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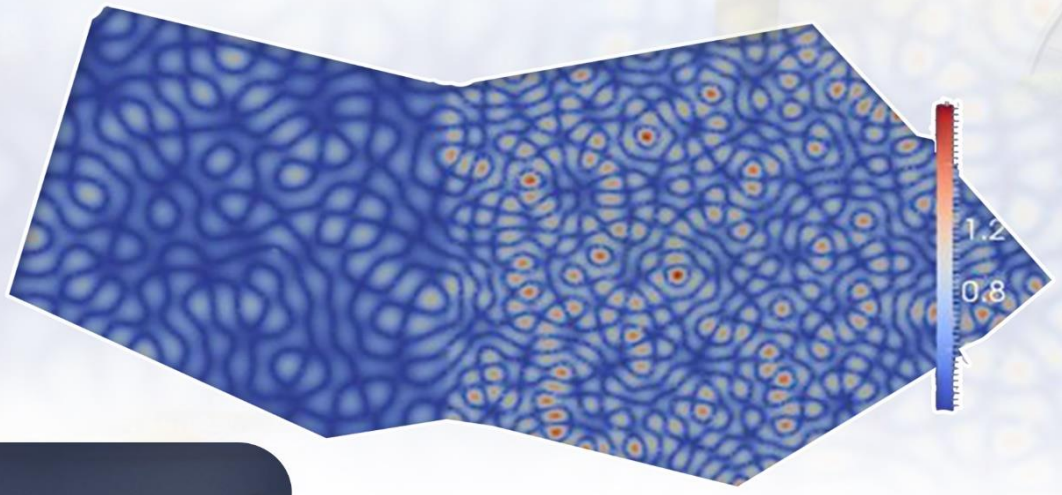

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Comparison of two Genomic DNA Extraction Protocols from Single Dray Seed of Barley Crop

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A B S T R A C T

Modern plant breeding studies are largely based on plant genetic engineering programs. Extraction of DNA with high quality plays as a key factor in most of plant genetic Studies, therefore two different DNA extraction protocols based on CTAB buffer and SDS buffer were tested for the purpose of selecting the best DNA extraction protocol from dry seeds of nine variety of barely plant . Barley seeds were taken from Libyan seeds gene bank .After Barely samples were prepared DNA was extracted directly using CTAB and SDS solutions .The quality of extracted DNA was assessed by spectrophotometric measurements and gel electrophoresis system. The results of this study showed that purity of extracted DNA by CTAB method was clearly batter compared with SDS method. CTAB method seemed to be more effective for extracting DNA from barely dry seeds. High quality DNA obtained through use of CTAB method, while CTAB had overall better A260/A280 ratio (1.736-1.932). SDS method seemed to be not suitable for DNA extraction from dry seeds of barely crop.

1 Introduction

Long-term food security management has recently become as a key issue in the polices of several countries aimed at ensuring and providing enough food with suitable quality available to everybody. In the meantime, agriculture production is the main source of human food along with animal production. Barley *(Hordeum vulgare* L*)*. (Poaceae) is one of the most important and fundamental growing crops can be used for providing food and somehow enhancement of human feeding. Currently barley is a significant crop plant globally and it is mainly exploited as feed or as a raw material for malt production (Ullaholopainen, 2015). Moreover, barley considered as one of the fourth most important cereals in the world in terms of quantity produced and planted areas (Caterina *et al*., 2016). Practically plant biotechnology considered as one of the most important methods has been largely used for plant enhancement. Recently plant DNA extraction become one of the most important factors for improving scientific researches in plant genetic engineering to increase plant productivity. Good quality DNA is a prerequisite for all experiments of DNA manipulation. However, extraction of high-quality DNA with high yield is a limiting factor in plants genetic analysis (Abdel-Latif and Osman, 2017). In plant molecular researches and experiments, a paramount needed is the extraction of DNA of good purity and efficiency suitable for other analyses (Sharma and Purohit, 2012). High quality extracted DNA from plants considered as one of the most important technologies to be hold in plant improvement field, while Purity, integrity and quality and is often needed (Daniel *et al*, 2017).

Several DNA extraction methods have been used and applied, but the most commonly used basic plant DNA extraction protocols are those of Dellaporta *et al*., 1983 and Saghai - Maroof *et al*., 1984 along with many others that are modifications of the components of these protocols (Sharma and Purohit, 2012). Whatever all plant DNA extraction protocols comprise of the basic

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steps of disruption of the cell wall, cell membrane and nuclear membrane to release the DNA into solution. Typical plant DNA isolation methods must go through some steps like breaking the cell wall which usually done by using SDS (sodium dodecyl sulfate) or CTAB (cetylrimethyl ammonium bromide), protection of DNA from the endogenous nucleases with EDTA, removal of protein from buffer/tissue and separate the protein from DNA (Daniel *et al*., 2017). In general plant DNA is mainly isolated by procedures derived from the hot CTAB and SDS methods of Saghai – Maroof *et al*., 1984 and Dellporta *et al*., 1983 respectively along with many others that are modified to be suitable for DNA extraction with high quality (Sharma and Purohit, 2012) It is generally quite difficult to extract and purify high quality DNA from cereals because of the occurrence of polysaccharides ,proteins, and DNA polymerase inhibitors in the extracts (kamel *et al*., 2011). Presence of these compounds reduces the quality and quantity of DNA which often make the sample non-qualified sample (Sarwat *et al*., 2006).

While polysaccharides are the most common contaminants found in plant extracts and can make DNA pellets slimy and difficult to handle (Kamel *et al*., 2011). On the other hand, difference among the DNA extraction methods should be tasted and observed related to the studied plant species. (Daniel *et al*., 2017).

DNA extraction from dry seeds instead of leaf tissues has some advantages which are seed can be analyzed during the non- field season, selected and prepared for the next breeding cycle and it is possible to send seed samples internationally for comparative studies this being difficult for leaf samples (Von, 2003). Furthermore most of used and tested plant DNA extraction methods were mainly based on use of CTAB and SDS, but there some factors must be taken in consideration before selecting the extraction method such as the plant type, stage of plant growth , plant tissue and cost of DNA extraction method. Therefore, the aim of this study was to compare two different plant DNA isolation protocols and obtained high quality extracted DNA using Barley dray seeds. DNA of Barley dray seeds will be extracted using CTAB and SDS protocols

2 Materials and Methods

2.1 Experimental Site

The experiment was conducted at Biotechnology Research Center laboratories (BTRC) which located in Tripoli Libya for the purpose of assessment of two different DNA extraction protocols to obtain high quality DNA with satiable yield from seeds of barley crop.

2.2 Sample Collection.

Seeds of nine barley varieties (table 3) were taken from national gene bank, which is located in Tajoura / Libya. Directly seeds were transferred to biotechnology research center laboratories for other analyses.

Table (1). List of used barley variety for DNA extraction

Number	Variety name
1	Beecher
2	Wadiyesterday
3	Boom
4	Erwan
5	Oxidation-176
6	Maimoon Valley
7	Wai Al-H
8	Basil 3
9	My Guide

2.3 DNA Isolation Buffers

Extraction of DNA with high quality and satiable quantity started with preparation of some buffers .For successfully arrival to final step of DNA extraction every step of extraction was conducted carefully due to that, every mistake well largely effect the final assessment of DNA extraction protocol and surely the final result of the experiment .DNA extraction buffers in this experiment includes : detergent : CTAB cetyltrimethyl ammonium bromide and SDS sodium dodecyl sulfate which helps to disrupts the membranes , ß mercaptoethanol which used for denaturing the proteins by braking the bonds and removing the polyphenols, EDTA added for magnesium ions needed for DNA activity, Tris at pH 8 and salts like sodium chloride for neutralizing the negative charges. First of all DNA extraction stage began with Preparation of the original solutions. Practically there were three main solutions were prepared first.

1- NaCl 5M

5M NaCl was prepared by dissolved 292.2g of NaCl in 1000ml of double distil water. 29.2 g of NaCl were dissolved in 100 ml double distil water.

2-Tris1M [PH8.0]

Normally preparation of 1M Tris obtained through dissolve 157.6g of Tris in 1000ml double distil water with use of 49ml of HCl and keeping pH at 8.

3-EDTA 0.5M

The preparation of EDTA conducted through dissolve 186.1g of EDTA in 100ml of double distil water with use of 20g NaOH. pH was kept at 8

2.3.1 2% CTAB Extraction Buffer.

CTAB for DNA extraction buffer was prepared as shown in table 2.

Table (2). CTAB Extraction buffer.

Amount for 50ml fine concentration	Amount for 100ml fine concentration	Reagent
1g 2%	2g 2%	CTAB
14ml (1.4M)	28ml (1.4m)	5M Na CL
2ml (20mM)	4ml (20mM)	0.5M EDTA [PH8.0]
5ml (100mM)	10ml (100mM)	1M Tris – CL
ß– mercaptoethanol 2% and proteinase k 100mg /ml		

2.3.2 SDS DNA Extraction Buffer.

SDS extraction buffer was prepared as shown in table 3.

Table (3). SDS extraction buffer.

Reagent	Amount for 25ml (fine concentration)
1M Tris – cl (PH7.0)	10ml (100Mm)
5M Na CL	28ml (1.4M)
0.5M EDTA (PH8.0)	4ml (20Mm)
10% SDS	1.25ml (0.5% or 0.125g) complete the size to 100ml
ß-mercaptoethanol 2% and proteinase k 100mg /ml just before use	Note /2% means (100ml /2g) and therefore (25ml/0.5g)

TE buffer. TE. buffer.was.prepared.as.shown.intable4.

Table (4). TE buffer.

Reagent	Amount for 100ml
1M Tris (pH8.0)	1ml(10mM)
0.5M EDTA (pH8.0)	0.2ml (1mM)

Complete the size to (100ml) with double distilled water

Table (5). 50X TAE electrophoresis buffer.

Reagent	Amount for 1000ml
Tris-base	242g
Glacial acetic acid	57.1ml
0.5 M EDTA (pH 8)	100ml

Table (6). 6X loading buffer.

Reagent	Amount for 100ml
Glycerol	30ml
Bromophenol blue	0.25g

Complete the volume to 100ml by double distilled water and kept in freezer degrees -20c◦.

2.4 DNA Extraction and Purification.

Plant DNA was extracted depending on following steps

1-Barley seeds were washed by sterilized distil water. Dried cleaned seeds were gently grind to fine powder with a pastel and mortar tools.

2- Fine powder of grinded seeds was transferred to Eppendorf tube for the next steps of analyses.

3-The samples were divided to two groups, CTAB group and SDS group, while CTAB extraction buffer was added to CTAB group and SDS extraction buffer was added to SDS group.

4- 2% CTAB and 0.5% SDS extraction solutions were added separately to Eppendorf tube in quantity of (600µl) with a satiable intermittently mixture using vortex. Samples were put in a water bath at temperature of 55c◦ for a period of 60 minutes.

Note for all samples; 600µl of extraction solutions were added with addition of 60µl proteinase k and 12µl of (ß-mercaptoethanol).

5- Additions of 10ml chloroform: isoamyl alcohol (24:1) and mixed well (inverting and spin). Samples were put in centrifuge for 5 minutes at a speed of 13000 rpm under temperature (24 c◦)

6- 400µl from the clear solution (up aqueous phase) were transferred using pipet to new Eppendorf tube

7-Re –add 400µl of chloroform isoamyl alcohol (24:1) and spin as previously then transferee to a new tube.

8- Add 800µl Ethanol to each sample (Eppendorf tube)

9-Spin for five minutes then carefully transferee to the ethanol without damaging the DNA (pellet)

10-Repeat the washing process three times by using ethanol 70% with volume of 600µl and spin or discard

11-Dry the samples using soft paper and cold air.

12-Addition of TE to pellet with volume of (20-50µl) depend on the size of pellet.

13-Addition of RNase (50µg/ml) then place the samples in water bath 37c◦ for 30 -60 minutes.

2.5. Gel Electrophoresis Test.

Gel electrophoresis test was applied on barely samples in order to find out and check if DNA had extracted by the tested DNA extraction methods or not. Gel electrophoresis buffer was prepared as shown in table (5) and (6) Gel electrophoresis was prepared by using TAE electrophoresis buffer (Tris acetate-EDTA buffer) with (Agarose 0.7%) then solution was transferred to microwave to dissolve the media. Finally Addison of (Ethidium bromide) whit concentration of (0.5 µg/ml). Place the samples in coolant, and then add each sample

with 4µl loading dye. Inject samples, which were added by loading buffer in the hole, which located in the prepared gel by quantity of 5-10. Marker will be injected in the first hole for comparison, followed by the rest of the samples. The electrolytic device is set to a period of 20 minutes from 70-65V. Images of extracted DNA were taken immediately to report the result.

2.6 Spectrophotometer Analyses.

Finally, samples were moved to spectrophotometer system for measuring the purity of extracted DNA to each tested extraction protocol (CTAB and SDS). The results were recorded for all used barley varieties in this study. While the spectrophotometer analyses began with calibration of the system through addition of TE solution with volume of 50µl and with (blank) at degree (260nm). 2- 5µl of sample were put in tube and added with 48µl of TE solution. Mix well until the solution has mixed well .A230 it means the carbohydrates, A260 means DNA and A280 it means protein z. Dilution process were conducted through enter 2µl of prepared sample then press sample in the system and record the results .The concentration or purity ratio is (A260/A280) , and absorbance ratio at 260–280 nm (A260/A230 ratio) , while the results were measured with a Thermo Scientific Nano Drop™ 1000 Spectrophotometer (Thermo Scientific, Germany) using 1µL of each sample. The spectra were recorded for a range of 220–750 nm.

3 Results

3.1 Gel Electrophoreses Analysis.

The presence of DNA was checked by gel electrophoresis. After samples had prepared directly put in gel electrophoresis system for the purpose of insuring that DNA has been extracted by used DNA isolation methods. The gel images of placed samples were observed and taken to report the results figure (1), while the gel image of barely samples proved that the DNA had extracted from both used methods CTAB and SDS method which mane that the extraction steps of barely DNA were successfully applied. The gel electrophoresis step proved that the barley extracted samples are ready for other analysis and tests

3.2 Spectrophotometer Measurements.

3.2.1 CTAB (DNA Extraction buffer) Assessment.

It is well known that the type of DNA extraction methods or protocols have a great influence on both quality and purity of obtained plant genomic DNA. The isolation of high quality genomic DNA for molecular analyses still the main challenge, also obtaining good quality DNA is a prerequisite for PCR and DNA manipulation studies. Many studies reported that use of CTAB in plant DNA extraction produce DNA with high quality, purity and suitable quantity. The results of this research figure (2). Showed that the best results were obtained in samples supplemented with CTAB extraction buffer, which mean that use of CTAB for DNA extraction from barley seeds was successful. According to the obtained results, CTAB extraction method is very suitable for DNA extraction from dry seeds of barely crop. CTAB had overall better A260/A280 ratio (1.736-1.932) .The results showed also among CTAB samples there were no significant differences have been found, which explain that all of the samples produced DNA with quality and purity in the same levels.

The results of our study were in agreement with Sharma and Purohit, (2012). The others in their study found that among several DNA extraction methods which have been tested, using of CTAB methods were successfully applied for the extraction of DNA from plants having secondary metabolites. The study also proved that there is a highly possibility to produce DNA with high quality and suitable quantity through use of CTAB DNA extraction methods. Our obtained results were also in agreement with Hasibe. (2005), the researchers reported that CTAB method is rapid and yields DNA sufficiently pure for PCR amplifications. Furthermore Behrooz et al .(2012) found use of 2% CTAB in modified Murray and Thompson method gave the best results for DNA extraction compared with other tested methods.

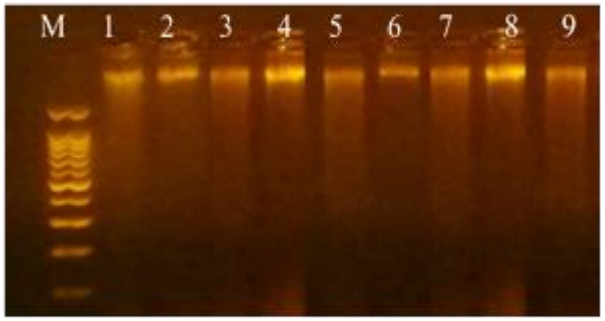

Figure (1). Agarose gel electrophoreses of DNA extracted from Barely varieties using M Bench Top PCR markers Lane 1, Beecher; Lane 2, Wadiyesterday; Lane 3, Boom; Lane 4, Erwan; Lane 5, Oxidation-176; Lane 6, Maimoon Valley; Lane 7, Wai Al-H; Lane 8, Basil 3; Lane 9, My Guide. Using cleaver scientific LTD. UV Transilluminator.

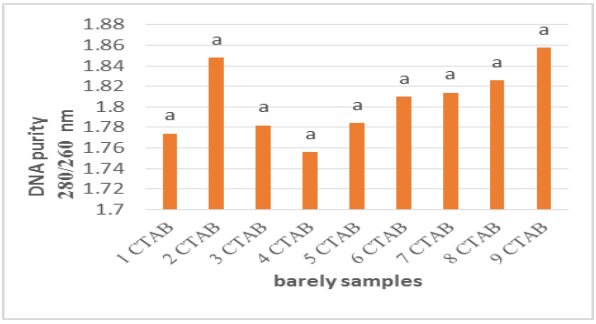

Figure (2). effect of CTAB method on purity of extracted DNA from barley seeds.

3.2.2. SDS (DNA Extraction Buffer) Assessment.

Successful molecular studies are depend on the quality of extracted DNA, while several DNA extraction methods and protocols have been involved in order to obtain high quality DNA. Plant DNA extraction methods have been modified by scientists time to time to achieve better results. SDS buffer has been used for plant DNA extraction since many years ago, and there are some studies reported that use of SDS for DNA extraction gives positive results and produce DNA with suitable quality. Furthermore, SDS methods have been successfully used for DNA extraction from microbial communities (Zhongtang and Mark, 2004). According to our obtained result figure (3), we observed that SDS method was not able to extract genomic DNA with high quality and purity from barley crop. The results of spectrophotometer analysis proved that the genomic DNA has been extracted through use of SDS but the purity level of extracted DNA through SDS was low, making this method not suitable for DNA extraction from barely plant. SDS had overall better A260/A280 ratio rich to 1.648 which mean that use of SDS method in this study was not successful.

The results showed also that among SDS samples there were no significant differences have been found in terms of DNA purity. Our results were in agreement with Behrooz *et a*l., (2012), the researcher found DNA extraction with SDS method had not good quality and quantity

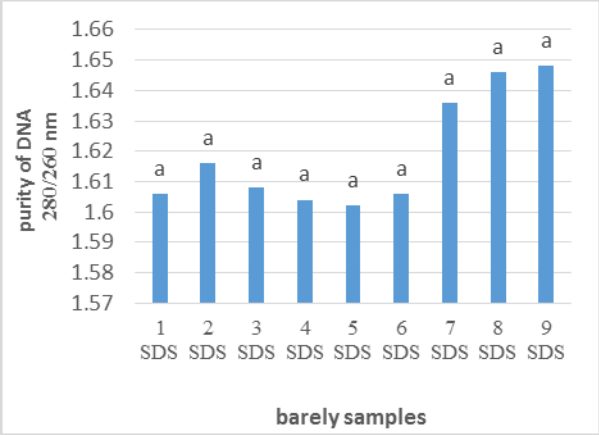

Figure (3). effect of SDS method on purity of extracted DNA from barely seeds

3.2.3. Comparison between CTAB and SDS (DNA Extraction Buffers).

As well known that high quality with satiable quantity are critical factors for successful PCR applications. The quality of DNA extractions highly infected by many compounds that inhibit DNA amplification such as polysaccharides, lipids and polyphenols (Daniel *et al*., 2017).

The comparative assessment of electrophoreses has been done to both used DNA extraction protocols (CTAB and SDS). As being reported two different DNA, extraction protocols were used to evaluate and select the best protocol for DNA extraction from cereal plants through use of seeds of barley crop. The first protocol was based on use of CTAB and second one was based on use of SDS buffer. The result figure (4) of the experiment showed that use of CTAB buffer for DNA extraction tend to be clearly better than use of SDS buffer. Furthermore the results of spectrophotometer analysis explained that the purity degree of extracted DNA through use of CTAB was is in good levels, which proved that CTAB method suitable for DNA extraction from barley plant .CTAB had overall better A260/A280 ratio (1.756-1.858), while SDS had ratio of (1.602-1.648). The results also proved that among the CTAB treatments there were no significant differences, also the same in SDS treatments there were no significant differences among the tested samples, which proved that the differences were found between the extraction methods which are CTAB and SDS but among the samples for each method there were no differences. This obtained results were in agreement with Daniel *et al*., (2017), the spectrophotometric analysis of their study resulted that CTAB extraction method had better results and batter DNA purity than SDS method, while CTAB method had ratio of (1.767 - 2.146).

Furthermore Behrooz *et al.*, (2012) found Murray and Thompson with use of 2% CTAB had the best results compared with other tested methods with SDS , while these results are very close and agree with what we had resulted in our experiment.

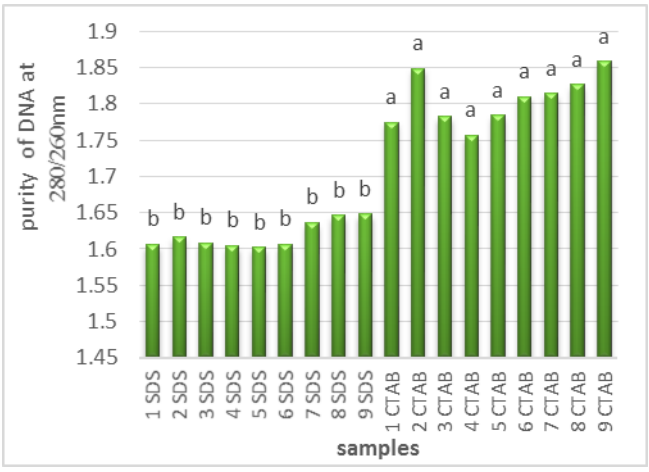

Figure (4). effect of CTAB and SDS on purity of extracted DNA.

4 Conclusions

Agriculture production become the main source for human food but more researches still needed to increase crop productivity .Plant productivity improvement is depend on plant genetic engineering and plant molecular studies .Extraction of plant DNA with high quality and suitable quantity is very important stage in molecular studies in order to produce scientific research which may help to improve plant yield .Several methods have been used for plant DNA extraction since DNA can be extracted and some of extraction methods have been modified to obtain high quality DNA. The result of this study proved that use of 2% CTAB gave the best results, and it can be successfully used for DNA extraction from cereal crops specially barely crop compared with SDS method. SDS method was not suitable for extracting DNA from barely crop.

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Conflict of interest: Members of this research certified that there are no conflicts of interest.

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