HAYATI
Journal of Biosciences
Vol. 33 No. 3, May 2026 750-763
DOI:10.4308/hjb.33.3.750-763
ISSN: 1978-3019
EISSN: 2086-4094

Research Article

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Detection of the PR-1 Resistance Gene and SSR-Based Genetic Diversity Analysis of S2 Lines in Local South Sulawesi and Introduced Maize

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ARTICLE INFO

Article history:
Received May 26, 2025
Received in revised form January 7, 2026
Accepted January 19, 2026
Available Online February 6, 2026

KEYWORDS:
Genetic Diversity,
PR-1 Gene,
SSR Markers

ABSTRACT

Maize cultivation in Indonesia faces significant challenges, particularly the prevalence of downy mildew. Plants infected by *Peronosclerospora* spp., the causal agents of this disease, increase the expression of pathogenesis-related (PR-1) proteins as an induced defense mechanism. Developing superior varieties through breeding programs requires robust germplasm management to maintain broad genetic diversity. Therefore, utilizing biomolecular methods to detect resistance genes and analyze genetic diversity is a strategic approach for maize genetic improvement. This study aimed to evaluate the basal expression of the *PR-1* gene associated with downy mildew resistance and analyze the genetic diversity of S2 lines derived from local South Sulawesi maize (Sinjai and Tana Toraja) and introduced maize (National Varieties and CIMMYT). Of the 30 lines used, consisting of 5 lines from local Sinjai maize, 15 lines from local Tana Toraja maize, and 10 lines of introduced maize, all were detected to have the *PR-1 gene*. Meanwhile, the genetic diversity analysis using 15 pairs of SSR primers from 10 chromosomal loci spread across corn obtained an average number of alleles of 10.27 alleles/locus and an average polymorphism information content (PIC) value of 0.76 (very informative). Cluster analysis shows a similarity coefficient value ranging from 0.68 to 0.96, with two main clusters at a similarity coefficient of 0.68. Cluster I consists of 27 lines, and Cluster II consists of 3 lines. The greatest genetic distance is possessed by the lines LK1 and BK5. The presence of the *PR-1* gene and the high genetic diversity observed in the S2 lines represent promising genetic materials for developing maize varieties with durable resistance to downy mildew.

1. Introduction

One of the important carbohydrate-producing crops in the world, besides rice and wheat, is corn. This plant is generally consumed as a staple food in many countries, including Central and South America, Africa, and Asia, especially in Africa and several regions of Indonesia (Ashan *et al.* 2020). In Indonesia, corn is cultivated in several regions, including North Sumatra, South Sumatra, Lampung, Central Java, East Java, Nusa Tenggara, North Sulawesi, South Sulawesi, and Maluku.

As an alternative staple food to rice, corn has a sufficient content of nutrients and dietary fibre, making it essential for human sustenance (Al-Hazemawi *et al.* 2024). Corn exhibits high genetic diversity due to its widespread cultivation. Regional differences in climate, water availability, soil types, topography, and other local

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environmental factors contribute to the distribution and differentiation of local germplasm. A local landrace is a cultivar of plants that is resistant to those local conditions (Ramlah *et al*. 2018). The diversity of this local landrace germplasm is highly valuable as a source of specific genetic traits that can be used to enhance desirable plant characteristics (Juhriah *et al*. 2012).

The development of corn in Indonesia faces many challenges, including increased cases of disease and pests that reduce agricultural production (Syahruddin *et al*. 2020). In addition, another factor contributing to the low productivity of corn in Indonesia is the limited number of corn germplasm, and the use of less-specific resistance genes makes it impossible to accurately identify the genes truly responsible for plant resistance to diseases and pests. As a result, plant breeding efforts to improve resistance to pests and diseases become less effective, resulting in reduced crop yields (Ashan *et al*. 2020).

One of the obstacles in maize cultivation is plant pests and diseases, including the fungus *Peronosclerospora* spp., which causes downy mildew. Plant cells use many proteins to regulate gene expression for survival and production. Thus, plant cells activate a large number of protective and defence genes, including cell wall proteins, lipoxygenase, and pathogenesis-related (PR) proteins (Zribi *et al*. 2021).

Pathogenesis-related proteins (PR) are plant proteins produced in leaves in response to pathogen infections, including viruses, viroids, fungi, and bacteria. Information on the presence or expression of PR in monocot plants remains very limited. PR-1 is a component of plant innate immunity and has been used as a marker to enhance disease resistance (Luo *et al*. 2023). The expression of the *PR-1* gene serves as a molecular marker to indicate the plant's defence response (Zribi *et al*. 2021).

Efforts to improve plant breeding require a deeper understanding of genetic resources that can be applied in conventional breeding or through molecular techniques using marker-assisted breeding (Annicet *et al*. 2022). Moreover, identification of downy mildew-causing microorganisms can be found using bimolecular techniques and microsatellite markers (Wang *et al*. 2022). Microsatellites (SSRs) are highly informative, easy to amplify using PCR techniques, highly polymorphic, codominant markers scattered throughout the corn genome due to their locus specificity, and have proven to be distinct from other molecular markers (Annicet *et al*. 2022; Zhao *et al*. 2023). SSR markers are one of the biomolecular methods that have been widely used in efforts to determine the characterization of certain traits as well as genetic diversity in plants such as oil palm (Riski 2021), sorghum (Habtegiorgis *et al*. 2025), corn (Abegunde *et al*. 2022; Mathiang *et al*. 2022), soybean (Khatun *et al*. 2021), and rice (Andarini *et al*. 2022).

The process of identifying disease resistance genes and characterising genes influencing superior, native traits and disease resistance through SSR markers can be carried out of produce and improve desired plant varieties (Sunarminto *et al*. 2018). In a sustainable agricultural environment, combining traditional knowledge, creativity, and modern technology may be the key to maximizing the potential of local corn.

Given the threat of downy mildew pathogens in corn, which impact yields, and the genetic characteristics of local corn that can be developed into superior varieties, research is needed to detect the *PR-1* gene for downy mildew resistance and to analyze genetic diversity in local corn using SSR molecular markers.

2. Materials and Methods

2.1. Plant Materials

The plant samples used in this study were S2 (second selfing) generation from South Sulawesi local maize (Sinjai and Tana Toraja), national varieties, and CIMMYT from the seed collection of the Laboratory the Center for Standardization of Cereal Crops Instrument Maros, with a total of 30 lines (Table 1), which had undergone PSY1 gene detection by Juhriah *et al*. 2012. The corn seeds were planted in planter bags measuring 25 × 25 cm and observed for 2 weeks. After 14 days after sowing (DAS), young leaves from each corn strain were collected at 50-100 mg/sample for RNA extraction and 0.4 grams/sample for DNA extraction.

2.2. Detection of the PR-1 Resistance Gene

2.2.1. RNA Extraction and cDNA Synthesis

In this study, RNA extraction was performed using the Total RNA Mini Kit (Plant) from Geneaid (2017). The RNA extraction process, according to the kit's procedure, involves cell lysis, RNA binding, washing, and RNA purification. The extracted RNA is then measured for purity and concentration using a spectrophotometer (Implen nanophotometer) at wavelengths of 260nm and 280nm (A_{260}/A_{280}). The extracted and purified RNA was used as a template for cDNA synthesis using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific 2013) according to the manufacturer's instructions. The RT reaction was carried out for 60

minutes at 42°C, followed by heating at 70°C for 5 minutes to terminate the reaction.

2.2.2. cDNA Amplification using PR-1 Primer

Amplification of cDNA using PCR reagents according to the procedure in Ambionet (2004) with a total volume of 25 µL, consisting of 3 µL of cDNA, KAPA2G Fast HotStart ReadyMix 2 × 12.5 µL (Kapa Biosystem), 7.5 µL nuclease-free water, and 1 µL each of *PR-1* gene amplification using a specific pair of PR-1 primers. The PCR reaction was performed using the RT-PCR program with the following steps: predenaturation for 5 minutes at 94ºC, denaturation for 30 seconds at 94ºC, primer annealing for 30 seconds at 55ºC, and extension for 1 minute at 72ºC. The denaturation extension cycle was repeated 39 times. Next, the final extension is carried out for 7 minutes at 72ºC, followed by cooling to 25ºC for 4 minutes.

2.2.3. Visualization of cDNA Bands

The process of visualizing cDNA bands for *PR-1* gene detection is carried out according to the procedure described by Ambionet (2004). Visualization of cDNA fragments was performed through electrophoresis on a 1% agarose gel in a 0.5× Tris-borate EDTA (TBE) buffer solution at 80 volts for 1.5 hours. Next, it is soaked in a 2% ethidium bromide solution for 15 minutes for observation through a UV transilluminator.

Table 1. List of sample numbers, codes, names, and origins

Sample code	Name and origin
BK1	
BK2	Bata Pulut Kuning
BK3	East Sinjai, South Sulawesi
BK4	
BK5	
LT1	
LT2	Lokal Toraja
LT3	Narrang-Tator, South Sulawesi
LT4	
LT5	
LK1	
LK2	Lokal Kandora
LK3	Tator, South Sulawesi
LK4	
LK5	
LB1	
LB2	Lokal Bebo
LB3	Sangalla-Tator, South Sulawesi
LB4	
LB5	
SR1	
SR2	Srikandi Kuning 1
SR3	National Variety
SR4	
SR5	
CM1	
CM2	Carotenoid Syn 3
CM3	CIMMYT, Mexico
CM4	
CM5	

2.3. Genetic Diversity Analysis

2.3.1. DNA Extraction

The DNA extraction procedure was performed using a modified CTAB buffer method (Ambionet 2004). First, a 0.4 g leaf sample was weighed and added to 1.4 mL CTAB buffer, then ground to a smooth paste. Next, 10 µL of β-mercaptoethanol was added, and the mixture was incubated in a microtube at 60ºC for 60 minutes. After incubation, the mixture was allowed to cool, chloroform-isoamyl alcohol was added, and the microtube was centrifuged at 11,600 rpm for 10 minutes. After centrifugation, the supernatant was transferred to a new microtube, and cold isopropanol was added. The tube was then spun to allow the DNA to form fine strands. Next, the DNA was precipitated by centrifugation for another 10 minutes. To wash the DNA pellet, 70% cold ethanol was added, and the mixture was incubated for 10 minutes. Finally, the DNA pellet was dried by inverting the tube over a tray lined with tissue paper. After drying, the tris-EDTA buffer is added to the DNA pellet tube, and the mixture is incubated for 60 minutes. After the DNA dissolves in the Tris-EDTA buffer, it is homogenized and centrifuged. The extracted DNA is then measured for purity and concentration using a spectrophotometer (Implen nanophotometer) at wavelengths of 260 nm and 280 nm (A_{260}/A_{280}).

2.3.2. DNA Amplification using 15 SSR Primers

1 µL of DNA solution in the microplate was added to 9 µL of reaction solution (PCR mix) consisting of @Primer Mix (F and R) at 5 µM (0.5 µL per reaction), KAPA2G Fast HotStart ReadyMix 2× (6.25 µL per reaction), and nuclease-free water (2.25 µL per reaction). Next, 1 drop of mineral oil is added, and the microplate is closed. This stage uses 15 genetic diversity primers (Table 2). The PCR program was pre-denatured for 5 minutes at 95ºC, denatured for 30 seconds at 95ºC, primer annealed at a temperature adjusted for each primer (Table 2) for 30 seconds, and extended at 72ºC for 30 seconds. The denaturation-extension cycle was repeated 35 times, followed by a final extension for 7 minutes at 72ºC and cooling at 25ºC for 4 minutes (Ambionet 2004).

Table 2. List of 15 SSR markers for genetic diversity analysis

Bin	Marka	Repeat	Primer sequence	Annealing temperature (^{0}C)
1.11	phi227562	ACC	F : TGATAAAGCTCAGCCACAAGG R : ATCTCGGCTACGGCCAGA	54
2.01	phi96100	ACCT	F : AGGAGGACCCCAACTCCTG R : TTGCACGAGCCATCGTAT	54
2.09	umc1736	(GCAT)6	F : CCATCCACCACTAGAAAGAGAGGA R : TTAATCGATCGAGAGGTGCTTTTC	56
3.02	phi374118	ACC	F : TACCCGGACATGGTTGAGC R : TGAAGGGTGTCCTTCCGAT	54
4.08	phi092	GCAA	F : GTGGGGGAGCCTACTACAGG R : GACGAGGCCATCATCACGGT	59
5.03	phi109188	AAAG	F : AAGCTCAGAAGCCGGAGC R : GGTCATCAAGCTCTCTGATCG	56
5.04	phi331888	AAG	F : TTGCGCAAGTTTGTAGCTG R : ACTGAACCGCATGCCAAC	54
6.05	bnlg1154	AG(27)	F : GGGTGATCACATGGGTTAGG R : AAATCAATGCTCCAAATCGC	53
7.02	phi034	CCT	F : TAGCGACAGGATGGCCTCTTCT R : GGGGAGCACGCCTTCGTTCT	54
7.04	phi328175	AGG	F : GGGAAGTGCTCCTTGCAG R : CGGTAGGTGAACGCGGTA	54
8.08	phi080	AGGAG	F : CACCCGATGCAACTTGCGTAGA R : TCGTCACGTTCCACGACATCAC	54
8.09	phi233376	CCG	F : CCGGCAGTCGATTACTCC R : CGAGACCAAGAGAACCCTC	54
9.03	phi065	CACTT	F : AGGGACAAATACGTGGAGACACAG R : CGATCTGCACAAAGTGGAGTAGTC	54
9.04	phi042	CATA	F : ATGTGGCCATCATTCAATGCTGTAGAC R : ACACATGCAGGTGCAGCCAGA	59
10.06	umc1061	(TCG)6	F : AGCAGGAGTACCCATGAAAGTCC R : TATCACAGCACGAAGCGATAGATG	53

Source: https://www.maizegdb.org/ (Maize genetics and genomics database)

2.3.3. Visualization of DNA Bands

Genetic diversity was assessed by visualizing DNA fragments through 8% polyacrylamide gel electrophoresis (PAGE) according to the protocol established by Ambionet (2004). For electrophoresis on 2 pages with 2 plates, a solution of 8% acrylamide (100 µL), ammonium persulfate (APS) (1,000 µL), and TEMED (100 µL) is used. The mixture of the solution was placed into a glass plate and set in a vertical electrophoresis apparatus containing 1× TE solution. 4 µL of PCR-amplified DNA samples were placed into each gel well, and 2 µL of marker was added as a marker in the first and last gel wells. The electrophoresis was carried out at an electric voltage of 100 V for 1 hour, until the first band reached the bottom of the gel. The result of electrophoresis on of polyacrylamide gel is washed with distilled water, then soaked in silver nitrate solution for 5-7 minutes, and rinsed in distilled water for about 2 seconds. It is then soaked in a NaOH solution containing 3,000 µL/l of formaldehyde and gently shaken for 3-5 minutes until DNA bands appear.

2.4. Data Analysis

The *PR-1* gene resistance to downy mildew can be analysed by observing the 345 bp DNA band formed after electrophoresis on a 1% agarose gel. Genetic diversity analysis was performed by observing the DNA bands formed after the electrophoresis process on an 8% polyacrylamide gel. Scoring bands are in the form of binary, where a score of 1 is for visible DNA bands, 0 for invisible bands, and a score of 9 for non-amplified bands or missing data. The Simple Machine Coefficient (SMC) method is used to measure the similarity between two operational taxonomic units (OTUs) based on the binary data obtained (Verma & Aggarwal 2019).

$$SMC = \frac{a + d}{a + b + c + d}$$

Where:
a : score 1.1
b : score 1.0
c : score 0.1
d : score 0.0

The scoring data were then analyzed using the Unweighted Pair Group Method with Arithmetic (UPGMA) and the Sequential Agglomerative Hierarchical and Nested (SAHN) program in NTSYS (Numerical Taxonomy System) version 2.1 (Rohlf 2009). The clustering feature in the NTSYS program was chosen to obtain groupings in the form of a dendrogram. Next, the heterozygosity values of the DNA bands generated by the 15 primers were analyzed using PowerMarker 3.25 (Liu & Muse 2005). The scoring data in the form of PIC (Polymorphism Information Content) generated by the markers used in the study were analyzed using the formula (Smith *et al*. 1997):

$$PIC = 1-\sum_{i}^{n} f_i^2$$

*fi*2 is the frequency of the i-th allele and i = 1, 2, 3,…*n*

3. Results

3.1. Concentration and Purity of RNA and DNA

The results of the RNA quality measurements yielded total RNA purity values for the 30 corn samples, ranging from 1.815 to 1.984, with the lowest value in sample LT3. In contrast, the lowest RNA concentration was in sample CM5, with a value of 356 ng/uL. Sample LB4 has the highest purity and concentration value, with 1.984 and 1218 ng/uL, respectively.

The results of the DNA quality results showed the lowest purity value in sample BK1 (1.675) and the highest in sample LB5 (2.038). Meanwhile, the highest DNA concentration was 4395 ng/uL in sample SR4, and the lowest was 238 ng/uL in sample BK5. The purity and concentration values of total RNA and DNA are fully presented in Table 3.

3.2. Visualization of The *PR-1* Gene

The visualization of the *PR-1* gene was visualized as a cDNA band after electrophoresis. The visualization results in Figure 1 show that all local corn samples from Sinjai and Tana Toraja, as well as national varieties and CIMMYT, exhibit a size of 345 bp cDNA band, consistent with the expected amplicon size from the PR-1 primer pair used.

Table 3. List of purity and concentration values of total RNA and DNA

Sample code	Purity (A_{260}/A_{280})		Concentration (ng/µL)	
	RNA	DNA	RNA	DNA
BK1	1.962	1.675	816	3053
BK2	1.937	1.967	678	3698
BK3	1.942	2.006	400	2418
BK4	1.978	1.996	538	2680
BK5	1.940	1.827	520	238
LT1	1.931	2.011	834	1398
LT2	1.893	2.000	458	815
LT3	1.815	1.958	1082	925
LT4	1.967	1.930	712	690
LT5	1.871	2.029	434	2070
LK1	1.942	1.925	540	2055
LK2	1.956	1.904	884	3780
LK3	1.948	1.819	756	1278
LK4	1.964	2.000	770	3245
LK5	1.938	1.878	438	1578
LB1	1.967	1.970	720	2985
LB2	1.953	1.945	1160	3685
LB3	1.962	2.011	1036	2262
LB4	1.984	1.857	1218	2395
LB5	1.961	2.038	498	1988
SR1	1.958	1.819	744	1333
SR2	1.915	1.813	452	2225
SR3	1.973	1.860	576	2428
SR4	1.906	1.946	446	4395
SR5	1.968	1.961	618	3872
CM1	1.905	1.921	560	1585
CM2	1.937	1.953	554	840
CM3	1.917	1.807	506	918
CM4	1.922	1.764	492	895
CM5	1.914	1.841	356	1498

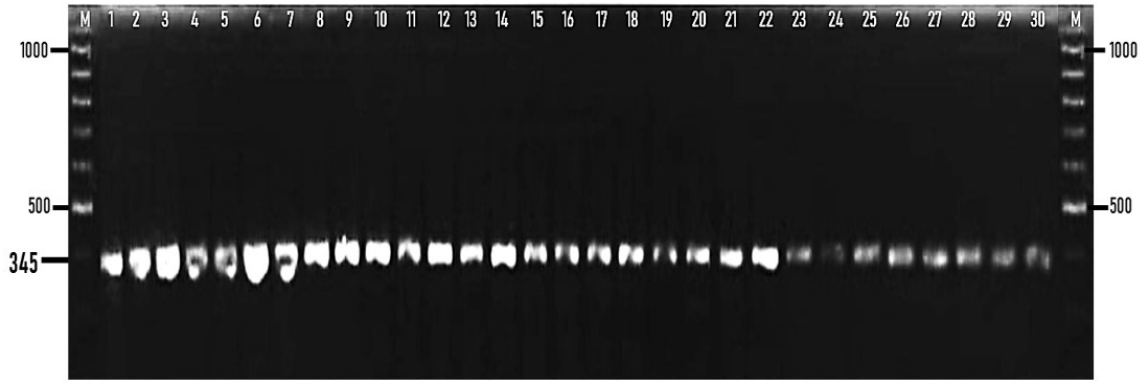

Figure 1. The results of cDNA amplification using the PR-1 primer pair with the forward primer 5'-AGGCTCGCGTGCCTCCTAGCTCT-3' and reverse primer 5'-GGAGTCGCGCCSCACCACCT-3'. Note: M: 100 bp ladder, 1-30: samples code number of BK1-CM5

3.3. DNA Visualization using 15 SSR Primers

Genetic diversity was assessed by observing the appearance of DNA bands following amplification with 15 SSR primers. Visualization of DNA bands on 15 primers (Figure 2) showed that the band positions varied in allele size from 24 bp on primer phi233376 to 540 bp on primer phi96100. In several markers, exhibit amplification failure (missing data), indicated by the absence of DNA bands on the polyacrylamide gel, as shown (Figure 2) with primer phi227562 (number samples 6, 12, 20, 21, and 23), primer phi96100 (number samples 5, 6, 7, and 18), primer phi374118 (number samples 2, 4, 19, and 22), primer phi331888 (number samples 14, 15, 29, and 30), primer phi034 (number sample 1), primer phi080 (number samples 5 and 8), primer phi065 (number sample 1), primer phi042 (number sample 4), and primer umc1061 (number sample 29). The number of bands ranged from 2 bands on primer phi042 to 16 bands on primers phi109188 and phi328175. There were 154 alleles with an average of 10.27 alleles/locus. The number and size of alleles on each primer are presented in Table 4.

3.4. PIC Value and Heterozygosity

The heterozygosity and PIC (Polymorphism Information Content) values obtained in this study were a total heterozygosity of 10.94 (average of 0.73) and a PIC of 11.43 (average of 0.76). The highest heterozygosity, 1.00, is observed with the primers umc1736 and phi092, and the highest PIC value, 0.89, is observed with the primer phi374118. Primer phi042 has the lowest heterozygosity and PIC values, respectively, of 0.03 and 0.10. The complete statistical data profile of the 15 marker primers is listed in Table 4.

3.5. Genetic Diversity

The kinship relationships of 30 S2 generation lines, based on the genetic similarity matrix values in Table 5, range from 0.56 to 0.96. The highest genetic similarity matrix value, which is 0.96, is held by the pair of accessions CM4 and CM5, while the lowest genetic similarity matrix value, which is 0.56, is held by the pair of accessions BK5 and LK1. This finding aligns with the dendrogram construction (Figure 3), which indicates that accessions CM4 and CM5, with a genetic similarity coefficient of 0.96, are grouped in the same sub-cluster, specifically sub-cluster IA, labelled as 'very close.' Meanwhile, the lowest genetic similarity coefficient is observed between the pair of accessions BK5 and LK1, which are in different sub-clusters: BK5 in sub-cluster IA and LK1 in sub-cluster IB.

The results of the cluster analysis of 30 S2 generation accessions using 15 SSR markers (Figure 3) show that the similarity coefficients range from 0.68 to 0.96, corresponding to 68%-96%. The cluster analysis divides the 30 S2 generation accessions into two main clusters, I and II, with a similarity coefficient of 0.68. Cluster I consists of 27 accessions, and Cluster II consists of 3 accessions. At a coefficient of 0.69, cluster I forms two sub-clusters, namely sub-cluster IA and sub-cluster IB. Sub-cluster IA consists of 25 accessions, namely BK1, BK2, BK3, LT1, LT2, LT4, LT5, LT3, LB1, LB2, LB3, LK2, LK4, BK5, BK4, SR1, CM3, SR2, SR5, SR3, SR4, CM1, CM2, CM4, and CM5, while sub-cluster IB consists of 2 accessions, namely LK1 and LK5. Similar to cluster I, cluster II, with a coefficient of 0.73, also forms 2 sub-clusters (sub-clusters IIA and IIB). Sub-cluster IIA consists of 1 accession, namely LK3, while accessions LB4 and LB5 are found in sub-cluster IIB.

4. Discussion

The process of extracting genetic material, both total RNA and DNA, is the initial step taken to detect the *PR-1* gene resistant to downy mildew and to analyze the genetic diversity of the S2 generation of local maize (Sinjai and Tana Toraja), national varieties, and CIMMYT. Corn leaves 14 days after sowing (DAS) were collected for extraction. The use of young leaves aged around 14 days after planting (DAP) for extraction is highly recommended, according to research (Yaikhom *et al*. 2018), because the process is simpler and yields purer DNA. Young leaves are usually easier to extract genetic material from because of their softer texture and lower compound content.

Extraction of DNA for the analysis of genetic diversity with the modified CTAB buffer. The modified CTAB method is often used to optimize DNA extraction, especially in plants that contain high levels of polysaccharides and secondary metabolites, such as polyphenols, found in corn plants. CTAB buffer is a commonly used DNA extraction reagent from various plant sources, as reported in the research (Irfan *et al*. 2013; Khatun *et al*. 2021; Jun *et al*. 2023; Kumari & Vinay 2025). Cetyltrimethylammonium bromide (CTAB) is a detergent agent that can degrade cell walls, damage cell membranes, separate carbohydrates, denature proteins, and dissolve DNA into solution.

The process of measuring purity and concentration of extracted RNA and DNA can be determined using a spectrophotometer at the absorbance ratio of 260 nm

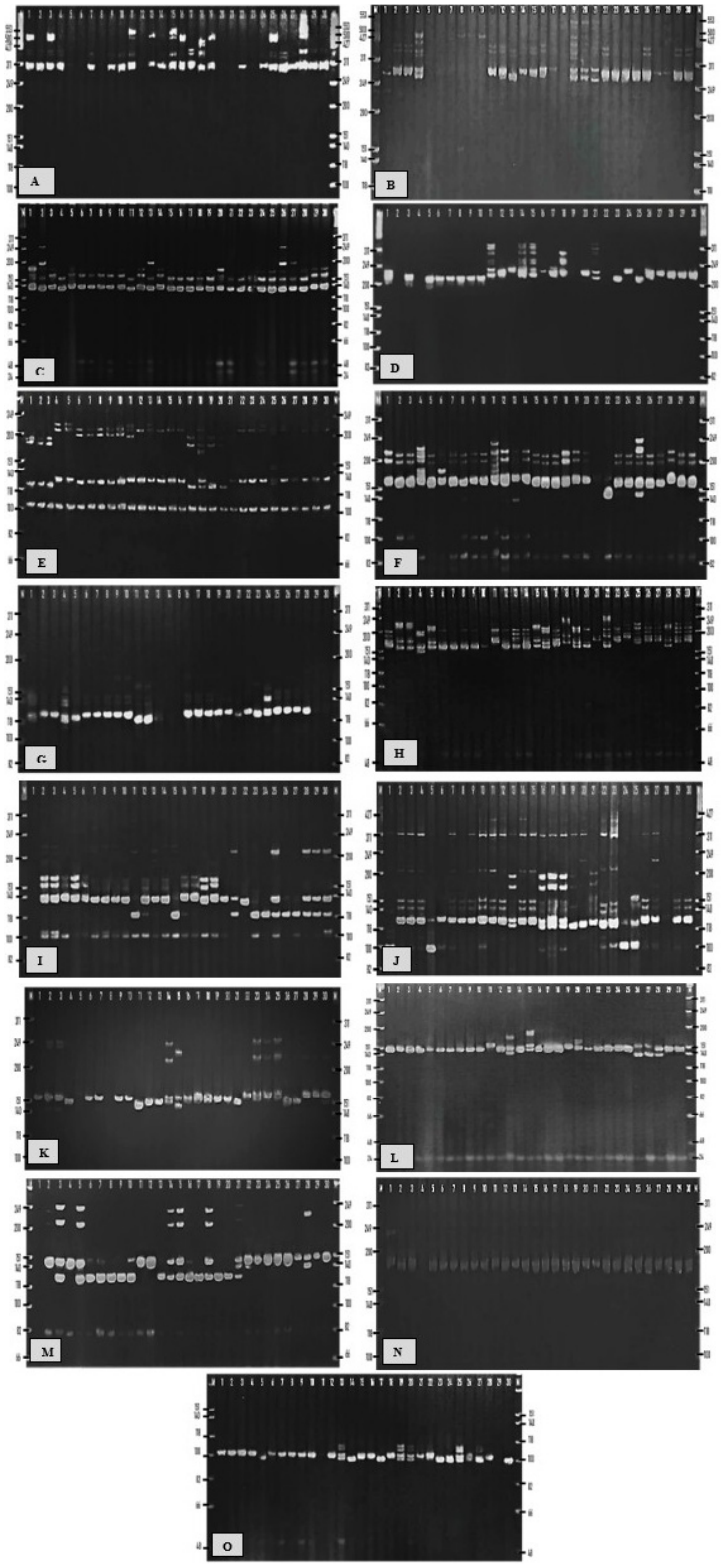

Figure 2. Visualization of DNA amplification results using 15 SSR primers; A.phi227562, B.phi96100, C.umc1736, D.phi374118, E.phi92, F.phi109188, G.phi331888, H.bnlg1154, I.phi034, J.phi328175, K.phi080, L.phi233376, M.phi065, N.phi042, O.umc1061 Note: M: Marker; 1-30: samples code number of BK1-CM5

Table 4. Data profile of 15 SSR markers for genetic diversity

Marka	Chromosome location	Number of bands	PIC	Heterozygosity	Alel size
phi227562	1.11	7	0.77	0.76	300-535
phi96100	2.01	12	0.87	0.65	151-540
umc1736	2.09	10	0.86	1.00	35-261
phi374118	3.02	11	0.89	0.54	195-355
phi092	4.08	12	0.83	1.00	103-227
phi109188	5.03	16	0.86	0.97	79-249
phi331888	5.04	8	0.82	0.69	118-173
bnlg1154	6.05	10	0.85	0.97	52-249
phi034	7.02	15	0.87	0.93	100-302
phi328175	7.04	16	0.88	0.97	91-410
phi080	8.08	11	0.78	0.36	140-257
phi233376	8.09	9	0.63	0.97	24-192
phi065	9.03	10	0.82	0.83	81-241
phi042	9.04	2	0.10	0.03	187-234
umc1061	10.06	5	0.60	0.28	52-114
Total		154	11.43	10.94	24-540
Average		10.27	0.76	0.73	

and 280 nm (A_{260}/A_{280}). The wavelength of 260 nm is the maximum absorption for nucleic acids to absorb light. In comparison, the wavelength of 280 nm is the maximum absorption for proteins or the maximum value of protein residues that can absorb light. In general, the minimum concentrations of RNA or DNA considered adequate for various research applications range from 100 ng to 1 µg, with acceptable purity values between 1.8 and 2.0 (Sambrook & Russell 2001). The concentration results for 30 local S2 generation corn strains show that both RNA and DNA are above 100 ng/µL, with total RNA concentrations ranging from 356-1218 ng/µL and DNA concentrations ranging from 238-4395 ng/µL. This indicates that the DNA and RNA samples are suitable for subsequent analysis. The RNA sample purity values ranged from 1.815 to 1.984, indicating they are free of protein contamination and are classified as pure according to the criteria established by Sambrook & Russell (2001). Meanwhile, the DNA purity values range from 1.675 to 2.038. In this study, samples BK1 and CM4 have purity values below 1.8, with 1.675 and 1.764, respectively. The results of this extraction indicate contamination by proteins, polysaccharides, or other contaminants that strongly absorb at or near 280 nm. The purity values below 1.8 in both samples, as reported by Ramlah *et al*. (2020), may be due to residual ethanol from incomplete drying and the presence of secondary metabolites in the extracted plant organs. The presence of these contaminants affects absorption at a wavelength of 280 nm, decreasing the absorbance ratio and resulting in the purity value falling below the established figure.

To analyze gene expression, including the *PR-1* gene, several steps are required: total RNA extraction, RNA quality and quantity testing, complementary DNA (cDNA) synthesis, and cDNA visualization by electrophoresis. After the extraction process and obtaining total RNA with a purity between 1.8 and 2.0, the RNA extract can be used as a template to form complementary DNA strands with the help of the enzyme reverse transcriptase and oligo dT primers. The cDNA formed is more stable than RNA, allowing it to be used in subsequent analyses, such as PCR (Polymerase Chain Reaction), to observe the expression of specific genes.

The visualization of cDNA on a 1% agarose gel by electrophoresis shows that 30 corn samples have bands visible at approximately 345 bp. This base-pair size is the size of the forward and reverse primer pairs for the PR-1 primer. Amplification of the *PR-1* gene, conferring resistance to downy mildew with these primer pairs, yielded the expected amplicon size, consistent with Morris *et al*. (1998) on induced resistance responses in maize using PR-1 and PR-5. The study (Hoerussalam *et al*. 2013) employed the PR-1 primer pair, which consists of the same forward and reverse primers across the entire maize genome, and obtained approximately 345 bp of *PR-1* gene amplification.

The frequent use of forward and reverse primer pairs targeting the *PR-1* gene, which confer resistance to downy mildew in various studies, yields amplicons of different sizes. As in the study by Saleem *et al*. (2024) on maize plants, which produced a PR-1 amplicon of approximately 440 bp, (Setiowati *et al*. 2025) identified

Table 5. Genetic similarity matrix from S2 generation of local South Sulawesi and introduced maize

	BK1	BK2	BK3	BK4	BK5	LT1	LT2	LT3	LT4	LT5	LK1	LK2	LK3	LK4	LK5	LB1	LB2	LB3	LB4	LB5	SR1	SR2	SR3	SR4	SR5	CM1	CM2	CM3	CM4	CM5
BK1	1.00																													
BK2	0.72	1.00																												
BK3	0.73	0.92	1.00																											
BK4	0.67	0.70	0.67	1.00																										
BK5	0.71	0.73	0.74	0.72	1.00																									
LT1	0.75	0.70	0.77	0.72	0.78	1.00																								
LT2	0.74	0.75	0.80	0.74	0.73	0.92	1.00																							
LT3	0.69	0.73	0.78	0.68	0.75	0.90	0.89	1.00																						
LT4	0.72	0.74	0.79	0.71	0.73	0.90	0.94	0.94	1.00																					
LT5	0.75	0.78	0.80	0.76	0.79	0.88	0.89	0.90	0.94	1.00																				
LK1	0.57	0.63	0.60	0.71	0.56	0.63	0.65	0.61	0.66	0.64	1.00																			
LK2	0.70	0.72	0.73	078	0.72	0.76	0.79	0.79	0.79	0.80	0.76	1.00																		
LK3	0.60	0.61	0.62	0.65	0.64	0.67	0.65	0.66	0.68	0.63	0.66	0.73	1.00																	
LK4	0.64	0.75	0.71	0.71	0.75	0.71	0.74	0.77	0.78	0.76	0.71	0.83	0.68	1.00																
LK5	0.64	0.77	0.69	0.74	0.70	0.68	0.72	0.69	0.73	0.73	0.80	0.72	0.62	0.75	1.00															
LB1	0.72	0.78	0.77	0.77	0.75	0.79	0.79	0.77	0.81	0.84	0.72	0.82	0.73	0.74	0.81	1.00														
LB2	0.77	0.75	0.72	0.72	0.71	0.76	0.75	0.75	0.77	0.81	0.62	0.71	0.67	0.71	0.70	0.85	1.00													
LB3	0.74	0.75	0.68	0.75	0.68	0.73	0.73	0.72	0.74	0.79	0.65	0.74	0.64	0.75	0.72	0.84	0.86	1.00												
LB4	0.69	0.62	0.62	0.65	0.63	0.74	0.72	0.70	0.73	0.73	0.65	0.68	0.73	0.69	0.65	0.76	0.78	0.78	1.00											
LB5	0.75	0.63	0.66	0.60	0.65	0.78	0.76	0.74	0.78	0.73	0.59	0.71	0.73	0.69	0.60	0.68	0.77	0.73	0.83	1.00										
SR1	0.64	0.60	0.63	0.59	0.66	0.71	0.64	0.65	0.66	0.65	0.61	0.65	0.73	0.65	0.65	0.67	0.69	0.66	0.70	0.74	1.00									
SR2	0.64	0.66	0.62	0.70	0.68	0.68	0.66	0.66	0.68	0.70	0.68	0.71	0.64	0.70	0.69	0.74	0.67	0.68	0.64	0.65	0.68	1.00								
SR3	0.70	0.69	0.68	0.69	0.74	0.73	0.73	0.74	0.75	0.77	0.65	0.71	0.67	0.73	0.72	0.77	0.76	0.68	0.60	0.67	0.68	0.80	1.00							
SR4	0.68	0.66	0.65	0.70	0.76	0.75	0.70	0.76	0.73	0.75	0.66	0.71	0.68	0.71	0.71	0.75	0.75	0.70	0.64	0.67	0.71	0.80	0.88	1.00						
SR5	0.67	0.67	0.67	0.65	0.67	0.72	0.70	0.71	0.71	0.74	0.62	0.66	0.63	0.62	0.68	0.75	0.74	0.66	0.64	0.65	0.68	0.68	0.80	0.79	1.00					
CM1	0.67	0.76	0.71	0.74	0.71	0.73	0.73	0.72	0.73	0.75	0.70	0.76	0.68	0.68	0.75	0.81	0.77	0.72	0.65	0.65	0.70	0.78	0.79	0.81	0.78	1.00				
CM2	0.65	0.69	0.68	0.65	0.71	0.71	0.71	0.72	0.73	0.73	0.65	0.71	0.72	0.70	0.68	0.73	0.78	0.68	0.69	0.76	0.77	0.71	0.79	0.80	0.75	0.87	1.00			
CM3	0.70	0.65	0.66	0.65	0.69	0.73	0.68	0.71	0.70	0.69	0.68	0.72	0.73	0.66	0.71	0.71	0.69	0.65	0.67	0.73	0.80	0.76	0.75	0.81	0.76	0.77	0.79	1.00		
CM4	0.69	0.72	0.74	0.70	0.77	0.79	0.80	0.78	0.79	0.78	0.72	0.78	0.70	0.70	0.73	0.79	0.73	0.67	0.62	0.70	0.71	0.75	0.81	0.85	0.79	0.84	0.84	0.81	1.00	
CM5	0.69	0.73	0.73	0.69	0.77	0.76	0.77	0.81	0.78	0.77	0.69	0.81	0.68	0.74	0.73	0.78	0.74	0.67	0.62	0.67	0.69	0.72	0.81	0.83	0.78	0.84	0.82	0.80	0.96	1.00

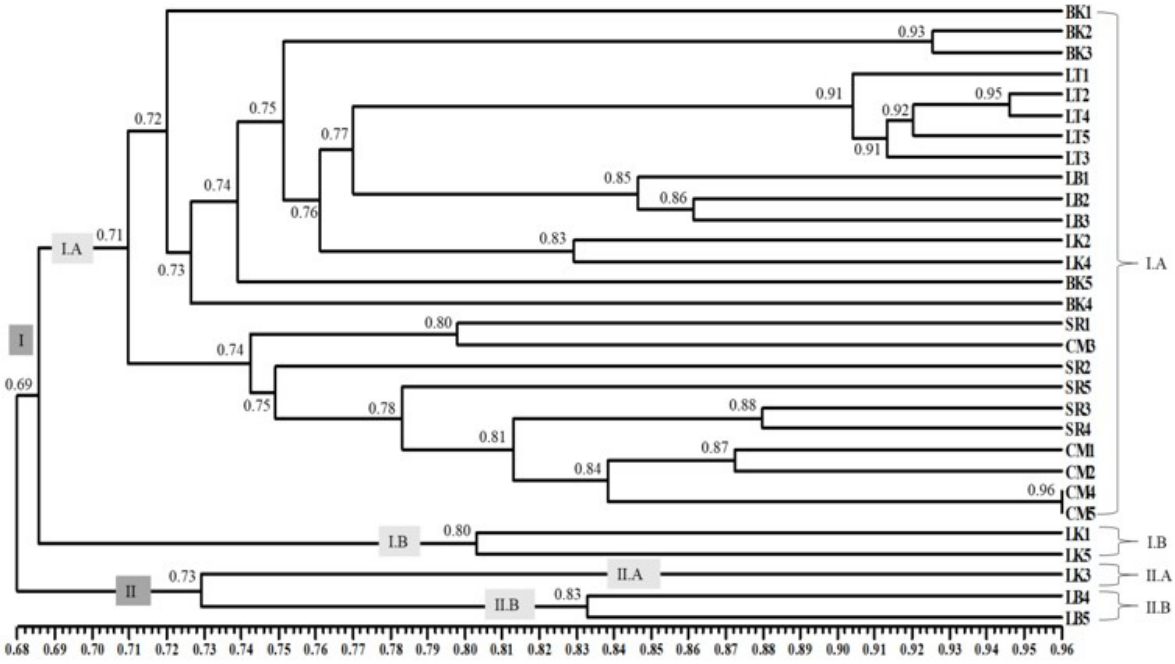

Figure 3. Dendrogram of kinship relationships based on similarity genetics from the S2 generation of 30 lines corn on 15 SSR markers. BK1-BK5: Bata Pulut Kuning, East Sinjai, South Sulawesi, LT1-LT5: Lokal Toraja, Tator, South Sulawesi, LK1-LK5: Lokal Kandora, Tator, South Sulawesi, LB1-LB5: Lokal Bebo, Tator, South Sulawesi, SR1-SR5: Srikandi Kuning 1, National Variety, and CM1-CM5: Carotenoid Syn 3, CIMMYT

the *PR-1* gene in *Theobroma cacao* plants with an expected amplicon size of around 400-500 bp, and in the study on *PR-1* gene expression in pumpkin plants conducted by (Guo *et al*. 2022) with an amplicon size was approximately 658 bp. Differences in primer pairs on a gene will affect the size of the amplicon (the amplified DNA fragment) because the distance between the 5' end of the forward primer and the reverse primer determines the length of the amplicon. In other words, if the primer pairs are spaced further apart, the resulting amplicon will be longer, and vice versa. The differences in the PR-1 primer pairs used align with the identification of 17 genomic families of the *PR-1* gene in maize plants in the study (Ma *et al*. 2022). Hence, the differences in the primer pairs result in distinct gene expression profiles across different amplicon sizes.

Plant pathogenesis-related proteins (PR) are usually induced by various pathogens, including viruses, bacteria, and fungi. These proteins play a role in the hypersensitive reaction, which accelerates the process of cell death to limit the spread of phytopathogens. Among these various proteins, there are pathogenesis-related proteins that can induce the innate immune system in plants (Dos Santos & Franco 2023). The *PR-1* gene in corn plays an important role in the plant's response against pathogen attacks, such as fungi. Research (Hoerussalam *et al*. 2013) shows that the *PR-1* gene remains in the progeny of self-pollinated resistant corn plants and can be induced by elicitors Bio1, Bio2, Abio1, and Abio2, which have the potential to enhance the plant's resistance to downy mildew.

The analysis of genetic diversity in this study used 15 SSR primers. A set of 15 SSR primers was employed to ensure adequate genome coverage and to achieve a high level of resolution in discriminating the genetic differences between local and introduced genotypes. This number of markers provides sufficient polymorphic information to construct a robust phylogenetic relationship and to assess the heterozygosity levels within the S2 population.

The PCR amplification results of each primer showed varying sizes of DNA bands and resulted in the presence of polymorphic bands. Polymorphic DNA bands provide information about genetic diversity among maize genotypes. This variability is due to differences in the DNA template binding sites recognized by the primers. Differences in the number and size of DNA bands affect the level of genetic diversity (Amzeri *et al*. 2022).

Some markers in this study exhibit amplification failure (missing bands). The missing bands indicate that the DNA template did not discover the corresponding base pairs on the used primers or that information about genetic variation at that location is not available. This is suspected to be due to the absence of the DNA template

binding site on the primer used. Some markers also show multiple bands in some samples, which is caused by the template DNA finding matching base pairs on the primers and detecting the number of repeats at different sizes from a single individual. Conditions like this are very likely due to individual variations within a species. Several important factors include the concentration and purity of the DNA template, PCR buffer conditions, the quality of primers and polymerase enzymes used, reaction temperature and time, and the condition of the PCR machine that influence the success of DNA amplification.

Based on the data profile from the analysis of 15 SSR marker loci (Table 4), a total of 154 alleles were detected, ranging from 2 alleles per locus to 16 alleles per locus, with an average of 10.27 alleles per locus. The average number of alleles in this study approaches the average number of alleles in previous research on genetic diversity conducted by (Vathana *et al*. 2019), who reported an average of 10.12 alleles per locus using 68 maize cultivars on 50 markers, and by (Islam *et al*. 2023), who identified 10.4 alleles per locus using 18 SSR markers on 22 maize inbred lines. Diversity studies often show a discrepancy between the number of detected alleles and the sample size, and different sampling techniques may influence this variation. However, the high allele frequency can also be caused by the application of dinucleotide SSRs, which is another factor that can influence allele frequency (Islam *et al*. 2023).

In genetic diversity, the PIC (Polymorphism Information Content) value is a measure of the extent of polymorphism at a genetic marker. The PIC value is interpreted into three groups: PIC≤0.25 (marker considered less informative), 0.25 < PIC≤0.5 (marker considered moderately informative), and PIC>0.5 (marker considered highly informative and can be used for genetic diversity studies). This value is explained as follows: low (0 to 0.10), moderate (0.10 to 0.25), high (0.30 to 0.40), and very high (0.40 to 0.50) (Serrote *et al*. 2020; Fernandez *et al*. 2023). The average PIC level in this study is 0.76, categorized as "very informative," with PIC values ranging from 0.10 for primer phi042 to 0.89 for primer phi374118. This indicates that the SSR markers are highly informative and have superior discriminatory power for identifying S2 maize populations. This high degree of polymorphism indicates a wide genetic basis from a breeding standpoint, offering a strong opportunity of select divergent parental lines. These results are essential for creating novel hybrid varieties that combine the high-yield potential and PR-1-mediated disease resistance of introduced genotypes with the local adaptability of South Sulawesi maize. This PIC range is close to the value ranges reported in the studies by Pandit *et al*. (2016) of 0-0.82) and Adu *et al*. (2019) of 0.32-0.85). Mukhlif *et al*. (2020) reported a PIC value range of 0.29-0.37, which is lower than the range reported in this study. Fourteen loci showed PIC values greater than 0.5, indicating that the SSR markers used in this study are highly informative. The PIC value increases with the number of detected alleles.

The heterozygosity values among the 15 markers used in this study averaged 0.73, with a range of 0.03 to 1.00. Additionally, 60% of the total markers used showed heterozygosity values (0.76-1.00) above 0.73, the average heterozygosity value. The average heterozygosity value obtained in this study is higher than that reported by Adu *et al*. (2019 of 0.54) and lower than that reported by Islam *et al*. (2023) with a value of 0.86). Heterozygosity measures the extent of genetic variation within a population. The high level of heterozygosity observed in several SSR markers indicates the need for further crossing to increase homozygosity while maintaining the desired genetic and phenotypic traits (Adu *et al*. 2019). The higher the heterozygosity value obtained, the more individuals have diverse genotypes (heterozygous), indicating greater genetic variability.

The kinship relationships of 30 S2-generation corn lines based on the genetic similarity matrix range from 0.56 to 0.96. The value of 0.56 appears in BK5 and LK1, while the similarity matrix value of 0.96 is found in CM4 and CM5. The range of similarity matrix values approaches the similarity matrix range in several studies, such as (Sutoro *et al*. 2017) which evaluated the genetic diversity of inbred corn based on ten simple sequence repeat markers with a similarity index of 0.62-0.93; (Shete *et al*. 2023) with a similarity range of 0.5-0.81 in the genetic diversity analysis of corn varieties (*Zea mays* L.) using five SSR markers; and the study on the assessment of genetic diversity based on microsatellite markers among native and exotic corn strains in Bangladesh (Islam *et al*. 2023) with a similarity range of 0.55-1.00.

The dendrogram of kinship relationships shows high genetic diversity among the 30 strains, with similarity coefficients ranging from 0.68 to 0.96. (Sutoro *et al*. 2017) reported a similar coefficient range of 0.68-0.93 in inbred corn. The similarity coefficients ranged from 0.86 to 0.96, as reported by Al-Hazemawi *et al*. (2024) on inbred corn in Iraq. The higher the similarity coefficient

value, the greater the similarity, and vice versa (Yani *et al*. 2022).

In conclusion, in plant defence systems, the presence of the *PR-1* gene is a determinant of a plant's potential to respond to pathogen attacks, allowing the selection of plants with greater resistance potential even before they are exposed to pathogens. The *PR-1* gene, which is one of the resistance genes against downy mildew, was found in 30 S2 lines of local maize (from Sinjai and Tana Toraja), as well as in national varieties and CIMMYT. The amplification results using the PR-1 marker pair showed the formation of DNA bands at the expected amplicon size. In addition to *PR-1* gene detection, a genetic diversity analysis was also conducted on the thirty corn samples, which showed high heterozygosity values. The emergence of diverse traits due to low levels of similarity can serve as a reference point for the development of superior plant varieties.

The detection of the *PR-1* gene conferring resistance to downy mildew and the high genetic diversity in local maize strains can serve as a reference for plant breeding programs to produce plants that are not only superior in phenotype but also resistant to pests and diseases.

Acknowledgements

The authors express their gratitude to the Department of Biology and the Head of the Biology Postgraduate Program at Hasanuddin University for their support in funding the research. Appreciation is also extended to the Head and analysts of the Molecular Biology Laboratory at the Center for Standardization of Cereal Crops Instrument, Maros, South Sulawesi, for the permission, facilities, and support provided during this research.

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