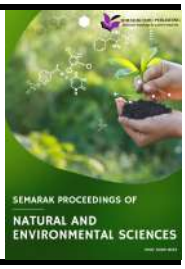
SEMARAK ILMU PUBLISHING

Seminar Biologi Kebangsaan 2025

https://semarakilmu.my/index.php/spnes/index
ISSN: 3083 - 8193



Genetic Diversity of Pineapple (*Ananas comosus*) Accessions Using ISSR Markers

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ABSTRACT

Pineapple is a member of Bromeliaceae family and a major tropical fruit crop with significant economic and nutritional importance globally. It contains high levels of vitamin C, sufficient to meet daily human requirements, making it is a highly nutritious fruit commonly consumed worldwide. However, the genetic diversity of pineapple accessions is not well-documented, hampering efforts to improve the crop through breeding programmes. Inter simple sequence repeat (ISSR) markers were used to assess the genetic diversity of 22 pineapple accessions obtained from MARDI Pontian. Five ISSR primers (IS21, IS34/1, UBC 822, UBC 824, UBC 827) amplified a total of 27 bands, of which 22 (81.5%) were polymorphic, with a mean Polymorphism Information Content of 1.236. ISSR primer IS21 and UBC 824 exhibited 100% polymorphism, whereas UBC 822 showed 60%. Jaccard's similarity coefficient ranged from 0.333 to 0.850, indicating extremely high genetic diversity among pineapple accessions. Cluster analysis was conducted using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to generate a dendrogram in NTSYS-pc software. The dendrogram based on UPGMA analysis grouped the 22 pineapple accessions into two main clusters. Cluster I comprised 21 accessions subdivided into two smaller clusters, whereas Cluster II contained a single accession. This study provides valuable data for utilizing genetic diversity in pineapple breeding and estimating heterosis. ISSR analysis proved to be an efficient method for assessing genetic variation, supporting both germplasm conservation, and breeding improvements.

Keywords: pineapple; ISSR markers; genetic diversity

1. Introduction

Globally, pineapple is a highly significant crop, especially in tropical and subtropical areas like Malaysia, where growing them boosts the local economy. The increasing demands for high fruit quality, high yield, and resistance to diseases and pests contribute to the development of numerous pineapple varieties. Genetic resources obtained from assessing genetic diversity within and among cultivars are essential for breeding, conservation, and cultivar identification. Thus, the effectiveness of genetic diversity assessment improves pineapple improvement programs for developing new varieties with enhanced traits. Morphological and molecular analysis can be closely interrelated, and integrating both approaches enables comparison of genetic similarities and genetic diversity among cultivars. However, molecular data are generally more specific and accurate than morphological observations, which can be time-consuming and influenced by environmental conditions (Ismail *et al.,* 2020) [1]. According to Thakur *et al.,* [2], molecular markers are suitable for rapid, cost-effective

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and higher genetic variation, reducing the likelihood of false positive test findings and providing precise data. Due to concurrent evolution, which can obscure phylogenetic reconstruction, molecular sequences are considered more reliable than morphological traits for constructing phylogenetic trees [3].

Inter-simple sequence repeat (ISSR) markers have been widely used in pineapple genetic diversity studies. Several studies reported clusters of pineapple accessions were identified, somaclonal variations was detected and moderate to high levels of polymorphism (48% to 93%) were observed among accessions (Wang *et al.;* Vanijajiva; Harahap *et al.,*) [4-6]. Hence, these markers are suitable for clustering cultivars into distinct groups, supporting their use in germplasm management and breeding programs. According to Hayati *et al.* (2024) [7], ISSR primers are informative for *Ananas comosus* with polymorphism information content (PIC) values ranging from 0.24–0.28.

Pineapple fiber is increasingly used in the textile industry for fabrics manufacturing, thermal insulation and biopolymer coatings. Due to latest eco-friendly technology and sustainable properties, demand for pineapple fiber is increasing. To ensure high quality fiber production, molecular markers have been used to assess the genetic information of different pineapple varieties. Souza *et al*., (2017) [8] studies reported that ISSR markers benefit in marker assisted selection for a high-quality fiber, generating 217 ISSR bands with a strong correlation value that supports early selection of pineapple genotypes. Therefore, efficiency, cost-effectiveness, and capacity to produce repeatable outcomes across a variety of plant species make ISSR markers beneficial. They are especially helpful in breeding programs for maintaining genetic diversity, choosing parents from a variety of backgrounds, and avoiding genotype misidentification.

2. Methodology

2.1 Plant Materials

For this study, a total of 22 pineapple accessions were used (Table 1). Young leaves from each accession were gathered from the Malaysian Agricultural Research and Development Institute (MARDI), Pontian, Johor.

Table 1
List of the 22 accessions of pineapple that were used in the study

No.	Accessions Name	Accessions Code
1.	SS Seedy Local	Seedy
2.	SS Bottle Neck	Bottleneck
3.	M Queen India	Queen
4.	SS Pontian	Pontian
5.	SCT2 Thailand	SCT2
6.	SC Mexico	Mexico
7.	M HQ2 Pakistan	Pakistan
8.	MD2	MD2
9.	M Local	Local
10.	N36	N36
11.	Maspine	Maspine
12.	SC Kucing	Kucing
13.	N Nangka	Namgka
14.	Mas Merah	Masmerah
15.	Johor 1	1
16.	SC Chalok	Chalok

17.	SC Giant	Giant
18.	SC1 Thailand	C1
19.	SS NTT2 Thailand	Thai
20.	Hawaiian Selection	Hawaiian
21.	M SPG4 Perak	Perak
22.	SC Charlotte	Charlotte

Source: MARDI Pontian.

2.2 Genomic DNA Extraction

A young pineapple leaf from each accession was collected and surface sterilized using 70% ethanol. An optimized, high efficiency protocol was then employed to isolate total genomic DNA from each plant sample (L. M. Schiebelhut *et al.* 2017) [9]. Liquid nitrogen was added to a mortar and the leaves were ground into a powder form. 600 µL of extraction buffer containing 10 mL of EDTA, 12.5 mL of 1 M Tris HCl, 20.5 g od NaCl, 7.5 g of CTAB powder and 0.2 µL of mercapto-ethanol were placed in an Eppendorf tube and incubated at 60°C. Ground leaves were transferred into the Eppendorf tube containing extraction buffer and then incubated at 60°C for 55 minutes. The mixture was then filled with a single volume of 24:1 chloroform: isoamyl alcohol. The tube was gently inverted to mix the mixture at room temperature. The tube was then centrifuged at 12,000 rpm for 15 minutes. The produced aqueous phase layer was transferred into a new Eppendorf tube. After adding two-thirds of the cold isopropanol, the tube was carefully inverted to mix and incubated on ice for 30 minutes, followed by centrifuge at 12,000 rpm for 30 minutes at 4°C using ThermoFisher Scientific, Micro 21R Microcentrifuge (United States). The produced pellets of DNA were washed with 70% ethanol and air-dried. Finally, 100 µL of TE buffer was added and incubated overnight at 4°C. On following day, RNAse (10 mg/mL) was heated at 65°C for 20 minutes to denature DNAse, then gradually cooled to room temperature. Subsequently, 1 µL of RNAse (10 mg/mL) was then added into the tube containing genomic DNA. The mixture was incubated at 37°C for 1 hour. The concentration and purity of the isolated DNA were determined using a NanoDrop®, Thermo Scientific's (United States) 2000 UV-vis Spectrophotometer was used to assess. The quality of extracted DNA was carried out using 1% agarose gel electrophoresis stained with florosafe and the gel was visualized under UV light using an Alpha Imager 2200 gel documentation system.

2.3 ISSR Amplification and Data Analysis

A total of five ISSR primers were used in the study (Table 2). Polymerase Chain Reactions (PCR) were performed in a 25 µL reaction volume consist of 12.5 µL of master mix, 1 µL of primer, 3 to 5 µL of DNA template and ddH2O was added to 25 µL. All samples were amplified using QIAGEN, QIAamplifier96 (German) in 0.2 mL tubes. Then, it was subjected to pre-denature for 5 minutes at 94°C, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 51°C for 45 seconds, and extension at 72°C for 90 seconds, with a final extension at 72°C for 5 minutes. The DNA sample volume used for each accession was based on the concentration of the genomic DNA obtained from the previous Nanodrop spectrophotometer readings. PCR products were separated in TAE buffer by 1.2% agarose gel electrophoresis at 100V for 1 hour. The results of the DNA banding patterns were transformed into binary data matrices that were rated according to whether each amplified band was present (1) or absent (0). The investigation only includes bands that are repeatable and clear.

Table 2
List of ISSR primers used in this study

Primer	Primer sequence
IS 21	$(AC)^8$
IS 34/1	$(GA)_8CT$
UBC 822	$(TC)_8A$
UBC 824	$(TC)_8G$
UBC 827	$(AC)_8G$

3. Results & discussion

All pineapple accessions displayed distinct banding patterns with all primers. Primer IS21 amplified a total of seven bands ranging from 500 bp to 1,400 bp, all of which were polymorphic (Figure 1). The amplified products across all primers ranged between 300 and 1,400 bp. A total of five ISSR primers produced 27 bands, and 22 of it were polymorphic and the primer's average EMR was 4.4 (Table 3). Primer UBC 827 has the lowest polymorphism percentage (50%), whereas IS 21 and UBC 824 has 100% polymorphism percentage. This indicates that primer IS 21 and UBC 284 are considered highly polymorphic. The results indicated that the average polymorphism percentage among the five ISSR primers was 79.14%.

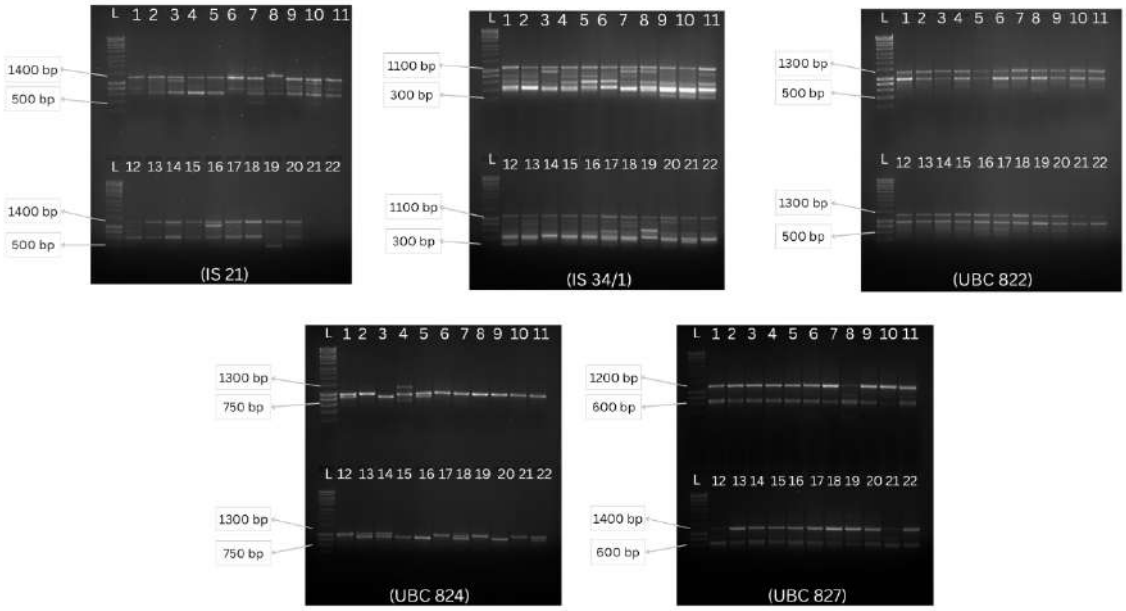

Fig. 1. The inter-simple sequence repeat (ISSR) profiles of IS 21, IS 34/1, UBC 822, UBC 824 and UBC 827 on 22 *Ananas comosus* accessions
Note: **L**: 1kb Ladder; **1**: SS Seedy Local; **2**: N36; **3**: M Queen India; **4**: Mas Merah; **5**: SS Bottle Neck; **6**: Maspine; **7**: SRK Chalok; **8**: SC Charlotte; **9**: SC Giant; **10**: SC1 Thailand; **11**: Johor 1; **12**: Hawaiian Selection; **13**: SS Pontian; **14**: SC Mexico; **15**: M HQ2 Pakistan **16**: M. Local; **17**: SC Kucing; **18**: N. Nangka; **19**: MD2; **20**: M SPG 4 Perak; **21**: SCT2 Thailand **22**: SS NTT2 Thailand

Polymorphic information content (PIC) and marker index (MI) were used to assess the effectiveness of markers in genetic diversity studies based on data gathered from ISSR profiles using the five primers (Table 3). The PIC readings showed an average of 1.7197 and ranged from 0.512 to

2.4196. This indicates that the locus has a significant degree of diversity among the pineapple accessions when PIC>0.5, the measure of gene variation (Igwe et al., 2017) [10]. The MI is a statistical tool that may be used to characterise each primer's ability to detect polymorphic loci among genotypes and determine the overall utility of the marker system (Bidyananda et al., 2024) [11]. Maximum MI (241.96) was produced by primer IS 21 with an average MI of 128.10. The positive association between PIC and EMR is reflected in MI, which is the product of these 2 variables. Polymorphic loci are comparable to EMR readings and associated with the degree of polymorphism.

Dendrogram was constructed using Jaccard's similarity coefficient (Table 4) to illustrate the genetic relationships of 22 pineapple accessions. Five ISSR primers were used to determine Jaccard's pairwise similarity coefficient using the NTSYSpc software. The Jaccard's similarity ranged from 0.333 (M Queen India and MD2) to 0.850 (SC1 Thailand and Johor 1) (Table 4). The resulting phylogenetic analysis is crucial for elucidating biological diversity and genetic classifications among 22 pineapple accessions, hence facilitating the development of future plant breeding systems. The coefficient values derived from dendrogram ranged from 0.58 to 0.89 (Figure 2). Table 4 indicates that there was a considerable degree of genetic heterogeneity among the accessions of a different group, with inter-group distances frequently being more than intra-group distances.

Table 3
ISSR data for molecular characterization 22 pineapple accessions

Primer name	NB	NPB	NMB	PIC value	PP (%)	MI
IS 21	7	7	0	2.4196	100.00	241.96
IS 34/1	7	6	1	2.3155	85.71	198.46
UBC 822	5	3	2	0.5120	60.00	30.72
UBC 824	4	4	0	1.7762	100.00	117.62
UBC 827	4	2	2	1.5750	50.00	78.75
Average	5.4	4.4^{EMR}	1	1.7197	79.14	128.10

Note: **NB**: Sum of bands; **NPB**: Number of Polymorphic Bands; **NMB**: Number of Monomorphic Bands; **PIC**: Polymorphic Information Content; **PP (%)**: percentage of polymorphism; **MI**: marker index

Table 4
Jaccard's similarity coefficients among 22 pineapple accessions studied

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1.000																					
2	0.611	1.000																				
3	0.632	0.429	1.000																			
4	0.591	0.700	0.500	1.000																		
5	0.824	0.667	0.684	0.636	1.000																	
6	0.600	0.722	0.435	0.609	0.571	1.000																
7	0.591	0.545	0.500	0.739	0.565	0.682	1.000															
8	0.591	0.417	0.565	0.600	0.565	0.609	0.667	1.000														
9	0.632	0.500	0.524	0.565	0.600	0.571	0.714	0.714	1.000													
10	0.500	0.455	0.478	0.583	0.478	0.591	0.727	0.652	0.789	1.000												
11	0.545	0.435	0.458	0.625	0.458	0.565	0.772	0.696	0.750	0.850	1.000											
12	0.571	0.524	0.478	0.520	0.545	0.522	0.583	0.652	0.700	0.565	0.682	1.000										
13	0.650	0.455	0.545	0.583	0.545	0.591	0.652	0.583	0.619	0.636	0.762	0.636	1.000									
14	0.550	0.500	0.600	0.565	0.684	0.571	0.636	0.636	0.600	0.545	0.522	0.619	0.545	1.000								
15	0.409	0.500	0.600	0.500	0.524	0.500	0.500	0.440	0.455	0.545	0.522	0.545	0.619	0.600	1.000							
16	0.476	0.429	0.684	0.500	0.600	0.435	0.500	0.500	0.600	0.545	0.591	0.619	0.700	0.600	0.778	1.000						
17	0.619	0.571	0.667	0.560	0.750	0.565	0.625	0.625	0.667	0.609	0.653	0.682	0.608	0.667	0.667	0.750	1.000					
18	0.684	0.722	0.500	0.681	0.737	0.789	0.682	0.512	0.571	0.591	0.565	0.522	0.667	0.650	0.650	0.571	0.714	1.000				
19	0.421	0.625	0.333	0.524	0.474	0.706	0.652	0.455	0.556	0.579	0.550	0.500	0.500	0.647	0.556	0.474	0.550	0.706	1.000			
20	0.375	0.391	0.619	0.520	0.470	0.458	0.600	0.520	0.478	0.636	0.609	0.500	0.565	0.619	0.789	0.700	0.682	0.591	0.500	1.000		
21	0.524	0.409	0.500	0.542	0.435	0.545	0.608	0.609	0.500	0.522	0.636	0.458	0.521	0.435	0.375	0.435	0.565	0.478	0.450	0.458	1.000	
22	0.588	0.529	0.474	0.391	0.647	0.450	0.391	0.391	0.400	0.429	0.409	0.500	0.500	0.556	0.474	0.474	0.550	0.526	0.500	0.429	0.381	1.000

Note: **L**: 1kb Ladder; **1**: SS Seedy Local; **2**: N36; **3**: M Queen India; **4**: Mas Merah; **5**: SS Bottle Neck; **6**: Maspine; **7**: SRK Chalok; **8**: SC Charlotte; **9**: SC Giant; **10**: SC1 Thailand; **11**: Johor 1; **12**: Hawaiian Selection; **13**: SS Pontian; **14**: SC Mexico; **15**: M HQ2 Pakistan **16**: M. Local; **17**: SC Kucing; **18**: N. Nangka; **19**: MD2; **20**: M SPG 4 Perak; **21**: **KIV 22**: SS NTT2 Thailand

Based on phylogenetic tree analysis, all pineapple accessions were grouped into two clusters with a 0.58 similarity coefficient (Figure 2). Cluster I was the largest group which consisted of 21 accessions and were substituted into two groups. Sub 1 consists of 9 accessions (Seedy, Bottleneck, Queen, Pontian, SCT2, Mexico, Pakistan, MD2 and Local), while sub 2 consists of 12 accessions (N36, Maspine, Kucing, Namgka, Masmerah, 1, Chalok, Giant, C1, Thai, Charlotte and Hawaiian). Cluster II only consists of 1 accession which is Perak. This demonstrated how similarly separate segments are to one another. In Sub 1, Seedy and Bottleneck has the highest similarity (0.89). Sub 2 shows high similarity between C1 and Thai, it was clear that these accessions had evolved close to one another and might have been impacted by comparable evolutionary causes. According to Zhao et al. (2021) [12] to comprehend how plants adapt to climate change and create efficient conservation and management plans, it is crucial to comprehend genetic variability and structure, adaptive genetic variation, and its link with environmental conditions. SS Seedy Local and Perak came from different cluster hence having low coefficient value of 0.375.

Furthermore, Wang *et al.,* (2017) [4] studies shows that 13 ISSR markers were 93.65% polymorphic among 36 different pineapple accessions, which suggest high genetic diversity in pineapple, and Harahap *et al.,* (2021) [6] reported that ISSR analysis can distinguish between major cultivars and somaclonal variation in tissue cultured plantlets. Pendi *et al.,* (2022) [13] stated that pineapple morphological abnormalities can be prevented by using genetic variation determination. ISSR analysis is valuable in a wide range of applications, including hybridisation and taxonomic studies, hence it also supports the use of ISSR markers in research on phylogeny, genetic diversity, genome mapping, gene tagging, and evolutionary biology (Murphy *et al.,* 2024) [14].

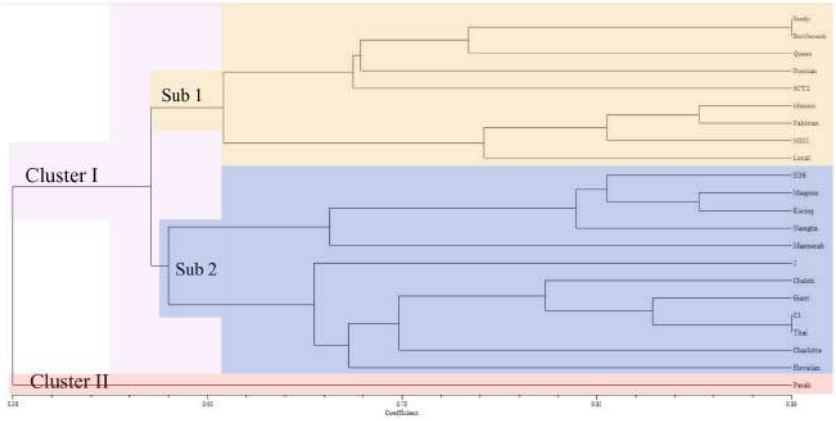

Fig 2. Unweighted Pair-Group Method with Arithmetic Averages (UPGMA) dendrogram estimating the genetic distance among 22 pineapple accessions
Note: Seedy: SS Seedy Local; Bottleneck: SS Bottle Neck; Queen: M Queen India; Pontian: SS Pontian; SCT2: SCT2 Thailand; Mexico: SC Mexico; Pakistan: M HQ2 Pakistan; MD2: MD2; Local: M Local; N36: N36; Maspine: Maspine; Kucing: SC Kucing; Namgka: N Nangka; Masmerah: Mas Merah; 1: Johor 1; Chalok: SRK Chalok; Giant: SC Giant; C1: SC1 Thailand; Thai: SS NTT2 Thailand; Charlotte: SC Charlotte; Hawaiian: Hawaiian Selection; Perak: M SPG4 Perak

4. Conclusions

This study effectively characterized the molecular diversity of pineapple accessions using ISSR primers. Each primer produced distinct banding patterns, and most of the bands were polymorphic, meaning they successfully captured differences among the accessions. Overall, 27 bands were amplified, 22 of which were polymorphic, resulting in an average polymorphism rate of 79.14%. The high average PIC value (1.7197), with all primers are PIC > 0.5, confirms substantial genetic diversity within the accessions. IS21 produced the highest MI value, presents its reliability in detecting informative loci. Phylogenetic analysis grouped all accessions into 2 major clusters (I and II). Overall, the high degree of polymorphism in pineapple found by the ISSR markers used in this study suggests that there are genetic variations present. By using the data from this study, it will be possible to overcome the limitations on pineapple genetic diversity, which will encourage more future research on pineapple.

Acknowledgement

This research was funded by a grant from Ministry of Higher Education of Malaysia (FGRS Grant 24-345-0954), This research is supported by MARDI Pontian by supplying pineapple leaves from each accession under the supervision of Halimaton Saadiah and Nurul Shamimi.

References

[1] Ismail, Siti Norhayati, Nurul Shamimi Abdul Ghani, Shahril Firdaus Ab. Razak, Rabiatul Adawiah Zainal Abidin, Muhammad Fairuz Mohd Yusof, Mohd Nizam Zubir, and Rozlaily Zainol. "Genetic Diversity of Pineapple (*Ananas comosus*) Germplasm in Malaysia Using Simple Sequence Repeat (SSR) Markers." *Tropical life sciences research* vol. 31, no. 3 (2020): 15-27. http://doi.org/10.21315/tlsr2020.31.3.2

[2] Thakur, Ritik, Vikram Singh, and Dhrubajyoti Banerjee. (2023). "Use of Molecular Marker in Fruit Crops for Their Traits and Genetical Diversity Analysis." *E3S Web of Conferences*. EDP Sciences. https://doi.org/10.1051/e3sconf/202345301020

[3] Zou, Zhengting, and Jianzhi Zhang. "Morphological and molecular convergences in mammalian phylogenetics." *Nature communications* vol. 7 12758. 2 (2016). https://doi.org/10.1038/ncomms12758

[4] Wang, Jian-Sheng, Jun-Hu He, Hua-Rui Chen, Ye-Yuan Chen, and Fei Qiao. "Genetic Diversity in Various Accessions of Pineapple [Ananas comosus (L.) Merr.] Using ISSR and SSR Markers." *Biochemical genetics* vol. 55, no. 5-6 (2017): 347-366. https://doi.org/10.1007/s10528-017-9803-z

[5] Vanijajiva, O. "Assessment of genetic diversity and relationships in pineapple cultivars from Thailand using ISSR marker."*International Journal of Agricultural Technology* 8, no. 5 (2012): 1829-1838. http://www.ijat-aatsea.com

[6] Harahap, Fauziyah, Annisa Afiva, Miftahul Jannah, and Eko Prasetya. "ISSR Based Analysis of Genetic Variability of Plantlets Culture of Pineapple (Ananas Comosus L.) from Sipahutar, North Sumatera, Indonesia," Biogenesis Jurnal Ilmiah Biologi 9, no. 1 (2021): 35. https://doi.org/10.24252/bio.v9i1.17068

[7] Hayati, Risyda, and Rina Sri Kasiamdari. "Genetic Diversity of Indonesian Pineapple (Ananas comosus (L.) Merr.) Cultivars Based on ISSR Markers.". *Pertanika Journal of Tropical Agricaltural science,* no. 4 (2024) https://doi.org/10.47836/pjtas.47.4.02.

[8] Souza, C., Ferreira, C., Souza, E., Neto, A., Marconcini, J., Ledo, C., & Souza, F. "Genetic diversity and ISSR marker association with the quality of pineapple fiber for use in industry." I*ndustrial Crops and Products*, (2017)104, 263-268. https://doi.org/10.1016/j.indcrop.2017.04.059.

[9] Schiebelhut, L., Abboud, S., Daglio, G., Swift, H., & Dawson, M. "A comparison of DNA extraction methods for high-throughput DNA analyses". *Molecular Ecology Resources*, 17. (2017) https://doi.org/10.1111/1755-0998.12620 .

[10] Igwe, D., Afiukwa, C., Ubi, B., Ogbu, K., Ojuederie, O., & Ude, G. "Assessment of genetic diversity in Vigna unguiculata L. (Walp) accessions using inter-simple sequence repeat (ISSR) and start codon targeted (SCoT) polymorphic markers." *BMC Genetics*, 18. (2017) https://doi.org/10.1186/s12863-017-0567-6.

[11] Bidyananda, Nongthombam, Imlitoshi Jamir, Karolina Nowakowska, Vanlalrinchhani Varte, Wagner A. Vendrame, Rajkumari Sanayaima Devi, and Potshangbam Nongdam. "Plant Genetic Diversity Studies: Insights from DNA Marker Analyses" *International Journal of Plant Biology* 15, no. 3 (2024): 607-640. https://doi.org/10.3390/ijpb15030046

[12] Zhao, Wei, Xiaolong Wang, Lin Li, Jiangnan Li, Hang Yin, Ying Zhao, and Xia Chen. "Evaluation of Environmental Factors Affecting the Genetic Diversity, Genetic Structure, and the Potential Distribution of Rhododendron Aureum Georgi Under Changing Climate." Ecology and Evolution 11, no. 18 (2021): 12294–306. https://doi.org/10.1002/ece3.7803.

[13] Murphy, Deanne, Nigel Paul Barker, and Arnold Frisby. "Automated ISSR Fingerprinting Is a Cost-Effective Way to Assess Genetic Diversity and Taxonomic Differentiation—A Case Study on the Encephalartos Eugene-maraisii Species Complex." Diversity 16, no. 8 (August 20, 2024): 507. https://doi.org/10.3390/d16080507.

[14] Pendi, Fifi Hafizzah, Hasnain Hussain, Faisal Ali Anwarali Khan, and Bala Jamel. "Genetic variations of in vitro propagated MD2 pineapples using DNA-based markers." *Journal of Sustainability Science and Management* 17, no. 8 (2022): 2-10.http://doi.org/10.46754/jssm.2022.08.002