BIODIVERSITAS
Volume 20, Number 9, September 2019
Pages: 2612-2619

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d200925

RAPD based genetic diversity, agronomic characters, and nutrition content of Timor Leste kidney bean (*Phaseolus vulgaris*) genotypes

MARCOS CORREIA VIDAL[1,2], ASEP SETIAWAN[3,♥], YUDIWANTI WAHYU[3]

[1]Graduate School, Institut Pertanian Bogor. Jl. Lingkar Akademik, Kampus IPB, Dramaga, Bogor 16680, West Java, Indonesia
[2]Ministry of Agriculture, Republic Democratic of Timor Leste, Rua Nicolai Lobato, Dili, Timor Leste
[3]Department of Agronomy and Horticulture, Faculty of Agriculture, Institut Pertanian Bogor. Jl. Meranti, Kampus IPB, Dramaga, Bogor 16680, West Java, Indonesia. Tel.: +62-251-8629354, Fax.: +62-251-8629352, ♥email: asetiawan_agh@yahoo.com

Manuscript received: 28 July 2019. Revision accepted: 22 August 2019.

Abstract. *Vidal MC, Setiawan A, Wahyu Y. 2019. RAPD based genetic diversity, agronomic characters, and nutrition content of Timor Leste kidney bean* (Phaseolus vulgaris) *genotypes. Biodiversitas 20: 2612-2619.* The productivity of kidney beans (*Phaseolus vulgaris* L.) in Timor Leste is relatively low and needs to be improved. The increase in kidney bean yield through plant breeding, however, can only be obtained if information the genetic diversity of breeding materials is available. This study aimed to characterize agronomic characters, to assess genetic diversity and nutrient content of kidney bean in Timor Leste. The materials used in this study were 13 Timor Leste and 2 Indonesian genotypes as controls. The experimental design for the field trial was a complete randomized block design with three replications. The treatment was 13 kidney bean genotypes. The results showed that there were significant variations in agronomic characters among the genotypes tested. Result from phylogenetic trees based on the Random Amplified Polymorphic DNA (RAPD) indicated that the genetic material understudied can be grouped into three main groups. The Indonesian genotype belongs to a different group from Timor Leste genotypes. Direct selection based on seed yields produced the best eight East Timor genotypes, TL-RO3, TL-LUN, TL-LM, TL-R10, TL-LL, TL-RW, TL-LB, TL-LB, and TL-Umabano. The nutritional content of the kidney bean genotype from Timor Leste was similar to that of the Indonesian genotype. The carbohydrate content of the Timor Leste genotype was considered high (average = 59.95%), while the average fat content is low (1.71%) and the average protein content was quite moderate (18.08%).

Keywords: Agronomic characters, landrace, RAPD marker

INTRODUCTION

Kidney beans (*Phaseolus vulgaris* L.) are members of the legume family which can be used as an alternative food source to meet the nutritional needs of people in developing countries including Timor-Leste. According to Rusilante (2007), kidney beans have high protein, mineral, vitamin, and fiber content. Gouveia et al. (2014) reported that 100 g of kidney bean seeds contained 3.64-5.67% ash, 0.57-2.86% fat, 18.55-29.69 protein, and 23.40-52.65% carbohydrate. The relatively high nutrient content made the bean play an important role in reducing protein deficiencies and contributes significantly to food sufficiency and community nutrition security. Ganesan and Xu (2017) considered that kidney beans also play a role in preventing various diseases such as diabetes mellitus, coronary heart disease, and cancer. Like other types of beans, kidney beans can increase soil fertility by N fixation through symbiosis with Rhizobium (Graham and Vance 2000). Many kidney beans are cultivated by East Timorese farmers, especially in the highlands. However, most East Timorese farmers cultivate local kidney bean cultivars that have low yields (MAP 2015).

The yield of kidney beans in Timor Leste is around 1000 kg ha-1 (Howeler et al. 2003) which is lower than the results in other countries such as Indonesia 1360-1500 kg ha-1 (Adie and Kurniawan 2002), and Brazil around 863-2084 kg ha-1 (Guimarães et al. 2011). Increasing the potential yield can be done through plant breeding activities (Poehlman 1991) that further requires information on genetic diversity of breeding materials.

Genetic diversity assessment of kidney bean based on phenotypic characters of plants has several disadvantages, including requiring considerable time and large environmental influences thereby increasing the chance of errors in the selection of genotypes (Lamadji et al. 1999). This constraint could be overcome with the use of molecular markers (Smith and Smith 1992). One of the most widely used molecular markers is the Random Amplified Polymorphic DNA (RAPD). The advantage of RAPD molecular markers compared to other markers is cheaper, easy to be performed, quickly to produces results, and produce a large number of DNA polymorphic bands (Skoric et al. 2012).

Identification and selection of the most promising genotypes for high yield from the existing genetic stocks need to be done before further breeding work initiated by selection process. Selection is the activity of choosing the best individual plants based on the desired character and will be effective if using the right character based on the values of genetic parameters such as heritability or correlation coefficient values (Phillips and Wolfe 2009). This research aimed to characterize the variability of agronomic characters, identify potential accession that produces high yield, estimate genetic diversity based on RAPD, and to analyze the nutritional content of Timor Leste kidney bean accessions.

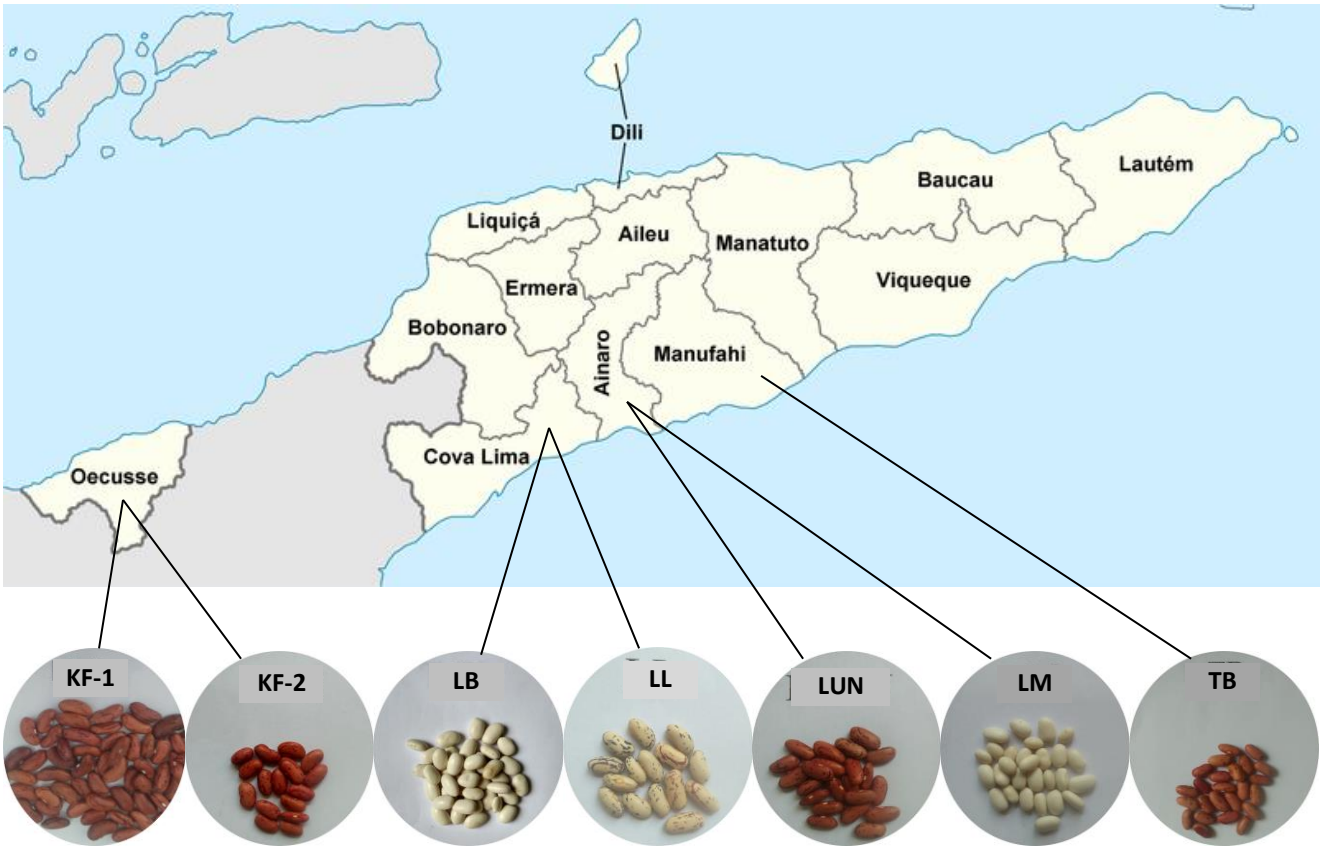

Figure 1. The seven Timor Leste kidney bean landraces and the district where the landraces were collected

MATERIALS AND METHODS

Agronomic trial

The study was conducted in the field-trial of Cipanas Ornamental Plants Research Institute, Cianjur, West Java (1100 m asl) started from June-October 2018. The plant materials used in this study were 13 East Timor kidney bean genotypes (7 landraces and 6 introductions) and 2 Indonesian genotypes (as controls) (Figure 1). The experimental design was a randomized complete block design with the genotype as a treatment, and the trial was replicated three times.

Agronomical characterization

Observations of agronomic characters were carried out following IBPGR (1983) descriptor for kidney beans, characters to be observed were plant height, stem diameter, number of branches per plant, leaf width, leaf length, stover weight per plant, days of flowering, number of flowers per plant, number of pods per plant, pod weight, pod length, weight of 100 seeds, and seed yield.

Molecular characterization

The molecular work was carried out in the Plant Molecular Biology Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University, Indonesia. The DNA extraction was done by taking as much as ±0.1 g of young leaves aged ±14 days after planting (DAP). The DNA was extracted using the modified Cetyltrimethylammonium bromide (CTAB) method (Briand et al. 1998). Amplification was carried out using 10 RAPD primers that were OPA8, OPE14 (Mienie et al. 2000), OPA17, OPB5 (Szilagyi et al. 2011). OPA07 (Bukhari et al. 2015), and OPA01, OPH5, OPA4, OPA9, OPE12 (Plant Molecular Biology Laboratory IPB). Thirty nanograms of genomic DNA were used in 10 uL reaction containing PCR Master mix 2 x (KAPA 2G Fast HotStart ReadyMix PCR Kit), and 25 pmol of RAPD primer. The Amplification was done in 10 uL reaction volumes in PCR machine (Esco Swift maxi thermal cycler). Amplification was done by using the following cycles: initial denaturation 94°C 5 minutes, denaturation program in cycles 94°C for 5 seconds, annealing 32°C for 30 'seconds, extensions 72°C for 60 seconds and final extension 72°C for 10 minutes. Amplicons were run on the horizontal 2% agarose gel electrophoresis and loaded with DNA ladder 100-3000bp (Vivantis). Scoring of the RAPD bands was done manually. A score of 0 was given if there was no amplification and score of 1 was given for the presence of band.

Proximate analysis

Nutritional content was determined by the proximate analysis at SIG (Saraswati Indo Genetech) laboratory of the Indonesian Molecular Biotechnology Company. The analysis was carried out using 200 g of dry seeds per sample based on Standard National Indonesia (SNI) 01-2891-1992 Item 8.2

Data analysis

Agronomic characters were analyzed using analysis of variance (ANOVA) by SAS software version 9.00 (SAS Institute, Inc., Cary, NC, USA). Estimation of heritability was performed following Singh and Chaundary (1979). RAPD data scores were used for cluster analysis based on unweighted pair group method with arithmetic mean (UPGMA) method using Ntsys (Numerical Taxonomy and Multivariate Analysis System) software version 2.2. (Rohlf 2000).

RESULTS AND DISCUSSION

Characterization of agronomic characters

Results of the analysis of variance (ANOVA) showed significant differences between the genotypes for the most observed characters, except for leaf width and leaf length (Table 1). This result is not much different from the research conducted by Razvi et al. (2017) who also showed that flowering days, number of branches, number of pods, number of seeds, the weight of 100 seeds and character of seed weight were also significantly affected by kidney bean genotypes. Characters that are significantly influenced by genotypes show variations in the genotype being tested, so that selection to improve character can be expected (Akhmadi et al. 2016). Hartati et al. (2012) also suggested that selection can be made effectively if there is a high variation in the population. Selection for most agronomic traits, therefore, can be carried out effectively in the population studied. Information about averages, standard deviations, and range values of each of the properties observed (Table 1) gives us overall insight into the genetic values of the genotypes studied.

The value of genetic material lies in its variability. The phenotypic and genotypic variants of all observed characters were presented in Table 2. Stover weight, leaf width, leaf length, stem diameter, and pod length are relatively low phenotypic and genotypic variants. In contrast, plant height, the weight of 100 seeds, pod yield per plant, seed yield per plant, number of flowers per plant, number of pods per plant, and number of leaves per plant were considered relatively high phenotypic and genotypic variants (Table 2). Characters have the highest variability cannot be judged by variance because variance has different units of measurement. To be able to compare the variability among these characters, we should use coefficient of variation as these statistics has no unit of measurement. The coefficient of genotypic variation is used to predict the extent of genetic variation of a character (Syukur et al. 2018). According to Murdaningsih et al. (1990), the coefficient of genotypic variation (GCV) is grouped into low (<25%), rather low (25% <GCV ≤50%), rather high (50% <GCV ≤75%) and high (> 75%). Most of the characters observed had a low coefficient of genetic variations except for the plant height, number of flowers per plant, number of pods per plant, pod yield per plant, and seed yield per plant (Table 2). The genotype coefficient of variation obtained in this study has a range between 0.00% (wide and length of leaf character) to 55.23% (plant height). If we excluded plant height, the results of this study were in accordance with the results of Razvi et al. (2017) and Raffi and Nath (2004). Razvi et al. (2017) showed that genotypic CV of 13 Kashmiri nuts was classified as low with a range of 0.97-15%, while Raffi and Nath (2004) reported that genotypic CV of 31 Bangladesh kidney beans was classified as low to moderate with a range of 8.0-47.6%. Characters with low and rather low genotypic variation coefficients (CV) are classified as characters of narrow genetic variation or tend to be homogeneous, which means that selection for characters can be more difficult (Syukur et al. 2018). On the other hand, characters with high and somewhat high genotypic variation coefficients are classified as characters with broad or heterogeneous genetic variations, the selection on these characters will be effective (Falconer and Mackay 1996). Because most genotypic CV from the observed characters was considered low to rather low, selection for those traits may be difficult. Therefore, it is necessary to enrich national germplasm genetic diversity. Introducing a better line, especially for the most important traits, such as yield, can be of an advantage.

Table 1. Summary of variance analysis (p-value), coefficient of variation, mean value and range of agronomic characters of kidney bean landraces from Timor Leste and control

Characters	p-value	Mean value±SD	Range value
Days of flowering (day)	0.00	42.33 ± 3.58	35.00-45.00
Stover weight (g/plant)	0.00	37.92 ±27.42	7.17- 80.11
Leaf width (cm)	0.91	6.45 ± 0.92	5.60-7.04
Leaf length (cm)	0.76	10.65 ± 1.55	9.66- 11.75
Plant height (cm)	0.00	158.43±82.71	23.11-255.11
Number of branches per plant	0.00	13.27 ± 3.44	8.72- 19.00
Number of leaves per plant	0.01	39.96 ±10.44	26.17- 57.00
Stem Diameter (cm)	0.02	0.49 ± 0.08	0.35- 0.58
Number of flowers per plant	0.00	19.88 ± 7.46	9.83- 30.61
Weight of 100 seeds (g)	0.00	42.36 ±10.58	20.00- 55.00
Pod length (cm)	0.09	11.16 ± 1.44	9.38- 12.81
Number of pods per plant	0.00	25.64 ± 9.63	8.61- 38.39
Pod yield per plant (g)	0.00	71.08 ±27.30	40.78-102.00
Seed yield per plant (g)	0.00	46.92 ±17.29	25.00- 65.83

Note: P<0.01: very significant difference, P<0.05: significantly difference, SD: standard deviation

Table 2. Estimated genetic variance (Vg), Phenotype variance (Vp), broad-sense heritability, and genetic coefficient variation of the observed characters

Characters	Vg	Vp	Heritability		Genotypic coefficient of variation	
			Value	Criteria	Value	Criteria
Days of flowering (day)	13.33	13.58	98.18	High	8.75	Low
Stover weight (g/plant)	0.01	0.01	64.04	High	20.76	Low
Leaf width (cm)	-0.18	0.88	0.00	Low	0.00	Low
Leaf length (cm)	-0.47	2.23	0.00	Low	0.00	Low
Plant height (cm)	6523.21	7130.68	91.48	High	55.23	Rather high
Number of branches per plant	7.38	12.33	59.86	High	20.97	Low
Number of leaves per plant	56.30	143.44	39.25	Moderate	19.83	Low
Stem diameter (cm)	0.00	0.01	32.26	Moderate	9.31	Low
Number of flowers per plant	34.84	49.77	70.01	High	29.88	Rather low
Weight of 100 seeds (g)	102.73	116.57	88.13	High	24.24	Low
Pod length (cm)	0.44	2.06	21.56	Moderate	5.94	Low
Number of pods per plant	53.48	231.33	23.12	Moderate	29.27	Rather low
Pod yield per plant (g	364.03	697.19	52.21	High	27.47	Rather low
Seed yield per plant (g)	176.93	282.19	62.70	High	29.10	Rather low

Note: Vg: genetic variance, Vp: phenotypic variance

Broad-sense heritability (H^2) can be used to estimate the influence of environmental variance to the genotypic variance of a certain character. The H^2 is the ratio of total genetic variance to total phenotypic variance (Acquaah 2012). The broad-sense heritability value of kidney bean genotypes was range between 0.00% (leaf width and leaf length) to 98.18% (days of flowering). Based on low to high criteria of H^2 (Syukur et al. 2018), the leaf width and leaf length were classified as the character with low heritability. Number of the leaf per plant, stem diameter, pod length, number of pods per plant were classified as characters with moderate heritability, and the date of flowering, stover weight per plant, plant height, number of branches per plant, number of flowers per plant, the weight of 100 seeds, pod yield per plant and seed yield per plant as the character with high heritability. Lestari et al. (2006) reported that selection can be made in the first generation if the heritability value is classified as high, but if the heritability value obtained is classified as moderate, the selection should be made in the later generation. Razvi et al. (2017) showed that the heritability of the observed characters of the 13 Kashmir germplasm was classified as high with a range of 58.3-99.8%. Raffi and Nath (2004) also reported a similar result where the heritability value of the observed characters of 31 kidney bean lines from Bangladesh was classified as high with a range of 54.8-90.9%. Estimation of H^2 for the important agronomic trait (flowering date, stover weight, plant height, number of branches, number of flowers, 100 seeds weight, pod yield, and seed yield) in this study was in agreement with the resulting study of Razvi (2017) and Raffi and Nath (2004). Since heritability is considered high, then the index selection will become the determining factor to increase response to selection.

Cluster analysis based on RAPD marker

The use RAPD primer of OPA1, OPA17 OPH5, OPB4, OPA5, OPA7, OPA8, OPA9, OPB5, OPE12, OPE14, OPH5 generated the different number of polymorphic and monomorphic bands (Table 3). Totally 174 polymorphic and 114 monomorphic RAPD bands were generated by the ten primers. Primer of OPA17 and OPA1 were the most two primers that generated the highest number of RAPD bands. Overall, the polymorphic level of the primers used in this study was considered good with a range between 37.5% to 68.75% polymorphic bands. In the case of RAPD, the difference in the length of the amplicon can be the result of insertion or deletion of the base sequence in the area between two primers landing sites or from point mutation, insertion or deletion in the primers landing sites.

Cluster analysis

Similarities of various traits are usually used to express the genetic distance between collections. The kinship relationship will be closer if the value of the similarity coefficient is close to 1. The similarity index value is used to compare the similarities found between the genotypes. If the value is close to 0%, the similarity level is low, and if the value is close to 100%, the similarity between genotypes is high. Cluster analysis (cut off value 0.50) has shown that the level of similarity among the genetic material under study can be grouped into three main groups. Cluster I consists of IND-M and IND-T, cluster 2 consists of TL-LUN, TL-KF1, TL-RW, TL-YELLOW, TL-MAC28, TL-R10, TL-KF2, TL-TB and cluster 3 consists of TL-LB, TL-UMABANO, TL-DCL, TL-R03, TL-LM, TL-LL. Genetic similarity coefficients based on RAPD markers ranged from 0.22 to 0.89. The lowest similarity in genetic values is between TL-R10 and TL-UMABANO (0.22), while the highest is found in TL-LUN and TL-KF1 (0.89) (Table 4). Cluster 3, 2 and 1 joint into single cluster at coefficient of similarity 0.41. This result indicates that genetic background of the Timor Leste genotypes is quite different from the Indonesian genotypes.

Cluster analysis carried out using RAPD marker data showed that the genetic material studied was grouped into three main groups, and the Timor Leste genotype formed a different group of Indonesian genotypes. The cluster

analysis, however, failed to show any correlation between genetic distances and the district where the landraces were collected. The TL-LUN and TL-LM, TL-KF1 and TL-KF2, TL-LL and TL-LB were collected from the district of Ainaro, Oecussi, and Covalima, respectively. The cluster analysis, however, showed that the landrace collected from the same district was clustered into a different cluster (Figure 2). RAPD markers are not influenced by the environment, analysis of diversity based on RAPD; therefore, reflects the genetic diversity of the population studied. The existence of high genetic diversity found in the population studied can be caused by different sources of seed origin or due to the influence of natural mutations and crosses. Knowledge of genetic distance, as presented in Figure 2, could be useful for germplasm management as well as for selecting parental plant of the crossing. Higher heterosis effect or hybrid vigor could be expected if the crossing parental plants have large genetic distance. In case germplasm fund limited, germplasm core collection can be done by reducing number of collections which have closer genetic distance.

Selection of Timor Leste kidney bean genotypes

The most important character of kidney bean is seed yield. The character, had a high heritability value with a genotypic coefficient of variation value of rather low (Table 2). Direct selection of seed yield character produced eight best Timor Leste kidney bean genotypes, namely TL-RO3, TL-LUN, TL-LM, TL-R10, TL-LL, TL-RW, TL-LB, and TL-Umabano (Table 5). The mean value of all selected Timor Leste kidney bean genotypes was higher than the controls, except for pod length character which has slightly shorter. The selected eight genotypes can be used for further breeding work to develop high yield variety of kidney beans.

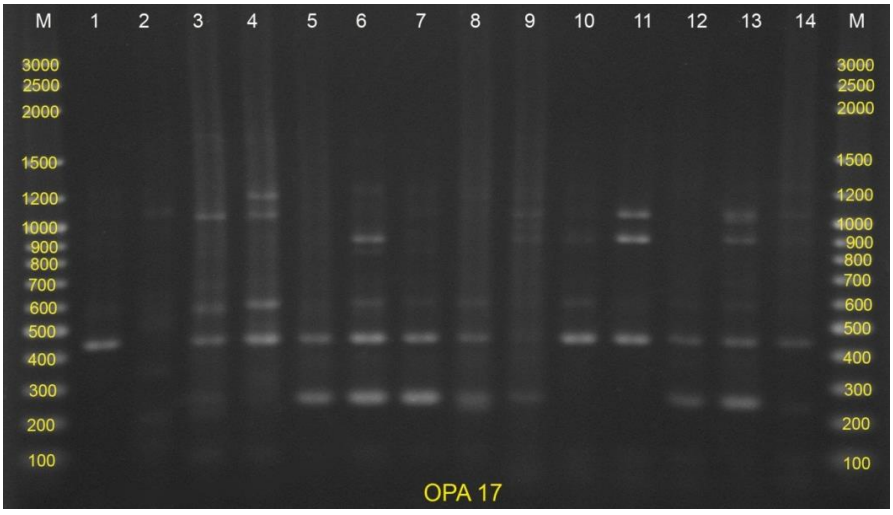

Figure 2. Electrophoregram DNA samples using OPA 17

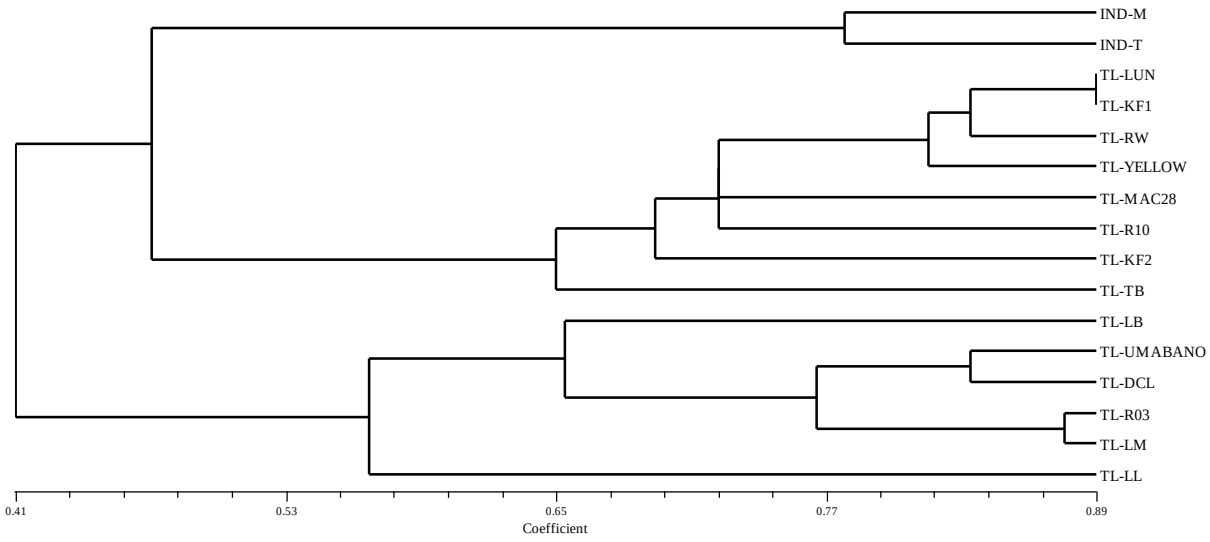

Figure 3. Dendrogram of Timor Leste kidney bean genotypes based on RAPD at coefficient of similarity 0.41

Table 3. DNA primers, base arrangements, and number of amplified loci on kidney bean landraces

Primers	Base sequence	Number of bands		Total	Polymorphism (%)
		Polymorphic	Monomorphic		
OPA1	CAGGCCCTTC	29	19	48	60.42
OPA17	GACCGCTTGT	30	18	48	62.50
OPA4	AATCGGGCTG	20	12	32	62.50
OPA7	GAAACGGGTG	7	9	16	43.75
OPA8	GTGACGTAGG	6	10	16	37.50
OPA9	GGGTAACGCC	19	13	32	59.38
OPB5	TGCGCCCTTC	11	5	16	68.75
OPE12	TTATCGCCCC	9	7	16	56.25
OPE14	TGCGGCTGAG	22	10	32	68.75
OPH5	AGTCGTCCCC	21	11	32	65.63
Total		174	114	288	

Table 4. Genetic similarity matrix of 14 kidney bean landraces from Timor Leste and controls based on RAPD markers

Genotype	IND-M	TL-LB	TL-LUN	TL-KF1	TL-TB	TL-KF2	TL-RW	TL-Umubano	TL-R03	IND-T	TL-Mac28	TL-LM	TL-LL	TL-DCL	TL-R10
TL-LB	0.33														
TL-LUN	0.44	0.33													
TL-KF1	0.44	0.33	0.89												
TL-TB	0.67	0.33	0.67	0.67											
TL-KF2	0.50	0.17	0.72	0.72	0.61										
TL-RW	0.56	0.22	0.78	0.89	0.67	0.83									
TL-Umabano	0.28	0.72	0.50	0.50	0.39	0.33	0.39								
TL-R03	0.33	0.56	0.56	0.56	0.33	0.50	0.44	0.72							
IND-T	0.78	0.44	0.33	0.33	0.56	0.39	0.33	0.28	0.44						
TL-Mac28	0.56	0.22	0.67	0.78	0.56	0.61	0.67	0.39	0.56	0.56					
TL-LM	0.38	0.56	0.69	0.63	0.44	0.50	0.50	0.81	0.88	0.38	0.56				
TL-LL	0.28	0.61	0.61	0.50	0.50	0.44	0.39	0.56	0.50	0.50	0.50	0.56			
TL-DCL	0.33	0.78	0.44	0.44	0.33	0.28	0.33	0.83	0.78	0.44	0.33	0.75	0.61		
TL-R10	0.61	0.28	0.61	0.72	0.61	0.67	0.83	0.22	0.28	0.50	0.72	0.38	0.44	0.28	
TL-Yellow	0.44	0.33	0.78	0.89	0.78	0.61	0.78	0.50	0.56	0.33	0.78	0.63	0.50	0.44	0.72

Table 5. The performance of selected Timor Leste kidney bean genotypes and the control genotypes

Genotypes	SW	LL	PH	JD	NB	W100	PL	NP	PY	SY
TL-RO3	27.78	11.01	209.17	31.83	17.50	21.67	11.31	34.94	102.00	65.83
TL-LUN	54.28	11.03	168.83	47.17	24.11	48.33	11.84	33.94	91.94	65.00
TL-LM	41.56	11.75	229.56	47.00	28.78	43.33	11.29	31.17	87.61	60.00
TL-R10	40.83	10.14	201.33	33.83	20.39	53.33	10.69	22.44	69.67	60.00
TL-LL	64.11	10.82	80.44	39.17	13.28	50.67	11.79	29.56	88.92	58.33
TL-RW	59.28	10.89	255.11	45.50	24.00	55.00	12.81	27.28	98.67	57.78
TLLB	15.06	10.84	114.92	26.17	9.83	35.00	10.24	30.11	69.83	47.22
TL-Umubano	11.17	10.87	190.61	47.00	30.61	20.00	11.25	38.39	76.52	45.56
IND-M	13.11	9.69	103.83	37.33	15.33	40.00	12.86	23.33	77.33	43.61
IND-T	15.39	10.25	30.22	28.00	20.94	36.67	10.36	18.17	40.50	31.94
$\bar{X}s$	39.26	10.92	181.25	39.71	21.06	40.92	11.40	30.98	85.65	57.47
$\bar{X}$	37.92	10.65	158.43	39.96	19.88	42.36	11.16	25.64	71.08	46.92
S	1.33	0.27	22.82	0.25	1.18	-1.44	0.25	5.34	14.56	10.54

Note : SW = strover weight per plant, LL = leaf length, PH = plant height, NL = number of leaves per plant, NB = number of branches per plant, W100 = weight of 100 seeds, PL = pod length, NP = number of pods per plant, PY = pod yield per plant, SY = seed yield per plant, $\bar{X}$ = mean value of unselected population, $\bar{X}s$ = mean value of selected population, S = differential selection

Table 6. The nutritional content of kidney bean genotypes from Timor Leste and the controls

Landraces	Water content (%)	Carbohydrate (%)	Total energy (kcal.100g^{-1})	Fat (%)	Protein (%)	Ash (%)
TL3-LB	15.82	56.83	329.76	1.60	22.01	3.74
TL-LUN	15.98	61.24	330.99	1.83	17.39	3.56
TL-KF1	15.36	59.51	332.04	1.52	20.08	3.53
TL-TB	15.69	59.22	332.43	1.99	19.41	3.69
TL-KF2	15.03	59.45	333.84	1.72	20.14	3.66
TL-Rw	16.55	63.18	328.96	1.76	15.1	15.84
TL-Umabano	15.81	62.09	321.86	0.30	17.7	4.1
TL-R03	15.22	63.40	336.75	2.27	15.68	3.43
TL-Mac28	18.32	57.73	320.48	1.60	18.79	3.56
TL-LM	17.32	59.69	324.14	1.74	17.43	3.82
TL-DCL	15.21	59.45	330.82	1.18	20.6	3.56
TL-R10	22.59	59.1	297.51	3.15	11.49	3.47
TL-Yello	17.25	58.52	324.74	1.54	19.20	3.49
IND-M	16.25	59.51	328.24	1.36	19.49	3.39
IND-T	13.82	58.72	339.91	1.91	21.96	3.59
Min	15.03	56.83	297.51	0.30	11.49	3.43
Max	22.59	63.40	336.75	3.15	22.01	15.84

Nutrient content of kidney bean genotypes from Timor Leste and controls

Kidney bean is consumed daily by Timor Leste people. They can be served as Kidney bean bray, or be mixed with other staple food such as rice or corn. Therefore, the kidney bean plays an important role in community nutrition improvement program, especially in the rural areas. There is so far no official information on the Nutrition content of the Timor Leste Kidney bean landraces. In response to such conditions, we conduct proximate analysis as a part of our study. The proximate analysis was performed to reveal carbohydrates, total energy, fat content, fat energy, protein, water content, and an ash content of the genetic materials under study. The seed moisture content of the samples was relatively equal, with ranged between 15.03-22.59%. Homogeneity of seed moisture content among genotypes could bias. The average carbohydrate content of Timor Leste Kidney bean genotypes was 59.95% with the range value of 56.8-63.40%, which is similar to the Indonesian Genotypes of IND-M and IND-T. Average energy total and range value of Timor Leste kidney bean genotypes were 326.49 kcal.100g^{-1} and 297.51 kcal.100g^{-1} to 336.75 kcal.100g^{-1}respectively. The energy total two Indonesian genotypes IND-M and IND-T was 328.24 kcal.100g^{-1} and 339.91 kcal.100g^{-1} respectively, which is a bit higher than the total energy content of Timor Leste Kidney bean genotypes. The average fat content of Timor Leste genotypes was 1.71%, and its range was 0.30-3.15%, while the average fat energy of Timor Leste kidney bean genotypes was 13.25 kcal.100g^{-1} with the range of 2.70-20.43% kcal.100g^{-1}. The protein content average of Timor Leste kidney bean genotypes was 18.08% with a range of 11.49-22.01%, which is close to the protein content of the Indonesian genotypes IND-M (19.49%) and IND-T (21.96%). These results were similar to the reports of Shimelis and Rakshit (2005) who examined the proximate content of 8 kidney bean genotypes from Ethiopia, they showed that the carbohydrate content was range from 56-61%, the fat content was range from 0.67-1.19%, the protein content was range from 17.96-22.07%, the fiber content was range from 4.66-5.95% and the ash content was range from 3.12-4.26%. To the best our knowledge, this is the first published information nutrient content of Timor Leste Kidney beans genotypes. This knowledge could benefit for many people or stakeholders especially the NGO or other agency work for community development in Timor Leste, nutritionist, health care service as well as the plant breeders who want to develop better nutritious of kidney beans.

In conclusion, genetic variations among the Timor Leste kidney bean genotypes were considered medium. Enriching genetic diversity by introducing new genotypes, therefore, still needs to be done. Cluster analysis based on RAPD marker generated of 3 main clusters. The Timor Leste genotypes form two separate clusters from the Indonesian genotypes. Direct selection based on seed yield per plant was able to identify the eight best Timor Leste kidney bean genotypes with high yield potential, namely TL-RO3, TL-LUN, TL-LM, TL-R10, TL-LL, TL-RW, TL-LB, and TL-Umabano. The nutritional content of the Timor Leste kidney bean genotype is similar to the nutrient content of kidney bean collections from other countries, where in general the average carbohydrate content is high (59.95%), low average fat content (1.71%), and average protein content quite moderate (18.08%).

ACKNOWLEDGEMENTS

This work was carried out with the support of SEARCA Scholarship. We also would like to thanks the AI-Com Project for supporting this research.

REFERENCES

Acquaah G. 2012. Principles of Plant Genetics and Breeding. John Wiley & Sons Ltd., Oxford

Adie MM, Kurniawan AD. 2002. Kacang Merah dan Peluang Pengembangannya di Indonesia. Puslitbang Tanaman Pangan. Balitbang Pertanian, Jakarta. http://bpatp.litbang.pertanian.go.id/ [Indonesian]

Akhmadi G, Purwoko BS, Dewi IS, Wirnas D. 2016. Karakter agronomi untuk seleksi pada galur-galur padi dihaploid hasil kultur antera. J Agron Indo 45 (1): 1-8. [Indonesian]

Briand L, Brown AE, Lenne JM, Teverson DM. 1998. Random amplified polymorphic DNA variation within and among bean landrace mixtures (*Phaseolus vulgaris* L.) from Tanzania. Euphytica 102: 371-377.

Bukhari A, Bahat MA, Ahmad M, Saleem N. 2015. Examination of genetic diversity in common bean (*Phaseolus vulgaris* L.) using random amplified polymorphic DNA (RAPD) markers. Division of Genetics and Plant Breeding, Shre-e-Kasmir University of Agricultural Sciences and Technology, Srinagar, India.

Falconer DS, Mackay TFC. 1996. Introduction to Quantitative Genetics 4th Edition. Longman, New York.

Ganesan K, Xu B. 2017. Food Science and Technology Program. Hong Kong Baptist University, Beijing, China.

Gouveia CSS, Freitas G, De Brito JH, Slaski JJ, Carvalho MAAP. 2014. Nutritional and mineral variability in 52 accessions of common bean varieties (*Phaseolus vulgaris* L.) from Madeira Island. Agric Sci 5: 317-329.

Graham PH, Vance CP. 2000. Nitrogen fixation in perspective an overview of research and extension needs. Field Crops Res 65: 93-106.

Guimarães GM, Stone LF, Del Peloso MJ, Oliveira JP de. 2011. Common bean genotypes under water stress. Rev Bras Engenharia Agríc Ambiental 15 (7): 649-656.

Hartati RS, Setiawan A, Heliyanto B, Sudarsono. 2012. Keragaman genetik, heritabilitas, dan korelasi karakter 10 genotipe terpilih jarak pagar (*Jatropha curcas* L.). J Littri 18: 74-80.

Howeler RH, Palmer B, Hartojo K, Piggin C. 2003. Evaluation of cassava and bean germplasm in East Timor. In: da Costs H, Piggin C, da Cruz CJ, Fox JJ (eds.) Proceedings of Aciar Agriculture: New Directions for a New Nation-East Timor (Timor-Leste), 2002 October.

Lamadji SL, Hakim, Rustidja. 1999. Akselarasi pertanian tangguh melalui pemuliaan non konvensional. In: Ashari (ed.) Prosiding Simposium Pemuliaan Tanaman, PERIPI Komda Jawa Timur. [Indonesian]

Lestari AD, Dewi W, Qosim WA, Rahardja M, Rostini N, Setiamiardja R. 2006. Variabilitas genetik dan heritabilitas karakter komponen hasil dan hasil lima belas genotipe cabai merah. Zuriat 17 (1): 97-98. [Indonesian]

Mienie CMS, Herselman L, RE Terhlanche. 2000. RAPD analysis of selected common bean (*Phaseolus vulgaris* L.) cultivars from three African countries. S A J Plant Soil 17 (2): 93-94.

Murdaningsih HK, Baihaki A, Satari G, Danakusuma T, Permadi AH. 1990. Variasi genetik sifat-sifat tanaman bawang di Indonesia. Zuriat. 1 (1): 32-36. https://doi.org/10.24198/zuriat.v1i1.6598 [Indonesian]

Phillips SL, Wolfe MS. 2009. Evolutionary plant breeding for low input systems. JAS 143: 245-254.

Poehlman JM. 1991. Germplasm: Resources, collection and cataloging. Westviw Pr, Colorado, USA.

Raffi SA, Nath UK. 2004. Variability, heritability, genetic advance and relationships of yield and yield contributing characters in dry bean (*Phaseolus vulgaris* L.). J Biol Sci 4 (2): 157-159.

Razvi SM, Khan MN, Bhat MA, Ahmad M, Ganaie SA, Sheikh FA, Najeeb S, Parry FA. 2017. Morphological variability and phylogenetic analysis in common bean (*Phaseolus vulgaris* L.). Legume Res 41 (2): 208-212.

Rohlf JF. 2000. NTSYSpc Numerical Taxonomy and Multivariate Analysis System Version 2.2 User Guide. Department of Ecology and Evaluation State University of New York, New York.

Rusilante KMK. 2007. Sehat dengan Makanan Berserat. Trans Info Media, Jakarta. [Indonesian]

Shimelis EA, Rakshit SK. 2005. Proximate composition and Physico-chemical properties of improved dry bean (*Phaseolus vulgaris* L.) varieties grown in Ethiopia. LWT 38: 331-338.

Singh RK, Chaudhary BD. 1979. Biometrical Method in Quantitative Genetic Analysis. 1st ed (Rev). Kalyani, New Delhi, India.

Skoric M, B Siler, Banjanac T, Zivkovic JN, Dmitrovic S, Misic D, Grubisic D. 2012. The reproducibility of RAPD profiles: Effects of PCR components on RAPD analysis of Centaurium species. Arch Biol Sci Belgrade.64 (1): 191-199.

Smith JSC, OS Smith. 1992. Fingerprinting crop varieties. Adv Agron 47: 85-129. https://repository.cimmyt.org/bitstream

Syukur M, Sujiprihati S, Yunianti R. 2018. Teknik Pemuliaan Tanaman. Penebar Swadaya, Jakarta. [Indonesia]

Szilagyi L, Tayyar S, Ciuca M 2011. Evaluation of genetic diversity in common bean (*Phaseolus vulgaris* L) using RAPD markers and morpho-agronomic traits. Department of Genetics and Plant Breeding, Faculty of Agriculture, University of Agronomical Sciences and Veterinary Medicine, Romania.