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Research Article

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Effects of Drying Methods, Tissue Types and Grinding Methods on Plant DNA Quality

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Abstract

Objective: This study systematically evaluated how different drying methods affect DNA quality in herbarium specimens, with a specific focus on the interaction between drying techniques, tissue types, and grinding methods.

Materials and Methods: DNA concentrations and purity were evaluated for three tissue types (leaf, flower, fruit), three drying methods (filter paper, newspaper, silica gel), and two grinding techniques (homogenizer, manual).

Results: The drying method significantly influenced DNA quality, with frequently changed filter paper consistently yielding the highest DNA concentration (23.26-27.72 ng/µL) and purity (1.78-1.90) across all tissues. Silica gel provided comparable results, offering a practical alternative when frequent material replacement is unfeasible. Tissue-specific responses were observed, with leaf tissue showing greater sensitivity to suboptimal drying conditions (concentration reduction of up to 84% with never-changed newspaper) compared to reproductive tissues. Principal component analysis captured 100% of the data variance (PC1: 89.30%, PC2: 10.70%), confirming distinct clustering patterns based on drying method and tissue type. A significant positive correlation between DNA concentration and purity ($r = 0.79$, $p < 0.001$) was observed overall, though this relationship varied according to tissue type. Grinding method effects were tissue-dependent, with homogenization generally improving DNA recovery, except in poorly dried leaf tissue.

Conclusion: These findings provide practical recommendations for optimizing preservation protocols according to target tissues, significantly enhancing the utility of herbarium specimens for long-term molecular research applications.

Keywords Plant DNA quality · Herbarium specimens · Drying methods · Tissue types · Grinding techniques · DNA concentration · DNA purity

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INTRODUCTION

Herbaria are curated collections of preserved plant specimens that serve as indispensable resources for taxonomy, systematics, phylogenetics, and biodiversity research. The phylogenetic classification system, initially shaped by the evolutionary theories of Lamarck and Darwin in the 19th century, continues to evolve with advancements in botanical science. The current botanical science approach has significantly enhanced this system through the integration of molecular data and bioinformatic approaches, allowing for more precise delineation of evolutionary relationships among plant taxa. Recent advancements in molecular studies have significantly supported plant classification, taxonomy, and plant identification through the integration of molecular data.[1] Herbarium specimens play a significant role in these investigations. However, field-based research often encounters challenges, including time and budget constraints, when target plants are extinct in their natural habitats, not in appropriate flowering or fruiting time, located in remote or inaccessible regions, or when accurate taxonomic identification is limited by insufficient expertise. Consequently, herbarium specimens are a significant resource for such studies.

The preparation and preservation of herbarium specimens are critical steps in ensuring the long-term usability of plant materials for molecular studies.[2] Among these steps, the drying process plays a pivotal role, as it directly impacts the quality and concentration of genomic deoxyribonucleic acid (DNA) extracted from the specimens. Most DNA damage in herbarium specimens originates from sample preparation. The different drying methods used cause varying degrees of damage.[3-5] Among the critical variables in specimen preparation, both the drying method and the frequency of drying material replacement can significantly impact DNA preservation, yet the latter has received limited attention in previous studies. Traditional drying methods, such as pressing plants between layers of paper, remain widely used; however, modern techniques like silica gel drying, have gained popularity due to their efficiency in preserving DNA integrity.[6] The determination of an appropriate drying methodology and the frequency of material replacement during the process can significantly influence the success of molecular analyses, making careful optimization essential for reliable results. These protocols directly affect nucleic acid quality, as improper drying can lead to the degradation of genetic material over time.

The extraction of DNA from herbarium specimens frequently results in significant degradation, which can impede the extraction of amplifiable genetic material. In addition to inhibiting amplification, this damage can generate sequence artifacts during Polymerase Chain Reaction (PCR). Damaged nucleotides can cause DNA polymerase enzyme misreading, leading to erroneous sequence data. Studies have confirmed that the most effective method for preserving DNA integrity is to ensure that plant material is dried properly. The preservation quality directly affects molecular analyses, with specimens that have undergone adequate desiccation yielding higher-quality genomic data for phylogenetic and taxonomic studies. Consequently, the implementation of optimal drying protocols is necessary to ensure the integrity of specimens for molecular research applications.[2,7] Plants exhibit significantly greater chemical diversity than other organisms, containing thousands of primary and secondary metabolites. Among them, polyphenols and polysaccharides have a substantial impact on DNA extraction. These compounds adversely affect the process by inducing DNA oxidation, forming covalent bonds with nucleotides, and inhibiting enzymatic reactions during PCR amplification.[8] Additionally, plant species do not respond uniformly to drying methods, as variations in their chemical composition and structural characteristics can lead to differing outcomes in DNA preservation and quality.[2] Different plant tissues vary significantly in their chemical composition, with leaves typically containing higher concentrations of secondary metabolites and polyphenols compared to reproductive tissues. These biochemical differences may influence the responses of tissues to preservation methods, making comparative analysis essential for developing optimized protocols.

This study focused on *Angelica sylvestris* L., a species with a widespread distribution that grows in forests, along streams, and damp grassy areas. Its preference for moist environments poses significant challenges during the drying process, as plants from such habitats are more difficult to preserve without risking DNA degradation.[9] These characteristics make *A. sylvestris* an ideal plant sample for this research, which aims to assess how different drying methods (filter paper, newspaper, silica gel) and drying frequencies affect the yield and purity of genomic DNA in herbarium samples. While the present study focused on *A. sylvestris* as a model organism because of its moisture-rich tissues and widespread distribution, the methodological findings are expected to be broadly applicable across taxonomically diverse plant groups, particularly those from humid environments. Additionally, this study compared the effectiveness of manual and mechanical homogenization in grinding plant tissues during DNA isolation and evaluated their impact on nucleic acid quality. By optimizing preservation and extraction protocols,

this research seeks to improve the reliability of molecular analyses of archived herbarium specimens, particularly those derived from moisture-rich plant species.

MATERIALS AND METHODS

Plant Material

This study focused on *A. sylvestris* species. Samples collected from various regions of Türkiye were identified, and voucher specimens were deposited in the Istanbul University Faculty of Pharmacy Herbarium (ISTE). For this research, multiple plant parts (leaf, flower, and fruit) from *A. sylvestris* specimens were utilized, with each specimen assigned a unique ISTE accession number. The specimens were subjected to various drying methods (filter paper, newspaper, and silica gel) with different replacement frequencies (frequently, rarely, never, or not applicable for silica gel) and were processed using different grinding methods (homogenizer or manual grinding) to evaluate the effects on DNA quality (Figure 1).

Drying Methods

Plant specimens were dried using four distinct methods during the study. The first method involved placing samples between frequently replaced filter papers (twice a day) to ensure effective moisture removal. The second method used filter papers that were replaced at longer intervals (once every 5-7 days), allowing for extended drying periods between changes. The third method used newspaper as the drying medium, with no replacement throughout the drying process. The fourth method used silica gel as the desiccant, which did not require replacement due to its sustained effectiveness in absorbing moisture. For the filter paper and newspaper methods, replacement was performed at predetermined intervals specified in the experimental protocol. Throughout the drying process, all specimens were maintained under controlled temperature and humidity conditions (22±2°C and 50±5% relative humidity) to minimize the influence of environmental variables. The drying process was continued under these same conditions for at least 3 months.

Grinding Methods

During the grinding stage, which is one of the initial steps of DNA isolation, samples were processed both mechanically and manually to ensure effective homogenization. For mechanical grinding, the samples were homogenized with three steel beads using the Allsheng Bioprep-6 device. The grinding process was performed in three cycles at 3000 rpm for 25 sec per cycle. For manual grinding, researchers ground the samples by hand to achieve a fine powder.

DNA Extraction

Genomic DNA was extracted using the GeneAll Plant SV Mini Kit (Seoul, Korea) according to the manufacturer's protocol. A total of 26 mg of dried plant material was used for each drying method tested in this study.

Assessment of DNA Quantity and Purity

The concentration of the isolated genomic DNA was quantified using a spectrofluorometric method with a DeNovix QFX Fluorometer. DNA purity was assessed spectrophotometrically using a Biotek-Epoch microplate reader. DNA concentration was recorded in ng/µL, and purity was determined based on the A260/A280 ratio.

Statistical Analysis

Statistical analyses were conducted to evaluate the effects of drying methods, tissue types, and grinding methods on DNA quality parameters. DNA concentration (ng/µL) and purity (A260/A280 ratio) data were analyzed using GraphPad Prism (Version 9). Principal Component Analysis (PCA) was performed to examine multivariate relationships between variables and identify patterns in the dataset. Before PCA, data were standardized using z-score normalization to account for the different scales of measurement between the DNA concentration and purity values. PCA was conducted using two principal components to capture the total variance in the dataset. To visualize the relationships between samples and variables, PCA biplots were generated, showing both sample positions and feature vectors. Additional visualizations

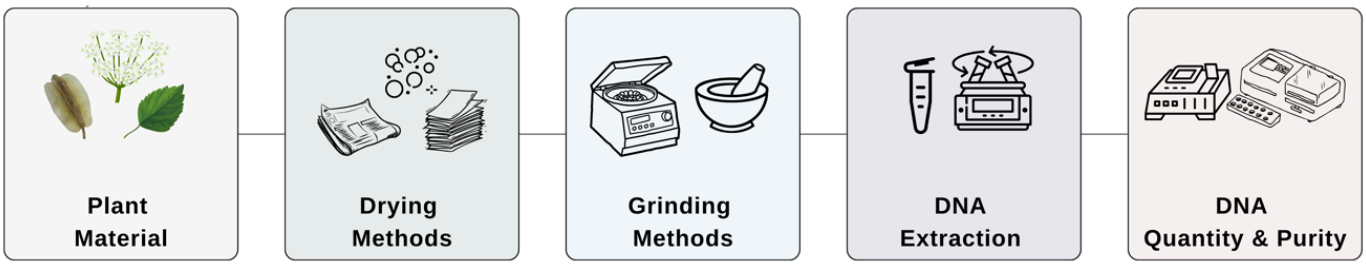

Figure 1. Experimental workflow for plant DNA quality assessment.

Table 1. DNA concentrations and purity values across different experimental variables including plant material types, drying methods, drying frequencies, and grinding methods.

ISTE No	Plant Material	Drying Type	Drying Frequency	Grinding Type	DNA Concentration (ng/µL)	DNA purity (A260/A280)
116697	Leaf	Silica Gel	-	Homogenizer	20.072	1.808
116697	Leaf	Filter Paper	Frequently	Homogenizer	21.631	1.775
116761	Fruit	Filter Paper	Frequently	Homogenizer	24.454	1.898
116803	Leaf	Silica Gel	-	Homogenizer	19.961	1.778
116803	Leaf	Filter Paper	Frequently	Homogenizer	19.392	1.739
116862	Leaf	Newspaper	Rarely	Homogenizer	15.098	1.468
116862	Flower	Newspaper	Rarely	Homogenizer	23.180	1.768
116862	Fruit	Newspaper	Rarely	Homogenizer	23.105	1.801
116862	Leaf	Newspaper	Rarely	Manuel	10.487	1.461
117142	Leaf	Filter Paper	Frequently	Homogenizer	28.759	1.811
117142	Flower	Filter Paper	Frequently	Homogenizer	27.736	1.801
117142	Flower	Filter Paper	Frequently	Manuel	27.694	1.792
117625	Leaf	Newspaper	Never	Homogenizer	4.293	1.463
117625	Flower	Newspaper	Never	Homogenizer	24.067	1.844
117625	Fruit	Newspaper	Never	Homogenizer	24.205	1.869
117625	Leaf	Newspaper	Never	Manuel	14.084	1.293
117625	Flower	Newspaper	Never	Manuel	19.210	1.850
117625	Fruit	Newspaper	Never	Manuel	22.122	1.869

included heatmaps of mean DNA concentration and purity values across tissue types and drying methods, scatter plots with regression analysis to examine correlations between DNA concentration and purity, and grouped bar charts to compare DNA quality parameters across experimental conditions. Statistical significance was assessed using analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons, independent samples t-test, and Pearson's correlation coefficient, with $p < 0.05$ considered statistically significant.

RESULTS

This study investigated the effects of different plant drying methods, tissue types, and grinding methods on DNA quality parameters using *A. sylvestris* specimens as representative material. The comprehensive dataset presented in Table 1 includes measurements of DNA concentration (ng/µL) and purity (A260/A280) across various plant tissues and drying methods.

Results revealed significant variations in DNA quality associated with drying methods, exhibiting distinct patterns among different tissue types. PCA indicated that drying method and tissue type were the primary determinants of DNA quality, whereas grinding technique had secondary, tissue-dependent effects. These findings highlight the importance of optimizing drying protocols for specific plant tissues, particularly for molecular studies requiring high-quality DNA.

Effects of Tissue Types on DNA Concentration and Purity

A comprehensive analysis of DNA extraction from different tissue types (leaf, flower, fruit) revealed significant variations in both concentration and purity between drying methods. Leaf tissue demonstrated the highest sensitivity to drying methods, and the DNA concentration (4.29 ng/µL) and purity (1.46) obtained from leaf samples decreased significantly. Flower and fruit samples were observed to be more resistant to poor drying methods. Flower and fruit samples maintained relatively higher DNA concentrations (24.07 ng/µL and 24.21 ng/µL, respectively) and purity values (1.84 and 1.87, respectively) even when dried in never-changed newspaper. This tissue-specific response persisted across suboptimal drying methods (rarely changed newspaper), with leaf samples consistently showing greater reductions in both DNA concentration and purity compared to reproductive tissues. In contrast, flower and fruit samples maintained relatively high DNA concentrations and purity values, demonstrating their greater resilience to suboptimal drying methods. This differential tissue response was clearly visualized in the PCA analysis, in which leaf samples showed greater dispersion across the principal component space, indicating higher

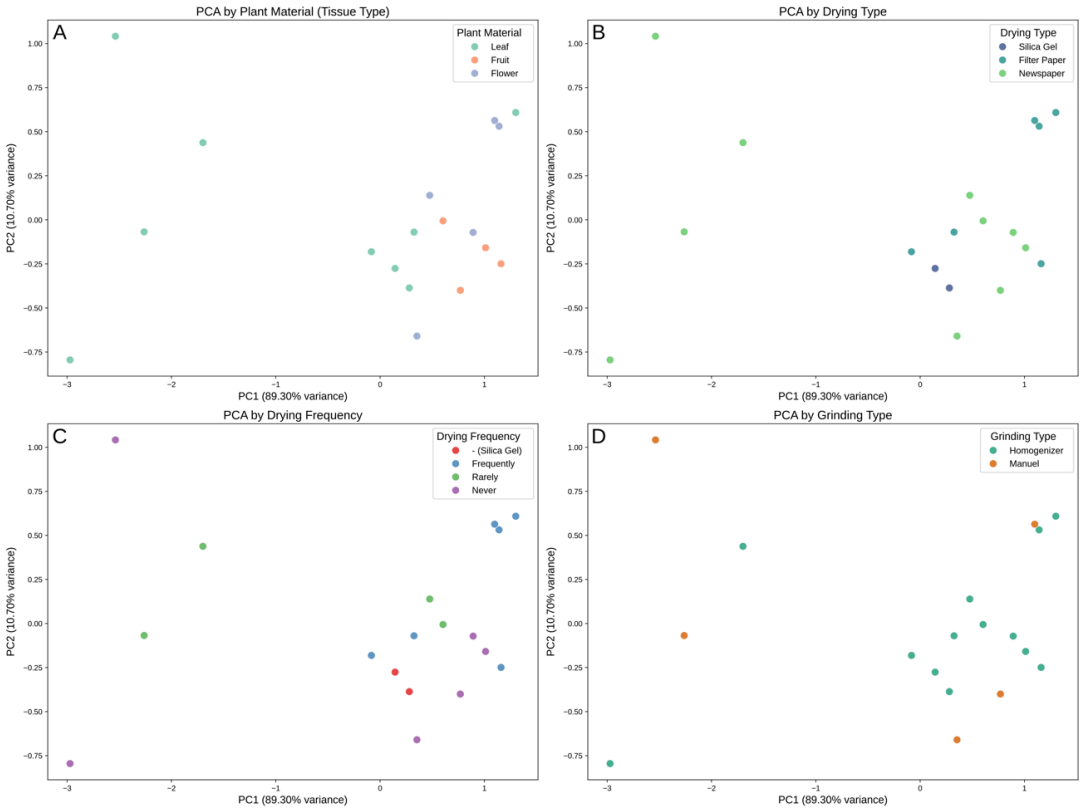

Figure 2. Principal Component Analysis (PCA) of DNA quality parameters grouped by experimental factors. (A) Plant material types showing distinct clustering of leaf, flower, and fruit tissues along PC1 and PC2 axes, which together explain 100% of the total variance. (B) Drying methods demonstrating separation between silica gel, filter paper, and newspaper drying methods. (C) Drying frequency categories revealing gradient distribution from never to frequently changed materials. (D) Comparison of grinding methods illustrating the differential impact of homogenizer versus manual grinding techniques on DNA quality parameters. The ellipses represent 95% confidence intervals for each group.

sensitivity to drying methods (Figure 2). A statistically significant difference was found in DNA purity among different tissue types (ANOVA, p = 0.024).

Effect of Grinding Method on DNA Concentration and Purity

Comparison of manual and mechanical grinding of tissues revealed that the method-specific effects varied by tissue type and drying method (Figure 2). Homogenizer use generally increased DNA yield for most sample types, with the notable exception of poorly dried leaf tissue. In leaf samples dried in never-changed newspaper, homogenizer processing resulted in substantially lower DNA concentration (4.29 ng/µL) compared with manual grinding (14.08 ng/µL). Conversely, homogenization improved DNA recovery in reproductive tissues. Flower samples dried in never-changed newspaper yielded 24.07 ng/µL DNA when processed with a homogenizer compared to 19.21 ng/µL with manual grinding. Although the grinding method had a minimal effect on DNA purity under all drying methods, the consistent increase in DNA concentration with homogenizer use (except in poorly dried leaf tissue) suggests that this method generally improves extraction efficiency. No statistically significant difference was observed in DNA concentration or purity with respect to drying method (p = 0.092 and p = 0.307, respectively), drying frequency (p = 0.203 and p = 0.440, respectively), or grinding method (p = 0.464 and p = 0.282, respectively).

Effects of Drying Methods on DNA Concentration and Purity

A quantitative analysis was conducted to determine the impact of the drying method on DNA concentration and purity in various tissue types. The highest DNA concentrations (leaf: 28.76 ng/µL; flower: 27.74 ng/µL; fruit: 24.45 ng/µL), and purity values (leaf: 1.81; flower: 1.80; fruit: 1.90) were consistently observed when using frequently changed filter paper across all tissue types.

Silica gel drying produced the second-highest quality results, with leaf tissue yielding 20.07 ng/µL DNA concentration and 1.81 purity. The most dramatic reduction in DNA quality was observed in leaf tissue dried with unchanged newspaper, which yielded the lowest concentration (4.29 ng/µL) and

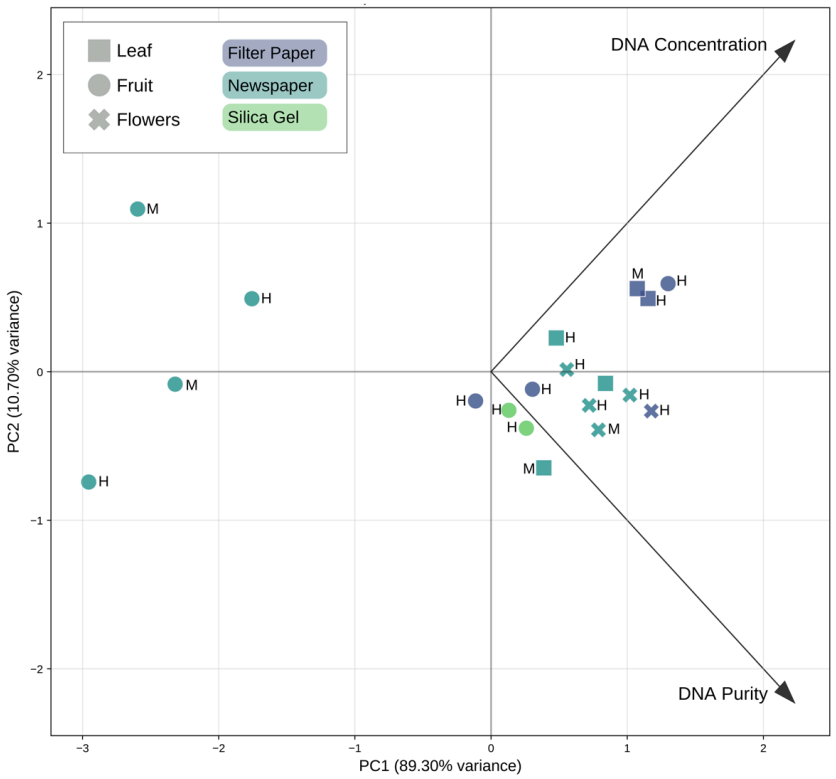

Figure 3. Principal Component Analysis (PCA) biplot of DNA quality parameters. The biplot illustrates the distribution of samples in the principal component space, with PC1 explaining 89.3% and PC2 explaining 10.7% of the total variance. Points represent individual samples, color-coded by drying method, and shaped according to plant tissue type. Black vectors indicate the contribution of DNA concentration and purity to the principal components. (H: Homogenization, M: Manuel Grinding).

purity (1.46) values recorded in this study, representing a concentration reduction of up to 84% compared with leaf tissue dried with frequently changed filter paper. The PCA biplot further supported the hypothesis that different drying methods vary in their effectiveness, demonstrating a clear separation between samples dried with filter paper or silica gel and those dried with newspaper (Figure 3).

Effect of Drying Frequency on DNA Concentration and Purity

The concentration and purity of DNA were significantly affected by the frequency at which the drying material was replaced (Figure 2). In addition to the type and replacement frequency of the drying material, the results were also significantly influenced by the specific plant tissue selected for DNA extraction (flower, leaf, fruit) (Figure 4). The highest DNA concentrations were observed when using frequently changed filter paper, with leaf tissue yielding 23.2 ng/µL (maximum 28.76 ng/µL), flower tissue 27.72 ng/µL, and fruit tissue 24.45 ng/µL. The corresponding purity values were also optimal (leaf: 1.78; flower: 1.80; fruit: 1.90). Silica gel drying although slightly less effective than frequently changed filter paper, produced comparable results (leaf: 20.07 ng/µL, purity: 1.81). The DNA concentration and purity obtained from silica gel-dried samples were the second-highest results, closely following those achieved with frequently changed filter paper and only slightly lower than the highest values obtained with always changed filter paper. This indicates its suitability as a practical alternative when frequent replacement of drying materials is not feasible.

The lowest DNA quality was observed in leaf tissue dried using newspaper that was never replaced, resulting in a marked reduction in DNA concentration (4.29 ng/µL) and purity (1.46) when processed with a homogenizer. Interestingly, manual grinding of the same tissue yielded higher DNA concentration (14.08 ng/µL) but lower purity (1.29), suggesting that mechanical damage may further compromise already degraded DNA in poorly dried leaf samples. Conversely, flower and fruit tissues demonstrated greater resilience under suboptimal drying methods (newspaper), maintaining relatively higher DNA concentrations and purity values (flower: 19.21-24.07 ng/µL, purity: 1.76-1.85; fruit: 22.1-24.21 ng/µL, purity: 1.80-1.87). These findings demonstrate that tissue-specific differences in DNA degradation susceptibility are associated with drying methods.

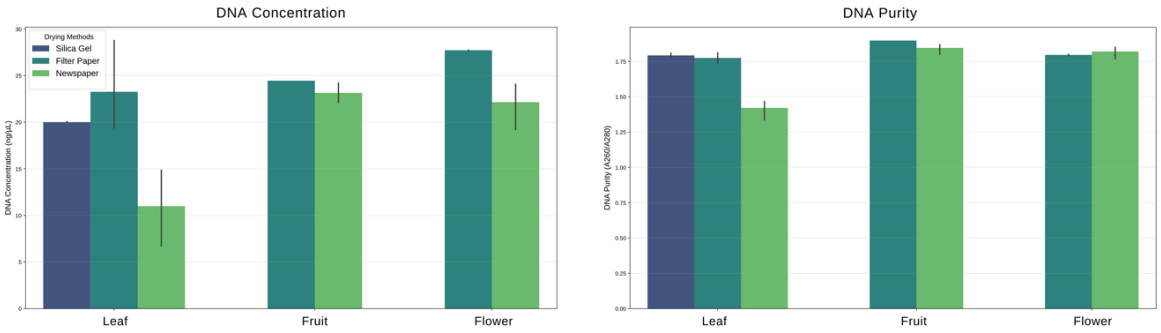

Figure 4. DNA concentration and purity according to plant tissue type and drying method. Bar plots show mean values with standard error bars.

Multivariate Analysis of DNA Quality Parameters

PCA was performed to evaluate the multivariate relationships among drying methods, tissue types, and DNA quality parameters. The analysis captured 100% of the data variance using two principal components, PC1 accounting for 89.3% and PC2 for 10.7% of the total variance. PCA revealed distinct clustering patterns according to tissue type and drying method (Figure 2A and Figure 2B). Samples dried using filter paper and silica gel clustered together, indicating similar DNA quality outcomes, whereas newspaper-dried samples formed separate clusters, particularly for leaf tissue. This separation demonstrates the significant impact of the drying method on DNA quality parameters. The loading plot indicated that both DNA concentration and purity contributed significantly to the principal components, with concentration having a stronger influence on PC1 and purity contributing more to PC2.

Tissue-specific responses to drying conditions were clearly visualized in the PCA plot. Leaf samples showed greater sensitivity to drying conditions than reproductive tissues (flower and fruit), as evidenced by their wider distribution across the principal component space. Samples from leaf tissue dried with never-changed newspaper were positioned furthest from the optimal cluster, confirming their significantly reduced DNA quality.

The frequency of drying material replacement was also identified as a critical factor in PCA. The frequently changed drying materials consistently produced samples with higher DNA quality, as shown by their distinct positioning in the PCA plot (Figure 2C). This pattern was particularly pronounced in leaf tissue, further supporting the tissue-specific response to preservation conditions observed throughout this study.

Statistical analysis of the relationship between DNA concentration and purity revealed a strong and statistically significant positive correlation (Pearson $r = 0.79$, $p < 0.001$), as visualized in the scatter plot (Figure 5). The strength of this correlation varied across tissue types, with leaf tissue showing a strong positive correlation ($r = 0.76$), fruit tissue showing a moderate positive correlation ($r = 0.40$), and flower tissue exhibiting a negative correlation ($r = -0.52$). This unexpected negative correlation in flower tissue suggests that different biochemical dynamics may be at play in reproductive tissues, where higher DNA concentrations may be associated with slightly lower purity values. When analyzed by drying method, samples dried with silica gel showed a perfect correlation between concentration and purity ($r = 1.00$), followed by newspaper ($r = 0.81$), whereas filter paper-dried samples exhibited a weaker correlation ($r = 0.39$). This pattern differs from the overall quality trends, implying that although filter paper yields higher absolute values for both parameters, the relationship between DNA quantity and quality varies according to the preservation method used.

Notably, some exceptions to this correlation pattern were observed, particularly in poorly dried leaf tissue. Samples from leaf tissue dried with never-changed newspaper occasionally exhibited relatively moderate DNA concentrations (14.08 ng/µL) despite suboptimal purity values (1.29), indicating that while some DNA quantity may be preserved under poor drying conditions, the integrity and quality of the extracted DNA still significantly compromised. This finding has important implications for molecular studies because it indicates that DNA concentration alone may not be a sufficient indicator of sample suitability for downstream applications.

The heatmap analysis of mean DNA concentration and purity by tissue type and drying method further illustrated these patterns. Leaf tissue dried with newspaper had the lowest mean DNA concentration (10.99 ng/µL) and purity (1.42), whereas the same drying method yielded substantially higher values for flower and fruit tissues. In contrast, filter paper drying consistently produced high DNA quality across all tissue types, with mean concentrations ranging from 23.26 ng/µL (leaf) to 27.72 ng/µL (flower) and mean purity values ranging from 1.78 to 1.90 (Figure 6).

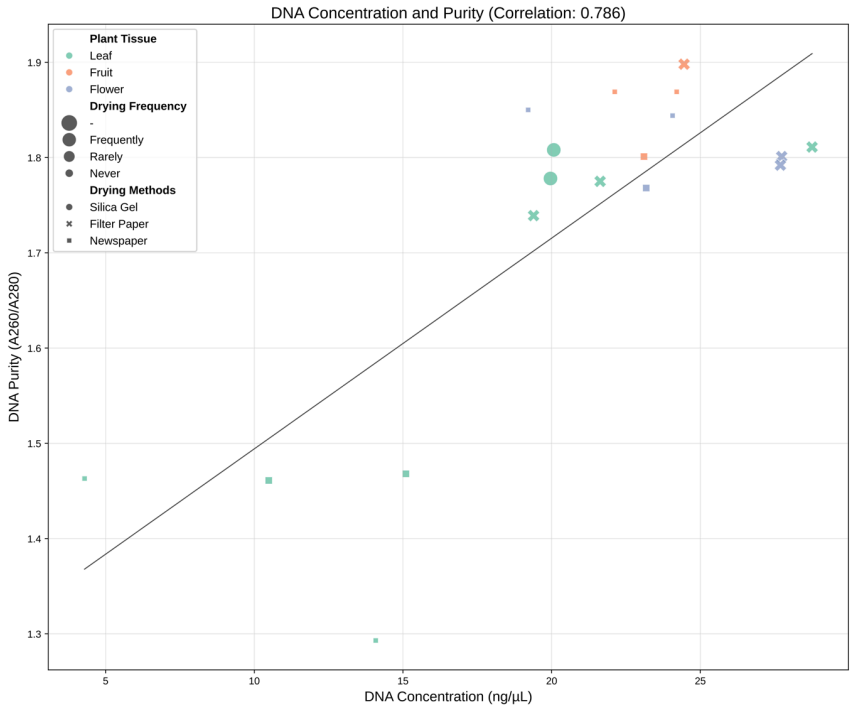

Figure 5. Correlation analysis between DNA concentration and purity (r = 0.79). Points are color-coded by plant tissue type and shaped according to drying method. The black line represents linear regression.

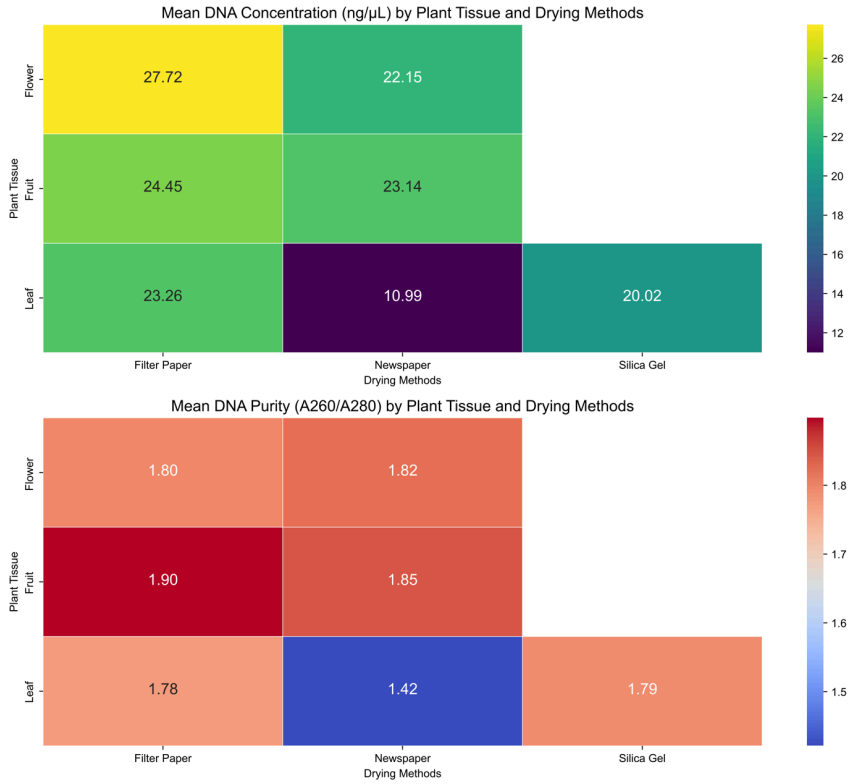

Figure 6. Heatmap analysis of mean DNA concentration (top) and purity (bottom) according to tissue type and drying method. Color intensity represents the magnitude of the values, with darker colors indicating higher values.

DISCUSSION

This comprehensive investigation evaluated the effects of drying methods, drying frequency, and grinding methods on DNA concentration and purity in plant tissues, providing valuable insights into optimal preservation strategies for herbarium specimens. The findings clearly demonstrated that DNA quality was significantly influenced by drying method, with notable variations observed among different tissue types and grinding methods. The frequently replaced filter paper consistently yielded the highest DNA concentrations and purity values across all examined tissues. This superior performance can be attributed to the rapid removal of moisture, which effectively minimizes enzymatic and microbial degradation processes.[6,10] Similarly, drying silica gel, which also facilitates rapid desiccation, produced comparable results, particularly in leaf tissue. Although frequently replaced filter paper remains the optimal drying method, silica gel drying produces the second-highest quality results.

In this study, a statistically significant difference was found in DNA purity among tissue types ($p = 0.024$), while no significant differences were observed in DNA concentration or purity with respect to drying method, drying frequency, or grinding method ($p > 0.05$ for all). Additionally, a strong positive correlation was found between DNA concentration and purity ($r = 0.79$, $p < 0.001$).

Silica gel effectively dries plant tissues, thereby preventing DNA degradation.[4,6] This finding is consistent with large-scale studies showing that silica gel-dried specimens generally yield higher quality and less degraded DNA compared to herbarium specimens. In particular, silica gel-dried samples provide better results in terms of DNA integrity and quality in both old (more than 24 years old) and recent (24 years old or less) herbarium specimens.[4] Notably, DNA was severely degraded in older herbarium samples, while the DNA quality in more recent herbarium specimens still remains lower than that of silica gel-dried material. However, since most herbarium specimen labels do not indicate how the samples were dried or what treatments they underwent, it is not possible to definitively attribute differences in DNA quality to specific preparation methods.[4] In another study comparing samples dried with silica gel and those dried between paper, it was reported that DNA extracted from samples dried using the paper method was often highly degraded, whereas DNA from silica gel-dried samples was much better preserved. However, a detailed protocol for the paper drying method was not provided in the study.[6] In different studies conducted, different drying methods did not significantly affect the quantity and quality of extracted DNA.[11] However, these studies have been characterized by the application of drying methods for varying durations across samples, with an absence of a standardized drying period. Furthermore, the relatively short drying durations (ranging from 7 to 38 days) limit the assessment of the long-term effectiveness of these methods, thus restricting the reliability and generalizability of the obtained results.[11] In the present study, frequent changing of the filter paper resulted in higher quality DNA compared to silica gel drying. Nevertheless, no studies in the literature have been encountered that provide information about the frequency of paper changes. Particularly in the field, where frequent material changes are impractical, silica gel is a reliable alternative. In this study, plant samples were subjected to four distinct drying methods under constant temperature and humidity conditions for a period of at least three months. The impact of drying material and replacement frequency on DNA quality was examined under controlled conditions. The findings of the research indicate that the frequency of replacement of the drying material exerts a substantial influence on the concentration and purity of DNA. It has been demonstrated that frequently changing filter paper provides the highest DNA concentration and optimal purity values in all tissue types. However, silica gel provides the second-best results. Conversely, leaf tissue dried using never-changed newspaper exhibited a dramatic decrease in DNA concentration and purity. These findings indicate that the type of drying material and the replacement frequency are of critical importance for preserving DNA quality.

The results of this study demonstrate clear tissue-specific responses, highlighting the necessity of customizing preservation protocols according to the target tissue. Specifically, leaf tissues required more stringent drying methods, whereas flower and fruit tissues exhibited greater flexibility under the tested conditions. The lowest DNA quality was observed in samples dried using newspaper that was never replaced, particularly in leaf tissue. This method failed to adequately remove moisture, resulting in prolonged exposure to conditions conducive to DNA degradation. Notably, flower and fruit tissues exhibited greater resilience under suboptimal drying methods, maintaining relatively higher DNA concentrations and purity values throughout the experiments. This differential response reflects intrinsic tissue-specific biochemical properties. Reproductive tissues generally contain more nuclei and fewer secondary metabolites than vegetative tissues, facilitating higher DNA yields even under suboptimal preservation conditions.[10] In contrast, leaf tissues are particularly rich in polysaccharides, polyphenols, and other secondary metabolites that readily oxidize DNA and form inhibitory complexes during extraction.[10] In a comparative study conducted on *Daucus*

carota, DNA extracted from flower and seed tissues showed higher concentrations compared to leaf tissue. In terms of purity, lyophilized leaf tissue yielded higher-quality DNA. The flower tissue used in this study was sourced from fresh samples. In another study, in plants with tough and fibrous leaf tissues, the presence of a thick cuticle and high levels of secondary metabolites resulted in low yield and quality when using standard DNA isolation protocols.[10,12] These findings suggest that flower and seed tissues generally contain more nuclei and fewer secondary metabolites, potentially making it possible to obtain higher DNA concentrations from these tissues. In contrast, leaf tissues are particularly rich in polysaccharides, polyphenols, and other secondary metabolites. These compounds can reduce the purity of the extracted DNA during isolation and negatively affect PCR success[10], thereby supporting this conclusion. In summary, considering the unique chemical and structural properties of each tissue, selecting the most appropriate DNA purification protocol is a critical step for ensuring the accuracy and reliability of the obtained results.

Different plant tissues respond differently to DNA isolation, and protocols should be optimized according to tissue type. The results of this study demonstrated that the effect of the grinding method on DNA quality varies depending on the tissue type and drying method used. In this study, although the use of a homogenizer increased the DNA concentration in flower and fruit samples, it may have damaged DNA in poorly dried leaf samples. This phenomenon can be attributed to the weakening of cell walls in poorly dried leaf tissues and the mechanical fragmentation of DNA during homogenizer use.[13] Indeed, tissue-specific responses emerged as a key finding of this study. Leaf tissue consistently demonstrated greater sensitivity to poor drying methods, experiencing significant reductions in DNA concentration and purity. This heightened sensitivity may be attributed to higher concentrations of secondary metabolites and phenolic compounds in leaves, which are known to interfere with DNA extraction and accelerate degradation under suboptimal preservation conditions.[14,15] In contrast, flower and fruit tissues exhibited greater resistance to degradation, possibly due to their lower metabolic activity and reduced levels of interfering compounds. Consequently, preservation protocols should prioritize rapid and effective drying methods for leaf tissues, whereas flower and fruit tissues may tolerate less stringent drying methods without substantial loss of DNA quality. The selection of the grinding methodology significantly impacted DNA recovery. Homogenizers generally use increased DNA concentrations in flower and fruit tissues. Conversely, in poorly dried leaf tissue, homogenizer application resulted in lower DNA concentrations than manual grinding. This reduction can be attributed to mechanical damage that exacerbates DNA degradation in already compromised samples. These findings underscore the importance of selecting grinding methods tailored to both tissue type and preservation conditions to optimize DNA recovery.

Overall, the results of this study provide clear practical guidance for optimizing DNA preservation in herbarium specimens. The finding that frequently replaced filter paper yields the highest DNA quality is consistent with previous reports emphasizing the importance of rapid and effective desiccation for preserving DNA integrity.[2,3,6] However, unlike earlier studies, which often did not specify the frequency of paper changes or standardized drying durations.[4,6,12] The present study demonstrates that not only the type of drying material but also the frequency of its replacement is a critical determinant of DNA quality. This highlights a methodological gap in the literature and indicates that more detailed protocols are necessary for reproducibility and optimal results. The observation that silica gel serves as a reliable alternative, particularly in field conditions where frequent material replacement is impractical, aligns with the findings of other studies reporting that silica gel-dried specimens generally yield higher quality and less degraded DNA compared to traditional herbarium methods.[4,6] Nevertheless, our results indicate that under controlled laboratory conditions, frequently changed filter paper can outperform silica gel in terms of both DNA concentration and purity, a nuance that has not been widely reported in previous comparative studies. The tissue-specific responses observed in this study, with leaf tissues exhibiting greater sensitivity to suboptimal drying and reproductive tissues (flower and fruit) showing greater resilience, are in agreement with previous research indicating that biochemical composition—such as higher levels of secondary metabolites and polyphenols in leaves—can negatively impact DNA extraction and quality.[10,11,14] Boiteux et al. similarly reported higher DNA concentrations from flower and seed tissues compared to leaves in *D. carota*, although lyophilized leaf tissue yielded higher purity DNA.[10] Our findings further support the notion that reproductive tissues, due to their lower content of inhibitory compounds, are more suitable for DNA extraction under less-than-ideal preservation conditions. Additionally, the strong correlation observed between DNA purity and PCR success in previous studies highlighted that DNA purity, rather than concentration alone, is a key predictor of successful amplification and sequencing.[3] This underscores the importance of optimizing both drying protocols and tissue selection to maximize the utility of herbarium specimens for molecular analyses.

CONCLUSION

This study demonstrated that drying methods, replacement frequency, and grinding techniques significantly influence DNA quality in plant tissues, with tissue-specific responses requiring customized preservation approaches. Frequently replaced filter paper provided optimal DNA quality, whereas silica gel was a practical alternative for field conditions. Leaf tissues exhibit greater sensitivity to suboptimal drying compared to reproductive tissues. These findings provide evidence-based recommendations for optimizing herbarium specimen preservation protocols and enhancing the utility of plant collections for molecular research and phylogenetic studies. Although this study provides valuable insights into preservation protocols, certain limitations should be acknowledged. The focus on a single species (*A. sylvestris*) may limit generalizability across taxonomically distant plant groups with different tissue compositions. Additionally, the three-month drying period examined here may not fully capture the long-term effects of preservation methods on DNA integrity over decades of storage, which is relevant for historical herbarium collections. Future studies should expand this investigation to include multiple plant families and longer preservation periods to further validate these findings across diverse taxonomic groups.

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