OPTIMIZATION OF DNA ISOLATION FROM HOP CONES AND PELLETS FOR MICROSATELLITE ANALYSIS

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Abstract

Reliable identification of hop (*Humulus lupulus* L.) cultivars is important for quality control and authentication in the brewing industry. DNA-based methods provide a powerful tool for this purpose, but isolation of high-quality DNA from processed hop materials such as cones and pellets can be challenging due to the presence of PCR inhibitors, including polyphenols, polysaccharides, and bitter acids. In this study we compared four cetyltrimethylammonium bromide (CTAB)-based DNA extraction protocols for hop cones and pellets, with the aim of improving yield and purity of DNA used for microsatellite genotyping. The tested methods included the standard CTAB protocol, CTAB supplemented with polyvinylpyrrolidone (PVP40), CTAB with PVP40 and activated charcoal, and CTAB with PVP10 and liquid nitrogen grinding. Additionally, a hexane pre-treatment step was evaluated with the aim to reduce the amount of PCR inhibitory compounds. DNA quality was assessed using NanoVue, Qubit, and agarose gel electrophoresis. Agarose gels showed intact high-molecular-weight DNA with minor RNA traces. Microsatellite genotyping confirmed consistent allele profiles across the first three extraction methods, thus confirming the suitability of CTAB-based methods for reliable hop genotyping.

Key words: hop, *Humulus lupulus*, DNA extraction, CTAB, genotyping

OPTIMIZACIJA IZOLACIJE DNA IZ STORŽKOV IN PELETOV HMELJA ZA ANALIZO MIKROSATELITOV

Izvleček

Možnost zanesljivega določanja sorte hmelja (*Humulus lupulus* L.) je pomembna za zagotavljanje kakovosti in avtentičnosti v pivovarski industriji. Metode, ki temeljijo na analizi DNA, so za ta namen učinkovito orodje, vendar je izolacija visokokakovostne DNA iz procesiranih oblik hmelja, kot so storžki in peleti, zahtevna zaradi prisotnosti inhibitorjev PCR, med katerimi so polifenoli, polisaharidi ter alfa in beta kisline. V tej raziskavi smo primerjali štiri protokole za izolacijo DNA na osnovi CTAB iz hmeljnih storžkov in peletov. Naš namen je bil izboljšati koncentracijo in čistost DNA, ki jo uporabimo za genotipizacijo hmelja z mikrosateliti. Preizkušene metode so bile: standardni CTAB protokol, CTAB z dodatkom polivinilpirolidona (PVP40), CTAB z dodatkom PVP40 in aktivnega oglja ter CTAB z dodatkom PVP10 in s predhodno homogenizacijo v tekočem dušiku. Poleg tega smo preizkusili tudi predobdelavo s heksanom, katere namen je bil v izolirani DNA zmanjšati količine spojin, ki zavirajo PCR. Kakovost izolirane DNA smo določali z instrumentoma NanoVue in Qubit ter z agarozno gelsko elektroforezo s katero smo pokazali prisotnost nepoškodovane DNA visoke molekulske mase z manjšimi sledovi RNA. Genotipizacija z mikrosateliti je pokazala skladne alelne vzorce pri prvih treh metodah izolacije, kar potrjuje primernost CTAB-protokolov za zanesljivo genotipizacijo hmelja, tako iz storžkov, kot iz peletov.

Ključne besede: hmelj, *Humulus lupulus*, izolacija DNA, CTAB, genotipizacija

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1 INTRODUCTION

The cultivar of hops (*Humulus lupulus* L.) plays a major role in their economic value, since different varieties provide unique brewing qualities such as aroma, bitterness, and essential oil content. Because of this, reliable identification of hop cultivars is important for both quality control and preventing mislabeling in the brewing industry. DNA-based methods have the potential to support this process, but authenticating cultivars in processed forms such as cones and pellets is still difficult. This is largely because this type of hop tissue contains high levels of PCR inhibitors, including polyphenols, polysaccharides, and bitter acids, which reduce the success and reliability of DNA amplification. Developing strategies to overcome these obstacles is essential for establishing consistent molecular tools for hop cultivar authentication.

Molecular markers have been widely used in hop genetics, breeding, and germplasm studies over the past decades. Early work with RAPD and microsatellite sequences showed that DNA markers can successfully differentiate hop cultivars (Brady et al., 1996). Later research at the Slovenian Institute of Hop Research and Brewing, Biotechnical faculty University of Ljubljana and partner institutions further developed these methods. Jakše et al. (2001) combined SSR and AFLP markers to study variation and differentiation between hop genotypes, later Jakše et al. (2004) showed large microsatellite diversity in wild and cultivated hops from Europe, Asia and North America with a clear genetic structure related to geographical origin. Murakami et al. (2006) confirmed these results on a larger set of wild hops, showing distinct genetic grouping among continental populations.

The development of new molecular markers further improved the precision of genetic analyses. Hadonou et al. (2004) and Štajner et al. (2005) described new polymorfic microsatellite loci with high information value. Similarly, Čerenak et al. (2004) developed a practical microsatellite-based system for identifying hop cultivars, that is able to separate clones and detect variation among geographically distinct varieties. Later Čerenak et al (2012) used RAPD and microsatellite markers to study the genetic diversity of wild and cultivated hops stored in the Slovenian hop gene bank, confirming their usefulness for evaluating genetic relationships between accessions.

More recent studies expanded the use of molecular tools by combining genetic and chemical analyses. Paquet et al. (2023) used microsatellite genotyping together with metabolomic and chemical profiling of wild hops from northern France, demonstrating strong correlation between genetic structure and chemical composition. Such combined approaches show the importance of molecular markers not only for cultivar identification but also for studying diversity, adaptation and the evolutionary history of hops.

The Slovenian Institute of Hop Research and Brewing uses an internal protocol for hop cultivar genotyping, which is based on the work of Pokorn (2011) where genotyping of *H. lupulus* cultivars is carried out with fluorescent microsatellite markers. In that study young leaves were used as the source of plant material for DNA isolation by the cetyltrimethylammonium bromide (CTAB)-based method, an extraction method which is commonly used in plant research. However, when this approach was extended to hop cones and pellets, the quality and reliability of genotyping results were lower compared to those obtained from leaves. Therefore, this study focused on optimizing DNA extraction for hop cones and pellets, in order to achieve accurate and consistent genotyping.

Korbecka-Glinka et al. (2016) developed a similar genotyping method of hops using CTAB DNA extraction protocol and six microsatellite loci. DNA of sufficient quality was obtained from leaves and cones, but also from highly processed pellets, with consistent results in the replicates. Most samples matched the declared cultivar, and the method was sensitive enough to detect mixtures as low as 3–5%. Similarly, Krofta and Patzak (2014) showed that DNA can be successfully isolated from both young leaves and dried hop cones of Czech cultivars using a CTAB-based method. With SSR, STS, and EST-SSR markers, they reliably identified all registered cultivars and detected admixtures as low as 5%. They showed that the method is suitable for authenticity and purity control of hop material. Patzak and Henychová (2018) confirmed that the CTAB method enables successful DNA isolation from young leaves, dried cones, and pellets of diverse hop cultivars. The protocols described involve grinding of leaf samples, incubation with CTAB extraction buffer, and several purification steps to remove PCR inhibitors. After extraction, DNA quantity and quality are always checked to confirm that the samples are suitable for molecular analysis. The DNA is then used for PCR amplification with fluorescently labelled microsatellite primers, and the resulting fragments are separated and analyzed by capillary electrophoresis. This provides a reliable identification of hop cultivars and is an effective tool for authentication and diversity studies.

In addition to DNA-based approaches, chemical analysis can also be used to determine hop variety. Ocvirk et al. (2016) used gas chromatography, HPLC, and FTIR spectroscopy combined with chemometric methods to

genotype hop samples and achieved nearly 100% correct results for five major Slovenian cultivars (Aurora, Savinjski golding, Bobek and Celeia).

We hypothesized that certain hop varieties may present greater challenges for genotyping due to their higher contents of α-acids, β-acids, and essential oils. Residual traces of these compounds can act as inhibitors and interfere with PCR amplification, thereby reducing the reliability of microsatellite analysis. To address this, our aim in this study was to evaluate several DNA extraction protocols and identify one that provides both reliable and efficient isolation of DNA from hop cones and pellets. We further assumed that a pre-isolation step involving incubation of samples in hexane could remove a substantial portion of these inhibitory compounds and in that way improving DNA purity and facilitating successful PCR and genotyping.

2 MATERIAL AND METHODS

2.1 Plant Material

Five hop varieties were included in this study, represented either as cones or vacuum-packed cones and pellets (Table 1, Picture 1). The selected samples covered a wide range of chemical characteristics, including α-acid, β-acid, and essential oil contents, which are known to influence both brewing quality and the efficiency of DNA isolation due to their potential role as PCR inhibitors. This diversity allowed us to evaluate DNA extraction performance across hop genotypes with different biochemical profiles and processing forms.

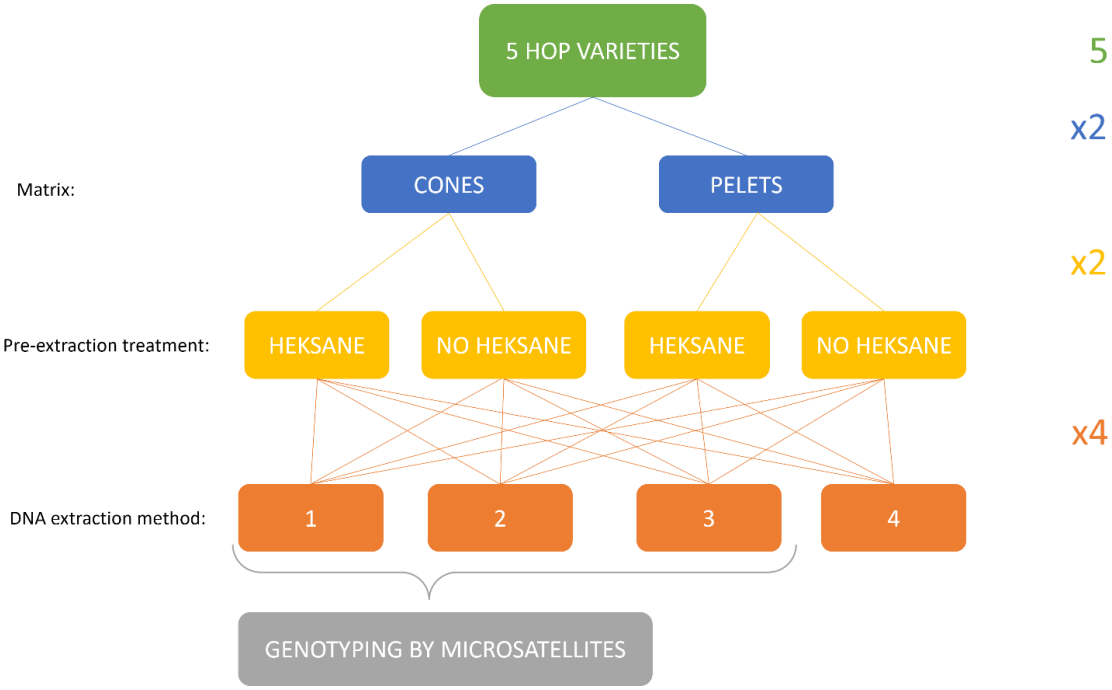

Figure 1: Workflow scheme.

Table 1: Characteristics of hop samples used in the study (Hop variety catalogues, IHPS).

No.	Variety	α-acids (% w/w)	β-acids (% w/w)	Essential oils (ml/100 g hops)
1	Celeia	3.0 – 6.5	2.0 – 3.3	1.5 – 3.6
2	Aurora	7.2 – 12.6	2.7 – 4.4	0.9 – 1.6
3	Styrian Wolf	13.5 – 18.5	5.0 – 6.0	3.0 – 4.5
4	Styrian Cardinal	10.0 – 15.0	3.2 – 4.6	3.0 – 4.0
5	Styrian Kolibri	4.0 – 6.0	3.8 – 5.4	1.0 – 2.0

Figure 2: Hop (A) cones and (B) pellets used in this study

2.2 Pre-Extraction Treatment with Hexane

For the pre-extraction treatment, 1,3 g of hop cones or pellets were placed in a 100 ml beaker and supplemented with 25 ml of hexane (Merck). The beaker was sealed with parafilm, and the mixture was stirred using a magnetic stirrer for 40 min. Following incubation, cones and hexane were separated using a metal colander and filter paper. The plant material was then left in a fume hood until complete evaporation of residual hexane. Subsequent DNA extractions were carried out using a portion of the pretreated samples.

2.3 DNA Extraction Protocols

We compared four CTAB-based DNA extraction protocols for hop cones and pellets performing all extractions both with and without hexane pre-treatment:

(1) Standard CTAB protocol (Kump & Javornik, 1996):
Approximately 50 mg of sample was homogenised in a mortar in 1ml CTAB buffer (2% CTAB, 100 mM Tris-HCl pH 8, 1.4 M NaCl, 20 mM EDTA pH 8, and 0.2% β-mercaptoethanol). 700 µl was transferred to a microcentrifuge tube and incubated at 68 °C for 1.5–2 h. After incubation, an equal volume of chloroform:isoamyl alcohol (24:1) was added, mixed vigorously, and centrifuged (16,000 × g, 15 min, 4 °C). The aqueous phase was transferred to a fresh tube, mixed with 0.1 volume 3 M sodium acetate (pH 5.2) and 1 volume cold isopropanol, and incubated at −20 °C for at least 30 min. DNA was pelleted (16,000 × g, 15 min, 4 °C), washed with 70% ethanol, dried, and resuspended in 100 µl TE buffer.

(2) CTAB with PVP40:
The extraction was performed as described in the above protocol (1), except that 1% (w/v) PVP40 was added to the CTAB buffer to bind phenolic compounds.

(3) CTAB with PVP40 and activated charcoal (modified from Križman et al., 2006):
Approximately 50 mg of plant tissue was homogenised in extraction buffer (100 mM Tris-HCl pH 8, 2.0 M NaCl, 20 mM EDTA pH 8, 2% CTAB, 1% PVP40, and 0.5% activated charcoal). Samples were incubated at 55 °C for 30 min with agitation and centrifuged (16,000 × g, 10 min, room temperature). The supernatant was extracted with chloroform:isoamyl alcohol (24:1) and centrifuged again; this step was repeated until the solution cleared. DNA was precipitated with 0.45 volumes of isopropanol, incubated for 1 h at 25 °C, and pelleted by centrifugation. Pellets were washed with 75% ethanol containing 15 mM ammonium acetate, air-dried, and resuspended in 100 µl TE buffer.

(4) CTAB with PVP10 and liquid nitrogen homogenisation (Patzak, 2025):
When using this protocol, samples were finely ground in liquid nitrogen prior to extraction. The CTAB buffer was added to 50 mg of the powderd samples and contained 1% (w/v) PVP10 in addition to the standard components (2% CTAB, Tris-HCl, NaCl, EDTA, β-mercaptoethanol). DNA isolation was then carried out as in protocol (1).

2.4 DNA Quality Assesment

The quality and quantity of the isolated DNA were evaluated using a combination of spectrophotometric, fluorometric, and electrophoretic separation methods.

Spectrophotometric measurements were carried out with a NanoVue instrument (GE Healthcare) to determine purity based on the A260/A280 and A260/A230 absorbance ratios, which provide an indication of protein and polysaccharide/phenolic contamination, respectively.

In parallel, DNA concentrations were quantified more accurately using fluorometry with the Qubit™ 4 Fluorometer and the Qubit™ dsDNA BR Assay Kit (Thermo Fisher Scientific), which is less affected by residual contaminants.

DNA integrity was examined by electrophoresis on 1 % agarose gels stained with ethidium bromide. Electrophoresis was carried out in a Sub-Cell Model 192 system (Bio-Rad Laboratories, Hercules, USA) at 90 V for 1 h. DNA bands were visualised under a UV transilluminator and documented using a G:BOX imaging system (Syngene).

2.5 Microsatellite Genotyping

Microsatellite analysis was performed following a protocol adapted from Pokorn (2011). DNA was diluted to a working concentration of 20 ng/µL. PCR reactions were prepared in a final volume of 10 µL, containing 1x PCR buffer, 2 mM $MgCl_2$, 0.8 mM dNTPs, 0.2 µM of each forward and reverse primer, 0.25 µM fluorescently labeled TAIL primer, 0.025 U Taq DNA polymerase, and 5 µl of template DNA, diluted to 20 ng/µl.

Amplifications were performed on a thermocycler under the following conditions: initial denaturation at 94 °C for 5 min; touchdown phase of 5 cycles with denaturation at 94 °C for 45 s, annealing at 60 °C (decreasing 1 °C per cycle) for 30 s, and elongation at 72 °C for 90 s; followed by 30 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 90 s; with a final extension at 72 °C for 8 min. PCR products were checked for successful amplification on agarose gel electrophoresis before submitting the samples for fragment analysis.

Four primer pairs (5-2, GA5-G3-10, 11a59, and GA7-A6-14) were used for genotyping (Table 2). Each forward primer contained a universal TAIL sequence for fluorescent labeling. Fluorescent dyes were assigned to the TAILs in this way: 6-FAM = blue, NED = yellow/black, VIC = green and PET = red.

Table 2: Primers used in this study

Primer name	Sequence (5' → 3')
5-2_F	TGTAAAACGACGGCCAGTCGAATGGTCCTAGATATCCCC
5-2_R	CAGTAAATGGATGCTTGAAGGC
GA5-G3-10_F	TGTAAAACGACGGCCAGTGCACAACCAGAGCTCCCTTA
GA5-G3-10_R	CTCGAAATCCCAACAACCAC
11a59_F	TGTAAAACGACGGCCAGTGCTTCAACCCTCTAATTTCTGACC
11a59_R	AGAAGGGATACACTCGGTTAATCC
GA7-A6-14_F	TGTAAAACGACGGCCAGTGGCAAGGCTAACCACCATTA
GA7-A6-14_R	CTGTTTCCCGCCAAATTAAA

For fragment analysis, 5 µl of PCR product from four different primer pairs were pooled (total volume 20 µl), centrifuged, and 3 µl of the combined sample was mixed with 0.5 µl GeneScan™ 600 LIZ™ dye size standard (Thermo Fisher Scientific) and 10.6 µL HiDi™ formamide (Applied Biosystems). Capillary electrophoresis was performed using an ABI genetic analyzer, and allele sizes were determined with GeneMapper Software v6.0 (Applied Biosystems). Raw data is available on demand.

3 RESULTS AND DISCUSSION

3.1 DNA Quality Assesment

Firstly, DNA quality was assessed using a NanoVue spectrophotometer, with particular attention to purity ratios. The absorbance ratio A260/A280 provides an estimate of protein contamination in nucleic acid samples. For double-stranded DNA the optimal ratio is approximately 1.8 (ranging from 1.7 to 1.9), lower values indicate protein contamination. In contrast, pure RNA samples typically exhibit higher ratios, around 2.1 (NanoVue, 2007; Koister & Cantor., 2019).

Since CTAB-based extraction protocols, although designed for DNA isolation, also co-extract RNA, the expected A260/A280 ratios for our samples should fall between 1.8 and 2.1. The average A260/A280 ratios obtained for DNA extracted using different CTAB-based protocols are presented in Figure 2. Across all treatments, the measured ratios ranged from 1.579 to 2.049. The optimal purity range for double-stranded DNA (1.7–1.9) is indicated by the green reference line. Most samples extracted with the (1) CTAB, (2) CTAB + PVP40, and (3) CTAB + PVP40 + activated charcoal protocols exhibited A260/A280 ratios close to or slightly above this optimal range, suggesting high DNA purity with minimal protein contamination. In contrast, pellet samples obtained using the (4) CTAB + N_2 method showed lower ratios (1.579), indicating possible protein or phenolic compound contamination. Generally, the addition of PVP40 or activated charcoal slightly improved the purity of extracted DNA, but the addition of liquid nitrogen homogenisation step did not enhance DNA sample quality.

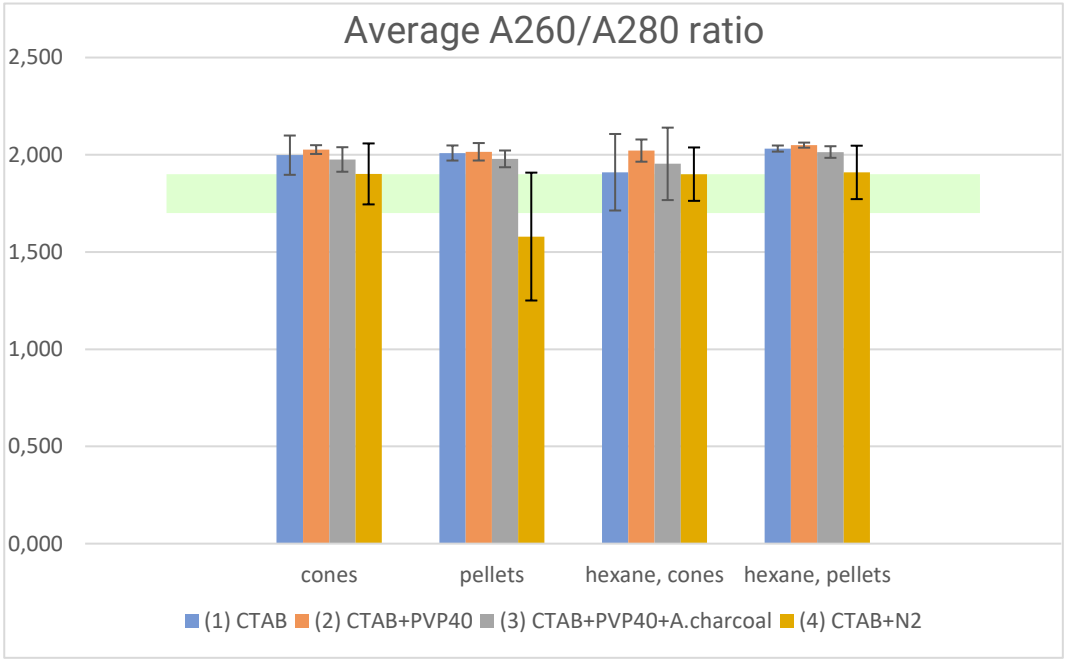

Figure 3: Average A260/A280 ratios indicating protein purity of the DNA samples of hop cones and pellets

Legend: The green line represents the optimal values for pure DNA (1,7-1,7)

The absorbance ratio A260/A230 serves as an indicator of contamination from organic compounds and salts that absorb strongly at 230 nm (e.g., guanidine, EDTA, Triton™ X-100, Tween® 20, phenol, polysaccharides and silica particles) (NanoVue, 2007; Koister & Cantor, 2019). dsDNA typically exhibits A260/A230 ratios between 2.0 and 2.2, while pure RNA samples range from 2.1 to 2.3. The results presented in Figure 3 show that most samples extracted using CTAB-based protocols displayed A260/A230 ratios below these ideal values, suggesting the presence of residual contaminants from the extraction process. Among the tested methods, (2) CTAB + PVP40 and (3) CTAB + PVP40 + activated charcoal yielded the highest ratios (up to 2.191 in cone samples), indicating improved removal of interfering compounds. Samples extracted using (4) CTAB + N_2 consistently exhibited the lowest ratios, with values as low as 0.697 in pellet samples, indicating contamination by salts, phenolic compounds, or polysaccharides.

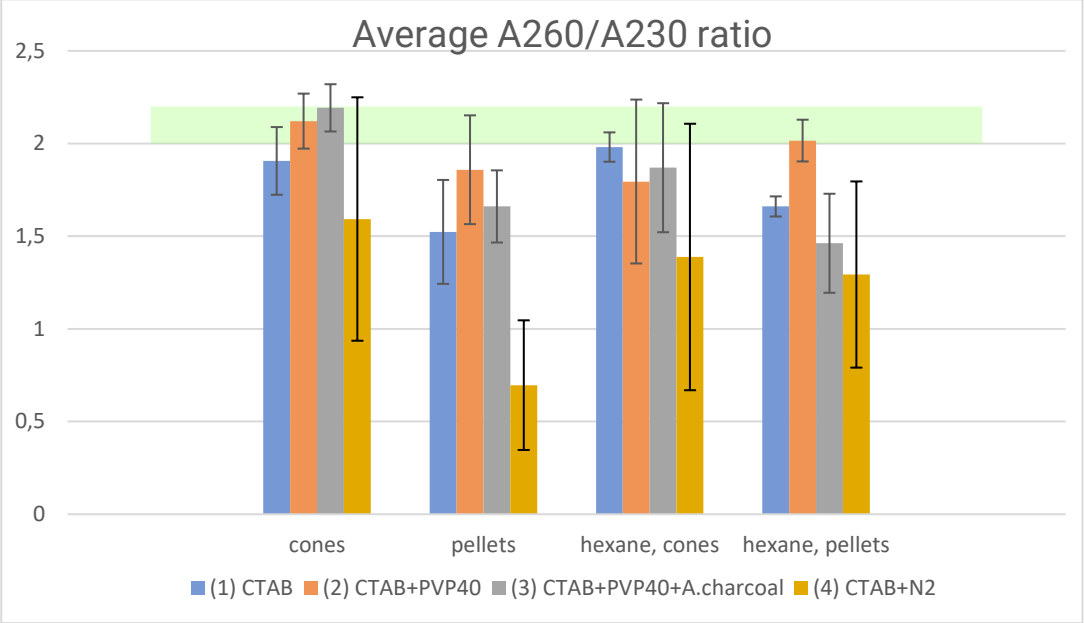

Figure 4: Average A260/A230 ratios indicating presence of residual contaminants in the DNA samples of hop cones and pellets

Legend: The green line represents the optimal values for pure DNA (2.0-2.2)

DNA concentrations were quantified using a Qubit fluorometer rather than the NanoVue spectrophotometer to avoid overestimation caused by RNA co-extraction when using CTAB-based methods. The results are presented in Figure 4. The average DNA concentrations varied among the extraction protocols, ranging from 13.66 to 173.92 ng/µl. The standard CTAB method consistently yielded the highest or comparable DNA concentrations across most sample types, with values up to 173.92 ng/µl in hexane-treated cone samples. The inclusion of PVP40 or activated charcoal slightly improved DNA recovery in some cases (notably reaching 172.4 ng/µL with (3) CTAB + PVP40 + activated charcoal in pellets), meaning that these additives can enhance extraction efficiency by binding phenolic compounds. In contrast, the (4) CTAB + N_2 protocol resulted in significantly lower DNA yields across all sample types, with concentrations below 45 ng/µL, indicating that the use of liquid nitrogen in this context did not improve extraction performance. Overall, this data demonstrates that the (1) standard CTAB and (3) CTAB + PVP40 + activated charcoal methods are the most effective for obtaining high-quality, high-yield DNA suitable for downstream molecular analyses.

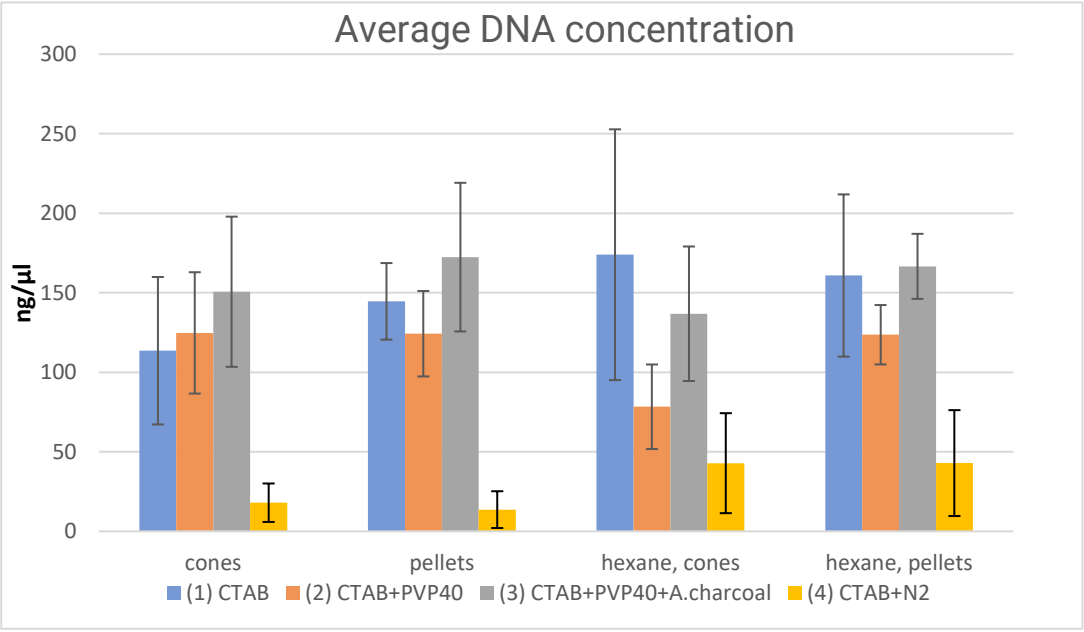

Figure 5: Average DNA concentration of hop cones and pellets in ng/µl

3.2 Assessment of DNA Integrity by Agarose Gel Electrophoresis

The quality and integrity of the extracted nucleic acids were further evaluated by agarose gel electrophoresis (Figure 6). Only samples obtained using the (1) CTAB, (2) CTAB + PVP40, and (3) CTAB + PVP40 + activated charcoal protocols were analyzed, as the (4) CTAB + N_2 extractions yielded DNA concentrations too low for visualization. Amongst all three tested methods we can observe clear high-molecular-weight bands that prove successful extraction of intact nucleic acids. However, as the CTAB-based protocols we used lacked a RNAse treatment step, we co-extract both DNA and RNA, therefore several samples demonstrate additional low-molecular-weight bands coming from undegraded ribosomal RNA (18S and 28S rRNA). No evident DNA degradation was detected and we confirmed that all three CTAB-based methods successfully isolated high-quality nucleic acids suitable for downstream molecular applications.

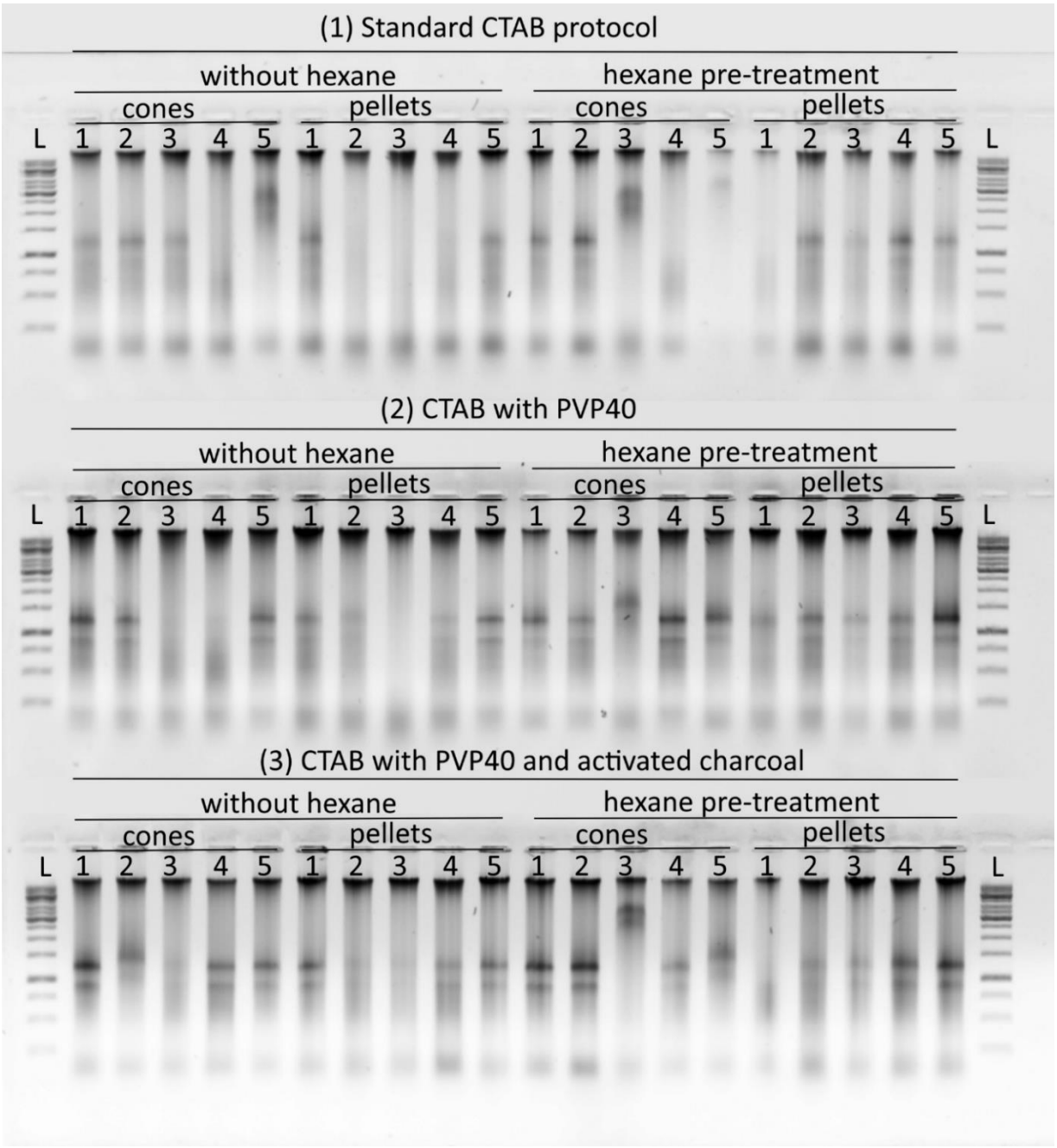

Figure 6: Results of agarose gel electrophoresis for cone and pellet hop DNA samples extracted with CTAB-based extraction methods with different pre-treatments

LEGEND: 1 = Celeia; 2 = Aurora; 3 = Styrian Wolf; 4 = Styrian Cardinal; 5 = Styrian Kolibri; L = GeneRuler 1 kb DNA Ladder (ThermoScientific)

3.3 Microsatellite Genotyping

Genotyping with fluorescent microsatellite markers (Pokorn, 2011) showed consistent and well visible allele patterns for DNA extracted using the first three protocols ((1) CTAB, (2) CTAB + PVP40, and (3) CTAB + PVP40 + activated charcoal) (Figure 7). A few samples did not amplify at certain loci, but these failures were random and not connected to any specific extraction method. Interestingly, rare or unexpected alleles were also detected. In the variety Kolibri, the majority of samples showed an additional 181 bp allele at the GA5-G3-10 locus, which has not yet been recorded in the reference database. Subsequent verification confirmed that this additional allele is genuine and represents a previously unreported fragment in Kolibri.

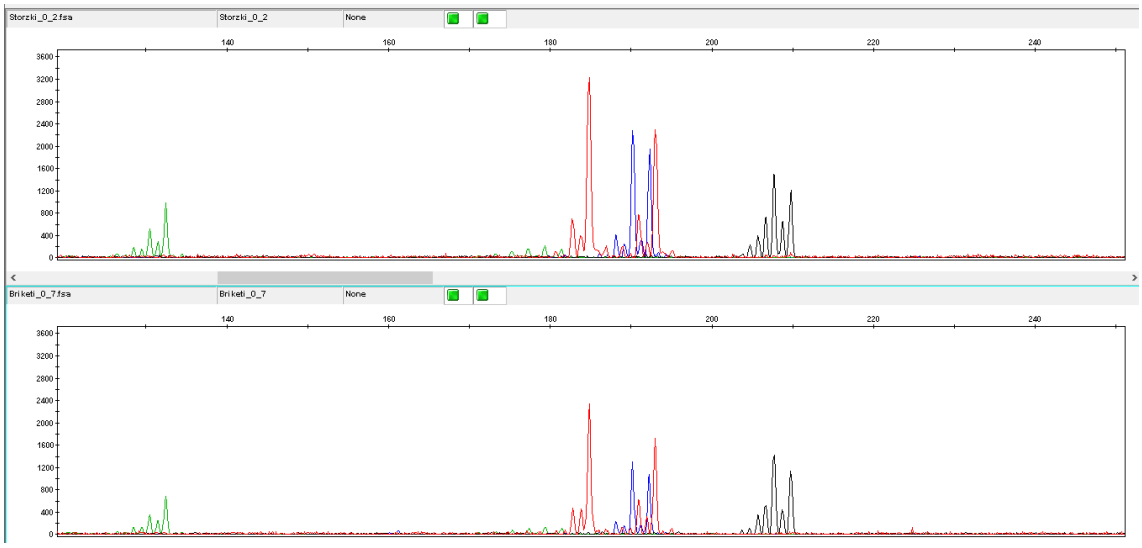

Figure 7: Representative electropherograms of microsatellite analysis from hop DNA samples. Capillary electrophoresis profiles showing microsatellite markers amplified from hop variety 'Aurora' DNA isolated from cones (up, Storzki_0_2) and pellets (down, Briketi_0_7). Both samples were processed using classical CTAB isolation method without hexane pre-incubation. The x-axis represents fragment size in base pairs, and the y-axis shows fluorescence intensity in relative fluorescence units (RFU). Different colours represent different fluorescent dyes used for distinct microsatellite markers.

The effect of different sample preparation methods on microsatellite peak heights was evaluated using normalized data to account for variety-specific variations (Figure 8). After z-score normalization within each variety, Kruskal-Wallis statistical analysis of microsatellite 11a59 peak height (1 allele per variety) revealed that the matrix type significantly influenced peak heights ($p = 0.0058$), with distinct patterns observed between cone and pellet samples. The DNA isolation method also showed significant effects ($p = 0.0266$), with the CTAB + PVP40 method demonstrating the most consistent results across varieties. Hexane pre-incubation exhibited a marginal effect ($p = 0.0719$), suggesting a subtle influence on peak heights that became more apparent after controlling for variety differences.

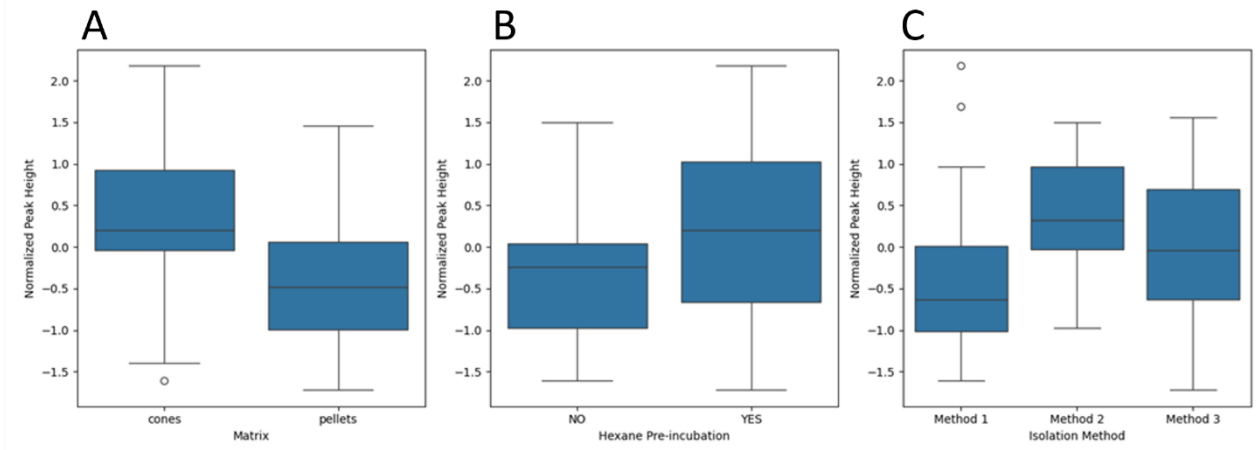

Figure 8: Impact of sample preparation methods on microsatellite peak heights in hop DNA analysis. Normalized peak heights compared across (A) matrix type, (B) hexane pre-incubation treatment, and (C) DNA isolation methods. Data normalized within varieties using z-score transformation.

These results additionally confirm that tested methods produced DNA of sufficient quality for reliable PCR amplification and genotyping with indication for improvement of results by hexane pre-incubation and isolation protocol 3 (classical CTAB with PVP40).

4 CONCLUSION

The results of this study show that three out of four tested CTAB-based extraction methods produced DNA of acceptable purity and integrity for downstream molecular analyses. The addition of PVP40 and activated charcoal slightly improved DNA purity by reducing contamination from phenolic compounds and polysaccharides, while the use of liquid nitrogen and PVP10 did not provide any benefit. DNA concentrations measured with Qubit ranged from 13.66 to 173.92 ng/µL, with the (1) standard CTAB and (3) CTAB + PVP40 + activated charcoal protocols giving the highest yields. Agarose gel electrophoresis confirmed that the extracted nucleic acids were largely intact, with no visible DNA degradation, although RNA bands were present due to the absence of RNase treatment.

Microsatellite genotyping further confirmed that DNA from the first three extraction protocols had sufficient quality for consistent PCR amplification and allele detection. Statistical evaluation of microsatellite peak heights revealed that both the matrix type and extraction method significantly influenced amplification intensity, while hexane pre-incubation showed a marginal but positive effect on signal strength. DNA from cone samples generally produced higher and more consistent fluorescence peaks than DNA from pellets. Among extraction protocols, the CTAB + PVP40 method demonstrated the most stable performance across varieties, suggesting improved removal of PCR inhibitors. In most cases, allele profiles matched reference data, although a new 181 bp allele was identified in the Kolibri variety, representing a previously unreported variant.

Overall, this study confirms that CTAB-based methods remain reliable for genotyping DNA from hop cones and pellets. These methods yield DNA of high quality and purity suitable for reliable microsatellite genotyping of hop tissues. The inclusion of PVP40 and activated charcoal improves DNA purity, and pre-incubation with hexane may further enhance PCR performance. Despite their reliability, CTAB-based extractions are time-consuming and labour-intensive, typically requiring around three hours. The duration can increase with larger amounts of sample, mainly due to the manual homogenization step. Therefore, future work will focus on developing a faster and more efficient DNA isolation protocol that maintains the quality required for accurate and reproducible hop genotyping.

Acknowledgment

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Data Availability

Data are available from the corresponding author upon reasonable request.

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