7)
6.	Leaf sheath color	Green (1), purple lines (2), light purple (3), purple (4)
7.	Ligule color	Absent (0), white (1), purple lines (2), purple (3)
8.	Ligule shape	Absent (0), acute to acuminate (1), cleft (2), truncate (3)
9.	Collar color	Absent (0), light green (1), green (2), purple (3)
10.	Auricle color	Absent (0), light green (1), purple (2)
11.	Culm strength at vegetative stage	Strong, no kneeling (1), moderate, most culm are kneeling (3), intermediate, most culms are intermediately kneeling (5), susceptible, almost all culms/tillers are drooping/kneeling (7), highly susceptible, all culms/tillers are kneeling (9)
12.	Culm strength at generative stage	Strong, no kneeling (1), moderate, most culm are kneeling (3), intermediate, most culms are intermediately kneeling (5), susceptible, almost all culms/tillers are drooping/kneeling (7), highly susceptible, all culms/tillers are kneeling (9)
13.	Culm node color	Green (1), light gold (2), purple lines (3), purple (4)
14.	Culm internode color	Green (1), light gold (2), purple lines (3), purple (4)
15.	Culm angle	Erect, <30° (1), intermediate, ~45° (3), open, ~60° (5), spreading, >60° (7), procumbent (9)
16.	Panicle type	Compact (1), intermediate (2), open (3)
17.	Panicle bases	Closed (1), open (2)
18.	Awning	Absent (0), short and partly awned (1), short and fully awned (5), long and partly awned (7), long and partly awned (9)
19.	Awn color	Awnless (0), straw (1), gold (2), brown/tawny (3), red (4), purple (5), black (6)
20.	Apiculus color	White (1), straw (2), brown/tawny (3), red (4), red apex (5), purple (6), purple apex (7)
21.	Stigma color	White (1), light green (2), yellow (3), light purple (4), purple (5)
22.	Lemma and palea color	Straw (0), gold (1), brown spots on straw (2), brown furrows on straw (3), brown/tawny (4), reddish to light purple (5), purple spots on straw (6), purple furrows on straw (7), purple (8), black (9), white (10)
23.	Lemma and palea pubesence	Glabrous (1), hairs on lemma keel (2), hairs on upper portion (3), short hairs (4), long hairs/velvety (5)
24.	Sterile lemma color	Straw/yellow (1), gold (2), red (3), purple (4)
25.	Grain shape	Bold (1), slender (2)
26.	Grain tip color	Straw (1), gold (2), red (3), purple (4)

Table 3. Observed qualitative characters of red and black upland rice accessions from ENT Province and the check varieties

Qualitative character		Accession number	%
Leaf blade pubescence	Glabrous	1, 4, 8, 10, 22, 34, 38	17.5
	Intermediate	2, 11, 15, 17, 21, 25, 27, 32, 40	22.5
	Pubescent	3, 5, 6, 7, 9, 12, 13, 14, 16, 18, 19, 20, 23, 24, 26, 28, 29, 30, 31, 33, 35, 36, 37, 39	60.0
Leaf blade surface	Smooth	1, 4, 7, 10, 12, 21, 22, 33, 34, 36, 38	27.5
	Rough	2, 3, 5, 6, 8, 9, 11, 13, 14, 15, 16, 17, 18, 19, 20, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 35, 37, 39, 40	72.5
Leaf blade attitude	Erect	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,28,39,40	100
Flag leaf attitude	Erect	7, 16, 19, 21, 25, 28, 31, 32, 33, 36, 37, 39, 40	32.5
	Intermediate	20, 23, 34	7.5
	Descending	1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 22, 24, 26, 27, 29, 30, 35, 38	60.0
Leaf blade color	Light green	1, 11, 13, 27, 32, 35	15.0
	Green	2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 28, 29, 30, 31, 33, 34, 36, 37, 38, 39, 40	85.0
Leaf sheath color	Green	1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40	90.0
	Purple lines	2, 20, 21, 22	10.0
Ligule color	White	1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 26, 27, 28, 29, 30, 32, 33, 34, 35, 36, 37, 28, 39, 40	92.5
	Purple	9, 24, 31	7.5
Ligule shape	Acute to acuminate	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,28,39,40	97.5
	Cleft	19	2.5
Collar color	Light green	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,28,39,40	100
Auricle color	Light green	1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 26, 27, 28, 29, 30, 32, 33, 34, 35, 36, 37, 28, 40	90.0
	Purple	9, 24, 32, 39	10.0
Culm strength at vegetative stage	Moderate, most culms are kneeling	1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15, 17, 19, 20, 21, 22, 23, 25, 26, 27, 30, 31, 32, 33, 37, 28, 39, 40	77.5
	Intermediate, most culms are intermediately kneeling	9, 16, 18, 24, 28, 29, 34, 35, 36	22.5
Culm strength at generative stage	Moderate, most culms are kneeling	14, 17, 20, 21, 23, 25, 30, 38, 39	22.5
	Intermediate, most culms are intermediately kneeling	2, 4, 5, 6, 7, 8, 11, 15, 22, 24, 26, 31, 36, 40	35.0
	Susceptible, almost all culms/ tillers are drooping/ kneeling	3, 10, 12, 13, 16, 18, 27, 28, 29, 32, 35, 37	30.0
	Highly susceptible, all culms/tillers are kneeling	1, 9, 19, 33, 34	12.5
Culm node color	Green	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 28, 39, 40	90.0
	Purple	1, 20, 21, 22	10.0
Culm internode color	Green	1, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 23, 25, 26, 27, 28, 30, 31, 32, 33, 34, 35, 36, 37, 3839, 40	87.5
	Light gold	29	2.5
	Purple lines	2, 24	5.0
	Purple	10, 22	5.0
Culm angle	Erect, <30°	1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34,35,36,37,28,39,40	95.0
	intermediate, ~45°	2, 24	5.0
Panicle type	Compact	5, 6, 12, 19, 21, 22, 24, 29, 32, 33, 34	27.5
	Intermediate	3, 14, 16, 18, 20, 23, 25, 30, 31	22.5
	Spreading	1, 2, 4, 7, 8, 9, 10, 11, 13, 15, 17, 26, 27, 28, 35, 36, 37, 38, 39, 40	50.0

Panicle basis	Closed	5, 6, 7, 11, 15, 17, 19, 26, 28, 29, 30, 31, 33, 36, 39, 40	40.0
	Open	1, 2, 3, 4, 8, 9, 10, 13, 14, 16, 18, 20, 21, 22, 23, 24, 25, 27, 32, 34, 35, 38	60.0
	Slender	1, 4, 6, 7, 8, 9, 10, 16, 17, 18, 21, 22, 23, 25, 26, 27, 28, 29, 31, 33, 35, 36, 37, 38, 40	62.5
Awning	Absent	1, 7, 10, 21, 35, 38	15.0
	Short and partly awned	2, 5, 12, 13, 16, 18, 19, 20, 23, 26, 27, 28, 33, 39	35.0
	Short and fully awned	29	2.5
	Long and partly awned	3, 4, 6, 7, 9, 10, 11, 14, 15, 17, 21, 22, 24, 25, 30, 31, 32, 34, 35, 36, 37, 38	42.5
	Long and fully awned	8, 40	5.0
Awn color	Awnless	10, 35	5.0
	Straw	1, 2, 3, 4, 5, 8, 9, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 23, 24, 25, 26, 27, 28, 20, 30, 32, 33, 34, 35, 36, 37, 39, 40	80.0
	Gold	6, 31, 38	7.5
	Purple	7, 15, 22	7.5
Apiculus color	White	1, 2, 8, 10, 13, 15, 18, 19, 20, 23, 27, 28, 29, 30, 33, 34, 36, 37, 40	42.5
	Straw	5, 11, 12, 14, 25, 32, 38	17.5
	Brownish	3, 9, 31, 35	10.0
	Red	4, 7, 16, 39	10.0
	Dark red	6, 10, 21, 22, 24	12.5
	Purple	17, 26	5.0
	Dark purple	20	2.5
Stigma color	White	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 15, 16, 17, 18, 23, 24, 25, 28, 30, 31, 33, 35, 36, 37, 39, 40	67.5
	Light green	14, 23, 38	7.5
	Yellow	10, 19, 20, 21, 22, 27, 29, 32, 34	22.5
	Purple	26	2.5
lemma and palea pubescence	Glabrous	1, 3, 7, 8, 9, 11, 13, 15, 17, 21, 24, 26, 27, 31, 32, 35	40.0
	Hairs on lemma keel	19, 22, 23, 29, 30, 36, 40	17.5
	Hairs on upper portion	2, 4, 18, 34, 37, 38, 39	17.5
	Short hairs	5, 6, 12, 14, 16, 19, 25, 28, 33	22.5
	Long hairs	10	2.5
Lemma and pallea color	Gold	2, 4, 8, 16, 19, 22, 25, 29, 37, 38, 39, 40	30.0
	Brown spots on straw	3, 32	5.0
	Brown furrows on straw	1, 5, 7, 10, 11, 12, 13, 14, 15, 17, 18, 23, 26, 27, 30, 31, 34, 35, 36	45.0
	Brown/tawny	6, 9, 20, 24, 28, 33	15.0
	Reddish to light purple	21	2.5
	Purple spots on straw	10	2.5
Sterile lemma color	Yellow	1, 2, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 19, 21, 22, 23, 25, 27, 28, 29, 30, 33, 34, 35, 39, 40	62.5
	Gold	26, 36, 37, 38	10.0
	Purple	3, 9, 11, 13, 15, 17, 20, 24, 27, 31, 32	27.5
Grain shape	Bold	2, 3, 5, 11, 12, 13, 14, 15, 19, 20, 24, 30, 32, 34, 39	37.5
	Slender	1, 4, 6, 7, 8, 9, 10, 16, 17, 18, 21, 22, 23, 25, 26, 27, 28, 29, 31, 33, 35, 36, 37, 38, 40	62.5
Grain tip color	Straw	1, 3, 5, 6, 7, 8, 9, 11, 13, 14, 15, 16, 18, 23, 24, 25, 29, 30, 31, 32, 34, 35, 36, 37, 40	57.5
	Gold	38	2.5
	Red	2, 4, 10, 16, 19, 21, 22, 27, 32, 39	25.0
	Purple	12, 17, 20, 26, 28, 33	15.0

Note: 1 (ADN-03), 2 (ADN-04), 3 (ADN-05), 4 (ALR-01, 5 (BLA-01), 6 (CBL-01), 7 (ISN-02), 8 (ISN-03), 9 (KMD-01), 10 (MANU-04), 11 (MGP-01), 12 (MGR-021), 13 (MGR-04), 14 (MK-01), 15 (NGR-011), 16 (NGR-012), 17 (NGR-021), 18 (NGR-022), 19 (PAU-01), 20 (PJ-01), 21 (PK-01), 22 (PKP-1), 23 (PM-01), 24 (PMK-01), 25 (PRA-01), 26 (SBD-01), 27 (SBD-02), 28 (SBD-03), 29 (SBD-04), 30 (SBD-05), 31 (SBR-01), 32 (SLR-07), 33 (SLT-01), 34 (TLB-04), 35 (TLB-05), 36 (Aek Sibundong), 37 (Asahan), 38 (Gajah Mungkur), 39 (IR-20), 40 (Kencana Bali)

The flowering date of the rice accessions varied from 62 days after planting (DAP) (Gajah Mungkur) to 111 DAP (PJ-01) (Table 4). More than one half the number of rice accessions flowered at no longer than 90 DAP. As with flowering date, we also observed substantial variation in harvesting date of the tested rice accessions, which ranged between 95 DAP (Gajah Mungkur) and 155 DAP (PJ-01) (Table 4). We found that flowering date and harvesting date were highly correlated (r = 0.82). This is in line with observation by Ahmad et al. (2015) that flowering date was strongly correlated with harvesting date, where rice accessions that flower earlier tended to mature earlier, and vice versa. Harvesting dates of most of the currently improved varieties in Indonesia are no longer than 120 days (IAARD 2012). About 60% local rice accessions evaluated in the present study matured at about 95 -120 days, which indicates that this rice germplasm is a potential source to select for short duration traits in red and black upland rice varietal development.

Number of tiller per plant at vegetative stage varied from six (PK-01) to 17 (TLB-04) while the productive tillers varied from four to 15 culms per plant with the highest number was observed in local accession TLB-04 (Table 5), which implies that about 67-89% of the total tillers produced at vegetative stage were able to produce panicle. These ranges of tiller number per plant are much higher than those reported from red and black rices from various countries of origin by Ahmad et al. (2015) who, in the same study, also found that tiller number was significantly correlated with grain yield per plant. In contrast to observation by Ahmad et al. (2015), we did find very weak correlation (R2 = 0.12) between number of productive tiller per plant and grain weight per plant. This implies that red and black upland rice accessions elucidated in the current study are unique, more specifically in the agronomical characters. Beside number of productive tiller, grain weight is also determined by other yield components such as number of grain per panicle and 100-seed weight. Accessions with low number of productive tiller per plant may have higher number of grain per panicle and/or 100-seed weight, and vice versa, which may likely cause weak or no correlation between each of these traits with the grain yield (grain weight per plant).

In the current study, we also observed substantial differences in the panicle length and the grain length. The maximum panicle length was 45.8 cm while the minimum panicle length was 17.5 cm (Table 5). This range of panicle length was much higher than that of 41 colored rice genotypes evaluated by Ahmad et al. (2015). The grain length varied from 4.5 mm (MGP-01) to 10.0 mm (Gajah Mungkur, TLB-05). This finding showed varying size of rice grain that covers short, medium, and long grain sizes of the tested rice accessions. This grain length variation correlates closely with the size of rice/caryopsis, which may correspond to the consumer's preference on the rice/caryopsis size. This local rice germplasm is, therefore, of a significantly useful genetic resource that can be used to select for rice accessions with the most preferred grain characters to the local consumers in ENT province in particular, and in Indonesia in general.

Our finding in the present study (Table 5) also revealed substantially variable grain width among the evaluated rice accessions. The grain width we observed ranged from 2.5 mm (BLA-01) to 4.3 mm (SBD-03, PJ-01), which indicates slender to bold size grain (Figure 2.B). This grain width range is a potential genetic resource that can be used to select for grain size that suit the consumer's preference.

The present study results also revealed that quantitative traits such as number of grain per panicle, weight of 100 seeds, and grain weight per-plant varied considerably among the rice accessions (Table 5). Data in Table 5 show a large variation in number of grain per-panicle, which ranged from 26 to 220 grain. The highest number of grain per-panicle was found in the local accession ISN-02 (220 grain). Twelve local accessions outnumbered the check varieties in terms of number of grain per-panicle, which indicates the superiority of local accessions in this trait.

As with number of grain per-panicle, we also observed significant variation in 100-seed weight among the tested rice accessions. The highest 100-seed weight was found in the local accession PRA-01 (4.5 g) while the lowest was observed in two local accessions, i.e. KMD-01and SBD-03, each produced only about 1.9 g per 100 grain (Table 5). Eleven local accessions were found to produce higher 100-grain weight than the check red rice variety, Aek Sibundong. Four of the eleven local accessions (PRA-01, PKP-01, PK-01, ISN-03) also produced higher 100-grain weight than the two white rice check varieties, i.e. Gajah Mungkur and Bali Kencana. Data analysis showed that there was no correlation between 100-seed weight and grain length (R2 = 0.04) and grain width (R2 = 0.04), which indicates that the rice accessions evaluated in this study are unique in these traits. These findings demonstrated wide grain size variability among the tested rice accessions indicating a wide genetic variability for selection of grain size suiting the consumer's preference. As a comparison, Ahmad et al. (2015) reported 100-seed weight range of red and black rice germplasm between 1.57 -3.21 g, which was lower than the present study results.

The present study results also revealed significant variation of grain weight per-plant ranging from 10.8 g (min.) to 28 g (max.) (Table 5). Local accession MGP-01 (Figure 2.A) from Sikka District produced the highest grain per-plant (28 g), followed by TLB-04 (26.4 g) (Figure 2.A), which is also collected from the same district, in the second place. We also found that each of 30 local accessions from ENT Province produced a higher grain weight per-plant than the check red rice variety, Aek Sibundong (13.6 g). Grain yield per-plant is influenced by other yield component traits such as number of productive tillers; panicle length, and 100-seed weight.

We also found in the current study that grain weight per plant showed no correlation with 100-seed weight (R2 = 0.04), panicle length (R2 = 0.003), and number of grain per panicle (R2 = 0.004). The panicle length was also found to be not correlated with number of grain per plant (R2 = 0.06). In a normal circumstance, we would expect strong correlations among these traits. Strong correlation between number of grain per-plant with number of productive tiller and panicle length had been reported by Ahmad et al.

(2015). Thus, the absence of correlation between grain yield and yield component characters observed in the present study suggests that the red and black upland rice accessions evaluated in the current study are unique in agronomical characters, more specifically the yield and yield component characters. Despite the absence of correlation among these characters, our finding demonstrates high grain yield potential and unique yield component characters of red and black upland rice accessions from ENT Province. These accessions are, therefore, invaluable genetic resources that can be exploited to assemble future high yielding red and black upland rice varieties.

Genetic diversity analysis

Results of this study revealed that the tested rice accessions exhibited significant variation in most of the observed qualitative and quantitative traits. These significantly differed traits were then used to assess the genetic diversity of the rice germplasm. Qualitative and quantitative characters were separately used in cluster analysis to generate genetic relationship dendograms.

Table 4. Mean leaf and culm characters and plant height of upland red and black rice accession from ENT Province and the check varieties

Rice accession	Leaf blade width (cm)*	Leaf blade length (cm)	Ligule length (mm)	Sterile lemma length (code**)	Basal culm internode diameter (cm)	Plant height at flowering (cm)	Plant height at harvesting (cm)	Flowering date (DAP)	Harvesting date (DAP)
ADN-03	$1.9^{bcdefgh}$	62.3^{abcde}	20^{abcd}	3.0^{a}	0.6^{abcde}	153.3^{j}	147.3^{ij}	$86.5^{cdefghij}$	111.0^{bc}
ADN-04	2.3^{h}	80.0^{e}	24^{bcde}	1.0^{a}	1.3^{k}	142.8^{ghij}	147.3^{ij}	$83.3^{cdefghi}$	118.3^{bcd}
ADN-05	2.3^{h}	70.8^{bcde}	21^{abcd}	3.0^{a}	0.5^{abcd}	143.8^{hij}	152.0^{j}	$81.8^{bcdefgh}$	111.0^{bc}
ALR-01	1.9^{cdefgh}	65.8^{abcde}	23^{abcde}	1.0^{a}	0.8^{cdef}	136.3^{fghij}	152.0^{j}	$84.0^{cdefghi}$	115.0^{bcd}
BLA-01	1.9^{defgh}	60.5^{abcde}	21^{abcd}	3.0^{a}	0.5^{abcde}	$122.5^{cdefghi}$	106.8^{d}	$97.3^{efghijkl}$	124.3^{cd}
CBL-01	1.5^{abcde}	50.0^{abc}	30^{ef}	3.0^{a}	0.4^{ab}	103.5^{abcd}	98.5^{bc}	$98.5^{fghijkl}$	124.8^{cd}
ISN-02	2.0^{efgh}	79.6^{e}	24^{bcdef}	1.0^{a}	1.3^{h}	137.0^{fghij}	153.5^{j}	74.0^{abcd}	112.8^{bcd}
ISN-03	2.2^{fgh}	75.5^{cde}	23^{bcde}	1.0^{a}	0.8^{bcdef}	135.3^{fghij}	143.8^{hij}	$89.3^{cdefghijk}$	126.5^{cd}
KMD-01	$1.8^{abcdefgh}$	44.8^{ab}	27^{def}	3.0^{a}	0.5^{ab}	105.3^{abcde}	103.8^{cd}	77.0^{abcde}	110.8^{bc}
MANU-04	2.1^{fgh}	80.3^{e}	26^{bcdef}	3.0^{a}	0.6^{abcde}	146.8^{ij}	139.8^{ghij}	81.8^{cdefgh}	108.0^{b}
MGP-01	$1.8^{abcdefgh}$	81.0^{e}	21^{abcd}	3.0^{a}	0.6^{abcde}	135.0^{fghij}	148.5^{ij}	$86.0^{cdefghij}$	111.8^{bcd}
MGR-012	$1.6^{abcdefg}$	60.5^{abcde}	23^{abcde}	3.0^{a}	0.5^{abcde}	107.8^{abcdef}	100.0^{bc}	99.5^{ghjkl}	120.3^{bcd}
MGR-04	2.2^{gh}	75.5^{cde}	25^{bcdef}	3.0^{a}	0.5^{abc}	$129.5^{defghij}$	138.0^{fghij}	$84.8^{cdefghi}$	113.0^{bcd}
MK-01	2.1^{fgh}	69.0^{bcde}	20^{abcd}	1.0^{a}	1.1^{ghijk}	132.0^{efghij}	139.8^{ghij}	108.5^{kl}	144.3^{efg}
NGR-011	2.0^{defgh}	73.4^{cde}	22^{abcde}	3.0^{a}	0.4^{ab}	$129.8^{defghij}$	136.0^{fghi}	$91.0^{cdefghijk}$	119.3^{bcd}
NGR-012	2.0^{defgh}	60.8^{abcde}	19^{abcd}	1.0^{a}	0.9^{fghij}	133.8^{fghij}	135.3^{fghi}	$95.5^{efghijkl}$	116.5^{bcd}
NGR-021	1.4^{abcde}	57.3^{abcde}	27^{def}	3.0^{a}	0.5^{abcd}	104.8^{abcde}	131.5^{fgh}	$88.3^{cdefghijk}$	118.8^{bcd}
NGR-022	1.6^{abcdef}	61.8^{abcde}	22^{abcd}	1.0^{a}	1.1^{hijk}	133.5^{fghij}	138.0^{fghij}	75.8^{abcde}	115.8^{bcd}
PAU-01	2.1^{efgh}	68.0^{bcde}	23^{abcde}	3.0^{a}	1.1^{ijk}	$130.8^{defghij}$	136.0^{fghi}	103.0^{hijkl}	135.3^{de}
PJ-01	2.2^{gh}	73.8^{cde}	19^{abcd}	1.0^{a}	1.1^{ijk}	$128.0^{defghij}$	135.8^{fghi}	111.3^{l}	155.3^{g}
PK-01	$1.8^{abcdefgh}$	58.3^{abcde}	21^{abcd}	1.0^{a}	1.1^{ijk}	134.0^{fghij}	131.5^{fgh}	103.5^{ijkl}	148.3^{fg}
PKP-01	$1.7^{abcdefgh}$	55.3^{abcde}	23^{abcde}	1.0^{a}	0.9^{fghij}	$115.0^{bcdefgh}$	116.8^{ce}	103.5^{ijkl}	141.3^{ef}
PM-01	1.9^{defgh}	69.5^{bcde}	18^{abc}	3.0^{a}	0.8^{defg}	$128.8^{defghij}$	130.5^{fgh}	101.5^{ghijkl}	134.0^{cd}
PMK-01	2.3^{h}	67.5^{abcde}	21^{abcd}	3.0^{a}	0.5^{ab}	142.3^{ghij}	130.5^{fgh}	99.8^{ghijkl}	120.5^{bcd}
PRA-01	2.0^{defgh}	60.1^{abcde}	20^{abcd}	3.0^{a}	1.0^{fghij}	114.0^{bcdefg}	134.3^{fghi}	105.8^{ijkl}	142.5^{ef}
SBD-01	$1.7^{abcdefgh}$	59.0^{abcde}	31^{f}	3.0^{a}	0.5^{abc}	$121.5^{cdefghi}$	104.5^{cd}	$79.5^{abcdefg}$	111.8^{bcd}
SBD-02	2.0^{efgh}	60.3^{abcde}	18^{ab}	3.0^{a}	0.5^{abcd}	$128.3^{defghij}$	124.5^{ef}	$94.3^{defghijkl}$	115.3^{bcd}
SBD-03	1.5^{abcde}	62.0^{abcde}	30^{ef}	3.0^{a}	0.4^{a}	96.5^{abc}	85.0^{a}	$93.0^{defghijkl}$	125.3^{cd}
SBD-04	$1.8^{abcdefgh}$	62.8^{abcde}	27^{def}	1.0^{a}	1.1^{fghijk}	110.0^{abcdef}	124.5^{ef}	63.8^{ab}	116.3^{bcd}
SBD-05	2.0^{defgh}	60.7^{abcde}	27^{cdef}	3.0^{a}	1.7^{l}	97.5^{abc}	104.8^{cd}	71.5^{abc}	114.8^{bcd}
SBR-01	$1.8^{abcdefgh}$	73.0^{cde}	24^{bcdef}	3.0^{a}	0.5^{ab}	135.0^{fghij}	104.8^{cd}	78.5^{abcdef}	112.5^{bcd}
SLR-07	$1.9^{bcdefgh}$	56.5^{abcde}	30^{ef}	3.0^{a}	0.4^{ab}	102.8^{abcd}	106.5^{cd}	99.0^{ghijkl}	122.3^{cd}
SLT-01	1.2^{a}	70.0^{bcde}	31^{f}	3.0^{a}	0.5^{abcd}	$125.5^{cdefghi}$	88.5^{ab}	$87.0^{cdefghij}$	123.5^{cd}
TLB-04	1.3^{ab}	77.3^{de}	25^{bcdef}	1.0^{a}	1.1^{hijk}	$115.0^{bcdefgh}$	118.8^{e}	79.3^{abcdef}	114.5^{bcd}
TLB-05	2.3^{h}	45.4^{ab}	25^{bcdef}	3.0^{a}	0.6^{abcde}	147.8^{ij}	152.8^{j}	83.0^{cdefgh}	110.5^{bc}
Aek Sibundong	$1.6^{abcdefg}$	52.5^{abcd}	23^{abcde}	3.0^{a}	0.8^{bcdef}	89.6^{ab}	94.4^{abc}	$80.5^{bcdefgh}$	111.3^{bc}
Asahan	$1.6^{abcdefg}$	41.8^{a}	23^{bcde}	3.0^{a}	1.2^{jk}	103.5^{abcd}	101.5^{c}	69.0^{ab}	113.8^{bcd}
Gajah Mungkur	1.4^{abcd}	45.9^{ab}	15^{a}	3.0^{a}	0.9^{efghi}	102.5^{abcd}	102.5^{c}	62.3^{a}	95.0^{a}
IR-20	1.3^{abc}	72.0^{cde}	23^{abcd}	3.0^{a}	0.5^{abc}	85.0^{a}	85.0^{a}	81.8^{cdefgh}	116.3^{bcd}
Kencana Bali	1.4^{abcd}	69.5^{cde}	39^{g}	3.0^{a}	0.8^{efgh}	$119.3^{cdefghi}$	126.8^{fg}	$91.0^{cdefghijk}$	127.3^{cd}
CV (%)	10.2	9.8	7.5	0.0	10.8	8.7	5.1	8.6	4.5

Note: DAP = Days After Planting. *Numbers within the same column with the same lower case are not significantly different at 0.05 DMRT. **1 = short (no longer than 1.5 mm), 3 = medium (1.6 -2.5 mm)

Qualitative trait-based genetic diversity

Prior to cluster analysis, all substantially-different qualitative data were first coded into quantitative scales according to the method of SES of rice (IRRI 2014). Clustering of the rice germplasm was based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) using Euclidean distance. Truncating the dendrogram at Euclidean distance of 6.0 resulted in four main clusters; each was further sub-grouped into two to four sub-clusters (Figure 3). The first cluster consisted of four accessions, the second consisted of 12 accessions, the third consisted of 15 accessions and the fourth cluster consisted of nine accessions. Cluster I is the smallest with only four accession members, i.e. Manu-04, PJ-01, PK-01 and SBD-01. Manu-04 was collected from TTU District while the last three accessions were collected from Southwest Sumba District. The cluster II was sub-divided into four sub-clusters containing 1 -5 accession members. PKP-01 accession from Southwest Sumba district stood alone as a sub-cluster of the cluster II. Cluster III comprised the largest accession members, and was sub-divided into five sub-clusters with two single-accession sub-clusters, i.e. TLB-4 from Sikka District in one sub-cluster and ADN-04 from East Flores District in the other sub-cluster. As with cluster III, cluster IV also consisted of two single-accession sub-clusters out of total four sub-clusters. The two single-accession sub-clusters contained NGR-021 from Nagekeo District in one sub-cluster and PMK-01 from Southwest Sumba in the other sub-cluster.

Table 5. Mean number of tiller, panicle length, grain length, grain width, # of grain per-panicle, 100-seed weight, and grain weight per-plant of red and black upland rice accessions from ENT Province and the check varieties

Rice accession	# of Tiller at vegetative stage (culm)	# of Productive tiller (culm)	Panicle length (cm)	Grain length (mm)	Grain width (mm)	# of Grain per-panicle	100-seed weight (g)	Grain weight per-plant (g)
ADN-03	$10.0^{abcdefg}$	7.0^{abcd}	34.8^{jklmn}	10^{g}	4^{de}	147.3^{hij}	2.6^{abcdef}	20.0^{cdefg}
ADN-04	7.3^{abcde}	9.5^{def}	$30.9^{fghijklm}$	7^{cd}	3^{abc}	131.0^{ghij}	3.6^{efg}	19.3^{cdef}
ADN-05	7.5^{abcdef}	6.5^{abcd}	37.8^{mn}	9^{ef}	4^{de}	98.3^{defg}	3.4^{bcdef}	17.6^{abcdef}
ALR-01	9.3^{abcdef}	6.3^{abcd}	$28.4^{bcdfghijk}$	8^{e}	3^{abc}	156.8^{ij}	2.9^{abcdef}	11.1^{ab}
BLA-01	7.0^{abcd}	4.3^{a}	$31.3^{ghijklm}$	8^{ef}	3^{a}	101^{efg}	2.8^{abcdef}	19.1^{cdef}
CBL-01	$10.8^{abcdefg}$	6.8^{abcd}	20.3^{ab}	9^{g}	4^{de}	70.3^{d}	2.1^{abc}	17.6^{abcdef}
ISN-02	9.3^{abcdef}	6.8^{abcd}	$29.1^{dfghijk}$	7^{c}	3^{ab}	220.3^{l}	3.5^{def}	19.2^{cdef}
ISN-03	8.0^{abcdef}	5.3^{ab}	33.6^{ijklmn}	9^{fg}	3^{abc}	122.3^{fghi}	4.4^{gh}	23.4^{fg}
KMD-01	14.0^{g}	11.8^{fg}	23.5^{abcdfg}	8^{ef}	4^{cd}	118.5^{efgh}	1.9^{a}	22.1^{defg}
MANU-04	8.8^{abcdef}	6.8^{abcd}	36.8^{lmn}	10^{g}	3^{bcd}	137.8^{ghij}	3.0^{abcdef}	13.4^{abc}
MGP-01	$10.8^{abcdefg}$	9.0^{cdef}	32.3^{ijklmn}	5^{a}	4^{de}	145.3^{hij}	2.1^{ab}	28.1^{h}
MGR-012	7.3^{abcde}	5.0^{ab}	45.8^{o}	9^{g}	4^{de}	84.5^{def}	3.1^{abcdef}	19.8^{cdef}
MGR-04	8.0^{abcdef}	6.0^{abcd}	36.8^{lmn}	9^{fg}	4^{de}	110.0^{efg}	3.1^{abcdef}	13.3^{abc}
MK-01	11.0^{bcdefg}	7.5^{abcde}	$29.6^{fghijkl}$	8^{de}	4^{de}	115.3^{efgh}	2.7^{abcdef}	10.9^{ab}
NGR-011	6.5^{ab}	5.0^{ab}	$31.0^{fghijklm}$	9^{g}	4^{de}	78.5^{de}	2.9^{abcdef}	17.8^{abcdef}
NGR-012	11.8^{cdefg}	7.5^{abcde}	$24.3^{abcdfgh}$	9^{ef}	3^{abc}	115.0^{efgh}	2.4^{abcde}	10.8^{a}
NGR-021	8.8^{abcdef}	5.0^{ab}	20.8^{ab}	10^{g}	4^{de}	60.8^{abcd}	3.1^{abcdef}	21.0^{defg}
NGR-022	$10.8^{abcdefg}$	8.0^{bcde}	$25.0^{bcdfghi}$	6^{b}	3^{abc}	137.0^{ghij}	2.2^{abc}	15.0^{abcde}
PAU-01	$10.8^{abcdefg}$	9.0^{cdef}	$27.3^{bcdfghijk}$	7^{cd}	4^{de}	113.0^{efgh}	3.4^{cdef}	12.6^{abc}
PJ-01	12.5^{fg}	9.5^{def}	$27.5^{bcdfghijk}$	8^{e}	4^{e}	79.3^{def}	3.3^{bcdef}	14.1^{abc}
PK-01	6.0^{a}	6.3^{abcd}	34.6^{jklmn}	10^{g}	3^{abc}	158.0^{ij}	3.8^{fg}	12.1^{abc}
PKP-01	8.8^{abcdef}	7.8^{abcde}	$31.7^{hijklmn}$	9^{ef}	3^{bcd}	194.3^{kl}	3.8^{fg}	13.2^{abc}
PM-01	12.3^{efg}	9.3^{def}	$30.6^{fghijklm}$	9^{fg}	3^{abc}	113.0^{efgh}	2.9^{abcdef}	14.2^{abcd}
PMK-01	6.5^{ab}	5.3^{ab}	$28.5^{cdfghijk}$	9^{fg}	4^{de}	66.0^{bcd}	2.6^{abcdef}	22.1^{defg}
PRA-01	7.5^{abcdef}	7.3^{abcd}	32.8^{ijklmn}	9^{fg}	3^{abc}	95.5^{defg}	4.5^{h}	12.6^{abc}
SBD-01	14.0^{g}	14.3^{gh}	$25.0^{bcdfghi}$	9^{ef}	4^{de}	113.3^{efgh}	2.3^{abcd}	21.8^{defg}
SBD-02	8.0^{abcdef}	6.8^{abcd}	$29.0^{dfghijk}$	10^{g}	4^{de}	84.0^{def}	2.9^{abcdef}	14.5^{abcd}
SBD-03	8.8^{abcdef}	5.5^{abc}	23.3^{abcdef}	10^{g}	4^{e}	81.5^{def}	1.9^{a}	18.2^{bcdef}
SBD-04	12.5^{fg}	12.3^{fgh}	$27.1^{bcdfghijk}$	8^{e}	3^{abc}	161.5^{jk}	3.0^{abcdef}	24.2^{efg}
SBD-05	$10.0^{abcdefg}$	7.5^{abcde}	$26.3^{bcdfghij}$	7^{c}	3^{abc}	113.8^{efgh}	2.5^{abcde}	14.1^{abc}
SBR-01	14.0^{g}	6.3^{abcd}	34.3^{jklmn}	8^{e}	4^{cd}	99.8^{defg}	2.0^{a}	17.2^{abcdef}
SLR-07	9.3^{abcdef}	5.3^{ab}	17.5^{a}	9^{fg}	4^{de}	67.5^{cd}	2.3^{abcd}	17.0^{abcdef}
SLT-01	7.3^{abcde}	12.0^{fg}	21.1^{abc}	9^{g}	3^{bcd}	39.5^{abcd}	2.0^{a}	19.7^{cdef}
TLB-04	17.0^{h}	15.0^{h}	21.5^{abcd}	7^{cd}	4^{de}	146.3^{hij}	2.5^{abcde}	26.4^{g}
TLB-05	7.8^{abcdef}	5.8^{abc}	38.8^{n}	10^{g}	3^{abc}	112.3^{efgh}	3.0^{abcdef}	16.9^{abcdef}
Aek Sibundong	$10.0^{abcdefg}$	9.3^{def}	$24.2^{abcdfgh}$	9^{fg}	3^{abc}	118.5^{efgh}	3.1^{abcdef}	13.6^{abc}
Asahan	11.8^{cdefg}	10.5^{ef}	$25.0^{abcdfghi}$	9^{fg}	3^{abc}	111.0^{efgh}	2.7^{abcdef}	16.3^{abcdef}
Gajah Mungkur	6.8^{abc}	4.3^{a}	22.5^{abcde}	10^{g}	3^{abc}	99.8^{defg}	3.6^{ef}	14.3^{abc}
IR-20	11.5^{bcdefg}	8.8^{cde}	21.0^{abc}	7^{cd}	3^{abc}	25.5^{a}	2.5^{abcde}	14.1^{abc}
Kencana Bali	12.3^{efg}	10.8^{ef}	23.6^{abcdfg}	9^{ef}	3^{abc}	128.8^{ghij}	3.6^{efg}	23.2^{fg}
CV (%)	10.0	10.7	6.8	4.6	8.0	8.5	0.4	8.7

Note: DAP = Days After Planting. **Numbers within the same coloumn with the same lower case are not significantly different at 0.05 DMRT

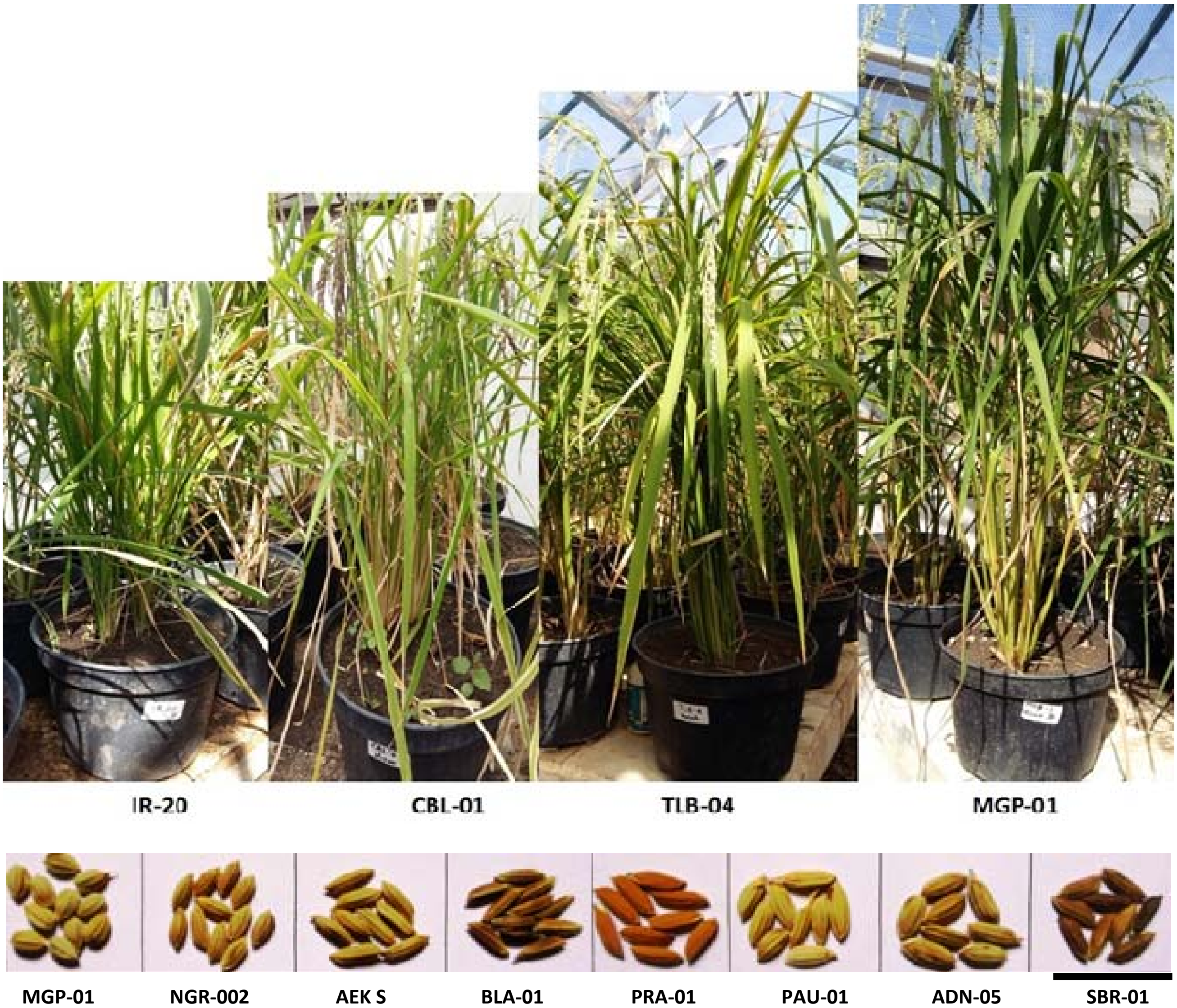

Figure 2. Samples of tested rice accession with a varying plant height at harvest (*above*) and grain size/grain shape (*below*). Bar = 2 cm

Overall, the present study results revealed that the rice accessions from ENT Province were distributed in four main clusters and fifteen sub-clusters (Figure 3). Six of the fifteen sub-clusters were single-accession sub-clusters, four of which contained accessions from Southwest Sumba District. These findings demonstrate that this rice germplasm is highly divergent in qualitative agro-morphological characters. As a comparison, previous study by Kristamtini et al. (2012) reported five taxonomic clusters of black rice accessions from several locations in Indonesia including one black rice accession from Manggarai District, ENT Province that was grouped in the same cluster with Pari Ireng, Cempo Ireng, and Jlitheng from Yogyakarta. This implies that red and black upland rice accessions from ENT, despite their origins in the same provincial location, was proved to be highly divergent that may had been partially caused by the divergent environmental factors of the province. The ENT Province is an archipelagic province with a varying degree of agro-climatic conditions. The climate type of the province is dominantly semi-arid but the west part of the province, especially the Flores Island, is sub-humid to humid climatic types. Average annual rainfall of the province is about 1500 mm but the range is large, i.e. from about 800 mm (in Sabu Raijua and East Sumba Districts) to about 4000 mm in the great Manggarai Districts. All these conditions have affected the development and the choice of rice varieties in the region.

Principal Component Analysis (PCA) was also used to further reveal the components mostly responsible for the observed variances. The PCA analysis involving qualitative traits resulted in 24 independent components that explained the total observed variance (100%). Five principal components explained about 75% variation in the characterized rice germplasm. The first component explained about 25% of the total variances while the second, the third, the fourth and the fifth accounted for, respectively, 18.9%, 13.8%, 10.3% and 7.4% of the variances. Scatter plots of PCA analysis involving 24 qualitative traits of the rice germplasm are presented in

Figure 4. This figure reveals both the distribution of rice accessions across the scatter plots and factor loadings of the qualitative traits mostly contributed to the variances in the principal component. Research results show that flag leaf attitude trait (loading factor 0.89) is mostly responsible for maximum variability in PC1, which explained 25% of the total observed variation in the data set. In PC2, three qualitative traits contributed the highest factor loadings, i.e. apiculus color (0.46), pallea-lemma color (0.43) and grain tip color (0.33). As with PC2, apiculus color also exhibited the highest loading factor (0.45) in PC3, besides flag leaf attitude (0.41) and awning (0.39). Pallea and lemma color, pallea and lemma pubescence and culm strength at vegetative stage are three agro-morphological traits that contributed the most for variation in PC4 and PC5, which, respectively, explained 10.3% and 7.4% of total variances in the data set.

The observed genetic variability of the tested rice accessions highlights their potential use as genetic resources to assemble new red and black upland rice variety that suits the consumer's preference. The high genetic divergence among the tested rice accessions allows members of one cluster to be crossed with member of other clusters to generate heterotic traits in preferred qualitative characters.

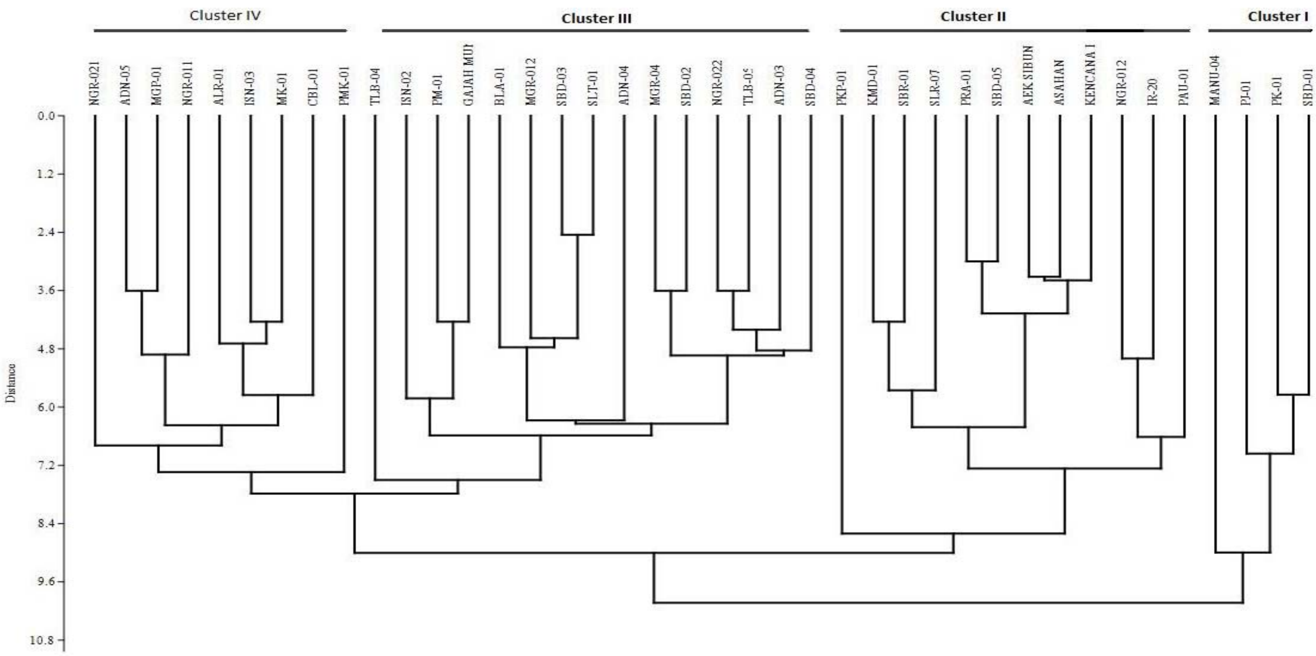

Figure 3. Dendogram of red and black upland rice accessions from ENT Province based on 24 qualitative agro-morphological traits

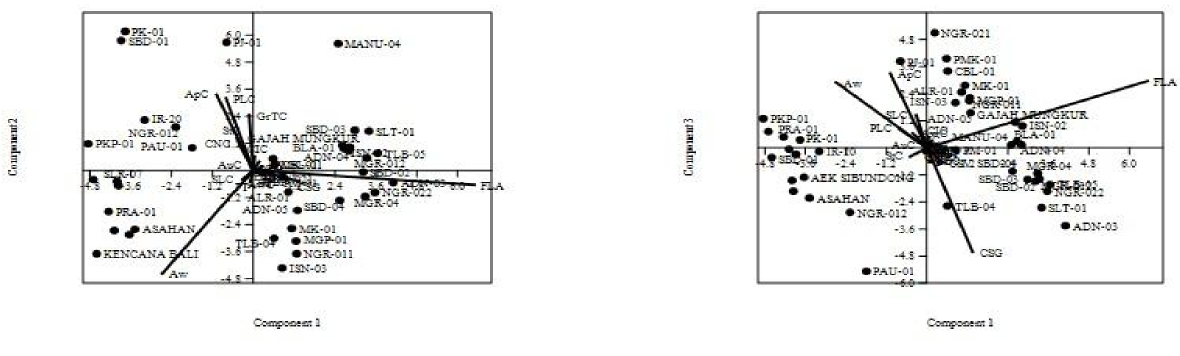

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Figure 4. Scatter plots showing distribution and traits contributed the most to the observed variances of red and black upland rice accessions from ENT Province. FLA = flag leaf attitude, Aw = awning, ApC = apiculus color), PLC = pallea-lemma color, GrTC = grain tip color, CSG = cum strength at generative stage. Note: A. PC-1 vs PC-2, B. PC-1 vs PC-3

Quantitative trait-based genetic diversity

Almost all quantitative agro-morphological characters observed in the present study, except sterile lemma length, were significantly affected by the treatment, i.e. rice accession. We then used these observed traits in a cluster analysis to group the rice accessions based on their taxonomic groups. As with qualitative characters, cluster analysis based on quantitative characters showed a great variability among the tested rice accessions with a Euclidean distance of up to 112 (Figure 5).

The 40 rice accessions were clustered into five main clusters when the taxonomic tree was truncated at Euclidean distance of 64 (Figure 5). Similar with grouping of the accessions using qualitative traits, the five clusters revealed by quantitative traits also comprised of various number of constituting members. The majority of tested rice accessions were grouped into clusters I and II, each consisted of 19 and 17 accessions, respectively. Interestingly, the cluster analysis revealed two single-accessions clusters and another cluster consisted only of two local accessions. The local accession ISN-02 from TTU District constituted the single-accession cluster III while two local accessions from Southwest Sumba District, i.e. PK-01 and PKP-01 constituted cluster IV. At last, the check white rice variety, IR-20, formed the last cluster (cluster V). The grouping of either ISN-02 or IR-20 into separate cluster may likely to be due to its unique quantitative characters. The local accession ISN-02 is very unique in its large number of grain per panicle (220 grain) (Table 4) while the check variety IR-20 possesses unique characters in both plant height (Table 4) and number of grain per panicle (Table 5). Further truncation of the dendogram tree at Euclidean distance of 45 further divided cluster I into five sub-clusters and cluster II into three sub-clusters. The grouping of local accessions PK-01 and PKP-01 into the same cluster (cluster III) indicates their high similarities in the observed quantitative characters. The similar place of origin/collection of PK-01 and PKP-01 (Southwest Sumba District) may be the likely indication that the two accessions are duplicate or may have derived from the same parental source.

Upland rice accessions used in this study were collected from 11 Districts in four main islands (Flores, Timor, Sumba and Alor) of East Nusa Tenggara Province. The present study results, as described in Figure 4, revealed that the rice accessions were clustered in various sub-clusters with mixed accession members from various district of origin except the clusters III and V that consisted only of a single accession. Accessions from the same district were not always grouped into the same cluster or sub-clusters, implying high quantitative trait diversity of red and black upland rice germplasm from ENT province. Similar results have been previously reported by other workers (do Nascimento et al. 2011; Rabara et al. 2014; Shinta et al. 2014).

Principal component analysis employing quantitative data yielded 16 independent components that explained the total observed variance of 100%. About 88% of the total variances in the data set were explained by three principal components. The first component explained about 58.5% of the total variances while the second and third components accounted for, respectively, 20.6% and 9.3% of the total variances. Scatter plots of PCA analysis involving 16 qualitative traits of the rice germplasm are presented in Figure 6.

PCA analysis results showed that number of grain per panicle (loading factor 0.91) is mostly responsible for maximum variability in PC1, which explained 58.5% of the total observed variation in the data set. In addition to number of grain per panicle, other factor that contributed to the variability in PC-1 was plant height at harvesting with a positive loading factor (0.31) and plant height at flowering (0.15). Four quantitative traits contributed the most for variability in PC-2, i.e. plant height at harvesting (0.67), plant height at flowering (0.50), flowering date (0.28) and harvesting date (0.18). In PC3, harvesting date, flowering date, number of grain per panicle and 100-seed weight exhibited the highest loading factors (0.71, 0.60, 0.16, 0.12, respectively), which explained about 9.3% of the total variances in the data set.

Overall, the present study results revealed that red and black upland rice germplasm from ENT Province is genetically divergent in both qualitative and quantitative agro-morphological characters. The wide genetic diversity of red and black rices have been previously reported by other workers (do Nascimento, 2011; Kristamtini et al. 2012; Mau et al. 2013; Rabara et al. 2014; Shinta et al. 2014; Ahmad et al. 2015). This germplasm from ENT Province is an invaluable genetic resource that can be optimally used to assemble new improved red and black upland rice varieties to meet the breeding objectives. Based on the study results, a number of superior agro-morphological traits can be selected from this germplasm, i.e. flag leaf attitude, plant height and maturity, number of productive tillers, panicle length, number of grain per panicle, grain size and shape, 100-seed weight and grain yield per-plant. Flag leaf attitude is an important trait as it determines the efficiency of photosynthesis, and hence the grain filling and grain yield (Rabara et al. 2014). Plant height and maturity are two important traits in crop improvement where shorter plant and shorter duration are mostly preferred by plant breeders. Number of productive tiller, number of grain per panicle and panicle length had been reported to be positively correlated with grain yield (Ahmad et al. 2015); therefore, these characters can be selected for production of high yielding red and black upland rice varieties. Wide variability of grain shapes and grain sizes observed in this germplasm are of important characters to meet the varying demand of rice consumers in rice shapes and sizes.

In conclusion, based on the results of the present study, we draw several conclusions as follows: (i) red and black upland rice accessions from East Nusa Tenggara Province showed a high diversity in both qualitative and quantitative agro-morphological traits. (ii) Cluster analysis using qualitative data put the 40 rice accessions into four main

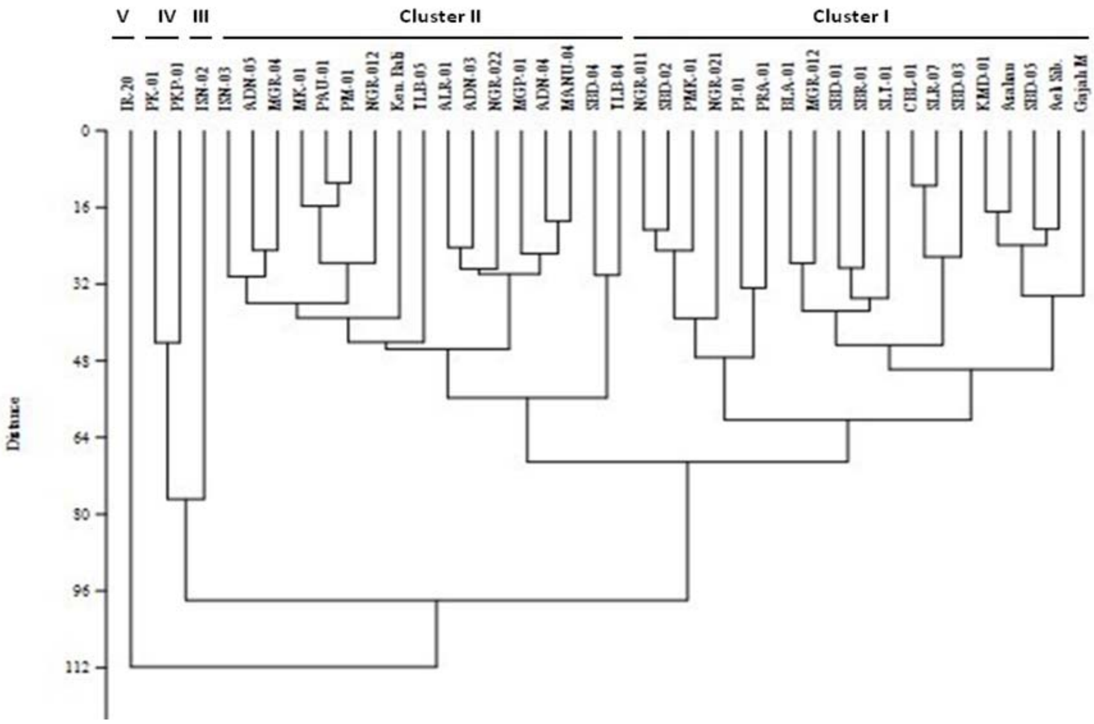

Figure 5. Dendogram of red and black upland rice accessions from ENT Province based on 16 quantitative agro-morphological traits

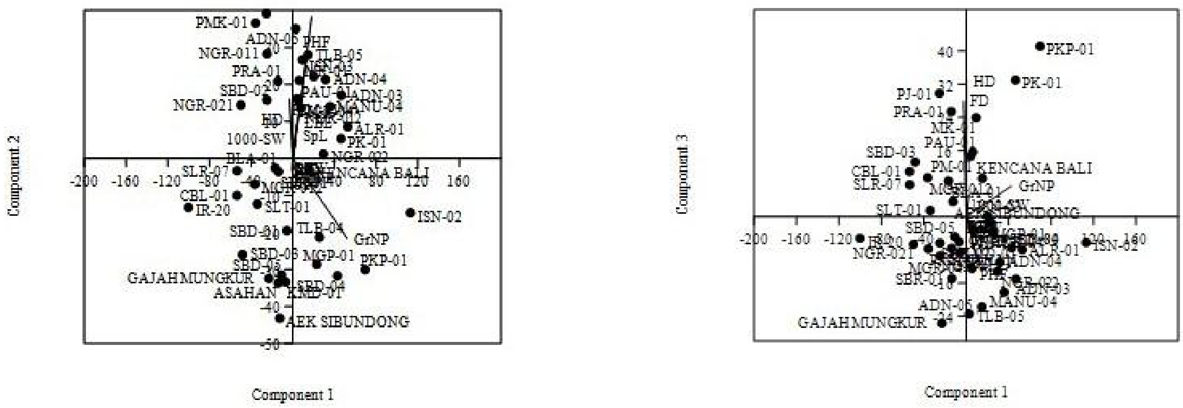

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Figure 6. Biplots showing distribution of red and black upland rice accessions from ENT Province based on 16 quantitative traits. GrNP = grain number per panicle, PHH = plant height at harvesting, PHF = plant height at flowering stage, FD = flowering date, HD = harvesting date. Note: A. PC-1 VS PC-2, B. PC-1 VS PC-3

clusters and 15 sub-clusters while the analysis using quantitative data grouped the accessions into five main clusters and 8 sub-clusters. (iii) Observed variances in qualitative data were mostly explained by flag leaf attitude, apiculus color, pallea-lemma color, grain tip color, and awning while that in quantitative data were mostly explained by number of grain per panicle, plant height at both flowering and harvesting, flowering date and harvesting date.

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REFERENCES

Abdel-Aal E-SM, Young JC, Rabalsk I. 2006. Anthocyanin composition in black, blue, pink, purple, and red cereal grains. J Agric Food Chem, 54: 4696-4704.

Ahmad F, Hanafi MM, Hakim AH, RafiMY, Arolu IW, Abdulla SNA. 2015. Genetic divergence and heritability of 42 coloured upland rice genotypes (*Oryza sativa*) as revealed by microsatellites marker and agro-morphological traits. PLoS One 10 (9): e0138246. DOI:10.1371/journal.

Chutipaijit S, Chaum S, Sompornpailin K. 2011. High contents of proline and anthocyanin increase protective response to salinity in *Oryza sativa* L. spp. *indica*. Austr J Crop Sci 5 (10):1191-1198.

Do Nascimento WF, da Silva EF, Veasey AN. 2011. Agro-morphological characterization of upland rice accessions. Sci Agric (Piracicaba, Braz.) 68 (6):652-660.

Hammer Ø, Harper DAT Ryan P.D. 2001. PAST: paleontological statistics software package for education and data analysis. http://palaeo-electronica.org/2001_1/past/issue1_01.htm.

Huang YP, Lai HM. 2016. Bioactive compounds and antioxidative activity of colored rice bran. J Food Drug Anal (2016), DOI: 10.1016/j.jfda.2016.01.004.

Hyun JW, Chung HS. 2004. Cyanidin and malvidin from *Oryza sativa* cv. Heugjinjubyeo mediate cytotoxicity against humanmonocytic leukemia cells by arrest of G2/M phase and induction of apoptosis. J Agric Food Chem 52: 2213-2217.

IAARD (Indonesian Agency for Agricultural Research and Development). 2012. Newly released superior rice varieties. Warta Penelitian Pertanian 34 (1): 8-9.

Indonesian Ministry of Agriculture. 2009a. The Release Of Local Upland Red Rice Variety Segreng as Superior Variety Segreng Handayani. Ministry of Agriculture Decree No.2226/Kpts/SR.120/5/2009, 19 May, 2009. Indonesian Ministry of Agriculture, Jakarta.

Indonesian Minsitry of Agriculture. 2009b. The release of local upland red rice variety Mandel as Superior Variety Mandel Handayani. Ministry of Agriculture decree No.2227/Kpts/SR.120/5/2009, 19 May, 2009. Indonesian Ministry of Agriculture, Jakarta.

IRRI (International Rice Research Institute). 2014. Standard Evaluation System for Rice. IRRI, Los Banos, the Philippines.

Kristamtini, Purwaningsih H. 2009. The potency of red rice development as germplasm of Yogyakarta. Jurnal Litbang Pertanian 28 (3): 88-95.

Kristamtini, Taryono, Basunanda P, Murti PH, Supriyanta, Setyorini W, Sutarno. 2012. Morphological of genetic relationships among black rice landraces from Yogyakarta and surrounding areas. ARPN J Agric Sci 7 (12): 982-989.

Kushwaha UKS. 2016. Black Rice. Springer International Publishing, Switzerland.

Lalel, HJD, Abidin Z, Jutomo L. 2009. Physico-chemical properties of local upland red rice from Ende District, East Nusa Tenggara. J Food Technol Industr 20 (2): 109-116.

Mau YS, Ndiwa AS, Oematan SS. 2013. Agronomic diversity of local upland rice accessions from Flores Island, East Nusa Tenggara Province. Journal Media Exacta 15 (2): 1720-1734.

Nam SH, Choi SP, Kang MY, Koh HJ, Kozukue N, Friedman M. 2006. Antioxidative activities of bran from twenty one pigmented rice cultivars. Food Chem 94: 613-620.

Nurhasanah, Sadaruddin, Sunary W. 2016. Diversity analysis and genetic potency indentification of local rice cultivars in Penajam Paser and Paser Districts, East Kalimantan. Biodiversitas 17 (2): 401-408.

Putra S, Suliansyah I, Ardi. 2010**.** Exploration and characterization of red rice germplasm from Solok and South Solok Districts, West Sumatera Province. Jerami 3 (1): 139-157.

Rabara RC, Ferrer MC, Diaz CL, Newingham MaCV, Romero GO. 2014. Phenotypic diversity of farmers' traditional rice varieties in the Philippines. Agronomy 4: 217-241.

Santika A, Rozakurniati. 2010. Evaluation technique of rice quality of several glutanous and red upland rice. Buletin Teknik Pertanian 15 (1): 1-5. [Indonesian]

Satue-Gracia M, Heinonen IM, Frankel EN. 1997. Anthocyanins as antioxidants on human low-density lipoprotein and lecithinliposome systems. J Agric Food Chem 45: 3362-3367.

Shao Y, Zhang G, Bao J. 2011. Total phenolic content and antioxidant capacity of rice grains with extremely small size. Afr J Argic Res 6 (10): 2289–2293.

Shinta, Indriyani S, Arisoesilaningsih E. 2014. Morphological variation of six pigmented rice local varieties grown in organic rice field in Sengguruh Village, Kepanjen District, Malang Regency. The Journal of Tropical Life Science 4 (2):149-160. http://www.jtrolis.ub.ac.id/index.php/jtrolis/article/view/171/207.

Sio-Se MA, Ahmadi A, Poustini K, Mohammadi V. 2006. Evaluation of drought resistance indices under various environmental conditions. Field Crop Res 98: 222-229.

Suliartini NWS, Sadimantara GR, Wijayanto T, Muhidin. 2011. Evaluation of anthocyanin content of upland red rice germplasm from Southeast Sulawesi. Crop Agro 4 (2): 43-48.

Tsuda T, Horio F, Osawa T. 2002. Cyanidin 3-*O*-*â*-glucoside suppresses nitric oxide production during a zymosan treatment in rats. J Nutr Sci Vitaminol 48: 305-310.

VSN International. 2009. GenStat 12th Edition for Windows. VSN International Ltd. www.vsni.co.uk

Zhao C, Giusti MM, Malik M, Moyer MP, Magnuson BA. 2004. Effects of commercial anthocyanin-rich extracts on colonic cancer and nontumorigenic colonic cell growth. J Agric Food Chem 52: 6122-6128.