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GENETIC STABILITY IN LOCAL GARLIC (*ALLIUM SATIVUM* L.) THROUGH *IN VITRO* PROPAGATED PLANTLETS USING SSR AND ISSR MARKERS

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SUMMARY

Genetic stability assessment of garlic (*Allium sativum* L.) regenerants is essential when true-to-type plants are the desired final product. In the following study, shoot tip explants totaling 23 genotypes, comprising 22 local garlic genotypes and one accession imported from China, underwent *in vitro* culturing as mother plantlets and sub-cultured ones. Genetic stability assessment among the individuals of identical genotypes between the mother plantlets and their first subculture used two different molecular markers—simple sequence repeat (SSR) and inter simple sequence repeat (ISSR). SSR and ISSR markers' analysis revealed a high degree of genetic monomorphism among individuals within the identical genotypes. The highest genetic stability, as indicated by identical genetic similarity coefficient values of 0.49 and 0.61 for SSR and ISSR markers, respectively, was evident among the mother plantlets and the first subculture. The individuals with identical genotypes, such as Eban NTT, Lokal Jawa, and Tes, appeared to be different, presumably due to genetic variations detected by SSR and ISSR markers. The SSR and ISSR markers together allowed the detection of higher polymorphism than either the set or the molecular markers alone. This successful clonal genetic stability assessment of micropropagated local garlic using SSR and ISSR markers demonstrates its potential for further applications.

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Keywords: Garlic (*A. sativum* L.), plantlets, genetic stability, genetic similarity, *in vitro*, ISSR and SSR, polymorphism

Key findings: In *in vitro* propagated garlic (*A. sativum* L.) plantlets, the genetic stability assessment was effective with the SSR and ISSR markers, which paved the way for further application. Required further studies could elucidate the factors contributing to genetic variations in *in vitro* propagation.

INTRODUCTION

Garlic (*Allium sativum* L.) belongs to the genus *Allium* and the family Alliaceae and has origins in Central Asia. Garlic has become a worldwide cultivated spice with considerable nutritional value, utilized both fresh and processed in the food and pharmaceutical industries (Ammarellou *et al*., 2022). In Indonesia, garlic stands as a potential strategic horticultural commodity, and its consumption level tends to rise with population growth. However, Indonesia still relies on imports from other producing countries like China to meet the nation's demands. Notably, the imported garlic bulbs seemed larger than the local ones and have a more pungent taste (Dianawati *et al*., 2022).

As a monocotyledonous diploid plant (2n = 16), garlic propagation is primarily clonal through bulbs and cloves due to complexities in flower induction, leading to a narrow genetic variability (Shemes-Mayer *et al*., 2013). Additionally, garlic bulbs have a short shelf life of 6 to 8 months. Garlic conservation programs typically proceed by cultivating them in the field annually, which is costly and leads to reduced yields due to the accumulation of various pathogens in the bulbs (Ayed *et al*., 2018). Addressing these issues can apply micropropagation techniques that could be a promising alternative for garlic conservation and mass propagation and producing virus-free plants. Shoot tips have succeeded in identification as the most suitable explants for regenerating virus-free garlic plants, demonstrating their effectiveness in plant regeneration (Benke *et al*., 2023).

Micropropagation, however, has the potential to alter the genetic properties of plant tissues, leading to somaclonal variations. This variation may manifest as changes in plant morphology, chromosome number, gene expression, protein profile, and DNA sequences (Parab *et al*., 2021; Siregar *et al*., 2023). The composition of the culture medium and the presence of phytohormones can promote this phenomenon (Smulders and de Klerk, 2011). Furthermore, mechanical factors, such as damage to the explant or exposure to sterilizing agents, as well as disturbances in humidity, lighting, and temperature that affect plant transpiration, can cause somaclonal variation (Pawelkowicz *et al*., 2021). However, the potential emergence of somaclonal variations is a concern during garlic micropropagation, particularly in producing true-to-type plants, which directly impacts genetic stability (Garcia *et al*., 2019).

A molecular marker-based approach is more efficient in rapidly and accurately assessing the genetic stability related to somaclonal variations in micropropagated garlic plants (Gimenez *et al*., 2016). Employing various PCR-based molecular markers, such as random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), and inter simple sequence repeat (ISSR), has been effective in determining the genetic stability of micropropagated plant species (Al-Quraini *et al*., 2018; Oliya *et al*., 2021). Although RAPD markers have been serving to evaluate the genetic stability of micropropagated garlic plants, however, they are less efficient than SSR and ISSR markers (Parvin *et al*., 2008).

Past studies demonstrated the SSR and ISSR markers emerged as cost-effective, easy to perform, and more efficient in detecting the genetic variations in crop plants (Serra *et al*., 2007; Buih and Susandarini, 2023). Several SSR markers developed have been successful in revealing the significant levels of genetic variations within the garlic species, determining relationships between different accessions, and identifying genetically distinct subgroups (Cunha *et al*., 2012). Therefore,

SSR and ISSR markers have become valid essential tools for assessing the genetic stability and homogeneity of micropropagated plants at the molecular level and thus ensuring the genetic uniformity of clonal plantlets. This study aimed to assess the genetic stability of micropropagated local garlic using cost-effective PCR-based molecular markers.

MATERIALS AND METHODS

Explants' source and surface sterilization

Garlic genotypes totaling 23, comprising 22 local garlic genotypes and one accession imported from China, served as samples in this study. Twenty-two local genotypes collected from nine provinces in Indonesia underwent cultivation at the Indonesian Vegetable Research Institute (IVEGRI), located at an altitude of 1,250 masl (Table 1). For comparative purposes, the collected bulbs of Chinese cultivar Liang as specimens came from the public market in Lembang, West Java, Indonesia. Cultivar Liang has broadly ovate bulbs and prominent basal plates.

Healthy cloves, carefully separated from the bulb, remained stored at 4 °C for two weeks to break the dormancy. Subsequently, the removal of protective dry sheaths of each clove occurred before thoroughly washing the cloves multiple times with pure water. The peeled cloves' disinfection by immersing them in 70% ethanol for 40 s, followed by 20% NaOCl solution for 20 min, ensured surface sterility. Finally, the cloves sustained rinsing 3 to 5 times with sterile distilled water.

Micropropagation and subculture

Following surface sterilization, the aseptic garlic cloves incurred transversal half-sectioning under a laminar airflow to expose the bud. The sterile shoot tips used as explants, approximately 2–5 mm in length, proceeded with careful excision from the buds before being used as explants. Individually placing each shoot tip progressed into a 20 mm × 150 mm test tube containing 4–5 ml of

Table 1. List of garlic genotypes used in this study.

Genotypes	Bulb	Basal plate	Origin (Province)
Saluyu Putih	Broadly ovate	Prominent	Bengkulu
Lumbu Putih Lembang	Broadly ovate	Prominent	West Java
Tes	Broadly ovate	Even	West Java
Lumbu Putih Cipanas	Broadly ovate	Prominent	West Java
Lokal Maja	Broadly ovate	Prominent	West Java
Lumbu Hijau Lembang	Broadly ovate	Even	West Java
Lumbu Hitam	Broadly ovate	Even	West Java
Lumbu Kuning Lembang	Broadly ovate	Even	West Java
Lumbu Hijau	Broadly ovate	Even	West Java
Sembalung	Broadly ovate	Even	West Java
Kesuna Kayu Agrihorti	Broadly ovate	Even	Bali
Lumbu Kuning Tegal	Broadly ovate	Even	Central Java
Lokal Jawa	Broadly ovate	Even	Central Java
Lumbu Kuning Temanggung	Broadly ovate	Even	Central Java
Lumbu Putih Tegal	Broadly ovate	Even	Central Java
Tawangmangu	Broadly ovate	Even	Central Java
Lokal Merek	Broadly ovate	Even	North Sumatra
Doulu	Broadly ovate	Even	North Sumatra
Lokal Palu	Broadly ovate	Prominent	Central Sulawesi
Sangga Sembalun	Broadly ovate	Even	West Nusa Tenggara
Eban NTT	Circular	Prominent	East Nusa Tenggara
Jangkiriah Adro	Broadly ovate	Prominent	Jambi
Liang	Broadly ovate	Prominent	Shandong, China

semi-solid Murashige and Skoog (MS) medium supplemented with MS vitamin, 30 g/L sucrose, 2 mg/L IAA, 2 mg/L cytokinin, 0.1 mg/L gibberellins, and solidified with 6.6 g/L agar, and adjusted to a pH of 5.7–5.8.

Each test tube contained a single mother plantlet, represented by the shoot tip from an individual garlic clove of a specific genotype, preparing 30 replicates for each genotype. The cultures maintained for five weeks had a temperature of 22 °C ± 4 °C under a cool daylight lamp (Philips TLD 36W/865), with a daily photoperiod of 16 h, and subsequently for the first subculture. Following this initial period, the first subculture succeeded by transferring whole parts of the mother plantlet, excluding any yellowing leaves, to a new culture medium containing identical composition.

Genomic DNA extraction and PCR amplification

The genomic DNA extraction from fresh and healthy leaves of randomly selected five mother plantlets (aged five weeks) and sub-cultured plantlets (aged 10 weeks) continued for each genotype. The conduct of the DNA extraction used the modified cetyl trimethyl ammonium bromide (CTAB) method. Genetic stability assessment utilized 15 pairs of SSR and 15 ISSR markers searched from several past reports (Cunha *et al*., 2012; Ipek *et al*., 2015; Kumar *et al*., 2019).

For each primer, the PCR amplification proceeded in 10 µL of the total reaction mixture containing 2 µL of 10 ng/µL DNA template, 5 µL of 2x MyTaq HS (Bioline, UK), 0.5 µL of each primer, and sterilized ddH_2O. The PCR reaction for SSR and ISSR primers, as performed in the T1 Thermocycler (Biometra, Germany), had the following program, i.e., initial denaturation at 95 °C for 30 s, followed by 35 cycles of denaturation at 94 °C for 30 s; annealing at 55 °C for one min; and extension at 72 °C for 1 min. The end of the reaction comprised a final extension at 60 °C for 15 min. The PCR products' separation used 2% agarose gel electrophoresis for ISSR primers and 6% polyacrylamide gel electrophoresis for SSR primers. The gels, stained with ethidium bromide (EtBr), reached visualization under the UV transilluminator (Biorad, USA). Repeating the amplification reactions of ISSR primers continued at least twice to ensure the reproducibility of the banding pattern.

Data analysis

The consistent and reproducible bands generated by SSR and ISSR primers received scores, with band intensity disregarded while scoring. The data pooled into a binary matrix relied on the presence (1) and absence (0) of the selected bands, with the number of alleles per locus determined. Moreover, the calculation of major allele frequency, gene diversity indices, heterozygosity, and polymorphism information content (PIC) ensued using PowerMarker V3.25. The binary data's further analysis utilized the unweighted pair group method with arithmetic mean (UPGMA)-sequential agglomerative hierarchical non-overlapping (SAHN) program in the NTSYS version 2.1. The genetic similarity matrices among the *in vitro* regenerated plants' computation used the simple matching coefficient in the SimQual subprogram for qualitative data. The genetic similarity coefficients obtained served to construct a dendrogram using the UPGMA method. The dendrogram visually represented the relationships and clustering patterns among the analyzed garlic genotypes. A comparison between the mother plantlets and their first subculture succeeded in evaluating the genetic stability.

RESULTS AND DISCUSSION

Indonesian garlic with geographical distribution

In Indonesia, the widespread cultivation of local garlic across different islands highlights its popularity in traditional farming practices, despite possessing superior agronomical traits. In observations, the local garlic genotypes exhibited significant genetic variability and unique characteristics, offering potential for garlic improvement. The genetic diversity

within local landraces' collection is crucial for effective conservation and breeding strategies for genetic improvement in the future (Vetriventhan *et al*., 2012). The presented study focused on local garlic genotypes from Java (West, Central, and East), along with those from Bali, Sumatera, Sulawesi, and Nusa Tenggara. Java, known for its fertility, has emerged as the main food-producing region for the country, while Nusa Tenggara's dry regions may have induced adaptation in garlic genotypes toward drought tolerance (Widiatmaka *et al*., 2016). The highlands of Sulawesi, with their cooler climate and volcanic soils, also provide favorable conditions for garlic production. Meanwhile, Sumatra, with its coastal areas, has influences from proximity to the sea and the availability of brackish water, affecting better cultivation practices of garlic.

Among the Indonesian garlic genotypes, the variations were noticeable in the shape of the garlic bulbs, which varied from broadly ovate to circular forms (Table 1). The garlic genotype Eban NTT, collected from East Nusa Tenggara, was the only genotype with a circular-bulb shape. The basal plate shape also distinguished the local garlic genotypes, with 15 genotypes having an even basal plate, while seven genotypes, along with an imported genotype from China, exhibited a prominent basal plate.

A dendrogram, based on the similarity of the garlic bulbs and basal plate characteristics, grouped the 22 local garlic genotypes into three distinct groups (Figure 1). Group I comprised seven genotypes with broadly ovate bulbs and prominent basal plates, while group II comprised 15 genotypes with broadly ovate bulbs and even basal plates. The genotype Eban NTT, with its circular-bulb shape, acquired plotting outside these two groups based on the dendrogram. Thus, these bulb characteristics were useful in investigating the genetic variations among the local garlic genotypes, as reflected by their phenotypic expression. However, it is crucial to consider that the observed phenotypic trait variations may not always reflect the real genetic variations due to environmental factors, genotype by environment interactions (GEI), and the genetic control. The DNA-level variations using molecular markers seem to complement the phenotypic traits and revealed a significant genetic diversity among the garlic genotypes.

Micropropagation and first subculture

In the presented study, shoot tips from 23 garlic genotypes reached micropropagation and culturing on supplemented MS medium. The percentage of healthy plantlets succeeded in recording for both the mother plantlets and the first subculture plantlets. The results showed up to 60% of the shoot tip explants of mother plantlets exhibited healthy growth without

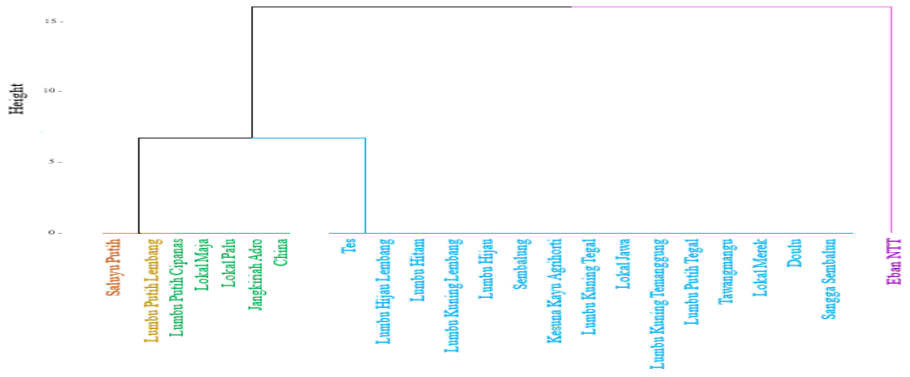

Figure 1. Dendrogram of genetic diversity of 23 garlic genotypes based on the phenotypic similarity of the garlic bulb and basal plate characteristics.

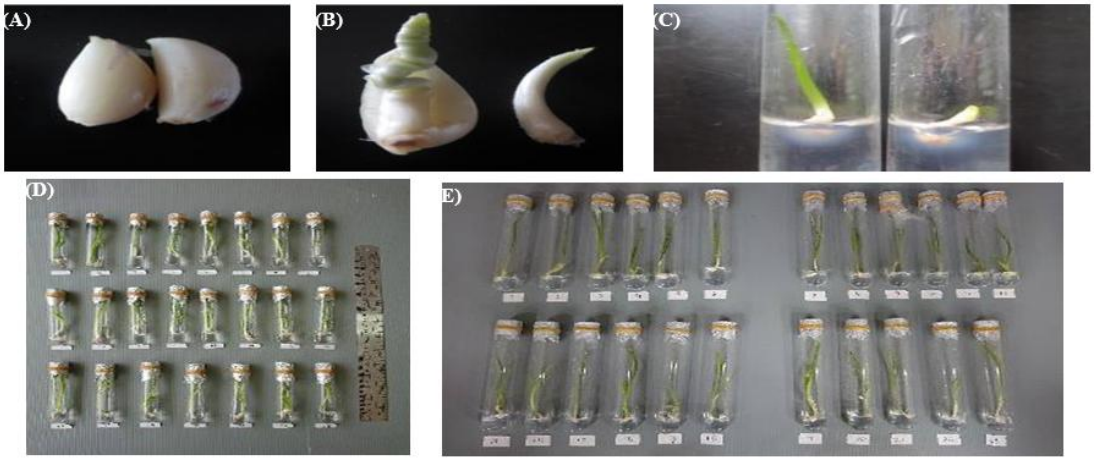

Figure 2. The micropropagation process and subculture of garlic genotypes. (A) garlic cloves, (B) shoot buds obtained from garlic cloves as the source of explants, (C) the initiation of shoot tips on the *in vitro* culture medium, (D) the mother plantlets of the 23 garlic genotypes after five weeks of culture, and (E) the first subculture plantlets of the 23 garlic genotypes after five weeks following subculture.

Table 2. The survival rates of mother plantlets and their first subculture plantlets of the 23 garlic genotypes.

Genotypes	Rates of surviving plantlets (%)		Genotypes	Rates of surviving plantlets (%)	
	Mother plantlets	1st subculture plantlets		Mother plantlets	1st subculture plantlets
Saluyu Putih	83.33^{ab}	80.00^{c}	Lokal Maja	83.33^{ab}	92.00^{a}
Lumbu Putih Lembang	90.00^{a}	85.19^{b}	Lumbu Hijau Lembang	86.67^{a}	92.30^{a}
Tes	83.33^{ab}	84.00^{bc}	Lokal Merek	80.00^{b}	95.83^{a}
Lokal Palu	80.00^{b}	87.50^{b}	Lumbu Hitam	76.67^{b}	91.30^{a}
Tawangmangu	86.67^{a}	92.30^{a}	Doulu	76.76^{b}	91.30^{a}
Lumbu Kuning Tegal	86.67^{a}	88.46^{b}	Lumbu Kuning Temanggung	80.00^{b}	91.67^{a}
Jangkiriah Adro	76.67^{b}	95.65^{a}	Lumbu Kuning Lembang	83.33^{ab}	92.00^{a}
Lokal Jawa	80.00^{b}	87.50^{b}	Lumbu Hijau Karanganyar	83.33^{ab}	88.00^{b}
Kesuna Kayu Agrihorti	83.33^{ab}	92.00^{a}	Sembalung	66.67^{c}	90.00^{a}
Sangga Sembalun	80.00^{b}	91.67^{a}	Eban NTT	66.57^{c}	80.00^{c}
Lumbu Putih Tegal	83.33^{ab}	96.00^{a}	China	63.33^{c}	78.94^{b}
Lumbu Putih Cipanas	83.33^{ab}	96.00^{a}			

Means followed by the same letters are not significantly different according to LSD test with α = 5%.

encountering any issues (Figure 2, Table 2). However, some plantlets also faced growth limitations due to contamination problems. In contrast, the first subculture demonstrated slight differences with positive nature, and 78% of the explants displayed healthy growth, characterized by the absence of necrosis.

Polymorphism profiles with SSR and ISSR

In this study, the SSR and ISSR markers effectively generated distinct and consistent polymorphic bands, allowing the evaluation of genetic stability (Table 3). The SSR primers amplified a total of 129 alleles, with an average

Table 3. Information of SSR and ISSR polymorphism observed in 23 garlic genotypes.

SSR markers	Maf	An	GdI	He	PIC	ISSR markers	Maf	An	GdI	He	PIC
AS440	0.45	7	0.84	1.00	0.78	UBC812	0.50	8	0.54	1.00	0.58
AS5944	0.34	14	0.88	1.00	0.87	UBC818	0.36	6	0.52	1.00	0.58
AS2655	0.36	7	0.83	1.00	0.81	UBC828	0.33	6	0.50	1.00	0.58
AS739	0.54	8	0.82	0.81	0.79	UBC834	0.13	12	0.65	1.00	0.80
AS987	0.58	6	0.76	0.92	0.70	UBC841	0.14	11	0.52	1.00	0.74
AS30	0.45	9	0.84	1.00	0.82	UBC844	0.13	5	0.49	1.00	0.50
ASTC-MGC	0.40	6	0.70	1.00	0.81	UBC814	0.13	7	0.45	1.00	0.50
EU909138	0.46	9	0.83	1.00	0.79	UBC822	0.27	8	0.46	1.00	0.60
ASA24	0.35	12	0.87	0.85	0.86	UBC848	0.20	6	0.44	1.00	0.68
GB-ASM-040	0.43	6	0.76	1.00	0.82	UBC813	0.47	9	0.40	1.00	0.61
GB-ASM-059	0.48	10	0.72	1.00	0.76	UBC820	0.22	6	0.53	1.00	0.62
GB-ASM-072	0.35	7	0.76	0.93	0.72	UBC824	0.14	7	0.56	1.00	0.68
GB-ASM-078	0.46	9	0.79	0.98	0.74	UBC830	0.15	9	0.51	1.00	0.61
AS6580	0.41	13	0.86	1.00	0.85	UBC842	0.18	9	0.63	1.00	0.65
AS449	0.44	6	0.83	0.96	0.82	UBC846	0.14	8	0.67	1.00	0.62
Mean	0.43	8.60	0.81	0.96	0.79	Mean	0.23	7.80	0.52	1.00	0.62
Total		129				Total		117			

Maf = Major allele frequency; An = Allele number; GdI = Gene diversity index; He = Heterozygosity; PIC = Polymorphism information content.

of 8.60 alleles per locus. The maximum number of alleles per locus (14 alleles) bore amplification by the SSR markers AS5944, AS987, ASTC-MGC, and GB-ASM-040; however, the marker AS449 amplified the minimum alleles (six alleles). The average major allele frequency for SSR markers was 0.43 (ranging from 0.34 to 0.58), and the SSR marker AS987 exhibited the highest value. Similarly, the ISSR markers amplified 117 alleles, with an average of 7.80 alleles per locus. The detected minimum number of alleles per locus (five alleles) came from ISSR marker UBC844, while the maximum (12 alleles per locus) resulted with the ISSR marker UBC834. The average major allele frequency produced by ISSR markers was 0.23 (ranging from 0.13 to 0.50), and the mean Nei's genetic diversity index, as calculated, was 0.52. These findings indicated a significant level of genetic diversity among the local garlic genotypes based on SSR and ISSR markers.

The PIC values further assessed the discriminatory power of the molecular markers. In the latest study, the 15 SSR markers displayed PIC values higher than 0.70, with an average PIC value of 0.79. Similarly, the mean PIC value for the 15 ISSR markers was 0.62, ranging from 0.50 to 0.80. The results affirmed the potential of SSR and ISSR markers as informative tools for evaluating the genetic stability in garlic. The promising results agreed with previous findings that have highlighted the effectiveness of SSR and ISSR markers in assessing the genetic diversity and stability in various plant species (Babu *et al*., 2018).

Genetic stability based on SSR and ISSR markers

Based on the number and intensity of amplified bands data, the 15 SSR markers generated distinct and reproducible bands among the individual plantlets within the same garlic genotypes and also among different genotypes for both mother plantlets and their first subculture. Most SSR primers produced more than two bands; however, a few genotypes exhibited a single band. In the mother plantlets, the 15 SSR markers generated reproducible and scorable bands, ranging from 31 to 42 in each garlic genotype. The amplified bands' size ranged from 100 to 400 bp across the garlic genotypes. Notably, the ISSR primer AS5944 produced the highest total of 70 scorable bands across all genotypes, whereas two primers, GB-ASM-059 and GB-ASM-070, produced the lowest total of 32 scorable bands each.

Most bands generated by 15 SSR markers displayed monomorphism within individual plantlets of the same genotype, indicating a high level of genetic homogeneity. The identical banding profiles observed in mother plantlets and their first subculture provide considerable evidence of genetic stability following the first subculture process. However, polymorphisms emerged within the individuals of the garlic genotype Eban NTT by eight SSR markers (AS2655, AS30, AS5944, AS739, ASA24, ASTC-MGC, GB-ASM-059, and GB-ASM072), as well as within the individuals of the genotypes Lokal Jawa and Tes, by the primer AS30. The presence of polymorphic bands could be ascribable to the environmental factors within *in vitro* culture conditions and the variations in the concentration of plant growth hormones, potentially inducing genetic variations. Similarly, low levels of genetic polymorphism detected through SSR markers have also come from previous studies on *in vitro* propagated garlic cultivars and Czech Bolting Garlic (Ovesná *et al*., 2014; Fišerová *et al*., 2016).

Genetic stability profiling based on SSR markers has been inexplicably effective for rapid clonal fidelity assessment among the micropropagated plantlets and seemed generally reliable for identifying genetic stability (Chinnappareddy *et al*., 2013). Nonetheless, caution is warranted due to the susceptibility of SSR markers to null alleles, where the intended PCR product fails to amplify because of mutations in the primer annealing sites. Consequently, it is essential to complement the SSR analysis with more advanced molecular markers. In the presented study, the SSR markers' analysis had supplementation with ISSR markers to enhance the assessment.

Fifteen ISSR markers successfully generated the clear and scorable amplified bands, ranging from 73 to 103 bands in each garlic genotype. In comparison with SSR markers, the amplified bands generated by ISSR markers exhibited remarkably larger sizes, ranging from 100 to 1000 bp per garlic genotype. The ISSR primer UBC841 generated the most scorable bands (216 bands) for all the garlic genotypes, whereas ISSR marker UBC844 produced the lowest count of 65 scorable amplified bands. The higher number of lengthy amplification bands observed with ISSR markers can refer to their broader distribution throughout the genome, allowing for the amplification of genomic DNA in a more critical number of fragments per locus as compared with the SSR markers (Babu *et al*., 2018). Thus, the ability of ISSR markers to bind to multiple sites within the genome enhances the chances of detecting variations and polymorphisms among the garlic genotypes, leading to the amplification of more scorable bands.

The amplified band profiles generated by ISSR markers revealed comparable levels of genetic stability between the mother plantlets and their first subculture. Notably, the polymorphic bands appeared among the garlic individuals Lumbu Kuning Lembang and Eban NTT plantlets using ISSR markers, signifying genetic variations within these genotypes. Additionally, the ISSR primer UBC812 exhibited polymorphisms within individual plantlets of the genotype Lumbu Kuning Temanggung, while primers UBC834 and UBC841 demonstrated polymorphisms among the individual plantlets of the garlic genotype Lumbu Hitam. In contrast, the ISSR primer UBC844 detected polymorphism among the individual plantlets of 13 out of the 23 garlic genotypes. Substantial genetic polymorphism may be due to the genetic variations, which could arise because of factors such as culture duration, the combination of plant growth hormones, and the stress induced by supplemented biochemicals (Parab *et al*., 2021). Compared with the amplified band profiles produced by SSR markers, the ISSR markers showed greater sensitivity in detecting polymorphism, particularly in assessing the genetic stability of individual plantlets within a genotype.

The UPGMA analysis of SSR and ISSR markers constructed a dendrogram that clustered each micropropagated plantlet of the 23 garlic genotypes, including both mother plantlets and their *in vitro* sub-cultured plantlets, into two distinct clusters. The clustering depended on a specific cut-off value for the genetic similarity coefficient of each

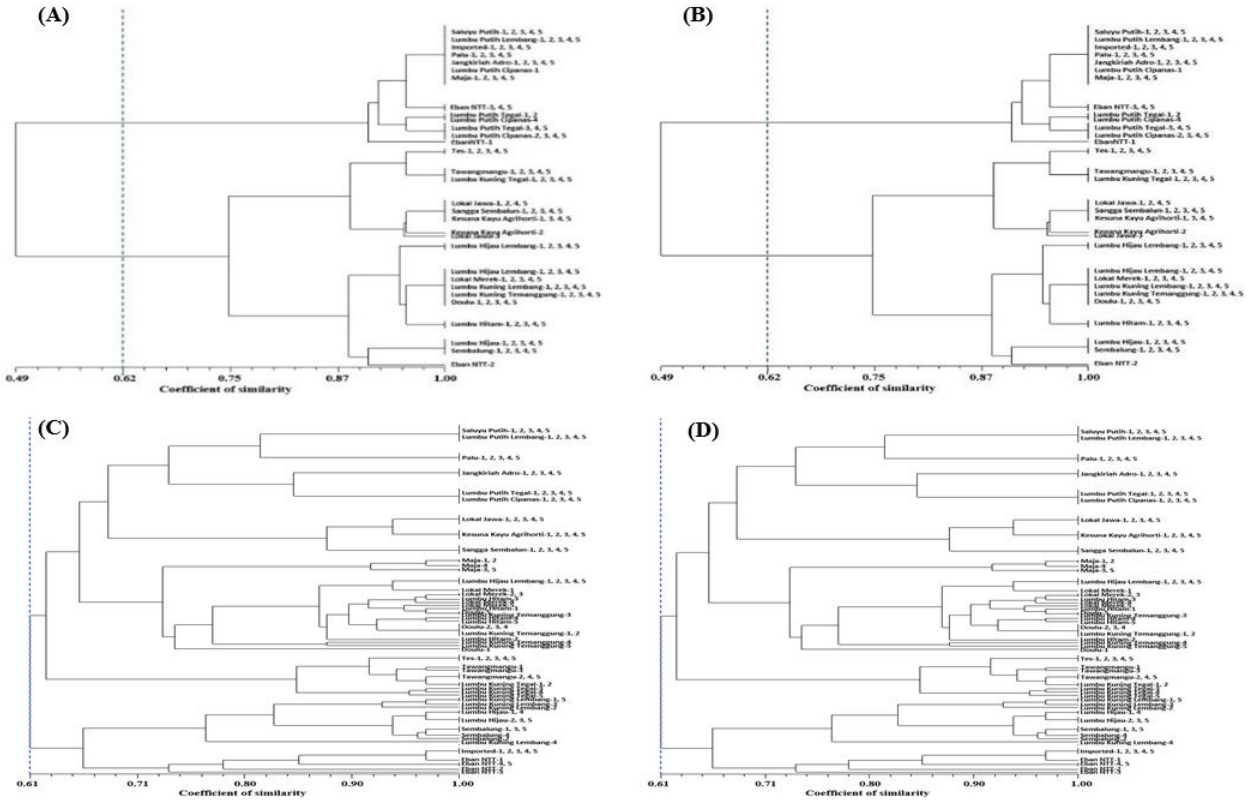

Figure 3. The UPGMA dendrogram represented the genetic relationship among mother plantlets (A) and their first subculture (B) of 23 garlic genotypes based on SSR markers. Furthermore, the dendrogram illustrated the genetic relationships among mother plantlets (C) and their first subculture (D) of 23 garlic genotypes based on ISSR markers.

dendrogram (Figure 3). The grouping of individual plantlets within the 23 garlic genotypes, as determined by SSR and ISSR markers, remained consistent between the mother plantlets and their subcultures, with identical genetic similarity coefficient values of 0.62 and 0.61 for SSR and ISSR markers, respectively. The dendrogram also revealed individual plantlets within the same garlic genotypes manifested a predominant grouping together, implying their close genetic identity. However, some individuals showed plotting farther away from their respective genotype groups, reflecting genetic variations caused by differences in the number and intensity of amplified bands. The results further demonstrated that SSR and ISSR markers effectively amplified the multiple loci and described the genetic diversity among the Indonesian local garlic genotypes. However, the ISSR markers exhibited greater potential and suitability for evaluating genetic stability than the SSR markers.

CONCLUSIONS

The presented study successfully assessed the genetic stability of 23 micropropagated garlic genotypes, comprising 22 local and one Chinese genotype as a comparison, using SSR and ISSR for the first time. The findings, which showed a high monomorphism, as divulged through SSR and ISSR markers' analysis, revealed the highest genetic stability in micropropagated garlic plants. Hence, this shows the maintenance of clonal fidelity among the *in vitro* regenerated garlic after the first subculture. However, some individuals within identical garlic genotypes exhibit differences,

which might be due to genetic variations detected by the SSR and ISSR markers. Therefore, further research is essential to investigate the underlying cause of these genetic variations among the individual plantlets within identical garlic genotypes in *in vitro* micropropagation.

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