

DNA POLYMORPHISM AND GENETIC DIVERSITY OF MANGO (*Mangifera sp.*) GERMPLASM IN TROPICAL ISLAND

Putu Suwardike^{1*}, I Nyoman Rai¹, Rindang Dwiyani¹, and Eniek Kriswiyanti²

¹Faculty of Agriculture, Udayana University, Jl. P.B. Sudirman, Denpasar, Bali, Indonesia

²Faculty of Mathematics and Natural Sciences, Udayana University, Jl. Raya Kampus Unud, Bukit Jimbaran, Badung, Bali, Indonesia

*Corresponding author: putu.suwardika@yahoo.co.id

ABSTRACT

Creation of new superior varieties of mango through the empowerment of local genetic resources requires information about the potential properties of mango germplasm, including DNA polymorphism and its genetic diversity. This research aimed to obtain basic data on DNA polymorphism, genetic relationship, genetic similarity level, and molecular accession of Bali's unique local mango. Sampling was conducted in four regencies in the Province of Bali. DNA preparation, PCR, and microsatellite analysis were carried out at the Laboratory of Genetics and Plant Breeding, Faculty of Agriculture, Gadjah Mada University. It found 44 accessions of mangoes in Bali. The test results with 10 pairs of SSR markers showed that all primers produced polymorphic loci. There were 825 amplified DNA bands. Primer of AY31 produced the highest number of loci, which were 14 loci, while AY21 produced the fewest loci, i.e., 3 loci. Forty-four mango accessions showed a genetic similarity coefficient of 0.27 to 0.97. At a coefficient of 0.27, accessions were divided into 2 major groups: group A and group B. Group A consisted of two accessions, namely, KRA-005 and BDG-006 (*Mangifera foetida* Lour.), which had a similar coefficient of 0.657. Group B was divided into two smaller groups, namely, groups B1 and B2, at a similarity coefficient of 0.342. Group B1 consisted of 39 accessions, while group B2 consisted of 3 accessions. Accessions Madu Anggur, Gading, Sambuk Mengwi, Kakul and Pakel Sulangai were identified as having unique alleles.

Keywords: Polimorphism, genetic similarity, Mango, DNA, Microsatellite

INTRODUCTION

Creation of new superior varieties (*varietas unggul baru* or VUB) of mango by empowering local genetic resources requires information about the potential properties of mango germplasm. Information about the potential properties is obtained through exploration and characterization of mango germplasm. Furthermore, the information obtained becomes the basis for consideration

in developing conservation, breeding, management and sustainable use of mango genetic resources.

Characterization of mango germplasm in Indonesia has been carried out by a number of researchers from Indonesian Tropical Fruit Research Institute and various universities in Indonesia, for example, characterizations that were conducted by Kostermans and Bompert (1993) in the West

Kalimantan and South Kalimantan regions, Sumiasri *et al.* (2006) in Madiun Regency, Rai *et al.* (2008) in the Bali region specifically for *Mangifera Caesia* Jack., Krisnawati (2008) in the Central Kalimantan region, Sumarsono *et al.* (2012) in Banyumas Regency, Fitawati *et al.* (2013) in the Central Sumatra region, and Oktavianto *et al.* (2015) in Kediri Regency. Characterization was also carried out on mango germplasm collection in the Mango Experimental Garden in Kampung Cukur Gondang, Pasuruan Regency (Maltuchah and Zainudin, 2007; Fitawati *et al.*, 2010; Zainudin *et al.*, 2010; Utami *et al.*, 2012; Tasliah *et al.*, 2013; Karsinah *et al.*, 2015). However, given the vast territory of Indonesia and the limited budget and availability of research resources, not all mango germplasm can be characterized, including those mango germplasm in the Province of Bali. Bompard and Schnell (1997) reported that there were 5 species of mangoes found in the Province of Bali. The five species are *Mangifera indica* L., *Mangifera foetida* Lour., *Mangifera caesia* Jack, *Mangifera odorata* Griff., and *Mangifera lalijiwa* Kosterm. A quite in-depth study of the characteristics of the *Mangifera Caesia* Jack was carried out by Rai *et al.* (2008). However, species of *Mangifera indika* L., *Mangifera foetida* Lour., *Mangifera odorata* Griff., and *Mangifera lalijiwa* Kosterm are still not

much studied so that basic data on DNA polymorphism, genetic similarity, genetic relationship, and the molecular uniqueness of mango germplasm in Bali are very limited.

This research aims to obtain basic data on DNA polymorphism, genetic relationship, and the level of genetic similarity, and to obtain accessions of unique local mangoes in Bali that molecularly and potentially may be developed as New Superior Varieties.

MATERIALS AND METHODS

Research Location and Time

Field research (on farm characterization) was conducted in 4 (four) regencies in the Province of Bali, Indonesia, namely, Buleleng, Karangasem, Badung, and Klungkung. Meanwhile, DNA preparation, PCR, and microsatellite analysis were conducted in the Laboratory of Genetics and Plant Breeding at the Faculty of Agriculture, Gadjah Mada University. The study was conducted for 13 months from July 2018 to August 2019.

Materials and Research Tools

Exploration equipments that were used included GPS, digital cameras, books of mango descriptors, scissors, label paper, plastic straps, paper bags, cool boxes, and stationery. Meanwhile, DNA analysis equipments were mortar, centrifugation

machines, micro pipettes, micro tubes, ovens, MJ Research PCT 100 PCR machines, gel electrophoresis, and Beckman Coulter® CEQ-8000 genetic analyzer. The materials used were young leaves, extraction buffer, EDTA, ice, lysis buffer, dithiothreitol, chloroform isoamil alcoholyl, sodium acetate, isopropanol, 70% ethanol, PCR buffer, forward primer, reverse primer, and Taq DNA Polymerase.

Determination of Sampling Location

Accession of Bali's local mangoes was obtained through exploration at predetermined sampling locations. The sampling location was determined by the stratified random sampling method (Chaudhuri and Stenger, 2005). Sampling was done in two stages. The first stage was to determine the location of the district. Meanwhile, the second stage was to determine the sub-district sampling locations in each selected district. The research sample was taken from 4 (four) districts that were sorted by the number of sub-districts with local mango accessions. Each district was chosen from 4 sub-districts with the highest number of villages with mango accession. Mango plant leaf samples were taken from each accession that was found in each

village. Plants that were selected were plants that were more than 10 years old, in healthy conditions, and classified as the best among plants on the same accession in the vicinity.

Scope of Bali's Local Mangoes

Determination of local mango accession refers to the scope proposed by Rai *et al.* (2016) that Bali's local mangoes belong to the Bali's local fruit group. As defined by Article 1 number 6 of the Bali Province Regional Regulation Number 3 of 2013, Bali's local fruit referred to in this research refers to all types of fruits developed and cultivated in Bali.

Molecular Characterization

Young leaf samples of 3 pieces per accession were wrapped in aluminum foil, placed in a cool box filled with dry ice, then taken to the laboratory. Molecular characterization used 10 pairs of microsatellite primers, which had been tested on mango plants (Schnell *et al.*, 2005; Tasliah *et al.*, 2013). A list of sequences and types of microsatellite primer repeats are listed in Table 1.

Table 1. List of Sequences and Repetition Types of Microsatellite Primers Used in the Research

Primer	Forward	Reverse	Repeats
AY942818	CCACGAATATCAACTGCTGCC	TCTGACACTGCTCTTCCACC	(CT/AG)11
AY942821	TGTAGTCTCTGTTTGCTTC	TTCTGTGTCGTCAAACCTC	(GTT/AAC)6
AY942825	CGAGGAAGAGGAAGATTATGAC	CGAATACCATCCAGCAAATAC	(CGG/CCT)7
AY942827	GTTTTCAATTCTCAAATGTGTG	CTTTCATGTTTCATAGATGCAA	(CT/AG)15
AY942828	CTCGCATTCTCGCAGTC	TCCCTCCATTTAACCCCTCC	(AG/CT)9
AY942829	GAACGAGAAATCGGGAAC	GCAGCCATTGAATACAGAG	(GTT/AAC)8
AY942831	TTTACCAAGCTAGGGTCA	CACTCTTAAACTATTCAACCA	(GA/TC)15
AJ635165	GATGAAACCAAAGAAGTCA	CCAATAAGAACTCCAACC	(TG)10
AJ635168	TTCTAAGGAGTTCTAAAATGC	CTCAAGTCCAACATACAATAC	(GT)9
AJ635176	TGCGTAAAGCTGTTGACTA	GACAAGATAAACAACCTGGAA	(TG)11

Source: Scanell *et al.* (2005); Tasliah *et al.* (2013)

DNA Isolation and PCR Analysis

A total of 1 to 2 g of young leaves from each accession was extracted until DNA samples were obtained. The DNA isolation procedure used cetyl trimethyl ammonium bromide (CTAB) buffer extraction, following the Doyle and Doyle (1990) protocols. Dithiothreitol was added to prevent oxidation of phenolic compounds during extraction. The PCR reaction was carried out in a total volume of 20 µl.

DNA multiplication stages were initiation of denaturation at 95°C for 3 minutes, followed by 34 cycles consisting of denaturation at 94°C for 1 minute, primer attachment at 50°C for 1 minute, and extension of base at 72°C for 2 minutes (Schnell *et al.*, 2005). The final stage of the PCR process was the final extension at 72°C for 5 minutes and incubation at 4°C. To see whether an amplified DNA appeared, electrophoresis using 2% agarose gel was performed. Primers, which produced bands in

agarose gel, were used for DNA fragment analysis.

Analysis of DNA Fragments

DNA fragments were detected on a genetic analyzer (Beckman Coulter® CEQ™ 8000). The procedure for preparing PCR product samples for loading and procedures for running CEQ 8000 followed the protocol established by Thomson (2004). Samples were analyzed by multiplexing, i.e., mixing PCR amplification products with two kinds of green and blue fluorescent primers in one well. PCR products with a certain volume (according to the optimization results) of each primer were mixed in one well, then 0.5 µl of CEQ internal size standard (400 bp) labeled in red and SLS with a final volume of 40 µl were added.

Fragment size analysis results on CEQ 8000 were processed using CEQ Fragment Analysis software. Then, binning was carried out. Binning is grouping DNA

fragments (alleles) based on the number of repeats of certain DNA motifs (for example, repeat of threads from two, three, or four bases pairs) flanked by a pair of microsatellite primers.

Data Analysis

DNA concentration and purity of Bali's local mango accession were measured using the Gene Quant 1300 spectrophotometer. DNA concentrations obtained were high, i.e., more than 100 ng/ μ L, even most DNA concentrations reached thousands of ng/ μ L. The purity of DNA compared to impurities, including protein impurity, was measured by the wavelength of λ 260/280, while the purity of DNA compared to impurity in the form of RNA was measured by the wavelength of λ 260/280. Good DNA purity ranges from 1.8 to 2.2.

Analysis of mango germplasm accessions was based on the level of allele similarity between accessions, using the genetic distance formula described by Nei *et al.* (1983). The dendrogram was generated from the binary data of the mango plant molecular scoring results using the unweighted pair group method with arithmetic mean (UPGMA) using NTSYS 2.02 software.

RESULTS AND DISCUSSION

Findings of Bali's Local Mango Accession

Exploration results found 44 accessions of Bali's local mangoes, with the following details: 29 accessions were found in Buleleng Regency, 6 accessions were found in Badung Regency, 5 accessions were found in Karangasem Regency, and 4 accessions were found in Klungkung Regency (Table 2). Thirty four of them, or 77.27%, were landrace accessions. The rest were non-landrace accessions. Landrace accessions include *Amplemsari (Legong)*, *Bila*, *Bikul*, *Gedang Bontihing*, *Santog*, *Manggis*, *Dodol*, *Depeha*, *Sanih*, *Kunyit*, *Poh Wani Kubutambahan*, *Gandarasa*, *Amplemsari Tejakula*, *Gula*, *Kunahan*, *Amplem Taluh*, *Pudak*, *Ijo Bukit*, *Gading*, *Sambuk Mengwi*, *Kakul*, *Gedang Kelan*, *Lambat Tianyar*, *Taluh Tianyar*, *Siap*, *Santen Banjarangkan*, *Lembongan*, *Pelem Nusa Penida*, *Sakti*, *Eni Bontihing*, *Eni Ban*, *Pakel Sibetan*, and *Pakel Sulangai*. Meanwhile, those that were included in non-landrace accessions were *Gedong Gincu*, *Brazil*, *Parkit*, *Shailon*, *Arumanis 143*, *Apel*, *Manalagi*, *Golek*, *Madu Anggur*, and *Lalijiwa*.

Table 2. Accessions of Bali's Local Mangoes

No	Field ID	Local Name	Origin	No	Field ID	Local Name	Origin
1	BLL-001	Amplemsari (Legong)	Buleleng	23	BLL-023	Gula Bengkala	Buleleng
2	BLL-002	Gedong Gincu	Buleleng	24	BLL-024	Kunahan	Buleleng
3	BLL-003	Brazil	Buleleng	25	BLL-025	Amplem Taluh	Buleleng
4	BLL-004	Parkit	Buleleng	26	BLL-026	Pudak	Buleleng
5	BLL-005	Shailon	Buleleng	27	BDG-001	Ijo Bukit	Badung
6	BLL-006	Arumanis 143	Buleleng	28	BDG-002	Gading	Badung
7	BLL-007	Bila	Buleleng	29	BDG-003	Sambuk Mengwi	Badung
8	BLL-008	Bikul	Buleleng	30	BDG-004	Kakul	Badung
9	BLL-009	Apel	Buleleng	31	BDG-005	Gedang Kelan	Badung
10	BLL-010	Manalagi	Buleleng	32	KRA-001	Lambat Tianyar	Karangasem
11	BLL-011	Gedang Bontihing	Buleleng	33	KRA-002	Taluh Tianyar	Karangasem
12	BLL-012	Santog	Buleleng	34	KRA-003	Siap	Karangasem
13	BLL-013	Manggis	Buleleng	35	KLK-001	Santen Banjarangkan	Klungkung
14	BLL-014	Golek	Buleleng	36	KLK-002	Lembongan (Nusa)	Klungkung
15	BLL-015	Dodol	Buleleng	37	KLK-003	Pelem Nusapenida	Klungkung
16	BLL-016	Depeha	Buleleng	38	KLK-004	Sakti Nusapenida	Klungkung
17	BLL-017	Sanih	Buleleng	39	BLL-027	Eni (Kweni) Bontihing	Buleleng
18	BLL-018	Kunyit	Buleleng	40	KRA-004	Eni (Kweni) Ban	Karangasem
19	BLL-019	Putih	Buleleng	41	KRA-005	Pakel Sibetan	Karangasem
20	BLL-020	Wani Kubutambahan	Buleleng	42	BDG-006	Pakel Sulangai	Badung
21	BLL-021	Ganda Rasa	Buleleng	43	BLL-028	Madu Anggur	Buleleng
22	BLL-022	Amplemsari Tejakula	Buleleng	44	BLL-029	Lalijiwa	Buleleng

Based on species, 44 accessions of Bali's local mangoes come from 4 species, namely, *Mangifera indica* L., *Mangifera odorata* Griff., *Mangifera foetida* Lour., and *Mangifera lalijiwa* Kosterm. Accession of the species of *Mangifera indica* L. dominates mango germplasm in Bali with 38 accessions or 86.36%. This finding corroborates the results of research by Bompard and Schnell (1997) that reported that there were 5 species of mangoes found in Bali. The five species were *Mangifera indica* L., *Mangifera foetida* Lour., *Mangifera caesia* Jack, *Mangifera odorata* Griff., and *Mangifera lalijiwa* Kosterm. Specifically on *Mangifera caesia* Jack, Rai *et al.* (2008) have conducted quite in-depth studies. However, on the other 4

species, no in-depth molecular studies have been carried out.

DNA Concentration and Purity

DNA concentration and purity of 44 Bali's local mango accessions were measured using the GeneQuant 1300 spectrophotometer. DNA concentrations obtained were high, i.e. more than 100 ng/ μ L. Most of concentrations even reached thousands of ng/ μ L. Good DNA purity ranges from 1.8 to 2.2. From the results of the quantification, it was known that the DNA purity of the 44 accessions of the mango analyzed was quite good because it was in the range 1.8 - 2.2.

DNA Polymorphism

Test results of 44 Bali's local mango accessions with ten pairs of SSR markers indicated that all primers that were used produced polymorphic loci. There were 825 DNA bands that were successfully amplified. The primer that produced the most loci was AY31, which produced as many as 14 loci, while the primer that produced the least locus was AY21, which produced as many as 3 loci only.

Genetic Relationship and Similarity Level

The dendrogram was generated from the binary data of the mango accession molecular scoring results, as presented in Fig. 1. From the dendrogram, it was known that the genetic similarity coefficient value of 44 accessions of Bali's local mango ranged from 0.27 to 0.97. The greater the similarity coefficient value is, the more similar DNA band patterns between accessions become,

and that means the accessions are increasingly similar. In this dendrogram, it can be seen that 44 mango accessions were divided into two large groups at a coefficient of 0.27, namely, group A (2 accessions) and group B (42 accessions). Group A consisted of two accessions, namely, KRA-005 and BDG-006 (species of *Mangifera foetida* Lour.), which had a similarity coefficient of 0.657. Group B was divided into two smaller groups, namely, groups B1 and B2 at a similarity coefficient of 0.342. Group B1 consisted of 39 accessions, while group B2 consisted of 3 accessions (BDG-002, BDG-003, and BDG-004). Among these 44 accessions, there were two very similar accessions, namely, BLL-006 and BLL-016, which were located in the similarity coefficient of 0.97.

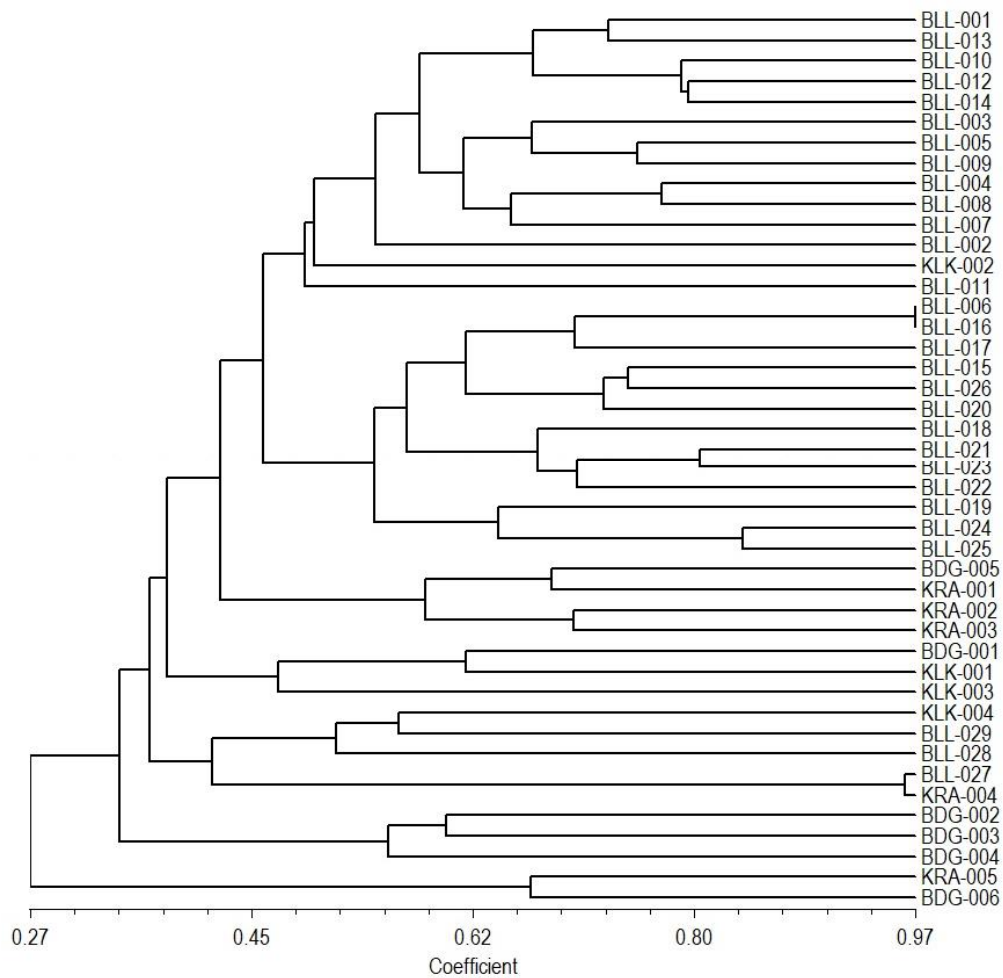


Fig. 1. Dendrogram of 44 Bali's local mango accessions
BLL, BDG, KRA, and KLK are accessions code

The principal coordinates analysis was used to find out the tendency of grouping of 44 Bali's local mango accessions based on their species. Forty-four accessions of Bali's local mangoes belong to the species of *Mangifera indica* L. (marked by a blue dot), *Mangifera foetida* Lour. (marked by an

orange dot), *Mangifera odorata* Griff. (marked by a gray dot), and *Mangifera lalijiwa* Kosterm (marked by a yellow dot), as can be seen in Fig. 2. The tendency of this grouping is generated from the value of Genetic Distance (GD) using GenAlEx software 6.503.

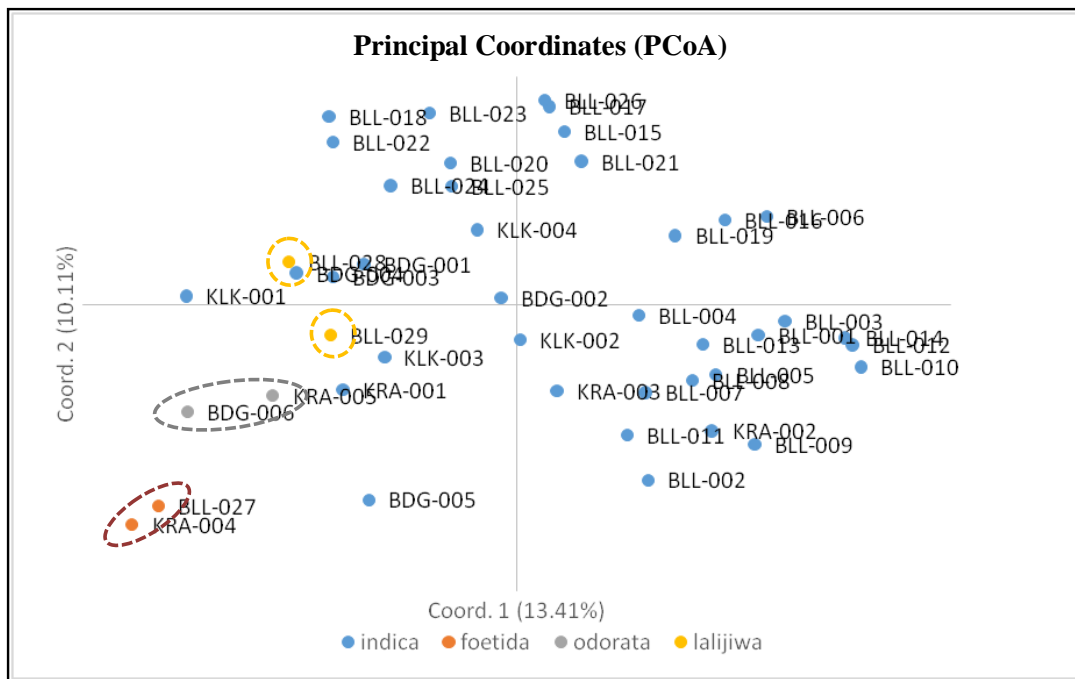


Fig. 2. Principal coordinates analysis of 44 Bali's local mango accessions with 10 SSR markers BLL, BDG, KRA, and KLK are accessions code

Fig. 2 shows that mango accessions generally tended to spread mainly to *Mangifera indica* L. species. However, *Mangifera foetida* Lour. species (marked by an orange dot) and *Mangifera odorata* Griff. (marked by a gray dot) are located close together according to their respective species groups. The *Mangifera lalijiwa* Kostermans species are located close together, but are among the *indica* accession groups.

The Unique Accession of Mango Germplasm

Among 44 accessions of Bali's local mangoes, there are five individuals that have one unique allele, as presented in Table 3.

The five accessions are BLL-028, BDG-002, BDG-003, BDG-004, and BDG-006. These alleles were mostly amplified by different primers, but some were identified in the same primers at different loci (AJ68 in accessions BDG-004 and BDG-003). Unique alleles are alleles that are only owned by one individual/accession at a specific locus size, and are not owned by another individual. BLL-028 (*Madu Anggur*) is a superior variety that has been released by the government or is classified as non-landraces. Whereas, BDG-002 (*Gading*), BDG-003 (*Sambuk Mengwi*), BDG-004 (*Kakul*), and BDG-006 (*Pakel Sulangai*) are accessions of landraces. In principle, accessions that have

unique alleles can be a characteristic of the accessions concerned and have the potential to be developed as plant breeding material and combined with other superior properties so that in the long run, they can be developed into New Superior Varieties.

Table 3. List of Mango Accessions that have Unique Alleles

Accession Code (Field ID)	Primer and Locus Size (bp)
BDG-002	AY27 (255 bp)
BLL-028	AY29 (450 bp)
BDG-006	AY31 (270 bp)
BDG-004	AJ68 (260 bp)
BDG-003	AJ68 (310 bp)

The findings of this study can facilitate improved management of local Bali mango germplasm conservation and help find strategies for future breeding activities. The same opinion was expressed by Bora *et. al.* (2018) based on the results of his study of 19 mango genotypes in India, Razak *et. al.* (2019) from the results of his research on mango germplasm in Malaysia, Riaz *et. al.* (2018) based on the results of mango research in Pakistan, and Yamanaka *et. al.* (2019) based on the results of his study of 120 mango genetic resources in Japan.

CONCLUSION

It can be concluded from this study that 44 mango accessions were found in Bali. Thirty four accessions or 77.27% are landrace accessions. The rest are non-landrace accessions. The accession of Bali's local mangoes originated from 4 species, namely, *Mangifera indica* L., *Mangifera odorata* Griff., *Mangifera foetida* Lour., and *Mangifera lalijiwa* Kosterm. Ten primers

produced polymorphic loci. There were 825 amplified DNA bands. AY31 Primer produced the highest number of loci, which was 14 loci, while AY21 Primer produced the fewest loci, i.e., as many as 3 loci. Forty-four mango accessions showed a genetic similarity coefficient of 0.27 to 0.97. At a coefficient of 0.27, it was divided into 2 major groups, namely, group A and group B. Group A consisted of two accessions, namely, KRA-005 and BDG-006 (species of *Mangifera foetida* Lour.), which had a similarity coefficient of 0.657. Group B was divided into two smaller groups, namely, groups B1 and B2 at a similarity coefficient of 0.342. Group B1 consisted of 39 accessions, while group B2 consisted of 3 accessions. Accessions Madu Anggur, Gading, Sambuk Mengwi, Kakul and Pakel Sulangai were identified as having unique alleles.

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