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Detection of the PR5 Gene Associated with Downy Mildew Resistance and Genetic Diversity Analysis of S2 Lines of Local Maize (*Zea mays* L.) From South Sulawesi Using SSR Markers

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ABSTRACT

A significant decline in corn production is often associated with downy mildew (*Peronosclerospora maydis*), while maize varieties with genetic resistance to this disease are still relatively limited. A molecular-based approach is needed, such as Phatogenesis Related (PR5) gene detection, to identify maize genotypes that are potentially resistant to downy mildew. This study aims to identify the presence of the PR5 gene and analyze genetic diversity in local maize from South Sulawesi, Srikandi kuning (national variety), and Carotenoid sync 3 from International Maize and Wheat Improvement Center (CIMMYT) to support the acceleration of downy mildew-resistant plant breeding programs. PR5 gene detection was carried out by extracting RNA according to the Total RNA Mini Kit Plant (Geneaid) procedure followed by PCR techniques with specific primers. Genetic diversity analysis was carried out using 15 polymorphic SSR primers. DNA amplification showed that 23 individuals were detected as containing the PR-5 gene from 30 samples tested based on the results of agarose gel electrophoresis. The PIC value obtained from the Simple Sequence Repeats (SSR) primers showed a high level of genetic diversity ranging from 0.64 to 0.93 with an average of 0.85. The genetic similarity matrix was calculated and analyzed using the UPGMA method using NTSYS version 2.2, producing a dendrogram with two main clusters. Cluster I has only one individual with a large genetic distance, while Cluster II is divided into two subclusters, IIA and IIB, reflecting the genetic closeness of most individuals. Individuals with PR5 genes and high genetic diversity were identified as potential candidates for use in a superior maize breeding program resistant to downy mildew disease.

1. Introduction

Maize (*Zea mays* L.) is one of the most important food commodities in Indonesia after rice, playing a strategic role in supporting national food security. In several regions such as Nusa Tenggara, Gorontalo, Madura, and parts of Sulawesi, maize serves as a staple food source. Nationally, it plays a dual role as both human food and a primary raw material for animal feed and bioenergy production (FAO 2021; Edhy *et al.* 2022). Approximately 75% of maize production is utilized as feed raw material, around 23% is used in various non-feed and food industries, and the remainder is allocated for direct household consumption and seed purposes (Prasetyo *et al.* 2024).

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The increasing population and the expansion of the food and feed industries have driven a growing demand for local maize, making it a strategic commodity in supporting food security. However, local maize production remains relatively low, being sufficient only to meet household needs. Local maize offers diverse benefits, ranging from serving as an alternative food source and livestock feed to being utilized in bioethanol production, which has recently become a global issue and a topic of scientific interest (Genesiska *et al.* 2020; Susanti & Supriyatna 2020). Its high carbohydrate content also makes it a promising raw material for bioethanol production (Bantacut *et al.* 2015).

Although maize plays a strategic role in maintaining national food security, the stability of its production continues to face serious threats, particularly from downy mildew disease. Maize productivity in various regions has significantly declined due to infections caused by *Peronosclerospora* species, such as *P. maydis*, *P. philippinensis*, and *P. sorghi* (Widiantini *et al.* 2015). Downy mildew disease in maize caused by *Peronosclerospora maydis* is one of the major factors contributing to yield losses. This disease can drastically reduce maize productivity, with losses reaching up to 90-100% (Widiantini *et al.* 2015; Setiadi Daryono *et al.* 2018; Kalqutny *et al.* 2020; Kasiamdari & Putri 2023). The pathogen is capable of infecting maize plants throughout all growth stages, thereby exerting a severe impact on overall productivity (Adhi *et al.* 2022).

High levels of downy mildew infestation in maize plants can be influenced by various factors, one of which is the lack of an effective resistance system in mayze varieties, making them susceptible to downy mildew (Wulandari *et al.* 2022). Therefore, the plant's ability to resist downy mildew is a crucial aspect in designing disease control strategies.

One of the most effective approaches to controlling downy mildew infection in maize is through the development of disease-resistant varieties using a breeding strategy based on resistance gene detection. Disease resistance improvement could be explored at the molecular level, ranging from marker-assisted selection to genomic selection (Miedaner *et al.* 2020; Zhu *et al*. 2021). This effort utilizes genetic resources that possess natural resistance, which are both adaptive to local environments and highly tolerant to downy mildew infection. Therefore, breeding disease-resistant varieties not only reduces dependence on pesticide use, but also supports sustainable increases in corn productivity (Limenie & Alehegn 2025). To assess variations in the ability of the resistance system, molecular markers of disease are often used (Bani *et al.* 2017; Ashan *et al.* 2020).

Disease resistance is a crucial trait in plant breeding because it influences crop quality and production (Herlinda *et al.* 2018). Genetic diversity plays a crucial role in maintaining the health of plant populations because it allows for the presence of different genes that can confer resistance to pests, diseases, or stressful environmental conditions (Salgotra & Chauhan 2023). One of the most frequently used molecular markers to estimate genetic relationships and diversity levels in maize is Simple Sequence Repeats (SSR) (Ramlah *et al.* 2018; Mukhlif *et al.* 2020). This opinion is supported by (Raghunath 2022; An *et al.* 2023), who reported that SSR-based markers are very suitable for gene mapping, genetic diversity studies, and specific gene identification due to their polymorphic characteristics and even distribution throughout the genome. The advantage of SSR also lies in its codominant nature, which is able to distinguish between heterozygous and homozygous individuals (Govindaraj *et al.* 2015).

Genetic diversity analysis is essential in detecting downy mildew resistance genes to identify maize genotypes carrying resistant alleles, thereby facilitating selection and breeding processes aimed at developing superior, adaptive, and sustainable varieties with resistance to pathogen infection.

Plant genetic diversity can be analyzed using molecular markers such as Simple Sequence Repeats (SSR), which enable the identification of genetic variations among accessions and are highly valuable in breeding programs for selecting superior varieties (Govindaraj *et al.* 2015). The development of resistant varieties requires a comprehensive understanding of the genetic diversity within maize germplasm, as well as the identification of genes that contribute to disease resistance.

With the advancement of biotechnology, the use of molecular markers has become an important approach for detecting downy mildew resistance genes. The PR5 (Pathogenesis-Related protein 5) gene is one of the genes activated in the plant defense response against pathogen infection. PR-5 is a that gene encodes a protein known as a thaumatin-like protein, due to its high sequence similarity to the thaumatin protein (Kitajima 1999). PR-5 has been reported to exhibit antifungal activity against fungi such as *Candida*

albicans, *Neurospora crassa*, *Trichoderma reesei*, *Fusarium oxysporum*, *Phytophthora infestans*, and *Alternaria solani* (Kitajima 1999). The expression of PR-5 in *Arabidopsis thaliana* is induced in response to salicylic acid (SA) and naphthalene acetic acid (NAA) signaling (Zhang *et al.* 2021).

Previous studies have demonstrated that PR-5 is involved in the plant defense system that acts against pathogen infections, for example, by inhibiting fungal spore germination or mycelial growth (Tachi *et al.* 2009; Hakim *et al.* 2018). The presence and expression of the PR5 gene have been shown to correlate with plant disease resistance mechanisms and exhibit antifungal activity, including against downy mildew (El-kereamy *et al.* 2011; Zaynab 2025). Therefore, the detection of the PR5 gene in maize can facilitate the rapid identification of downy mildew–resistant varieties.

Local maize varieties hold strategic value as germplasm resources rich in adaptive genes to local environmental conditions, making their conservation essential as a source of genetic diversity. In addition, the detection of genes conferring resistance to downy mildew can be used to confirm the presence of resistance traits in local maize lines. The combination of information derived from SSR markers and resistance gene detection enables breeders to develop superior local maize varieties that are more adaptive to specific environments and resistant to major diseases, particularly downy mildew.

The study on the presence of downy mildew resistance genes using PR-5 primers, along with genetic diversity analysis based on SSR markers, was conducted on local maize from South Sulawesi, the national variety Srikandi Kuning, and maize introduced from CIMMYT (Mexico), to support breeding programs aimed at developing superior disease-resistant varieties.

2. Materials and Methods

2.1. Plant Material and Growth Condition

Plant materials used in the research This S2 generation of maize local originating from South Sulawesi, National Varieties and CIMMYT-Mexico which were planted using planting media from mixture soil, husk and fertilizer pen with 1:1:1 ratio. After plant 14 days old, then 5 samples leaf maize from each source taken For DNA and RNA extraction. There are 30 samples material plants used presented in Table 1.

2.2. Detection of PR-5 Gene

2.2.1. RNA Extraction and cDNA Synthesis

RNA extraction was carried out following the standard protocol of the Total RNA Mini Kit Plant (Geneaid 2017). The primer pair used to detect the downy mildew resistance gene PR-5 consisted of the forward sequence TCTCTCTCATCAGTCCTTTT and the reverse sequence GCTCATTCTTCATCTTCCTC. The procedure began with grinding 0.1 g of young maize leaves in liquid nitrogen using a mortar and pestle until fine powder was obtained. The sample was then transferred into a 1.5 mL microcentrifuge tube. Cell lysis was performed by adding 500 µL of RB/PRB buffer and 5 µL of β-mercaptoethanol, followed by vortexing and incubation at 60°C for 5 minutes. The mixture was transferred to a filter column, centrifuged, and the resulting filtrate was collected for the next step. For RNA binding, the filtrate was mixed with absolute ethanol and transferred into an RB column, then centrifuged to

Table 1. Plant materials maize from South Sulawesi, National Variety and CIMMYT

Sample code	Corn name	Prigin
PK1	Pulut kuning	Bone, South Sulawesi
PK2		
PK3		
PK4		
PK5		
SL1	Soppeng lokal	Soppeng, South Sulawesi
SL2		
SL3		
SL4		
SL5		
BD1	Batara didi	Selayar, South Sulawesi
BD2		
BD3		
BD4		
BD5		
BP1	Batara didi Pamatata	Pamatata, South Sulawesi
BP2		
BP3		
BP4		
BP5		
SK1	National Variety	Indonesia
SK2		
SK3		
SK4		
SK5		
KS1	Carotenoid syn 3	Mexico
KS2		
KS3		
KS4		
KS5		

allow RNA binding to the membrane. The washing step was performed three times sequentially using W1 buffer and Wash buffer (with ethanol) to remove contaminants, each followed by centrifugation. The column matrix was then dried by centrifugation to eliminate residual buffer. In the final step, RNA was eluted by adding 50 µL of RNase-free water to the center of the membrane, incubated for 2 minutes, and centrifuged to obtain purified total RNA. The purified RNA was subsequently used as a template for cDNA synthesis using the RT-PCR reagents according to the standard protocol of the cDNA Synthesis Kit (Thermo Fisher 2013). The reverse transcription (RT) reaction was carried out at 42°C for 60 minutes, followed by enzyme inactivation at 95°C for 5 minutes. The resulting cDNA was then used as the template for PCR amplification.

2.2.2. PCR Amplification of PR-5 Gene

The cDNA amplification was carried out according to the standard protocol of KAPA Biosystems PCR Reagents, with a total reaction volume of 25 µL consisting of 3 µL of cDNA, 12.5 µL of KAPA2G Fast HotStart ReadyMix (2×), 7.5 µL of nuclease-free water, and 1 µL each of the PR-5 specific forward and reverse primers. PCR amplification was performed using an RT-PCR thermal cycling program comprising an initial denaturation at 94°C for 5 minutes, followed by 39 cycles of denaturation at 94°C for 30 seconds, primer annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute. A final extension was conducted at 72°C for 7 minutes, followed by cooling at 25°C for 4 minutes (KAPA Biosystem 2025). The PCR products were visualized by electrophoresis on a 1% agarose gel in 0.5× Tris–borate EDTA (TBE) buffer at 80 V for 1.5 hours. After electrophoresis, the gel was stained with ethidium bromide at a concentration of 0.5 µg/mL for 15 minutes and visualized under a UV transilluminator (Lee *et al.* 2012). The presence of visible DNA bands indicated successful amplification of the PR-5 gene, while the absence of bands indicated that the gene was not detected.

2.3. Genetic Diversity Analysis

2.3.1. DNA Extraction

DNA extraction was performed using a modified CTAB buffer method (Khan *et al.* 2004). Young maize leaves (0.4 g) were finely ground in CTAB buffer and supplemented with 10 µL of β-mercaptoethanol. The mixture was incubated in a water bath at 60°C for 60 minutes, with gentle inversion every 15 minutes. After incubation, the samples were cooled to room temperature, followed by the addition of chloroform:isoamyl alcohol (24:1). The mixture was homogenized using a vortex mixer for 10 minutes and centrifuged at 11,600 rpm for 10 minutes. The supernatant was precipitated with cold isopropanol, washed twice with 70% ethanol, and air-dried. The DNA pellet was dissolved in Tris-EDTA buffer and incubated at 60°C for 60 minutes to obtain purified DNA for further analysis. The SSR primers used for the genetic diversity analysis are listed in Table 2.

2.3.2. SSR Marker Amplification

DNA solutions measured by spectrophotometer were diluted to a concentration of 10 ng/µL, and 1 µL of the diluted DNA was added to a microplate. The PCR reaction mixture (9 µL) consisted of 2.25 µL nuclease-free water, 0.5 µL primer mix (forward and reverse, 5 µM), and 6.25 µL KAPA2G Fast HotStart ReadyMix 2× enzyme. One drop of mineral oil was added before sealing the microplate. The amplification process was carried out using 15 SSR primers. The PCR program consisted of pre-denaturation at 95°C for 5 minutes, denaturation at 95°C for 30 seconds, primer annealing at primer-specific temperatures (Table 2) for 30 seconds, and extension at 72°C for 30 seconds. This cycle was repeated 35 times, followed by a final extension at 72°C for 7 minutes and cooling at 25°C for 4 minutes (Kapa Biosystem 2025).

2.3.3. Fragment Analysis

The PCR products were visualized through electrophoresis on 8% polyacrylamide gel (PAGE) at 100 V for approximately 1 hour. The gel was then stained with silver nitrate solution, rinsed, and developed in NaOH solution containing formaldehyde until DNA bands became visible. Visualization was performed using a camera placed over a white light transilluminator.

Fragment analysis was performed by scoring the DNA bands on the gel. The analysis was conducted to identify the size and banding pattern of amplified DNA fragments using Simple Sequence Repeat (SSR) markers. The resulting DNA fragments reflected allele length variations at specific loci among the tested genotypes. Differences in fragment sizes indcated polymorphisms, which served as the basis for determining the level of genetic diversity among individuals. The fragment sizes were determined by comparing the position of the sample DNA bands to a 100 bp DNA ladder used as a molecular size reference. Each band observed at a locus was considered as one allele, with a score of

Table 2. SSR primers used for diversity genetic South Sulawesi corn,national varieties, and CIMMYT

Primary	Location chromosome	Sequence		Temperature annealing
		Forward	Reverse	
Phi227562	1.11	TGATAAAGCTCAGCCACAAGG	ATCTCGGCTACGGCCAGA	54
Phi96100	2.01	AGGAGGACCCCAACTCCTG	TTGCACGAGCCATCGTAT	54
Umc1736	2.09	CCATCCACCACTAGAAAGAGAGGA	TTAATCGATCGAGAGGTGCTTTTC	56
Phi374118	3.02	TACCCGGACATGGTTGAGC	TGAAGGGTGTCCTTCCGAT	56
Phi 092	4.08	GTGGGGGAGCCTACTACAGG	GACGAGGCCATCATCACGGT	59
Phi 109188	5.03	AAGCTCAGAAGCCGGAGC	GGTCATCAAGCTCTCTGATCG	56
Phi331888	5.04	TTGCGCAAGTTTGTAGCTG	ACTGAACCGCATGCCAAC	54
Bnlg1867	6.01	CCACCACCATCGTAGGAGTT	CAGTACACAGCAGGCAGCTC	56
Bnlg 1154	6.05	GGGTGATCACATGGGTTAGG	AAATCAATGCTCCAAATCGC	53
Nc013	6.05	AATGGTTTTGAGGATGCAGCGTGG	CCCCGTGATTCCCCTTCAACTTTC	59
phi034	7.02	TAGCGACAGGATGGCCTCTTCT	GGGGAGCACGCCTTCGTTCT	54
Phi328175	7.04	GGGAAGTGCTCCTTGCAG	CGGTAGGTGAACGCGGTA	54
Phi233376	8.09	CCGGCAGTCGATTACTCC	CGAGACCAAGAGAACCCTCA	54
Phi065	9.03	AGGGACAAATACGTGGACACAG	CGATCTGCACAAAGTGGAGTAGTC	54
Umc1061	10.06	AGCAGGAGTACCCATGAAAGTCC	TATCACAGCACGAAGCGATAGATG	53

Source: https://www.maizegdb.org/

"1" assigned for the presence of a band and "0" for its absence. Samples that failed to amplify were assigned a score of "9" and treated as missing data. The binary data from all SSR loci were compiled into a matrix for further analysis.

2.4. Data Analysis

The binary data obtained Next will counted similarity coefficient with use formula simple matching coefficient (SMC) (Verma & Aggarwal 2019).

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

where a: score 1.1; b: score 1.0; c: score 0.1; d: score 0.0

Result data scoring furthermore analyzed using the Unweighted Pair-Group Method with Arithmetic (UPGMA)- Sequential Agglomerative Hierarchial and Nested (SAHN) program on the device NTSYS software version 2.2 (Rohlf 2015).

Grouping done with choose clustering feature in NTSYS program version 2.2 for to obtain grouping in form dendogram. From dendogram will know genotype that has large genetic distance. Furthermore, the data results scoring analyzed use Power Marker 3.25 software (Liu & Muse 2005) to analysis statistics like mark heterozygosity, and Polymorphism Information Content (PIC) values generated by the markers used in the study. The PIC value was calculated according to formulation (Hildebrand *et al.* 1992) as following.

$$PIC_j = 1 - \sum_{i-1}^{n} pi^2$$

PIC j is Polymorphism Information Content marker value to -j, is frequency allele to -I on the mark to -j, and n is amount alleles on markers to -j

3. Results

3.1. The Quality and Quantity of S2 Generation DNA from Various Source Local Maize

RNA and DNA quality and quantity tests from a number of sample individual corn local shown in Table 3.

The results of RNA and DNA measurements using the A_{260}/A_{280} spectrophotometer show the lowest purity mark for RNA was 1.893 (Pulut kuning) and the highest was 2.250 (Batara didi), while for DNA the lowest purity value is 1.76 (Local Soppeng) and the highest is 2.04 (Pulut kuning). The lowest RNA concentration value obtained is 108 ng/µL (Batara didi and Batara didi Pamatata), while the highest DNA concentration is 948 ng/µL(Pulut kuning).

Based on table results for purity and concentration of DNA samples from a number of source corn, in a way overall, it is known that from thirty isolated DNA samples, there is one sample showing marked purity not enough from 1.8 to 2.0, namely, the number code KS4 sample with marked purity 1.77, and the value of the highest purity is on the number code PK1 sample with marked purity 2.04. This shows that the DNA sample possibly contains contaminants.

Table 3. Purity and concentration of DNA and RNA of local corn from Bone, Soppeng, Selayar, Srikandi Kuning (National Variety) and Syn Carotenoid (CIMMYT)

Sample code	DNA		RNA	
	Concentration (ng/µL)	Purity (OD) 260/280	Concentration (ng/µL)	Purity (OD) 260/280
PK.1	2315	2.04	284	1,893
PK.2	1168	2.03	816	1,971
PK.3	3732	1.95	948	1,975
PK.4	555	1.98	876	1,955
PK.5	2660	1.91	482	1,913
SL.1	1210	1.82	780	1,980
SL.2	3208	1.88	740	1,968
SL.3	1763	1.76	550	1,937
SL.4	2355	1.81	654	2,006
SL.5	1265	2.00	584	1,921
BD.1	3388	1.87	338	2,096
BD.2	2805	1.98	108	2,250
BD.2	3320	1.98	300	2,043
BD.3	2090	1.99	218	2,057
BD.4	1945	1.85	388	2,064
BP.1	1955	1.82	108	2,250
BP.2	1230	1.82	422	2.02
BP.3	2090	1.83	602	2,007
BP.4	2920	1.87	360	2.00
BP.5	558	2.02	358	1,904
SK.1	1333	1.82	744	1,958
SK.2	2225	1.81	452	1,915
SK.3	2428	1.86	576	1,973
SK.4	4395	1.95	446	1,906
SK.5	3872	1.96	618	1,968
KS.1	1585	1.92	560	1,905
KS.2	840	1.95	554	1,937
KS.3	918	1.81	506	1,917
KS.4	895	1.77	492	1,922
KS.5	1498	1.84	356	1,914

3.2. PR-5 Gene Visualization

Amplification of the PR-5 downy mildew resistance gene using the PR-5 primer pair on agarose gel produces DNA fragments. The result is indicated by the bands expressed from the in the agarosa gel electrophoresis results ,as shown in Figure 1.

According to Figure 1, thirty samples tested detected 23 samples that have resistant characters to downy mildew disease. This is indicated by the band expressed by electrophoresis results on agarose gel. The appearance of the band on the agarose gel indicates that the sample contains the gene for downy mildew resistance. The twenty-three corn samples detected have downy mildew resistance genes, namely, PK2, PK3, PK4, PK5, SL1, SL2, SL3, SL4, SL5, BD1, BD4, BP3, BP4, BP5, SK2, SK3, SK4, SK5, KS1, KS2, KS3, KS4, and KS5.

Meanwhile, the other 7 samples were not detected to have downy mildew resistance genes; this is indicated by the absence of bands on the agarose gel against the tested samples. The seven samples that were not found to have downy mildew resistance genes are PK1, BD2, BD3, BD5, BP1, BP2, and SK1.

3.3. Visualization of DNA Bands of Local Corn Samples, Bone, Soppeng, Selayar, Yellow Srikandi (National Variety) and Syn Carotenoid (CIMMYT) using SSR Markers

The PCR amplification results of each primer after being visualized through electrophoresis on 8 % polyacrylamide gel, shows the presence of DNA bands of varying sizes, results obtained as shown in Figure 2.

Observation results the position and number of DNA bands from the 15 SSR primers show that the DNA bands on each primer are different position and number. The number of bands formed is at least 6 and at most 25. The heterozygosity is 0.40 to 1, and the position of the bands varies. between 73 bp to 553 bp, as shown in Table 4.

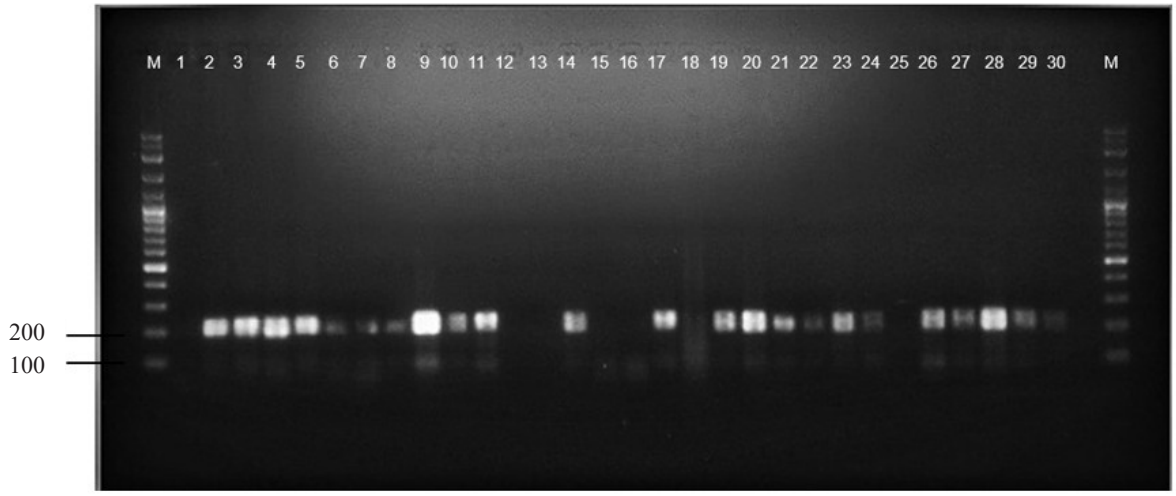

Figure 1. Visualization of the PR-5 gene for downy mildew resistance in 30 individuals of local corn, Srikandi Kuning and Carotenoid Syn. Ket: M Ladder 100 bp

The PIC value is indicators used for measure level polymorphism or diversity genetics a mark molecular. In research This using SSR (Simple Sequence Repeats) primers. PIC values range from 0.64 to 0.93. PIC reflects ability a mark for differentiate between individual in population, providing information about allele diversity and frequencies among individuals, In general, the greater the number of alleles at a locus, the higher the level of polymorphism.

In Table 4 it can be seen that mark Heterozygosity is in the range of 0.40 to 1. This is means 15 SSR primers were used in study corn this own level sufficient polymorphism high and high heterozygosity. Heterozygosity is proportion individual in a population that has allele different (heterozygous) in a locus certain. High heterozygosity show high diversity because lots of individuals who have combination different alleles. The DNA bands on each primer are then arranged in binary data table (0 and 1) which is next processed with the NTSYS program for count coefficient similarity genetics use Simple Matching Coefficient (SMC) formula as shown in Table 5.

Genetic kindship analysis using 15 SSR molecular markers on 30 maize genotypes yielded a genetic similarity matrix with values ranging from 0.53 to 0.90, indicating a moderate to high level of genetic diversity among the genotypes analyzed. The similarity values were calculated based on the proportion of shared alleles between pairs of individuals and reflected the degree of genetic relatedness among them. The genetic kindship among local maize genotypes, the Yellow Srikandi (national varieties), and introduced genotypes from CIMMYT exhibited a relatively wide range of variation, as presented in Table 5. The highest similarity value of 0.90 was observed between the pairs (KS.4 vs KS.5) and (BP.4 vs BP.3), which correspond to the carotenoid Syn-3 genotype from CIMMYT and the local maize Batara Didi Pamatata from Selayar, South Sulawesi, respectively. Meanwhile, the lowest genetic similarity value of 0.53 was found in the pair BD2 and PK5, both local genotypes originating from Selayar and Bone, indicating a relatively large level of genetic difference. Based on the genetic similarity matrix values between individuals, grouping was carried out in the form of a dendogram and the results obtained were as in Figure 3.

The analysis of genetic diversity among 30 local maize individuals was conducted using Simple Sequence Repeat (SSR) markers. The genetic relationships among the individuals were further assessed through cluster analysis, and the results are presented in the form of a dendrogram (Figure 3). Based on the dendrogram, the 30 individuals were classified into two main clusters according to their genetic similarity coefficients, which ranged from 0.64 to 0.90. These coefficients indicate the degree of genetic similarity between genotypes, with higher values representing closer genetic relationships. Cluster I consists of a single individual, PK3, which exhibited the lowest similarity coefficient (0.64), indicating relatively distant genetic characteristics compared to the others. In contrast, the remaining 29 individuals are grouped into Cluster II, which is further subdivided into two sub-clusters: Sub-cluster IIA, which includes Batara Didi and Batara Didi Pamatata, and sub-cluster IIB consists of all yellow sikandi individuals, maize from CIMMYT, local Soppeng maize and some yellow glutinous maize.

4. Discussion

In research molecular biology, quality and quantity are crucial factors that affect success analysis molecularly, including the purity and concentration of extracted DNA and RNA. Nano spectrophotometers are used to measure the results. Successful DNA and RNA extraction is not only determined by the amount (quantity) of nucleic acid

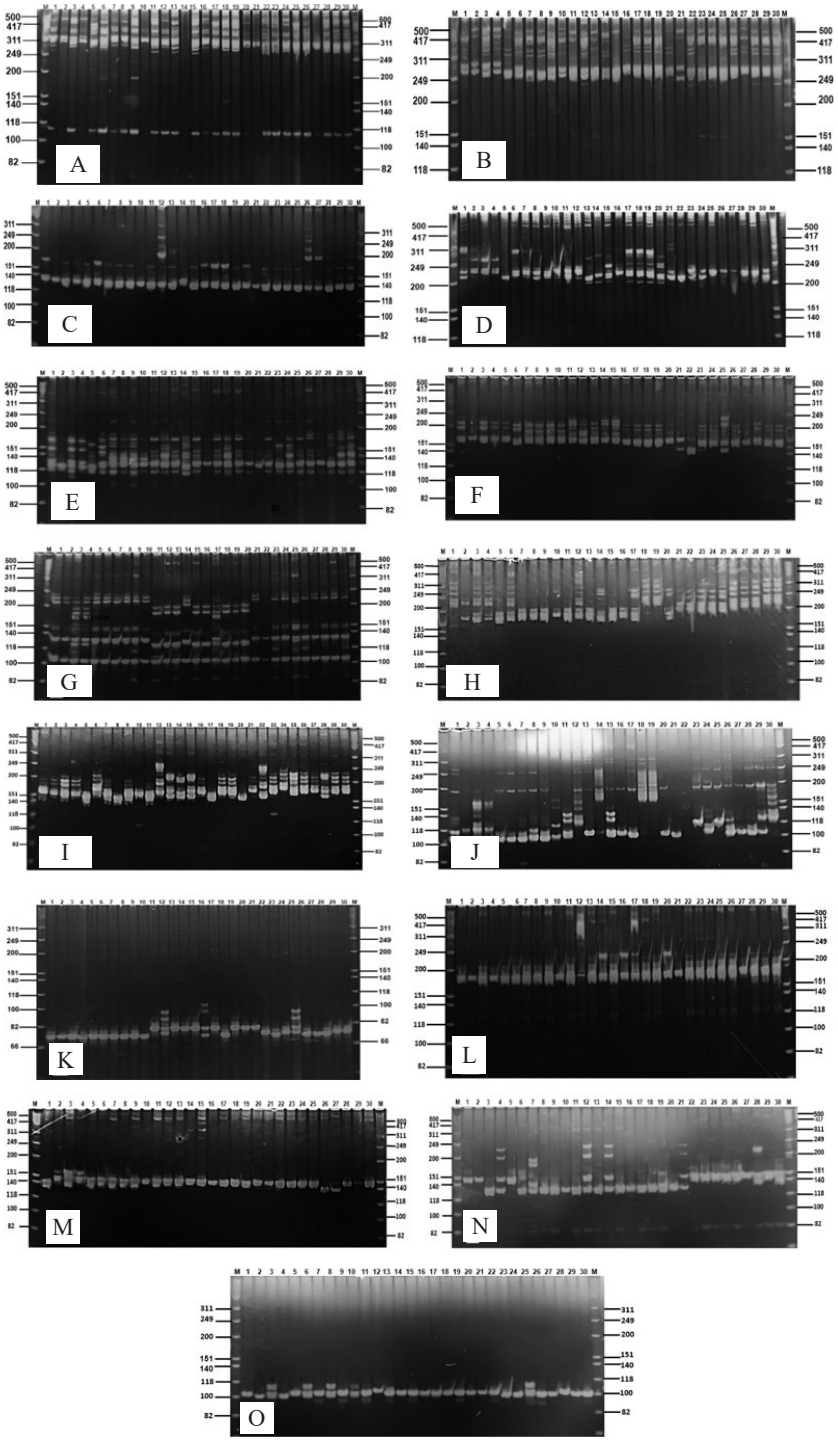

Figure 2. Visualization of DNA bands from 30 corn samples using 15 SSR primers on 8% polyacrylamide Gel. A: Phi 227562, B: Phi 96100, C: Umc 1736 , D: Phi 374118, E: Phi 092, F: Phi 109188, G: Phi 331888, H: Bnlg 1867, I: Bnlg 1154, J: Nc103, K: Phi 034, L: Phi 328175, M: Phi 233376, N: Phi 065, O: Umc 1061

Table 4. Data profile of 15 SSR primers used in 30 maize individuals : PK1-PK5: Pulut kuning, Bone, South Sulawesi, SL1-SL5: Local Soppeng South Sulawesi, BD—BD5: Batara Didi Selayar South Sulawesi, BP1-BP5: Batara Didi Pamatata Selayar, South Sulawesi, SK1-SK5: Srikandi kuning (National Variety) and KS1-KS5: Syn 3 Carotenoids (CIMMYT)

Primary	Location chromosome	Amount allele	PIC	Heterozygosity	Size allele
Phi227562	1.11	16	0.88	1.00	113-553
Phi96100	2.01	11	0.92	1.00	239-500
Umc1736	2.09	10	0.77	0.83	133-311
Phi374118	3.02	15	0.91	0.90	200-500
Phi092	4.08	20	0.92	0.93	100-500
Phi109188	5.03	11	0.90	1.00	145-354
Phi331888	5.04	25	0.91	1.00	82-445
Bnlg1867	6.01	11	0.90	0.97	167-458
Bnlg1154	6.05	13	0.90	0.93	104-235
Nc013	6.05	19	0.93	0.97	104-377
Phi034	7.02	7	0.75	0.73	73-107
Phi328175	7.04	9	0.85	0.80	169-535
Phi233376	8.03	11	0.79	0.83	134-500
Phi065	9.03	14	0.86	0.50	123-417
Umc1061	10.01	6	0.64	0.40	91-112
Total		198	12.75	12.79	73-553
Average		13.20	0.85	0.85	

obtained but also by the level of its purity. Therefore, that evaluation purity becomes an important indicator to ensure that the extracted DNA and RNA are truly worthy used in molecular analysis.

The concentration and purity of DNA and RNA were measured using a nanodrop spectrophotometer with an absorbance ratio at a wavelength of 260 nm (A_{260}) and 280 nm (A_{280}). Based on instructions, testing RNA purity is performed with an observed ratio absorption at a length of 260/280 nm wave. Measurement results RNA quality with a ratio between 1.8 and 2.0 is considered optimal for analysis to continue. Ratio absorbance on both long waves has been used as indicator level of purity in the extraction process of sour nucleic acid and protein. The A_{260}/A_{280} ratio is generally ±1.8, accepted as a pure DNA indicator, whereas a ratio of ±2.0 indicates pure RNA. DNA quality is seen from the purity test value, while its quantity is seen from its concentration value (Harahap 2017; Pangaribuan *et al.* 2022).

In research biology molecular, quality and quantity sour nucleic is factor crucial that affects success analysis molecular, including purity and concentration of extracted DNA and RNA Then measured using nano spectrophotometers. Success DNA and RNA extraction is not only determined by the amount (quantity) of acid nucleic acid obtained, but also by the level of its purity. Therefore that, evaluation purity become indicator important for ensure that the extracted DNA and RNA truly worthy used in analysis molecular.

The concentration and purity of DNA and RNA were measured using a nano spectrophotometer with an absorbance ratio at a wavelength of 260 nm (A_{260}) and 280 nm (A_{280}). Based on Instructions tools, testing RNA purity is performed with observe ratio absorption at length 260/280 nm wave. Measurement results RNA quality with ratio between 1.8 to 2.0 is considered optimal for analysis more continue. Ratio absorbance on both long wave this has used as indicator level purity in the extraction process sour nucleic and protein. The A_{260}/A_{280} ratio is generally ±1.8 accepted as pure DNA indicator, whereas ratio ±2.0 indicates pure RNA. DNA quality is seen from the purity test value while its quantity is seen from its concentration value (Harahap 2017; Pangaribuan *et al.* 2022).

The DNA purity results are said to be good if the optical density (OD) value of 260/280 nm is obtained in the range of 1.8-2.0. DNA purity of less than 1.8 indicates the presence of other contaminants such as protein, phenol, or others that are strongly absorbed at a wavelength of 280 nm, while if the DNA purity value is higher than 2.0, the DNA can be said to be contaminated by RNA (Wardana & Mushlih 2021). Based on this statement, the results of DNA quality and quantity tests from several individual local corn samples (Table 3), show that the DNA concentration obtained is generally good with a concentration value above 100, with purity in the range of 1.8-2.0 except for sample code numbers PK1, PK2, BP5 and KS4 with values of 2.04

Table 5. Genetic Similarity Matrix of 30 individuals corn on 15 SSR primers. PK1-PK5: Jagung Pulut , Bone, South Sulawesi, SL1-SL5: Local Soppeng, South Sulawesi, BD—BD5: Batara didi Selayar South Sulawesi, BP1-BP5: Batara didi Pamatata Selayar , South Sulawesi, SK1-SK5: Srikandi kuning (National Variety) and KS1-KS5: Syn 3 Carotenoids (CIMMYT)

	PK1	PK2	PK3	PK4	PK5	SL1	SL2	SL3	SL4	SL5	BD1	BD2	BD3	BD4	BD5	BP1	BP2	BP3	BP4	BP5	SK1	SK2	SK3	SK4	SK5	KS1	KS2	KS3	KS4	KS5
PK1	1.00																													
PK2	0.70	1.00																												
PK3	0.66	0.67	1.00																											
PK4	0.71	0.87	0.76	1.00																										
PK5	0.72	0.72	0.69	0.66	1.00																									
SL1	0.73	0.65	0.69	0.66	0.77	1.00																								
SL2	0.70	0.67	0.67	0.63	0.79	0.72	1.00																							
SL3	0.70	0.71	0.68	0.66	0.83	0.74	0.88	1.00																						
SL4	0.73	0.66	0.68	0.61	0.78	0.72	0.88	0.81	1.00																					
SL5	0.69	0.80	0.65	0.76	0.74	0.66	0.71	0.76	0.72	1.00																				
BD1	0.62	0.66	0.64	0.62	0.71	0.63	0.70	0.69	0.73	0.69	1.00																			
BD2	0.56	0.55	0.54	0.58	0.53	0.58	0.58	0.58	0.60	0.58	0.68	1.00																		
BD3	0.59	0.62	0.60	0.58	0.69	0.61	0.68	0.67	0.73	0.66	0.86	0.68	1.00																	
BD4	0.59	0.64	0.57	0.65	0.70	0.68	0.67	0.67	0.66	0.66	0.66	0.66	0.72	1.00																
BD5	0.58	0.59	0.59	0.56	0.64	0.59	0.65	0.65	0.68	0.64	0.86	0.67	0.87	0.72	1.00															
BP1	0.67	0.76	0.61	0.73	0.71	0.65	0.68	0.71	0.68	0.77	0.69	0.59	0.68	0.64	0.69	1.00														
BP2	0.66	0.64	0.62	0.65	0.66	0.65	0.68	0.69	0.74	0.69	0.75	0.64	0.73	0.67	0.76	0.76	1.00													
BP3	0.67	0.60	0.65	0.62	0.64	0.70	0.65	0.66	0.70	0.64	0.71	0.68	0.72	0.68	0.71	0.73	0.80	1.00												
BP4	0.64	0.58	0.63	0.60	0.62	0.67	0.63	0.63	0.69	0.63	0.76	0.66	0.77	0.67	0.77	0.71	0.83	0.90	1.00											
BP5	0.64	0.72	0.62	0.72	0.71	0.63	0.67	0.71	0.67	0.73	0.73	0.62	0.75	0.69	0.73	0.79	0.75	0.72	0.72	1.00										
SK1	0.72	0.80	0.60	0.76	0.73	0.68	0.68	0.73	0.66	0.81	0.67	0.57	0.65	0.66	0.64	0.73	0.66	0.59	0.60	0.75	1.00									
SK2	0.69	0.71	0.66	0.66	0.76	0.72	0.75	0.72	0.76	0.76	0.77	0.63	0.74	0.67	0.70	0.71	0.70	0.72	0.73	0.74	0.76	1.00								
SK3	0.69	0.66	0.65	0.63	0.74	0.74	0.69	0.70	0.70	0.69	0.68	0.59	0.67	0.60	0.66	0.67	0.65	0.70	0.70	0.67	0.72	0.80	1.00							
SK4	0.70	0.69	0.64	0.65	0.75	0.73	0.71	0.69	0.72	0.67	0.74	0.63	0.67	0.62	0.67	0.69	0.67	0.70	0.69	0.68	0.68	0.79	0.86	1.00						
SK5	0.68	0.61	0.65	0.60	0.72	0.75	0.66	0.70	0.68	0.70	0.69	0.58	0.67	0.64	0.66	0.66	0.69	0.70	0.73	0.67	0.69	0.80	0.84	0.78	1.00					
KS1	0.71	0.63	0.60	0.62	0.68	0.69	0.63	0.64	0.66	0.63	0.63	0.59	0.62	0.58	0.59	0.64	0.70	0.72	0.72	0.65	0.63	0.72	0.77	0.74	0.78	1.00				
KS2	0.75	0.80	0.63	0.76	0.70	0.73	0.64	0.69	0.65	0.73	0.61	0.60	0.60	0.61	0.57	0.75	0.69	0.70	0.67	0.72	0.80	0.73	0.73	0.71	0.70	0.82	1.00			
KS3	0.70	0.68	0.61	0.65	0.74	0.76	0.69	0.68	0.70	0.68	0.67	0.60	0.66	0.66	0.63	0.69	0.68	0.73	0.70	0.69	0.70	0.78	0.73	0.73	0.76	0.78	0.81	1.00		
KS4	0.72	0.66	0.60	0.65	0.67	0.72	0.63	0.66	0.66	0.66	0.61	0.61	0.61	0.63	0.60	0.67	0.70	0.73	0.70	0.68	0.68	0.71	0.72	0.76	0.75	0.81	0.82	0.84	1.00	
KS5	0.69	0.63	0.58	0.62	0.65	0.67	0.62	0.61	0.61	0.61	0.60	0.61	0.58	0.59	0.56	0.64	0.63	0.71	0.66	0.64	0.63	0.71	0.74	0.77	0.73	0.79	0.77	0.82	0.90	1.00

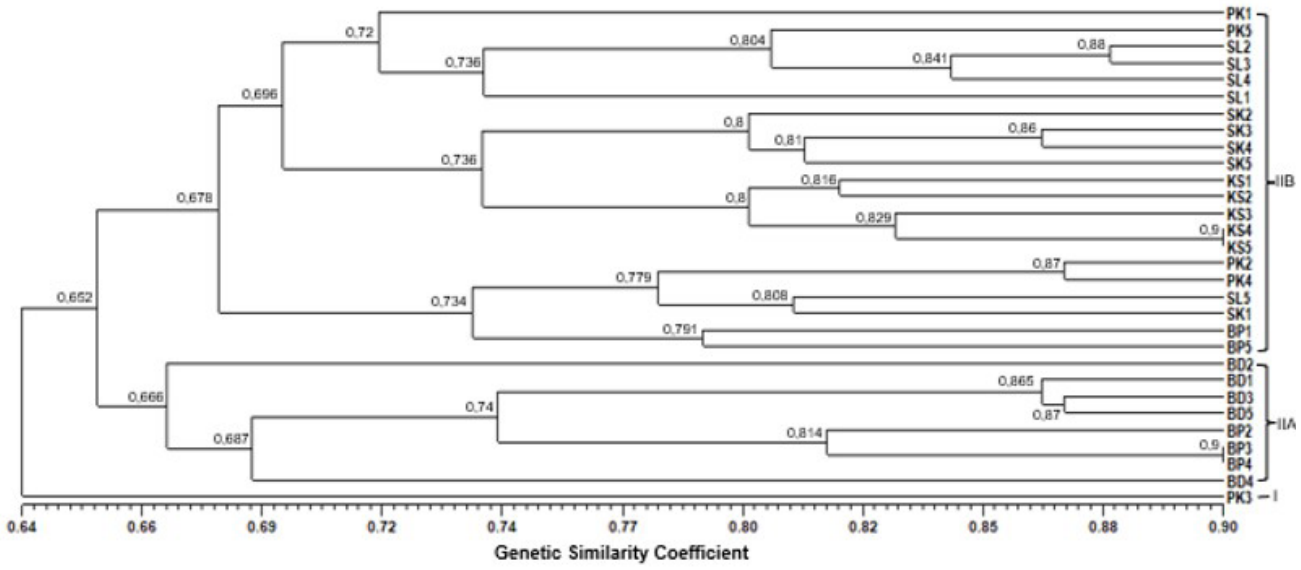

Figure 3. Dendrogram based on similarity genetics of 30 individual corn on 15 primary SSR.PK1-PK5: pulut kuning Bone, South Sulawesi, SL1-SL5: Local Soppeng, South Sulawesi, BD—BD5: Batara Didi Selayar, South Sulawesi, BP1-BP5: Batara Didi Pamatata Selayar, South Sulawesi, SK1-SK5: Srikandi kuning (National Variety) and KS1-KS5: Syn 3 Carotenoids (CIMMYT)

respectively; 2.03, 2.02 and 1.77. Based on the table of purity and concentration results of DNA samples from several corn sources as a whole, it is known that out of thirty isolated DNA samples, there is one sample that shows a purity value of less than 1.8 to 2.0, namely sample code number KS4 with a purity value of 1.77, and the highest purity value is in sample code number PK1 with a purity value of 2.04. This value indicates that the DNA sample contains contaminants or impure DNA. The values obtained are in line with the results of the study (Pratiwi & Widodo 2020), which states that samples containing nucleic acid have a range of absorbance ratio values of 260/280 obtained of 1.8-2.0; if more than that, then the sample is contaminated with protein.

Figure 1's visualization on an agarose gel reveals that the PR5 gene was not present in all tested samples. Of the 30 samples tested, there were 7 individuals who did not show any PR-5 gene expression, which was indicated by the absence of electrophoresis bands. Some individuals who did not express the PR-5 gene were from the Batara Didi (BD) sample, even though they belonged to the same variety and were grown in a similar environment. Such an outcome indicates the presence of genetic variation or other factors that influence the expression of the gene. Genetic variation between varieties can influence the ability of PR-5 gene expression (Yang *et al.* 2023).

PR-5 is a response gene to stress or pathogens (pathogenesis-related gene), so there is the possibility of this gene not being expressed if the plant does not experience biotic pressure (e.g., infection by fungi or viruses). The same thing in the study (De Jesus Miranda *et al.* 2017).

PR-5 gene expression in corn increases in a way that is significant after infection by pathogens, indicating that the expression of this gene is highly dependent on the presence of pressure biotic. PR accumulation helps plants prevent reinfection, resulting in the development of systemic acquired resistance (Zhang *et al.* 2021). (Huo *et al.* 2021a), in the report, show that PR-5 gene expression as a response to various biotic and hormonal stresses defense-like sour salicylate and ABA, indicating that regulation of PR-5 expression can be influenced by transcription factors and environmental conditions. Other research results show that PR-5 has antifungal activity, including spore lysis, inhibition of hyphal growth, and reduced spore germination (Agung *et al.* 2016). Of the 30 corn samples tested, there were 23 individuals that expressed the PR-5 gene (Figure 1). This indicates that the presence of the PR-5 gene in corn plants shows potential resilience to various biotic stress such as pathogens infection. This is in line with research results (Huo *et al.* 2021b; Yang *et al.* 2025), which concluded that the PR-5 gene plays a direct role in regulating the expression of genes related to the plant defense system, thereby strengthening its role in the plant's immune response mechanism, increasing plant resistance to

various environmental stresses and pathogen attacks. Therefore, identifying individual corn that is consistent with optimally expressing the PR-5 gene can become a strategic step in glorifying plants in order to produce seeds superior to those more adaptive and resilient to attack disease. In the analysis of diversity genetics, value heterozygosity and polymorphism information content (PIC) values are important parameters that reflect the level of variation genetics in a population (Kanaka *et al.* 2023). Heterozygosity indicates the proportion of individuals who have an allele different on a locus, while the PIC value reflects the level of informativeness of a genetic marker in differentiating genotypes. The taller the mark heterozygosity and PIC value, the greater the diversity of genetics that are owned by both populations (Zhu & Shen 2024). Mark, this is very closely related to the amount of alleles. The more alleles detected in a locus, generally is to be followed by increasing marker heterozygosity and PIC, because the opportunity for genetic variation also increases. Therefore, analysis of these three parameters is very important in evaluating potential genetics, conservation source power genetics, as well as selection in the breeding program (Kanaka *et al.* 2023).

Based on Table 4, the level of polymorphism (polymorphism information content) of the 15 SSR markers used ranged from 0.64 to 0.93. The lowest PIC was owned by the Umc1061 primer, which was 0.64 with the number of alleles 6, and the highest PIC was owned by the Nc013 primer, which was 0.93 with the number of alleles 19. The average PIC value obtained was 0.85, and the average allele was 13.20. The PIC value can be used as a standard for evaluating genetic markers to show how well a genetic marker can distinguish individuals in a population. The PIC value criteria are classified according to Dalimunthe *et al.* (2020) and Hildebrand *et al.* (1992), where PIC>0.75= very informative, PIC>0.5 = moderate, and PIC<0.5= low. Based on this statement, the PIC value obtained in this study was very informative, with an average value of 0.85. Of the 15 primers used, there was one primer that showed a medium category value, namely Umc 1061 with a PIC value of 0.64. A high PIC (Polymorphism Information Content) value indicates that the genetic marker used has the potential to detect high genetic variation among individuals in the population. A similar study was reported (Muhammad *et al.* 2017), PIC ranged from 0.17 to 0.47, obtained from 190 alleles with an average value of 9.5 alleles using 20 primers. The PIC value reported (Jhansi Rani & Vanisri 2022) using 50 SSR molecular markers ranged from 0.164 to 0.672, with an average PIC of 0.345, while in Salazar *et al.*'s (2017) study, the average PIC value obtained was 0.55 from 10 primers used, all of which were polymorphic with 162 identified alleles. Furthermore, (Islam *et al.* 2023), in their research, found the average PIC value to be 0.85 from 18 markers used for 22 hybrid corn with an average of 10.4 alleles per locus. PIC is used to evaluate diversity in every marker, while matrix similarity genetics is used to describe the connection between genotypes based on allelic patterns. The results of the cluster analysis of 30 corn individuals using 15 SSR markers in the form of a dendrogram based on UPGMA (unweighted pair group method with arithmetic) on the genetic similarity matrix (Figure 3) showed that the genetic similarity coefficient value ranged from 0.64 to 0.90, which is divided into two main clusters. The main clusters are clusters I and II. Cluster I, with a similarity coefficient of 0.64, consists of only 1 individual, namely PK3, and at a coefficient of 0.67, it forms cluster II, consisting of 29 individuals. In cluster II, 2 sub-clusters form, namely clusters IIA and IIB. Cluster IIA consists of 8 individuals, namely BD1, BD2, BD3, BD4, BD5, BP2, BP3, and BP4. all of which are local corn groups: Batara Didi and Batara Didi Pamatata, while sub-cluster IIB consists of PK1, PK2, PK4, PK5, SL1, SL2, SL3, SL4, SL5, SK1, SK2, SK3, SK4, SK5, KS1, KS2, KS3, KS4, and KS5. Individuals of Srikandi kuning (SK) and Carotenoid Syn (KS), along with some individuals of pulut kuning and local Soppeng, are all in the same sub-cluster, namely IIB. Individuals in Cluster I and sub-cluster IIB have the same genetic source. The value of the genetic similarity coefficient in individuals of Cluster I is very different from Cluster IIA so that it forms a different cluster. Based on this dendrogram, it shows that there is high genetic diversity among individuals of local corn, national varieties, and CIMMYT without being influenced by their geographical origin.

Similar research by Islam *et al.* (2023) analyzed the genetic diversity matrix in 22 inbred corn lines using 18 SSR molecular markers, with genetic diversity coefficient values of 0.07-0.44. (Yani *et al.* 2022), in their research, reported the genetic similarity matrix value was 0.29-0.65 in 8 local corn cultivars using 5 SSR primers. Similar research was reported by Andayani *et al.* (2020) in 15 local corn varieties using 34 SSR markers with genetic similarity coefficient values of 0.37-0.86. Local South Sulawesi corn from yellow sticky rice individuals (PK2, PK3, PK4 and PK5), local soppeng (SL1-SL5), batara didi (BD1 and BD4), batara didi pamataa (BP3-BP5), national variety srikandi kuning (SK) and Syn

carotenoid (KS) were detected to contain the downy mildew resistance gene (PR5). These individuals also have high genetic diversity and have the potential to be used as selection materials in efforts to breed disease-resistant plants.

The maize identified as containing the PR5 gene with high genetic diversity values show significant genetic potential and can be used as superior genetic sources in plant breeding programs, especially to increase resistance to downy mildew.

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References

Adhi, S.R., Widiantini, F., Yulia, E., 2022. Variasi morfometri dan patogenisitas *Peronosclerospora* spp. penyebab penyakit bulai jagung di Pulau Jawa, Indonesia. *Jurnal Fitopatologi Indonesia*. 17, 173182. https://doi.org/10.14692/jfi.17.5.173-182

Agung, M.B., Budiarsa, I.M., Suwastika, I.N., 2016. Analisa "In Silico" gen kakao (*Theobroma cacao* L.) yang terlibat dalam sistem ketahanan terhadap hama dan penyakit. *Natural Science: Journal of Science and Technology*. 5, 234-250. https://doi.org/10.22487/25411969.2016.v5.i2.6710

An, Y., Xia, X., Zheng, H., Yu, S., Jing, T., Zhang, F., 2023. Multi-genome comprehensive identification of SSR/SV and development of molecular markers database to serve *Sorghum bicolor* (L.) breeding. *BMC Genomic Data*. 24, 1-11. https://doi.org/10.1186/s12863-023-01165-y

Andayani, N.N., Isnaini, M., Aqil, M., Muis, A., Pabendon, M.B., Azrai, M., 2020. Evaluation of genetic diversity of QPM, provit-A, and elite maize inbreds resistant to downy mildew disease using simple sequence repeats. *Jurnal Penelitian Pertanian Tanaman Pangan*. 4, 161. https://doi.org/10.21082/jpptp.v4n3.2020.p161-170

Ashan, M.D., Miftahudin., Reflinur., 2020. Identifikasi marka molekuler terpaut gen ketahanan bulai pada tanaman jagung (*Zea mays* L.) [Skripsi]. IPB Repository, Bogor: IPB University.

Bani, P.W., Daryono, B.S., Purnomo, P., 2017. Penanda molekuler inter simple sequence repeat untuk menentukan ketahanan tanaman jagung terhadap penyakit bulai. *Jurnal Fitopatologi Indonesia*. 13, 127-135. https://doi.org/10.14692/jfi.13.4.127

Bantacut, T., Firdaus, Y.R., Akbar, M.T., 2015. Pengembangan jagung untuk ketahanan pangan, industri dan ekonomi corn development for food security, industry and economy. *Jurnal Pangan*. 24, 135-148. http://www.jurnalpangan.com/index.php/pangan/article/view/29

Dalimunthe, S.R., Siregar, L.A.M., Putri, L.A.P., Chairunnisa, T., Hairmansis, A., 2020. Polymorphism levels of some SSR markers (Simple Sequence Repeat) for parental line identification on low temperature tolerance. *IOP Conference Series: Earth and Environmental Science*. 454. https://doi.org/10.1088/1755-1315/454/1/012165

De Jesus Miranda, V., Porto, W.F., Da Rocha Fernandes, G., Pogue, R., Nolasco, D.O., Guerra Araujo, A.C., Cota, L.V., De Freitas, C.G., Dias, S.C., Franco, O.L., 2017. Comparative transcriptomic analysis indicates genes associated with local and systemic resistance to *Colletotrichum graminicola* in maize. *Scientific Reports*. 7, 1-14. https://doi.org/10.1038/s41598-017-02298-8

Edhy, S., Budiana, K., Kurniawati, R., Nurhayati, Y., Bowo, K.A., Herdyanti, I.F., 2022. *Statistik Ketahanan Pangan 2021*. Pusat Data dan Informasi Pangan Badan Pangan Nasional, Jakarta.

El-kereamy, A., El-sharkawy, I., Ramamoorthy, R., Taheri, A., Errampalli, D., Kumar, P., Jayasankar, S., 2011. *Prunus domestica* pathogenesis-related protein-5 activates the defense response pathway and enhances the resistance to fungal infection. *PLoS ONE*. 6, 1-11. https://doi.org/10.1371/journal.pone.0017973

FAO., 2021. World Food and Agriculture – Statistical Yearbook 2021. FAO, Rome.

Geneaid., 2017. Total RNA Mini Kit (Plant). www.geneaid.com

Genesiska, G., Susanto, B., Mulyono, M., 2020. Karakter fenotip tanaman jagung (*Zea mays* L.) lokal varietas pulut Sulawesi di Daerah Istimewa Yogyakarta. *PLANTROPICA: Journal of Agricultural Science*. 5, 85-94. https://doi.org/10.21776/ub.jpt.2020.005.1.10

Govindaraj, M., Vetriventhan, M., Srinivasan, M., 2015. Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. *Genetics Research International*. 2015, 14. https://doi.org/10.1155/2015/431487

Hakim., Ullah, A., Hussain, A., Shaban, M., Khan, A.H., Alariqi, M., Gul, S., Jun, Z., Lin, S., Li, J., Jin, S., Munis, M.F.H., 2018. Osmotin: A plant defense tool against biotic and abiotic stresses. *Plant Physiology and Biochemistry.* 123, 149-159. https://doi.org/10.1016/j.plaphy.2017.12.012

Harahap, A.S., 2017. Uji kualitas dan kuantitas DNA beberapa populasi pohon kapur Sumatera. *Journal of Animal Science and Agronomy Panca Budi*. 2, 1-6.

Herlinda, G., DAS, S.S., Syafi, S., 2018. Keragaman dan heritabilitas genotip jagung merah (*Zea Mays* L.) lokal. *Techno: Jurnal Penelitian*. 7, 191. https://doi.org/10.33387/tk.v7i2.793

Hildebrand, C.E., Torney, D.C., Wagner, R.P., 1992. Informativeness of polymorphic DNA markers. *Los Alamos Science*. 20, 100-102.

Huo, T., Wang, C., Li, M., Zhao, D., 2021a. Ekspresi berlebihan ZmWRKY65 faktor transkripsi dari jagung memberikan ketahanan terhadap stres pada Arabidopsis transgenik. 1-15.

Huo, T., Wang, C.T., Yu, T. F., Wang, D. M., Li, M., Zhao, D., Li, X.T., Fu, J. D., Xu, Z. S., Song, X.Y., 2021b. Overexpression of ZmWRKY65 transcription factor from maize confers stress resistances in transgenic Arabidopsis. *Scientific Reports*. 11, 115. https://doi.org/10.1038/s41598-021-83440-5

Islam, M.A., Alam, M.S., Maniruzzaman, M., Haque, M.S., 2023. Microsatellite marker-based genetic diversity assessment among exotic and native maize inbred lines of Bangladesh. *Saudi Journal of Biological Sciences*. 30, 103715. https://doi.org/10.1016/j.sjbs.2023.103715

Jhansi., Rani, K., Vanisri, S., 2022. Genetic diversity assessment of inbred lines in maize through SSR markers. *The Pharma Innovation Journal.* 11, 765-770.

Kalqutny, S.H., Pakki, S., Muis, A., 2020. Potensi pemanfaatan teknik molekuler berbasis DNA dalam penelitian penyakit bulai pada jagung. *AGROSAINSTEK: Jurnal Ilmu Dan Teknologi Pertanian*. 4, 17-27. https://doi.org/10.33019/agrosainstek.v4i1.107

Kanaka, K.K., Sukhija, N., Goli, R.C., Singh, S., Ganguly, I., Dixit, S.P., Dash, A., Malik, A.A., 2023. On the concepts and measures of diversity in the genomics era. *Current Plant Biology*. 33, 100278. https://doi.org/10.1016/j.cpb.2023.100278

Kapa Biosystem., 2025. Kapa Biosystems PCR Reagents. In Biosystems. pp. 1-2.

Kasiamdari, R.S., Putri, R.K., 2023. Ketahanan tiga varietas jagung (*Zea mays* L.) terhadap infeksi jamur penyakit bulai *Peronosclerospora maydis*. *Berkala Ilmiah Biologi.* 14, 2131. https://doi.org/10.22146/bib.v14i1.5658

Khan, I. A., Awan, F. S., Ahmad, A., Khan, A.A., 2004. A modified mini-prep method for economical and rapid extraction of genomic DNA in plants. *Plant Molecular Biology Reporter*. 22, 89-89. https://doi.org/10.1007/BF02773355

Lee, P.Y., Costumbrado, J., Hsu, C.Y., Kim, Y.H., 2012. Agarose gel electrophoresis for the separation of DNA fragments. *Journal of Visualized Experiments*. 62, 1-5. https://doi.org/10.3791/3923

Limenie, A.D., Alehegn, M., 2025. Genetic engineering for cereal crop yield improvement and disease resistant breeding. *Scientific World Journal.* 2025, 40225354. https://doi.org/10.1155/tswj/6743917

Liu, K., Muse, S.V., 2005. PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics*. 21, 2128-2129. https://doi.org/10.1093/bioinformatics/bti282

Miedaner, T., Boeven, A.L.G.C., Gaikpa, D.S., Kistner, M.B., Grote, C.P., 2020. Genomics-assisted breeding for quantitative disease resistances in small-grain cereals and maize. *International Journal of Molecular Sciences*. 21, 1-22. https://doi.org/10.3390/ijms21249717

Muhammad, R.W., Qayyum, A., Ahmad, M.Q., Hamza, A., Yousaf, M., Ahmad, B., Younas, M., Malik, W., Liaqat, S., Noor, E., 2017. Characterization of maize genotypes for genetic diversity on the basis of inter simple sequence repeats. *Genetics and Molecular Research*. 16, 1-9. https://doi.org/10.4238/GMR16019438

Mukhlif, F.H., Hammadi, H.J., Latiff, N.I., 2020. Genetic diversity analysis in maize (*Zea Mays* L.) using Ssr markers. *Biochemical and Cellular Archives*. 20, 2215-2220. https://doi.org/10.35124/bca.2020.20.1.2215

Pangaribuan, O., Hanafiah, D.S., Setiado, H., Simamora, J.R.M., Sari, N.W., 2022. Quantity and quality test of DNA marigold plants (*Tagetes erecta* L.) for the suistainability of plant breeding. *IOP Conference Series: Earth and Environmental Science*. 977, 0-5. https://doi.org/10.1088/1755-1315/977/1/012047

Prasetyo, R., Sari, M.K., Lestari, Y. kurnia., 2024. Penguatan ekosistem jagung: Isu, tantangan, dan kebijakan. *Policy Brief Pertanian, Kelautan, Dan Biosains Tropika*. 6, 749-753. https://doi.org/10.29244/agro-maritim.0601.749-753

Pratiwi, E., Widodo, L.I., 2020. Kuantifikasi hasil ekstraksi gen sebagai faktor kritis untuk keberhasilan pemeriksaan Rt Pcr. *Indonesian Journal for Health Sciences*. 4, 1. https://doi.org/10.24269/ijhs.v4i1.2293

Raghunath, S., 2022. Application of bioinformatics resources for mining of simple sequence repeats (SSRs) marker in plant genomes : An overview. *Research Journal of Biotechnology.* 17. 136-143. https://doi.org/10.25303/1708rjbt1360143

Ramlah, R., Aziz, I.R., Muthiadin, C., Masri, M., Mustami, M.K., Pabendon, M.B., 2018. Genetic diversity of local maize germplasm of Tana Toraja South Sulawesi using SSR (Simple Sequence Repeat) markers. *Ilmu Pertanian (Agricultural Science*). 2, 144. https://doi.org/10.22146/ipas.33085

Rohlf, F.J., 2015. NTSYS-pc-Numerical Taxonomy and Multivariate Analysis System Version 22. Stony Brook, Newyork.

Salazar, E., González, M., Araya, C., Mejía, N., Carrasco, B., 2017. Genetic diversity and intra-racial structure of Chilean Choclero corn (*Zea mays* L.) germplasm revealed by simple sequence repeat markers (SSRs). *Scientia Horticulturae*. 225, 620-629. https://doi.org/10.1016/j.scienta.2017.08.006

Salgotra, R.K., Chauhan, B.S., 2023. Genetic diversity, conservation, and utilization of plant genetic resources. *Genes*. 14, 174. https://doi.org/10.3390/genes14010174

Kitajima, S., Sato, F., 1999. Plant pathogenesis-related proteins: molecular mechanisms of gene expression and protein function. *J. Biochem*. 125, 1-8. https://doi.org/10.1093/oxfordjournals.jbchem.a022244

Setiadi., Daryono, B., Parazulfa, A., Purnomo, P., 2018. uji ketahanan tujuh kultivar jagung (*Zea mays* L.) terhadap penyakit bulai (*Peronosclerospora* spp.). *Biogenesis: Jurnal Ilmiah Biologi*. 6, 11-17. https://doi.org/10.24252/bio.v6i1.4175

Susanti, A., Supriyatna, A., 2020. Outlook Jagung 2020: Komoditas Pertanian Subsektor Tanaman Pangan. Pusat Data Dan Sistem Informasi Pertanian Kementrian Pertanian, 1-78. http://epublikasi.setjen.pertanian.go.id

Tachi, H., Fukuda-Yamada, K., Kojima, T., Shiraiwa, M., Takahara, H., 2009. Molecular characterization of a novel soybean gene encoding a neutral PR-5 protein induced by high-salt stress. *Plant Physiology and Biochemistry*. 47, 73-79. https://doi.org/10.1016/j.plaphy.2008.09.012

Thermo Fisher, S., 2013. Product information: RevertAid First Strand cDNA Synthesis Kit, #K1621. Thermo Fisher Scientific Inc. www.thermoscientific.com/onebio

Verma, V., Aggarwal, R.K., 2019. A new similarity measure based on simple matching coefficient for improving the accuracy of collaborative recommendations. *International Journal of Information Technology and Computer Science*. 11, 37-49. https://doi.org/10.5815/ijitcs.2019.06.05

Wardana, A.C., Mushlih, M., 2021. Comparison the quality of template DNA isolated by column method with and without centrifugation: Perbandingan kualitas DNA template yang diisolasi dengan metode kolom dengan dan tanpa sentrifugasi. *Indonesian Journal of Innovation Studies*. 15, 10-21070. https://doi.org/10.21070/ijins.v15i.552

Widiantini, F., Yulia, E., Purnama, T., 2015. Morphological variation of *Peronosclerospora maydis*, the causal agent of maize downy mildew from different locations in Java-Indonesia. *Journal of Agricultural Engineering and Biotechnology*. 2015, 23-27. https://doi.org/10.18005/jaeb0302002

Wulandari, E., Prasetyo, J., Nurdin, M., & Maryono, T., 2022. Penyakit bulai dan pertumbuhan tanaman jagung effect of *Mefenoxam* and *Trichoderma* sp. of downy. *Jurnal Agrotek Tropika*. 10, 43-49.

Yang, Z., Cao, Y., Shi, Y., Qin, F., Jiang, C., & Yang, S., 2023. Genetic and molecular exploration of maize environmental stress resilience: Toward sustainable agriculture. *Molecular Plant*. 16, 1496-1517. https://doi.org/10.1016/j.molp.2023.07.005

Yang, W., Cai, H., Zhang, Y., Hao, J., Ma, Y., He, Q., Zhao, K., Li, H., Fang, A., Hou, D., & Ren, X., 2025. Functional analysis of the ZmPR5 gene related to resistance against *Fusarium verticillioides* in maize. *Plants*. 14. https://doi.org/10.3390/plants14050737

Yani, A., Chasani, A.R., & Daryono, B.S., 2022. Genetic diversity of eight maize (*Zea mays*) cultivars from East Nusa Tenggara (Indonesia) based on inter simple sequence repeat markers. *Biodiversitas*. 23, 4124-4130. https://doi.org/10.13057/biodiv/d230833

Zaynab, M., 2025. The role of PR-5 genes in inducing toxicity and resistance in potato against phytophthora infestans. 1-21.

Zhang, Y., Chen, W., Sang, X., Wang, T., Gong, H., Zhao, Y., Zhao, P., & Wang, H., 2021. Genome-wide identification of the thaumatin-like protein family genes in *Gossypium barbadense* and analysis of their responses to verticillium dahliae infection. *Plants*. 10. https://doi.org/10.3390/plants10122647

Zhu, M., Tong, L., Xu, M., & Zhong, T., 2021. Correction to: Genetic dissection of maize disease resistance and its applications in molecular breeding. *Molecular Breeding*. 41, 32. https://doi.org/10.1007/s11032-021-01250-z

Zhu, B., & Shen, C., 2024. Tools and techniques of using NGS platforms in forensic population genetic studies. In: *Next Generation Sequencing (NGS) Technology in DNA Analysis*. Elsevier. pp. 121-171.