http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (11): 1436-1442, 2008
ISSN 1028-8880

Separation of DNA for Molecular Markers Analysis from Leaves of the *Vitis vinifera*

Nafiseh Rigi Nazhad and Mahmood Solouki
Department of Plant Breeding-Biocentre, Faculty of Agriculture, Zabol University, Zabol, Iran

Abstract: In the present study, three DNA extraction procedures were examined to determine which might yield DNA from Grape leaves suitable for molecular analysis for RAPD, SSR. AFLP and etc analysis. The three methods examined were: the miniprep procedure and the modified CTAB for difficult species and protocol CTAB. Only the modified CTAB method consistently yielded DNA suitable for Polymerase Chain Reaction (PCR) amplification, regardless of plant growing conditions or leaf age. The quality and quantity of extracted genomic DNA gained from these methods are deliberated by means UV biophotometer, electrophoresis in 1.2% agarose gel and PCR. In this regard, application chosen for young and mature leaves, the most value of qualified DNA, is extracted from fully expanded leave when PVP was added to the extraction buffer. This same procedure also yielded PCR-amplifiable DNA from various other perennial, woody species and from other fruit species such as apple (*Malus domestica*), cherry (*Prunus avium*), peach (*Prunuspersica*), plum (*Prunus domestica*). DNA yield from this procedure is high (up to 1 mg g^{-1} of leaf tissue). DNA is completely digestible with restriction endonucleases and amplifiable in the Polymerase Chain Reaction (PCR).

Key words: DNA isolation, RAPD analysis, SSR analysis, AFLP analysis, horticultural crops, quality and quantity of DNA, Iraman grapes, PCR

INTRODUCTION

Vitis vinifera taxonomically belongs to the Ampelidaceae or Vitaceae family. Grapevine is an ancient fruit species in Iran and its domestication goes back to 5000 years old (McGovern, 2003). Recently raisins of seeded and seedless grapevine were found by Iranian and Italian archeologists in Shahre Sokhta (Sistan, Iran).

The long history of grapevine growth has determined a complex picture in which many biotypes or even cultivars are misidentified or called by different names in different areas. This often makes genetic identification difficult. So it takes for serious to collect the varieties of all its genotypes, to assay and classify them. For this movement extraction and preparation of proper DNA samples is certainly of importance. Classifying the genetic resources and molecular methods play significant roles to manage and hoard such precious priceless collections.

Vitis vinifera and related species has been the subject of extensive genetic studies due to their worldwide cultivation and importance. Recently this plant has been used for gene mapping (Akkurt *et al*., 2007; Kikkert *et al*., 2005; Troggio *et al*., 2007), genetic transformation (Wang *et al*., 2005; Baribault *et al*., 1990; Hébert *et al*., 1993) and DNA fingerprinting (Di Gaspero *et al*., 2007; Adam-Blondon *et al*., 2004) However, molecular markers require DNA of suitable purity for the enzymatic PCR and it is often difficult to separate DNA from naturally occurring plant cell contaminants.

The relatively small genome size of *Vitis vinifera* (0.50 pg C^{-1}) compared to many other perennial plant species (Lodhi and Reisch, 1995; Arumuganathan and Earle, 1991) should facilitate molecular genetic studies of *Vitis*. However, DNA extraction from grapevine has been difficult due to the presence of contaminants such as polyphenols and polysaccharides. In particular, polysaccharides (Do and Adams, 1991; Murray and Thompson, 1980; Fang *et al*., 1992) and polyphenolic compounds (Newbury and Possingham, 1977; Katterman and Shattuck, 1983; Couch and Fritz, 1990; Howland *et al*., 1991; Collins and Symons, 1992) can form a complex with and become irreversibly bound to nucleic acids during extraction (Varadarajan and Prakash, 1991).

Some of these contaminants can inhibit the activity of DNA modifying enzymes (Draper and Scott, 1988). This study indicates that extraction of DNA is not always simple or routine and that published protocols are not necessarily reproducible for all species (Stein, 1993; Rogers, 1994). In this laboratory, simple extraction protocols such as suggested by Wang *et al*. (1993), did not yield DNA from mature grape leaves. Other published protocols included the use of ultracentrifugation with cesium chloride (Baker *et al*., 1990), protoplast isolation

Corresponding Author: Nafiseh Rigi Nazhad, Department of Plant Breeding-Biocentre, Faculty of Agriculture, Zabol University, Zabol, Iran

(Deragon and Landry, 1992), detergents such as SDS (Dellaporta *et al*., 1983; Varadarajan and Prakash, 1991) and CTAB (Murray and Thompson, 1980; Doyle and Doyle, 1990), or Modified CTAB (Lodhi *et al*., 1994). In addition, PVP have been used to remove phenolic compounds (Doyle and Doyle, 1990) and excess polysaccharides (Do and Adams, 1991).

Respectively Successful extraction of PCR-amplifiable DNA can lead to the establishment of DNA fingerprints for individual grape cultivars. These fingerprints will be used as a diagnostic tool for cultivar identification. Grape cultivars are vegetatively propagated; therefore, a requirement of the DNA extraction method is that it not be influenced by leaf age or growing conditions that generated the leaf. The objectives of this study were to determine which of the three common DNA extraction methods (SDS, CTAB, or modified CTAB) might yield PCR-suitable DNA from Grapevine leaves, to evaluate the need for PVP in the extraction process, to examine the effect of leaf age and growing conditions on DNA extraction and to examine the reproducibility of the amplification products.

MATERIALS AND METHODS

Plant material: The plant material used for comparison of DNA extraction methods included 20 grape cultivars (Table 1) which all belong to *Vitis vinifera* were hardly collected from the demolished vineyards of different area of Iran. Leaf material of these 20 cultivars was available from 6 to 7 year-old field-grown material, 1 to 2-year-old rooted greenhouse cuttings in the Zabol University glass house and micropropagated material that was sub cultured a regular basis in the Biocenter of Zabol University.

Culture media: The powder media (4.2 g L^{-1}) of Murashige and Skoog (1962) obtained from Sigma, 20 g L^{-1} sucrose, 0.1 mg L^{-1} IAA for rooting and 1.5 mg L^{-1} BAP were used to produce enough shoots and leaves *in vitro*. The leaf materials from jars and plants in pots were utilized for DNA isolation during the study.

DNA extraction: Leaf material included the blade and petiole from field and greenhouse-grown material and stems and leaves of micropropagated shoots. The leaf material was washed to remove any source of foreign DNA, such as insects or insect eggs on the field- or greenhouse-grown leaves. 0.5 to 1 g of leaf material was then frozen in liquid nitrogen, ground with a mortar and pestle and subjected to one of the following extraction procedures.

Table 1: Comparison between means of DNA concentration from leaf samples of 20 cultivars that collected from different area of Iran

Local names	Collected from	Means of DNA concentration (ng μL^{-1})
Ssangak	Sistan	32.567^{n}
Laal	Sistan	70.733^{i}
Yahghooti Sefeid	Sistan	34.367^{3}
Fakhri	Sistan	101.100^{h}
Cheshm Gavi	Sistan	105.950^{g}
Tokhmeh Tehrani	Shiraz	40.800^{m}
Hossein Sefeid	Ferdows	104.367^{gh}
Shahani	Qazvin	169.450^{b}
Aaskari Sefeid	Shiraz	157.167^{c}
Molaei	Shahryar	127.750^{f}
Yahgooti Ghermez	Sistan	8.663^{o}
Bedaneh Sefeid	Gazvin	65.900^{j}
Polet	Varamin	132.633^{e}
Khalili Bedaneh	Rezvan	51.900^{l}
Khalili	Varamin	58.400^{k}
Askari Kardinal	Varamin	199.167^{a}
Shahani	Ghasre Shirin	144.667^{d}
Bedaneh Ghermez	Gazvin	68.333^{ij}
Aaskari Sefeid	Varamin	144.850^{d}
Yahghooti Ghermez	Shiraz	12.450^{o}

Mean separation within columns by Duncan's Multiple Range Test. Mean with the same alphabets are nonsignificant but mean with the different alphabets have significant difference

Table 2: Analysis of variance for different methods of DNA extraction, leaf type (young, mature) and growing condition (field, greenhouse, tissue culture) from *Vitis vinifera*

		Means of square	
SOV	Degree of freedom	DNA concentration (ng μL^{-1})	Absorption ratio (260/280 nm)
Extraction method (A)	2	13364.7**	2.5727**
Growing condition (B)	2	325.23**	0.1539**
Leaf type (C)	1	105.84**	0.0535*
A*B	4	11.50**	0.4100**
A*C	2	1.44ns	0.2881**
B*C	2	0.13ns	0.0185ns
A*B*C	4	0.50ns	0.0488*
Error	36	2.37	0.0216
CV (%)		3.97	6.26

*Significant at p = 0.05, **Significant at p = 0.01

Three DNA extraction methods, referred to as the SDS, CTAB and CTAB modified methods, were examined. The first method was the miniprep procedure (= SDS) described by Dellaporta *et al*. (1983). The second method was described by Doyle and Doyle (1990). The third method was modified Protocol CTAB suggested by Lodhi *et al*. (1994). The soluble PVP was added (wt:vol) in to the extraction buffer (Table 2) and after that, DNA was solubilized in TE buffer (Tris 10 M, EDTA 1 mM and pH 8.0) and stored at -20°C.

Data analysis: Three replications of a 3×3×2 factorial, completely randomized design were used in the analysis of interaction between extraction methods, growing conditions and leaf age (Table 2). The effect of the insoluble PVP (0, 50 mg Polyvinyl Pyrrolidone) on the

DNA yields and purity of 20 cultivars (Table 3) were analyzed using three replications of a factorial completely randomized design. Six cultivars were native and other cultivars were collected from other regions.

Estimating of DNA quantity and quality: Two DNA quantification methods were used. The first method was biophotometer (Eppendorf AG.Hamburg) measurement with UV absorption at a wavelength of 260 nm (as nucleic acids absorbing wavelength of light) and also in wavelength of 280 nm (as the proteins absorbing wavelength of light). The second method was the minigel method using ethidium bromide fluorescence and a lambda DNA standard, as described by Sambrook *et al.* (1989).

Measures of DNA purity were determined by the A260:A280 and A260:A230 ratios. These ratios provide indications of protein and polyphenol and carbohydrate contamination, respectively (Manning, 1991). Absorption ratios were determined using the UV biophotometer. The samples with optimal values ranging from 1.8-2.0 were used for the amplification and the DNA concentration of 10 ng was formed to be used in the laboratory.

The electrophoresis of DNA on 1.2% agarose gel, Showed quality of DNA bands for each sample. Each one contained 8 μL of extracted DNA and 2 μL loading buffer, that put into the agarose gel sumps and loaded off in TBE buffer condition. Agarose gel was under a steady voltage of 75 for 1 h and then stained by ethidium bromide. The results were visualized and photographed by Gel Documentation system (Vilber Lourmat-France) under UV lighting.

DNA amplification: The ability of amplification of extracted DNA strands was proved by 2 randomized 10-nucleotide primer (Sinagen). Genomic DNAs were amplified using 2 different primers (5′- CGGCCCACGT-3′ and 5′-CCCACTACTG-3′) after optimization of amplification reaction. Polymerase chain reaction (PCR) was performed using a Gradient Eppendorf Termal Cycler based of what reported by Solouki *et al.* (2007). Each PCR reaction was carried out more than three times to be observed the repeatability of amplification patterns.

The reaction mixture with total volume of 25 μL containing of 7xPCR buffer (10 mM Tris-HCl, pH 8.0, 50 mM KCl, Sinagene), 0.1% triton x 100, 2 mM $MgCl_2$, 200 μM dNTPs, 2U of *Taq* DNA polymerase (Sinagene), 0.4 μM primer and 10 ng genomic DNA was used. The Termal Cycler with the following profile was programmed (i) 40 cycles of 30 sec at 94°C, 1 min at 36°C and 2 min ramp until 72°C. (ii) Final step of 72°C for 8 min and then cooled to 4°C. The PCR products were separated by electrophoresis on 1.4% agarose gel in 0.5 x TAE buffer (0.045 M Tris, 0.001 M EDTA, Acetic acid) and stained with ethidium bromide. *EcoR1/HindIII* digested lambda DNA was used as molecular size marker. The gels were visualized by Gel Documentation system (Vilber Lourmat-France) and image of gels were printed.

Table 3: Analysis of variance for the effect of insoluble PVP (0, 50 mg Polyvinyl Pirrolidone) on 20 grape cultivars

	Means of square		
SOV	Degree of freedom	DNA concentration (ng μL^{-1})	Absorption ratio (260/280 nm)
Cultivar (A)	19	16264.16**	2.4726**
Soluble PVP (B)	1	38159.76**	0.1269**
Cultivar* soluble PVP (A*B)	19	457.73**	0.0535*
Error	80	938.33	0.4129
CV (%)		11.78	9.47

*Significant at p = 0.05, **Significant at p = 0.01

RESULTS AND DISCUSSION

The number of PCR-amplifiable DNA samples, resulting from the SDS, CTAB and modified CTAB extraction methods are given in Table 4 and 5.

The results has indicates that Only the CTAB modified (with PVP in buffer extraction) consistently yielded DNA suitable for PCR-amplification from all growing conditions. Generally these results are accordance with those of Alaey *et al.* (2005).

DNA extracted by the SDS and CTAB methods was considered unsuitable because: (1) DNA yields were extremely low and (2) only a few of these DNA samples were amplified by the PCR using 2 single primers. Quality and quantity of extracted DNA were tested in two biophotometer- electrophoresis methods and also PCR. In due to the fact, when the ratio of absorption 260/280 nm is between 1.8 and 2, it shows the most absorption is presented by nucleic acids and therefore extracted DNA is well-qualified and its purity is acceptable.

Many of DNA samples obtained using from two methods SDS and CTAB did not produce enough DNA for minigel quantification because of extremely low yields. In these cases, DNA was quantified by UV absorption and some of these samples were amplified by the PCR. The other SDS and CTAB extracted DNA samples were quantified using the minigel method, but all were not PCR amplifiable.

It seems, Non-amplification of these DNA samples could be due to the extremely low yields and residual contaminants which interfere with the PCR. In contrast, yields for all modified CTAB extracted samples were quantified using the minigel method and amplified by

Table 4: Number of sample amplified by PCR for DNA extracted by the SDS, CTAB and CTAB modified methods From field, greenhouse and micropropagation plant material

Extraction method	Source of leaf material		
	Micropropagation	Field	Greenhouse
SDS	1/3	0/3	1/3
CTAB	2/3	1/3	2/3
CTAB modified	3/3	3/3	3/3

Table 5: Mean DNA yield and number of samples amplified by PCR for CTAB extraction method with and without the inclusion of PVP purification column

Extraction method	Source of leaf material	
	Yield (mg g^{-1})	No. of samples amplified by PCR
CTAB	<0.5	1/3
CTAB + PVP in buffer extraction	>1.0	3/3
CTAB + PVP in TE buffer	≤0.5	2/3
CTAB + PVP in buffer extraction and TE buffer	≤1.0	2/3

PCR. So these results confirm that extracted DNA executed by modified CTAB method from young and mature leaves possess better quality and quantity in compare with the other methods. Experiments using PVP in conjunction with the SDS extraction method did not increase the number of DNA samples which could be amplified by the PCR (data are not shown).

The addition of PVP on CTAB modified extracted DNA did not interfere with amplification of the DNA sample. The average yield for CTAB modified extracted DNA was 0.5±1.0 mg g^{-1} fresh weight of leaf material. Yields were determined using the minigel method and are similar to those using variety of DNA extraction procedures found in the literature (Lodhi *et al*., 1994; Dellaporta *et al*., 1983; Rogers and Bendich, 1985), including ultracentrifugation with CsCl (Murray and Thompson, 1980). This study also indicates the recovery of high molecular weight with less shearing and easy mobility. The procedure is fast and simple that 30 to 40 DNA samples may be processed in a single day. It is also evident that the uncut DNA exhibits little shearing and is suitable for Southern hybridization (Southern, 1975). The DNA is also amplifiable in PCR using the RAPD technique (Williams *et al*., 1990).

Comparison of the two methods of quantifying DNA indicates that estimation of yield by UV absorption was greater than indicated by the minigel method. Although, Doyle and Doyle (1990) suggested that CTAB interferes with UV absorption at 260 nm, there was interference in the SDS-extracted samples also. Interference by RNA is possible, but agarose gel electrophoresis of extracted DNA samples did not reveal contamination by RNA. Another possible source of UV interference could be cellular compounds which not removed during the extraction process. Both polysaccharide (Draper and Scott, 1988) and phenolic compounds (Manning, 1991) can interfere with DNA extraction and quantification by UV absorption. Many polyphenolic compounds absorb in the 260 to 280 nm range (Harborne, 1963) and therefore could interfere with DNA quantification. The use of PVP discussed above, did not reduce the absorption interfering contaminants (data are not shown). Consequently, because of UV interference by an unknown contaminant, the validity of absorption ratios were questioned and with the exceptions indicated above, DNA yields were determined by the minigel method. The reproducibility of amplification products were examined for CTAB modified extracted DNA. Amplification products from three replications of a single DNA sample and from leaf samples that differ in age and source.

Similarly, differences in product reproducibility occur among cultivars. The results showed that products generated for some cultivars are different than others. Using 1 g leaf material in CTAB modified method with 0.50 mg PVP gained the most DNA extraction (177.2-227 Ng μL^{-1}) of Askari Kardinal in compare with native grape namely Yahghooti Ghermez (7.9 -17.8 Ng μL^{-1}). The results showed in Table 1.

Proper choice of leaf tissue is very important for DNA extraction. The use of very young leaf tissues has resulted in poor yields. We found that partially expanded leaves are the best material. This is consistent with the results reported by Di Gaspero *et al*. (2007), in which the best results were obtained from rapidly expanding leaves, one to two nodes from the shoot tip. With fully expanded leaves the yield was low and the DNA was not completely digestible. However, we were able to get equally good results with fully expanded leaves when PVP was added to the extraction buffer. The results presented only for two cultivars that had the least DNA concentration (Table 6-11). PVP has been used to remove polyphenols from mature, damaged and improperly stored leaf tissues (Rogers and Bendich, 1985; Doyle and Doyle, 1987; Howland *et al*., 1991). PVP forms complex hydrogen bonds with polyphenolic compounds which can be separated from DNA by centrifugation (Maliyakal, 1992). The presence of polyphenolic compounds can be reduced by keeping plant material frozen before extraction and by using PVP in the DNA extraction procedure.

The developmental stage of the plant is also important. The optimal time for leaf collection was during the period of active shoot elongation following bud break. Later in the season DNA extraction was difficult and the DNA obtained was unstable for long term storage. Remnant polysaccharides are a group of chemicals which

Table 6: Comparison between quantity of extracted DNA from leaf samples of Sistan Yaghooti Ghermez cultivar

	Field		Greenhouse		Tissue culture	
Extraction methods	DNA conc. young leaves	DNA conc. mature leaves	DNA conc. young leaves	DNA conc. mature leaves	DNA conc. young leaves	DNA conc. mature leaves
	(ng μL^{-1})					
Dellaporta *et al.* (1983)	11e	7f	19e	12f	6f	11ed
Doyle and Doyle (1987)	24c	18d	30c	24d	10de	17c
Lodhi *et al.* (1994)	62a	55b	69a	58b	32b	41a

Mean separation within columns by Duncan's Multiple Range Test. Mean with the same alphabets are nonsignificant but mean with the different alphabets have significant difference

Table 7: Comparison between quantity and qualities of extracted DNA from leaf samples of Shiraz Yahghooti Ghermez cultivar

	Field		Greenhouse		Tissue culture	
Extraction methods	DNA conc. young leaves	DNA conc. mature leaves	DNA conc. young leaves	DNA conc. mature leaves	DNA conc. young leaves	DNA conc. mature leaves
	(ng μL^{-1})					
Dellaporta *et al.* (1983)	15e	10f	20e	14f	7f	10e
Doyle and Doyle (1987)	30c	22d	35c	27d	12d	21c
Lodhi *et al.* (1994)	80a	74b	86a	79b	41b	52a

Mean separation within columns by Duncan's Multiple Range Test. Mean with the same alphabets are nonsignificant but mean with the different alphabets have significant difference

Table 8: Comparison between qualities of extracted DNA from leaf samples of Sistan Yaghooti Ghermez. cultivar in the present of PVP

	Field		Greenhouse		Tissue culture	
Extraction methods	Absorption ratio (260/280) young leaves	Absorption ratio (260/280) mature leaves	Absorption ratio (260/280) young leaves	Absorption ratio (260/280) mature leaves	Absorption ratio (260/280) young leaves	Absorption ratio (260/280) mature leaves
Dellaporta *et al.* (1983)	0.70f	0.9ed	1.05f	1.21ed	1.20c	1.49bc
Doyle and Doyle (1987)	1.02d	1.29c	1.13d	1.40c	1.59b	1.79a
Lodhi *et al.* (1994)	1.32a	1.67a	1.73b	1.80a	1.79a	1.84a

Mean separation within columns by Duncan's Multiple Range Test. Mean with the same alphabets are nonsignificant but mean with the different alphabets have significant difference

Table 9: Comparison between qualities of extracted DNA from leaf samples of Shiraz Yahghooti Ghermez cultivar in the present of PVP

	Field		Greenhouse		Tissue culture	
Extraction methods	Absorption ratio (260/280) young leaves	Absorption ratio (260/280) mature leaves	Absorption ratio (260/280) young leaves	Absorption ratio (260/280) mature leaves	Absorption ratio (260/280) young leaves	Absorption ratio (260/280) mature leaves
Dellaporta *et al.* (1983)	1.01d	1.29cb	1.11d	1.31c	2.13bc	2.03ab
Doyle and Doyle (1987)	1.21bc	1.31b	1.68b	1.78b	2.01ab	1.96ab
Lodhi *et al.* (1994)	2.01a	1.99a	1.99a	1.88a	1.92a	1.89a

Mean separation within columns by Duncan's Multiple Range Test. Mean with the same alphabets are nonsignificant but mean with the different alphabets have significant difference

Table 10: Comparison between qualities of extracted DNA from leaf samples of Sistan Yaghooti Ghermez cultivar without the present of PVP

	Field		Greenhouse		Tissue culture	
Extraction methods	Absorption ratio (260/280) young leaves	Absorption ratio (260/280) mature leaves	Absorption ratio (260/280) young leaves	Absorption ratio (260/280) mature leaves	Absorption ratio (260/280) young leaves	Absorption ratio (260/280) mature leaves
Dellaporta *et al.* (1983)	0.30f	0.62ed	0.70f	1.21ed	1.07c	1.23bc
Doyle and Doyle (1987)	0.87d	1.29c	0.91d	1.41c	1.39b	1.69a
Lodhi *et al.* (1994)	1.42b	1.68a	1.53b	1.78a	1.76a	1.79a

Mean separation within columns by Duncan's Multiple Range Test. Mean with the same alphabets are nonsignificant but mean with the different alphabets have significant difference

decrease DNA quality. To omit these materials, NaCl 5 M is recommended (Zhang *et al.*, 2003). In this procedure, β-mercaptoeyhanol works as an antioxidant agent and forbids oxidation in polyphenols (Porebski *et al.*, 1991; Bushra *et al.*, 1999) and this can also elevate the extraction process during the method of (Lodhi *et al.*, 1994).

In conclusion, only the modified CTAB method used in this study consistently yielded DNA suitable for PCR amplification from Grape leaves, regardless of plant growing conditions or leaf age. In addition, the application of this method to various species yielded PCR-amplifiable DNA, which suggests this procedure could yield suitable DNA from many other species. These

Table 11: Comparison between qualities of extracted DNA from leaf samples of Shiraz Yahghooti Ghermez cultivar without the present of PVP

	Field		Greenhouse		Tissue culture	
Extraction methods	Absorption ratio (260/280) young leaves	Absorption ratio (260/280) mature leaves	Absorption ratio (260/280) young leaves	Absorption ratio (260/280) mature leaves	Absorption ratio (260/280) young leaves	Absorption ratio (260/280) mature leaves
Dellaporta *et al.* (1983)	0.85d	1.24cb	1.07d	1.23c	1.99ab	2.02b
Doyle and Doyle (1987)	1.41bc	1.63b	1.60b	1.75b	1.90a	1.98a
Lodhi *et al.* (1994)	1.79a	1.99a	1.83a	1.89a	1.88a	1.89a

Mean separation within columns by Duncan's Multiple Range Test. Mean with the same alphabets are nonsignificant but mean with the different alphabets have significant difference

results are concordance with those of Rogers (1994), who demonstrated the superiority of CTAB-based extraction methods over those containing SDS or SDS and CTAB, for a number of plant and fungal species. The addition of PVP was necessary to enhance the generation of reproducible molecular markers.

Distinguishing between 20 Grape cultivars is possible using the CTAB modified -extraction and amplification protocols presented in this study. The yield and molecular weight of the CTAB modified-extracted DNA are comparable with DNA extracted using other more complex procedures.

ACKNOWLEDGMENT

Thank are due to the Zabol University and Biocenter for financial assistance.

REFERENCES

Adam-Blondon, A.F., C. Roux, D. Claux, G. Butterlin and D. Merdinoglu *et al.*, 2004. Mapping 245 SSR markers on the *Vitis vinifera* genome: A tool for grape genetics. Theor. Applied Genet., 109: 1017-1027.

Akkurt, M., L. Welter, E. Maul, R. Topfer and E. Zyprian, 2007. Development of SCAR markers linked to powdery mildew (Uncinula necator) resistance in grapevine (*Vitis vinifera* L. and *Vitis* sp.). Mol. Breed., 19: 103-111.

Alaey, M., R. Naderi, A. Vezvaei, A. Khalichi and A. Salami, 2005. Comparing study between four different methods of genomic DNA extraction from *Cyclamen persicum* Mill. Int. J. Agric. Biol., 7 (6): 882-884.

Arumuganathan, K. and E.D. Earle, 1991. Nuclear DNA content of some important plant species. Plant Mol. Biol. Rep., 9: 208-218.

Baker, S.S., C.L. Rugh and J.C. Kamalay, 1990. RNA and DNA isolation from recalcitrant plant tissues. BioTechniques, 9 (3): 268-272.

Baribault, T.J., K.G.M. Skene, P.A. Cain and N.S. Scott, 1990. Transgenic grapevines: Regeneration of shoots expressing β-glucuronidase. J. Exp. Bot., 41 (8): 1045-1049.

Bushra, C., Y. Afshan, H. Tayyab and S. Riazuaain, 1999. Mini-Scale genomic DNA extraction from cotton. Plant Mol. Biol. Rep., 17: 1-7.

Collins, G.G. and R.H. Symons, 1992. Extraction of nuclear DNA from grape vine leaves by a modified procedure. Plant Mol. Biol. Rep., 10: 233-235.

Couch, J.A. and P.J. Fritz, 1990. Isolation of DNA from plants high in polyphenolics. Plant Mol. Biol. Rep., 8: 8-12.

Dellaporta, S.L., J. Wood and J.B. Hicks, 1983. A plant DNA minipreparation: Version II. Plant Mol. Biol. Rep., 1: 19-21.

Deragon, J.M. and B.S. Landry, 1992. RAPD and other PCR based analyses of plant genomes using DNA extracted from small leaf disks. PCR Meth. Applied, 1: 175-180.

Di Gaspero, G., G. Cipriani, A.F. Adam-Blondon and R. Testolin, 2007. Linkage maps of grapevine displaying the chromosomal locations of 420 microsatellite markers and 82 markers for R-gene candidates. Theor. Applied Genet., 114: 1249-1263.

Do, N. and R.P. Adams, 1991. A simple technique for removing plant polysaccharide contaminants from DNA. BioTechniques, 10 (2): 162-166.

Doyle, J.J. and J.L. Doyle, 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissues. Phytochem. Bull., 19: 1-15.

Doyle, J.J. and J.L. Doyle, 1990. Isolation of plant DNA from fresh tissue. Focus, 12: 13-15.

Draper, J. and R. Scott, 1988. The Isolation of Plants Nucleic Acids. In: Plant Genetics Transformation and Gene Expression: A Laboratory Manual, Draper, J., R. Scott, P. Armitage and R. Walden (Eds.). Blackwell Scientific Publications, Oxford, pp: 199-236.

Fang, G., S. Hammar and R. Rebecca, 1992. A quick and inexpensive method for removing polysaccharides from plant genomic DNA. BioTechniques, 13 (1): 52-56.

Harborne, J.B., 1963. Ultraviolet Spectroscopy of Polyphenols. In: Methods in Polyphenol Chemistry, Pridham, J.B. (Ed.). Peragamon Press, New York, pp: 13-36.

Hébert, D., J.R. Kikkert, F.D. Smith and B.I. Reisch, 1993. Optimization of biolistic transformation of embryogenic grape cell suspensions. Plant Cell Rep., 12 (10): 585-589.

Howland, D.E., R.P. Oliver and A.J. Davy, 1991. A method of extraction of DNA from birch. Plant Mol. Biol. Rep., 9: 340-344.

Katterman, F.R.H. and V.I. Shattuck, 1983. An effective method of DNA isolation from the mature leaves of *Gossypium* species that contain large amounts of phenolic terpenoids and tannins. Preparative Biochem., 13: 347-359.

Kikkert, J.R., M.J. Striem, J.R. Vidal, P.G. Wallace, J. Barnard and B.I. Reisch, 2005. Long-term study of somatic embryogenesis from anthers and ovaries of twelve grapevine (*Vitis* sp.) genotypes. *In vitro* Cell. Dev. Biol. Plant, 41: 232-239.

Lodhi, M.A., G.N. Ye, N.F. Weeden and B.I. Reisch, 1994. A simple and efficient method for DNA extraction from grapevine cultivars, *Vitis* species and *Ampelopsis*. Plant Mol. Biol. Rep., 12 (1): 6-13.

Lodhi, M.A. and B.I. Reisch, 1995. Nuclear DNA content of *Vitis* species, cultivars and other genera of the *Vitaceae*. Theor. Applied Genet., 90: 11-16.

Maliyakal, E.J., 1992. An efficient method for isolation of RNA and DNA from plants containing polyphenolics. Nucl. Acids Res., 20: 23-81.

Manning, K., 1991. Isolation of nucleic acids from plants by differential solvent precipitation. Anal. Biochem., 195: 45-50.

Mc Govern, P.E., 2003. Ancient Wine: The Search for the Origins of Viniculture Princeton University Press, Princeton.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15: 473-497.

Murray, M.G. and W.F. Thompson, 1980. Rapid isolation of high molecular weight DNA. Nucl. Acids Res., 8: 4321-4325.

Newbury, H.J. and J.V. Possingham, 1977. Factors affecting the extraction of intact ribonucleic acid from plant tissues containing interfering phenolic compounds. Plant Physiol., 60: 543-547.

Porebski, S., L. Grant Bailely and B.R. Boum, 1991. Modification of a CTAB DNA extraction protocol from plants containing high polysaccharide and polyphenol components. Plant Mol. Biol. Rep., 15: 8-15.

Rogers, S.O. and A.J. Bendich, 1985. Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. Plant Mol. Biol., 5: 69-76.

Rogers, S.O., 1994. Phylogenetic and Taxonomic Information from Herbarium and Mummified DNA. In: Conservation of Plant Genes II: Utilization of Ancient and Modern DNA, Adams, R.P., J.S. Miller, E.M. Goldenberg and J.E. Adams (Eds.). Missouri Botanical Garden, St. Louis, MO, pp: 47-67.

Sambrook, J., J. Fritsch and T. Maniatis, 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Press, New York.

Solouki, M., N. Rigi Nazhad, R. Vignani, B.A. Siahsar, H. Kamaladini and A. Emamjomeh, 2007. Polymorphism of some native sistan grapes assessed by long and short primers for RAPD markers. Pak. J. Biol. Sci., 10 (12): 1996-2001.

Southern, E.M., 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol., 98: 503-517.

Stein, D.B., 1993. Isolation and Comparison of Nucleic Acids from Land Plants: Nuclear and Organellar Genes. In: Molecular Evolution Producing the Biochemical Data, Zimmer, E.A., T.J. White, R.L. Cann and A.C. Wilson (Eds.). Methods Enzymol., 224: 153-167.

Troggio, M., G. Malacarne, G. Coppola, C. Segala and D.A. Cartwright *et al*., 2007. A dense single-nucleotide polymorphism-based genetic linkage map of grapevine (*Vitis vinifera* L.) Anchoring pinot noir bacterial artificial chromosome contigs. Genetics, 176: 2637-2650.

Varadarajan, C.S. and C.S. Prakash, 1991. A rapid and efficient method for the extraction of total DNA from the sweet potato and its related species. Plant Mol. Biol. Rep., 9: 6-12.

Wang, H., Q. Meiging and A.J. Cutler, 1993. A simple method of preparing plant samples for PCR. Nucl. Acids Res., 21: 4153-4154.

Wang, Q., P. Li, U. Hanania, N. Sahar and M. Mawassi *et al*., 2005. Improvement of Agrobacterium-mediated transformation efficiency and transgenic plant regeneration of *Vitis vinifera* L. by optimizing selection regimes and utilizing cryopreserved cell suspensions. Plant Sci., 168: 565-571.

Williams, J.G.K., A.R. Kubelik, J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res., 18: 6531-6535.

Zhang, J., M.B. McDonald and P.M. Sweeney, 1997. Testing for genetic purity *Peturia* and *Cyclamon* seed using random amplified polymorphic DNA markers. Am. Soc. Hort. Sci., 32: 246-247.