RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
56 (6) 2184-2195, 2024
http://doi.org/10.54910/sabrao2024.56.6.1
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

GENETIC RELATIONSHIP AMONG THE LOCAL MAIZE ACCESSIONS BASED ON SSR MARKERS IN BALI, INDONESIA

N.L.M. PRADNYAWATHI[*1], I.N. RAI[1], R. DWIYANI[1], G. WIJANA[1], and I.G.P.M. ARYANA[2]

[1]Department of Agroecotechnology, Faculty of Agriculture, Udayana University, Indonesia
[2]Department of Agroecotechnology, Faculty of Agriculture, Mataram University, Indonesia

*Corresponding author's email: pradnyawathi@unud.ac.id
Email addresses of co-authors: rainyoman@unud.ac.id, rindangdwiyani@unud.ac.id, muliarta1@yahoo.co.id, gedewijana@unud.ac.id

SUMMARY

Characterization using molecular markers is not influenced by environmental factors; hence, it can provide an accurate picture of kinship relationships between species and distant relatives. The presented research aims to determine the genetic diversity and kinship relationship among 12 local Balinese maize (*Zea mays* L.) accessions, resulting from the exploration of one drought-resistant and high-yielding maize cultivar (Lamuru) with simple sequence repeat (SSR) markers. The DNA amplification carried out by the study used a BIO-RAD T100 PCR instrument. The calculation of 10 SSR markers information utilized the iMEC online software, with the data analyzed using NTSYS 2.02 software to produce a dendrogram (phylogenetic analysis). The dendrogram development engaged the Jaccard similarity coefficient and the UPGMA clustering method. After studying their genetic profiles, the local Balinese maize accessions were genetically diverse. Assessing 12 local Balinese maize accessions and one superior cultivar revealed locations at a Jaccard similarity coefficient of 0.28 to 0.65. Maize accession Putih Bayung Gede (Karo) was the most distinct genotype, separating at a coefficient of 0.28. The separation of two other maize accessions, Berondong Lokal, and Kuning/Panes Daup, from the other 10 maize genotypes had a coefficient of 0.35. Accession Ketan Belok Sidan had the most similar DNA profile to the high-yielding maize cultivar, Lamuru, with a similarity coefficient of 0.65.

Keywords: Maize (*Z. mays* L.), dendrogram, exploration, genetic diversity, Jaccard similarity coefficient, kinship relationships

Communicating Editor: Dr. Anita Restu Puji Raharjeng

Manuscript received: March 02, 2024; Accepted: May 20, 2024

Citation: Pradnyawathi NLM, Rai IN, Dwiyani R, Wijana G, Aryana IGPM (2024). Genetic relationship among the local maize accessions based on SSR markers in Bali, Indonesia. *SABRAO J. Breed. Genet.* 56(6): 2184-2195. http://doi.org/10.54910/sabrao2024.56.6.1.

Key findings: Local Balinese maize (*Z. mays* L.) accessions occurred highly diverse regarding their genetic profile. Twelve local Balinese maize accessions, i.e., Lokal Landih, Barak Bayung Gede, Putih Bayung Gede (Karo), Lokal Bangli, Barak Keliki Kintamani, Pengootan, Berondong Lokal, Kuning/Panes Daup, Injin Daup, Bali Malet Gusti, Barak Belok Sidan, and Ketan Belok Sidan and one high-yielding and drought-resistant cultivar (Lamuru) underwent evaluation, and they laid between Jaccard similarity coefficients of 0.28 and 0.65.

INTRODUCTION

Maize (*Zea mays* L.) local cultivars and landraces are long-cultivated crops and have adapted to the existing environmental conditions and the farming community's cultivation methods (Palumbo *et al*., 2017; Di-Pasquale *et al*., 2024), and are diverse and heterogeneous populations (Mathiang *et al*., 2022). Maize genotypes with wide genetic diversity proved useful for plant breeding purposes and yield trait improvement (Navvaro *et al*., 2017; Nashath *et al*., 2024). For developing elite hybrids, the source of genetic diversity mainly comes from local maize genotypes (Zhang *et al*., 2018; Arca *et al*., 2023; Di-Pasquale *et al*., 2024). Future breeding advancement is highly dependent on the genetic diversity of the available genetic resources thus, exploration of genetic diversity is vital for selecting suitable parental genotypes applicable in hybridization and the formation of heterotic hybrids, in addition to the characterization and conservation of maize genetic resources (Sharma *et al*., 2018).

The release of high-yielding varieties of maize have been widespread in Indonesia, including the Bali Province. With the growing new varieties' use by farmers, the pressure on local varieties' (landraces) less use increases and could lead to extinction, with even some already destroyed. However, so far, no report has come out on the types of local maize in Bali and their characteristics. The exploration and characterization of local Balinese maize needs carrying out for further use as a plant breeding material. Characterization seeks specific characteristics possessed by the crop plants, used to distinguish between the types and individuals within one crop (Miswarti *et al*., 2014). Characterization defines as an account for inherited traits both morphologically, agronomically, and molecularly (Lutatenekwa *et al*., 2020). At the molecular level, research on the molecular characters of inbred lines provides certainty on genotypic diversity and relies on the selection of elite genetic resources (Sathua *et al*., 2018). Sharma *et al*. (2018) stated that, as compared with morphological data analysis, DNA-based molecular markers provide more accurate and repeatable results to assess the levels of genetic diversity of a particular species, with no influence from the environment.

Similarly, reports stated that the levels of genetic diversity and kinship of a population could be more visible through molecular markers (Syafii and Ruswandi, 2019). Furthermore, molecular characterization is necessary to support the morphological markers' description. DNA is a genetic material used in characterizing with molecular markers, with no environmental factor influences, and can provide an accurate description of kinship relationships among the species and distant relatives.

Among molecular markers, simple sequence repeat (SSR) markers are ideal for genetic diversity analysis (Rohini *et al*., 2020; Mathiang *et al*., 2022). The SSR is one of the most widely used molecular markers in analyzing the genetic relationship between inbred strains and the levels of genetic diversity in maize crops (Adeyemo *et al*. 2011). They are highly accurate (Ramlah *et al*., 2017), highly polymorphic and reproducible (Sathua *et al*., 2018), very informative, and easily detected by the PCR (Sharma *et al*., 2018). Alsaleh (2022) stated that SSR markers have many advantages, such as codominance, high concentration levels of polymorphism, chromosome specificity, and high reproducibility.

The SSR markers also proved beneficial in identifying and monitoring the target traits in various crop cultivars and in assessing the

genetic diversity of maize genetic resources (Ramlah *et al*., 2017). Characterizing the maize inbred lines explored the genetic diversity of elite inbred lines (Sathua *et al*., 2018; Sharma *et al*., 2018). Jaishreepriyanka *et al*. (2020) also analyzed the genetic variety of several maize-inbred strains and found remarkable outcomes. The presented study sought to determine the genetic diversity and kinship relationship among the local Balinese maize accessions and one high-yielding cultivar (Lamuru), resulting from exploration.

MATERIALS AND METHODS

Plant source

The latest study procured 12 local Balinese maize (*Z. mays* L.) accessions in Central Bali (Pradnyawathi *et al*., 2022). These are Local Landih, Barak Bayung Gede, Putih Bayung Gede, Local Bangli, Barak Keliki Kintamani, Pengootan, Berondong Local Bali, Kuning/Panes Daup, Injin Daup, Bali Malet Gusti, Barak Belok Sidan, and Ketan Belok Sidan (Figures 1 and 2) and one high-yielding new maize cultivar (Lamuru) for their evaluation.

DNA isolation

The DNA extraction began with 2% CTAB and a leaf sample weight of 0.1 g from each type of maize (Doyle and Doyle, 1990). The DNA amplification continued by PCR reaction to duplicate the DNA sequence based on the primers' compatibility with the template DNA. The primers used in the study are available in Table 1. The conduct of PCR reactions had a total volume of 12.5 µL for each PCR tube. Each PCR reaction consisted of 6.25 µL PCR mix Go Taq® Green (Promega), 0.25 µL 10 µM primers (IDT) each for Forward and Reverse primers, 2.5 µL sample DNA (template*)* and 3.00 µL nuclease-free water.

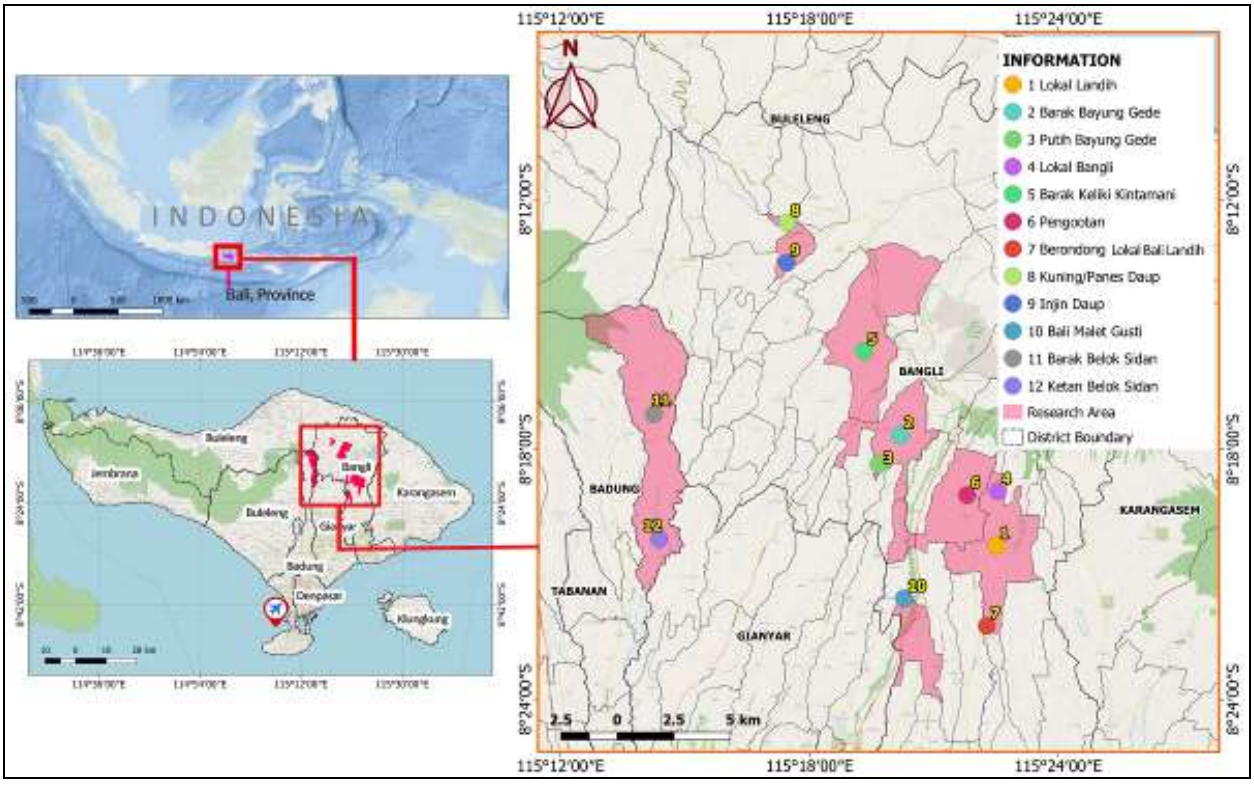

Figure 1. The research proceeded in Bali, Indonesia and focused on the spatial distribution of 12 local Balinese maize (*Z. mays* L.) accessions.

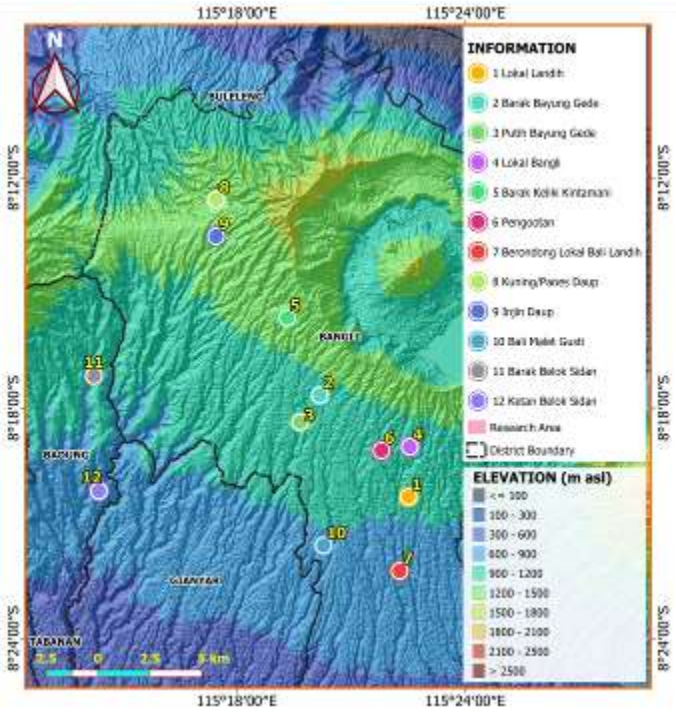

Figure 2. Spatial distribution of 12 local Balinese maize (*Z. mays* L.) accessions with different elevations.

Table 1. The SSR primers used in the PCR reaction.

No.	Primary Code	Primer Sequence (5' - 3')	Annealing Temperature (°C)
1	bnlg1017 Forward	ATT GGA AGG ATC TGC GTG AC	61
	bnlg1017 Reverse	CAG CTG GTG GAC TGC ATC TA	
2	bnlg1189 Forward	CGT TAC CCA TTC CTG CTA CG	58
	bnlg1189 Reverse	CTT GCT CGT TTC CAT TCC AT	
3	phi015 Forward	GCA ACG TAC CGT ACC TTT CCG A	60
	phi015 Reverse	ACG CTG CAT TCA ATT ACC GGG AAG	
4	phi034 Forward	TAG CGA CAG GAT GGC CTC TTC T	60
	phi034 Reverse	GGG GAG CAC GCC TTC GTT CT	
5	phi112 Forward	TGC CCT GCA GGT TCA CAT TGA GT	60
	phi112 Reverse	AGG AGT ACG CTT GGA TGC TCT TC	
6	Phi 8175 Forward	GGG AAG TGC TCC TTG CAG	62
	Phi 8175 Reverse	CGG TAG GTG AAC GCG GTA	
7	umc1630 Forward umc1630	CAG ACC TTC GAG GGC AAG AAC T	60
	Reverse	AGT TTT GGC TTC TTC TCC CAA GTC	
8	umc1641 Forward umc1641	CTC CCT TCG TCT CCC GAC TC	60
	Reverse	CAG ATC GGC TCA GCC ACA AC	
9	umc1653 Forward umc1653	GAG ACA TGG CAG ACT CAC TGA CA	62
	Reverse	GCC GCC CAC GTA CAT CTA TC	
10	umc2013Forward	GGG ACG AGA GTC TGT TGT TGT TG	56
	umc2013 Reverse	GTT GAT GCA TGT GAC TCT GGA AAC	

DNA amplification

The DNA amplification proceeded with a BIO-RAD T100 PCR tool. The initial denaturation stage commenced at 95 °C for three minutes, then followed by 34 cycles with the temperature and time in each cycle as follows: denaturation at 94 °C for one minute, annealing for one minute, and elongation at 72 °C for two minutes, followed by final elongation at 72 °C for five minutes, and a storage temperature of 12 °C. Annealing temperature data appears in Table 1.

DNA visualization

The DNA from PCR underwent electrophoresis using a horizontal electrophoresis tank with 2% (b/v) agarose. Dissolving 0.6 g of agarose in 30 mL of 1x TBE continued using a microwave. Afterward, the agarose solution received a 5 µL of florosafe DNA stain. Pouring the agarose into a mold had a capacity of 17 wells. After the agarose solidified, the sample loading into each agarose well ensued. The SMOBIO AccuBand™ ladder served as a standard for the size of the DNA band profile when running the electrophoresis. Electrophoresis ran with a voltage of 100 volts and an electric current of 400 mA for 70 minutes. Then, the electrophoresis results' visualization utilized a UV transilluminator and photographed with a Nikon D3400 DSLR.

The SSR markers information used in this study bore calculations using the iMEC online software developed by Amiryousefi *et al*. (2018). Marker information includes expected heterozygosity (H), polymorphism information content (PIC), effective multiplex ratio (E), mean heterozygosity (H_{avp}), marker index (MI), discriminating power (D), and resolving power (R).

Data analysis

DNA banding patterns on agarose gels after visualization received scoring via the binary data based on the presence (1) and absence (0) of the DNA bands at a certain base pair size. Furthermore, data analysis used the NTSYS 2.02 software to carry out the phylogenetic analysis and develop a dendrogram (Rohlf, 1998). Dendrograms' construction employed the Jaccard similarity coefficient and the UPGMA clustering methods.

RESULTS AND DISCUSSION

Marker information, including expected heterozygosity (*H*), polymorphism information content (PIC), effective multiplex ratio (E), mean heterozygosity (H_{avp}), marker index (MI), discriminating power (D), and resolving power (R) ran tests on local Balinese maize accessions using 10 pairs of SSR markers (Table 2) (Amiryousefi *et al*., 2018).

Table 2. Information on 10 SSR markers tested on Balinese local maize accessions.

No.	Primary	H	PIC	E	H.av	MI	D	R
1	bnlg 1017	0.473	0.361	1.154	0.012	0.014	0.858	0.923
2	bnlg 1189	0.444	0.346	1.000	0.011	0.011	0.895	2.000
3	phi 015	0.426	0.335	1.231	0.008	0.010	0.910	2.308
4	Phi 034	0.484	0.367	1.769	0.012	0.022	0.659	1.846
5	Phi 112	0.473	0.361	1.231	0.018	0.022	0.631	0.462
6	Phi 8175	0.492	0.371	2.615	0.006	0.016	0.813	3.538
7	Umc 1630	0.497	0.374	1.077	0.019	0.021	0.720	0.769
8	Umc 1641	0.497	0.374	1.615	0.013	0.021	0.717	1.692
9	Umc 1653	0.399	0.319	1.923	0.004	0.008	0.927	3.846
10	Umc 2013	0.488	0.369	1.154	0.019	0.022	0.677	0.308

In genetic diversity studies, the PIC value of the marker is an indicator to measure the marker's quality. A marker's PIC value indicates its ability to detect polymorphism between individuals in the tested population (Chesnokov and Artemyeva, 2015). The higher the discrimination capacity of a marker, the greater the PIC value. The PIC value for codominant markers is between 0 and 1, where it is zero when monomorphic and 1 when certain alleles show the same frequency. According to the classification of Botstein *et al*. (1980), SSR markers (codominant) are considerably very informative if the PIC value is >0.5. PIC is moderately informative if the value is between 0.25 to 0.5 and slightly informative if the value is <0.25.

The PIC value of SSR markers for each population was different, depending on the pattern of DNA bands produced in each population (Chesnokov and Artemyeva, 2015; Fernandez *et al*., 2023). Specific SSR markers can be informative in one population, but may not necessarily be informative when tested in other populations. The PIC values of SSR markers tested on local Balinese maize populations ranged from 0.319 to 0.374 (Table 2), which are considered moderately informative. The lowest PIC appeared in the SSR marker umc 1653 (0.399), while the highest and the same PIC value (0.374) resulted in umc1630 and umc 1641 (Table 2). Various past studies reported the PIC values, i.e., 0.36 (Sharma *et al.*, 2017), PIC ranges of 0.13-0.32 (Jaishreepriyanka *et al*., 2020), PIC ranged from 0.14 to 0.36 (Rani *et al.*, 2020), average PIC value of 0.345 (Joshi *et al*., 2020), PIC ranged from 0.32 to 0.57 (Emam, 2023), and average PIC 0.379 (Fernandez *et al*., 2023).

Aside from the high PIC values in the SSR markers umc1630 and umc1641, they also gave the highest heterozygosity (H) value of 0.497 each. The SSR marker umc1630 has also shown the maximum average heterozygosity (H.av) value (0.019). However, the lowest PIC value emerged in the SSR marker umc1653, followed by the lowest H, H.av, and MI values (0.399, 0.004, and 0.008, respectively). Expected heterozygosity (H) refers to the condition where individuals carry two different alleles at a given locus, while mean heterozygosity (H.av) measures the average heterozygosity of all loci in the population (Amiryousefi *et al*., 2018). The results further revealed that H values ranged from a low (0.399) in marker umc1653 to a high (0.497) in SSR markers (umc1630 and umc1641). The H.av values ranged from a low (0.004) in umc1653 to a high and the same value (0.019) in SSR markers umc2013 and umc1630. In the research of Abebe *et al*. (2020), the H values ranged from 0.09 to 0.61 with an average value of 0.45, while in the studies of Amiryousefi *et al*. (2018), the H values ranged from 0.2188 to 0.4998, and H.av values ranged from 0.0030 to 0.0026.

The effective multiplex ratio (E) refers to the efficiency of a marker in analyzing multiple loci simultaneously in the PCR reaction. The higher the E value, the more efficient the marker used (Amiryousefi *et al*., 2018). In the presented study, the E values range from the low (1.000) in SSR marker bnlg 1189 to the high (2.615) in SSR marker phi 8175. According to Amiryousefi *et al*. (2018), the E values ranged from 0.2500 to 0.9896. In genetic analysis, the marker index (MI) can help identify the relevant SSR markers. The higher the MI value, the better the quality of the marker (Setiawan *et al*., 2023). The MI values ranged from the lowest (0.008) in marker umc1653 and the highest (0.022) in the SSR markers umc2013, phi 034, and phi 112). Amiryousefi *et al*. (2018) reported in studies that the MI values ranged from 0.2461 to 5.7229.

The discriminating power (D) of the SSR markers is the ability of the markers to distinguish crop genotypes (Setiawan *et al*., 2023). The greater the D value, the more diverse the markers and genotypes. In the relevant study, the D value of the markers ranged from the lowest (0.631) in phi 112 to the highest (0.927) in the marker umc1653. Amiryousefi *et al*. (2018) reported that the D values ranged from 0.4877 to 0.9849. Resolving power (R) is a measure of a marker's ability to distinguish the alleles at a particular locus, and it is the measure of a marker's effectiveness in distinguishing populations (Prevost and Wilkinson, 1999). In this study,

the R values ranged from a low (0.308) in the marker umc 2013 to a high (3.846) in the marker umc1653. Amiryousefi *et al*. (2018) observed R values with a range of 0.3750 to 9.8125. The electrophoresis results of local maize analyzed using 10 primers occur in Figure 3. The number of loci, the number of polymorphic loci, and the number of DNA bands that appeared can be seen in Table 3.

The percentage of polymorphic loci (PPL) in the local Balinese maize population ranged from 50% to 100%. Emam *et al.* (2023) found a range of 50% to 67% polymorphism. Abebe *et al*. (2020) stated that the degree of polymorphism varied from population to population, with a range of 96.43% to 100%. The high and wide range of polymorphism indicates high genetic diversity and can be a good source of diversity for future breeding programs. The highest number of loci surfaced in the marker umc1653, with seven loci, all of which were polymorphic. However, the fewest appearing loci were 2 loci in four markers, phi 015, phi 112, umc1630, and umc2013. The most DNA bands appeared on the marker phi 8175, with 34 DNA bands, while the fewest bands (6) arose on the marker phi 015. More DNA bands can also authenticate the higher genetic diversity.

The Jaccard coefficient served as a similarity coefficient, as was the UPGMA clustering method. The similarity coefficient ranged from 0.00 to 1.00. Sunaryo *et al*. (2020) stated that the wide range of similarity coefficient values indicates the highest genetic diversity among the samples. The similarity coefficient value will affect the genetic relationship among the studied samples. By reading the DNA profiles of all the maize samples using 10 pairs of SSR markers, the following dendrogram resulted, as shown in Figure 4.

The local Balinese maize accessions and one high-yielding new cultivar proved very diverse in terms of their genetic profiles. Although, the accessions tested were not from the distant regions of Central Bali, Indonesia (Bangli Regency, i.e., Local Landih, Barak Bayung Gede, Putih Bayung Gede, Local Bangli, Barak Keliki Kintamani, Pengootan, Berondong Local Bali, Kuning/Panes Daup, Injin Daup, and Bali Malet Gusti, and two from the Petang area of Badung Regency, i.e., Barak Belok Sidan, and Ketan Belok Sidan). Prasanna (2012) stated that local landraces, cultivated plants, have distinct origins and identities. They are genetically diverse, lacked formal improvement, locally adapted, and associated with traditional farming systems. Their findings also added that mutations could lead to new variations.

In the presented study, 12 local Balinese maize accessions and one new high-yielding drought-resistant cultivar (Lamuru) reached assessment, within a Jaccard similarity coefficient of 0.28 to 0.65. The dendrogram showed that local Balinese maize accessions were grouped into five clusters, i.e., cluster 1 (Lokal Landih, Barak Keliki Kintamani, Barak Belok Sidan), cluster 2 (Barak Bayung Gede, Injin Daup, Bali Malet Gusti, Ketan Belok Sidan), cluster 3 (Local Bangli, Pengootan), cluster 4 (Berondong Lokal, Kuning/Panes Daup), and cluster 5 (Putih Bayung Gede). The maize accession Putih Bayung Gede has genetic characteristics that are very different from other land races. The reason is the genotype Putih Bayung Gede has the furthest genetic distance from those of other cultivars (with a coefficient of 0.28).

The local Balinese maize accession, Belok Sidan, has the closest genetic relationship to the new cultivar, Lamuru (with a similarity coefficient of 0.65). The same research has also progressed on the local maize 'Tana Toraja Sulawesi.' Ramlah *et al*. (2017) reported that the local maize 'Tana Toraja Sulawesi' showed a genetic similarity coefficient with a range of 0.47 to 0.85. The two clusters formed from four accessions at a genetic similarity coefficient of 0.47. Yani *et al*. (2022) reported eight local maize cultivars in East Nusa Tenggara, Indonesia, which had the highest diversity with similarity indices between 0.29–0.65 and splitting into two clusters at a similarity index of 0.43. Aci *et al*. (2013) grouped 15 local Algerian accessions with 18 SSR markers, resulting in three main clusters related to geographical origin. Ignjatovic-Micic *et al*. (2013) disclosed about

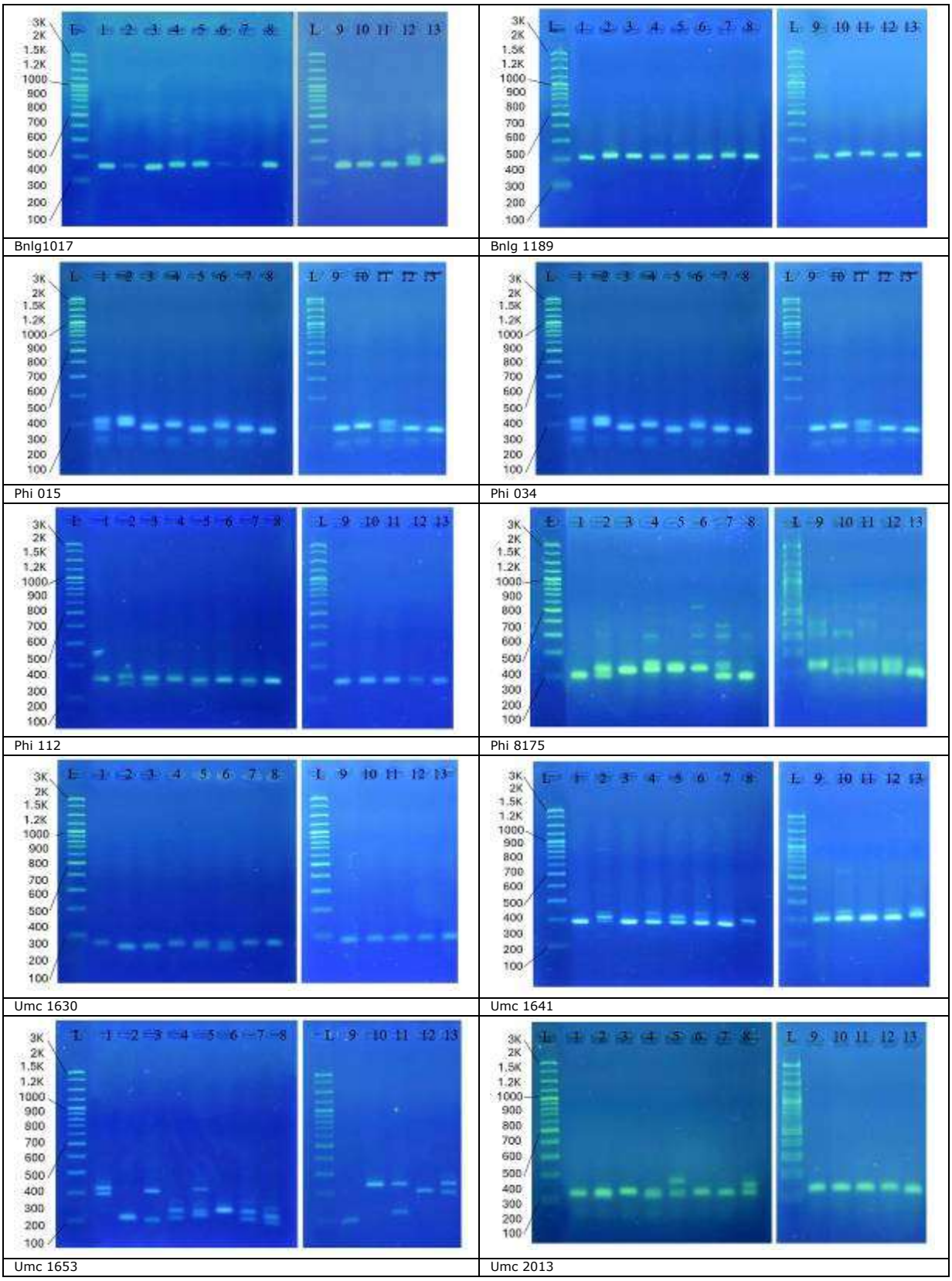

Figure 3. Electrophoresis results of each primer.

Table 3. Number of loci, polymorphic loci, and merging DNA bands.

No.	Primary	Number of loci	Number of polymorphic Loci	Percentage of Polymorphic Loci	Number of DNA bands that appeared
1	Bnlg 1017	3	3	100	15
2	Bnlg 1189	3	3	100	13
3	Phi 015	2	2	100	6
4	Phi 034	3	3	100	23
5	Phi 112	2	1	50	16
6	Phi 8175	6	6	100	34
7	Umc 1630	2	2	100	14
8	Umc 1641	3	3	100	21
9	Umc 1653	7	7	100	25
10	Umc 2013	2	2	100	15

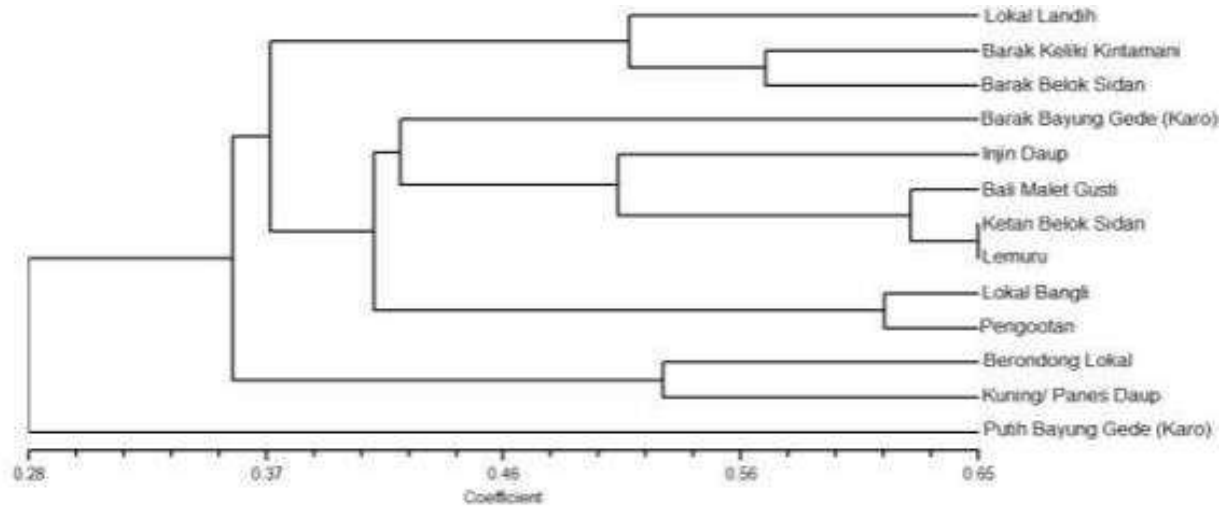

Figure 4. Dendrogram of Bali local maize accessions.

the local maize from Former Yugoslavia, for which the value of genetic similarity ranged from 0.30 to 0.81.

Dividing the dendrogram resulted in five clusters, with two accessions separated from the 12 accessions and one new high-yielding drought-resistant cultivar. The level of genetic diversity in plants is essential to the plant breeding process. A broad genetic distance between the candidate crossing parents will grant a better chance of producing hybrids with higher compatibility and fertility (Martínez-Fortún *et al*., 2022). Genetic diversity in this population seemed to be caused by a series of evolutions due to the effects of migration, selection, and geographic isolation (Hassan *et al.,* 2014; Ali and Alshugeairy, 2023; Mukhlif *et al*., 2023), as well as backcrossing, inbreeding, and outbreeding events (Fatchiyah *et al*., 2011). Vu *et al*. (2022) also added that genetic content had ongoing modification, resulting from spontaneous mutations, transcription errors, induced mutations, transposon activity, meiotic crosses, and cross-fertilization processes (Vergauwen and Smet, 2017). Thus, genetically promising genotypes are a supporting factor in the success of plant breeding programs. The research proved very beneficial in selecting the Bali local maize accessions to produce superior cultivars through breeding.

CONCLUSIONS

Local Balinese maize (*Z. mays* L.) accessions proved highly diverse based on their genetic profile. Twelve local Balinese maize accessions and one high-yielding and drought-resistant cultivar (Lamuru) showed between the Jaccard similarity coefficients of 0.28 and 0.65. Maize accession Putih Bayung Gede (Karo) emerged as the most divergent individual with a coefficient of 0.28. Accessions Berondong Lokal and Kuning/Panes Daup were also distant from the other 10 maize landraces at a coefficient of 0.35. The Balinese maize accession Ketan Belok Sidan and the new cultivar Lamuru were prominent with the most similar DNA profile, with a similarity coefficient 0.65.

ACKNOWLEDGMENTS

The research team would like to thank the Head of Udayana University for providing funds to help carry out the research. Thanks also to the Head of the Institute for Research and Community Service, Udayana University, and staff for all the help to conduct the research smoothly.

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