SHORT COMMUNICATION

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PCR compatible, genomic DNA extraction procedure for *lasora* (*Cordia myxa* (Roxb.)

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Cordia myxa (Roxb.) is commonly known as *"lasora"*. It is an under-exploited fruit and herbal tree in rural India. It belongs to the family *Boraginaceae* and probably originated in the Indian sub-continent (Samadia, 2005). It is widely distributed in the arid and semi-arid regions of India and yields fruit during April-May. Immature fruits of *lasora* are used for making pickles and vegetable curries, and matured fruits are very nutritious, rich in carbohydrates and total ash. This fruit tree is not a commercial crop but it grows naturally in the non-cultivable or waste lands, backyards, on roadsides and farm boundaries (Samadia, 2005). Conservation and understanding genetic diversity of this under-exploited crop is the primary requirement for identification of superior genotypes for its improvement and exploitation. Considerable diversity on the basis of morphological parameters has been reported in the naturally growing population of *lasora* in Rajasthan (Samadia, 2007). No report is available on the genetic diversity of *lasora* germplasm using DNA markers. Good quality, PCR compatible genomic DNA is essential for most of the PCR based markers. Our experience showed that the genomic DNA extracted from leaf tissue of *lasora* using many commercial kits did not yield amplicons with random hexaprimers (RAPD primers) in PCR (unpublished data). Therefore, standardization of a PCR-compatible DNA extraction procedure is essential for *lasora*. Here, we report the standardized PCR-compatible genomic DNA extraction procedure from the leaf tissue of *lasora*.

Young leaf tissues (2nd leaf from top of the twig) were collected from a single *lasora* tree and stored in an ice-box and brought to the laboratory immediately. One hundred mg of leaf tissue was weighed and stored at -20°C from eighteen samples (three samples for each treatment) (Table 1). Each sample was ground separately in a pestle and mortar with liquid nitrogen and transferred to 1.5 ml microcentrifuge tube. One ml of DNA extraction buffer (Dellaport *et al.*, 1983) (Table 1) was added to three samples separately and processed. These samples were incubated at 65 °C in a water bath for one hour. Samples were then removed from the water bath and brought to room temperature. To remove protein and other organics, 0.6 volume of chloroform: isoamyl alcohol (24:1) was added to the sample and mixed for 20 minutes and centrifuged at 10000 rpm for 10 minutes at 4 °C. The supernatant was removed and transferred to a fresh microcentrifuge tube and again chlorofom: isoamylalcohol (24:1) was added, mixed and centrifuged at 10000 rpm for 10 minutes at 4 °C. The supernatant was removed and transferred to a fresh microcentrifuge tube. To the supernatant, 0.8 volume of isopropanol was added and mixed gently and centrifuged at 10000 rpm for 10 minutes at 4 °C. The supernatant was decanted and to the pellet 400µl of 70 % ethanol was added and centrifuged for 10 minutes. The supernatant was decanted and the pellet was dried at room temperature for 30 minutes. 100µl of sterile double distilled water was added to the pellet and allowed to dissolve for two hours at room temperature or overnight at 4 °C. After dissolving, an aliquot of 50µl was taken from each sample and transferred to a fresh micro-centrifuge tube. Two µl of RNase A (10mg/ml) (MBI Fermentas) was added to each sample and kept at 37 °C for 30 minutes. The volume in each sample was made up to 200 µl with sterile double distilled water. Equal volume of chloroform: isoamyl alcohol (24:1) was added, mixed and centrifuged as described above and the supernatant was removed and transferred to a fresh microcentrifuge tube. To the supernatant, 1/10th volume of sodium acetate (pH 4.8) followed by 0.8 volume of isopropanol were added and mixed gently. The subsequent steps are same as described previously.

Four µl from each tube was loaded on a 0.7 % agarose gel with 10µl sterile double distilled water and 2µl of 6x loading dye and electrophoresed. After electrophoresis the gel was viewed under a UV transilluminator to check the quality of DNA. Quantification of DNA was done spectrophotometrically. The genomic DNA extracted from *lasora* was used in PCR to check its compatibility. A 50 as well as 25 µl PCR reaction volume was set up containing 100 ng of template DNA, 3mM $MgCl_2$, 20 pmol random primer (OPBE 07, Operon Technologies, USA), 1U *Taq* DNA polymerase, 0.2mM dNTP (MBI Fermentas) with the following thermal profile: initial denaturation of 94°C for 5

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Table 1 Effect of different treatments on DNA yield, quality and its compatibility in PCR

S.No.	Buffer details	DNA Yield µg/ µl	Quality of DNA	Compatibility in PCR*
Without RNase A treatment				
1.	DNA extraction buffer only	0.40-0.45	Sheared DNA with degraded RNA	Yes
2.	DNA extraction buffer + 0.1% β-ME	0.45-0.48	Less sheared DNA with degraded RNA	Yes
3.	DNA extraction buffer + 0.5% β-ME	0.75-0.80	No shearing of DNA with degraded RNA	Yes
4.	DNA extraction buffer + 1.0% β-ME	0.75-0.80	No shearing of DNA with degraded RNA	Yes
5.	DNA extraction buffer + 0.1% SDS	0.60-0.63	No shearing of DNA with degraded RNA	Yes
6.	DNA extraction buffer + 1% PVP	0.65-0.70	No shearing of DNA with degraded RNA	Yes
With RNase A treatment				
7.	DNA extraction buffer only	0.35-4.00	Sheared DNA, no RNA	Yes
8.	DNA extraction buffer + 0.1% β-ME	0.44-0.46	Less sheared DNA, no RNA	Yes
9.	DNA extraction buffer + 0.5% β-ME	0.72-0.77	No shearing of DNA, no RNA	Yes
10.	DNA extraction buffer + 1.0% β-ME	0.71-0.75	No shearing of DNA, no RNA	Yes
11.	DNA extraction buffer + 0.1% SDS	0.52-0.64	No shearing of DNA, no RNA	Yes
12.	DNA extraction buffer + 1% PVP	0.60-0.63	No shearing of DNA, no RNA	Yes

DNA extraction buffer- 100mM Tris-HCl (pH 8.0) + 1.4 M NaCl + 20mM EDTA + 2% CTAB

'*' DNA yield measured at 260 nm through spectrophotometer

'#' PCR compatibility checked with RAPD primer, OPBE-7

β-ME: β-mercaptoethanol; **SDS**: Sodium dodecyl sulphate; **PVP**: Polyvinyl pyrrolidone

min followed by 35 cycles of 94°C for 30 seconds, 36°C for 1 min and 72°C for 2 min 30 seconds. Final extension was set at 72°C for 10 min. The PCR products were loaded on a 1.4 % agarose gel pre-stained with ethidium bromide, electrophoresed and viewed under a UV transilluminator. The standardized DNA extraction and PCR procedure using random primers were validated with 22 lasora genotypes available at the experimental farm of Central Institute for Arid Horticulture.

On the basis of different treatments in the extraction of PCR compatible genomic DNA from *lasora*, higher amounts of DNA (0.75-0.80 µg/µl) was obtained with DNA extraction buffer containing 0.5 or 1.0 % β-ME without RNase A treatment followed by additives like SDS or PVP (Table 1). Similarly, the quality of DNA was also better in the β-ME treated samples. The quality of DNA in these samples was equally good even after RNase treatment, except for a slight reduction in the yield of DNA (~ 0.5 µg/ µl). Though, the yield and quality of DNA varied with different additives used with DNA extraction buffer, all the DNA samples with or without RNase treatment were found to be PCR compatible. The results obtained indicated that the quality of DNA may be improved by DNA extraction buffer alone which could easily remove the PCR inhibitors present in the leaf tissue. The *lasora* leaves contain high polysaccharides/mucilaginous substances (Samadia, 2005) and phenolics which inhibit *Taq* DNA polymerase activity (Fang *et al.*, 1992) in PCR. PCR incompatibility of genomic DNA extracted through commercial kits might have been due to the ineffective removal of inhibitors. However, this problem has been addressed by our current method. It was observed that the additives used in the present study could improve the DNA yield greatly. DNA extracted using 0.5 to 1.0 % β-ME added to the extraction buffer and without RNase A treatment was found superior to all other treatments. The reason for higher DNA yields may be due to the fact that β-ME is a reducing agent and it probably inhibited the activity of DNA degrading nucleases (Lodhi *et al.*, 1994). Among the treatments, DNA extraction buffer with 0.5% β-ME without RNase treatment was found to be the best for DNA extraction and 25 µl of final volume in PCR is sufficient. Genomic DNA extracted from 22

genotypes of *lasora* available at the CIAH farm using this procedure was found PCR compatible with different RAPD primers. This procedure could be very useful in studying its genetic diversity through DNA based markers and also for its improvement. To our knowledge, this is the first report on a DNA extraction procedure for the mucilaginous fruit tree, *lasora*.

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