SUPPLEMENTARY MATERIAL

Table S1. Detailed steps of each procedure tested for DNA extraction of *Mimosa tenuiflora*.

Protocol				
Steps	Murray and Thompson (1980)	Haberer *et al.* (1996)	Bonato *et al.* (2002)	Faleiro *et al.* (2003)
Rupture of cell walls	Maceration in liquid N2 using 2.0 mL tubes and glass stirring rod.	Maceration in liquid N2 using 2.0 mL tubes and glass stirring rod.	Maceration of 300 mg of leaf tissue in liquid N2 using a porcelain crucible followed by transference to 2.0 mL tubes.	Maceration of 300 mg of leaf tissue in liquid N2 using a porcelain crucible followed by transference to 2.0 mL tubes.
Rupture of cell membranes	Extraction buffer: 1 M Tris pH 8.0; 0.5 M EDTA; 5 M NaCl; 2% CTAB; H2O to a final volume of 50 mL.	Extraction buffer: 1 M Tris pH 8.0; 0.5 M EDTA; 5 M NaCl; 1% CTAB; H2O to a final volume of 50 mL.	Extraction buffer (add 900 µL to each tube): 100 mM Tris-HCl; 20 mM EDTA; 1.4 M NaCl; 0.2% β-mercaptoethanol; 2% CTAB pH 8; 1% PVP.	Extraction buffer (add 800 µL to each tube): 100 mM Tris-HCl pH 8; 20 mM EDTA; 1.3 M NaCl; 1.3% CTAB; 0.2% β-mercaptoethanol; 1% PVP.
Protein digestion	Add 620 µL of buffer solution + 1.56 µL of proteinase K + 69 µL of 20% SDS. Incubate at 65°C for 1 hour, inverting the tubes each 20 min. After cooling, add 315 µL of phenol and 315 µL of chloroform:isoamyl alcohol (24:1), inverting the tubes to form an emulsion. Centrifuge for 5 min at 13,273 rpm and transfer the supernatant to a new tube.	Add 650 µL of buffer solution. Incubate at 65°C for 30 min, inverting the tubes each 10 min. After cooling, add 550 µL of chloroform:isoamyl alcohol (24:1), inverting the tubes for 5 min. Centrifuge for 5 min at 10,753 rpm. Transfer the supernatant to a new tube.	Incubate at 65°C for 1 hour, inverting the tubes each 10 min. After cooling, centrifuge for 10 min at 4,500 rpm. Add 500 µL of phenol-chloroform (1:1 v/v), inverting the tubes for 10 min. Centrifuge for 10 min at 4,500 rpm. Transfer the supernatant to a new tube and repeat the procedure.	Incubate at 70ºC for 60 min, inverting the tubes each 10 min. After cooling, add 700 µL of chloroform:isoamyl alcohol (24:1), inverting the tubes for 10 min. Centrifuge for 10 min at 4ºC using 18,845 rpm. Transfer the supernatant to 2.0 mL tubes. Add 55 µL of 7% CTAB and repeat the deproteinization process.
DNA precipitation	Add cooled isopropanol up to 2/3 of sample volume, followed by gentle mixing (up to visualization of DNA cloud). Centrifuge for 6 min at 5,206 rpm to form a DNA pellet.	Add RNAse (10mg/mL) in a ratio of 17% of supernatant volume Incubate at 37°C for 60 min, followed by adding isopropanol in a proportion of 67% of supernatant volume, with gentle mixing. Incubate overnight at 4ºC (for at least 5 hours) or at -20ºC for 15 min. Centrifuge for 30 min as abovementioned., wash the pellet and let it dry.	Transfer the supernatant of each sample to 1.5 mL tubes. Add 600 µL of cooled (-20ºC) isopropanol. Incubate at -20ºC for 30 min. Centrifuge at 10,000 rpm for 5 min.	Add 700 µL of cooled isopropanol. Store the tube at -20ºC for 2 hours and centrifuge as abovementioned.
DNA washes	Wash the pellet in 500 µL of cooled 70% ethanol. Centrifuge for 3 min. Discard the supernatant and let the DNA dry at 37°C for 2 hours.		Wash the pellet twice in 70% ethanol (v/v) and let it dry at room temperature for about 60 min. Add 400 µL of TE buffer. Precipitate again the DNA by adding 20 µL of 5 M NaCl and 800 µL of 100% ethanol (-20ºC), following gentle mixing. Centrifuge at 10,000 rpm for 5 min. Let the pellet dry and add 100 µL of TE containing 6 µL of RNAse (10 mg/mL) and store at 37º C for 30 min.	Discard the supernatant and wash the pellet twice in 70% ethanol (v/v). Let it dry at room temperature for about 60 min.
Degradation of RNA	Add 50 µL of 0.1x TE with RNAse. Incubate for 30 min at 37°C.			Add 150 µL of water with RNAse (40 µg/mL) and incubate at 37ºC for 60 min.
DNA reprecipitation	Precipitate the DNA in 5 µL of cooled 3 M sodium acetate and 100 µL of 95% ethanol. Store at -20°C for 10 min.			Precipitate the DNA again. Centrifuge and add 150 µL of water. Store the extracted DNA at a -20ºC.
Storage of DNA solution	Centrifuge for 5 min. Wash the pellet in 300 µL of 70% ethanol. Add 50 µL of 0.1x TE.	Add 30 µL of 0.1x TE pH 8 0 or milliQ water.	Store the DNA at -20º C.	

Protocol				
Steps	Promega kit	Roy *et al.* (1992)	Doyle and Doyle (1987)	Optimized protocol
Rupture of cell walls	Maceration in liquid N2 using 2.0 mL tubes and glass stirring rod.	Maceration in liquid N2 using 2.0 mL tubes and glass stirring rod.	Maceration with 700 µL of extraction buffer using 2.0 mL tubes and glass stirring rod.	Maceration in liquid N2 using 2.0 mL tubes and glass stirring rod.
Rupture of cell membranes	Extraction buffer: 600 µL of Lysis Solution.	Extraction buffer (600 µL per tube): 100 M Tris pH 8.0; 0.02 M EDTA; 1.4 M NaCl; 2% CTAB; 0.5% β-mercaptoethanol; Proteinase k (10 mg/ml).	Extraction buffer (700 µL per tube): 2% CTAB; 1.4 M NaCl; 100 mM Tris-HCl; 20 mM EDTA, pH 8.0; 1% PVP; 0.2% β-mercaptoethanol; 50 $g.ml^{-1}$ of proteinase k.	Extraction buffer (600 µL per tube): 100 M Tris pH 8.0; 0.02 M EDTA; 2.5 M NaCl; 3% CTAB; 5% β-mercaptoethanol; 2.5 % PVP; Proteinase k (10 mg/ml).
Protein digestion	Vortex for 1-3 seconds to homogenize the solution. Incubate at 65°C for 15 min. Add 200 µL of Protein Precipitation solution and homogenize for 20 seconds using vortex. Centrifuge for 3 min at 13,000 rpm.	Incubate at 65ºC for 30 min, inverting the tubes gently each 10 min. Add 700 µL of chloroform:isoamyl alcohol (24:1). Mix gently the samples for 10 min. Centrifuge for 15 min at 14,000 rpm.	Incubate at 60ºC for 30 min. Add 600 mL of chloroform:isoamyl alcohol (24:1). Centrifuge for 5 min at 14,000 rpm.	Incubate at 60ºC for 30 min, inverting the tubes gently each 10 min. After cooling, centrifuge the samples at 13,000 rpm for 10 min. Add 350 µL of phenol and 350 µL of chloroform:isoamyl alcohol (24:1). Invert the tubes for 10 min. Centrifuge at 13,000 rpm.
DNA precipitation	Transfer the supernatant to a new tube containing 600 µL of isopropanol at room temperature. Invert gently and centrifuge at 13,000 rpm for 1 min at room temperature.	Transfer the supernatant to 2 mL tubes and add 600 µL of cooled isopropanol. Store the tubes at -20ºC for 24 hours. Centrifuge at 14,000 rpm for 20 min.	Transfer the supernatant to a new tube containing 10% CTAB, 1.4 M NaCl, and homogenize for 5 min. Centrifuge at 12,000 rpm for 5 min.	Transfer the supernatant to 2 mL tubes. Add cooled isopropanol (v/v). Store the tubes at -20ºC for 24 hours. Centrifuge at 14,000 rpm for 20 min.
DNA washes	Wash the pellet in 600 µL of 70% ethanol. Remove the ethanol and let it dry for 15 min. Add 100 µL of Rehydration solution and incubate at 65°C for 1 hour. Add 3 µL of RNAse solution and incubate at 37°C for 15 min. Store the DNA at 2-8°C.	Wash twice in 200 µL of 70% ethanol (v/v) and let it dry at room temperature for about 60 min.	Transfer the supernatant to a new tube and add cooled isopropanol in a proportion of 2/3 of the supernatant volume. Store at -20°C for 1 to 2 hours. Centrifuge at 7,500 rpm for 5 min. Wash the pellet twice in 1 mL of 70% ethanol, followed by another wash in 1 mL of 95% ethanol.	Wash the pellet twice in 200 µL of 70% ethanol (v/v) and let it dry at room temperature for about 60 min.
Degradation of RNA		Add 100 µL of milliQ water and 6 µL of RNAse (10 mg/mL). Store at room temperature.	Add 50 to 100 mL of TE with RNAse. Store at 37ºC for 2 hours or overnight at 4ºC.	Add 50 µL of TE and 3 µL of RNAse. Incubate at 37ºC for 2 hours. Store the DNA at 2-8°C.
DNA reprecipitation				
Storage of DNA solution				