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DNA QR Code Using Internal Transcribed Spacer 1 (ITS1) Region of Commercial Cucumber Varieties (*Cucumis sativus* L.)

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ABSTRACT

DNA Quick Response (DQR) is an accurate, efficient, fast, and cost-effective alternative to PCR-based or other sequencing methods. DQR is the method integrated seamlessly with digital systems. The DQR could be apply as effective approach to preventing seed counterfeiting, for example in cucumber. This study aimed to identify DNA barcodes based on the *ITS*1 region and develop DQR for commercial cucumber varieties in Indonesia. Two cucumber varieties, namely cv. Mars and cv. Makka were used. The genomic DNA was extracted using commercial DNA purification kit. The DQR for these two cucumber varieties were developed based on experimental data from the *ITS*1 region. The efficiency and characterization of *ITS*1 region were evaluated through homology analysis using BLAST-NCBI and DQR development. The DQR of cucumber cv. Mars and cv. Makka based on *ITS*1 region were successfully developed. The length of the *ITS*1 region encrypted in the QR code was 496 bp and 437 bp for cv. Mars and cv. Makka, respectively. The A-T and G-C proportions for cv. Mars were 45.2% and 54.8%, respectively, while for cv. Makka, the A-T and G-C proportions were 42.6% and 57.4%, respectively. Our findings are valuable for genetic labeling and authentication of horticultural crops, especially cucumber.

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INTRODUCTION

Cucumber (*Cucumis sativus* L.), a member of Cucurbitaceae family, is a widely popular horticultural commodity. Genetically, cucumbers exhibit significant diversity in growth habit, fruit shape and color, sexual expression, and seed characteristics (Guo et al., 2020; Kaur & Sharma, 2021). The edible portion of the cucumber is the fruit, which is consumed either fresh or processed, for example, as pickles (Pan et al., 2020; Grumet et al., 2023). Globally, cucumber production in 2022 was estimated to reach around 94 million tons, with Indonesia being the largest cucumber-producing country in Southeast Asia, contributing around 444 thousand tons. Currently, the area of cucumber harvest land in the world is predicted to be around 3.4 million hectares, with around 41 thousand hectares located in Indonesia (FAO, 2024). The large area of cucumber cultivation land in Indonesia is due to the high consumption. According to Ministry of Agriculture, Republic of Indonesia (2022), the availability of cucumbers in Indonesia in 2022 reached 281 thousand tons with 98% of of this quantity used as food ingredients.

The high production of cucumber is is attributable to the nutritional value contained in their fruit. According to Abiodun & Adeleke (2010), the nutritional composition of cucumbers consists of 96.4% water, 2.6% carbohydrates, 0.4% protein, 0.3% minerals, and 0.1% fat. In addition, cucumber seeds are reported to have high protein, fat, and mineral content. Cucumbers contain vitamins (A and C), folic acid, and several important minerals, such as Calcium (Ca), Potassium (K), and Magnesium (Mg). According to Chakraborty & Rayalu (2021), cucumber is among the most popular commodities due to their various health benefits, such as regulation of cell hydration, maintenance blood pressure and sugar balance, alleviation of

digestive problems, and support in losing weight. Not only is it used as a food ingredient, but cucumber is also used in the cosmetics and soap industries (Kaur & Sharma, 2021).

Indonesia cultivates a diverse range of cucumber varieties, with seeds primarily sourced from seed companies, including Mars, Saturnus, Expo, Model 21, Makka, Baby, Mercy, and others. The high demand for cucumbers makes farmers' seed purchases highly vulnerable to counterfeiting. According to FAO (2020), fake seeds are a significant problem in Indonesia, Thailand, Vietnam, and Cambodia. In Indonesia, several cases of seed counterfeiting have been reported, including garlic (Department of Agriculture and Food of Badung Regency 2018), tomatoes (Regional Government of West Java Province 2022), and corn (Saputra, 2022). Seed counterfeiting poses risks to farmers, producers, and consumers alike (Nehal et al., 2021; Liandhajani, 2022). According to the International Seed Federation (2018), counterfeit seed practices can lower seed quality, including reselling seeds from hybrid plants, using packaging with fraudulent variety names, and failing to meet minimum standards for variety purity and germination rates. These actions must be mitigated, including through the use of molecular biology approaches. This strategy is particularly useful in ensuring the authenticity of products. Traditional authentication methods rely on the plant's flowering and fruiting stages. However, this approach has several limitations, such as the required traits being visible only at certain times and the need for expert evaluation based on extensive experience. Advances in plant and animal genetics have led to the development of species authentication technologies (Zhu et al., 2022).

DNA barcoding is a molecular biology technique that uses short, standardized DNA sequences for species identification, conservation support, and further applications (Kress, 2017; Antil et al., 2023; Turhadi & Hakim, 2023). DNA barcodes provide a fast, accurate, and effective method for species identification, especially when morphological characteristics are difficult to asses (Taberlet et al., 2007; Trivedi et al., 2020). Various genomic regions have been utilized for plant identification, including both chloroplast DNA (cpDNA) and nuclear DNA (nrDNA), include *rbcL*, *matK*, *trnK*, *trnL-trnF*, *atpF-atpH*, *psbK-psbI*, *trnH*, *psbA*, *ITS*, and *ETS* (Ho et al., 2020; Ahmed et al., 2022; Faza et al., 2024).

Ensuring the authenticity of products in the market is crucial for the seed industry. According to Krishna & Dugar (2016), industrial products can use holograms and barcodes to minimize counterfeiting. In agriculture, DNA QR code (DQR) technology can be employed to ensure product authenticity. DQR technology is an innovative approach that converts DNA sequences obtained through DNA barcoding into a Quick Response (QR) code, enhancing product security with encrypted messages (Liu et al., 2012; Sharief et al., 2021). DQR technology is highly effective in combating product counterfeiting and can be easily accessed by the public through digital devices, such as smartphones (Krishna & Dugar, 2016; Yu et al., 2016; Khan et al., 2017). DQR technology works by converting the DNA base sequence of a specific DNA barcode, which has been widely used for plant identification to prevent product counterfeiting into QR code. In China (Zhu et al., 2022) and Thailand (Urumarudappa et al., 2022), DQR technology has been applied to authenticate raw materials in traditional herbal medicine products.

In Indonesia, the Ministry of Agriculture has implemented a barcode/QR code-based quality assurance system for seeds. This system is designed to be fast, effective, efficient, and transparent in preventing counterfeit seeds (Communication and Information Service of East Java Province 2020). Additionally, Vanany et al. (2014) developed an electronic tracking method using barcodes to combat fruit plant counterfeiting in Indonesia. However, DQR technology has yet to be utilized for product authentication in the country. Given this potential, DQR technology could be effectively applied in Indonesia, making research essential for ensuring product authenticity, particularly in horticultural plant seeds. This study aimed to identify DNA barcodes based on the ITS1 region and generate DNA QR codes (DQR) for commercial cucumber varieties in Indonesia.

MATERIALS AND METHODS

Genetic Materials

This study used two cucumber varieties (*Cucumis sativus*): cv. Makka and cv. Mars. The cv. Makka is a superior variety from PT. Benih Citra Asia (BCA) (Var KE 4769, Kepmentan No. 037/Kpts/SR.120/D.2.7/4/2016), while cv. Mars is a variety from the Vegetable Plant Standard Instrument Testing Center (BPSI) (SK Mentan No. 741/Kpts/TP.240/6/1999).

Genomic DNA Extraction

A total of 40 mg of fresh cucumber leaves were used for genomic DNA extraction, following the Wizard® Genomic DNA Purification Kit protocol (Promega, USA). The quality and quantity of the extracted DNA were assessed using agarose gel electrophoresis and a NanoPhotometer® NPOS 6.6c (Implen, Inc., USA). The DNA was then stored at -30 °C for further analysis.

DNA Barcode Amplification and DNA Sequencing

DNA amplification was performed using primers for the *ITS*1 region. The PCR reaction had a final volume of 25 µL, consisting of 12.5 µL GoTaq® Green Master Mix (Promega, USA), 0.5 µL of each forward and reverse primer (10 pmol/µL), 10.5 µL nuclease-free water (Promega, USA), and 1 µL genomic DNA (100 ng/µL). Amplification was conducted using the TakARa PCR Thermal Cycler Dice Gradient (Takara Bio, Inc., USA) with specific primer pairs: 5'-TCCGTAGGTGAACCTGCGG-3' (forward) and 5'-GCTGCGTTCTTCATCGATGC-3' (reverse).

The target region was amplified through 35 PCR cycles under the following conditions: pre-denaturation at 95 °C for 1 minute, denaturation at 95 °C for 15 seconds, annealing at 60 °C for 15 seconds, extension at 72 °C for 10 seconds, and a final extension at 72 °C for 5 minutes. The PCR products were visualized on a 1% agarose gel using electrophoresis at 80 volts for 35 minutes. Gel imaging was performed with a UV transilluminator (Gel Documentation System, Major Science Co., Ltd., USA). The resulting PCR products were then sequenced using the Sanger sequencing method on a Genetic Analyzer 3730XL (Thermo Fisher Scientific Inc., USA) at Macrogen, Singapore.

Identification of Specific DNA Barcode and Its Conversion into QR Code

DNA sequences from each sample were aligned using multiple sequence alignment (MSA) in MEGA X software version 10.0.5 (Kumar et al., 2018). Based on the MSA results, specific DNA sequences were identified and designated as unique barcodes for *C. sativus*. These DNA barcode sequences were then converted into QR codes using open-source DNA barcode and QR code generators (Yu et al., 2016; Khan et al., 2017; Turhadi et al., 2024). The generated QR codes were scanned and read using smartphones equipped with QR code scanners (Ma et al., 2017).

RESULTS AND DISCUSSION

DNA samples from two cucumber varieties, cv. Mars and cv. Makka, were successfully extracted (Figure 1) with good concentration and purity levels (Table 1). DNA concentrations ranged from 34.65 to 112.15 ng/µl, with purity values between 2.066 and 2.137. The extracted DNA was then used to amplify the *ITS*1 region. PCR successfully amplified the *ITS*1 region in all six samples of cv. Mars and cv. Makka, producing an amplicon of approximately 290 bp (Figure 2).

Bi-directional sequencing of the *ITS*1 region in cucumber (*C. sativus*) samples, cv. Mars (MR) and cv. Makka (MK), was successfully performed (Figure 3). The sequencing results for both varieties produced clear chromatograms, indicated by a single chromatogram in the target region. These high-quality chromatogram profiles allow for further DNA sequence analysis. All six *ITS*1 sequences obtained in this study were deposited in the NCBI GenBank database with the following accession numbers: PQ722132 (MK2), PQ722133 (MK3), PQ722134 (MK5), PV170691 (MR1), PQ722135 (MR2), and PQ722136 (MR5).

The contig sequences obtained from chromatogram pre-processing showed relatively consistent lengths across samples. The *ITS*1 region in cv. Mars ranged from 559 to 575 bp, while in cv. Makka, it ranged from 541 to 553 bp. Homology analysis using the BLAST-NCBI database confirmed that both cv. Mars and cv. Makka belonged to *C. sativus*, with similarity values ranging from 98.12% to 100.00% and 96.68% to 99.63%, respectively (Table 2).

This study successfully identified the specific *ITS*1 regions for cv. Mars and cv. Makka, with sequence lengths of 496 bp and 437 bp, respectively. These sequences were encrypted into DNA QR codes (DQR) for cv. Mars (Figure 3A) and cv. Makka (Figure 3B). Both DQRs can be scanned using a QR code scanner on a digital device. Additionally, sequence characterization of the *ITS*1 region revealed distinct differences between cv. Mars and cv. Makka (Table 3). These results suggest that these genetic characteristics can serve as unique identifiers for each cucumber variety.

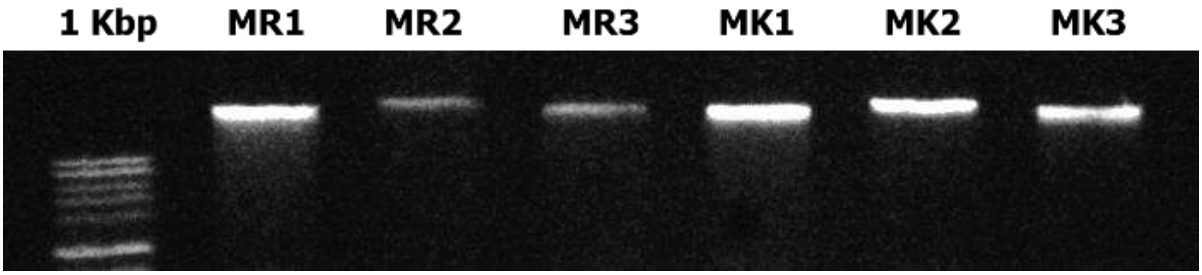

Figure 1. Electrophoregram bands of DNA samples of Cucumber (*C. sativus*) cv. Mars (MR) and cv. Makka (MK)

Table 1. DNA concentration and purity level of cucumber (*C. sativus*) cv. Mars (MR) and cv. Makka (MK)

Varieties	Sample codes	DNA concentration (ng/µl)	DNA purity level (A260/280)
Mars	MR1	72.500	2.1
	MR2	34.650	2.1
	MR3	64.000	2.1
Makka	MK1	86.650	2.1
	MK2	54.850	2.1
	MK3	112.15	2.1

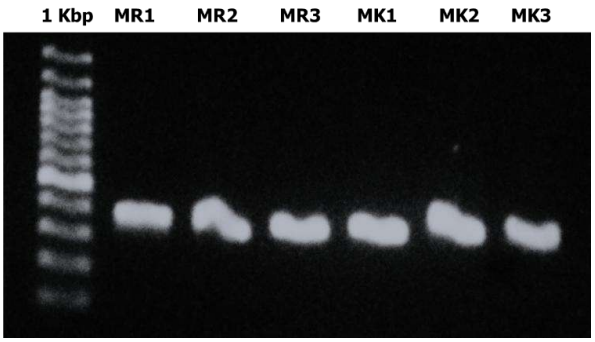

Figure 2. PCR results of *ITS1* region on cucumber's DNA samples (*C. sativus*) cv. Mars (MR) and cv. Makka (MK)

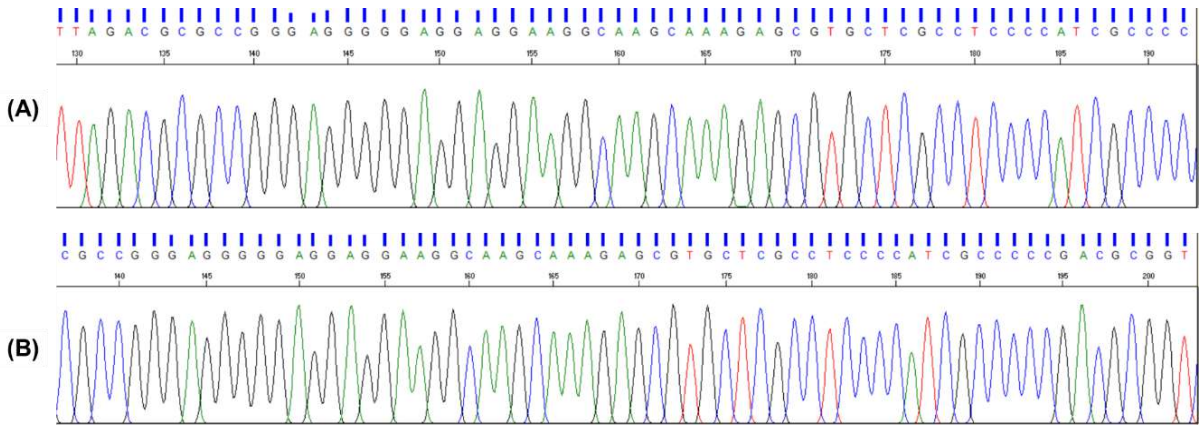

Figure 3. Representative chromatograms of *ITS1* region of cucumber (*C. sativus*) cv. Mars (A) and cv. Makka (B) samples

Table 2. Homology analysis of *ITS*1 regions of cucumber samples cv. Mars and cv. Makka against the NCBI database

Variety	Sample codes	GenBank/BLAST	Max. ID (%)	Accession number
Mars	MR1	*Cucumis sativus*	99.04	KX231332.1
	MR2	*Cucumis sativus*	98.12	KX231332.1
	MR3	*Cucumis sativus*	100.00	OP978788.1
Makka	MK1	*Cucumis sativus*	99.63	OP978788.1
	MK2	*Cucumis sativus*	96.68	KX231332.1
	MK3	*Cucumis sativus*	97.77	KX231332.1

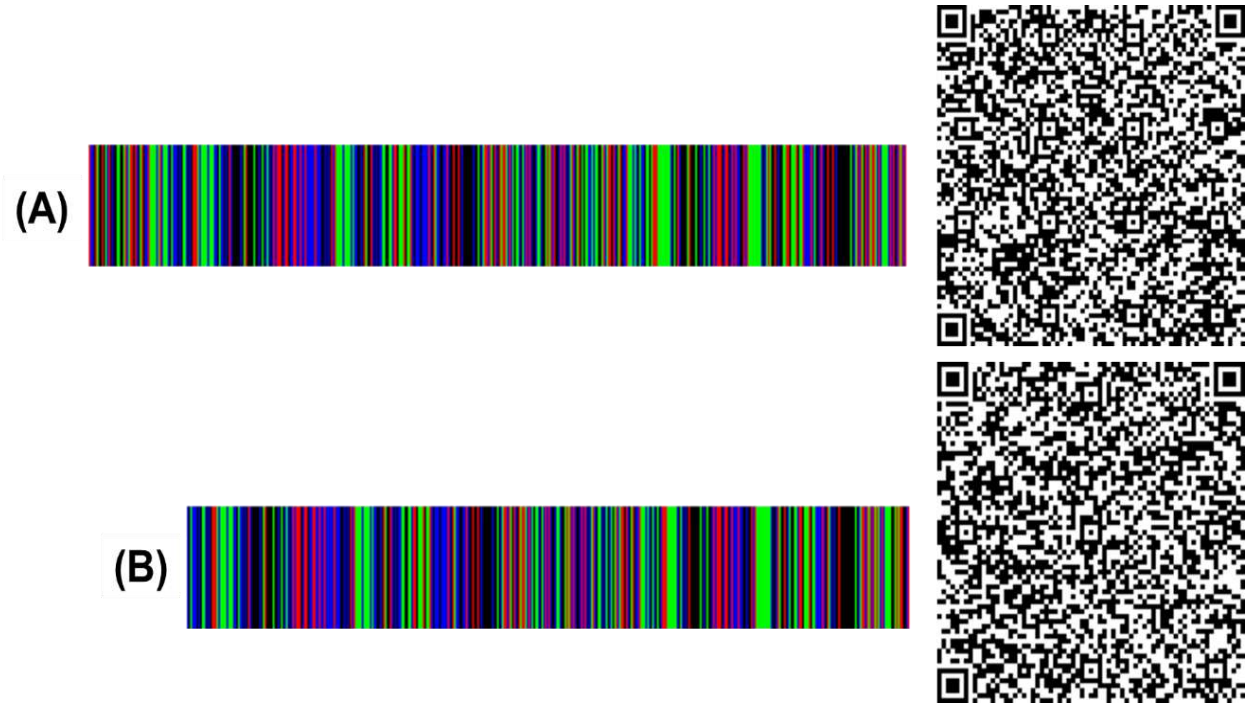

Figure 3. DNA QR code (DQR) based on *ITS*1 region for cucumber cv. Mars (A) and cv. Makka (B)

Table 3. Nucleotide composition of DNA QR code (DQR) based on *ITS*1 region for cucumber cv. Mars and cv. Makka

Varieties	A (%)	T (%)	G (%)	C (%)	A-T (%)	G-C (%)
Mars	25.0	20.2	27.6	27.2	45.2	54.8
Makka	23.3	19.2	28.8	28.6	42.6	57.4

Cucumber cv. Mars has A-T and G-C proportions of 45.2% and 54.8%, respectively, while cv. Makka has A-T and G-C proportions of 42.6% and 57.4%. Overall, both cucumber varieties share a similar *ITS*1 sequence pattern, with nucleotide proportions in ascending order: Thymine (T), Adenine (A), Cytosine (C), and Guanine (G).

The two-dimensional DNA barcode generated in this study serves as a highly accurate genetic label for cucumber cv. Mars and cv. Makka. According to Yu et al. (2016), this barcode facilitates species identification and can also be used as a product safety protection technique. The generated barcode can be scanned using a mobile

phone with a barcode scanner. This will display specific DNA sequence information, enabling accurate species identification (Khan et al., 2017). Various studies have successfully created specific DNA barcodes and converted them into QR codes, offering broader benefits to users. The QR codes can be scanned using a mobile phone equipped with a barcode scanner as reported in similar previous studies (Lv et al., 2020; Li et al., 2021; Jiang et al., 2022; Jiang et al., 2023; Yao et al., 2022; Gogoi et al., 2020; Yu et al., 2016; Cai et al., 2016; Ma et al., 2017). QR codes have been developed for plant identification across multiple taxonomic levels, including the family level, such as Apocynaceae (Lv et al., 2020), Orchidaceae (Li et al., 2021), Theaceae (Jiang et al., 2022), and Apiaceae (Jiang et al., 2023); the genus level, such as *Syringa* (Yao et al., 2022) and *Clerodendrum* (Gogoi et al., 2020); and the species level, such as *Trachelospermum jasminoides* (Yu et al., 2016) and *Panax ginseng* (Cai et al., 2016). QR codes have also been applied to plant-derived products, such as Shi-Liang tea, which is made from processed leaves of *Chimonanthus salicifolius* and *Chimonanthus zhejiangensis* (Ma et al., 2017).

CONCLUSION

The DNA QR code (DQR) for cucumber cv. Mars and cv. Makka, based on the *ITS*1 region, has been successfully developed. The *ITS*1 sequences encoded in the QR codes were 496 bp for cv. Mars and 437 bp for cv. Makka. The cv. Mars had A-T and G-C proportions of 45.2% and 54.8%, respectively, while cv. Makka had A-T and G-C proportions of 42.6% and 57.4%.

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