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DNA Isolation and Purification Protocols for DNA Amplification Enhancement in *Monochoria vaginalis* PCR Analysis

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Abstract

***Monochoria vaginalis* contains phenolic chemicals that can disrupt DNA extraction and downstream molecular processes. Phenolics are known to bind to DNA and hinder enzymatic processes, which affects procedures like PCR. The goal of this study was to create an effective DNA extraction and purification method for obtaining high-quality genomic DNA from *M. vaginalis*. The DNA was extracted using a modified CTAB technique and purified with 30 µL sodium acetate and 1,000 µL 70% ethanol. The purification procedure enhanced DNA quality, as seen by less smearing and cleaner bands in electrophoresis, particularly in Sukra, Rawamerta, East Karawang, Patokbeusi, and Ciasem. After purification, DNA concentrations reduced to 6.12-37.45 µg/mL. Spectrophotometric measurements revealed higher purity, with A_{260}/A_{280} ratios of 1.43-2.31 and A_{260}/A_{230} ratios of 0.04-0.30. The 1,931 bp *MvALS1* gene was successfully amplified across all ecotypes, demonstrating the purification method's efficacy in yielding DNA appropriate for molecular biology applications.**

Keywords: CTAB extraction, DNA purification, DNA quality, Pickerel weed

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Introduction

Monochoria vaginalis (Burm. f.) C. presl. is an annual weed commonly seen in rice fields, as well as a herbaceous aquatic plant endemic to temperate and tropical climates that predominantly self-pollinates. *Monochoria vaginalis* is widely found in the US and tropical and subtropical Asia, including Indonesia (Widianto *et al.*, 2022). *Monochoria vaginalis* has straight stems, fibrous roots, and white roots. Young leaves elongate before widening. Its flowers are purple, with tubular petals and six stamens of different lengths, five of which are short and have yellow anthers.

Monochoria vaginalis is especially harmful because it has a high nitrogen uptake in the soil and competes with rice for nitrogen absorption in the early stages of development (Nozoe *et al.*, 2022). Among the weeds that grow in rice paddies, *M..vaginalis* is a main concern because it is officially listed as one of the top three harmful weeds in rice fields in Indonesia, Japan, and Taiwan (Widianto *et al.*, 2022). This weed can take over up to 60% of the broadleaf weed population in affected areas (Sulaiman *et al.*, 2025), and when it grows in large amounts, it can reduce rice crop yields by as much as 82% (CABI, 2016). Unlike grass weeds like *Echinochloa crus-galli*, which can be controlled with different types of herbicides, *M. vaginalis* and other broadleaf weeds that are sensitive to ALS (like *Scirpus juncoides*) have the same target site that is prone to resistance (Park *et al.*, 2022). Furthermore, *Monochoria vaginalis* seeds can remain alive for almost a decade (Tazawa *et al.*, 2021). Metsulfuron-methyl is a type of sulfonylurea herbicide that works by blocking the acetolactate synthase (ALS) enzyme.This enzyme is necessary for plants to make branched-chain amino acids, which are important for their growth (Kudoyarova *et al.*, 2024). A previous study found that *Monochoria vaginalis* from Rawamerta, Karawang had intermediate resistance to metsulfuron-methyl (resistance ratio 6.00), however the Patokbeusi, Subang biotype had

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low resistance (ratio 3.89) compared to susceptible biotypes. A nucleotide alignment of the *MvALS1* gene revealed amino acid changes in Rawamerta and Patokbeusi (Ser-64-Ala, Asp-66-Glu, Asn-240-Asp, Glu-426-Asn, Ser-469-Asn) (Putri *et al.*, 2024). Resistance mechanisms are further divided according to the extent of target protein involvement: target site resistance (TSR) and non-target site resistance (NTSR) (Jugulam and Shyam, 2019). The TSR mechanism is mostly based on mutations at the herbicide target region (Murphy and Tranel, 2019). Therefore, analyzing target-site resistance (TSR) helps identify changes in the *MvALS1* gene that can confirm the results from the bioassay regarding resistance. This analysis involves extracting and cleaning DNA from plant tissue, then using PCR to amplify the specific gene area, and finally sequencing the DNA to find changes in the nucleotides linked to herbicide resistance.

A high quality of DNA extraction is essential for molecular investigations. Cetyl Trimethyl Ammonium Bromide (CTAB) is a widely used technique for extracting DNA from plants rich in polysaccharides and phenolic chemicals (Liu *et al.*, 2025). DNA extraction consists of three steps: cell wall disintegration (lysis), DNA separation from solid materials like cellulose and protein, and DNA purification (Barbosa *et al.*, 2016). CTAB is different from common methods that use SDS or commercial kits because CTAB, which is a type of positively charged detergent, creates a solid mix with sugars when there is a lot of salt. This allows the sugars to be separated from the DNA (Gautam, 2022) — sugars that would usually come down with the DNA in SDS methods or block the silica columns in commercial kits and stop the DNA from attaching (Vilanova *et al.*, 2020). Since *Monochoria vaginalis* is a sticky, water-loving plant that contains a lot of polysaccharides, using CTAB to extract DNA gives better purity and is cheaper and easier to scale up compared to commercial kits.These kits have a limit because they rely on a fixed volume of the column (Turaki *et al.*, 2017). However, standard CTAB protocols might not work well for very mucilaginous tissues because leftover polysaccharides can settle with the DNA and lower its purity (Feng *et al.*, 2024). Therefore, this study used a modified CTAB method with a higher concentration of NaCl and included PVP to reduce contamination from polysaccharides and phenolics even more, and then performed another purification step to achieve better DNA purity, which is necessary for downstream PCR amplification and sequencing of the *MvALS1* gene.

According to Narathota & Jayasiri (2020), *Monochoria vaginalis* contain a variety of chemical substances including carbohydrates, proteins, amino acids, alkaloids, saponins, phenolic compounds, tannins, flavonoids, glycosides, phytosteroids, oils and fats. The ethanolic leaf extract of *Monochoria vaginalis* contains phenols (16.91 g GAE/100 g) and tannins (11.5 g GAE/100 g). The ethanolic extract has the highest total flavonoid concentration in the leaf, with a value of 24.33 g QE/100 g. Furthermore, according to previous studies, the solvent utilized has a significant impact on the antioxidant activity, total phenol content, total flavonoid content, and total tannin level of *Monochoria vaginalis* extract. Ethanol is the ideal solvent for extracting *Monochoria vaginalis* since it contains antioxidant activity (IC50 = 0.49 mg/mL), a yield of 26.17%, a yield of 26.17%, a total phenol content of 11.12 mg GAE/g, a total flavonoid content of 8.26 mg QE/g, and a total tannin content of 5.18 mg TAE/g (Padmawati *et al.*, 2020).

Phenolics, including terpenoids and tannins, rapidly oxidize and attach to the phosphate backbone of DNA, causing browning of leaf material (Wagay *et al.*, 2020). Both phenolics prohibit the use of DNA for molecular biology purposes, such as PCR or sequencing since it blocking the operation of polymerases or endonucleases (Dini & Grumetto, 2022). Earlier research has looked at how different strains of M.vaginalis resist metsulfuron-methyl, using bioassays, and found changes in the MvALS1 gene linked to resistance at the target site (Putri *et al.*, 2024). However, these studies have not tackled the technical problem of getting high-quality genomic DNA from the sticky, sugary leaves of *M.vaginalis*, which can affect the accuracy of PCR and sequencing if regular extraction methods are used. The suggested methods for extracting high-quality DNA are reliable and can be consistently reproduced. To ensure that PCR amplification works properly without any obstacles, genomic DNA samples must be

purified to a high enough quality, no matter which method is used for the molecular analysis (Lim *et al.*, 2016; Sajali *et al.*, 2018). Therefore, this study seeks to develop a revised CTAB-based method for extracting and purifying DNA, tailored for the sticky leaf tissue of *M. vaginalis*, to produce high-quality genomic DNA that can be used for dependable PCR amplification and precise sequencing of the *MvALS1* gene, which has not been explored in prior research on resistance in this species.The proposed procedures for high-quality DNA purification are reproducible and trustworthy. To eliminate inhibitions or interference during PCR amplification, genomic DNA samples should be purified to a sufficient purity level, regardless of the molecular technique used (Lim *et al.*, 2016; Sajali *et al.*, 2018). Thus, this research will cover effective and efficient DNA isolation and purification techniques, which are still capable of producing high-quality, uncontaminated DNA, resulting in clear PCR findings.

Method

Time and Place of Research

The study was done in January 2025. The research was conducted at Bogor Agricultural University's Plant Molecular Biotechnology Laboratory 2 in the Faculty of Agriculture.

Tools and Materials

The following tools were utilized in this study: PCR machine (Thermal Cycler Esco Swift Maxi), electrophoresis machine, Gel Doc EZTM (Bio Rad, USA), micropipette, glassware, waterbath, mortar, analytical balance, centrifuge, desiccator, spatula, and others. The materials used are *Monochoria vaginalis* leaf samples that are resistant and sensitive to metsulfuron-methyl, the sampling sites included Patrol and Sukra in Indramayu Regency; Karawang Timur and Rawamerta in Karawang Regency; as well as Patokbeusi, Ciasem, and Tambakdahan in Subang Regency (Putri *et al.*, 2024), CTAB (Cyltrimethylammonium Bromide) 2%, Tris HCl 100 mM pH 8, 1.4 M NaCl, 20 mM EDTA, 1% PVP, Chloroform-Isoamyl Alcohol (CIA), agarose powder, gel red 10,000x in water, VC 100bp Plus DNA Ladder, ddH_2O, primers (forward, reverse, and inner), Taq polymerase 2x KAPA2G Fast ReadyMix Kit, and Ethidium Bromide (EtBr).

DNA Extraction and Purification

Genomic DNA was extracted from leaf samples of the *Monochoria vaginalis* ecotype utilized in the initial experiment. 0.1-0.5 g of leaves were crushed with 700 µL of DNA extraction buffer solution (100 mM Tris HCl (pH 8), 1.4 M NaCl, 20 mM EDTA, 2% CTAB, and 1% PVP) following a modified procedure (Turaki *et al.*, 2017). The samples were incubated in a water bath for 10 minutes at 52°C before adding 700 µL of chloroform-isoamyl alcohol (CIA) to the tube. The samples were combined and centrifuged at 11,500 rpm for 5 minutes. The supernatant was then transferred to a new microcentrifuge tube for DNA precipitation. The sample was gently inverted after being doubled in volume with 100% ethanol. The sample was incubated for ten minutes before being centrifuged for two minutes at 11,500 rpm. The supernatant was discarded, and the DNA precipitate dried. The DNA was frozen after being dissolved in 100 µL of ddH_2O. Purification involved adding 30 µL of sodium acetate and 1,000 µL of 70% ethanol to the DNA precipitate. The sample was centrifuged for 5 minutes at 11,500 rpm. The DNA precipitate was dried and dissolved in 50 µL ddH_2O. The DNA quality was then assessed after purification with 1% agarose at 90 volts for 45 minutes in 1x Tris-acetate-EDTA buffer. The purity and concentration were then evaluated again.

Measurement of DNA quality and quantity. The quality of the extracted DNA was assessed using a 1% agarose gel at 90 volts for 45 minutes in 1x Tris-acetate-EDTA buffer. The purity and concentration of 2 µL of isolated DNA stock solution were tested using a Thermo Scientific™ µDrop™ and a Multiskan SkyHigh Microplate Spectrophotometer. The acquired data included the ratio of DNA concentration (µg/mL) and purity of each sample.

DNA Amplification

The primer set was designed using complete sequences from the *MvALS1* Genebank: AB243613.1, and the 1,931 base pair (bp) *MvALS1* gene was amplified from the

unpurified and purified genomic DNA of *Monochoria vaginalis* using primers designed by Song *et al.* (2010) and Putri *et al.* (2024). A 50 µL PCR mixture was prepared by adding 25 µL of 2x KAPA2G Fast ReadyMix Kit, 2.5 µL of 10 µM forward and reverse primers, 12.5 µL of unpurified and purified *Monochoria vaginalis* genomic DNA at a working solution concentration of 2 ng/µL, and 7.5 µL of Nuclease Free Water (NFW). The PCR process included an initial denaturation at 94 °C for 5 min, 40 cycles of 94 °C for 5 s, 60 °C for 30 s, and 72 °C for 1 min, and a 10-minute final extension at 72 °C. Amplicons were observed using electrophoresis on a 1.5% agarose gel (5 µL sample) at 90 V for 85 min in 1× TAE buffer (Putri *et al.*, 2024).

Results and Discussion

DNA Quality Test

The quality of genomic DNA obtained through the extraction procedure confirms the technique's efficacy and the purity of the resultant DNA. Figure 1 shows electrophoresis patterns of unpurified DNA (A) and purified DNA (B). The results of non-purified DNA isolation revealed a distinct band with a high smear intensity. In contrast, the quality of DNA after purification was good, as evidenced by electrophoresis data, with high DNA band intensity in samples from Sukra, Rawamerta, East Karawang, Patokbeusi, and Ciasem and low smear intensity. However, as compared to the quality of the sensitive and Patrol ecotype DNA, the DNA bands were thinner and had lower smear intensity (Figure 1). These results show that the DNA is of good quality and has not been fragmented. Smears represent fragmented nucleotide bases or impurities, whereas bands represent DNA (Matsuo *et al.*, 2018). According to (Singh *et al.*, 2025), the lack of DNA electrophoresis stains implies that the DNA is pure. This is significant because undamaged DNA yields more reliable PCR results. Secondary metabolites such as polysaccharides, tannins, pigments, alkaloids, and flavonoids are common forms of plant DNA contamination.

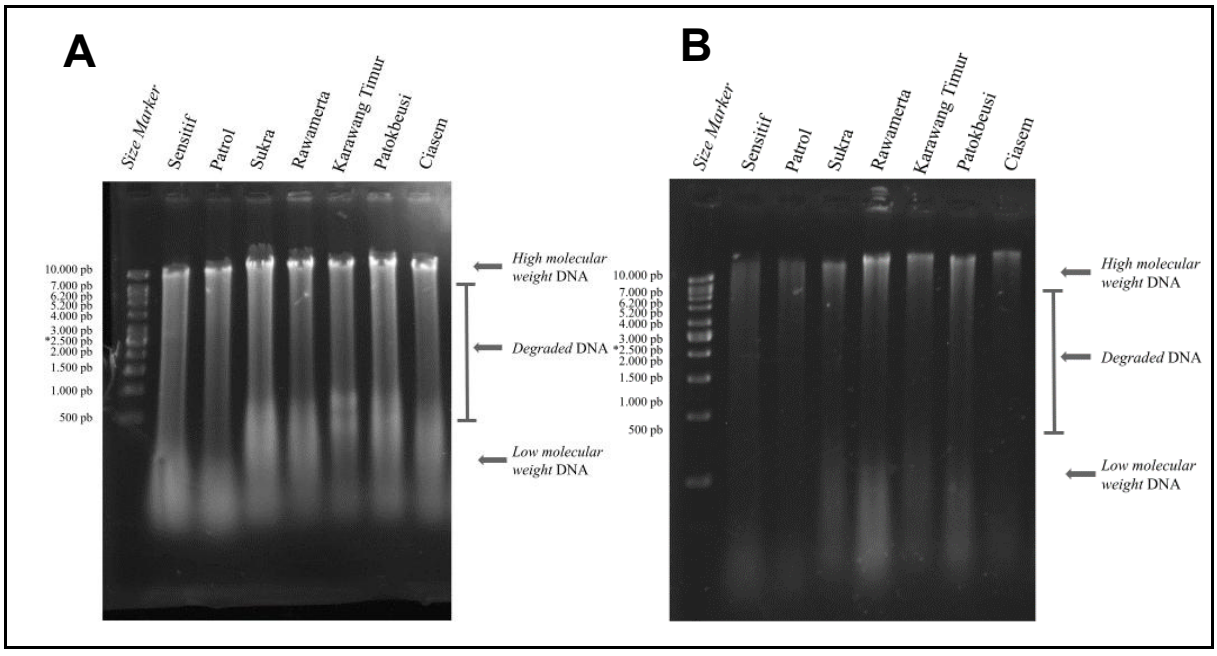


Figure 1. Visualization of the purity of DNA stock recovered from seven ecotypes of *Monochoria vaginalis*: A) Non-purified, B) Purified (bp= base pairs)

DNA Quantity Test

Table 1 show the DNA spectrophotometer results for *Monochoria vaginalis* DNA before and after purification. Purification significantly reduced DNA content in all ecotypes (Table 1), with the sensitive ecotype experiencing a reduction from 1,276 µg/mL to 22.24 µg/mL. This drop reflects the removal of non-DNA impurities during the purifying process. A high concentration value does not necessarily imply high purity. If the A_{260} value, which represents DNA, is high, the concentration value will also be high, and vice versa. However, for purity values, even a high concentration value does not always imply a high purity level. This is because purity values are influenced by the A_{280} value (contaminant value). In addition to observable DNA bands, genomic DNA electrophoresis data reveal smears that are carried over and appear thick (Iqbal *et al.*, 2016).

However, the A_{260}/A_{280} ratio, which shows DNA purity, did not rise considerably, such as *Monochoria vaginalis* from Sukra (1.76 to 1.64) and East Karawang (1.86 to 1.43), indicating that some DNA components may have been lost or contaminated. The A_{260}/A_{230} ratio, which should be greater than 2, remained low across all ecotypes, indicating the presence of pollutants such as salts or phenols. Overall, purification reduced DNA concentration, but the quality improvement was not perfect.

Table 1. Spectrophotometer results of non-purified and purified DNA of *Monochoria vaginalis*

***M. vaginalis* Ecotype**	**Condition**	**Absorbance**				**(µg/mL)**	**DNA Quality**	
		A_{230}	**A_{260}**	**A_{280}**	**A_{320}**		**$A_{260/280}$**	**$A_{260/230}$**
Sensitive	Non-purified	2.19	1.95	1.40	0.70	1,276	1.77	0.84
	Purified	0.16	0.04	0.03	0.02	22.24	1.86	0.15
Patrol	Non-purified	2.15	1.77	1.29	0.74	1,050	1.87	0.73
	Purified	0.17	0.09	0.07	0.05	37.45	1.90	0.30
Sukra	Non-purified	2.47	2.38	1.87	1.20	1,199	1.76	0.93
	Purified	0.20	0.09	0.08	0.06	35.20	1.64	0.24
Rawamerta	Non-purified	0.64	0.64	0.37	0.13	529.10	2.11	1.01
	Purified	0.19	0.04	0.04	0.03	6.12	2.31	0.04
Karawang Timur	Non-purified	1.75	1.07	0.80	0.49	598.30	1.86	0.47
	Purified	0.18	0.08	0.07	0.05	32.45	1.43	0.25
Patokbeusi	Non-purified	2.30	1.81	1.30	0.80	1,032	2.02	0.68
	Purified	0.27	0.09	0.08	0.06	29.49	1.62	0.14
Ciasem	Non-purified	1.32	0.92	0.68	0.43	503.30	1.92	0.55
	Purified	0.21	0.07	0.06	0.04	26.02	1.62	0.16

Concentration (µg/mL) formula: $(A_{260}-A_{320})*(50/0.049)$ a µDrop plate: 0.049 cm pathlength; Purity (A_{260}/A_{280}) formula: $(A_{260}-A_{320})/(A_{280}-A_{320})$ source step: blank subtraction; Purity (A_{260}/A_{230}) formula: $(A_{260}-A_{320})/(A_{230}-A_{320})$ source step: blank subtraction

Moreover, the DNA of the seven *Monochoria vaginalis* samples was of good quality following purification, as evidenced by spectrophotometer quality tests (Table 1). The DNA recovered ranged from 6.12 to 37.45 µg/mL, sufficient for PCR analysis and usage. Meanwhile, the quality of the DNA collected fell within the range of figures that define pure DNA, namely 1.43-2.31 (A_{260}/A_{280}) and 0.038-0.30 (A_{260}/A_{230}). Genomic DNA isolated from samples with low contamination from polysaccharides, phenolic compounds, and proteins has a high A_{260}/A_{280} ratio of 1.8-2.0 and an A_{260}/A_{230} ratio of more than 2.0 (Abdel-Latif and Osman, 2017; Kaya and Şahin, 2025). Moreover, Turaki *et al.* (2017) found that DNA readings with an A_{260}/A_{230} ratio less than 1.6 suggest a high polysaccharide content.

Amplification of the *MvALS1* gene. Amplification of the *MvALS1* gene yielded an amplicon of around 1,931 bp in all seven *Monochoria vaginalis* ecotypes (Figure 3). Different base pairs may be read due to changes in DNA quality or unreadable sections close to the primer annealing sit (Crossley *et al.*, 2020). The low amplicon quantity is attributed to poor DNA quality during the PCR cycle. However, increasing the number of PCR cycles can increase the quantity of amplicons (Prosser *et al.*, 2016)

This study used leaf samples from seven different types of *Monochoria vaginalis* plants to extract DNA and perform PCR.These included one type that is known to be sensitive to metsulfuron-methyl, and six other types collected from the field.These six were previously found to be resistant or had been exposed to metsulfuron-methyl.The resistant types came from Patrol and Sukra in Indramayu Regency, Karawang Timur and Rawamerta in Karawang Regency, and Patokbeusi and Ciasem in Subang Regency (Putri *et al.*, 2024). The type that is easily affected by the herbicide, marked as "Sensitive" in Table 1 and Figure 3, comes from the Tambakdahan accession located in Kertajaya Village, Tambakdahan District, Subang Regency.This population was first created as a reference for herbicide sensitivity by Putri *et al.*in 2024. Using the same plant samples that were already studied in the earlier bioassay research made it possible to connect the molecular findings here like DNA quality, amount, and success of the *MvALS1* gene amplification directly to the known resistance traits of each plant type, instead of using unconfirmed field samples.

To check how DNA purification affects PCR results, amplification was also tried with genomic DNA that wasn't purified. The results showed no clear band, and instead there was a continuous smear seen close to the loading wells in all the sample lanes (Figure 2).This suggests that leftover polysaccharides and phenolic substances prevented the primers from binding properly and/or stopped the Taq polymerase from working effectively. In contrast, when using purified DNA, a clear band of about 1,931 bp appeared (Figure 3), showing that the DNA purification step was really important for successfully amplifying the *MvALS1* gene.

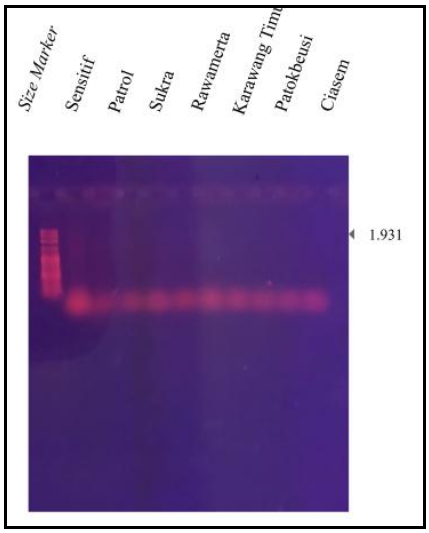


Figure 2. PCR amplification using non-purified DNA

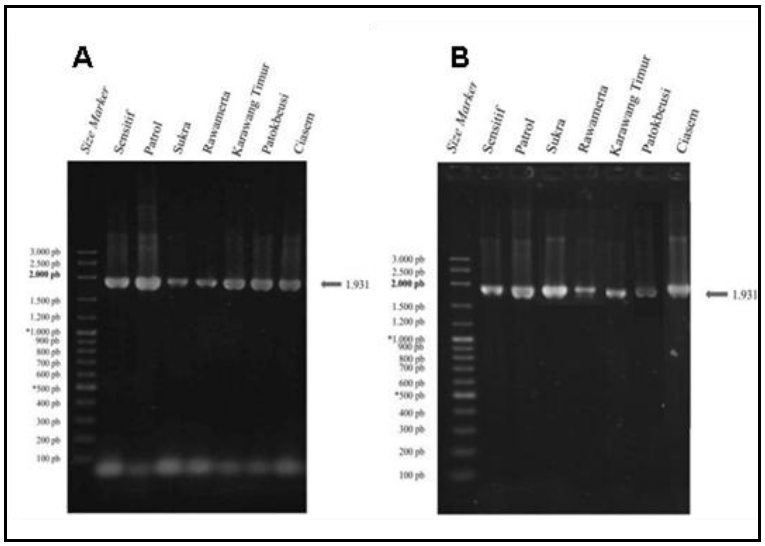


Figure 3. Visualization of DNA quality of PCR results in seven ecotypes of *Monochoria vaginalis*. Volume: A) 10 µL, B) 50 µL

Plant samples with significant phenolic content may disrupt the PCR process. Leaf DNA is difficult to get if the material is old, contains interfering substances, or takes a lengthy amplification time (Marengo *et al.*, 2019). Purification with 30 µL of sodium acetate and 1,000 µL of 70% ethanol resulted in a satisfactory amplicon of PCR for *Monochoria vaginalis* in this study.

Conclusion

Purification with 30 µL of sodium acetate and 1,000 µL of 70% ethanol enhanced DNA purity. Electrophoresis results revealed lower smear intensity and clearer DNA bands in purified samples, particularly those from Sukra, Rawamerta, East Karawang, Patokbeusi, and Ciasem. Purification lowered DNA content in all *Monochoria vaginalis* ecotypes from baseline values to a post-purification range of 6.122-37.450 µg/mL, but yields were still sufficient for PCR analysis. Spectrophotometric measurements indicated higher DNA purity, with A_{260}/A_{280} ratios ranging from 1.43 to 2.31 and $A_{260}/_{A230}$ ratios from 0.04 to 0.30. The successful amplification of the *MvALS1* gene 1,931 bp in all seven ecotypes demonstrates the effectiveness of the purifying method. The confirmed *MvALS1* mutations provide a useful method for quickly testing resistance using PCR, letting farmers find resistant *M. vaginalis* strains before applying herbicides and helping them manage weeds more effectively and sustainably in rice fields in West Java. Future research might improve the purification process to achieve a better A_{260}/A_{230} ratio and study a bigger group of *M. vaginalis* samples to look at both the TSR mutations found here and other ways the organism might become resistant to the treatment, called NTSR. The confirmed *MvALS1* mutations might also be used to create a quick diagnostic test based on molecular markers, which can help check crop populations for resistance before applying herbicides.

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