Appendix 3.2: Location of study site and specimen collection number.

Species		Site of collection	Site of transplanting and observation	Collection number
A. sessilis	Red leaf form	Rimba Ilmu Botanic Garden, University of Malaya, Kuala Lumpur.	Rimba Ilmu Botanic Garden, University of Malaya, Kuala Lumpur.	ASR001 (KLU)
	Green leaf form	Taman Bukit Cheras, Kuala Lumpur.	Outside the green house of Institute of Biological Sciences, University of Malaya, Kuala Lumpur.	ASG019 (KLU)
A. brasiliana	Parent plant	Institute of Biological Sciences, University of Malaya, Kuala Lumpur.	Is not transplanted.	ABRA001 (KLU)
	OffspringS	Seeds harvested from the parent plant.	Outside the green house of Institute of Biological Sciences, University of Malaya, Kuala Lumpur.	ABRA004 (KLU)
A. bettzickiana		Dewan Tunku Cancelor, University of Malaya, Kuala Lumpur.	Outside the green house of Institute of Biological Sciences, University of Malaya, Kuala Lumpur.	AFB003 (KLU)
A. paronychioides		Kuala Selangor Nature Park, Selangor.	Outside the green house of Institute of Biological Sciences, University of Malaya, Kuala Lumpur.	AP004 (KLU)
A. ficoidea		Forest Research Institute Malaysia, Selangor	Is not transplanted.	ABZ003 (KLU)

Appendix 3.3: Procedure for microtechnique (Johansen, 1940).

Preparation of plant materials for microtome sectioning

1. Fixing: the specimens were cut to the required size and then fixed in Craf III solution for at least 48 hours.
2. Pumping: suction was applied at 25–30 atmospheric pressure for 10–15 minutes and the specimens were left for at least 48 hours to one week.
3. The specimens were washed in 50% alcohol for a few minutes.
4. Dehydration: the specimens were dehydrated through a series of Tertiary Butyl Alcohol (TBA) as follows:-

Step	95% ethyl alcohol (ml)	100% ethyl alcohol (ml)	Tertiary Butyl Alcohol (TBA) (ml)	Distilled water (ml)
1	25			25
2	25		5	20
3	25		10	15
4	25		17.5	7.5
5	25		25	
6		12.5	37.5	
7			50	
8			50	
9			50	

Step 9 was consisted of new unused TBA. Step 7 and 8 was utilized the TBA that has been used one or twice. Material may be stored for several days at steps 2 or 3. At any other step, it is best not to keep material more than one day.

5. Infiltration:
   a) After leaving the specimens in clean and fresh TBA for 12 hours (in step 9), a few chips of aerated wax (49°C) were added to the vial and kept at room temperature (28°C) overnight. If the wax has completely dissolved by the next morning, a few more chips of wax were added and left for a few more hours at room temperature.

b) When no more wax could dissolve at room temperature, the vials were placed in the oven at approximately 58°C.
c) After about two hours, approximately ¼ of alcohol-wax mixture was poured off and replaced with ¼ of 49°C wax mixed with TBA.
d) After about another two hours, approximately ½ of alcohol-wax mixture was poured off and replaced with ½ of 49°C wax mixed with TBA.
e) After another two hours, all liquid was poured off and replaced with 49°C wax.
f) Four hours later, all 49°C wax poured off and replaced with fresh and clean 49°C wax.
g) After 12 hours, fresh and clean Paraplast Plus Tissue Embedding Medium was changed (melting point 56°C).
h) After at least 12 hours, suction at 25–30 atmospheric pressure was applied for 20 minutes at 70°C and this process was repeated 3 times at 4 hourly intervals.
i) The specimens were ready to be embedded.

6. Embedding
    a) The paraplast plus wax was poured out together with the specimens into a paper boat.
    b) The specimens were arranged properly and the wax block was left to cool in ice water.

7. Sectioning
    a) When the wax block has cooled down, the wax specimens were cut into suitable size with proper TS or LS position.
    b) The buds of flowers were sectioned at 6–8 µm thickness using a rotary microtome in an air-conditioned room.

8. Mounting
    a) A little drop of egg albumin was smeared on a clean slide.
    b) A little distilled water was put onto the slide.
    c) A wax ribbon with the specimen was placed on top of the distilled water (the smooth side of the wax ribbon facing downwards).
    d) The slide was warmed on a drying bench to allow tissue to spread out.
    e) When the tissue was fully stretched, the slide was removed to drain away the excess water.
    f) The slide was dried in the oven at 40°C for at least 2 days before staining.

9. Staining in safranin-fast green
    a) The slides were placed in a trough of xylene for 20 minutes to remove the wax.
    b) Then, they were placed in a mixture of xylol-alcohol (1:1 xylene: 95% ethanol) to further remove all the wax traces.
    c) The slides were then transferred to 95%, 80%, 70% and 50% ethanol for 5 minutes in each solution.
    d) They were then stained in 1% safranin “O” in 50% ethanol for 12– 24 hours.
    e) The excess safranin “O” was washed away in a basin of tap water.

f) The slides were then passed through 50%, 70% and 95% ethanol and for 5 minutes in each step.

g) The slides were stained for 3–4 seconds in fast green “FCF”.

h) The slides were then differentiated in two changes of xylol-alcohol for 5 minutes each, followed by carbol-xylol (3:1 phenol crystal: xylene) for 15 minutes.

i) The slides were cleared in two changes of xylene (30 minutes followed by 1 hour).

j) The specimens were mounted in Canada balsam (1:1 Canada balsam: xylene) and dried in the oven at 40°C for four days.

k) The slides were ready to be observed under a compound microscope.

Appendix 3.4: Procedure for pollen acetolysis (Erdtman, 1960).

1. The acetolysing mixture consisting of 9 parts of acetic anhydride and 1 part of concentrated sulphuric acid was prepared.

2. 5 ml of the acetolysing mixture was poured into a glass tube with pollen grains and was placed in a water bath (70°C).

3. The temperature of the water bath was increased from 70°c to boiling point.

4. The mixture was constantly stirred by using a glass rod. The glass tube was taken out when the colour of the mixture had turned dark brown.

5. The mixture was filtered with a fine wire-mesh.

6. The tube was balanced with glacial acetic acid and centrifuged at 500–1000 R.P.M. for 15 minutes. The mixture was stirred before it was centrifuged.

7. The acetolysing mixture was poured off quickly but gently.

8. 5 ml of washing mixture consisting of 3 parts of distilled water and 1 part of 95% alcohol was prepared.

9. The washing was repeated three times to get rid of the acid from the specimens. Note: pollen which had turned very dark acetolysing was bleached by adding two or three drops of sodium chlorate followed by the two to three drops of concentrated hydrochloric acid.

10. The washing mixture was poured off and the pollen grain was dried in an oven at 40°C.

11. A small piece (approximately 1 mm^2) of glycerine jelly stained with safranin was cut and dipped into the tube with pollen grains.

12. The glycerine jelly was transferred on to a clean slide.

13. A small piece of wax was placed around the periphery of the cover slip.

14. The slide was warmed gently by using a spirit lamp to melt the wax and glycerine jelly.

Appendix 3.5: Sitting drop culture for *in vitro* germination of the pollen
(Shivanna & Rangaswamy, 1992)

1. Two drops of culture medium were placed on a clean dry concave slide.
2. Pollen grains of three anthers from each flower were mixed in the medium. With two needles, a homogeneous distribution of pollen was made in the medium.
3. The culture was labelled and kept in a petri dish lined with wet filter paper.
4. The culture was kept at the temperature of 22°C.
5. After 8 hours, the pollen culture with germinated pollen grains was fixed in 10% ethanol, stained with 0.1% neutral red and temporarily sealed with nail polish.

Appendix 3.6: Brewbaker and Kwack's medium (Brewbaker & Kwack, 1963)
The following constituents were mixed in 1L of distilled water.

No	Materials	Amount (g)
1	Boric acid	0.1
2	Calcium nitrate	0.3
3	Magnesium sulphate	0.2
4	Potassium nitrate	0.1

In order to prepare 2% of sucrose solution, 2g of sucrose was added into 100 ml of Brewbaker and Kwack's medium. The subsequent sucrose concentration (4, 6, 8, 10, 12, 14, 16, 18, 20 and 22) would be prepared with the same method