APPENDIXES

Appendix A: Critical Point Drying Procedures – preparation of specimens for scanning electron microscopy

1. Specimens were fixed in Craft III solution.
2. 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. After 48 hours, specimens were then fixed in alcohol 50% and prepared to use.
4. Specimens were washed in distilled water, 15 minutes with two changed.
5. After washing, specimens were soaked in glutaraldehyde + Sorensen's phosphate buffer (ratio 1:1) for one hour.
6. The solution was then discarded and changed to Sorensen's phosphate buffer + distilled water (1:1)
7. Discarded the solution and changed to osmium + distilled water (1:3) for 14 hours in a fume hood.
8. The specimens were dehydrated through a graded ethanol series from 10% to 90% (15 minutes in each of 10% steps).
9. The specimens were replaced by 95% ethanol and soaked for 15 minutes.
10. The specimens were changed with absolute ethanol for twice, 15 minutes each time.
11. Then specimens were soaked in 100% ethanol + 100% acetone solutions for 20 minutes, each at the ratio of 3:1, 1:1 and 1:3 and finally into pure acetone (100%) for 20 minutes.
12. The specimens were then left for one hour with three changes.
13. The specimens were then critical point dried.
14. The specimens were transferred on to a stub very gentle on a small amount of Electrodag 915 or Silverdag.
15. The specimens were coated with gold using a diode sputter coater.

16. The specimens were ready and can be scanned using a JEOL JSM-6400 scanning electron microscope.

Appendix B: Preparing the solutions for anatomical and embryological studies

1. Craf III Solution, fixing the specimen

1% Chromic acid	30 ml
10% Acetic acid	20 ml
40% Formaldehyde	10 ml
Distilled water	40 ml

2. Safranin "O"

   4 grams of the dye was dissolved in 200 ml of 2-methoxyethanol. Then 100 ml of 95% alcohol and 100 ml of distilled water were added.
   4 grams of sodium acetate in 8 ml formalin was added.

3. Fast green "FCF"

Fast green	1 g
Clove oil	30 ml
2-methoxyethanol	30 ml
Absolute alcohol	30 ml

4. Carbol-xylol

Xylene	300 ml
Phenol crystals	100 g

5. Xylol-alcohol

Xylene	100 ml
95% alcohol	100 ml

6. Egg albumen
   (a) Egg white was cutted with scissors.
   (b) An equal volume of distilled water and glycerine was added, followed by 0.5% sodium benzoate (1% of total volume).
   (c) The mixture was stirred and filtered.
   (d) The solution was then stored in a refrigerator.

7. Alcohol solutions

   50% alcohol = 50 ml 95% alcohol + 45 ml distilled water

   70% alcohol = 70 ml 95% alcohol + 25 ml distilled water

   80% alcohol = 80 ml 95% alcohol + 15 ml distilled water

8. Aerated wax

   (a) 49 ℃ wax was poured into a clean paper box

   (b) The wax was stirred until it became hard to introduce air into the wax.

Preparation of plant materials for microtome sectioning

1. Fixing: The specimen was 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 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 once or twice.

   Materials 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 ℃) were added to the vial and kept at room

temperature (28 ℃) 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) After about two hours, approximately 1/4 of alcohol-wax mixture was poured off and replaced with 1/4 49 ºC wax mixed with TBA.

(d) After about another two hours, approximately 1/2 of alcohol-wax mixture was poured off and replaced with 1/2 49 ℃ wax mixed with TBA.

(e) After another two hours, all liquid was poured off and replaced with 49 ℃ wax.

(f) Four hours later, all 49 ℃ wax was poured off and replaced with fresh and clean 49 ℃ wax.

(g) After 12 hours, fresh and clean Paraplast Plus Tissue Embedding Medium was changed (Melting point 56 ℃).

(h) After at least 12 hours, suction at 25–30 atmospheric pressure was applied for 20 minutes at 70 ℃ 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 sizes with proper TS or LS position.

(b) The buds or flowers were sectioned at 6–8 µm thickness while fruits at 8–10 µm using a rotary microtome in an air-conditioned room.

8. Mounting

(a) A little drop of egg albumin (Appendix 1) 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 the 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 ℃ 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 (Appendix 1) 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" (Appendix 1).

(h) The slides were then differentiated in two changes of xylol-alcohol (5 minutes each) followed by carbol-xylol (3:1 phenol crystals: xylene) and 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 ℃ for four days.

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

Appendix C: Procedure for pollen acetolysis (Erdtman, 1960)

1. The acetolysing mixture was prepared by adding 9 parts of acetic anhydride and 1 part of concentrated sulphuric acid (9:1).
2. Approximately 5 ml of the acetolysing mixture was poured into a clean tube with pollen grains and the tube was put into a water bath (70 ℃).
3. The temperature of the water bath was raised slowly from 70 ℃ to boiling point.
4. The tubes were left in the water bath for about 5 to 10 minutes and the content was stirred occasionally with the glass rod.
5. The tubes were taken out of the water bath when the colour of the solution had turned dark brown.
6. The solution was filtered with a fine wire-mesh and centrifuged at 500-1000 rpm for 15 minutes.
7. The acetolysing mixture was poured off quickly but gently.
8. 5 ml of washing mixture consisting of 3 parts distilled water and 1 part 95% ethanol was added. The tube was shaken and then centrifuged.
9. This washing was repeated for 3 times to get rid of the acid from the specimens.
   Note: Pollen grains which had turned very dark after acetolysis were bleached by adding two or three drops of sodium chlorate, followed by two to three drops of concentrated hydrochloric acid. Chlorine gas evolved would instantly bleach the dark pollen grains. These pollen grains were rinsed three to four times with the washing mixture to get rid of the acid.
10. The washing fluid was poured off and the tubes were inverted on a blotting paper and placed in the oven at 40 ℃ for approximately 10 to 20 minutes to dry.
11. A small piece of glycerine jelly stained with safranin was cut and dipped into the tube containing the pollen grains.
12. The small piece of glycerine jelly was transferred on the centre of a clean slide.
13. A coverslip was put on the centre of the clean slide and a small piece of wax was put around the periphery of the coverslip.
14. The slide was warmed gently above a spirit lamp to allow the wax and glycerine jelly to melt under the coverslip.
15. When the jelly was spread out, the pollen grains were sealed in by the wax.

Appendix D: Preparation of media and solutions for cytotoxic activity

Basic RPMI 1640 medium, DMEM and MEM media

Medium was prepared by dissolving one bottle of medium powder, 10.4 g, (Sigma) in one litre sterile distilled water. Two grammes of sodium bicarbonate (Merck) and 0.5026 g of N-2-hydroxylethyl-piperazine-N'-2-ethane-sulfonic acid (HEPES, Sigma) were added. The medium was stirred and the pH was adjusted to pH 7.4. The medium was filter sterilized using 0.22 µm filter membrane (Schleicher & Schuell) and stored at 4 ℃ up to a shelf life of 2 months.

10% supplemented RPMI 1640 medium, DMEM and MEM media

100 ml of 10% supplemented medium was prepared by mixing 90 ml of basic medium, 10 ml of heat inactivated foetal bovine serum (FBS was heated at 56 ℃ for 30 min, PAA lab), 2 ml of penicillin/streptomycin (PAA lab) and 1 ml of fungizone (PAA lab). The medium was filter sterilized using 0.22 µm filter membrane and stored at 4 ℃ up to 2 weeks.

20% supplemented RPMI 1640 medium, DMEM and MEM media

50 ml of 20% supplemented medium was prepared using 45 ml of 10% supplemented medium and 5 ml of heat inactivated FBS in a beaker. The medium was filter sterilized using 0.22 filter membrane and stored at 4 ℃ up to 2 weeks. This 20% supplemented medium was used to revive cells.

Freezing RPMI 1640 medium, DMEM and MEM media

The freezing medium consisted of 50% inactivated FBS, 40% of medium and 10% of DMSO (Sigma). The solution was mixed well using a 20 ml disposable syringe (Terumo) and filter sterilized using 0.22 filter membrane and stored at 4℃. Freshly prepared freezing medium was using for cryopreservation of cells.

Phosphate buffer saline (PBS) pH 7.2

The PBS was prepared using 1.52 g of disodium hydrogen orthophosphate anhydrous (Na_2HPO_4, BDH), 0.58 g of potassium dihydrogen orthophosphate (KH_2PO_4, BDH) and 8.5 g of sodium chloride (NaCl, BDH), which were dissolved in 1 litre of sterile distilled water. The solution was stirred and the pH was adjusted to pH 7.2. The solution was then filter into a sterilized bottle using filter paper (Whatman 541). The solution was autoclaved and kept at room temperature.

0.4% Tryphan blue

0.4% Tryphan blue was prepared by dissolving 0.2 g of powdered tryphan blue (sigma) in 50 ml sterile distilled water. The solution was kept at room temperature.

Neutral red cytotoxic activity assay

Preparation of solutions

Neutral red stock solution

0.04 g of neutral red (ICN, Biomedicals Inc.) was dissolved in 10 ml of sterile distilled water to provide a final concentration of 4 mg/ml. The stock solution was wrapped with aluminium foil and stored at 4℃.

Neutral red medium

The neutral red stock solution was further diluted (1:80) in treatment culture medium to give a final concentration of 50 µg/ml in a centrifuge tube. The solution was wrapped with aluminium foil and centrifuge twice at 1500 rpm for 10 minutes to remove any precipitate of dye crystals.

Neutral red washing solution

The washing solution consisted of 1 ml of formaldehyde (Sigma) and 1 g of calcium chloride ($CaCl_2$, Sigma), which were dissolved in 100 ml distilled water and stored at 4 ℃.

Neutral red resorb solution

The resorb solution was prepared by adding 1 ml of glacial acetic acid (Merck) into a mixture of 50 ml absolute ethanol (Hamburg) and 49 ml distilled water. The solution was stored at 4 ℃.

Appendix E: Pollen morphology in heterostylous variety of *P. chinensis* var. *chinensis*

Pin flower, highland

	Length of style (mm)	thickness of stigma (mm)	width of stigma (mm)	length of filaments, mm (distinct stamen)	length of filaments, mm (epipetalous stamen)
1	2.41	0.125	0.242	1.68	0.99
2	2.38	0.146	0.21	1.62	1.04
3	2.23	0.119	0.256	1.56	1.03
4	2.39	0.141	0.245	1.61	1.01
5	2.36	0.144	0.234	1.59	1.00
6	2.45	0.118	0.223	1.58	0.98
7	2.17	0.129	0.223	1.64	1.04
8	2.29	0.142	0.219	1.60	1.02
9	2.36	0.133	0.212	1.57	1.02
10	2.58	0.147	0.246	1.57	1.03
11	2.67	0.152	0.267	1.64	1.01
12	2.57	0.145	0.225	1.58	1.05
13	2.28	0.154	0.269	1.57	1.02
14	2.29	0.138	0.263	1.56	0.97
15	2.48	0.142	0.223	1.62	0.99
16	2.49	0.141	0.244	1.61	1.00
17	2.4	0.15	0.225	1.61	1.05
18	2.63	0.135	0.23	1.63	1.10
19	2.33	0.133	0.232	1.59	1.12
20	2.67	0.156	0.226	1.63	1.09
21	2.43				
22	2.37				
23	2.34				
24	2.3				
25	2.36				
26	2.41				
27	2.43				
28	2.41				
29	2.32				
30	2.42				
STDEV	0.122	0.011	0.018	0.032	0.040
Mean	2.41	0.140	0.236	1.603	1.028

Thrum flower, highland

	Length of style (mm)	thickness of stigma (mm)	width of stigma (mm)	length of filaments, mm (distinct stamen)	length of filaments, mm (epipetalous stamen)
1	1.17	0.171	0.255	2.16	1.52
2	1.16	0.172	0.266	2.10	1.72
3	1.13	0.197	0.265	2.06	1.62
4	1.06	0.172	0.261	2.19	1.50
5	1.12	0.156	0.268	2.21	1.48
6	1.18	0.204	0.282	2.09	1.63
7	1.06	0.137	0.255	2.02	1.52
8	1.17	0.159	0.268	2.28	1.58
9	1.14	0.161	0.225	2.26	1.63
10	0.99	0.175	0.277	2.13	1.52
11	1.04	0.17	0.258	2.16	1.50
12	1.03	0.17	0.285	2.14	1.64
13	1.00	0.164	0.272	2.05	1.69
14	1.00	0.174	0.25	2.21	1.70
15	1.09	0.179	0.23	2.08	1.54
16	1.15	0.19	0.252	1.99	1.62
17	1.13	0.173	0.257	2.06	1.52
18	1.08	0.183	0.268	2.15	1.57
19	1.14	0.174	0.273	2.01	1.64
20	1.11	0.162	0.275	2.15	1.62
21	1.03				
22	1.08				
23	1.08				
24	1.04				
25	1.04				
26	1.04				
27	1.03				
28	1.06				
29	1.09				
30	1.02				
STDEV	0.056	0.015	0.015	0.081	0.073
Mean	1.08	0.172	0.262	2.125	1.588

Thrum flower, lowland

	Length of style (mm)	thickness of stigma (mm)	width of stigma (mm)	length of filaments, mm (distinct stamen)	length of filaments, mm (epipetalous stamen)
1	0.859	0.184	0.292	2.42	2.07
2	0.871	0.192	0.265	2.41	1.99
3	0.828	0.180	0.285	2.77	2.15
4	0.847	0.199	0.267	2.6	2.01
5	0.849	0.196	0.315	2.73	2.02
6	0.891	0.200	0.305	2.73	2.12
7	0.908	0.152	0.289	2.62	1.91
8	0.91	0.175	0.290	2.62	2.2
9	0.899	0.171	0.258	2.5	2.22
10	0.886	0.177	0.277	2.61	2.12
11	0.871	0.181	0.286	2.74	2.03
12	0.882	0.172	0.262	2.68	1.98
13	0.891	0.167	0.285	2.73	2.21
14	0.926	0.162	0.282	2.62	2.2
15	0.872	0.180	0.273	2.57	2.18
16	0.913	0.175	0.268	2.71	2.09
17	0.865	0.165	0.290	2.67	2.1
18	0.908	0.200	0.307	2.69	2.08
19	0.882	0.184	0.278	2.63	2.13
20	0.902	0.178	0.294	2.62	2.16
21	0.91				
22	0.873				
23	0.877				
24	0.854				
25	0.881				
26	0.862				
27	0.909				
28	0.902				
29	0.904				
30	0.892				
STDEV	0.023	0.013	0.015	0.100	0.087
Mean	0.88	0.180	0.283	2.634	2.099

	Pin flower, highland		Thrum flower, highland		Thrum flower, lowland	
	polar length	equatorial diameter	polar length	equatorial diameter	polar length	equatorial diameter
1	36.083	42.764	40.874	47.499	45.220	49.091
2	35.420	42.201	40.231	46.891	45.555	47.993
3	36.374	41.272	37.752	46.546	41.002	47.117
4	37.063	41.949	42.473	46.009	43.017	48.970
5	36.987	40.683	41.232	46.104	44.956	48.791
6	36.077	41.783	42.517	46.131	44.753	49.473
7	35.400	42.390	41.407	48.978	42.884	47.161
8	37.818	39.329	39.927	45.933	46.231	46.369
9	35.664	42.143	41.461	47.798	43.655	48.070
10	35.826	41.060	41.030	45.176	44.767	49.002
11	36.520	40.674	41.740	47.598	46.442	47.564
12	37.400	41.407	41.867	45.312	42.181	47.110
13	37.833	41.798	41.575	47.730	44.953	49.980
14	37.374	41.534	41.760	45.828	45.527	43.654
15	36.148	40.794	41.029	47.599	46.001	50.646
16	35.717	41.274	42.862	46.116	47.013	45.540
17	37.278	41.035	42.122	46.602	45.940	49.065
18	37.932	43.997	40.110	45.234	44.736	50.004
19	36.935	41.477	41.065	45.449	44.942	50.228
20	37.672	40.869	36.035	45.063	43.521	43.643
21	34.268	40.123	39.006	45.834	45.701	51.752
22	35.754	40.044	41.808	46.927	45.698	47.554
23	35.056	41.050	41.820	47.200	44.653	43.691
24	34.876	41.779	41.897	45.383	42.420	48.009
25	34.232	40.505	41.334	45.882	47.636	48.771
26	37.297	41.344	40.639	47.099	47.851	49.656
27	34.292	40.563	39.790	45.975	47.691	49.799
28	36.907	42.127	41.767	45.160	49.200	47.802
29	36.557	42.644	40.403	47.368	43.471	49.164
30	36.160	41.944	39.217	46.435	45.223	48.140
31	37.148	40.591	40.414	45.549	45.908	51.689
32	36.068	41.960	42.836	46.299	47.985	51.597
33	35.440	42.126	39.709	46.431	41.140	46.312
34	36.999	41.869	42.401	46.147	42.422	47.270
35	35.090	42.102	42.499	46.846	44.691	45.604
36	35.732	41.081	40.162	45.688	42.918	44.404
37	35.421	40.559	39.160	44.729	42.560	48.617
38	36.023	41.604	39.964	45.484	41.341	49.510
39	37.212	41.416	40.526	47.826	44.575	50.197
40	35.297	41.892	40.328	46.782	44.119	48.265
STDEV	1.022359	0.854936189	1.39842712	0.952472	1.964752	2.098153
Mean	36.234	41.444	40.869	46.366	44.762	48.182

Welch Two Sample t-test (Table 3.8 & 3.9)

length of style (highland pin vs highland thrum)
t = 54.1446, df = 40.867, **p-value < $2.2e^{-16}$ (P<0.0001)**
confidence interval = 1.275895 1.374772
mean of pin = 2.407333, mean of thrum = 1.082000

thickness of stigma (highland pin vs highland thrum)
t = -8, df = 34.921, **p-value = $2.089e^{-9}$ (P<0.0001)**
confidence interval = -0.04093608 -0.02436392
mean of pin = 0.13950, mean of thrum = 0.17215

width of stigma (highland pin vs highland thrum)
t = -5.0566, df = 37.269, **p-value = $1.164e^{-5}$ (P<0.0001)**
confidence interval = -0.3697599 -0.01582401
mean of pin = 0.2357, mean of thrum = 0.2621

filament - distinct (highland pin vs highland thrum)
t = -26.7669, df = 24.695, **p-value < $2.2e^{-16}$ (P<0.0001)**
confidence interval = -0.5621897 -0.4818103
mean of pin = 1.603, mean of thrum = 2.125

filament - epipetalous (highland pin vs highland thrum)
t = -30.2256, df = 29.315, **p-value < $2.2e^{-16}$ (P<0.0001)**
confidence interval = -0.597875 -0.522125
mean of pin = 1.028, mean of thrum = 1.588

number of pollen grains in one anther (highland pin vs highland thrum)
t = 17.1585, df = 29.308, **p-value < $2.2e^{-16}$ (P<0.0001)**
confidence interval = 46.3146 58.8433
mean of pin = 151.947, mean of thrum = 99.368

polar length of pollen grain (highland pin vs highland thrum)
t = -16.9222, df = 71.426, **p-value < $2.2e^{-16}$ (P<0.0001)**
confidence interval = -5.181057 -4.088893
mean of pin = 36.23375, mean of thrum = 40.86872

equatorial diameter of pollen grain (highland pin vs highland thrum)
t = -24.3225, df = 77.107, **p-value < $2.2e^{-16}$ (P<0.0001)**
confidence interval = -5.325058 -4.519142
mean of pin = 41.4439, mean of thrum = 46.3660

length of style (highland thrum vs lowland thrum)
t = 17.7738, df = 38.769, **p-value < $2.2e^{-16}$ (P<0.0001)**
confidence interval = 0.1753448 0.2203886
mean of highland thrum = 1.082, mean of lowland thrum = 0.8841

thickness of stigma (highland thrum vs lowland thrum)
t = -1.6652, df = 37.555, **p-value = $1.042e^{-4}$ (P<0.0001)**
confidence interval = -0.016289066 0.001589066
mean of of highland thrum = 0.17215, mean of lowland thrum = 0.17950

width of stigma (highland thrum vs lowland thrum)
t = -4.3962, df = 38, **p-value = $8.574e^{-5}$ (P<0.0001)**
confidence interval = -0.03110845 -0.01149155
mean of of highland thrum = 0.2621, mean of lowland thrum = 0.2834

filament – distinct (highland thrum vs lowland thrum)
t = -17.646, df = 36.455, **p-value < $2.2e^{-16}$ (P<0.0001)**
confidence interval = -0.5669178 -0.4500822
mean of of highland thrum = 2.1250, mean of lowland thrum = 2.6335

filament – epipetalous (highland thrum vs lowland thrum)
t = -20.1143, df = 36.845, **p-value < $2.2e^{-16}$ (P<0.0001)**
confidence interval = -0.5619321 -0.4590679
mean of of highland thrum = 1.5880, mean of lowland thrum = 2.0985

polar length of pollen grain (highland thrum vs lowland thrum)
t = -10.2115, df = 70.445, **p-value = $1.547e^{-15}$ (P<0.0001)**
confidence interval = -4.654162 -3.133338
mean of of highland thrum = 40.86872, mean of lowland thrum = 44.76248

equatorial diameter of pollen grain (highland thrum vs lowland thrum)
t = -4.9841, df = 54.419, **p-value = $6.685e^{-6}$ (P<0.0001)**
confidence interval = -2.546158 -1.085542
mean of of highland thrum = 46.36600, mean of lowland thrum = 48.18185

Appendix F: Pollination experiment

Open pollination, population H2

No.	Flower	fruit
1	91	52
2	58	41
3	24	14
4	110	34
5	104	31
6	71	12
7	91	19
8	110	69
9	138	66
10	75	39
11	92	26
12	78	36
13	79	61
14	24	12
15	34	23
16	41	24
17	136	68
18	38	21
19	93	50
20	82	45
21	46	11
22	15	10
23	13	4
24	102	18
total	1745	786

population H3

No.	Flower	fruit
1	106	52
2	70	5
3	46	34
4	53	11
5	74	27
6	117	53
7	112	74
8	39	8
9	140	78
10	79	35
11	44	24
12	130	75
13	73	35
14	62	44
15	113	38
16	89	37
Total	1347	630

Population H5

No.	Flower	fruit
1	28	14
2	98	55
3	71	52
4	133	83
5	39	5
6	83	42
7	47	3
8	50	31
9	69	30
10	131	65
11	109	56
12	44	29
13	66	31
14	143	86
Total	1111	582

Bagging experiment, population H2

No.	Flowers	fruits
1	58	2
2	51	2
3	41	7
4	43	9
5	63	17
6	49	9
total	305	46

Population H3

No.	Flowers	fruits
1	31	6
2	30	5
3	64	12
4	59	11
5	48	8
6	37	3
total	269	45

Population H5

No.	Flowers	fruits
1	92	20
2	49	9
3	40	6
4	36	4
5	81	16
6	79	13
7	40	5
8	30	4
9	102	19
10	49	10
11	53	11
12	42	9
13	62	16
total	755	142

Appendix G: Cytotoxic activity of the highland and lowland varieties of *P. chinensis* var. *chinensis*

concentration (µg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude methanol extracts of the highland variety *Persicaria chinensis* var. *chinensis* against CaSki cell line		
	leaves	stem	whole plants
100	67.06 ±1.86	76.52 ±0.63	75.00 ±3.17
75	58.06 ±2.13	48.43 ±0.90	64.48 ±2.57
50	48.71 ±0.65	31.68 ±1.45	44.71 ±1.17
25	28.25 ±2.29	19.44 ±0.80	23.89 ±0.67
10	15.16 ±2.34	9.21 ±0.51	11.78 ±0.58
1	6.09 ±2.48	3.15 ±0.14	3.92 ±0.43

concentration (µg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude methanol extracts of the lowland variety *Persicaria chinensis* var. *chinensis* against CaSki cell line		
	leaves	stem	whole plants
100	85.91 ±0.91	68.44 ±0.93	77.42 ±1.12
75	74.52 ±0.88	36.94 ±0.22	67.08 ±1.04
50	70.75 ±1.08	21.55 ±1.73	46.35 ±0.49
25	60.16 ±2.02	12.15 ±0.11	30.04 ±2.45
10	48.72 ±2.14	8.22 ±0.92	16.84 ±2.04
1	34.96 ±1.14	1.71 ±1.79	6.18 ±1.26

concentration (µg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude dichloromethane extracts of the highland variety *Persicaria chinensis* var. *chinensis* against CaSki cell line		
	leaves	stem	whole plants
100	45.62 ±0.93	90.52 ±1.42	65.02 ±5.12
75	29.55 ±5.34	59.72 ±3.54	43.06 ±1.52
50	21.19 ±3.80	35.33 ±4.31	35.35 ±5.08
25	12.01 ±4.40	18.00 ±2.51	25.61 ±3.10
10	7.64 ±3.99	12.46 ±4.24	10.78 ±3.56
1	1.02 ±1.21	7.94 ±2.17	6.40 ±3.48

concentration (µg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude dichloromethane extracts of the lowland variety *Persicaria chinensis* var. *chinensis* against CaSki cell line		
	leaves	stem	whole plants
100	77.51 ±2.60	73.94 ±2.07	75.53 ±1.86
75	56.01 ±2.00	63.60 ±2.12	65.43 ±1.07
50	29.68 ±0.87	42.56 ±3.56	46.12 ±3.50
25	21.65 ±3.29	28.89 ±2.23	34.68 ±3.76
10	8.24 ±5.10	18.18 ±1.08	22.10 ±3.87
1	2.38 ±0.79	9.65 ±1.58	13.00 ±1.51

concentration (μg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude water extracts of the highland variety *Persicaria chinensis* var. *chinensis* against CaSki cell line		
	leaves	stem	whole plants
100	49.08±2.57	35.77±2.78	62.91±5.22
75	46.29±3.50	28.17±4.85	50.23±4.13
50	29.31±2.47	17.10±1.04	30.90±3.99
25	21.70±1.12	8.68±2.24	16.84±4.78
10	14.25 ±3.80	3.57±2.65	7.93±4.23
1	5.44 ±1.83	2.02±1.54	4.21±1.64

concentration (μg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude water extracts of the lowland variety *Persicaria chinensis* var. *chinensis* against CaSki cell line		
	leaves	stem	whole plants
100	37.59±3.09	32.09±0.39	52.20±4.70
75	32.66±0.86	21.86±2.16	35.14±0.62
50	28.12±1.91	14.71±3.33	24.20±3.06
25	17.05±1.12	9.66±2.07	14.25±1.92
10	6.38±0.96	2.21±1.13	7.86±1.30
1	2.09±0.28	1.05±0.80	1.26±0.82

concentration (μg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude methanol extracts of the highland variety *Persicaria chinensis* var. *chinensis* against SKOV-3 cell line		
	leaves	stem	whole plants
100	91.43±0.50	86.81±1.17	89.85±0.46
75	90.55±0.65	77.47±1.73	79.53±0.89
50	82.89±1.10	61.48±4.51	62.12±0.57
25	59.82±1.42	29.97±1.90	54.16±1.35
10	33.57±1.78	27.39±1.60	35.21±1.63
1	13.14±0.18	14.94±0.96	16.60±0.61

concentration (μg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude methanol extracts of the lowland variety *Persicaria chinensis* var. *chinensis* against SKOV-3 cell line		
	leaves	stem	whole plants
100	80.38±1.72	80.11±0.43	76.02±0.82
75	78.46±0.25	57.21±0.32	67.86±2.53
50	66.20±1.20	26.12±1.31	55.81±1.47
25	28.22±2.97	17.80±0.91	15.35±1.36
10	18.76±1.69	13.87±1.34	4.62±1.48
1	15.26±0.90	2.52±0.26	2.82±0.64

concentration (μg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude dichloromethane extracts of the highland variety *Persicaria chinensis* var. *chinensis* against SKOV-3 cell line		
	leaves	stem	whole plants
100	63.79±1.17	59.15±0.71	60.72±1.19
75	39.44±0.59	33.43±1.32	35.06±0.87
50	32.59±1.02	12.89±0.93	22.32±0.95
25	18.71±1.78	9.89±1.91	12.73±0.63
10	7.99±1.67	5.32±0.62	6.67±1.41
1	3.37±0.07	1.07±0.40	3.76±0.35

concentration (μg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude dichloromethane extracts of the lowland variety *Persicaria chinensis* var. *chinensis* against SKOV-3 cell line		
	leaves	stem	whole plants
100	64.71±0.10	59.69±1.53	61.05±0.69
75	48.52±1.34	46.23±1.94	47.46±3.16
50	36.91±2.06	30.56±2.69	31.33±1.77
25	19.00±1.81	13.91±1.28	21.63±1.27
10	14.84±2.96	8.12±1.31	11.08±2.20
1	5.23±0.45	3.35±0.28	4.47±0.69

concentration (μg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude water extracts of the highland variety *Persicaria chinensis* var. *chinensis* against SKOV-3 cell line		
	leaves	stem	whole plants
100	45.17±0.87	41.38±1.09	45.53±0.66
75	30.74±0.74	22.00±1.51	37.06±1.21
50	16.69±1.64	15.68±0.82	29.35±0.88
25	6.32±0.92	10.60±1.52	15.69±1.32
10	3.35±1.45	4.56±0.58	3.13±0.45
1	1.04±0.46	3.09±0.80	1.97±0.21

concentration (μg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude water extracts of the lowland variety *Persicaria chinensis* var. *chinensis* against SKOV-3 cell line		
	leaves	stem	whole plants
100	81.34±2.11	31.46±3.11	69.72±1.90
75	69.21±1.33	17.97±1.48	51.37±3.62
50	27.13±2.65	12.75±1.77	35.77±1.66
25	21.03±2.81	8.17±1.00	26.31±1.30
10	15.49±0.62	6.48±1.74	18.94±1.33
1	9.96±0.89	4.95±0.98	13.64±0.54

concentration (µg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude methanol extracts of the highland variety *Persicaria chinensis* var. *chinensis* against HT-29 cell line		
	leaves	stem	whole plants
100	75.83±2.09	87.75±0.71	76.56±2.08
75	65.59±2.56	77.43±2.44	67.44±0.98
50	40.91±2.65	61.20±2.27	51.61±2.00
25	26.80±3.57	34.07±1.64	38.16±2.58
10	15.31±1.10	22.73±2.52	18.32±3.46
1	5.65±1.89	9.05±1.00	9.96±0.96

concentration (µg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude methanol extracts of the lowland variety *Persicaria chinensis* var. *chinensis* against HT-29 cell line		
	leaves	stem	whole plants
100	71.73±1.75	66.58±3.43	68.83±4.81
75	65.42±4.42	44.12±2.16	58.13±4.08
50	47.94±1.93	32.11±1.73	41.52±1.52
25	34.42±0.35	23.60±1.56	23.73±3.18
10	16.06±3.38	13.42±2.87	14.25±2.05
1	6.52±1.68	3.40±1.05	5.49±0.46

concentration (µg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude dichloromethane extracts of the highland variety *Persicaria chinensis* var. *chinensis* against HT-29 cell line		
	leaves	stem	whole plants
100	77.40±1.73	90.86±1.26	77.34±2.97
75	59.50±2.12	89.54±1.15	73.90±3.01
50	47.01±1.48	83.90±2.91	69.52±2.83
25	33.67±2.88	70.26±3.18	54.79±5.09
10	15.91±2.20	44.58±1.76	31.89±1.99
1	5.29±0.87	19.26±0.50	15.60±0.99

concentration (µg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude dichloromethane extracts of the lowland variety *Persicaria chinensis* var. *chinensis* against HT-29 cell line		
	leaves	stem	whole plants
100	73.06±2.32	63.35±3.87	62.42±1.04
75	61.16±3.68	56.22±2.51	51.99±1.63
50	37.83±0.09	38.64±3.31	41.56±1.35
25	22.23±0.48	13.15±1.82	33.97±2.55
10	5.87±0.78	4.11±0.83	13.53±0.72
1	2.66±0.35	1.27±0.72	3.72±1.41

concentration (μg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude water extracts of the highland variety *Persicaria chinensis* var. *chinensis* against HT-29 cell line		
	leaves	stem	whole plants
100	31.94±1.42	29.31±1.85	34.68±2.51
75	24.01±1.93	21.96±1.04	25.46±3.02
50	14.15±1.74	17.95±1.88	21.01±2.97
25	11.18±1.96	12.50±1.11	9.98±1.64
10	3.43±2.78	7.79±0.68	6.30±1.02
1	2.01±1.57	3.54±1.56	3.37±0.76

concentration (μg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude water extracts of the lowland variety *Persicaria chinensis* var. *chinensis* against HT-29 cell line		
	leaves	stem	whole plants
100	40.00±1.17	34.06±0.51	38.53±0.62
75	33.53±1.87	24.73±2.31	29.59±0.88
50	25.81±0.95	16.98±1.70	20.64±0.95
25	19.13±0.19	10.65±3.03	10.34±2.66
10	7.55±0.66	3.33±1.10	6.34±2.22
1	3.08±0.97	1.15±0.92	2.70±1.87

concentration (μg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude methanol extracts of the highland variety *Persicaria chinensis* var. *chinensis* against A549 cell line		
	leaves	stem	whole plants
100	66.06±3.77	52.55±2.30	60.76±0.92
75	55.64±2.59	31.88±1.95	51.20±4.92
50	44.61±2.77	20.35±1.54	38.80±1.17
25	21.40±1.31	14.40±1.75	17.51±3.09
10	15.79±0.50	6.13±1.68	5.26±1.30
1	12.90±0.91	3.03±0.96	2.43±0.85

concentration (μg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude methanol extracts of the lowland variety *Persicaria chinensis* var. *chinensis* against A549 cell line		
	leaves	stem	whole plants
100	76.27±3.39	65.57±2.89	75.27±2.36
75	68.92±3.28	49.69±1.69	64.59±2.27
50	52.85±0.54	21.38±0.44	40.03±2.17
25	20.69±1.09	15.23±0.60	24.26±1.56
10	13.84±2.24	12.01±1.48	13.30±1.88
1	5.63±0.47	5.71±0.47	8.06±0.56

concentration (μg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude dichloromethane extracts of the highland variety *Persicaria chinensis* var. *chinensis* against A549 cell line		
	leaves	stem	whole plants
100	66.95±1.50	65.74±0.57	54.71±2.08
75	62.36±1.64	54.18±2.30	33.69±1.41
50	48.94±0.27	47.26±1.68	24.79±1.84
25	44.16±0.53	27.19±0.49	19.67±0.52
10	35.76±1.17	18.15±2.82	15.16±1.66
1	23.85±1.04	8.14±0.27	8.88±0.52

concentration (μg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude dichloromethane extracts of the lowland variety *Persicaria chinensis* var. *chinensis* against A549 cell line		
	leaves	stem	whole plants
100	49.78±0.10	55.70±1.53	52.73±0.69
75	42.86±1.34	51.58±1.94	43.79±3.16
50	34.27±2.06	41.72±2.69	37.01±1.77
25	15.71±1.81	33.04±1.28	26.76±1.27
10	8.20±2.96	19.13±1.31	11.47±2.20
1	4.16±0.45	10.78±0.28	6.31±0.69

concentration (μg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude water extracts of the highland variety *Persicaria chinensis* var. *chinensis* against A549 cell line		
	leaves	stem	whole plants
100	19.38±1.76	30.22±0.44	26.30±0.66
75	12.56±0.63	18.70±1.38	20.04±0.51
50	8.76±1.17	12.62±0.58	16.81±1.84
25	7.43±1.68	9.49±0.28	9.61±1.41
10	3.34±0.86	5.33±1.58	6.55±0.60
1	1.20±0.50	1.54±0.36	2.54±0.48

concentration (μg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude water extracts of the lowland variety *Persicaria chinensis* var. *chinensis* against A549 cell line		
	leaves	stem	whole plants
100	22.84±0.60	29.28±1.26	26.04±0.65
75	15.87±1.25	22.95±1.51	21.64±0.80
50	11.56±0.72	14.05±1.23	10.22±1.34
25	7.95±1.72	6.97±0.45	9.31±3.75
10	3.80±1.75	4.30±1.38	3.17±1.08
1	1.65±0.53	1.90±0.75	1.14±0.92

concentration (µg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude methanol extracts of the highland variety *Persicaria chinensis* var. *chinensis* against MCF7 cell line		
	leaves	stem	whole plants
100	71.70±3.20	90.22±0.25	73.97±3.73
75	65.76±3.44	84.88±2.16	61.78±0.71
50	49.20±3.05	50.32±1.63	42.85±2.32
25	34.24±1.18	36.26±2.92	36.06±2.99
10	18.88±1.22	23.88±1.14	22.41±3.07
1	11.09±3.65	12.63±1.92	13.19±2.02

concentration (µg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude methanol extracts of the lowland variety *Persicaria chinensis* var. *chinensis* against MCF7 cell line		
	leaves	stem	whole plants
100	76.96±1.63	74.30±4.43	81.59±1.49
75	72.76±1.68	55.38±2.29	71.11±2.86
50	69.35±2.95	31.82±2.89	34.77±4.05
25	46.01±3.15	23.67±1.86	26.53±4.09
10	29.11±1.41	13.23±1.35	20.38±4.68
1	10.12±3.34	7.95±2.44	10.01±3.26

concentration (µg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude dichloromethane extracts of the highland variety *Persicaria chinensis* var. *chinensis* against MCF7 cell line		
	leaves	stem	whole plants
100	79.68±1.44	88.78±2.08	65.38±2.14
75	54.80±0.89	56.46±2.25	37.99±3.46
50	30.91±1.95	41.19±1.84	39.37±4.03
25	21.41±2.68	15.78±1.26	18.95±2.64
10	12.75±4.17	5.78±0.83	4.17±1.57
1	5.07±3.30	2.34±0.27	0.93±0.37

concentration (µg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude dichloromethane extracts of the lowland variety *Persicaria chinensis* var. *chinensis* against MCF7 cell line		
	leaves	stem	whole plants
100	48.70±2.36	47.17±3.54	43.01±2.70
75	34.57±3.04	43.22±2.90	37.58±2.74
50	19.47±1.35	32.82±2.15	24.35±1.67
25	12.29±1.80	18.40±1.25	16.30±4.11
10	6.62±1.44	10.27±2.02	7.20±2.21
1	5.23±1.03	7.00±2.56	3.17±1.58

concentration (µg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude water extracts of the highland variety *Persicaria chinensis* var. *chinensis* against MCF7 cell line		
	leaves	stem	whole plants
100	58.28±2.13	39.70±3.29	64.13±4.11
75	46.60±1.74	28.34±5.11	59.88±4.66
50	37.18±1.69	20.39±3.49	39.12±3.57
25	26.83±1.41	10.48±2.61	24.39±1.33
10	6.08±0.67	3.98±1.27	3.87±3.21
1	1.60±0.24	0.45±0.26	1.85±2.51

concentration (µg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude water extracts of the lowland variety *Persicaria chinensis* var. *chinensis* against MCF7 cell line		
	leaves	stem	whole plants
100	55.64±3.37	45.86±1.24	59.60±3.81
75	47.80±4.18	40.08±1.25	46.67±2.91
50	32.13±0.86	24.57±3.27	35.20±2.26
25	22.71±2.23	11.78±0.75	22.67±4.05
10	13.15±1.89	4.26±0.99	11.09±2.88
1	5.62±1.29	1.18±0.57	2.91±1.72

concentration (µg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude methanol extracts of the highland variety *Persicaria chinensis* var. *chinensis* against MRC-5 cell line		
	leaves	stem	whole plants
100	47.78±1.81	55.58±3.45	48.51±2.17
75	40.25±3.95	46.11±0.10	33.10±2.43
50	31.50±2.65	39.87±3.98	23.83±0.58
25	22.07±1.29	26.96±1.60	8.87±0.31
10	10.73±1.01	14.25±0.63	4.24±0.99
1	0.78±0.17	5.63±3.84	2.54±0.95

concentration (µg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude methanol extracts of the lowland variety *Persicaria chinensis* var. *chinensis* against MRC-5 cell line		
	leaves	stem	whole plants
100	35.90±2.26	41.71±1.00	38.12±3.12
75	26.39±1.51	30.58±0.68	26.29±1.31
50	20.24±1.36	22.42±0.73	19.20±1.96
25	13.85±3.66	16.50±0.32	13.41±1.14
10	5.07±2.67	11.20±0.69	5.52±0.21
1	2.04±1.56	7.08±1.20	1.79±0.37

concentration (µg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude dichloromethane extracts of the highland variety *Persicaria chinensis* var. *chinensis* against MRC-5 cell line		
	leaves	stem	whole plants
100	56.13±2.59	73.22±2.22	61.41±1.98
75	42.88±1.11	55.20±1.89	45.43±3.66
50	28.54±1.30	43.69±1.01	32.66±2.62
25	19.76±1.46	29.90±0.49	23.59±2.77
10	6.46±0.55	10.81±0.51	13.53±0.72
1	3.06±0.96	4.49±1.14	3.48±0.40

concentration (µg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude dichloromethane extracts of the lowland variety *Persicaria chinensis* var. *chinensis* against MRC-5 cell line		
	leaves	stem	whole plants
100	53.21±1.13	47.19±3.23	48.32±1.51
75	44.66±1.50	39.05±2.86	42.75±1.07
50	41.40±1.32	30.77±1.17	35.48±1.05
25	30.43±0.81	22.83±1.41	26.81±1.32
10	20.20±0.95	13.50±0.60	16.93±1.42
1	6.08±2.63	3.67±0.45	4.44±0.89

concentration (µg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude water extracts of the highland variety *Persicaria chinensis* var. *chinensis* against MRC-5 cell line		
	leaves	stem	whole plants
100	58.28±2.13	39.70±3.29	64.13±4.11
75	46.60±1.74	28.34±5.11	59.88±4.66
50	37.18±1.69	20.39±3.49	39.12±3.57
25	26.83±1.41	10.48±2.61	24.39±1.33
10	6.08±0.67	3.98±1.27	3.87±3.21
1	1.60±0.24	0.45±0.26	1.85±2.51

concentration (µg/ml)	Average percentage (%) and standard deviation of 3 tests of growth inhibition by crude water extracts of the lowland variety *Persicaria chinensis* var. *chinensis* against MRC-5 cell line		
	leaves	stem	whole plants
100	34.76±1.24	40.78±1.16	38.09±1.34
75	27.39±1.88	29.12±0.64	27.04±1.93
50	17.88±1.26	22.04±0.54	19.98±0.29
25	12.06±1.19	13.10±1.33	11.67±0.70
10	3.27±0.62	3.77±1.25	3.46±2.13
1	1.23±0.14	2.23±0.53	1.14±0.64