I J T A
© Serials Publications
National Academy of Agricultural Science (NAAS) Rating : 3.03

Isolation of High Quality Genomic DNA from Different Date Palm (*Phoenix dactylifera* L.) Varieties Using Plant DNAzol Reagent

M. S. Rafi[1], A. K. Garg[1*] and M. Al Shamisi[2]

ABSTRACT: *Date palm (Phoenix dactylifera L.) is a cultivated fruit tree species with socio-economic impacts on millions of people in the arid regions of the world. The extraction of total genomic DNA from the rigid and fibrous date palm leaves is notoriously difficult. This is attributed to the high content of polyphenolic compounds and polysaccharides in the tissue. To solve this problem, we have developed a simple and reliable genomic DNA isolation method using Plant DNAzol reagent that uses a guanidine-detergent lysing solution. From homogenized leaf tissues from 30 different varieties, DNA was extracted and quantified using a spectrophotometer and confirmed using agarose gel electrophoresis. Interestingly, our modified DNAzol method isolated high-quality genomic DNA from all the established date palm varieties with an A260/A280 absorbance ratio greater than 1.6. The genomic DNA yield from the 27 female plants ranged from 23 to 87 µg/g leaf tissue, and it ranged from 36 to 114 µg/g leaf tissue for the three male plants. Furthermore, the isolated DNA can be used for PCR, DNA sequencing, Southern blot analysis, and a wide range of molecular biology and biotechnology applications.*

Key words: *Date Palm, genomic DNA, Phoenix dactylifera, Plant DNAzol reagent*

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a dioecious, monocot plant that has been cultivated across the Middle East and North Africa for over 5000 years, and it remains as one of the oldest known fruit crops (Chou and Krueger, 2007). The production of dates has increased steadily in recent years, with annual global production of over 7.6 million tons of date fruits harvested in 2012. However, there is a need to rapidly develop high yielding, superior quality date varieties (Al-Mssallem *et al.* 2013). This can only be accomplished by obtaining complete genome sequencing of hundreds of date palm varieties to obtain a detailed view of genome-wide structural parameters of genes, genetic diversities of cultivars, gene annotation, and characterization of functional genes for genetic improvement.

Date palm leaves are hard and fibrous, and the extraction of genomic DNA from these leaves is a challenging task. Most of the published DNA isolation procedures for date palm used the conventional phenol-chloroform or the CTAB extraction method,which is time consuming and tedious (Aitchitt *et al.* 1993; Al Shayji *et al.* 1994; Ouenzar *et al.* 1998; Haymes *et al.* 2004). Therefore, in an era of plant genomics, it is desirable to optimize high-throughput, simple genomic DNA isolation protocols for precise molecular and biotechnological applications (Karakousis and Peter, 2003). Thus, the aim of our study was to develop an efficient genomic DNA extraction method from date palm using Plant DNAzol, a nontoxic and ready-to-use reagent (Invitrogen, USA). The principle behind this reagent is based on the use of a novel guanidine-detergent lysing solution, which hydrolyzes RNA and allows the selective precipitation of DNA from the lysate (Chomczynski *et al.* 1997). In general, low genomic DNA yield from the leaf tissues is mainly due to the persistent problem of polyphenolic and protein contamination. Moreover, removal of phenolic compounds binding to DNA using conventional methods is a difficult task (John, 1992). However, the Plant DNAzol reagent is able to overcome the interference created by the production of secondary

[1] Department of Arid Land Agriculture, College of Food and Agriculture,UAE University, P.O. Box 59267, Al Ain, United Arab Emirates: *Principal Investigator: **E-mail: ajay.garg@uaeu.ac.ae*

[2] Date Palm Research & Development Unit, UAE University, P.O. Box 81908, Al Ain, United Arab Emirates.

metabolites that cause the quality and yield of the DNA to deteriorate. Considering these challenges, we have established a reliable protocol for the isolation of high quality genomic DNA from date palms.

MATERIALS AND METHODS

Plant material: Samples of relatively young leaf tissue from 30 different date palm varieties (27 female plants and 3 male plants) were collected from the Date Palm Research & Development Unit and Al Foah Experimental Farm at the UAE University (Table 1). Altogether, about 15 leaves from each variety were collected separately in plastic bags and subsequently frozen in liquid nitrogen. Later, each leaf was cut into small pieces and pulverized in liquid nitrogen using a mortar and pestle until a homogenous powder was obtained. Ground leaf samples were stored in falcon tubes at -80°C until use.

Table 1
Genomic DNA yield and purity of 30 date palm (27 females and 3 males) varieties that were extracted using DNAzol method

Sample ID	*Variety name*	*Gender*	*OD Ratio A260/ A280*	*DNA Conc. (µg/µl)*	*DNA Yield (µg/g tissue)*
1	Al Ain City date male	Male	1.56	1.82	114
2	Dabas	Female	1.72	0.74	46
3	Madain	Female	1.64	0.74	46
4	Nadra	Female	1.67	0.81	50
5	Chichi	Female	1.60	0.75	47
6	Bagla Bint Nafeh	Female	1.65	0.66	41
7	Sakee	Female	1.72	0.81	51
8	Jadmi	Female	1.69	0.80	50
9	Hilali	Female	1.77	0.88	55
10	Sheham	Female	1.62	0.85	53
11	Medjool	Female	1.71	0.96	60
12	Nimishi	Female	1.68	0.91	57
13	Fahal Madsari	Male	1.55	0.57	36
14	Jesh Ramli	Female	1.60	0.43	27
15	Barhee	Female	1.68	1.36	85
16	Zamli	Female	1.65	1.39	87
17	Fardh	Female	1.62	1.21	76
18	Ashal Al Hassa	Female	1.57	0.37	23
19	Kadrawi	Female	1.66	1.09	68
20	U.S date male	Male	1.64	1.17	73
21	Hiri	Female	1.64	0.64	40
22	Jabri	Female	1.65	0.94	59
23	Aboumaan	Female	1.68	0.92	58
24	Nawader	Female	1.72	1.01	63
25	Nagal	Female	1.61	0.86	54
26	Khenizi	Female	1.64	1.06	66
27	Nabtat Saif	Female	1.72	1.02	64
28	Khalas	Female	1.74	0.97	61
29	Lulu	Female	1.71	0.96	60
30	Sultana	Female	1.74	0.98	61

Reagents and solvents: Plant DNAzol reagent; chloroform; ethanol and TE buffer (10 mM Tris, 1mM EDTA, pH 8.0).

Isolation of total genomic DNA using the modified DNAzol method: We transferred 2g of homogenous date palm leaf powder to a 15ml falcon tube. We then added 5ml of Plant DNAzol reagent and mixed the solution thoroughly several times and placed the tube gently on a rotator for 20-30 minutes at room temperature. We immediately added 5ml of chloroform and again placed the tube on a rotator for 15-20 minutes. Following extraction steps, the tubes were centrifuged at 4000 rpm for 7 minutes and the resulting supernatant was collected carefully in a fresh tube. The DNA was precipitated by mixing the aqueous phase with 2/3 volume of 100% ethanol. Tubes were gently mixed and placed in a rotator for about 15 minutes. The precipitated DNA in the tube was centrifuged at 4000 rpm for 7 minutes and the supernatant was discarded. The pellet was allowed to air dry for 5 minutes and the DNA pellet was dissolved in 125 µl of TE buffer. The solubilized DNA solution was cloudy and viscous.

DNA quantification and agarose gel electrophoresis: The purity and quantity of isolated genomic DNA from 30 varieties of date palm were determined using a Nanodrop 2000c (Thermo Scientific, USA) based on the absorbance at a ratio of A260/A280 nm. The extracted DNA was electrophoresed on 1% agarose gels at 100 volts, run for 1h, and later visualized using a Typhoon™ FLA 9500 bimolecular imager (GE Healthcare Life Sciences, USA).

RESULTS AND DISCUSSION

We made modifications to the manufacturer's protocol for Plant DNAzol to increase the yield of DNA extracted from the date palms. We had initially tested homogenization of date palm leaf samples directly using the DNAzol reagent, but this resulted in decreased yield and low molecular weight DNA (data not shown); instead, grinding the leaf tissue in liquid nitrogen prior to DNA isolation consistently produced high yield, high quality, and high molecular weight DNA. The modified DNAzol method that was tested on different date palm varieties produced a DNA yield between 23 to 87 µg/g leaf tissue in the 27 female plants and 36 to 114 µg/g leaf tissue in the 3 male plants. The A260/A280 absorbance ratio was greater than 1.6 in all date palm samples. In comparison with routine phenol-chloroform or the

CTAB extraction method, our protocol took less time (less than 2 hours from DNA extraction to DNA solubilization steps) and gave higher yields of genomic DNA and a desirable $A_{260/280}$ ratio. Agarose gel electrophoresis confirmed the presence of high molecular weight genomic DNA in the gel with a clearer banding pattern and no evidence of substantial band shearing or RNA contamination (Figure.1). Thus, no additional RNA purification step was necessary, because the Plant DNAzol reagent contained a guanidine-detergent lysing solution that can easily hydrolyze the RNA contamination. Using this method, high quality genomic DNA was extracted in a large number of date palm samples (Figure 1). DNA extraction using DNAzol reagent has already been successfully employed in other monocot plants such as *Oryza sativa* (Garg *et al.* 2002) and *Poa pratensis* (Lickfeldt *et al.* 2002). Now, the isolated DNA can be used in next generation DNA sequencing machines or to rapidly determine gender in date palms using DNA-based assays (Al-Mssallem *et al.* 2013).

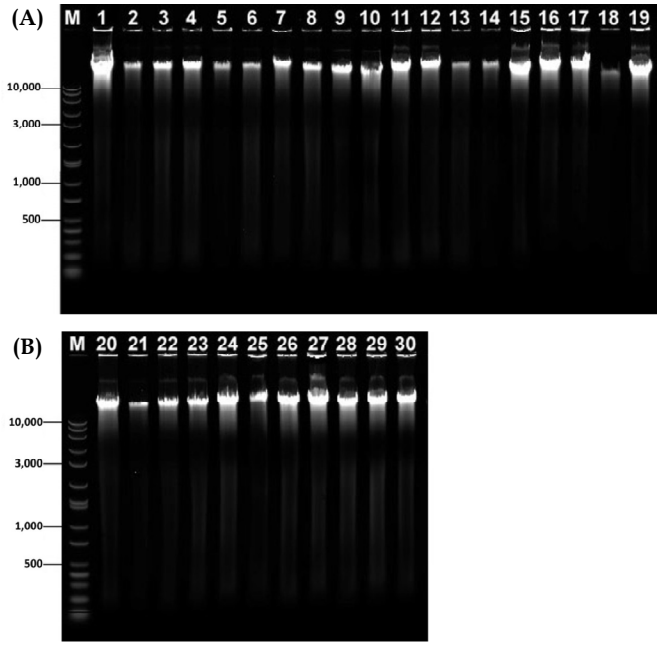

Figure 1: Genomic DNA isolated by DNAzol method was resolved by 1% agarose gel electrophoresis. The DNA samples of 30 date palm varieties were loaded as follows: (A): 1. Al Ain City date male 2. Dabas 3. Madain 4. Nadra 5. Chichi 6. Bagla Bint Nafeh 7. Sakee 8. Jadmi 9. Hilali 10. Sheham 11. Medjool 12. Nimishi 13. Fahal Madsari 14. Jesh Ramli 15. Barhee 16. Zamli 17. Fardh 18. Ashal Al Hassa 19. Kadrawi. (B): 20. U.S. date male 21. Hiri 22. Jabri 23. Aboumaan 24. Nawader 25. Nagal 26. Khenizi 27. Nabtat Saif 28. Khalas 29. Lulu 30. Sultana. M: DNA Marker with fragment sizes represented in base pairs.

CONCLUSION

In summary, our results successfully demonstrated the isolation of high quality and a reasonably good yield of genomic DNA from different date palm varieties using the Plant DNAzol reagent. Thus, our modified DNAzol method will allow isolation of genomic DNA from diverse plant species for sensitive molecular research and for a wide range of biotechnological applications.

ACKNOWLEDGMENTS

We are grateful to National Research Foundation for supporting our UAEU-NRF Research Project (Grant No. 31F025). We would like to thank Eng. Arshed for his assistance in collecting the date palm leaf samples from Al Foah Experimental Farm and undergraduate students Abdullah I. Albloushi and Firas A. Amirafor their technical assistance in the lab.

REFERENCES

Aitchitt M., Ainsworth, C. C., and Thangavelu M. (1993), A rapid and efficient method for the extraction of total DNA from mature leaves of the data palm (*Phoenix dactylifera* L.), *Plant Molecular Biology Reporter*, **11**(4): 317-319.

Al-Mssallem I. S., Hu S., Zhang X., Lin Q., Liu W., Tan J. *et al.* and Yu J., (2013), Genome sequence of the date palm *Phoenix dactylifera* L, *Nature communications.*, **4**: 2274.

Al Shayji Y., Saleem M., Al Amad S., Al Awadhi S. and Al Salameen F., (1994), Isolation and analysis of the total genomic DNA from the date palm (*P. dactylifera* L.) and related species, *Acta biotechnological.*, **14**(2): 163-168.

Chao C. T. and Krueger R. R., (2007), The date palm (*Phoenix dactylifera* L.): overview of biology, uses, and cultivation, *HortScience.*, **42**(5): 1077-1082.

Chomczynski P., Mackey K., Drews R. and Wilfinger W., (1997), DNAzol: a reagent for the rapid isolation of genomic DNA, *Biotechniques.*, **22**(3): 550-553.

Garg A. K., Kim J. K., Owens T. G., Ranwala A. P., Do Choi Y., Kochian L. V. and Wu R. J., (2002), Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses, *Proceedings of the National Academy of Sciences.*, **99**(25): 15898-15903.

Haymes K. M., Ibrahim I. A., Mischke S., Scott D. L. and Saunders J. A., (2004), Rapid isolation of DNA from chocolate and date palm tree crops, *Journal of Agricultural and Food Chemistry.*, **52**(17): 5456-5462.

John M. E., (1992), An efficient method for isolation of RNA and DNA from plants containing polyphenolics, *Nucleic Acids Research.*, **20**(9): 2381.

Karakousis A. and Langridge P., (2003), A high-throughput plant DNA extraction method for marker analysis, *Plant Molecular Biology Reporter.*, **21**(1): 95-95.

Lickfeldt D. W., Hofmann N. E., Jones J. D., Hamblin A. M. and Voigt T. B., (2002), Comparing three DNA extraction procedures for cost, efficiency, and DNA yield, *HortScience.*, **37**(5): 822-825.

Ouenzar B., Hartmann C., Rode A. and Benslimane A., (1998), Date palm DNA mini-preparation without liquid nitrogen, *Plant Molecular Biology Reporter.*, **16**(3): 263-269.