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FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

Comparison of six genomic DNA extraction methods for molecular downstream applications of apple tree (*Malus X domestica*)

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Abstract: Extraction of high quality DNA is crucial for any molecular genetic analysis. However, it is difficult to be obtained from problematic plant tissue, high in phenolic compounds, such as apple leaves. Despite the variety of commercially available kits for DNA isolation, no study has been done so far evaluating their potential for apple tree. We have tested six different kits and compared their performance on five to ten samples of apple tree (*Malus X domestica*) leaves. Genomic DNA was extracted following manufacturers' protocols and amplified by touchdown PCR using 12 different SSR markers. The quality of DNA and PCR products was proven on agarose gel; additionally, DNA concentrations were measured using fluorimeter. Results showed high level of variation for concentrations and DNA purities; the highest yield (more than 512 ng/µL) was obtained with E.Z.N.A. SP Plant DNA Kit (Omega bio-tek), although DNA was not absolutely pure. The highest DNA sample purity was obtained using the DNeasy Plant Pro Kit (Qiagen); however, it resulted in the lowest DNA concentration (13 ng/µL). Despite big differences in DNA yields, all kits performed well for further PCR amplification. We conclude that choosing suitable method for DNA extraction of the

ABOUT THE AUTHOR

Our research group is active in the fields of environmental genetics, trait-associated molecular marker studies, plant breeding, new NGS based applications and molecular biology studies in general. The research team belongs to the Genetics laboratory at Agricultural institute of Slovenia, led by Barbara Pipan, the corresponding author of this study. Group collaborates with common bean, potato and buckwheat breeding programmes and facilitates application of molecular markers to the breeding processes and as well performs genetic diversity and population genetics studies of agronomically important plant species. There are different kinds of tissues from agronomically important plants (common bean, potato, buckwheat, wheat, grapevine, brassicas, blueberries, sweet potato, ambrosia, clovers, dandelion…) analysed in our laboratory. Therefore, there is a need to have well optimised and effective DNA extraction methods with good yields of pure DNA for further applications. This study supplied basic information related to quick and effective plant DNA extraction methods.

PUBLIC INTEREST STATEMENT

The process of DNA isolation represents a basic step for genetic research since all of the molecular analyses require quality DNA. In our research, we have often met with problematic samples that require additional optimisation of protocols and methods. Plant samples, for instance, contain high concentrations of organic compounds that can hinder further analysis. Many manufacturers are producing a number of commercial kits addressing problematic samples. The goal of our study was to test six different DNA extraction kits from four different manufacturers using apple tree leaves. We have compared the quality of isolated DNA, its purity and performance in the polymerase chain reaction. We have observed different concentrations and DNA purity between kits used, although no correlation was observed. All kits resulted in a sufficient DNA quality for successful polymerase chain reaction. We conclude that commercial kits differ in produced yield and purity of DNA; therefore, optimisation for specific plant species is required beforehand.

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particular sample plays a big role for the quality of DNA and its downstream applications. Extraction with DNeasy Plant Pro Kit (Qiagen) was the most efficient, as it resulted in the purest DNA. Despite its relatively low DNA yield, concentrations were still high enough for further PCR amplification. Obtained results indicate the optimal DNA extraction method used for problematic plant species in molecular studies.

Subjects: Environment & Agriculture; Bioscience; Food Science & Technology

Keywords: Malus X domestica; DNA extraction; PCR amplification; simple sequence repeat primers; problematic plant tissues

1. Introduction

Apple (*Malus X domestica* Brokh.) with its numerous varieties is one of the most widely cultivated fruit species worldwide (Kikuchi, Kasajima, & Morita et al., 2017). Since molecular marker technology is evolving into a more and more valuable tool for creation of new plant cultivars (Dayteg, Tuvesson, Merker, Jahoor, & Kolodinska-Brantestam, 2007), it is important to provide good quality, high-yield DNA and a consistent methods for its extraction. The reliable DNA sample is a basis for further molecular genetic analyses (Abdel Latif & Osman, 2017), for instance, PCR and real-time PCR analysis, Southern blotting, restriction enzyme digestion and other genotyping procedures.

A number of commercial kits for DNA extraction are available in the market nowadays, differing in isolation technology, sample type and amount; time needed per run, elution volume, DNA yield and potential downstream applications. Most commonly these kits are based on solid-phase nucleic acid purification (Tan & Yiap, 2018) and performed by using a spin column, operated under centrifugal force (Gjerse, Hoang, & Hornby, 2009). That results in a fast and efficient DNA purification in comparison to the conventional methods, such as CTAB or SDS method (Tan & Yiap, 2018).

However, plant samples usually contain high amounts of secondary metabolites whose content varies between species. Different commercial kits or DNA extraction methods will thus give different results when used with different plant species or tissues for further SSR applications (Derlink et al., 2014; Maras, Pipan, & Šuštar Vozlič et al., 2015; Pipan, Šuštar Vozlič, & Meglič, 2013; Pipan, Žnidarčič, & Kunstelj et al., 2017; Pipan, Žnidarčič, & Meglič, 2017; Rusjan, Pelengić, & Pipan et al., 2015; Sinkovič, Pipan, & Meglič et al., 2017); therefore, the extraction methods need to be optimised for each material to ensure the best possible outcome (Sahu, Thangaraj, & Kathiresan, 2012). Apple leaves contain various polyphenolic compounds, such as flavonoids (Mikulic Petkovsek, Slatnar, Stampar, & Veberic, 2010), phenolic acids and hydroxycinnamic acids (Liaudanskas, Viškelis, & Raudonis et al., 2014; Mikulic Petkovsek et al., 2010). Phenolic compounds bind irreversibly to nucleic acids, making them resistant to different modifying enzymes (Manoj, Tushar, & Sushama, 2007). This can lead to DNA degradation, contamination and low yield (Azmat, Khan, & Cheema et al., 2012) and therefore interfere with its use in various types of analyses (Souza, Muller, & Brandão et al., 2012).

Despite the abundance of commercial kits available in the market, no thorough study has been performed so far focusing on optimising DNA extraction protocol for apple tree (*Malus X domestica*). The aim of this study was to evaluate six comparable and commercially available kits for DNA extraction from problematic apple tree tissue based on DNA yield and suitability of extracted DNA for further molecular applications.

2. Materials and methods

2.1. Plant material

DNA was extracted from young frozen (−20°C) leaves of apple tree (*Malus X domestica*). The amount of tissue used was as stated in instructions for a particular kit (40–90 mg). Five to eight samples from the same accession were used for DNA isolation.

2.2. DNA isolation kits

DNA isolation was performed using six comparable genomic DNA isolation kits for plant tissues from four different manufacturers (Table 1). All kits use spin columns operated under centrifugal force.

2.3. Homogenisation

Homogenisation was performed using Retsch TissueLyser (Qiagen). For kits 1–5, Mixer Mill MM 400 Adapter Set 2 × 5 (Retsch) was used. Two stainless steel beads, lysis buffer, RNase and proteinase (if included in the kit) were added to the starting material before grinding. Samples were mixed with vortex and homogenised at frequency 30 /s for 5 min. When material was not completely homogenised above step was repeated two (kit 3) or three times (kit 1). For kit 6, homogenisation was performed using TissueLyser Adapter Set 2 × 24 (Qiagen) as described by the kit protocol. Prior to homogenisation, 450 µL of Solution CD1 and 50 µL of Solution PS were added as suggested for samples high in phenolic compounds.

2.4. DNA extraction

DNA extraction was performed following manufacturers' instructions for each kit. If not stated differently, volume of elution buffer used was 70 µL. For Kit 1, samples were incubated for 5 min after adding elution buffer. For Kit 3, duration of starting incubation at 65°C was increased to 30 min; five samples were treated with Lysis Buffer PL1 and five samples with Lysis Buffer PL2. For Kit 4, starting incubation at 65°C was 50 min long. Lysozyme and RNase A (marked as optional in the protocol and not included in the kit) were not added. Elution step was repeated two times, each time with 70 µL of elution buffer. For Kit 5, Buffer P3 was added before homogenisation due to analyst's mistake. For Kit 6, 250 µL of Solution CD2 was added, following the recommendations for problematic samples.

2.5. PCR amplification

Extracted DNA samples were used as a template for amplification by 12 different species-specific SSR markers (Gianfranceschi, Seglias, Tarchini, Komjanc, & Gessler, 1998; Guilford et al., 1997; Hokanson, Szewc-McFadden, & Lamboy et al., 1998, Liebhard et al., 2002), listed in Table 2. PCR reaction mixture was prepared as described by Pipan et al. (2017, 2017), containing 1 µL of template DNA. The forward primer of each SSR marker was appended with 18 bp tail sequence 5′-TGTAAAACGACGGCCAGT-3′ (M13(−21) as described by Schuelke (2000). Additionally, two non-template controls (N) were included. Amplification reactions were carried out using Veriti™ 96-Well Thermal Cycler (Applied Biosystems) and SureCycler 8800 Thermal Cycler (Agilent

Table 1. Commercial kits and samples used for DNA extraction from *Malus X domestica*. Kit 3 includes two different lysis buffers and both of them were tested, each with five samples

Kit no.	Commercial name	Manufacturer	Amount of starting material [mg]	Number of samples
1	E.Z.N.A. SP Plant DNA Kit	Omega Bio-tek	80–90	5
2	E.Z.N.A. Plant DNA DS Mini Kit	Omega Bio-tek	40–50	5
3a	NucleoSpin Plant II —Lysis Buffer PL1	Macherey-Nagel	80	5
3b	NucleoSpin Plant II —Lysis Buffer PL2	Macherey-Nagel	80	5
4	Invisorb Spin Plant Mini Kit	Stratec Biomedical AG	80	5
5	DNeasy Plant Mini Kit	Qiagen	80	5
6	DNeasy Plant Pro Kit	Qiagen	50	8

Table 2. SSR primers used for PCR amplification of DNA extracted from *Malus X domestica* (Gianfranceschi et al., 1998; Guilford et al., 1997; Hokanson et al., 1998; Liebhard et al., 2002). PCR protocols: 1—Pipan et al. (2013), 2—Pipan et al. (2017)

Marker name	Forward sequence	Reverse sequence	Expected product length [bp]	Reference of sequence	PCR protocol
02b1	ccg tga tga caa agt gca tga	atg agt ttg atg ccc ttg ga	238	Guilford et al. (1997)	1
05g8	cgg cca tcg att atc tta ctc tt	gga tca atg cac tga aat aaa cg	121	Guilford et al. (1997)	2
GD96	cgg cgg aaa gca atc acc t	gcc agc cct cta tgg ttc cag a	152–197	Hokanson et al., 1998)	1
GD147	gcc agc cct cta tgg ttc cag a	aaa ccg ctg ctg ctg aac	124–156	Hokanson et al. (1998)	2
CH01h01	gaa aga ctt gca gtg gga gc	gga gtg ggt ttg aga agg tt	114–134	Gianfranceschi et al. (1998)	2
CH01c06	ttc ccc atc atc gat ctc tc	aaa ctg aag cca tga ggg c	146–188	Liebhard et al. (2002)	1
CH01d09	gcc atc tga aca gaa tgt gc	ccc ttc att cac att tcc ag	134–172	Liebhard et al. (2002)	1
CH01f12	ctc ctc caa gct tca acc ac	gca aaa acc aca aca ggc ata ac	145–162	Liebhard et al. (2002)	1
CH02b10	caa gga aat cat caa gac aa	caa gtg gct tcg gat agt tg	121–159	Gianfranceschi et al. (1998)	1
CH02c02a	ctt caa gtt cag cat caa gac aa	tag ggc aca ctt gct ggt c	129–176	Liebhard et al. (2002)	1
CH03g04	atg tcc aat gta gac acg caa c	ttg aag atg gcc taa cct tgt t	122–144	Liebhard et al. (2002)	1
CH03g07	aat aag cat tca aag caa tcc g	ttt ttc caa atc gag ttt cgt t	119–181	Liebhard et al. (2002)	1

Technologies) with two touchdown PCR protocols; protocol 1 as described by Pipan et al. (2013) and protocol 2 as described by Pipan et al. (2017, 2017).

2.6. Visualisation and DNA quantification

Quality of DNA bands was checked on agarose gel with 0.5X Tris-borate-EDTA (TBE), stained with ethidium bromide. Obtained DNA samples were run on 2% agarose gel at 100V for 45 min. 5–6 µL of each sample and 3–5 µL of loading buffer (XC+BB, Thermo Scientific) were used alongside with GeneRuler 1 kb DNA Ladder (Fermentas). PCR products were separated on 1.4% agarose gel at 90V for 90 min, samples containing 4 µL of each product and 6 µL of loading buffer (XC+BB, Thermo Scientific). Size of products was assessed using GeneRuler 100 bp DNA Ladder (Thermo Scientific). Gels were visualised under UV light using GeneGenius Gel Imaging System (Syngene). DNA concentrations of extracted samples were measured using Qubit 3.0 fluorometer with dsDNA Broad Range Assay Kit (Thermo Scientific).

3. Results and discussion

The goal of our study was to evaluate and compare different commercially available kits for DNA extraction from apple plant tissue (*Malus X domestica*), which can be difficult to isolate due to its high levels of phenolic compounds. All of the kits used in the study (Table 1) are based on silica membranes, combined with spin column technology. They contain six to eight buffers and reagents with comparable functions during the extraction; cell lysis, DNA binding, washing of the membrane and DNA elution. They do, however, differ in the presence of RNase (not included in Kit 4) and proteinase (included only in Kits 2 and 4). Regarding convenience of the protocols used, it is worth mentioning that Kits 3 and 4 require

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transferring all of the homogenised material into a column at the beginning of the extraction. This step is rather time consuming yielding thick lysate, difficult to transfer without losing some of the material.

According to the visual appearance of obtained DNA solutions, all impurities were successfully removed; solutions transparent without any yellowish or dark contaminants indicating the absence of phenolic compounds (Souza et al., 2012). Electrophoresis of DNA samples showed significant differences in their purity and levels of degradation (Figure 1). Some of the obtained DNA exhibited very high purity (Kit 6), while presence of smears in others indicated presence of short genetic material fragments (Kits 1 and 4). This could be caused by either degradation of DNA or by the presence of RNA fragments due to the insufficient RNase activity or its absence in the kit. Observed smearing in the samples extracted using Kit 2 and 3, was probably a consequence of a very high DNA concentration.

The measurements of DNA concentrations ranged from 0.9 ng/µL (Kit 5) to over 1000 ng/µL (Kit 1 and 2) (Table 3). The highest concentration on average (more than 512.9 ng/µL) was observed for samples extracted using Kit 1, whereas Kit 6 yielded the lowest average concentrations (13 ng/µL). Overall, the highest measured yield was more than 1000 times higher than the lowest one, proving differences between protocols to be really significant.

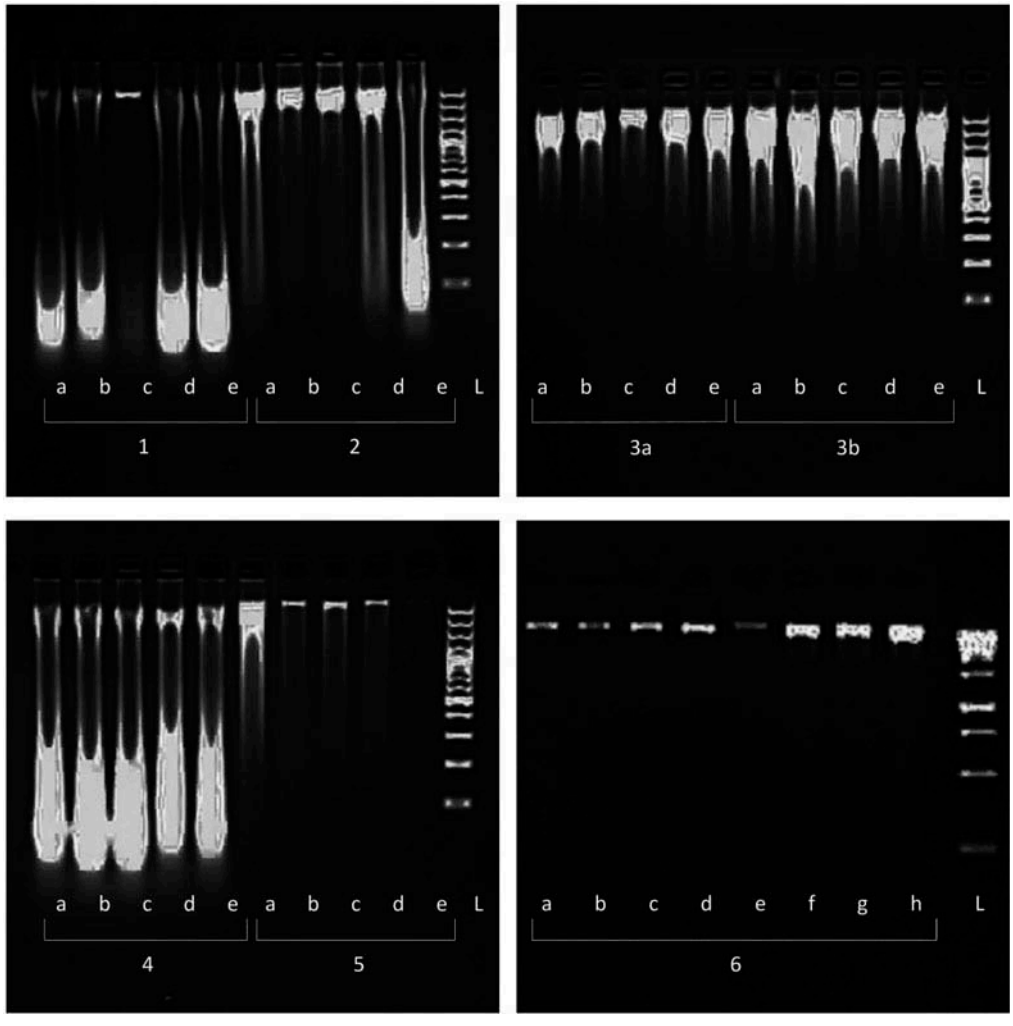

Figure 1. Electrophoresis of total genomic DNA of *Malus X domestica* on 2% agarose gel.

DNA was extracted with the following commercial kits: 1—E.Z.N.A. SP Plant DNA Kit (Omega Bio-tek), 2—E.Z.N.A. Plant DNA DS Mini Kit (Omega Bio-tek), 3—NucleoSpin Plant II (Macherey-Nagel); 3a—Lysis Buffer PL1, 3b—Lysis Buffer PL2, 4—Invisorb Spin Plant Mini Kit (Stratec Biomedical AG), 5—DNeasy Plant Mini Kit (Qiagen), 6—DNeasy Plant Pro Kit (Qiagen). Lines a–h—DNA samples, line L—1 kb DNA Ladder.

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Table 3. DNA concentrations and PCR amplification ratios from samples of *Malus X domestica*, extracted with different commercial kits. 1—E.Z.N.A. SP plant DNA kit (Omega Bio-tek), 2—E.Z.N.A. Plant DNA DS mini kit (Omega Bio-tek), 3—NucleoSpin plant II (Macherey-Nagel); 3a—lysis buffer PL1, 3b—lysis buffer PL2, 4—Invisorb spin plant mini kit (Stratec Biomedical AG), 5—DNeasy plant mini kit (Qiagen), 6—DNeasy plant pro kit (Qiagen). Concentrations were measured on Qubit 3.0 fluorometer with dsDNA broad range assay Kit (Thermo Scientific) with a range from 2 to 1000 ng/µL, therefore concentrations exceeding this were not precisely determined. Amplification ratio was calculated by dividing the number of samples, successfully amplified during reaction, with number of all samples, taking into account 12 SSR markers used in the study. Average DNA concentration and amplification ratio is based on five (kits 1–5) or eight samples (kit 6)

	DNA concentration [ng/µL]			
DNA isolation kit	**Min**	**Max**	**Average**	**Amplification ratio**
1	10.7	>1000	>512.9	47%
2	92.8	>1000	>503.2	88%
3a	19.2	81.2	61.3	98%
3b	95.6	232.0	138.7	95%
4	48.0	74.4	63.4	95%
5	0.9	75.6	19.5	78%
6	10.7	>1000	13.0	92%

Agarose electrophoresis of PCR products is depicted in Figure 2. For evaluation of PCR efficiency, amplification ratio (Table 3) was calculated by dividing the number of successfully amplified samples with total number of samples. The amplification ratio for Kits 2–6 ranged between 78% and 98%, while amplification ratio for Kit 1 was 47%. There was no clear correlation between DNA concentration, purity and performance of polymerase chain reaction. Generally, the highest amplification ratio was achieved with Kits 3 and 4 with a relatively high DNA concentration (Table 3). On the other hand Kit 1 had the highest average concentration but the lowest amplification ratio. This might be caused by a too high amount of DNA template, which could consequently increase the amount of potential PCR inhibitors, or by higher DNA fragmentation extracted using Kit 1 (Figure 1). However, Kit 4 showed even higher levels of fragmentation combined with lower DNA concentration when compared to Kit 1, and yet amplification was in many cases more efficient with Kit 4 than with Kit 1. There are many factors apart from quality and amount of DNA template we should consider, when evaluating PCR efficiency, such as pcontamination of reaction mixture, efficiency of a thermal cycler, optimisation of temperature profiles, quality of reagents and suitability of primers (Degen, Deufel, & Eisel et al., 2006). The DNA yield from all four kits was sufficient to perform a successful PCR amplification despite big differences in concentrations and purity.

Ideally, a DNA isolation protocol should be quick, efficient, safe and easy to perform, and yielding sufficient levels of high-quality DNA suitable for application in molecular analysis (Biteau et al., 2012). Conventional methods like CTAB are often time consuming and require use of toxic substances (Karaaslan, Akel, & Ünlü et al., 2014). Compared to those, commercial DNA isolation kits have advantages of limited and smaller amounts of chemical use, practical methodology, shorter isolation steps and faster achievement of results (Akkurt, 2012). On the other hand, costs of commercial kits are high and in a few studies DNA yields were found to be lower than those obtained with conventional methods (Akkurt, 2012; Sousa, Gomes, & Lopes et al., 2014; Stefanova, Taseva, Georgieva, Gotcheva, & Angelov, 2013).

4. Conclusions

In our comparative study using apple tree leaves for DNA extraction, all four tested kits gave sufficient quality and quantity of DNA to be used for further SSR analyses. DNeasy Plant Pro Kit (Qiagen) produced the purest product and had the lowest level of DNA degradation and contamination. However, to perform PCR only, relatively small quantities of DNA are required. For other

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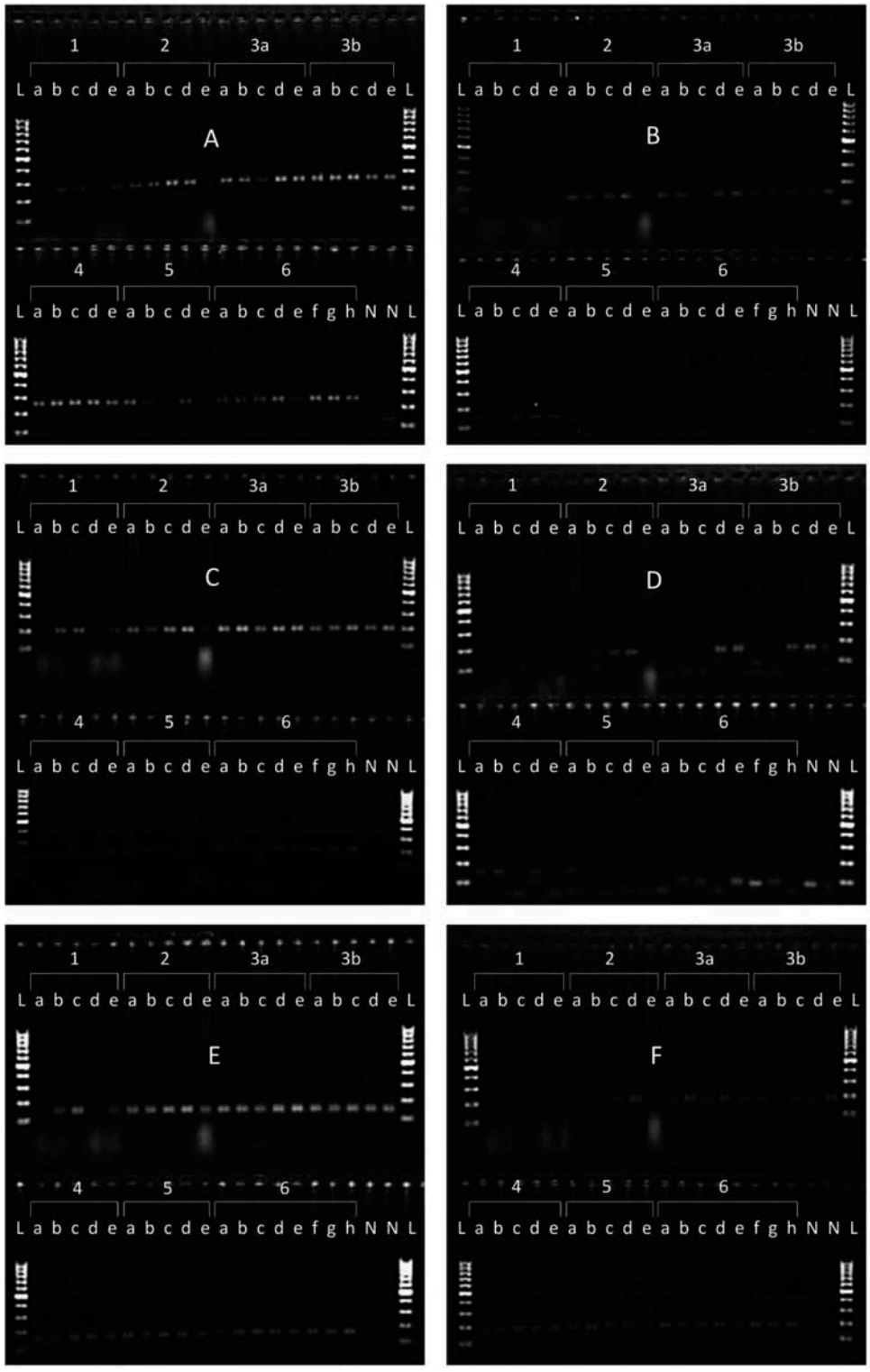
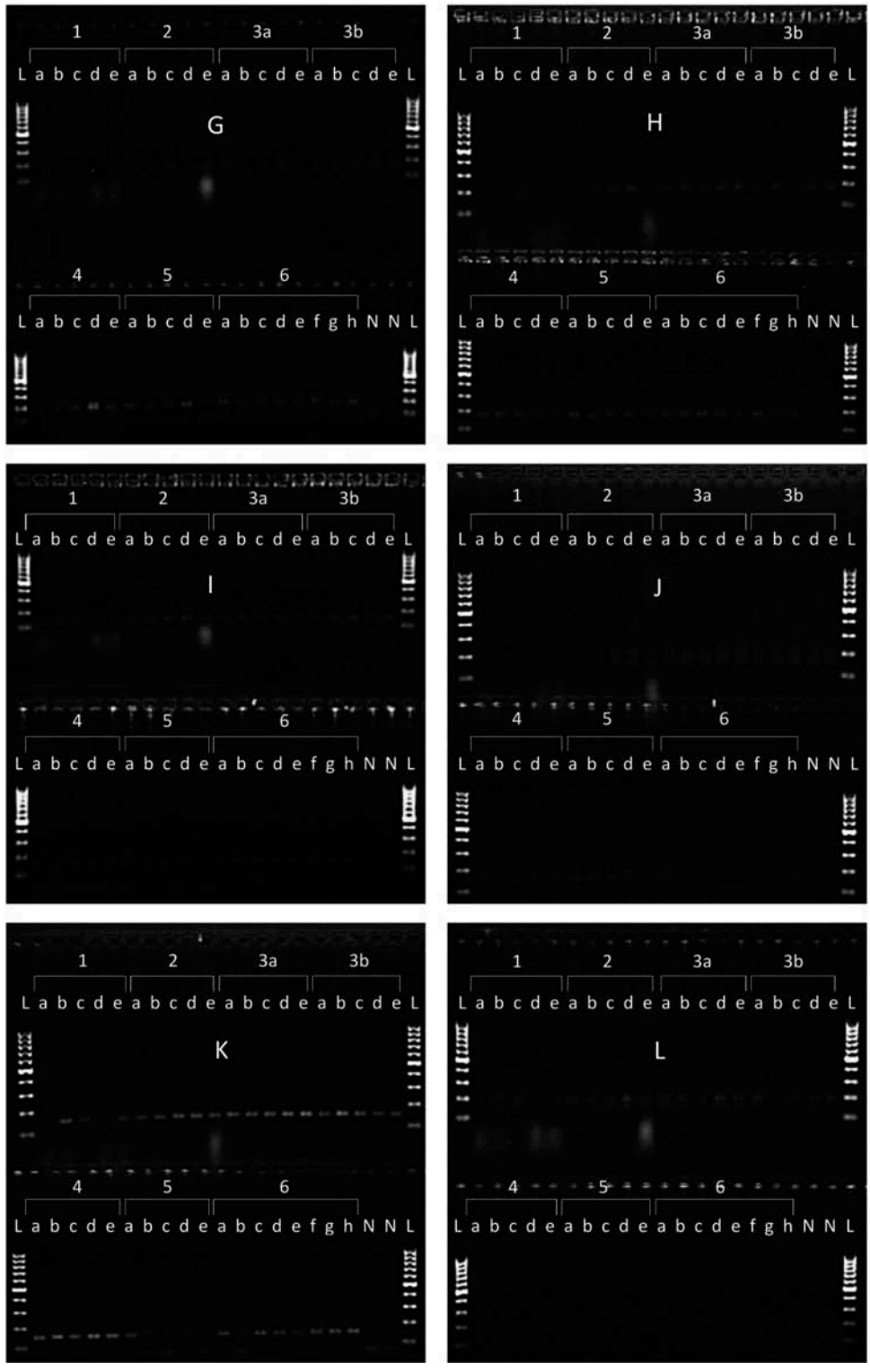


Figure 2. PCR amplification of 12 SSR markers from *Malus X domestica*. Products were run on 1.4% agarose gel.

DNA was extracted with the following commercial kits: 1—E.Z.N.A. SP Plant DNA Kit (Omega Bio-tek), 2—E.Z.N.A. Plant DNA DS Mini Kit (Omega Bio-tek), 3—NucleoSpin Plant II (Macherey-Nagel); 3a—Lysis Buffer PL1, 3b—Lysis Buffer PL2, 4—Invisorb Spin Plant Mini Kit (Stratec Biomedical AG), 5—DNeasy Plant Mini Kit (Qiagen), 6—DNeasy Plant Pro Kit (Qiagen). Lines a–h—DNA samples, line L—100 bp DNA Ladder, line N—non template control. Markers: A—02b1 (Guilford et al., 1997), B—05g8 (Guilford et al., 1997), C—GD96 (Hokanson et al., 1998), D—GD147 (Hokanson et al., 1998), E—CH01h01 (Gianfranceschi et al., 1998), F—CH01c06 (Liebhard et al., 2002), G—CH01d09 (Liebhard et al., 2002), H—CH01f12 (Liebhard et al., 2002), I—CH01b10 (Gianfranceschi et al., 1998), J—CH02c02a (Liebhard et al., 2002), K—CH03g04 (Liebhard et al., 2002), L—CH03g07 (Liebhard et al., 2002).



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downstream applications, such as Southern blot analysis that needs larger quantities of non-degraded DNA (Bitencourt, Roratto, Bartholomei-Santos, & Santos, 2007), required DNA quantity needs to be decided before selecting the most suitable DNA extraction method. Based on our results, kits used should be suitable as well for preparation of material to be used for the next-generation sequencing-based application. Moreover, required time and cost of a particular kit should not be ignored, especially when dealing with a big number of samples.

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Competing interests
The authors declares no competing interests.

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