



Research Article

A simple and efficient protocol for high quality of DNA from *Vitis quadrangularis* L.

Nidhi M. Nakum, Viralkumar B. Mandaliya, Rohan V. Pandya, Vrinda S. Taker

Centre for Advanced Studies in Plant Biotechnology and Genetic Engineering, Department of Biosciences, Saurashtra University, Rajkot – 360 005 (Gujarat), India.

Email: casprogramme@gmail.com

(Received:12 Nov 2010; Accepted:31 Jan 2011)

Abstract:

Vitis quadrangularis L., popularly known as *Hadsankal* in Saurashtra region, exhibits quadrangular and flat stems structure. The present study was aimed to establish efficient protocol for DNA extraction and to evaluate taxonomic status of morphological variants using molecular characterization method RAPD. Three protocols were tested and the final protocol was modified and optimized for high quality DNA. Third protocol was CTAB and SDS based method, developed by adding higher concentration of CTAB and NaCl with an aim to remove polysaccharides, and extra PVP as a phenols and polyphenols remover. It resulted into high quality DNA with average purity of 1.8 and yield 200 µg per gm stem tissue that appropriate for molecular characterization. For molecular marker analysis total 20 decamer primers of OPA series were tested and among them four primers successfully revealed 42.10 % polymorphism. Thus, the results adequately discriminate the morphological variants.

Key words: *Vitis quadrangularis* L., DNA extraction, Molecular Characterization, RAPD

Abbreviations: CI - Chloroform : Iso-amyl alcohol (24:1), CO - Chloroform : Octanol (24:1), PCI - Phenol: Chloroform: Iso-amyl alcohol (25:24:1), PVP - polyvinyl pyrrolidone

Introduction

Vitis quadrangularis is tendril climber with angled or winged stem morphology which mainly varied by quadrangular and flat stem structure. It is popularly known as *Hadsankal* in Saurashtra region (Figure 1). Different plant phenotype is directly and indirectly related with genotype. The variation in morphology and taxonomy of the plant made its identification difficult. In spite of presence of distinct characters for classification and differentiation of these variants, they are not constant and sometimes are ambiguous. It is essential to know the existing differences between morphological variants and to establish genetic similarities.

The conventional methods of plant identification and classification rely mainly on morphological and anatomical features. There are various screening techniques established for plant variety identification such as biochemical and molecular methods. The characterization of plant species by isoenzyme pattern was in practice since long but molecular marker techniques were more reliable and accurate

compared to others (Vural, 2009). With molecular markers, it is theoretically possible to exploit the entire diversity in DNA sequence that exists. Recent developments of marker technology have made great progress towards faster, cheaper, and reliable. There are numerous molecular markers including Restriction Fragment Length Polymorphism (RFLP) (Juskeviciute and Paulauskas, 2003), Random Amplified Polymorphism DNA (RAPD), Simple Sequence Repeats (SSRP), Amplified Fragment Length Polymorphism (AFLP), Arbitrarily Primed PCR (AP-PCR) and DNA Amplified Fingerprinting (DAF) (Almeida et al, 2000).

Among all molecular markers; RAPD is rapid, easy, accurate and economic and used in several phylogenetic studies, varietal identification etc. (Gabrielsen et al, 1997; Tollefsrud et al, 1998). RAPD-PCR is a wide-spread method for genetic fingerprinting of eukaryotes developed by Welsh and McClelland & Williams et al. in 1990 (Cheng et al, 1997). Small sample of the subject's DNA which is then selectively amplified a million fold thus

producing a large amount of concentrated DNAs (PCR products). PCR products can be separated into bands of different molecular weight DNAs by agarose gel electrophoresis. Banding patterns from an individual or population can then be used as a “fingerprint” that may distinguish it from other genetic types and are inherited as Mendelian markers in a dominant fashion and scored as present/absent (Rana and Bhatt, 2005).

This technique is a multilocus which allows obtaining information on the general polymorphism, screening large number of loci and individuals without prior knowledge of genome. It is low expensive, efficient in developing a large number of DNA markers in a short time and required less sophisticated equipment. The technique is simple and has wide applicability that require small amount of DNA without the requirement of cloning, sequencing made it as valuable tool for molecular characterization (Bardakci, 2001; Nuchprayoon et al, 2007).

Basic requirement for molecular identification of any species is good quality and sufficient quantity of DNA. There were several plant DNA isolation and purification protocols described in literature based on various application and needs. These protocols are categorized as per their different mode and approach during cell lyses step during extraction and purification i.e. i) Detergent extraction ii) Enzymatic degradation of cell wall iii) Spin column extraction etc. There were several DNA extraction protocols reported for the family *Vitaceae*; in which majority of them dealt with Grape (*Vitis vinifera* L.) species (Lodhi et al, 1994; Hanania et al, 2004). However, specific DNA extraction protocol for *Vitis quadrangularis* is not reported in the earlier literature.

Vitis quadrangularis have several primary and secondary metabolites. Dry plants contain about 13.1 % moisture content, along with other primary metabolites i.e. proteins 12.8 %, fats and wax 1%, carbohydrates 36.6 %, ash 18.2 %, mucilage and pectin 1.2 % (Kapoor, 2000). Percent distribution of primary metabolites indicates higher levels of protein and carbohydrate present in this species. There are few reports indicating the presence of polysaccharides and polyphenols in *Vitis quadrangularis* (Kapoor, 2000). These primary metabolites (i.e. protein and carbohydrate) are key factors that mainly hinder during DNA extraction. Moreover, the presence of polyphenols, which are powerful oxidizing agents, can reduce the yield and

purity of extracted DNA (Loomis, 1974; Porebski et al, 1997).

Considering the biochemical constituents of *Vitis quadrangularis* and available protocols for other members of *Vitaceae*; three basic DNA extraction protocols were tested and high quality DNA subjected to RAPD analysis. Amongst all three protocols for DNA extraction, Protocol 1 was specific for Grape species that belonged to the same genus whereas, Protocols 2 was an altered protocol with several modification steps derived from Protocol 1. Protocol 3 was specific for removal of higher polysaccharide and polyphenols content and which was tested for same genus. In this 3rd protocol, cationic detergent CTAB and anionic detergent SDS were used to eliminate polysaccharides. PVP helped to purge polyphenol content. The high concentration of NaCl enhances solubility of polysaccharides. The resulted DNA was suitable for molecular characterization like RAPD. The high quality DNA from two *Vitis quadrangularis* morphological variants were examined by RAPD aiming development and establishment of simple and reliable plant genetic marker that conform to the taxonomic status of variants, since no such protocols are available in the literature.

Material and Methods:

Plant Material

Two morphological variants (flat and square stem) of *Vitis quadrangularis* (Figure 1) were grown in Botanical garden, Department of Biosciences, Saurashtra University, Rajkot. Collected plant material was cleaned with running tap water followed by distilled water wash and surface water was blotted dry. Fresh plant material was weighed, crushed with mortal pestle in liquid nitrogen and preserved at -20 °C. Further, fresh plant material was also lyophilized and stored at -20 °C.

DNA Extraction

Total three different DNA extraction protocols were evaluated for the better quality and quantity of DNA and their further application in molecular characterization. In each protocol, the plant material was processed in triplicate for DNA extraction.

Protocol 1

From the reviewed literature very little information regarding biochemical constituents of *Vitis quadrangularis* were found. Present protocol of DNA extraction was reported by (Lodhi et al, 1994) from several Grapes species belonging to the same family of *Vitis quadrangularis*. This protocol was designed

especially to overcome the interfering metabolites such as phenols and polysaccharides. In brief, preserved plant tissue (0.5 g) was crushed using mortar and pestle in 5 ml of extraction buffer [100 mM Tris-HCl (pH 8.0), 20 mM EDTA, 1.4 M NaCl, 2.0 % (w/v) CTAB, 50 mg PVP, 0.2 % β -mercaptoethanol (added fresh)]. Samples were incubated at 60 °C for 25 minutes and allowed to cool down at room temperature. Samples were extracted twice using 6 ml CI by inverting tubes to form emulsion and centrifuged at 3380 xg for 15 minutes at 4 °C. Then, DNA was precipitated by first addition of 0.5 volume of 5 M NaCl and double volume of chilled 95 % ethanol, and incubated at 4 °C for overnight. On the following day, samples were centrifuged at 2350 xg for 10 minutes at 4 °C. Resulted pellet was washed twice with chilled 76% ethanol, air dried and dissolved in 200 μ l TE buffer. RNase treatment were given by addition of 4 μ l RNase stock solution (2 mg/ml) for 15 minutes by incubation at 37 °C, then centrifuged at 3380 xg for 15 minutes at 4 °C and supernatant was preserved at 4 °C.

Protocol 2

This protocol was alteration of the Protocol 1 tested to improve DNA quality. The modified protocol was termed as Protocol 2. In brief, preserved plant tissue (2 g) was crushed using mortar and pestle in 5 ml of extraction buffer [100 mM Tris-HCl (pH 8.0), 20 mM EDTA, 1.4 M NaCl, 2 % (w/v) CTAB, 200 mg PVP, 0.2 % β -mercaptoethanol (added fresh)]. Samples were incubated at 60 °C for 25 minutes and allowed to cool down at room temperature. Samples were extracted first using double volume of PCI and then twice using double volume CI by inverting tubes to form emulsion and centrifuged at 3380 xg for 15 minutes at 4 °C. Then, DNA was precipitated by first addition of 0.5 volume of 5 M NaCl and double volume of chilled 95 % ethanol, and incubated at 4 °C for overnight. On the following day, samples were centrifuged at 2350 xg for 10 minutes at 4 °C. Resulted pellet was washed twice with chilled 76% ethanol, air dried and dissolved in 200 μ l TE buffer. RNase treatment was given by addition of 4 μ l RNase stock solution (2 mg/ml) for 15 minutes by incubation at 37 °C, then centrifuged at 3380 xg for 15 minutes at 4 °C and supernatant was preserved at 4 °C.

Protocol 3

Higher polysaccharide and polyphenol content are major factors hindering DNA isolation process and that requires special measures. This protocol was prescribed for isolation of DNA from Sugarcane

tissue dealing with same challenges (Aljanabi et al, 1999). Several modifications were made on the basis of earlier experimental work. In brief, lyophilized plant tissue (0.5 g) was crushed using mortar and pestle in 5 ml of extraction buffer [100 mM Tris base (pH 8.0), 20 mM EDTA, 1.4 M NaCl, 1.75 gm PVP, 0.2 % β -mercaptoethanol (added fresh)]. Samples were incubated at 60 °C for 30 minutes and allowed to cool down at room temperature. Samples were centrifuged at 13520 xg for 15 minutes at 4 °C. Supernatant was mixed with 2 ml each of warm 20 % CTAB and 5% SDS, 0.5 gm PVP and 5 μ l of β -mercaptoethanol. These samples were incubated for 1 h at 70 °C water bath and centrifuged at 13520 xg for 15 minutes at 4 °C. The aqueous phase were extracted first using double volume of PCI and then twice using double volume CI by inverting tubes to form emulsion and centrifuged at 13520 xg for 15 minutes at 4 °C. Then, DNA was precipitated by first addition of 0.5 volume of 5 M NaCl and equal volume of chilled IPA, and incubated at 4 °C for overnight. On the following day, samples were centrifuged at 13520 xg for 15 minutes at 4 °C. Resulted pellet was washed twice with chilled 76% ethanol and 3 M Sodium acetate, air dried and dissolved in 200 μ l TE buffer. RNase treatment was given by addition of 4 μ l RNase stock solution (2 mg/ml) for 1 hr at room temperature and 30 minutes at 4 °C, then centrifuged at 21130 xg for 15 minutes at 4 °C and supernatant was proceeded for the proteinase K treatment same as RNase treatment.

DNA Quantification

The DNA concentration was determined at 260 nm, and purity was determined by calculating the ratio of absorbance at 260 nm to that of 280 nm (Matasyoh et al, 2008) using the β Quant microplate reader, Bio-Tek instruments incorporation, USA. The fragmentation state and RNA contamination of the extracted DNA were evaluated by agarose gel electrophoresis.

RAPD-PCR

Twenty random decamer primers of OPA series (OPA-01 to OPA-20) were tested for PCR amplification. Primers were purchased from Operon Biotechnologies GmbH, Germany. All primers were primarily screened for *Vitis quadrangularis* square variant. Four primers were finalized for RAPD analysis of both variants according to resulted amplification profile on gel electrophoresis. RAPD analysis of *Vitis quadrangularis* was performed according to Mandaliya et al (2010). Each reaction mixture consists of total 25 μ L. PCR amplification

was carried out in 3 stages in a Veriti (96 Well Fast Thermal Cycler), Applied Biosystems, USA.

Agarose gel electrophoresis

Electrophoresis was carried out on 2 % agarose gel at constant voltage of 50 V in 1x TAE buffer. 100 bp DNA ladder and Lambda DNA EcoRI+HindIII double digest marker, both from Bangalore Genei, India, were used as molecular size standards.

Data analysis

The RAPD products were scored according to Vafaie-Tabar et al, 2004 for each primer-hybrid combination and % polymorphism was calculated.

Results and Discussion:

DNA extraction

From available review of literature DNA extraction protocol for *Vitis quadrangularis* was not recorded, so this work was initiated to develop simple and efficient protocol of DNA extraction from this species. The protocol (Protocol 1) was reported to isolate DNA from different Vitis species such as *Vitis acerifolia*, *Vitis berlandieri*, *Vitis cinerea*, *Vitis labrusca*, *Vitis rupestris*, *Vitis vinifera* cv. cabernet sauvignon (Lodhi et al, 1994). This protocol was performed with minute variation at low molecular protein removal step where CI was used instead of CO. Protocol 1 gave the purity of DNA about 1.32 to 1.46 and concentration of 44 to 100 $\mu\text{g/gm}$ (Table 1). There was no DNA band observed on agarose gel which indicates the poor quality of DNA. The protocol was further repeated several times but the result was same. Probable reason of poor results was the original protocol was designed for DNA extraction from leaf source while in this protocol the plant material used was stem part.

On the bases of results from the earlier protocol, a second protocol (Protocol 2) was tested. Protocol 2 was modified method from the Protocol 1. Protocol 2 yielded low amount (0.5 to 7.5 $\mu\text{g/gm}$) of DNA with average purity of 1.3 (Table 1). The probable reason for decrease in DNA yield could be the repeated washing treatment of samples using PCI. Though PCI steps were added to enhance purity, but the purity ratio was 1.3. It suggested no effect of PCI purification steps which might be because of co-isolation of polysaccharides present in the tissue (Fang et al, 1992). No DNA bands were observed while performing the agarose gel electrophoresis which indicated a need to develop a high quality DNA extraction protocol for this species.

Based on the earlier results third protocol was tested, which deals with effective removal of polysaccharides and polyphenol constituents. The Protocol 3 resulted in high yield of DNA (100 to 200 $\mu\text{g/gm}$) with highest purity ratio (1.8) among all tested protocols (Table 1). The use of lyophilized plant material has certain advantages. The method uses lyophilized plant tissue, permitting storage of field collections and eliminating the need for fresh material (Guinn, 1966; Stein and Thompson, 1978). The cationic detergent CTAB and anionic detergent SDS were used in this protocol that enhanced DNA yield by providing effective cell lysis (Sambrook et al, 2001). Furthermore, according to Aljanabi et al (1999) CTAB in high concentration was used to eliminate polysaccharides. PVP used to eliminate polyphenols during DNA purification (Lodhi et al, 1994). PVP forms complex hydrogen bonds with polyphenolic compounds which can be separated from DNA by enhanced centrifugation (Maliyakal, 1992). Application of high concentration of NaCl in this protocol enhances solubility of polysaccharides in ethanol and helps in effective removal of contaminants (Fang et al, 1992). In this protocol, the integrity of DNA conformed by agarose gel electrophoresis (Figure 2) that proved the successful product outcome.

In brief, the balance combination of lyophilization, application of both detergents (CTAB and SDS), higher amount of PVP and NaCl in Protocol 3 helped effectively in removing contaminant from DNA samples during extraction from *Vitis quadrangularis* and made it high quality that was suitable for molecular characterization studies.

RAPD Analysis

RAPD is rapid, easy, accurate and economic technique used in phylogenetic, varietal identification studies (Gabrielsen et al, 1997; Tollefsrud et al, 1998). Application of OPA primers for discrimination of individuals of *Vitaceae* family was previously reported (Aras et al. 2005) where total nine primers had successfully generated the amplification profile. In the present study, the genetic relationships between two morphological variants of *Vitis quadrangularis* (square and flat stem) were studied by RAPD.

Total 20 decamer primers of OPA series (OPA-01 to OPA-20) were tested to discriminate genetic similarity and variation among both. Among them total four different arbitrarily decamer primers (OPA-03, OPA-04, OPA-09 and OPA-10) were revealed 42.10 % polymorphism. The primer OPA-09 showed

the highest 80 % polymorphism, with 1 monomorphic and 4 polymorphic band in the molecular weight range from 0.5 kb to 1.2 kb (Figure 3). Highest total 6 bands resulted with OPA-03 amplification, where total number of monomorphic bands was 5 and 1 were polymorphic with 16.67 % polymorphism (Table 2). The OPA-03 also showed the bands to molecular weight range of 0.25 kb to 1.4 kb (Figure 3). Maximum versatile range of molecular weight bands were observed with OPA-10 in range of 0.8 kb to 5.0 kb (Figure 3), where total 2 monomorphic and 1 polymorphic bands were recorded with 33.33 % polymorphism. OPA-04 has showed the total 3 monomorphic and 2 polymorphic band in 0.5 kb to 1.3 kb size range (Figure 3) with 40 % polymorphism (Table 2).

The results establish RAPD as a useful predictive tool to identify plant individual and their levels of genetic variability. Genetic polymorphism detected with RAPD reveals one allele per locus which corresponds to the amplification product visualized (Figure 3). Accordingly, the number of RAPD polymorphism identified in this study may be sufficient to permit an estimation of genetic diversity and similarity among both tested morphological variants (Table 2). The present molecular characterization work upon *Hadsankal* (*Vitis quadrangularis*) was the first time to be performed and there were no RAPD-PCR studies found in the literature using these species.

There could be moderate genetic variation among the both tested morphological variants for component traits and their combination could be the reason for moderate level of polymorphism. However, the results adequately discriminate the two tested *Vitis quadrangularis* morphological variants. For further DNA polymorphism it is necessary to add more number of primers.

Acknowledgments:

The authors would like to thank the State Government of Gujarat for financial support for Center for Advanced Studies in Plant Biotechnology and Genetic Engineering (CPBGE) programme, and University Grant Commission (UGC), New Delhi for financial support as a meritorious scholarship.

References:

Aljanabi, S.M., Forget, L. and Dookun, A. 1999. An Improved and Rapid Protocol for the Isolation of Polysaccharide- and Polyphenol-Free Sugarcane DNA. *Plant Mol. Biol. Rep.*, **17** : 1-8.
Almeida, F.C., Moreira, M.A.M., Bonvicino, C.R. and Cerquerira, R. 2000. RAPD analysis of *Nectomys*

squamipes (Rodentia, Sigmodontinae) populations. *Gene Mol. Biol.*, **23(4)** : 793-797.
Bardakci, F. 2001. Random amplified polymorphic DNA (RAPD) markers. *Turk J. Biol.*, **25** : 185-196.
Cheng, K., Chang, H., Su, C. and Hsu, F. 1997. Identification of dried rhizomes of *Coptis* species using random polymorphic DNA. *Bot. Bull. Acad. Sin.*, **38** : 241-244.
Fang, G., Hammar, S. and Rebecca R. 1992. A quick and inexpensive method for removing polysaccharides from plant genomic DNA. *BioTechniques*, **13** : 52-56.
Gabrielsen, T. M., Bachmann, K., Jakobsen, K. S. and Brochmann C. 1997. Glacial survival does not matter: RAPD phylogeography of Nordic *Saxifraga oppositifolia*. *Mol. Ecol.*, **6** : 831-842.
Guinn, G. 1966. Extraction of Nucleic Acids from Lyophilized Plant Material. *Plant Physiol.*, **41** : 689-695.
Hanania, U., Velcheva, M., Sahar, N. and Perl, A. 2004. An Improved Method for Isolating High-Quality DNA From *Vitis vinifera* Nuclei. *Plant Mol. Biol. Rep.*, **22** : 173-177.
Juskeviciute, E. and Paulauskas, A. 2003. Application of fragment length polymorphism analysis for identification of small rodents. *Ekologija*, **1** : 21-23.
Kapoor, L.D. 2000. In: Hand book of Ayurvedic medicinal plants CRC Press, Boca Raton, Florida.
Lodhi, M.A., Ye, G.N., Weeden, N.F. and Reisch, B.I. 1994. A simple and efficient method DNA extraction from grapevine cultivars and vitis species. *Plant Mol. Biol. Rep.*, **12** : 6-13.
Loomis, M.D. 1974. Overcoming problems of phenolics and quinones in the isolation of plant enzymes and organelles. *Methods Enzymol.*, **31** : 528-544.
Maliyakal, E.J. 1992. An efficient method for isolation of RNA and DNA from plants containing polyphenolics. *Nucleic Acids Res.*, **20** : 2381.
Mandaliya, V.B., Pandya R.V. and Thaker, V.S. 2010. Genetic diversity analysis of Cotton (*Gossypium*) hybrids using RAPD markers. *J. Cotton Res. Dev.*, **24(2)** : 127-132.
Matasyoh, L.G., Wachira, F.N., Kinyua, M.G., Muigai, A.W.T. and Mukiyama, T.K. 2008. Leaf storage conditions and genomic DNA isolation efficiency in *Ocimum gratissimum* L from Kenya. *African J. Biotech.*, **7(5)** : 557-564.
Nuchprayoon, S., Junpee, A. and Poovorawan, Y. 2007. Random Amplified Polymorphic DNA (RAPD) for differentiation between Thai and Myanmar strains of *Wuchereria bancrofti*. *Filaria J.*, **6** : 6.
Porebski, S., Bailey, L.G. and Baum, B.R. 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Mol. Biol. Repr.*, **15** : 8-15.
Rana, M.K. and Bhat, K.V. 2005. RAPD markers for genetic diversity study among Indian cotton cultivars. *Current Science*, **88(12)** : 1956-1961.



- Sambrook, J., MacCallum, P. Russell, D. 2001. In: Molecular Cloning: A Laboratory Manual (Third Edition) CSHL press.
- Stein, D. B. and Thompson, W. F. 1978. Isolation of DNA from tannin-containing plants. *Plant Science Letters*, **11(4)** : 323-328.
- Tollefsrud, M.M., Bachmann, K., Jakobsen, K. S. and Brochmann, C. 1998. Glacial survival does not matter—II: RAPD phylogeography of Nordic *Saxifraga cespitosa*. *Mol. Ecol.*, **7** : 1217–1232.
- Vafaie-Tabar, M., Chandrashekar, S., Rana, M.K. and Bhat, K.V. 2004. RAPD Analysis of Genetic Diversity in Indian Tetraploid and Diploid Cotton (*Gossypium* spp). *J. Plant Biochem. Biotech.*, **13** : 81-84.
- Vural, H.C. 2009. Genomic DNA isolation from aromatic and medicinal plants growing in Turkey. *Scientific Research and Essay*, **4(2)** : 059-064.

Table 1. Comparison of different DNA isolation protocols

Protocol	Source Tissue	DNA Yield ($\mu\text{g/gm}$)	DNA Purity (260/280)
Protocol 1	Fresh stem	44-100	1.39
Protocol 2	Fresh stem	0.5-7.5	1.30
Protocol 3	Lyophilized stem	100-200	1.8

Table 2. RAPD primes and degree of polymorphism

Primer	Primer Sequence (5' to 3')	TNB		TMB	TPB	% Polymorphism
		Square	Flat			
OPA-03	AGTCAGCCAC	6	5	5	1	16.67
OPA-04	AATCGGGCTG	5	3	3	2	40
OPA-09	GGGTAACGCC	1	5	1	4	80
OPA-10	GTGATCGCAG	3	2	2	1	33.33

[TNB - Total No. of Bands, TMB - Total No. of Monomorphic Bands, TPB - Total No. of Polymorphic Bands]



Square Stem



Flat Stem

Figure 1. Two morphological variants (square and flat stem) of *Vitis quadrangularis* (Hadsankal).

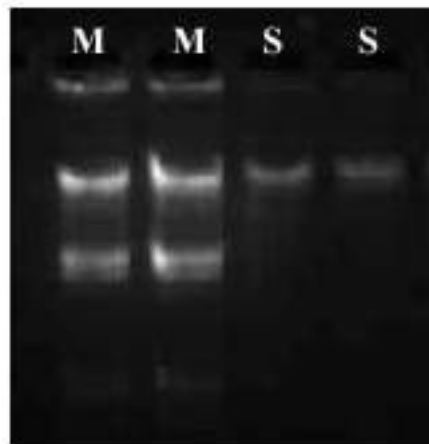


Figure 2. Agarose gel electrophoresis of *Vitis quadrangularis* genomic DNA with Protocol 3 [M: Marker, S: Sample genomic DNA].

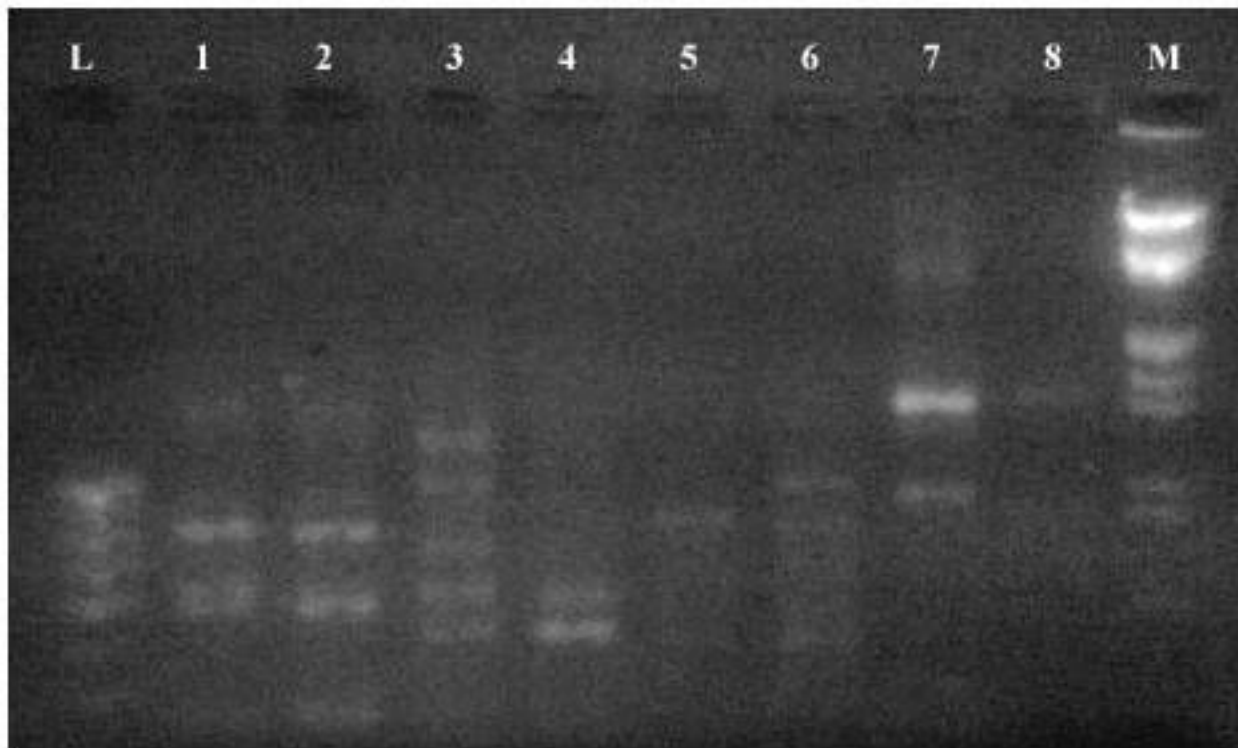


Figure 3. RAPD amplification profile of *Vitis quadrangularis*. The odd number given to square while even number to flat stem variants of *Vitis quadrangularis*. [L - 100 bp DNA ladder, M - Lambda DNA EcoRI+HindIII double digest DNA marker, 1 & 2 - OPA 03, 3 & 4 - OPA 04, 5 & 6 - OPA 09, 7 & 8 - OPA 10 amplification profile].