Comprehensive Journal of Science
Volume (11), Issue (41), (JUNE, 2026)
ISSN: 3014-6266

مجلة العلوم الشاملة
المجلد (11) العدد 41 يونيو 2026
ردمد: 3014-6266

Evaluation of the Purity of Plant Genetic Material of Dry Seeds and Modern Leaves of Some Local Barley (*Hordeum vulgare* L.) Varieties using CTAB, SDS and Mixture Extraction Solutions (CTAB and SDS)

Elmundr Abugnia [1], Mohamed A. Elahmar[1], Wagdi Ghaleb [1], Naser Mohamed Al. M. Abdalgader[2] , Talal Emhemed Yahia[1]
[1] Libyan Biotechnology Research Center, Tripoli, Libya
[2] Engineering Department, Libya Open University, Tripoli, Libya

Elmundr Abugnia Orcid 0009-0004-7776-4153

Mohamed A. Elahmar Orcid 0009-0005-1501-4653
abugnia.elmundr@lbcr.ly, mohamed.elahmar@lbtrc.edu.ly, ghaleb.wagdi@lbcr.ly, Dr.naser.almhdey@mhesr.gov.ly

تاريخ الاستلام:2026/4/01 –تاريخ المراجعة:2026/05/2– تاريخ القبول: 2026/05/14– تاريخ للنشر2026/06/01

Abstract
The extract of genomic DNA from dry seeds as well as young leaves of various local *Hordeum vulgare*, or barley plants, the research evaluates the efficiency of three plant DNA isolation methods such as CTAB, SDS, and the integration of both. The CTAB extraction procedure effectively eliminated impurities that are linked to inhibitory chemicals, as evidenced by spectrophotometric studies. This procedure also yielded the highest DNA purity for both fresh leaves and dry seeds. At electrophoresis exposed at 70 V for 60 minutes, for DNA migration patterns, which showed intact and recoverable genomic DNA, the results validated the successful extraction of plant genetic material. The spectrophotometric experiments indicated that the CTAB protocol of extraction was successful in the removal of contaminants associated with inhibiting compounds. Furthermore, this approach resulted in the maximum DNA purity for fresh leaves and dried seeds. The research outcome in the earlier stage corroborated CTAB efficiently, which separated DNA from secondary metabolite-rich plants. Furthermore, lower purity values (about 1.30) have been obtained utilizing the combined method (CTAB + SDS), indicating the presence of residual impurities. Because of limited effectiveness in tissues containing polysaccharides as well as phenolic compounds, SDS alone yielded very low-quality DNA (purity ~1.65).
Key words; Extraction DNA, Barley Plant, Purity, Genomic DNA,

Introduction
Recently, regarding the development of genetic engineering techy have made them indispensable to enhance the genetic traits, which is important for crops like barley (*Hordeum vulgare* L.). These techies are frequently employed for the improvement of grain nutritional quality, which is associated with bioactive chemical content. Furthermore, increase production of the plant to be more resistant to environmental challenges, for instance, drought, which is associated with salinity (Shlibak et al., 2021). Moreover, gene-transfer techies, such as Agrobacterium tumefaciens-mediated transformation, which is associated with particle bombardment (sometimes known as "gene guns"), are among the most crucial instruments in contemporary plant biotechnology, which makes it possible to introduce genes that confer disease resistance as well as tolerance (Dunwell, 2000; Shlıbak and Zencirci, 2021; and Soliman et al., 2026). Under drought (high saline conditions), the plant can survive by genes

that produce anti-stress proteins, such as DREB or HVA1 (Shlibak and Dalla, 2020; Chaves et al., 2009). Particularly CRISPR/Cas9 has the development of barley as declared by Lawrenson et al. (2015). The significance of bioactive phenolic chemical compounds, whose developments have raised barley's economic which is associated with nutrition (Dalla et al., 2025; Matros et al., 2017). Moreover, with the comparison of the production of 140–150 million tons, barley is the fourth most significant cereal crop in the world after rice, wheat, as well as maize, respectively (FAO, 2023). Barley continues to be an important crop for food security in arid and semi-arid areas because of its high degree of environmental adaptability (Ullrich, 2011; Soliman et al., 2026). Phenolic components, such as ferulic acid, catechins, and flavonols, have strong antioxidant activity linked to lowering the risk of diabetes, cancer, and cardiovascular disease (Zielinski & Kozlowska, 2000; Guo et al., 2014). Extracting high-quality DNA for molecular analysis is still difficult because secondary compounds included in barley plants are rich in polysaccharides as well as phenols. Utilizing three extraction methods such as CTAB and SDS, which is associated with a 1:1 combination of CTAB and SDS, as well as the current study, sought to develop a dependable procedure for obtaining high-quality genomic DNA from the dry seeds as well as early leaves of five Libyan barley cultivars, for instance, (Dulaimi, Sayd, Rayhan, Wadi Zart, as well as Wadi Al-Hay). CTAB-based extraction method are effective to remove polyphenols as well as obtaining high-quality DNA for molecular applications as declared by Dellaporta et al., (1983); Al-Saghir et al., (2020); (Daniel et al., 2017); (Faraj, 2017). The difficulties in extracting DNA from barley tissues that are high in polysaccharides, lignin, which is associated with phenolic compounds all of which frequently impede PCR as well as other molecular analyses as announced by Sarwat et al., (2006); Abd-Elsalam et al., (2011). **Materials and Methods**

To evaluate a successful procedure for obtaining plant DNA from the dry seeds as well as immature leaves of a few regional barley cultivars, the study was carried out at the Libyan Biotechnology Research Centre in Tripoli, Libya.

Sample collection

Seeds of five barley cultivars (Table 1) were obtained from the Department of Crop Sciences, Faculty of Agriculture, University of Tripoli, Libya, and were then directly transferred to the laboratories of the Libyan Biotechnology Research Center for the purpose of conducting laboratory experiments.

Table 1. Descriptions of the genotypes of barley utilized in the study

Genotypes of *Hordeum vulgare*	Lineage	Source	Date of derivation or introduction
Al-Rayhan	Atlas46/Arivat//Athénaïs ICB-76 2L-1AP-0AP	ICARDA	1987
Al-Dulaimi	Landraces	Libya	Old Local
Al-Sayd	-	Libya	Entrance
Wadi Al-Hay	Asse/Jaidor	Libya	1998
Wadi Zart	Arizona 5908/Aths//Lignee 460/5/aths/4/Pro/Tolli/3/5106	Libya	1998

Solutions for material isolation preparation

DNA extraction solutions in this study included 2% cetyltrimethyl ammonium bromide (CTAB) extraction solution, 2% sodium dodecyl sulfate (SDS) extraction solution.

Extraction solution, Acetyl Trimethyl Ammonium Bromide (CTAB)

CTAB, commonly utilized at 2%, is an effective cationic detergent that breaks down cell walls as well as bonds between proteins and nucleic acids, precipitates polysaccharides, and stabilizes nucleic acids in the presence of sodium chloride (NaCl). β-mercaptoethanol is also

added, acting as an antioxidant to prevent nucleic acid breakdown caused via oxidation of phenolic compounds during the extraction process as reported via Doyle and Doyle, (1987); (Ben Dalla, 2021; Ben Dalla, 2021).

Extraction solution Sodium Dodecyl Sulfate (SDS)

SDS extraction solution is an anionic detergent that works specifically to break down the plasma membrane and cell organelles, as well as remove proteins bound to nucleic acids that aid in protein degradation by breaking hydrogen bonds, facilitating the separation of nucleic acids in a more pure form as documented by Aljanabi and Martinez, (1997).

Extraction solution Sodium Dodecyl Sulfate (SDS)

SDS extraction solution is an anionic detergent that works specifically to break down the plasma membrane and cell organelles, and remove proteins bound to nucleic acids that aid in protein degradation by breaking hydrogen bonds, facilitating the separation of nucleic acids in a more pure form (Aljanabi & Martinez, 1997).

Procedure for obtaining plant DNA from fresh leaves and dry grains

1. After being cleaned with sterile distilled water, the dried barley seeds and young leaves were ground gently in a mortar that had been well dried. Liquid nitrogen was added to help with the grinding process until the seeds and young leaves were reduced to a fine powder.
2. Transfer the fine powder of dry seeds and fine leaves to a 2 ml centrifuge tube and place 100 mg of powder in each tube.
3. The samples were separated into three groups: one that received CTAB, one that received SDS, and one that received a 1:1 blend of CTAB and SDS extraction solution. Every treatment has five replicates, and each group stands for a treatment.
4. Extraction solutions (600 µL), β-mercaptoethanol (12 µL), and Proteinase K (60 µL) were added with good intermittent mixing using a shaker. The samples were then placed in a water bath at 55°C for 60 minutes.
5. After adding 600 µL of chloroform isoamyl alcohol (24:1) and thoroughly mixing the mixture for a while, the samples are centrifuged for five minutes at 13,000 rpm at 24°C.
6. The residual organic residue (organic phase) is disposed of after the clear solution at the tube's top is moved to a fresh centrifuge tube using a 400 µL pipette.
7. Repeat the previous procedure with 400 µL of chloroform-isoamyl alcohol (24:1) and then move to a fresh tube.
8. Add 800 microliters of ethanol, which was previously in the freezer at a 70% concentration, and double the 400 microliters that were previously added. The samples are then centrifuged for five minutes at 13,000 rpm at 24°C.
9. Gently pour the solution so that the precipitated genetic material does not sink to the bottom of the tube. Stir the genetic material as you add 600 µL of ethanol, and then put the tubes in the centrifuge as previously.
10. Three times, repeat the preceding washing procedure with 600 µL of 70% ethanol.
11. Place the samples in a water bath at 37°C for 60 minutes after adding 50 µL of RNase or DNase enzyme to extract RNA.
12. Cold air is used inside the insulation compartment to dry the tubes after the solution has been carefully poured to prevent the precipitated genetic material from sinking to the bottom.
13. The tubes are filled with 30 µl of TE solution. After that, the samples are either stored in a freezer at -20°C for extended periods of time or at -4°C for short-term storage Martinez and Aljanabi (1997)

Adjustment of Electrophoresis

The electrophoresis apparatus was prepared by preparing a gel containing 0.7% agarose with added ethidium bromide and filling the apparatus container with electrophoresis solution

(TAE), then the solution is poured into a special mold with a comb to form the sample holes and left to solidify. The gel is placed inside the transfer chamber and immersed in the aforementioned transfer solution, which acts as a medium for conducting the electrical current and maintaining the pH. The holes inside the aforementioned gel are injected with a loading dye of 4 microliters, and 2 microliters of plant genetic material (DNA) are added to it using micropipettes for injection, and the electrophoresis device is adjusted (Sambrook & Russell, 2001: Green & Sambrook, 2012). To compare the sizes of the genetic material employed, a molecular ladder (DNA ladder) was also made. The electrical relay device was connected so that the black negative pole at the DNA openings had a negative charge and was pointed in the direction of the positive pole. Usually, the voltage is set between 70 and 120 V, and the device monitors the migration for 30 to 60 minutes (Alberts et al., 2014). Following the completion of the migration, the gel is taken out and inspected under a UV lamp to look for bands (Ausubel et al., 2002).

DNA Purity Assessment

One of the primary criteria used to judge the effectiveness of different extraction solutions and the quality of the work is the purity of the genetic material, which enables continuing working on various biological applications, such as polymerase chain reaction (PCR), molecular hybridization, or gene sequencing. This purity is often measured using UV spectrophotometry or the NanoDrop device (Wilfinger et al., 1997). This measurement is based on the absorption of nucleic acids in ultraviolet light at a wavelength of 260 nanometers (nm), while proteins absorb most at 280 nm. Therefore, the ratio of absorbance at 260 to 280 nm (A260/A280) is used as an indicator of DNA purity (Glasel, 1995). To determine sample contamination with abrasives such as carbohydrates, EDTA, or polyphenols, the ratio is usually between 2.0 and 2.2 (Wilfinger et al., 1997). The results were recorded for all barley varieties used in this study. The Nanodrop device was first zeroed and calibrated with a single drop (5 µL) of TE solution, which preserved the genetic material. For analysis, 5 µL of sample was placed in the system and mixed thoroughly until homogeneous. Sample purity was calculated based on the percentage (A260/A280) and the absorbance ratio at 260-280 nm (A260/A230 ratio), and the results were measured using a Thermo Scientific Nano Drop spectrometer.

Statistical analysis of the Results

A spectrophotometer operating at wavelengths between 260 and 280 nm was used to evaluate the DNA purity. As the graph illustrates, we had 15 treatments for dry seeds and 15 treatments for young leaves. There were ten duplicates of each therapy. Completely Randomized Design (CRD) was used for statistical analysis, and the Duncan test was used to compare the means at the 0.05 level.

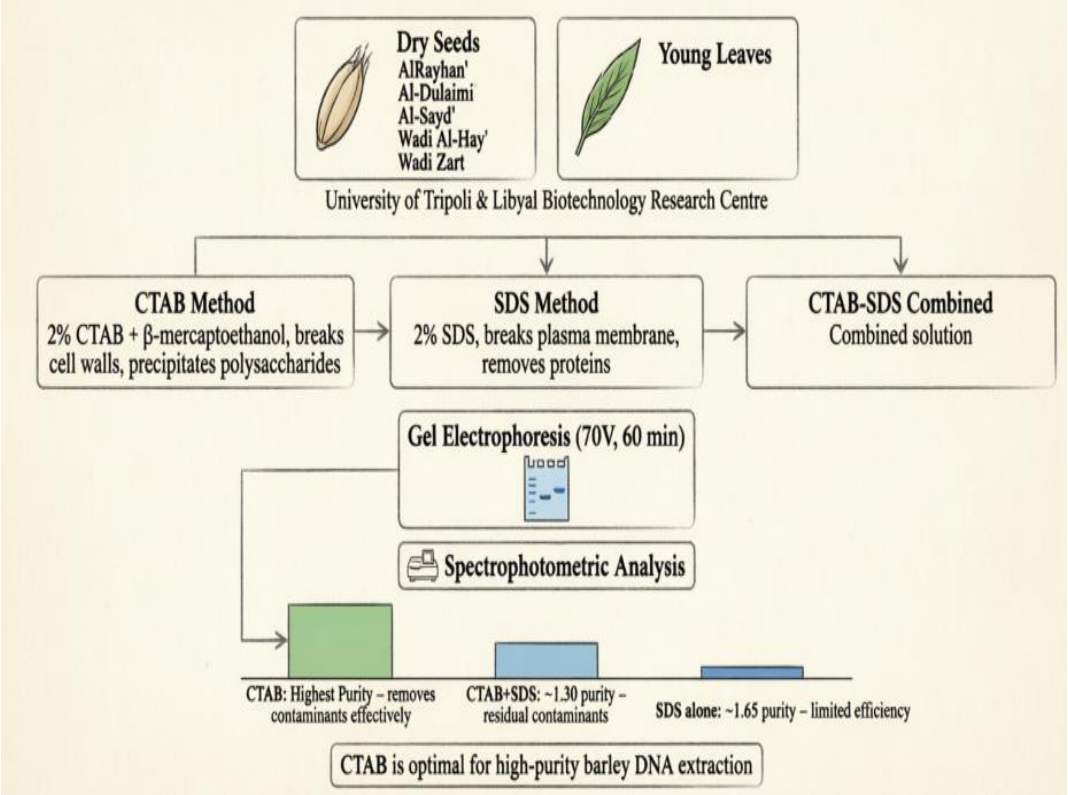


Figure (1) DNA Extraction Workflow of Barley Varieties from Seeds and Leaves

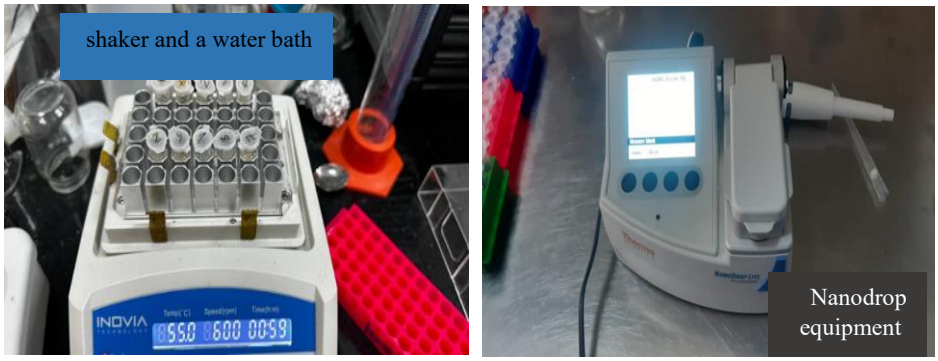


Figure (2) Preparation and quality Assissement of Extracted DNA

Results and Discussion

The experiment provided initial evidence for the presence of plant genetic material from several local barley varieties following electrophoretic transfer. The procedure involved preparing the electrophoresis apparatus, filling the tank with TAE buffer, installing the comb, and loading the extracted DNA into the wells. At an applied voltage of 70 V, successful migration of the samples through the gel was observed. The movement of the DNA bands was continuously monitored throughout the 60-minute run, as illustrated in Figure 3 (Alberts et al., 2014).

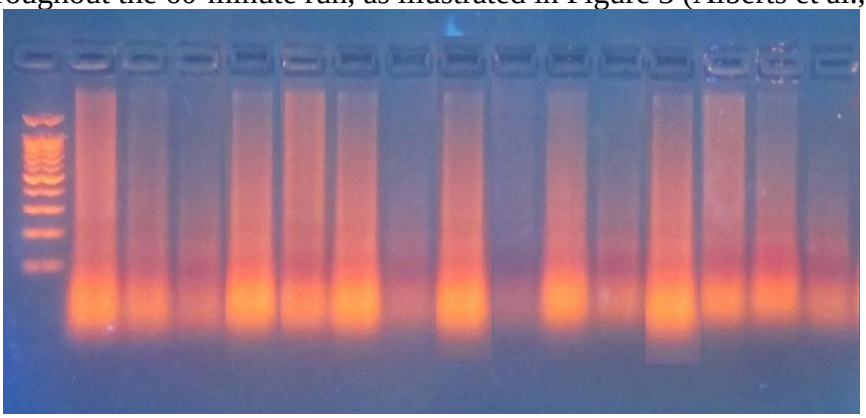
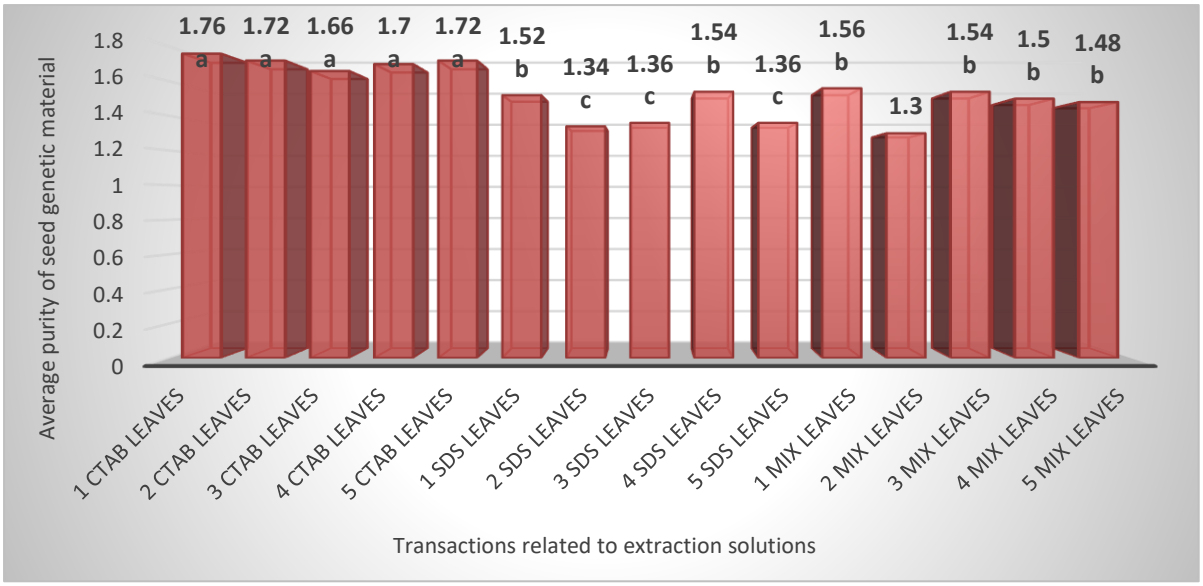


Figure (3) illustrates the use of different extraction solutions, CTAB extracts included (1 Al-Dulaimi, 2 Al-Sayd, 3 Al-Rayhan, 4 Wadi Zart, and 5 Wadi Al-Hay), SDS extracts included (6 Al-Dulaimi, 7 Al-Sayd, 8 Al-Rayhan, 9 Wadi Zart, and 10 Wadi Al-Hay), while the combined CTAB & SDS extracts included (11 Al-Dulaimi, 12 Al-Sayd, 13 Al-Rayhan, 14 Wadi Zart, and 15Wadi Al-Hay).

Spectrophotometric analysis (Figures 4 and 5) further demonstrated that the CTAB extraction method was highly effective for isolating DNA from dry barley seeds and young leaves. The A260/A280 ratio of DNA extracted using CTAB ranged from 1.66 to 1.76 for dry seeds and from 1.86 to 1.92 for leaf tissues, indicating high purity and minimal protein contamination. These findings confirm the effectiveness of CTAB in removing inhibitory compounds, particularly in leaf samples. Moreover, no significant variation was observed among the CTAB-treated samples. These findings are consistent with the results reported by Sharma and Purohit (2012), who identified CTAB as one of the most reliable DNA extraction methods for plant tissues. Similar conclusions were previously reported by Murray and Thompson (1980).

The CTAB method was very successful in extracting DNA from plants. In the same context, our results also agreed with (Murray and Thompson 1980).
The efficiency of DNA extraction differed between dry seeds and young leaves. The results indicated that DNA extracted from young leaves exhibited higher purity compared to that obtained from dry seeds. The purity values ranged between 1.66 and 1.96, confirming that leaf tissue is generally a more suitable source for high-quality DNA extraction
Figure 4 shows the spectrophotometric analysis results for young leaves following extraction using CTAB, SDS, and CTAB & SDS across the same five barley varieties

Figure (4) shows the results of the spectrophotometer analysis of dry seeds after using the spectrophotometric results for dry seed samples extracted using CTAB, SDS, and the combined CTAB & SDS methods across the five barley varieties, (Al-Dulaimi, Al-Sayd, Al-Rayhan, Wadi Zart, and Wadi Al-Hay).



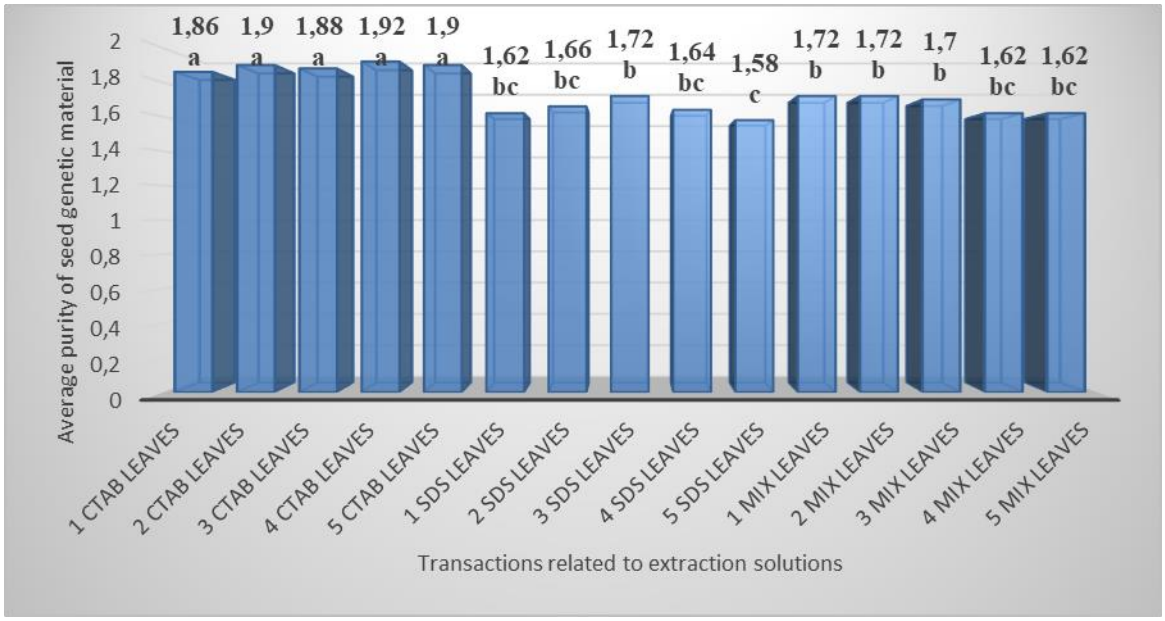


Figure (5) shows the results of the spectrophotometer analysis of the young leaves after using the extraction solution (CTAB) as follows: (1 Al-Dulaimi , 2 Al-Sayd, 3 Al-Rayhan, 4Wadi Zart, 5 Wadi Al-Hay) and extraction solution (SDS) (6 Al-Dulaimi 7 Al-Sayd, 8Al-Rayhan,

9Wadi Zart 10Wadi Al-Hay) and extraction solution (CTAB & SDS) (11Al-Dulaimi, 12 Al-Sayd, 13 Al-Rayhan, 14Wadi Zart 15Wadi Al-Hay)

SDS has been employed for genomic DNA extraction in several plant species, with variable success. However, additional studies are required to evaluate its effectiveness, particularly for plants rich in lipids and polysaccharides. Although some studies have reported that SDS can yield high-quality DNA, the present study demonstrated that SDS-extracted genomic DNA showed comparatively low purity (Figures 4 and 5). The overall A260/A280 ratio obtained using the SDS method averaged 1.65, reflecting suboptimal DNA purity. These results indicate that the SDS method is not suitable for extracting high-quality DNA from either barley dry seeds or young leaves. This finding agrees with Chen and Ronald (1999), who reported that SDS extraction yields DNA of low quality and quantity in plant tissues. Furthermore, no statistically significant differences were observed among the SDS-treated samples in the present study.

The combined extraction method (CTAB & SDS) produced DNA of moderate quality. This outcome is likely due to the interference between the highly effective CTAB reagent and the less suitable SDS solution when used together for barley seed and leaf DNA extraction.

Conclusion

CTAB proved to be the most efficient extraction method for obtaining high-purity DNA from both dry seeds and young leaves of local barley cultivars. SDS and the mixed CTAB–SDS method yielded DNA of lower quality, reflecting the challenges posed by barley's high polysaccharide and phenolic content. CTAB is therefore recommended for downstream molecular applications including PCR, sequencing, and genetic diversity analysis.

References

Abd-Elsalam, K ., Bahkali, A., Moslem,M., Amin, O & Niessen ,L .(2011). An optimized Protocol for DNA Extraction from wheat Seeds and Loop-Mediated Isothermal Amplification (LAMP) to Detect Fusarium graminearum Contamination of wheat Grain. International Journal of Molecular Sciences.,Sciences. 3459-3472.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2014). Molecular Biology of the Cell (6th ed.). Garland Science.

Aljanabi, S. M., & Martinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Research, 25(22), 4692–4693.

Al-Saghir, M., Hassan, R., & Abdel-Fatah, A. (2020). DNA Extraction Methods and Molecular Markers Applications in Barley. Journal of Genetic Engineering and Biotechnology, 18(1), 40.

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., & Struhl, K. (2002). Short Protocols in Molecular Biology. Wiley.

Ben Dalla, L, O, F. (2021). Literature review (LR) on the dominant of Research methodology. Conference (LRDRMC) (pp. 1-14). IEEE. https://doi.org/10.6754/LRDRMC56412.2020.45987623

Ben Dalla, L, O, F. (2021). Literature review (LR) on the powerful of Research methodology processes life cycle. In 2021 The Powerful of Research Methodology Processes Life Cycle Conference (TPRMPLCC) (pp. 1-10). IEEE. https://doi.org/10.16543/TPRMPLCC 50717.2020.92876580

Chaves, M. M., Flexas, J., & Pinheiro, C. (2009). Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. Annals of Botany, 103(4), 551–560.

Chen, C., & Ronald, P. C. (1999). A modified SDS method for isolating high-quality genomic DNA from barley seeds and young leaves. Plant Molecular Biology Reporter, 17(3), 249–255. https://doi.org/10.1007/BF02823455

Dalla, L. O. B., Karal, Ö., & Degirmenciyi, A. (2025). Leveraging LSTM for Adaptive Intrusion Detection in IoT Networks: A Case Study on the RT-IoT2022 Dataset

implemented On CPU Computer Device Machine.5 th International Conference on Engineering, Natural and Social Sciences, April 15-16, 2025: Konya, Turkey, 2025. Published by All Sciences Academy. https://www.icensos.com/

Daniel Cristina , Matilda and Calina . (2017). Comparison of four genomic DNA isolation methods from single dry seed of wheat , barley and rye . Agro life scientific journal . ISSN 2285-5718.

Dellaporta, S. L., Wood, J., & Hicks, J. B. (1983). A plant DNA minipreparation: Version II. Plant Molecular Biology Reporter, 1(4), 19–21.

Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 19, 11–15.

Dunwell, J. M. (2000). Transgenic barley. Journal of Cereal Science, 31(2), 99–107. https://doi.org/10.1006/jcrs.1999.0280

FAO. (2023). FAOSTAT – Barley Production Statistics. منظمة الأغذية والزراعة.

Faraj, L. O. (2017). observations on evolution of lean software development(LSD).pages.https://tez.yok.gov.tr/UlusalTezMerkezi/tezDetay.jsp?id=R_EJxYiWWNffOuWM4F4eXQ&no=fiwArXgOvJPKmFC-nX3H-w/

Glasel, J. A. (1995). Validity of nucleic acid purities monitored by 260nm/280nm absorbance ratios. BioTechniques, 18(1), 62–63.

Green, M. R., & Sambrook, J. (2012). Molecular Cloning: A Laboratory Manual (4th ed.). Cold Spring Harbor Laboratory Press.

Guo, D., Yu, X., & Zhang, X. (2014). Phenolic composition and antioxidant activity of barley grains. International Journal of Food Science & Technology, 49(5), 1236–1243.

ICARDA. (2022). Barley Production and Improvement in Dry Areas. International Center for Agricultural Research in the Dry Areas.

Lawrenson, T., Shorinola, O., Stacey, N., Li, C., Østergaard, L., Patron, N., ... & Harwood, W. (2015). Induction of targeted, heritable mutations in barley and Brassica oleracea using RNA-guided Cas9 nuclease. Genome Biology, 16(1), 258. https://doi.org/10.1186/s13059-015-0826-7

Matros, A., Peshev, D., Peukert, M., Mock, H. P., & Van den Ende, W. (2017). Sugars as hydroxyl radical scavengers: Proof-of-concept by studying the fate of sucralose in Fenton reaction. Scientific Reports, 7, 9844. https://doi.org/10.1038/s41598-017-09943-9

Murray, M. G., & Thompson, W. F. (1980). A rapid isolation procedure for high-molecular-weight plant DNA. Nucleic Acids Research, 8(19), 4321–4325.

Sambrook, J., & Russell, D. W. (2001). Molecular Cloning: A Laboratory Manual (3rd ed.). Cold Spring Harbor Laboratory Press.

Sarwat, M., Singh Negi, M., Lakshmikumaran, M., Kumar Tyagi, A., Das, S., & Shankar Srivastava, P. (2006). A standardized protocol for genomic DNA isolation from Terminalia arjuna for genetic diversity analysis. Electronic Journal of Biotechnology, 9(1), 0-0.

Sharma, P., & Purohit, S. D. (2012). An improved method of DNA isolation from polysaccharide rich leaves of Boswellia serrata Roxb.

Shlibak, A. A. A., & Dalla, L. O. F. B. (2020). The sustainable research Long while between bee pollen and honey bee diversity in Libya: Literature review. *International Journal of Social Sciences and Management Research, 7*(1), 2545-5303.

Shlibak, A. A., Örgeç, M., & Zencirci, N. (2021). Wheat landraces versus resistance to biotic and abiotic stresses. In *Wheat Landraces* (pp. 193-214). Cham: Springer International Publishing.

Shlıbak, A., & Zencirci, N. (2021). Wheat: Biotrophic Fungi and Resistance Genes. *Uluslararası Anadolu Ziraat Mühendisliği Bilimleri Dergisi, 3(1), 10-20*.

Soliman, A., Shlibak, A., & ELfaraikh, S. (2026). Response of Two Libyan Wheat Cultivars (Triticum turgidum L. and Triticum aestivum L.) to Cadmium Stress: Growth Parameters, Germination, and Seedling Vigor. *Scientific Journal for Publishing in Health Research and Technology*, 262-272..

Soliman, A., Shlibak, A., & Zencirci, N. (2026). Wheat Fungal Diseases: A Review. *Wadi Alshatti University Journal of Pure and Applied Sciences*, 191-198.

Ullrich, S. E. (2011). Barley: Production, Improvement, and Uses. Wiley-Blackwell.

Wilfinger, W. W., Mackey, K., & Krug, D. E. (1997). RNA integrity determination using spectrophotometry. BioTechniques, 22(3), 556-563.

Zielinski, H., & Kozlowska, H. (2000). Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. Journal of Agricultural and Food Chemistry, 48(6), 2008–2016.