An efficient method for plant DNA extraction from dry seeds of wheat crop

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طريقة فعالة لاستخلاص الحمض النووي DNA من البذور الجافة لنبات القمح

المستخلص:

علوم الأحياء الجزيئية في النبات تشهد اهتماماً متزايداً خصوصاً في هذه الأيام لغرض تحسين إنتاجية النباتات بشكل عام. استخلاص الحمض النووي DNA بجودة عالية بات حاجة ملحة لإجراء البحوث التي تساعد في الرفع من إنتاجية النبات. في هذه الدراسة تم مقارنة طريقتين لاستخلاص المادة الوراثية النباتية DNA بالاعتماد أساساً علي مركبات الـ SDS وCTAB. تم استخدام جهاز الترحيل الكهربي للتأكد من استخلاص الـ DNA في حين تم استخدام جهاز قياس الطيف الضوئي لقياس نقاوة الحمض النووي المستخلص. نتائج هذه الدراسة برهنت على أن استخدام طريقة الـ CTAB كانت أفضل من طريقة الـ SDS في استخلاص المادة الوراثية من بذور القمح الجافة مما يؤكد نجاح الـ CTAB في استخلاص المادة الوراثية (DNA) عالي النقاوة من بذور الشعير مقارنة بـ SDS. النتائج بينت أيضاً أن المعدل A260/A280 للمادة الوراثية المستخلصة باستخدام الـ CTAB كانت بين 1.78 to1.85nm مما يبرهن على قدرة استخلاص الـ CTAB للمادة الوراثية بجودة عالية بعكس استخدام الـ SDS الذي استخلص المادة الوراثية بمعدلات نقاوة منخفضة.

الكلمات المفتاحية: *بذور القمح، استخلاص المادة الوراثية، CTAB، SDS.*

Abstract

Recently plant molecular studies have an increased attention throughout the world in order to improve plant productivity. High quality extracted DNA is extremely needed for plant improvement studies , therefore two different DNA isolation methods based on use of CTAB buffer and SDS buffer were used to find the best DNA isolation method from dry seeds of wheat plant .Spectrophotometer measurements and gel electrophoresis systems were used for estimation of DNA purity and quality. The results of this study showed that purity of extracted DNA by CTAB method was better than SDS method. CTAB method was clearly satiable for isolating high quality DNA from dry seeds of wheat plant. Whereas the A260/A280 ratio of extracted DNA from wheat seeds by CTAB was in ranged from 1.78 to1.85nm. On the other hand SDS method did not give good results compared with CTAB method which indicated that SDS method was not satiable for DNA isolation from dry seeds of wheat plant.

Keywords: *Wheat seeds, DNA extraction, CTAB, SDS.*

Introduction:

Survival of agriculture crops and sustainable food production are the most important challenges for human have to face in order to provide food with enough quantities specially with dally increasing of starvation rates in some countries throughout the world. It is well known that crops are frequently exposed to many stresses such as drought, salt, heat, low temperature, oxidative stress and heavy metal toxicity (Simataheri, 2011). Plant breeding and plant genetic engineering are the most important practices must gave more attention to increase plant yield through obtaining new varieties with some characteristics such as pest and disease resistance, drought resistance, salt tolerance and satiable productivity. However high yield potential under drought stress for example is one of the most important targets of crop breeding (Blum, 1996). Wheat crop largely effected by environmental conditions specially drought.Therefore plant breeding and genetic engineering are necessary, to obtain new wheat variety. Moreover the availability of wheat cultivars tolerant to the water shortage in the late season is essential to the sustainable production of this important food crop (Nouri-Ganbalani et al., 2009). Wheat (genus *Triticum*) belongs to the grass family of Poaceae. It is a cool-season crop, widely cultivated under varied agro-ecologic conditions and cropping systems throughout the world. Wheat considered as an old crop and wheat has been used by human as food since many years ago. Wheat mainly provides proteins, minerals, B-group vitamins and dietary fiber more in quantity than other cereal crops. Recently in the global market there is an increasing demand on wheat crop production in order to provide enough food to every person throughout the world (Peter and Sandra,2015).While about 70% carbohydrates, 12% water, 2% fat, 12% protein, 1.8% minerals, and 2.2% crude fiber are found in wheat grain kernel (Sana Afzal et al., 2013). Wheat grain also contain phosphorus, magnesium, manganese, zinc, selenium, iron, potassium and copper (Liu *et al.*, 2012). However plant breeding based on molecular biology and plant tissue culture technology is being carried out by using molecular genetics tools (Daniel et al., 2017). Plant DNA extraction step came as one of the most important steps due to that obtaining high quality DNA with enough yield is critical step for other molecular and PCR studies. extraction of good quality DNA with high yield is a limiting factor in plants genetic analysis (Abdel latif and Gamal Osman, 2017). Usually every typical plant DNA extraction conducts mainly by breaking the plant 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). The extraction of genomic plant DNA usually conducted by using protocols based on Saghai- Maroof et al ., 1984 or Dellporta et al ., 1983along with many others that are modified to be suitable for DNA extraction with high quality (Sharma and Purohit,2012). Generally extracted of high purity and quality plant DNA is extremely needed in plant improvement sector (Daniel et al., 2017). Therefore the aim of this study was verify and Foundation of satiable protocol for extracting high quality DNA through use of two different DNA isolation protocols from dry seeds of wheat crop. In fact number of DNA isolation methods have been used and tested whereas the challenges are often low DNA yields or recovering DNA free of inhibitory substances (Zhongtang and Mrk, 2004), but all plant DNA extraction protocols go through braking the cell wall, cell membrane and nuclear membrane in

order to release the DNA into the isolation buffer. Extraction of high quality DNA from plant tissues contain amount of resins, gums, polyphenols, polysaccharides and tannins remains as the main challenge (Katterman and Shattuck,1983). However polysaccharides are the main component in wheat grain cell wall followed by lignin, fructan, and resistant starch (Peter and Sandra , 2015).

The presence of these compounds in plant tissue largely inhibits the quality and quantity of isolated DNA, which makes the sample non-amplifiable (Sarwat et al ., 2006). Furthermore polysaccharide considered as the main contaminates existed in extracts can make DNA pellets slimy and difficult to handle (KamelAbd-esalam et al., 2011). Thereby higher CTAB concentration and addition of PVP prevents the browning of DNA cased by polyphenol whereas, precipitation of DNA with 2.5 *M*NaCl facilitated the removal of polysaccharides and addition of sodium acetate was useful (Sharma and Purohit, 2012). In fact CTAB extremely able to separate partial nucleic acid form poluphenol and release clean extracted genomic DNA .On the other hand selection of plant tissue is very important point that must be taken in consideration during plant DNA extraction to obtain high quality DNA. Dry seeds are used for DNA extraction because seeds have the potential to be available all the time due to ability of storage. Using dry seeds for plant DNA extraction instead of other plant tissues facilitates the DNA extraction process for the following reasons dry seeds are available at the whole year and it is possible to send seed samples internationally for comparative studies this being difficult for leaf samples (Von Post, 2003).

Material and methods:

experimental site.

The study was conducted at Libyan center for biotechnology research laboratories which located in Tripoli Libya for the purpose of evaluating the response of single dry seeds of wheat crop to different plant DNA extraction methods.

sample collection.

Seeds of nine wheat varieties have been brought from national gene bank which located in Tripoli / Libya , then directly seeds were moved to biotechnology research center laboratories for the next stages of experimental work.

Preparation of DNA isolation buffers.

DNA extraction buffers in this study includes: detergent: 2%CTAB cetyltrimethyl ammonium bromide and 0.5% 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,1M NaCl,EDTA added for magnesium ions needed for DNA activity, Tries at pH 8, proteinase k and other salts such as sodium chloride for neutralizing the negative charges . Plant DNA has been extracted depending on following steps:

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

2- Transfer the fine product of grinded dry seeds to eppendorf tube for the next steps of analyses.

3- The samples were divided to two groups, CTAB group and SDS group. CTAB extraction buffer will be added to CTAB group and SDS extraction buffer will be added to SDS group. While CTAB extraction buffer will be added to CTAB group and SDS

extraction buffer will be added to SDS group.

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

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

6- Add 600 µl chloroform : isoamyl alcohol (24:1) and mixed well for some time (inverting and spin). Then place the samples in centrifuge for 5 minutes at a speed of 13000 rpm under temperature (24 c°).

7- Transfer the clear solution on top tube (up aqueous phase) p by using pipet 400µl to new eppendorf tube and eliminate the remaining organic residue (organic phase).

8- Re –add 400µl of chloroform isoamyl alcohol by (24:1) and spin as previously then move to a new tube, this step can be canceled if the solution is very transparent and pure then samples moved to the centrifuge.

9- Add double amount of the presented quantity which is 400µl. Add Ethanol (800 µl), then samples were put again in the centrifuge for a buried of 5 mints time.

10- Spin for 5 minutes then move the ethanol carefully without damaging the DNA (pellet).

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

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

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

14- Addition of TE to pellet with volume of (20-50µl) depend on the size of pellet. Put samples in -4C° or in the freezer - 20C°.

Gel electrophoresis test.

Gel electrophoresis test figure (1) was applied on wheat samples in order to find out and check if DNA had extracted by the tested DNA extraction methods or not. Gel electrophoresis has been worked for both DNA extraction methods (CTAB and SDS samples).

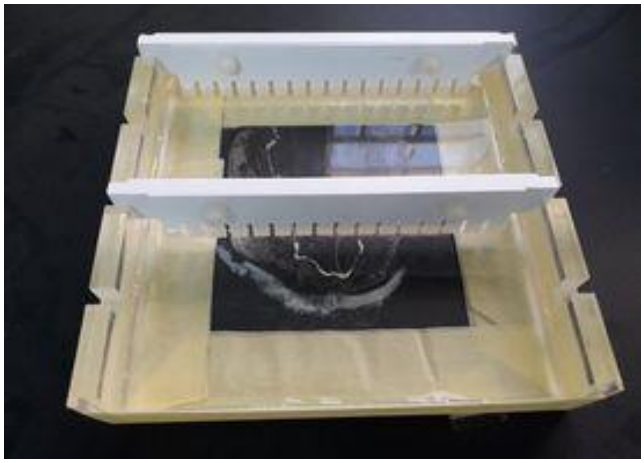

Figure (1): gel electrophoresis system

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 wheat 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.

Results and discussion:

Gel electrophoreses analysis

Gel electrophoreses analysis was done as initial step before conducting the other analysis to check The presence of DNA figure (2). After samples had prepared directly put in gel electrophoresis system for the purpose of insuring that DNA has been extracted or not. The gel images of wheat samples were, while the results showed that the DNA had extracted from both used methods CTAB and SDS method. The gel electrophoresis step proved that the wheat extracted samples are ready for next analysis.

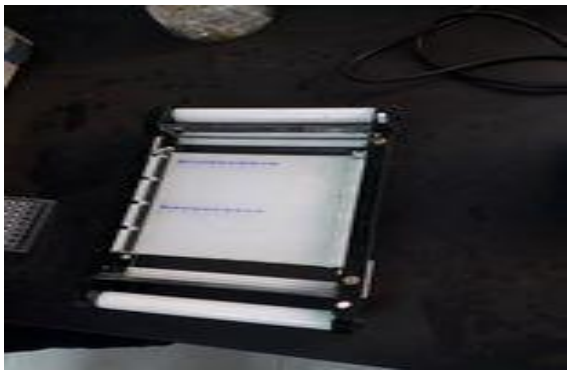

Figure (2): Gel electrophoresis photo of DNA isolated from seeds of barely plant

Spectrophotometer measurements

CTAB (DNA extraction buffer).

CTAB method is commonly used for plant genomic DNA extraction . However several studies reported that use of CTAB method for plant DNA extraction is really successful and usually gives good results.The results of this research proved that use of CTAB method for extracting DNA from wheat seeds is very successful and the best results were obtained from samples supplemented with CTAB extraction buffer. Our results figure (3) proved also that use of CTAB buffer is very suitable for DNA isolation from dry seeds of wheat plant .The A260/A280 ratio for DNA extracted by CTAB method was between 1.78-1.85nm,which mean that CTAB performance for eliminating the inhibitor compound was high and clearly observed. Furthermore the results showed that among

CTAB samples there were no significant differences have been found. 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 have been tested, using of CTAB methods were very successful for extracting DNA from plants having secondary metabolites. In the same line our obtained results were also in agreement with (Hasibe and Abdolkadir, 2005).

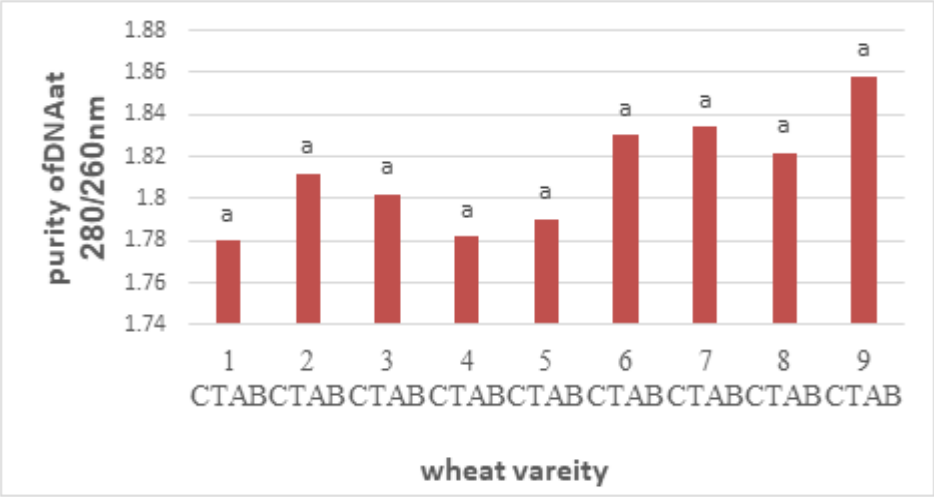

Figure (4) : effect of CTAB method on purity of extracted DNA from wheat seeds

SDS (DNA extraction buffer).

Efficiency of DNA extraction method is different from plant to another. SDS has used for DNA extraction from several plant species and different results have been recorded. However more studies are needed to assess the use of SDS specially on plants contains polysaccharides and lipids. Furthermore there are some research reported that use of SDS for DNA extraction produce DNA with good quality. Our results figure (4) showed that genomic plant DNA has been extracted using SDS, but not with high quality. DNA.The results of this study explained also that SDS method was able to extract plant genomic DNA but the purity degree of extracted DNA through use of SDS was low.SDS had overall better A260/A280 ratio rich to 1.65.According to our obtained results proved that SDS method was not satiable for isolating DNA from dry seeds of wheat plant. Our results were in agreement with (Behrooz et al., 2012) , the researcher found that the plant genomic DNA extracted by SDS method had low quality and quantity.The results of our study proved that among SDS samples there were no significant differences have been found.

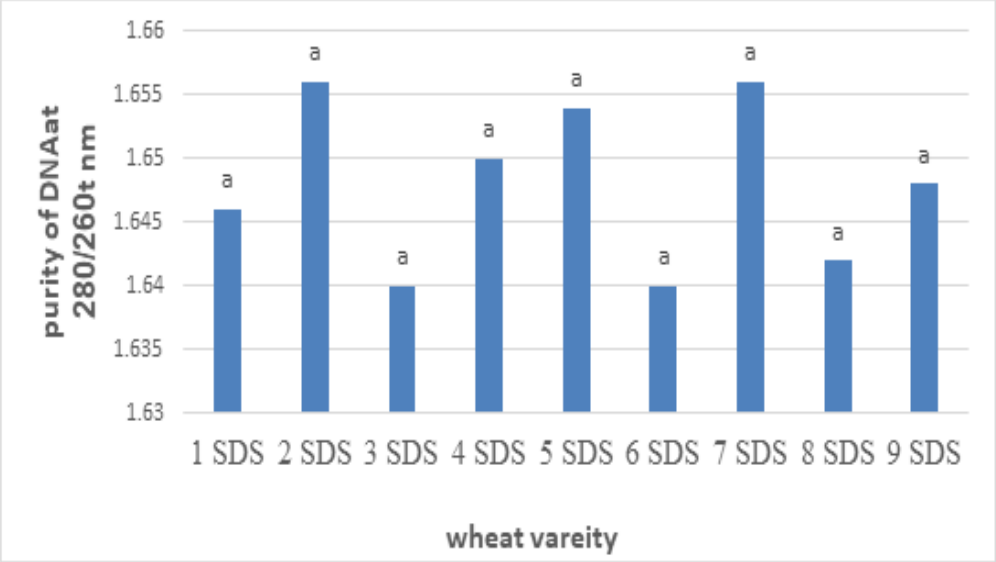

Figure (5) : effect of SDS method on purity of extracted DNA from wheat seeds

Compression of CTAB and SDS (DNA extraction methods).

Assessments of DNA extraction methods are important steps to select the best method for extracting high quality DNA specially when genomic plant DNA is extracted from plants rich in polysaccharides and polyphenols.In this experiment comparative assessment of electrophoreses system results has been done for both used DNA extraction methods (CTAB and SDS) to reported the best protocol for DNA isolation from dry seeds of wheat plant. The result figure (5) of this experiment showed that use of CTAB buffer for DNA extraction was significantly better than use of SDS buffer. Furthermore the results of spectrophotometer analysis showed that the purity of extracted DNA through use of CTAB was better than SDS, which proved that CTAB method is very adapted for DNA extraction from wheat plant. The A260/A280 ratio for CTAB method samples was (1.78-1.85), while SDS had ratio of (1.64-1.648). This obtained results were in agreement with (Behrooz et al., 2012). Our results were also in agreement with (Daniel et al., 2017) who reported that after comparison of four genomic DNA isolation methods based on CTAB and SDS from single dry seeds of wheat , barley and rye . Daniel et al ., 2017 fond that CTAB method had the best results among the other treatments CTAB samples had overall better A 260/ A208 ratio (1.767 -2.146) followed by SDS 1 (1.693-1.860) . While these results are very close with what have been found in our results .According to our results CTAB method is the best choice for extracting high quality DNA from dry seeds of wheat plan which proved that CTAB was able to eliminate the effect of inhibitor compound .Our results were in full agreement with (Shaaban et al., 2022) who found that use of CTAB method was successful for extracting high quality from dry seeds of barley plant.

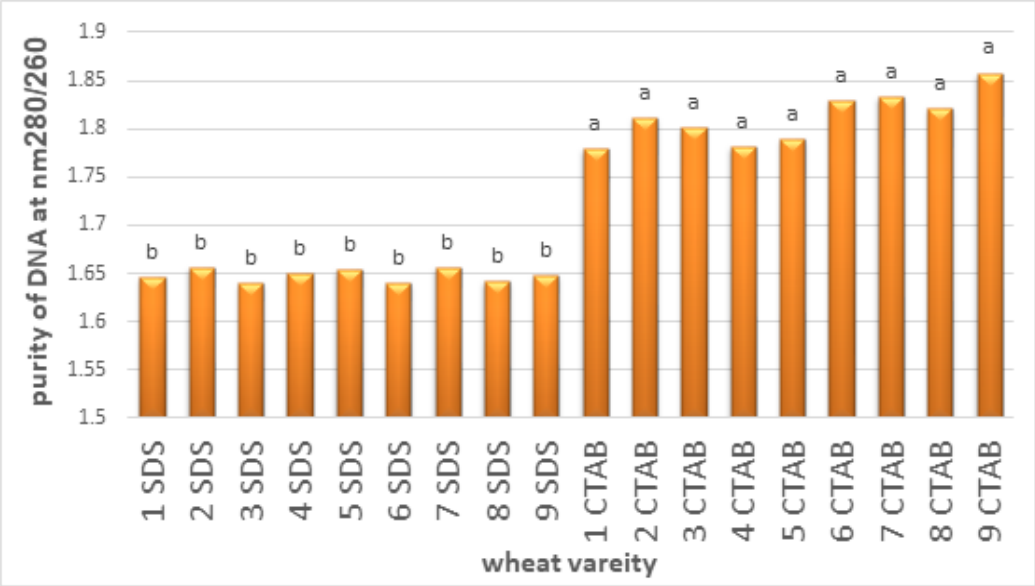

Figure (6): effect of CTAB and SDS on purity of extracted DNA from wheat dry seeds.

Conclusion and Recommendations:

Plant genetic engineering is highly contribute the agriculture movement now days. In fact one of the main stages of plant genetic engineering is extracting high quality DNA whereas high quality extracted DNA is extremely needed .Thereby Assessment of DNA isolation methods is critical point in molecular studies in order to obtain the best results. Our results proved that use of CTAB protocol for DNA isolation from dry seeds of wheat plant was better than method .According to our results CTAB method is the best choice for extracting high quality DNA from dry seeds. However back to our obtained results for extracting DNA from dry seeds we recommend to use CTAB method instead of SDS method. This experiment should be repeated on different plant seeds to ensure this obtained results.

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