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APPENDICES

APPENDIX A

PROTOCOL FOR DNA EXTRACTION OF HERBARIUM SPECIMENS

This is a protocol for DNA extraction from herbarium specimens using a combination of CTAB method (Agrawal et al., 1992) and QIAquick PCR Purification Kit (QIAGEN).

Materials:

1. Pestles and mortars
2. 1.5 µl microcentrifuge tubes
3. CTAB buffer
4. β-mercaptoethanol
5. Chloroform : isoamylalcohol (24 :1) mixture
6. QIAquick PCR Purification Kit (QIAGEN)
7. Water-bath
8. Liquid nitrogen
9. RNase A (100mg/ml)

Preparation:

1. Preheat CTAB buffer in water-bath to 65°C
2. Set the microcentrifuge tube filled with extraction buffer containing 650 µl CTAB buffer, 20 µl β-mercaptoethanol and 10 µl RNase A at 65 °C
3. Clean leaf with 70% alcohol, then remove midrib and weigh 50 mg leaf material of each sample

Extraction:

1. Add liquid nitrogen to a pestle containing leaf material and grind to powder with a mortar.
2. Transfer powder to extraction buffer in a microcentrifuge tube, vortex 10 second, and incubate 65 °C for 3 hours, mixing every 10 minutes.
3. Add 650 µl of Chloroform : Isoamylalcohol mixture, invert gently 5 times and incubate by gently shaking at room temperature. Leave for at least 1½ hours
4. Centrifuge at 10,000 rpm at RT for 10 minutes
5. Transfer supernatant into a new microcentrifuge tube
6. Add 1ml Buffer PB (provided in QIAquick PCR Purification Kit) and mix by pipetting
7. Place a QIAquick spin column in a collecting tube
8. To bind DNA, apply the sample from step 6. to the QIAquick column and centrifuge for 30-60 second
9. Discard flow-through and place the QIAquick column back into the same tube
10. To wash, add 500 µl Buffer PE to the QIAquick column and centrifuge for 30-60 second
11. Discard flow-through and place the QIAquick column back into the same tube. Centrifuge the column for an additional 1 minute
12. Place QIAquick column in a clean microcentrifuge tube
13. To elute DNA, add 30 µl Buffer EB or H₂O to the center of the QIAquick membrane, let the column stand for 1 minute and centrifuge the column for 1 minute
14. DNA is ready to use

APPENDIX B

CHARACTER SCORING FOR MORPHOLOGICAL DATA MATRIX

1. Habit: (0) shrubs or small trees; (1) large trees
2. Indument of young primary shoots: (0) generally glabrous to hairy;
(1) invariably densely hairy/velutinous
3. Glossiness of leaf lamina (adaxially): (0) matt; (1) nitid
4. Prominence of secondary veins (adaxially):
(0) impressed to slightly prominent; (1) distinctly prominent
5. Tertiary vein arrangement: (0) reticulate; (1) percurrent
6. Flower position: (0) axillary; (1) (slightly) supra-axillary
7. Occurrence of flower fascicles: (0) absent; (1) present
8. Flower position on branches: (0) young growth only; (1) also older growth
9. Flower position on trunk: (0) not exclusively at base; (1) exclusively at base
10. Number of flowers: (0) solitary (occasionally paired); (1) invariably paired
11. Flower orientation: (0) pendent; (1) erect
12. Flower pedicel length: (0) up to 20 mm maximum; (1) 21-50 mm maximum;
(2) 51-110 mm maximum
13. Sepal fusion: (0) free; (1) (sometimes) basally connate
14. Sepal venation: (0) indistinct; (1) (sometimes) distinct
15. Sepal reflexion: (0) not reflexed; (1) reflexed
16. Outer petal length: (0) up to 45 mm maximum; (1) 50-85 mm maximum;
(2) over 100 mm maximum
17. Shape of outer petal base: (0) not distinctly clawed; (1) distinctly clawed
18. Indument of basal adaxial region of outer petals:
(0) glabrous or sparsely hairy; (1) velutinous
19. Shape of inner petals: (0) without extensive contiguous area:
(1) extensive contiguous area
20. Indument of inner petal (adaxially): (0) glabrous; (1) velutinous; (2) woolly
21. Presence of glabrous basal flanges on inner petal claw:
(0) absent; (1) present

22. Stamen number per flower: (0) up to 320 maximum; (1) 450-570 maximum
23. Staminal connective shape: (0) truncate; (1) apiculate;
(2) very long apiculate
24. Apiculate staminal connective shape:
(0) not distinctly tapered; (1) distinctly tapered
25. Carpel number per flower: (0) up to 60 maximum; (1) 90-105 maximum
26. Ovary indument: (0) essentially glabrous; (1) hairy
27. Style indument: (0) glabrous; (1) hairy
28. Stigma shape: (0) funnel-shaped or subulate; (1) large, convoluted
29. Stigma indument: (0) (sub-) glabrous; (1) hairy
30. Sepal persistence in fruit: (0) caducous; (1) persistent
31. Monocarp shape: (0) not moniliform; (1) slightly moniliform (when > 2 seeds)
32. Monocarp width: (0) up to 15 mm maximum; (1) 15-25 mm maximum;
(2) 26-30 mm maximum
33. Occurrence of longitudinal ridge on monocarp: (0) absent; (1) present
34. Monocarp ornamentation: (0) smooth; (1) verrucose
35. Pericarp thickness: (0) thin; (1) (medium-) thick
36. Seed width: (0) up to 16 mm maximum; (1) 17-20 mm maximum
37. Seed number per monocarp: (0) 1-2 (-3); (1) 4-5
38. Seed shape around micropyle: (0) not elongated; (1) elongated
39. Indument of seed testa: (0) glabrous; (1) hairy
40. Seed micropylar plug: (0) sunken; (1) flush; (2) protruding
41. Mucilage around seeds: (0) slight; (1) copious
42. Inner petal arrangement: (0) not mitriform; (1) mitriform
43. Stamen septation: (0) aseptate; (1) septate

BIOGRAPHY

Maliwan Nakkuntod was born in Nakhon Ratchasima Province, Thailand, on 7 January 1975. She earned her Bachelor Degree in Science in Biology from the Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, in 1995. In 1998, she received her Master of Science Degree in Botany from Department of Botany, Kasetsart University, Bangkok. After graduation, she worked as lecturer at Department of Biology, Faculty of Science, Naresuan University, Phitsanulok. Since June 2001, she pursued her Ph.D. study in Biological Science Ph.D. program, Faculty of Science, Chulalongkorn University, Bangkok.

