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Genetic characterization of cassava (*Manihot esculenta* Crantz) cultivars using ISSR molecular marker

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Abstract The genetic diversity of 22 cassava varieties using the inter-simple sequence repeat (ISSR) molecular marker was investigated. Genetic similarity was derived from the matrix of simple similarity coefficients using the unweighted pair group method with arithmetic average (UPGMA). The genetic similarity coefficient ranged from 0.73 to 0.94. The similarity coefficient at 0.73, cluster analysis was clustered to be 22 cassava varieties into two significant groups. The first group was the sweet variety of cassava used for consumption, consisting of Rayong2, Ha-na-tee and variegated leaves used for decoration. Another group, the bitter variety of cassava used for industry, consisted of Rayong1, Rayong3, Rayong5, Rayong7, Rayong9, Rayong11, Rayong15, Rayong60, Rayong72, Rayong86-13, Rayong90, Kasetsart50, Kasetsart72, Huay Bong60, Huay Bong80, Huay Bong90, Pirun1, Pirun2 and unknown. The sweet and bitter variety contained different levels of cyanide content. So, the ISSR marker might be able characterize two major cassava varieties.

Keywords: Cassava, *Manihot esculenta*, ISSR, Molecular marker

Introduction

Cassava (*Manihot esculenta* Crantz) is a crop abundant in carbohydrates, offering versatile uses. It serves as a food source for humans, animal fodder, a raw material for fuel ethanol production, and a vital component in diverse industries, contributing to the manufacturing of alcohol, citric acid, clothing, medicines, paper, and chemicals. So, cassava is becoming a high-value crop, the world's 5th most important crop, after corn, wheat, rice, and potatoes. Thailand

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is the world's third largest cassava producer after Nigeria and Congo, and it is the largest exporter of cassava. Cassava propagation can occur through either sexual seeds or asexual stem cuttings. Nevertheless, farmers' predominant and widely adopted method is using stem cuttings, which serve as a conventional and straightforward approach for multiplication and replanting. This propagation creates offspring that are genetically identical to their parent. It creates cassava genotypes that do not respond differently to diverse environments. However, cassava is grown in almost all country areas with various environments. Therefore, sexual seeds in breeding programs are required.

Many breeding programs aim to broaden the genetic foundation and enhance the selection of genetically diverse parents in crossbreeding initiatives. The goal is to generate new clones that exhibit desirable traits based on agricultural demands, such as enhancing yield, quality, adaptability, stress tolerance, and resistance to drought, pests, and diseases. The diversity of genotypes can be assessed using markers derived from morphological, biochemical, and molecular data. Morphological traits exhibit significant polymorphism and are strongly influenced by environmental factors, posing considerable challenges in distinguishing genotypes solely through morphological characters.

Molecular markers serve as a valuable tool in addressing the intricate genetic diversity of cassava, revealing detailed genetic differences without being influenced by environmental factors. In examining cassava, various molecular techniques have been employed to investigate genetic diversity. Marmey *et al*. (1994), Zacarias *et al*. (2004), and Rimoldi *et al*. (2010) have utilized random amplified polymorphic DNA (RAPD), while Beeching *et al*. (1993) employed restriction fragment length polymorphism (RFLP). Amplified fragment length polymorphism (AFLP) has been applied by Roa *et al*. (1997) and Wong *et al*. (1999). Additionally, inter-simple sequence repeats (ISSR) have been studied by Zayed *et al*. (2013), Vidal *et al*. (2015), Tiago *et al*. (2016), Afonso *et al*. (2019), Asha *et al*. (2019), and Asha *et al*. (2020). Microsatellite or simple sequence repeats (SSR) have been explored by Lyimo *et al*. (2013), Mohan *et al*. (2013), Asha *et al*. (2019), and Adjebeng-Danquah *et al*. (2020), while single nucleotide polymorphism (SNP) analysis has been conducted by Prempeh *et al*. (2020).

Within Thailand, genetic diversity assessments of certain cassava cultivars and germplasm have employed various techniques, including RAPD (Thanananta *et al*., 2012), HAT-RAPD (Rangsiruji *et al*., 2019), and SSR (Ruttawat *et al*., 2011; Wangsomnuk *et al*., 2013). The Inter Simple Sequence Repeat (ISSR) is a marker technique utilizing microsatellite core sequences as primers in a polymerase chain reaction (PCR). This method enables the simultaneous analysis of multiple loci within a single reaction. ISSR seamlessly

integrates the benefits of AFLP and SSR analyses while retaining the simplicity and speed of RAPD. Therefore, it is a straightforward, rapid, and cost-effective molecular technique. The objective was to showcase the application of ISSR markers for assessing the genetic diversity among cassava cultivars gathered from the germplasm collection at the Rayong Field Crops Research Center, Department of Agriculture, in Rayong province, Thailand.

Materials and methods

Plant materials and DNA extraction

The study entailed the analysis of leaves from twenty Thai cassava varieties sourced from the cassava germplasm collection at the Rayong Field Crops Research Center, Department of Agriculture, Rayong province, Thailand. Additionally, two cassava varieties with unknown genetic backgrounds were collected from farms in Suphan Buri province, Thailand. This study used 22 Thai cassava variety samples of three groups: the first group is the sweet variety of cassava used for consumption, consisting of Rayong2 (R2), Pirun2 (PR2), and Ha-na-tee (M5), and the second group has variegated leaves (D1) used for decoration. The third group, the bitter variety of cassava used for industry, consists of Rayong1 (R1), Rayong3 (R3), Rayong5 (R5), Rayong7 (R7), Rayong9 (R9), Rayong11 (R11), Rayong15 (R15), Rayong60 (R60), Rayong72 (R72), Rayong86-13 (R86-13), Rayong90 (R90), Kasetsart50 (KU50), Kasetsart72 (KU72), Huay Bong60 (HB60), Huay Bong80 (HB80), Huay Bong90 (HB90), Pirun1 (PR1), and unknown (PC2). The fresh and healthy uninfected leaf samples were collected, transported to our laboratory, washed with clean water, air-dried and kept at -80°C until DNA extraction. The modified cetyltrimethylammonium bromide (CTAB) method extracted the genomic DNA from leaf material. The precipitated DNA was purified using the GF-1 AmbiClean Kit (Vivantis). Finally, gel electrophoresis and absorbance evaluate the high-quality genomic DNA concentration.

Inter Simple Sequence Repeat (ISSR) marker

Two cassava varieties with two different principal types, sweet cassava (Ha-na-tee, M5) and bitter cassava (Rayong7, M7), were selected for primer screening in a preliminary study. Thirty-nine ISSRs (UBC-University of British Columbia) were initially screened for genetic profile analysis as seen in Table 1. The PCR reactions were carried out in a total volume of 20 µl, consisting of 100 ng of high-quality genomic DNA, 0.5 µM of each primer, 0.20 mM dNTP mix,

2.0 mM $MgCl_2$, 1U of *Taq* DNA polymerase, and 1× PCR buffer, following the established protocols outlined by Mouhamady *et al.* (2020). The PCR amplification process commenced with an initial denaturation step at 94°C for 5 minutes, followed by 35 cycles. Each cycle comprised 45 seconds of denaturation at 94°C, 50 seconds of annealing in the temperature range of 49-55°C, and 1 minute of elongation at 72°C. The amplification concluded with a final extension step lasting 10 minutes at 72°C. The ISSR fragments obtained were then separated on a 2% agarose gel, which was stained with ethidium bromide to establish the ISSR profile.

Data scoring and analysis

Data scoring and analysis of ISSR markers involved comparing banding patterns, where a score of 1 was assigned for presence and 0 for absence, using a binary character system. The simple matching coefficients among the 22 cassava varieties were calculated to evaluate genetic similarity. The dendrogram was constructed using UPGMA by the NTSYSpc 2.11X software package based on genetic similarity matrices.

Table 1. ISSR primer codes and their corresponding sequences (5'-3') employed in this investigation

ISSR primer	Sequence (5'-3')	ISSR primer	Sequence (5'-3')
UBC801	ATATATATATATATATT	UBC834	GAGGAGAGAGAGAGAG(CT)T
UBC807	AGAGAGAGAGAGAGAGT	UBC835	GAGGAGAGAGAGAGAG(CT)C
UBC808	AGAGAGAGAGAGAGAGC	UBC836	AGAGAGAGAGAGAGAGCA
UBC809	AGAGAGAGAGAGAGAGG	UBC842	GAGAGAGAGAGAGAGAYG
UBC810	GAGAGAGAGAGAGAGAT	UBC844	CTCTCTCTCTCTCTCTGC
UBC811	GAGAGAGAGAGAGAGAC	UBC847	CACACACACACACACARC
UBC813	CTCTCTCTCTCTCTCTT	UBC849	GTGTGTGTGTGTGTGTYC
UBC814	CTCTCTCTCTCTCTCTA	UBC855	ACACACACACACACAC(CT)T
UBC815	CTCTCTCTCTCTCTCTG	UBC856	ACACACACACACACACYA
UBC817	CACACACACACACACAA	UBC857	ACACACACACACACACTG
UBC818	CACACACACACACACAG	UBC861	ACCACCACCACCACCACC
UBC819	GTGTGTGTGTGTGTGTA	UBC862	AGCAGCAGCAGCAGCAGCT
UBC820	GTGTGTGTGTGTGTGTC	UBC863	AGTAGTAGTAGTAGTAGT
UBC821	GTGTGTGTGTGTGTGTT	UBC866	CTCCTCCTCCTCCTCCTC
UBC822	TCTCTCTCTCTCTCTCA	UBC868	GAAGAAGAAGAAGAAGAA
UBC824	TCTCTCTCTCTCTCTCG	UBC870	TGCTGCTGCTGCTGCTGC
UBC825	ACACACACACACACACT	UBC873	GACAGACAGACAGACA
UBC826	ACACACACACACACACC	UBC880	TCTCTCTCTCTCTCTCAA
UBC827	ACACACACACACACACG	UBC881	GGGTGGGGTGGGGTG
UBC828	GTGTGTGTGTGTGTGTA		

Results

ISSR marker profile

The aim was created the dependable and swift detection markers and explore the genetic diversity among cassava varieties utilizing ISSR markers. In a preliminary study, two varieties, M5 (Ha-na-tee) and R7 (Rayong7), which have different types, were selected to be studied for primer screening. Initially, thirty-nine ISSR primers underwent screening for genetic profiling, with only 30 primers yielding reproducible fragments. Most primers showed reproducible DNA fragments with given polymorphisms (Figure 1A) and monomorphic bands (Figure 1B). However, some primers showed non-specific amplification bands that showed a smear in the gel (Figure 1C) and could not amplify (Figure 1D). From 39 ISSR primers, only twelve primers showed reproducible fragments with easily recordable bands and gave polymorphisms. However, only six primers (UBC808, UBC814, UBC822, UBC835, UBC844, and UBC856) showed easily recordable bands among 22 cassava varieties used to construct the dendrogram. The examples of the ISSR profiles from 22 cassava varieties revealed by the UBC856 primer are shown in Figure 2.

The count of recordable loci varies for each primer, ranging from 11 (UBC822) to 20 (UBC808). The average number of bands per primer is 16.33, and their sizes span from 250 (UBC835 and UBC844) to 2,500 (UBC822) base pairs (bp). These six primers produced up to 98 bands, of which 61 (62.87%) were polymorphic, and the UBC856 primer gave the highest number of polymorphic fragments and percent polymorphism. The examination of polymorphic information content (PIC) revealed an average PIC value of 0.43, ranging from a 0.17 (minimum) to 0.75 (maximum). Table 2 summarizes the primers and amplified products obtained in this study.

ISSR relationship between varieties

The pair-wise genetic similarity estimates among the 22 genotypes varied from 0.62 to 0.94. The highest genetic similarity of 0.94 was observed between Huay Bong80 (HB80) and Pirun1 (PR1), indicating a closer relationship. Conversely, the lowest genetic similarity of 0.62 was noted between variegated leaves (D1) and Rayong15 (R15). The simple matching coefficient among 22 cassava varieties was used to calculate and construct a dendrogram (Figure 3) by UPGMA analysis. Based on a cut-off point of 0.73, the population was divided into two major groups. The major group I related to the bitter variety of cassava used for the industry was divided into three sub-groups and one individual

(Rayong1: R1). The first sub-group comprises two cassava varieties: Rayong3 (R3) and Rayong5 (R5). Inside the second sub-group were ten cassava varieties: Rayong7 (R7), Rayong9 (R9), Rayong11 (R11), Rayong60 (R60), Rayong72 (R72), Rayong90 (R90), Rayong86-13 (R86-13), Kasetsart50 (KU50), Kasetsart72 (KU72) and Huay Bong60 (HB60), with close relationship to third sub-group consist of six cassava varieties: Rayong15 (R15), Huay Bong80 (HB80), Pirun1 (PR1), Huay Bong90 (HB90), Pirun2 (PR2) and unknown (PC2). Group II is the sweet variety of cassava used for consumption, consisting of Rayong2 (R2), Ha-na-tee (M5), and variegated leaves (D1) used for decoration.

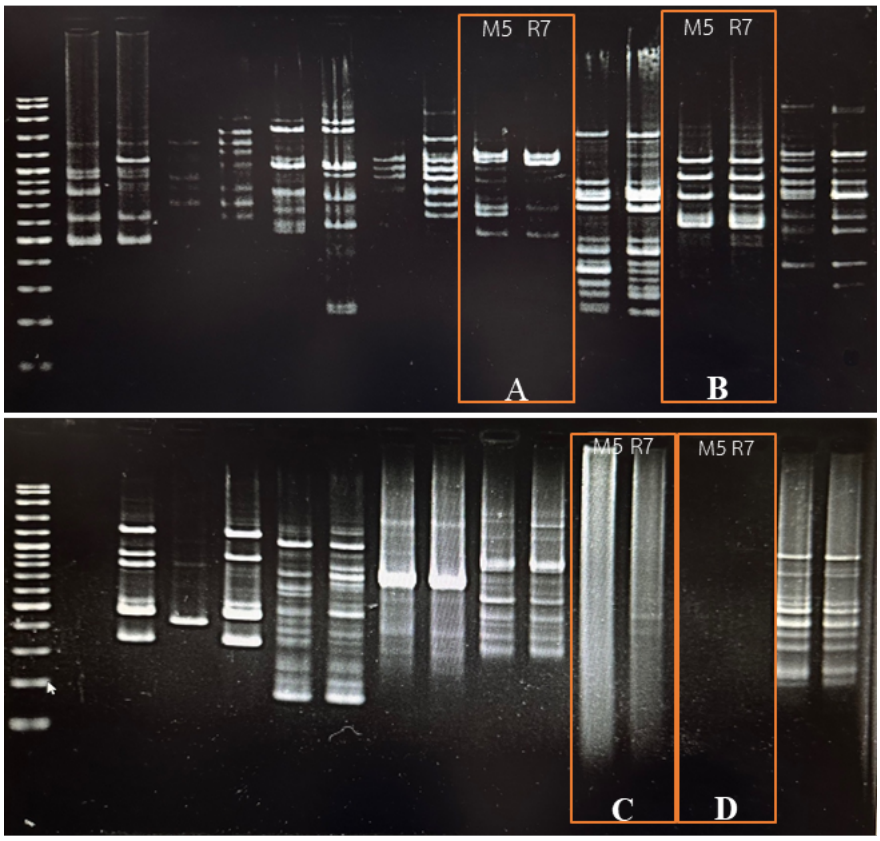

Figure 1. ISSR profiles of two different cassava varieties: sweet cassava (Ha-na-tee, M5) and bitter cassava (Rayong7) A: polymorphic bands, B: monomorphic bands, C: non-specific bands, D: no band

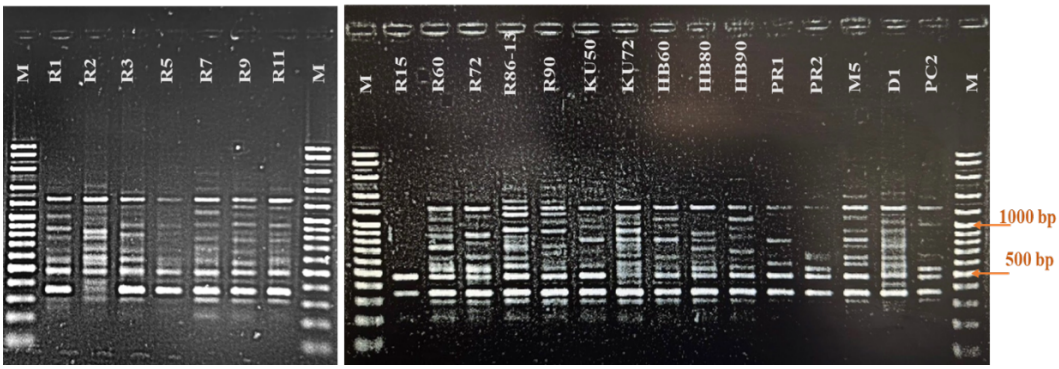

Figure 2. ISSR profiles generated by the UBC856 primer for 22 cassava varieties (Lane M: VC 100 bp plus DNA Ladder) were presented

Table 2. Analysis of ISSR markers in 22 cassava varieties provided details on primer codes, fragment sizes, total amplified fragments, polymorphic fragments, percentage of polymorphism, and polymorphism information content

Primers	Fragment size (bp)	Number of total amplified fragments	Number of polymorphic fragments	Percent polymorphism	Polymorphism information content
UBC808	320-2250	20	9	45	0.17
UBC814	320-2250	14	8	57.14	0.60
UBC822	450-2500	11	8	72.73	0.75
UBC835	250-1500	17	7	41.18	0.40
UBC844	250-1400	18	12	66.67	0.33
UBC856	300-1500	18	17	94.44	0.33
Total		98	61	377.16	2.58
Average		16.33	10.17	62.87	0.43

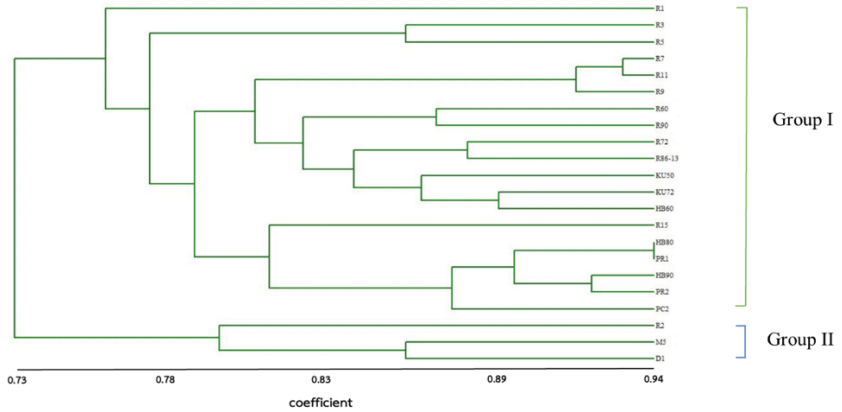

Figure 3. A dendrogram illustrating the relationships among 22 cassava varieties was generated from ISSR data utilizing the UPGMA method and relying on the simple matching coefficient

Discussion

Asexual propagation of cassava through stem cuttings resulted in genetic uniformity for a simple method for farmers to propagate cassava. This can be advantageous for maintaining desirable traits but poses risks regarding vulnerability to diseases or environmental changes, and it also facilitates the spread of diseases (Chavarriaga-Aguirre *et al.,* 2016). Thai farmers have encountered challenges which related to pathogen contamination in the plants they intend to cultivate, with a particular emphasis on the prevalence of cassava leaf spot disease. On the other hand, sexual seed propagation introduces genetic diversity, allowing for adaptation to various environmental conditions and potential resistance to diseases. This method is suitable for breeding programs research on promoting cassava cultivation, which is essential for developing new cassava varieties with improved traits such as disease resistance, higher yields, and nutritional content.

Genotype diversity can be determined which based on markers of morphological, biochemical, and molecular information. Enhancing cassava varieties through breeding programs using morphology is a time-consuming process. The morphological traits exhibit significant polymorphism and are greatly influenced by environmental factors, making the differentiation of genotypes based on these characteristics extremely challenging. Consequently, the exploration of biological molecules becomes imperative in this context, like Mbanjo's research (Mbanjo *et al*., 2021).

Molecular markers provide a more precise and reliable means of identifying genetic differences among cassava varieties. This saves time and ensures that the selected traits are accurately passed on to the next generations. While various molecular markers have been employed in cassava research, presently, microsatellite or SSR and SNP markers are the most frequently utilized in numerous molecular studies, particularly in the fields of population genetics, linkage mapping, association studies, and diversity analysis (Tiago *et al*., 2017; de Albuquerque *et al*., 2018; Albuquerque *et al*., 2019; Adjebeng-Danquah *et al*., 2020; Prempeh *et al*., 2020). SSR and SNP analysis can reveal information about the evolutionary history, population structure, and adaptation of cassava varieties. It can also assist in identifying genes associated with important traits such as disease resistance, yield, and nutritional content. However, SSR and SNP genotyping technologies can be expensive for large-scale studies. The initial investment in equipment reagents and labor-intensive can be a barrier for some research groups. For ISSR markers, the studies by Zayed *et al*. (2013), Vidal *et al*. (2015), Tiago *et al*. (2016), Asha *et al.* (2019; 2020), and Afonso *et al*. (2019) are valuable tools for researchers studying genetic diversity, population structure, and breeding strategies in cassava, such as the genetic fidelity and variability of

micropropagated cassava plants, which has practical implications for agricultural practices and crop improvement programs. ISSR markers are characterized by their simplicity, cost-effectiveness, and ability to generate highly polymorphic and reproducible results.

In Thailand, the molecular genetic studies conducted on cassava have utilized a range of techniques, including RAPD, HAT-RAPD, and SSR markers (Ruttawat *et al*., 2011; Thanananta *et al*., 2012; Wangsomnuk *et al*., 2013; Rangsiruji *et al*., 2019). Each technique has its advantages and limitations. No recorded studies conducted in Thailand specifically employ ISSR markers for cassava research. In this manuscript, the research team aims to investigate the application of ISSR markers in discerning the genetic diversity of cassava varieties. Initially, cassava samples sourced from the Rayong Field Crops Research Center, Department of Agriculture, Rayong Province, Thailand, were employed for the study.

PCR-based molecular tools known as ISSR markers are designed to amplify regions between microsatellite motifs within the genome. In the analysis of genetic profiles, ISSR markers employ a variety of primer numbers, types, and positions. This study characterized molecular variability among the 22 cassava varieties using six selected ISSR primers (UBC808, UBC814, UBC822, UBC835, UBC844, and UBC856). The amount and type are not different from the previous study by Asha *et al.* (2019), which used six selected ISSR primers (UBC807, UBC808, UBC811, UBC817, UBC836, and UBC845) and five selected ISSR primers (UBC808, UBC811, UBC817, UBC835, and UBC845) by Asha *et al.* (2020). However, some papers that used about 10 to 24 primers that revealed satisfactory levels of polymorphic loci were selected. UBC808 yielded the highest number of bands (20) among the selected primers, while UBC822 generated the lowest number of bands (11), with an average of 16.33 bands per primer. UBC808 consistently exhibited polymorphisms in multiple studies (Tiago *et al*., 2016; Asha *et al*., 2019; 2020). Nevertheless, the PIC for individual markers varied from 0.17 (UBC808) to 0.75 (UBC822), with an average of 0.43. Primers UBC822 and UBC814 exhibited the highest PIC values (0.75 and 0.60, respectively), making them the most informative loci in the analysis.

This metric aids in prioritizing primers according to their efficiency in detecting polymorphisms, serving as a criterion for primer selection. Generally, PIC values greater than 0.5 indicate highly informative markers, values between 0.25 and 0.5 indicate moderately informative markers, and values below 0.25 suggest slightly informative markers. The markers unveiled an overall polymorphism of 62.87% (61 out of 98 bands), averaging 10.48% polymorphic fragments per primer. This indicates the presence of genetic diversity among the

examined genotypes. Comparable findings were reported by Vidal *et al*. (2015), revealing a total polymorphism of 57.1% (100 out of 175 bands) in their assessment of 22 cassava accessions from the Germplasm Bank of Embrapa Cassava and Fruit Farming of Cruz das Almas BA, Brazil. Tiago *et al*. (2016) observed a total polymorphism of 61.67% (74 out of 120 bands) when evaluating the genetic diversity of cassava landraces cultivated by farmers in the north of Mato Grosso State, Brazil. Additionally, Zayed *et al.* (2013) found a total polymorphism of 54.43% among Indonesian, Brazilian, Thai (Rayong 60), and Thai (Huay Bong 60) cassava genotypes. However, they found more significant polymorphism (93.24%) in their evaluation of cassava genotypes of cassava crops in Angola (Afonso *et al*., 2019).

The simple matching coefficient was employed for cluster analysis to calculate and form a dendrogram through UPGMA analysis for 22 cassava varieties. The population was categorized into two primary groups using a 0.73 coefficient cut-off point for the 5 ISSR markers. Major group I corresponds to the bitter cassava variety utilized in the industry, while group II comprises the sweet cassava variety consumed directly. This group includes Rayong2 (R2), Ha-na-tee (M5), and variegated leaves (D1), the latter used for decorative purposes. Based on this study, the grouping appears to be delineated based on the hydrocyanic acid (HCN) levels present in the cassava roots. Cassava cultivars are broadly categorized into two primary types, bitter and sweet, determined by HCN levels that can convert to cyanide. Sweet cassava varieties have roots containing less than 50 mg/kg of HCN on a fresh weight basis, while bitter varieties may have up to 100 mg/kg of HCN. As an illustration, Rayong2 and Ha-na-tee, representing the sweet cassava variety, exhibit approximately 52.80 and 31.23 mg/kg of HCN, respectively. In contrast, Kasetsart50 representing the bitter cassava variety, show approximately 500.00 mg/kg of HCN. Nevertheless, the diversity of cassava aside, hydrogen cyanide levels are influenced by factors such as altitude, geographic location, harvest times, and seasonal conditions. (Ndam *et al.,* 2019). Even the sweet cassava with little cyanide can be effectively reduced with peeling and thorough cooking. In this study, Pirun2 (PR2), which belongs to the sweet cassava variety, will be excluded. So, the ISSR marker might be able characterize two major cassava varieties.

Thirty-nine ISSR primers were initially screened for genetic profiles. However, only six primers (UBC808, UBC814, UBC822, UBC835, UBC844, and UBC856) showed easily recordable bands among 22 cassava varieties used to construct the dendrogram. At 0.73 coefficient, cluster analysis clustered 22 cassava varieties into two groups, the sweet and bitter. The result indicated that the ISSR primers selected for the present study will be helpful for future molecular profiling of cassava germplasm.

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References

Adjebeng-Danquah, J., Manu-Aduening, J., Asante, I. K., Agyare, R. Y., Gracen, V. and Offei, S. K. (2020). Genetic diversity and population structure analysis of Ghanaian and exotic cassava accessions using simple sequence repeat (SSR). Heliyon, 6:1-9.

Afonso, S. D. J., Moreira, R. F. C., de Silva, C. A. L., Ferreira, C. F., da Silva Santos, V. and Muondo, P. A. (2019). Genetic structure of cassava populations (*Manihot esculenta* Crantz) from Angola assessed through (ISSR) markers. African Journal of Biotechnology, 18:144-154.

Albuquerque, H. D. Y. G., Carmo, C. D. D., Brito, A. C. and Oliveira, E. D. J. (2018). Genetic diversity of *Manihot esculenta* crantz germplasm based on single-nucleotide polymorphism markers. Annals of Applied Biology, 173:271-284.

Albuquerque, H. D. Y. G., Oliveira, E. D. J., Brito, A. C., Andrade, L. D. R. B., Carmo, C. D. D., Morgante, C. V., Vieira, E. A., Moura, E. and Faleiro, F. G. (2019). Identification of duplicates in cassava germplasm banks based on single-nucleotide polymorphisms (SNPs). Scientia Agricola, 76:328-336.

Asha, K. I. Varghese, A. M., Radhika, N. K., Koundinya, A. V. V. and Krishnan B. S. P. (2019). Molecular profiling of selected cassava (*Manihot esculenta* Crantz) germplasm using ISSR and SSR markers. Journal of Root Crops, 45:24-32.

Asha, K. I., Philip, R. R. and Mohan, N. (2020). Molecular profiling of landraces of Cassava (*Manihot esculenta* Crantz) using ISSR markers. International Journal of Research in Engineering, Science and Management, 3:343-349.

Beeching, J. R., Marmey, P., Galvada, M. C., Noirot, M., Haysom, H. R., Hughes, M. A. and Charrier, A. (1993). An assessment of genetic diversity within a collection of cassava (*Manihot esculenta* Crantz) germplasm using molecular markers. Annals of Botany, 72:515-520.

Chavarriaga-Aguirre, P., Brand, A., Medina, A., Prías, M., Escobar, R., Martinez, J., Díaz, P., López, C., Roca, W. M. and Tohme, J. (2016). The potential of using biotechnology to improve cassava: a review. In Vitro Cellular and Developmental Biology – Plant, 52:461-478.

Lyimo, L. D., Tairo, F. M. and Rweyemamu, C. L. (2013). Morphological and molecular characterization of Cassava (*Manihot esculenta* Crantz) landraces using SSR markers. International Journal of Integrative Biology, 13:71-78.

Marmey, P., Beeching, J. R., Hamon, S. and Charrier, A. (1994) Evaluation of cassava (*Manihot esculenta* Crantz) germplasm collections using RAPD markers. Euphytica, 74:203-209.

Mbanjo, E. G. N, Rabbi, I. Y., Ferguson, M. E., Kayondo, S. I., Eng, N. H., Tripathi, L., Kulakow, P. and Egesi, C. (2021). Technological innovations for improving cassava production in Sub-Saharan Africa. Frontiers In Genetics, 11:623736

Mohan, C., Shanmugasundaram P. and Senthil, N. (2013). Identification of true hybrid progenies in cassava using simple sequence repeat (SSR) markers. Bangladesh Journal of Botany, 42:155-159.

Mouhamady, A. B. A. E., Kordy, M. A. A. and Elewa, T. A. F. (2021). Elucidation of genetic diversity among some accessions of sugar beet (*Beta vulgaris* L.) using inter-simple sequence repeats (ISSR) markers. Bulletin of the National Research Centre, 45:166-193.

Ndam, Y. N., Mounjouenpou, P., Kansci, G., Kenfack, M. J., Meguia, M. P. F., Natacha, N. S., Eyenga, N., Akhobakoh, M. M. and Nyegue, A. (2019). Influence of cultivars and processing methods on the cyanide contents of cassava (*Manihot esculenta* Crantz) and its traditional food products. Scientific African, 5:e00119.

Prempeh, R. N. A., Manu-Aduening, J. A., Quain, M. D., Asante, I. K., Offei, S. K. and Danquah, E. Y. (2020). Assessment of genetic diversity among cassava landraces using single nucleotide polymorphic markers. African Journal of Biotechnology, 19:383-391.

Rangsiruji, A., Pringsulaka, O. and Binchai, S. (2019). HAT-RAPD Fingerprinting analysis of Thai cassava germplasm and economic cultivars of farmers' preferences. Science Essence Journal, 35:59-74.

Roa, A. C., Maya, M. M., Duque, M. C., Tohme, J., Allem, A. C. and Bonierbale, M. W. (1997). AFLP analysis of relationships among cassava and other *Manihot* species. Theoretical and Applied Genetics, 95:741-750.

Rimoldi, F., Filho, P. S. V., Kvitschal, M. V., Gonçalves-Vidigal, M. C., Prioli, A. J., Pinto Prioli, S. M. A. and Ribeiro da Costa, T. (2010) Genetic divergence in sweet cassava cultivars using morphological agronomic traits and RAPD molecular markers. Brazilian Archives of Biology and Technology, 53:1477-1486.

Ruttawat, B., Wangsomnuk, P. P. and Wangsomnuk, P. (2011). Genetic studies of selected Thai cassava by SSR markers. The 12th Khon Kaen University graduate research conference. 28 January 2011. Khon Kaen University. p. 564-571. (in Thai)

Tiago, A. V., Rossi, A. A. B., Tiago, P. V., Carpejani, A. A., Silva, B. M., Hoogerheide, E. S. S. and Yamashita, O. M. (2016). Genetic diversity in cassava landraces grown on farms in Alta Floresta-MT, Brazil. Genetics and Molecular Research, 15:gmr.15038615

Tiago, M. G., Pedro, S. V. F., Maria, C. G. V., Rebecca, C. U. F., Vanesca, P. C. R., Alex, H. T. O., Moiana, L. D. and Kvitschal, M. V. (2017). Genetic diversity and population structure of traditional sweet cassava accessions from Southern of Minas Gerais State, Brazil, using microsatellite markers. African Journal of. Biotechnology, 16:346-358.

Thanananta, N., Charoenchai, M. and Charoenchai, T. (2012). Study on the genetic relationship in cassava using RAPD technique. Thai Journal of Science and Technology, 1:127-133. (in Thai)

Vidal, Á. M., Vieira, L. J., Ferreira, C. F., Souza, F. V. D., Souza, A. S. and Ledo, C. A. S. (2015). Genetic fidelity and variability of micropropagated cassava plants (*Manihot esculenta* Crantz) evaluated using ISSR markers. Genetics and Molecular Research, 14:7759-7770.

Wangsomnuk, P. P., Ruttawat, B. and Wongtiem, P. (2013). Identification of genetically distinct cassava clones from on-farm plantations to widen the Thai cassava breeding gene pool. American Journal of Plant Sciences, 4:1574-1583.

Wong, H. L., Yeoh, H. H. and Lim, S. H. (1999). Customisation of AFLP analysis for cassava varietal identification. Phytochemistry, 50:919-924.

Zacarias, A. M., Botha, A. M., Labuschagne, M. T. and Benesi, I. R. M. (2004). Characterization and genetic distance analysis of cassava *(Manihot esculenta* Crantz) germplasm from Mozambique using RAPD fingerprinting. Euphytica, 138:49-53.

Zayed, E. M., Shams, A. S. and Kamel, A. S. (2013). Genetic diversity in introduced cassava using inter simple sequence repeat markers (ISSRs). Geneconserve, 12:23-33.

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