International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2020; 8(1): 486-489

Received: 09-09-2019
Accepted: 13-10-2019

Raimani Hembrom
Division of Floriculture & Medicinal Crops, ICAR-IIHR, Bengaluru, Karnataka, India

T Manjunatha Rao
Division of Floriculture & Medicinal Crops, ICAR-IIHR, Bengaluru, Karnataka, India

M Manamohan
Division of Biotechnology, ICAR-IIHR, Bengaluru, Karnataka, India

S Sriram
Division of Plant Pathology, ICAR-IIHR, Bengaluru, Karnataka, India

Rajiv Kumar
Division of Floriculture & Medicinal Crops, ICAR-IIHR, Bengaluru, Karnataka, India

Corresponding Author:
Raimani Hembrom
Division of Floriculture & Medicinal Crops, ICAR-IIHR, Bengaluru, Karnataka, India

Isolation of quality DNA from gladiolus corms using different methods

Raimani Hembrom, T Manjunatha Rao, M Manamohan, S Sriram and Rajiv Kumar

DOI: https://doi.org/10.22271/chemi.2020.v8.i1g.8302

Abstract
DNA is the principal material for numerous molecular studies in plants. Leaves are most commonly used for extraction of DNA pertaining to the ease of isolation. In this study, we have tried two different methods for isolation of quality DNA from gladiolus corms of two genotypes i.e. *Psittacinus* hybrid and Pink Friendship. Gladiolus corms are rich in polysaccharides and polyphenols which make extraction of DNA difficult. The two DNA isolation methods i.e. method 1 and method 2 gave different results for quality and quantity of DNA. DNA yield was found to be better in method 2 for both *Psittacinus* hybrid and Pink Friendship genotypes as compared to method 1. DNA bands were also found to be less smeared in method 2 with respect to method 1. Good quality DNA obtained from uncommonly used plant part like corms with a suitable method can be successfully employed for various molecular studies.

Keywords: Corm, DNA, gladiolus, polysaccharides, polyphenols

Introduction
Molecular breeding is a non-conventional method of crop improvement where DNA is the base material for several analyses. Genomic DNA is subjected to various molecular techniques like polymerase chain reactions (PCR), genetic marker analysis, southern blot analysis, DNA fingerprinting and association mapping, etc. A good quality genomic DNA is crucial for carrying out molecular studies pertaining to research in areas of crop improvement, conservation of genetic materials and diversity analysis (Tan and Yiap, 2009) [1]. Suitable methods for extraction of DNA largely depend upon the nature and complexity of the plant material (Kumari *et al*., 2012) [2]. However, isolation of good quality DNA from plant parts such as bulbs, corms and tubers that are rich in polysaccharides proves to be difficult than DNA extraction from leaves. Several authors have studied different DNA isolation protocols for various plant parts like leaves, flower buds, fruits, seeds, roots and tubers (Sharma *et al*., 2008 [3], Hwang Bo *et al*., 2010 [4], Amani *et al*., 2011 [5], Kumari *et al*., 2012) [2]. Various chemicals such as Polyvinylpyrrolidone (PVP), proteinase K, sodium dodecyl sulfate (SDS), etc., were utilized at different steps for improving the efficiency of DNA isolation protocols.
Gladiolus is an important bulbous flower crop in the world. Molecular studies in gladiolus are mainly carried out with DNA isolated from its leaves, whereas other plant parts like corms are rarely used. Corms are modified underground stem which act as a storage structure and also used as propagating material (Ghamsari *et al*., 2007) [6]. Corms consist of polysaccharides, polyphenols and secondary metabolites like alkaloids, tannins, saponins, glycosides, flavonoids and carbohydrate (Ameh *et al*., 2011) [7]. These compounds impede in extraction of DNA (Bandaranayake, 2002) [8] and render the DNA samples non-amplifiable (Sarwat *et al*., 2006) [9]. Polysaccharide impurities can inhibit the activity of enzymes, such as polymerases, ligases and restriction endonucleases (Sarwat *et al*., 2006) [9] whereas oxidized polyphenols bind to DNA and degrade its keeping quality (Katterman and Shattuck, 1983) [10]. Therefore, in this study, an easy and rapid protocol for extraction of genomic DNA from the corms of gladiolus has been identified. The quality DNA thus obtained from corm tissues utilizing suitable method can be used for various molecular techniques like polymerase chain reaction with random primers.

Materials and Methods

Plant material

Gladiolus genotypes *Psittacinus* hybrid and Pink Friendship were used in this study. Corms were collected from the field and were thoroughly washed under tap water followed by washing with sterile water. The samples were stored at 4 ˚C until use.

DNA isolation procedures

The protocols for isolation of DNA from corms of gladiolus were carried out according to Sharma *et al*., (2013) [11] and Lodhi *et al*. (1994) [12] with few modifications as follows:

Method 1: Sharma *et al*., (2013) [11]

Before grinding, the corms were heated at 65°C for 30 min. 100 mg of tissue were homogenized with mortar and pestle and 500 µL of extraction buffer [100 mM Tris-HCl pH 8.0, 10 mM Ethylenediaminetetraacetic acid (EDTA), 1 M NaCl, 1 % sodium dodecyl sulfate (SDS), 2 % PVP, 1 % β-mercaptoethanol, 0.05 mg/mL proteinase K and 4 % (w/v) polyethylene glycol (PEG)] was added to it. The mixture was transferred to 2 mL micro centrifuge tube and mixed well. The sample was then incubated at 65°C for 30 min in a water bath with intermittent gentle shaking. Then the mixture was centrifuged at 13000 g at room temperature for 10 min. The supernatant was taken into a fresh 1.5 mL micro centrifuge tube. Two-third volume of Isopropanol was added to it and mixed gently. Then it was kept for incubation at – 20 °C for 1 hour followed by centrifugation at 13000 g for 10 min. The supernatant was discarded and DNA pellet was washed with 500 µL of ice cold 70% ethanol by centrifuging at 13000 g for 5 min. After discarding the supernatant the pellets were air dried at room temperature until the traces of ethanol was gone. DNA pellets were suspended in 100 µL of TE buffer (10 mM Tris-HCl pH 8.0 and 1.0 mM EDTA). DNA was treated with RNAse A (10 µg/100 µL) and kept at 37°C for 1 hour and stored at – 20 °C until further use.

Method 2: Modified Lodhi *et al.* (1994) [12]

100 mg of fresh weight of tissues was cut from the corm and pulverized into fine powder with autoclaved mortar and pestle in liquid nitrogen. 1 mL of extraction buffer [100 mM Tris-HCl pH 8.0, 10 mM EDTA, 1.5 M NaCl, 2% cetyl trimethylammonium bromide (CTAB)] and 10 mg of PVP (P6755; Sigma-Germany) were added to the ground sample. CTAB was dissolved by heating at 65 °C and 2% β-mercaptoethanol was added to the extraction buffer just before its use. The slurry was then transferred to 2 mL micro centrifuge tubes and mixed thoroughly. Then it is kept for incubation in a water bath at 65 °C for 30 min and mixed intermittently. After cooling at room temperature the homogenized mixture is centrifuged at 10000 g for 5 min and supernatant was collected separately in a fresh 2 mL micro centrifuge tube. Equal volumes of chloroform: octanol was added to the supernatant and mixed gently by inverting the tube to form an emulsion. Then it was again centrifuged at 10000 g for 5 min at room temperature. The top aqueous phase was transferred to 1.5 mL micro centrifuge tube. 0.1 volume of 3M sodium acetate was added to it and mixed gently. Then two volumes of cold (- 20 °C) absolute alcohol was also added to the tube and gently mixed by inverting the tubes. The solution was kept at - 20 °C for 1 hour. It was then centrifuged at 10000 g for 10 min at room temperature. The supernatant was discarded and DNA pellets were washed with 100 µL cold (- 20 °C) 70 % ethanol by spinning at 10000 g for 5 min. The supernatant was discarded and the pellets were air dried until smell of ethanol was gone completely. Pellets were then dissolved in 50 µL TE buffer. DNA solution was then treated with RNAse A (10 µg/100 µL) and kept at 37°C for 1 hour to remove traces of RNA. The sample was then stored at - 20 °C until further use.

Quantitative and qualitative analysis of DNA samples

The yield of genomic DNA samples and presence of impurities in it were analyzed with Nanodrop spectrophotometer by measuring absorbance at 260 nm and 280 nm in the form of A_{260}/A_{280} ratio. Absorbance at 260 nm indicates protein impurities whereas absorbance at 280 nm shows presence of polyphenols or polysaccharides impurities in the DNA samples. An aliquot of 2 µL DNA samples were also analyzed through gel electrophoresis in 0.8 % agarose gel (SeaKem LE Agarose, Lonza) and DNA bands were visualized under UV spectrophotometer to check for quality of DNA.

Results

Isolation of genomic DNA

DNA was isolated from the corms of *Psittacinus* hybrid and Pink Friendship. The A_{260}/A_{280} ratio in method 1 for *Psittacinus* hybrid and Pink Friendship was 0.86 and 1.14 respectively, indicating presence of protein, phenol or other contaminants. The DNA yield was found to be 217.20 ng/µL and 63.60 ng/µL for *Psittacinus* hybrid and Pink Friendship respectively (Table 1). In method 2, the A_{260}/A_{280} ratio for *Psittacinus* hybrid and Pink Friendship were 2.05 and 2.01 respectively, whereas DNA yield was 854.90 ng/µL and 481.40 ng/µL respectively. The agarose gel analysis of genomic DNA samples from the two methods showed quality of DNA extracted according to the two methods. Compacts bands of DNA were found in method 2 whereas method 1 showed smeared DNA bands (Fig. 1).

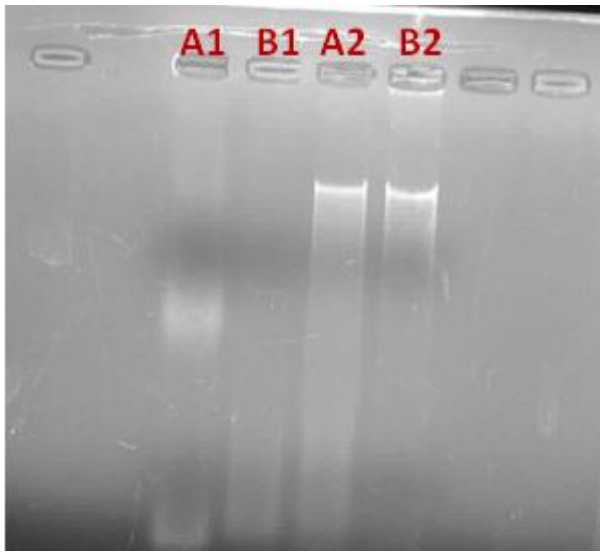

Fig 1: Gel picture of DNA isolated from gladiolus corm using different protocols. A1= DNA isolated from *Psittacinus* hybrid using Method 1, B1= DNA isolated from Pink Friendship using Method 1, A2= DNA isolated from *Psittacinus* hybrid using Method 2, B2= DNA isolated from Pink Friendship using Method 2.

Table 1: Quantification of genomic DNA isolated using different protocols

Protocol for DNA isolation	A260/A280 ratio	DNA yield (ng/µL)
Method 1		
Psittacinus hybrid	0.86	217.20
Pink Friendship	1.14	63.60
Method 2		
Psittacinus hybrid	2.05	854.90
Pink Friendship	2.01	481.40

Discussion

The yield of DNA for *Psittacinus* hybrid and Pink Friendship obtained in method 2 was found to be better than method 1 which is suitable for different molecular techniques. A_{260}/A_{280} ratio for DNA samples isolated from *Psittacinus* hybrid and Pink Friendship ranged from 2.01 to 2.05 which showed insignificant amount of contaminants. Pure DNA has 1.8 as the ratio of absorbance at 260/280 nm. A_{260}/A_{280} ratio ranging from 1.8 to 2.0 shows uncontaminated DNA sample (Kasem *et al*., 2008 [13] and Osman *et al*., 2015 [14]). A_{260}/A_{280} ratio less than 1.8 indicates protein contamination whereas more than 2.0 A_{260}/A_{280} ratio shows presence of RNA in the sample (Varma *et al*., 2007) [15]. The cetyl trimethyl ammonium bromide (CTAB) based DNA isolation protocol is the most commonly used method for different plant materials (Kumari *et al*., 2012) [2]. Polysaccharides present in tuberous plant parts form tight complexes with nucleic acid and makes DNA inaccessible to the enzymes (Sharma *et al*. 2002) [16]. CTAB is a cationic surfactant added to extraction buffer that prevents co-purification of polysaccharides from plant tissues and averts embedding of DNA (Moreira *et al*., 2011) [17] and also selectively precipitates DNA from histone proteins (Khan *et al*., 2007) [18]. To improve efficiency of DNA isolation protocol polyvinylpyrrolidone (PVP) was incorporated during the process. PVP and β-mercaptoethanol helps in sequestering polyphenols form the sample (Khanuja *et al*., 1999 [19] and Sahu *et al*., 2012) [20]. Addition of NaCl to extraction buffer effectively inhibits co-precipitation of polysaccharides with DNA by increasing its solubility in ethanol (Ribeiro *et al*., 2007) [21]. EDTA in the extraction buffer has the ability to sequester ions such as Mg^{2+} and Ca^{2+} present in cellular membranes. The bands obtained in method 2 after gel electrophoresis appeared to be compact with least smear showing little degradation during isolation process and absence of impurities. In method 1, the yield and quality of DNA were poor. Smearing of DNA during gel electrophoresis might be due to degradation of DNA during the process of extraction.

Conclusion

In this study, two DNA extraction methods were used to isolate high quality DNA that can be used for various molecular analyses. Generally fresh leaf samples are preferred for DNA extraction protocols. Isolation of DNA from corms will help in carrying out molecular studies even when there is no standing crop. CTAB method of DNA isolation was found to be effective for complex plant tissues like corms which are rich in polysaccharides and polyphenols. Therefore this method is reliable for isolation of quality DNA from gladiolus corms and there is no need to go for special techniques or commercial kits which might be expensive, time consuming and labour-intensive for obtaining high throughput DNA.

Acknowledgments

The authors are thankful to ICAR-Indian Institute of Horticultural Research, Bengaluru for providing lab facilities and financial support.

References

1. Tan SC, Yiap BC. DNA, RNA, and protein extraction: the past and the present. BioMed Research International, 2009, 10p.
2. Kumari V, Bansal A, Aminedi R, Taneja D, Das N. Simplified extraction of good quality genomic DNA from a variety of plant materials. African J. Biotech. 2012; 11(24):6420-6427.
3. Sharma K, Mishra AK, Misra RS. A simple and efficient method for extraction of genomic DNA from tropical tuber crops. African J. Biotech. 2008; 7(8):1018-1022.
4. HwangBo K, Son SH, Lee JS, Min SR, Ko SM, Liu JR *et al*. Rapid and simple method for DNA extraction from plant and algal species suitable for PCR amplification using a chelating resin Chelex 100. Plant Biotech. Reports. 2010; 4(1):49-52.
5. Amani J, Kazemi R, Abbasi AR, Salmanian AH. A simple and rapid leaf genomic DNA extraction method for polymerase chain reaction analysis. Iranian J. Biotech. 2011; 9(1):69-71.
6. Ghamsari L, Keyhani E, Golkhoo S. Kinetics properties of guaiacol peroxidase activity in *Crocus sativus* L. corm during rooting. Iranian Biomedical J. 2007; 11(3):137-46.
7. Ameh SJ, Obodozie OO, Olorunfemi PO, Okoliko IE, Ochekpe NA. Potentials of Gladiolus corms as an antimicrobial agent in food processing and traditional medicine. J. Microbiology and Antimicrobials. 2011; 3(1):8-12.
8. Bandaranayake WM. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands Ecology and Management. 2002; 10(6):421-52.
9. Sarwat M, Negi M, Lakshmikumaran M, Tyagi A. A standardized protocol for genomic DNA isolation from *Terminalia arjuna* for genetic diversity analysis. Electron J Biotechnol. 2006; 9:86-91.
10. Katterman FR, Shattuck VI. An effective method of DNA isolation from the mature leaves of *Gossypium* species that contain large amounts of phenolic terpenoids and tannins. Preparative Biochemistry. 1983; 13(4):347-59.
11. Sharma K, Bhattacharjee R, Sartie A, Kumar PL. An improved method of DNA extraction from plants for pathogen detection and genotyping by polymerase chain reaction. African J. Biotech. 2013; 12(15):1894-1901.
12. Lodhi MA, Ye GN, Weeden NF, Reisch BI. A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Molecular Biology Reporter. 1994; 12(1):6-13.

13. Kasem S, Rice N, Henry R. DNA extraction from plant tissue. In: Henry RJ, editor. Plant genotyping II: SNP technology. Wallingford: CAB International, 2008, 219-271p.
14. Osman GH, Assem SK, Alreedy RM, El-Ghareeb DK, Basry MA, Rastogi A *et al*. Development of insect resistance maize plants expressing the chitinase gene of *Spodoptera littoralis*. Nat Sci Rep. 2015; 5:18067–18077.
15. Varma A, Padh H, Shrivastava N. Plant genomic DNA isolation: an art or a science. Biotechnology Journal: Healthcare Nutrition Technology. 2007; 2(3):386-92.
16. Sharma AD, Gill PK, Singh P. DNA isolation from dry and fresh samples of polysaccharide-rich plants. Plant Molecular Biology Reporter. 2002; 20(4):415.
17. Moreira PA, Oliveira DA. Leaf age affects the quality of DNA extracted from *Dimorphandra mollis* (Fabaceae), a tropical tree species from the Cerrado region of Brazil. Genetics and Molecular Research. 2011; 10(1):353-358.
18. Khan S, Qureshi M, Kamaluddin A, Abdin M. Protocol for isolation of genomic DNA from dry and fresh roots of medicinal plants suitable for RAPD and restriction digestion. African J. Biotech. 2007; 6:175-178.
19. Khanuja SP, Shasany AK, Darokar MP, Kumar S. Rapid isolation of DNA from dry and fresh samples of plants producing large amounts of secondary metabolites and essential oils. Plant Molecular Biology Reporter. 1999; 17(1):1-7.
20. Sahu S, Thangaraj M, Kathiresan K. DNA extraction protocol for plants with high levels of secondary metabolites and polysaccharides without using liquid nitrogen and phenol. ISRN Mol Biol. 2012; Article ID 205049, 1-6. doi:10.5402/2012/205049.
21. Ribeiro R, Lovato M. Comparative analysis of different DNA extraction protocols in fresh and herbarium specimens of the genus *Dalbergia*. Genet Mol. Res. 2007; 6:173-187.