Journal of Agriculture and Environmental Sciences
December 2015, Vol. 4, No. 2, pp. 172-180
ISSN: 2334-2404 (Print), 2334-2412 (Online)

Published by American Research Institute for Policy Development
DOI: 10.15640/jaes.v4n2a20
URL: http://dx.doi.org/10.15640/jaes.v4n2a20

Enhanced Protocol for Isolation of Plant Genomic DNA

Muhammad Youssef[1], Ruby Valdez-Ojeda[2], José Roberto Ku-Cauich[3]&Rosa María Escobedo-GraciaMedrano[4]*

Abstract

A reproducible method for extraction of high-quality genomic DNA (gDNA) suitable for application in several PCR-based methods was developed after modifications to the Dellaporta method. Changes to the extraction buffer include the use of a higher concentration of NaCl, substitution of β-mercaptoethanol with sodium-metabisulfite, and the use of polyethylene glycol for DNA precipitation. Compared to the original method and two other protocols tested, our improved protocol resulted in the isolation of a good yield and purity of gDNA. The content of extracted DNA was spectrophotometrically evaluated, and the quality was analyzed by Amplified Fragment Length Polymorphisms (AFLP). AFLP profiles of the DNA obtained with our protocol were comparable to those of a commercial kit for plant DNA extraction. The potential of this improved method relies on its successful use with different molecular markers using gDNA extracted from fresh and frozen tissues of a variety of vascular plants, including banana in this paper, and proven in wheat, guava, sugarcane, and bean, as well as from microalgae. Therefore, the new protocol is an adequate, convenient and economical choice for use and study of various fields of genomics.

Keywords: AFLP, DNA purity, Genotyping, Safety Protocol.

1. Introduction

Isolation of genomic DNA is always an important issue in the field of plant molecular biology. Ideally, the method should be simple, rapid, efficient, and reproducible. In addition, it must be suitable for extracting multiple types of samples and generate a high quality and quantity of DNA with minimal risk for the operator. Quality is of critical concern for genomic studies and most amplification-based analyses because DNA amplification can be influenced by the presence of co-purifying inhibitors, which reduce subsequent PCR efficiency (Jara et al. 2008; Anuradha et al. 2013).

Numerous protocols for isolation of plant DNA have been published (e.g., Doyle and Doyle 1990; Gawel and Jarret 1991; Scott and Playford 1996; Li et al. 2001; Mogg and Bond 2003; Haymes et al. 2004). Commercially available DNA isolation kits provide higher throughput and reduced labor time, though this is not always true for all plant materials.Additionally, their availability and high cost can be limiting in certain developing countries, especially when handing a large number of samples and considering experiments with limited financial resources (Bashalkhanov and Rajora 2008; Niu et al. 2008).

[1]Genetics Department, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt
[2]Unidad de Energía Renovable, Centro de Investigación Científica de Yucatán, A.C.Mérida, Yucatán, México.
[3]Unidad de Bioquímica y Biología Molecular de Plantas, Centro de Investigación Científica de Yucatán, A.C.
[4]Unidad de Bioquímica y Biología Molecular de Plantas, Centro de Investigación Científica de Yucatán, A.C., Calle 43, No. 130, Chuburna de Hidalgo, 97200, Mérida, Yucatán, México.*Correspondingautor E-mail: escobedo@cicy.mx, **Tel:** 52 (999) 942 83 30 ext. 240**, Fax:** 52 (999) 981 39 00

Molecular markers based on PCR amplification and/or relying on digestion of DNA with restriction enzymes require good-quality DNA that is relatively free of contaminants (Jobes et al. 1995). Several plant species contain naturally high amounts of polysaccharides, polyphenols and other secondary metabolites, substances known for binding firmly to nucleic acids during DNA isolation and interfering with subsequent reactions (Pirttilä et al. 2001; Takakura and Nishio 2012).

Reliability, achievability and reproducibility of molecular genetics studies rely on the preliminary step of DNA isolation. Obtaining high-quantity and -quality DNA from small amounts of tissue is often a difficult task (Pereira et al. 2011). The most commonly used plant DNA isolation methods employ toxic and hazardous chemicals (e.g., phenol, chloroform, β-mercaptoethanol), which require special equipment to minimize exposure and may limit their use in certain environments (Niu et al. 2008). For instance, β- mercaptoethanol, which is being used in some protocols, e.g.,Doyle and Doyle (1990) protocol, identified as CTAB protocol (for CetylTrimethyl Ammonium Bromide), causes severe eye irritation, is harmful if swallowed and may cause skin and respiratory tract irritation. Furthermore, it targets the central nervous system, respiratory system and eyes (Fisher Scientific Co.).

In the present study, published observations were considered during the improvement of the method, i.e., a high molar concentration of NaCl inhibits the co-precipitation of polysaccharides and DNA (Ribeiro and Lovato 2007). Polyethylene glycol (PEG) has been used in DNA precipitation to avoid the presence of plant metabolites (glycosides, polyphenols, etc.), which would inhibit Taq DNA polymerase activity (Agundo et al. 1995). Different studies have shown that the best indicator of good DNA quality, a predictor of PCR success, was PCR amplification amounts with respect to DNA concentrations and most commonly used UV absorbance ratio measurements. However, 260/280 ratios below approximately 1.3 and above 2.3 also indicate poor DNA quality in amplification (Llongueras et al. 2012)

The main objective of this study was to establish a reproducible and efficient method for isolation of pure,high-yield DNA from plant tissues. In addition, the method is affordable, demands less specialized lab equipment (a fume hood) and produces less toxic waste, although caution is always advised. Several factors affecting DNA isolation were investigated, including buffer composition, replacement of some reagents, and addition of components that enhanced the DNA quality. The quantity of DNA obtained with the modified protocol was comparable to the DNA extracted by the original Dellaporta's method, (Dellaporta et al. 1983), MATAB (Gawel and Jarret 1991) and CTAB (Doyle and Doyle 1990) protocols, based on UV-spectrophotometry, and the quality was also compared to a commercial kit protocol by the banding pattern obtained by Amplified Fragment Length Polymorphism (AFLP) technique.

2. Materials and Methods

2.1. Plant material

Banana young cigar leaves were collected from *Musa acuminata*Colla cv. Grand Naine (AAA) plants. Plants were grown in Cambisol (CMX)-type soil at the Uxmal Experimental Site of the InstitutoNacional de InvestigacionesForestalesAgrícolas y Pecuarias (INIFAP) in Yucatán, México (20◦24'27.72"Lat. N, and 89◦45'06.66"Long. W, elevation 44.0 meters above sea level), with a tropical wet-dry climate (AW0).

2.2. Reagents and solutions

- Extraction buffer (Fresh preparation):
  - 100 mM Tris-HCl(pH 8)
  - 50 mMEDTA (pH 8)
  - 1.5 M NaCl
  - 1.0% (w/v) Polyethylene Glycol8000(PEG)
  - 0.5% (w/v) Sodium metabisulfite
  - 2% (w/v) Polyvinyl pyrrolidone (PVP), used in the case of high polyphenols content plants
- Sodium dodecyl sulfate (SDS) 20% (w/v)
- 5 M Potassium acetate (pH 8)

- 3 M Sodium acetate (pH 5.2)
- RNAase 10 mg/ml
- Isopropanol, -20ºC
- 70% ethanol, -20ºC
- TE buffer: 10 mM Tris HCl (pH 8), 1 mM EDTA (pH 8)
- Distilled autoclaved water

2.3. Protocol

The protocol described here was developed from Dellaporta et al. (1983), after major modifications (Table 1).

1- Grind 75-100 mg of plant tissue using liquid nitrogen (in the case of high secondary metabolites, use PVP); transfer the powder into a 2 ml Eppendorf tube.
2- Add 1 ml of pre-heated extraction buffer (65°C) plus 100 µl of SDS (20%) to each sample, shake vigorously and let stand on ice.
3- Incubate in water bath at 65°C for 30 min (with inversion each 5 min).
4- Leave samples at the room temperature (RT), and add a half volume of 5 M potassium acetate (pH 8) with mixing; incubate on ice for 30 min.
5- Leave samples at RT and centrifuge at 12200 × g (13000 rpm) for 30 min at 10°C.
6- Transfer the supernatant to a new 2 ml tube and add 5 µl of RNAase (10 mg/ml); incubate at 37°C for 30 min.
7- Add an equal volume of cold isopropanol, mix gently and incubate -20°C for 1 h.
8- Centrifuge at 12200 × g (13000 rpm) for 20 min at 4°C.
9- Discard the supernatant and wash the pellet 2 times with ethanol 70%, (500 µl, 9500 × g (10000 rpm), 5 min).
10- Let the pellet completely dry and dissolve in 600 µl of TE (incubation at 45°C can be used for acceleration).
11- Centrifuge at 12200 × g (13000 rpm) for 10 min at 10°C.
12- Transfer the supernatant to a new 1.5 ml tube, add an equal volume of cold isopropanol with 1/10 volume sodium acetate (3 M, pH5.2) and invert the tube gently.
13- Incubate at -20°C for 1 hr.
14- Repeat steps 8 and 9.
15- Let the pellet completely dry, dissolve in 50 µl of ddH_2O.

Table 1: Modifications of the Dellaporta (et al. 1983) DNA extraction buffer made to improve the new protocol

Component	Original Dellaporta	New protocol
NaCl	500 mM	1.5 M
β-mercaptoethanol	10 mM	Excluded
Sodium meta-bisulfate	-	0.5% (w/v)
Polyethylene glycol	-	10% (w/v)
Polyvinylpyrrolidone	-	1- 2% (w/v)[1]

[1] Used in the case of high polyphenols content plants.

2.4. Comparison of the extraction protocols for efficacy.

A comparison between the developed protocol and some published DNA extraction protocols, i.e., Dellaporta (et al. 1983), CTAB (Doyle and Doyle 1990) and MATAB (Gawel and Jarret 1991), was performed using the same plant material. DNA quality and quantity were determined using a spectrophotometer according to Stulnig and Amberger (1994). The DNA of the different extracted samples was evaluated at 230, 260, 280 and 320 nm wavelengths to check their concentration and purity. An AFLP molecular marker was used to evaluate the integrity and efficiency of the extracted DNA using the novel protocol, and its results were compared with the other extraction methods mentioned plus a commercial DNA extraction kit (Qiagen).

2.5. AFLP-PCR amplification

AFLP analysis was carried out according to Vos et al. (1995) with some modifications. Two AFLP primers were used in the selective amplification (Eco-1/Mse-15 and Eco-3/Mse-15, Youssef et al. 2011).

2.6. Statistical data analysis

Three replicates from each sample were used for DNA extraction, and spectrophotometer measurements were taken for the investigated protocols. Analysis of variance (ANOVA) was performed to check the significance of differences amongst the absorbance ratios from the spectrophotometer from the tested protocols as well as the effect of adding PEG, with the MSTAT-C statistical program (Nissen 1984). Averages were compared using Duncan's multiple-range test.

2.7. Molecular data analysis

A binary data matrix indicating the presence (1) or the absence (0) of bands was made from the AFLP profiles of the banana samples. Only strong, reproducible and clearly distinguished bands were used for the analysis. The software NTSYSpc ver. 2.20s was used, and genetic similarities were computed using Jaccard's coefficient (1980) of similarity. Cluster analysis was carried out on similarity estimates using the unweighted pair-group method with arithmetic averages (UPGMA). One thousand repetition counts were used to generate the bootstrapping using the FreeTree program.

3. Results and Discussion

DNA extraction is one of the most relevant step for the molecular analysis of plants at the genetic, phylogenetic and genomic levels. Several plant DNA extraction protocols have been used and compared to find the best protocol that isolates high-quality, amplifiable DNA. During the extraction, the aim is to lessen the effect of endogenous metabolites such as high content of polyphenols and polysaccharides on DNA, particularly in hard-to-extract species and herbarium samples (Pirttilä et al. 2001; Coyle et al. 2003; Ribeiro and Lovato 2007; Bashalkhanov and Rajora 2008; Jara et al. 2008; Karthikeyan et al. 2010; Sandip 2013). In the current study, four different DNA extraction protocols, including a commercial kit, were compared with our protocol developed after major modifications to the Dellaporta method based on the quality of DNA, PCR and AFLP reproducibility. The comparison included analysis of absorbance at various wavelengths using a spectrophotometer for the isolated DNA (concentration and purity), effect of polyethylene glycol on the DNA quality and an evaluation using AFLP molecular marker.

3.1. Assessment of DNA quality and yield

Total concentration and purity of the banana DNA isolated by the different protocols were measured spectrophotometrically at different wavelengths (230, 260, 280 and 320 nm). Values at 320 were subtracted from the other readings for background corrections, and ratios of absorbance at 260/280 and 260/230 were then estimated (Table 2). In the improved protocol presented here, the modifications to the protocol were efficient in improving the original Dellaporta method, giving higher-quality DNA compared with other common protocols. We increased the concentration of NaCl according to Ribeiro and Lovato (2007), who reported that a high molar concentration of NaCl inhibits the co-precipitation of polysaccharides and DNA. The analysis of variance showed no significant differences among the tested protocols in the 260/280 ratio, except for the value of DNA extracted with the CTAB protocol, which showed a high ratio (260/280=2.02) indicating the likely presence of phenols in the extracted DNA. On the other hand, the DNA absorbance 260/230 ratio showed highly significant differences ($p < 0.01$) amongst the examined protocols, which were 0.94 (CTAB), 1.37 (MATAB), 0.88 (Dellaporta), 1.99 (our new protocol with PEG), and 1.23 (new without PEG). PEG addition to the extraction buffer improved the absorbance ratios both at 260/280 from 1.60 to 1.75 and 260/230 from 1.23 to 1.99 (Table 2). In this regard, Stulnig and Amberger (1994) reported that significant absorbance at 230 nm indicates contamination by phenolate ions, thiocynates and other organic compounds. The absorbance at 230 nm should be half the value at 260 nm to yield a ratio (260/230) of almost 2. Moreover, the addition of PEG in this study enhanced the quality of DNA at both 260/280 and 260/230 absorbance ratios. These results are in agreement with those of Agundo et al. (1995) who used PEG in DNA precipitation in the presence of plant metabolites (glycosides, polyphenols, etc.).

Table 2: Differences in spectrophotometer absorbance ratios for DNA purity, depending on the method used for DNA extraction

Method	Abs. ± SE 260/280	Abs. ± SE 260/230	Concentration ± SE ng/µl	Yield± SE µg/100mg
CTAB	2.02±0.03a	0.94±0.07c	923.33±113.64	46.17±5.68
MATAB	1.67±0.08b	1.37±0.10b	544.17±193.72	27.21±9.69
Dellaporta	1.70±0.19b	0.88±0.27c	172.50±2.50	8.63±0.13
New with PEG	1.75±0.04b	1.99±0.13a	380.00±17.50	19.00±0.88
New without PEG	1.60±0.00b	1.23±0.06bc	235.0±10.90	11.75±0.55

Means within columnsthat have the same letters are statistically similar (Duncan's multiple range test, $P< 0.01$). The values reported are means ± standard error, SE (n = 3).

Several types of endogenous metabolite contaminations are released during DNA extraction protocols depending on the origin of the biological sample (Pereira et al. 2011). Regarding secondary metabolites, Varma et al. (2007) reported that the most frequently encountered problem in plant DNA isolation protocols is the degradation of genomic DNA due to the presence of polyphenols, i.e., an oxidized form of polyphenols covalently binds to DNA that causes damage of DNA and/or inhibits enzymatic reactions. A strong reducing reagent, β-mercaptoethanol, is often included in DNA extraction buffers to prevent oxidation of polyphenols present in the crude plant extract (Kawata et al. 2003; Varma et al. 2007). However, it is harmful and targets the central nervous system, respiratory system, and eyes (Fisher Scientific Co.). The replacement of β-mercaptoethanol with sodium meta-bisulfate performed in this study made the new protocol safer for laboratory work (Jara et al. 2008; Anuradha et al. 2013). Furthermore, this replacement did not affect the purity of DNA. However, in some plant species, which contain excessive polyphenols, e.g., guava, the use of polyvinilpyrrolidone (PVP) is recommended in addition to PEG.

3.2. Evaluation of the new developed protocol using AFLP marker

Llongueras et al. (2012) suggested that 260/280 ratios below approximately 1.3 and above 2.3 are indicative of DNA quality too poor to be amplified. Therefore, 260/280 measurements are not useful to identify samples that are likely to be amplified, but can be used to exclude samples that likely will not amplify, reducing the cost for unnecessarily subjecting samples to PCR. Furthermore, they demonstrated that the ability to amplify by PCR was the best indicator of extracted product quality. In this study, we used an AFLP marker to evaluate the quality of DNA isolated from the leaves of *Musa acuminata*Colla cv. Grand Naine (AAA) by comparing the new extraction protocol with that of other protocols and a commercial kit.Since the AFLP marker depends on restriction enzymes digestion and PCR amplification (Vos et al. 1995), it would be more influential in evaluating both processes.Results showed that, all DNA samples were amplified successfully; however, the AFLP pattern obtained from DNA isolated by the new protocol was very similar to that of the commercial kit (Fig 1).

The total numbers of amplified bands resulted from DNA template extracted by Dellaporta, CTAB, MATAB, commercial kit and with the new protocol were 49, 61, 61, 83 and 75, respectively (Table 3). These results showed that our modifications to the Dellaporta method were efficient in amplifying 26 extra bands than the original method, which made the new protocol more similar to the commercial kit. Additionally, there were some bands generated distinctively by each protocol, i.e., one band for Dellaporta, one band for CTAB, two bands for MATAB and four bands for the commercial kit, while the newly developed protocol did not generate unique bands (Table 3). The percentage of similarity amongst the examined protocols calculated using Jaccard's coefficient isshown in table (4). The highest similarity was 81.61% between the commercial kit and our new protocol, while the lowest similarity was 40.43% between the commercial kit and Dellporta. The AFLP pattern resulting from our new protocol and that of the commercial kit shared 10 unique and specific bands that appeared only when these two protocols were used, while they disappeared with the other protocols (Fig. 1, Table 4).

Additionally, only two bands were unique and specific for the commercial kit and both CTAB and MATAB protocols, while the commercial kit and the original Dellporta protocol did not share any unique bands (Table 4).

Figure (2) showed the relationship amongthe investigated protocols demonstrated in a dendrogram generated from AFLP results based on Jaccard's coefficient. The cluster analysis placed the new protocol and the commercial kit in the same group that was closer in the dendrogram. On the other hand, both CTAB and MATAB methods gathered in one subgroup, while the Dellaporta method was on a single branch (Fig2).

Table 3: Summary of the total number and specific bands generated from the test protocols

	Dellaporta	CTAB	MATAB	Kit	New
TNB	49	61	61	83	75
UB	1	1	2	4	0

TNB: Total number of bands, UB: unique bands for each protocol.

Table 4: Number of shared unique bands (above diagonal) and percentage of similarity according to AFLP pattern (below diagonal) amongst the tested DNA extraction protocols

Method	Del	CTAB	MATAB	Kit	New
Dellaporta	-	2	1	0	3
CTAB	46.67	-	1	2	0
MATAB	42.86	64.87	-	2	0
Kit	40.43	63.64	60.00	-	10
New with PEG	49.40	60.00	58.14	81.61	-

The CTAB method has demonstrated the greatest yield and probably remains the method of choice when large amounts of DNA are required (Bashalkhanov and Rajora 2008). However, we found that our new protocol gave higher-quality DNA than the CTAB method, and the results from that method indicated it was more similar to the AFLP results of the commercial kit in terms of profile reproducibility and number of bands. Likewise, the CTAB extraction buffer uses harmful chemicals, e.g., β-mercaptoethanol and chloroform, which makes it an unfavorable protocol due to the chemical hazardous waste. CTAB extraction can yield variable results depending on the experience of the scientist and the quality of the plant material (Coyle et al. 2003). In addition, Kawata et al. (2003) demonstrated that CTAB, as a component of DNA extraction buffer, was not required in the buffer unlike other reagents with successful PCR amplification.

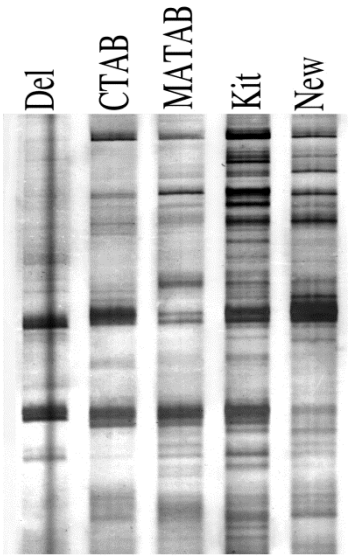

Fig 1: AFLP pattern generated with DNA of banana samples extracted by the different protocols evaluated in this study.

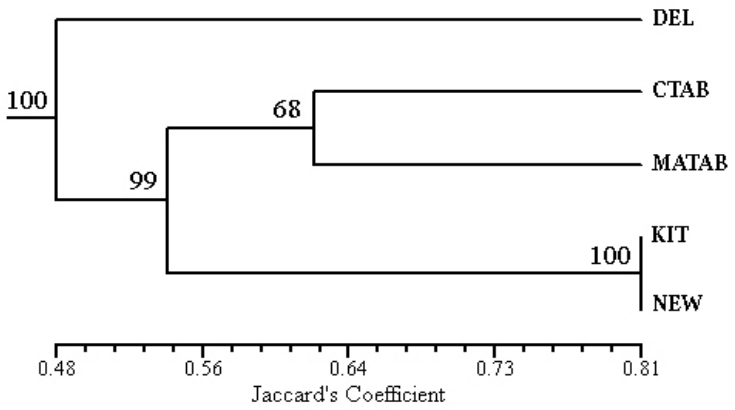

Fig 2: Dendrogram of Jaccard's similarities among the investigated DNA extraction protocols using AFLP–UPGMA cluster analysis. Numbersindicate bootstrap support

Moreover, our new protocol has been used with several plant species including banana (Valdez-Ojeda et al. 2014), sugarcane (Fawaz et al. 2013), guava (Youssef et al. 2013), wheat (El-Rawy and Youssef 2014) and bean (unpublished data), as well as microalgae (Valdez-Ojeda et al. 2015). The resulting DNA from the mentioned species exhibited high quality for amplification of several molecular markers (AFLP, SRAP, TRAP, RAPD and ISSR), and this process has been performed successfully, with excellent amplification for sequencing purposes (Mahendiran et al. 2014).

The isolation protocol presented here is economical, efficient and a good choice in different molecular biology fields that require high-quality plant genomic DNA.

Acknowledgements

Funding for this research work was provided by Secretaria de Agrícultura, Ganadería, RecursosPesqueros y Alimentos (SAGARPA) and the ConsejoNacional de Ciencia y Tecnología (CONACYT) through the research grant 0048160 (RME). The first author (Dr. Youssef M) is the recipient of a Postdoctoral fellowship from CONACYT-TWAS (The World Academy of Science) award # 3240267275. The authors acknowledge Jorge M. Itzá-Can, Rosa Grijalva-Arango, and Miguel A. Keb-Llanes for technical support. All the co-authors included had an active part in the final manuscript.

References

Agundo, L., Gavidia, I., Bermudez, P. and Segura, J. (1995). PEG precipitation, a required step for PCR amplification of DNA from wild Plants of *Digitalis obscura* L. Biotechniques, 18,766-768.

Anuradha, H.J., Vijayan, K., Nair, C.V. and Manjula, A. (2013). A novel and efficient protocol for the isolation of genomic DNA from mulberry (*Morus* L.). Emirates Journal of Food and Agriculture, 25, 124-131.

Bashalkhanov, S. and Rajora, O.P. (2008). Protocol: A high-throughput DNA extraction system suitable for conifers. Plant Methods, 4, 1-6.

Coyle, H.M., Shutler, G., Abrams, S., Hanniman, J., Neylon, S., Ladd, C., Palmbach, T. and Lee, H. (2003). A Simple DNA Extraction Method for Marijuana Samples Used in Amplified Fragment Length Polymorphism (AFLP) Analysis. Journal of Forensic Sciences, 48, 1-5.

Dellaporta, S.L., Wood, J. and Hicks, J.B. (1983). A plant DNA mini-preparation: version II, Plant Molecular Biology Reporter, 1, 19–21.

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

El-Rawy, M.A. and Youssef, M. (2014). Evaluation of drought and heat tolerance in wheat based on seedling traits and molecular markers. Journal Crop Science Biotechnology, 17, 183-189.

Fawaz, A.W., Nasr, M.I., El-Aref, H.M., Hemeida, A.A., Abouel-Khier, A.S.M. (2013) Genetic Variability among Sugarcane Genotypes Based on TRAP Markers of Candidate Drought Genes and Some Agronomic Traits. Assuit Journal of Agricultural Sciences, 44, 46-62.

Gawel, N.J. and Jarret, R.L. (1991). A modified CTAB DNA extraction procedure for *Musa* and *Ipomoea*. Plant Molecular Biology Reporter, 9, 262-266.

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, 52,5456-5462.

Jaccard, P. (1980). Nouvellesrecherchessur la distribution florale. Bulletin de la Sociéte vaudoise des sciences naturelles,44, 223–270.

Jara, C., Mateo, E., Guillamón, J.M., Torija, M.J. and Mas, A. (2008) Analysis of several methods for the extraction of high quality DNA from acetic acid bacteria in wine and vinegar for characterization by PCR-based methods. International Journal of Food Microbiology, 128, 336-321.

Jobes, D.V., Hurley, D.L. and Thien, L.B. (1995) Plant DNA isolation: a method to efficiently remove polyphenolics, polysaccharides, and RNA. Taxon 44, 379-386.

Karthikeyan, V., Patharajan, S., Palani, P., Spadaro, D., Gullino, M.L. and Garibaldi, A. (2010). Modified simple protocol for efficient fungal DNA extraction highly suitable for PCR based molecular methods. Global Journal of Molecular Sciences, 5, 37-42.

Kawata, M., Matsumura, Y., Oikawa, T., Kimizu, M., Fukumoto, F. and Kuroda, S. (2003). Analysis of DNA extraction buffer components from plant tissue by polymerase chain reaction. Analytical Biochemistry, 318, 314–317.

Li, H., Luo, J., Luo, J., Hemphill, J.K., Wang, J. and Gould, J.H. (2001). A rapid and high yielding DNA miniprep for cotton (*Gossypium* spp.). Plant Molecular Biology Reporter, 19,183a-183e.

Llongueras, J.P., Nair, S., Salas-Leiva, D. and Schwarzbach, A. (2012). Comparing DNA Extraction Methods for Analysis of Botanical Materials Found in Anti-Diabetic Supplements. Molecular Biotechnology, 53, 249-56.

Mahendhiran, M., Ramirez-Prado, J.H., Escobedo-GraciaMedrano, R.M., Canto-Canché, B., Tzec-Simá, M., Grijalva-Arango, R. and James-Kay, A. (2014). Single nucleotide polymorphisms in partial sequences of the gene encoding the large sub-units of ADP-glucose pyrophosphorylase within a representative collection of 10 *Musa* genotypes. Electronic Journal of Biotechnology, 17, 137–147.

Mogg, R.J. and Bond, J.M. (2003). A cheap, reliable and rapid method of extracting high-quality DNA from plants. Molecular Ecology Notes, 3, 666-668.

Nissen, O. (1984). MSTAT: A microcomputer program for statistical analysis of experiments and surveys. In: H. Riley and A. O. Skjelvag (Eds.), The impact of climate on grass production and quality, (pp. 555–559). Proceedings of the 10th General Meeting of the European Grassland Federation. Aas Norway. Norwegian State Agricultural Research Stations.

Niu, C., Kebede, H., Auld, D.L., Woodward, J.E., Burow, G. and Wright, R.J. (2008) A safe inexpensive method to isolate high quality plant and fungal DNA in an open laboratory environment. African Journal of Biotechnology, 7, 2818-2822.

Pereira, J.C., Chaves, R., Bastos, E., Leitão, A. and Guedes-Pinto, H. (2011). An efficient method for genomic DNA extraction from different Molluscs Species. International Journal of Molecular Science, 12, 8086-8095.

Pirttilä, A.M., Hirsikorpi, M., Kämäräinen, T., Jaakola, L. and Hohtola, A. (2001). DNA isolation methods for medicinal and aromatic plants. Plant Molecular Biology Reporter, 19, 273a-273f.

Ribeiro, R.A. and Lovato, M.B. (2007). Comparative analysis of different DNA extraction protocols in fresh and herbarium specimens of the genus *Dalbergia*. Genetics and Molecular Research, 6, 173-187.

Sandip, M.S. (2013). A Reliable and High Yielding Method for Isolation of Genomic DNA from *Ammimajus*. International Research Journal of Biological Sciences, 2, 57-60.

Scott, K.D. and Playford, J. (1996). DNA extraction technique for PCR in rain forest plant species. Biotechniques, 20, 974-979.

Stulnig, T.M. and Amberger, A. (1994). Exposing contaminating phenol in nucleic acid preparations. BioTechniques, 16, 403–404.

Takakura, K. and Nishio, T. (2012). Safer DNA extraction from plant tissues using sucrose buffer and glass fiber filter. Journal of Plant Research, 125, 805-807.

Valdez-Ojeda, R., James-Kay, A., Ku-Cauich, J. and Escobedo-GraciaMedrano, R.M. (2014). Genetic relationships among a collection of Musa germplasm by fluorescent-labled SRAP. Tree Genetics and Genomes, 10, 465-476.

Valdez-Ojeda, R., González-Muñoz, M., Us-Vázquez, R., Narváez-Zapata, J., Chavarria-Hernandez, J.C., López-Adrián, S., Barahona-Pérez, F., Toledano-Thompson, T., Garduño-Solórzano, G. and Escobedo-GraciaMedrano, R.M. (2015). Characterization of five fresh water microalgae with potential for biodiesel production. Algal Research, 7, 33-44.

Varma, A., Padh, H. and Shrivastava, N. (2007). Plant genomic DNA isolation: An art or a science. Biotechnology Journal, 2, 386-392.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research, 23, 4407–4414.

Youssef, M., James, A.C., Rivera-Madrid, R., Ortiz, R. and Escobedo-GraciaMedrano, R.M. (2011). *Musa* genetic diversity revealed by SRAP and AFLP. Molecular Biotechnology, 47,189-199.

Youssef, M., Ibrahim, R.A. and Amein, K.A. (2013). Comparison of phenotypic and molecular assessment of genetic diversity in guava. Presentation 25th-27th September. Riva del Garda: III International Symposium on Molecular Markers in Horticulture.