

THE REPRODUCTIVE BIOLOGY AND CYTOTOXIC ACTIVITY OF
PERSICARIA CHINENSIS (L.) H. GROSS VAR. *CHINENSIS*
(POLYGONACEAE)

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ABSTRACT

Persicaria chinensis var. *chinensis* is a native variety in Malaysia and found in the tropics growing in lowland and lower montane forests. Growth habit and morphological differences were observed between the highland and lowland varieties. The anthesis of the highland wild population was at 0600–0730 hours whereas that of the lowland variety was at 0745–0930 hours.

The anther is tetrasporangiate and the wall development conforms to the Monocotyledonous type. Cytokinesis in the microspore mother cells is simultaneous forming tetrahedral and decussate tetrads in the highland variety and mostly tetrahedral tetrads in the lowland variety. The mature pollen grains are shed at the three-cell stage. The ovule is orthotropous, bitegmic and crassinucellate and the micropyle is formed by both the outer and inner integuments. The development of the embryo sac conforms to the monosporic *Polygonum* type. An occurrence of twin eggs within an ovule was observed in the lowland variety. In the highland variety, the development of the endosperm is *ab initio* Nuclear type.

The highland variety is heterostylous; pin flowers have taller style, shorter filaments, less thick and less wide stigma than the thrum flower. The number of pollen grains in a pin flower is higher than that in a thrum flower. The pollen grains of the thrum flowers are bigger than those of the pin flowers. The pin and thrum pollen grains are 3-colpate, medium-sized, suboblate to oblate and have reticulate exine with thick, narrow muri and free clavae in the deep lumina.

The lowland variety is observed to have only thrum flower. They have shorter style, longer filaments, thicker and wider stigma than those of the highland thrum flowers. The pollen grains of the lowland variety are bigger than those of the highland thrum flowers. Their lumina were less wide and the muri were thicker, less narrow and shorter than those of the highland thrum pollen with free clavae.

In the highland variety, the optimum sucrose and PEG concentration for pollen germination was 15% and 30% respectively. Bursting of pollen grain occurred in the medium without PEG. Pollen grains reached maximum germination (50%) in the modified medium with an average pollen tube length of 140 μm .

The highland variety of *P. chinensis* var. *chinensis* was self-compatible. Open pollination produced three times more fruits than self pollination and they are cross-compatible and show outbreeding. The pollen-ovule ratio suggested the breeding system was facultative xenogamy. The most common insect visitors are from the Order of Hymenoptera. Seed germination is epigeal showing 77% viability. The average percentage of germination for the fresh seeds was 47% in the natural highland environment and 34% in the lowland laboratory environment.

In Malaysia, *P. chinensis* var. *chinensis* has been used traditionally as one of the main ingredients in Chinese traditional medicine to treat various lung ailments including cancer. The *in vitro* neutral red cytotoxicity assay was successfully applied to screen the cytotoxic activity against 18 crude methanol, dichloromethane and water extracts from the highland and lowland varieties of *P. chinensis* var. *chinensis* derived from the leaf, stem and whole plant. Overall results showed that the methanol extracts from the leaf samples of the highland and lowland varieties possessed greater cytotoxicity effect against cancer cell lines. Two active extracts obtained from the methanol leaf extracts of the highland and lowland varieties were more effective in suppressing the expression of CaSki and SKOV-3 cells. The dichloromethane extract from the stem sample of the highland variety also showed significant cytotoxic effect against HT-29 cells. Since both varieties showed signs of the selectivity for CaSki, SKOV-3 and HT-29 cells, these findings suggest that *P. chinensis* var. *chinensis* should be further researched to isolate and identify the active compound(s) from the active extracts which demonstrated significant cytotoxic activities.

ABSTRAK

Persicaria chinensis var. *chinensis* ialah varieti tempatan yang terdapat di tropik dan tumbuh di tanah pamah dan hutan hujan gunung bawah. Perbezaan dalam sifat pertumbuhan dan morfologi dapat diperhatikan di antara varieti tanah tinggi dan pamah. Masa antesis populasi varieti tanah tinggi ialah 0600–0730 sementara varieti tanah rendah ialah 0745–0930. Dehis anter dan stigma reseptiviti adalah serentak.

Anter adalah tetrasporangiat dan pembangunan dinding anter mematuhi jenis monokot. Sitokinesis serentak berlaku dalam sel-sel ibu mikrospora dan membentuk kedua-dua jenis tetrad ‘tetrahedral’ dan ‘decussate’ dalam varieti tanah tinggi dan kebanyakannya tetrad ‘tetrahedral’ dalam varieti pamah. Debunga matang terbentuk di peringkat tiga sel. Ovul ialah ortotropus, bitegmik dan krasinuselat dan mikropil terbentuk daripada kedua-dua integumen luar dan dalam. Perkembangan pundi embrio mematuhi jenis yang monosporik *Polygonum*. Kejadian telur kembar dalam ovul diperhatikan dalam varieti tanah rendah. Dalam varieti tanah tinggi, pembangunan endosperma mengikuti jenis *ab initio* Nukleus.

Bunga-bunga varieti tanah tinggi ialah heterostil; bunga pin mempunyai stil yang lebih tinggi, filamen yang lebih pendek, stigma yang kurang tebal dan kurang luas dibanding dengan bunga thrum. Bilangan butir debunga pada bunga pin lebih banyak daripada bunga thrum. Debunga bunga thrum lebih besar daripada bunga pin. Butir debunga pin dan thrum adalah 3-kolpat, ukuran sederhana, daripada suboblat kepada oblat dan mempunyai eksin retikulat dengan muri yang tebal, sempit dan klavae yang bebas di antara lumina.

Bunga thrum tanah pamah mempunyai stil yang lebih pendek, filamen yang lebih panjang, stigma yang lebih tebal dan lebih luas daripada bunga thrum varieti tanah tinggi. Ukuran debunga varieti tanah pamah adalah lebih besar daripada debunga thrum varieti tanah tinggi. Lumina debunga thrum tanah rendah adalah kurang luas

daripada debunga thrum tanah tinggi dan Muri debunga thrum varieti tanah pamah adalah lebih pendek daripada debunga thrum varieti tanah tinggi.

Dalam varieti tanah tinggi, sukrosa optima dan kepekatan PEG untuk percambahan masing-masing adalah 15% dan 30%. Debunga terpecah berlaku dalam medium tanpa PEG. Debunga mencapai percambahan maksimum (50%) dalam medium yang diubah suai dengan tiub debunga mempunyai purata panjang 140 μm .

Varieti tanah tinggi *P. chinensis* var. *chinensis* berupaya berkacuk sendiri. Pendebungaan terbuka yang dihasilkan adalah tiga kali ganda lebih banyaknya daripada pendebungaan sendiri dan mereka berupaya berkacuk luar dan perkacukan asing telah ditunjukkan. Nisbah debunga-ovul mencadangkan sistem pembiakan adalah xenogami fakultatif. Pelawat serangga yang paling biasa adalah terdiri daripada Order Hymenoptera. Percambahan biji adalah epigeal dan menunjukkan viabiliti bijinya sebanyak 77%. Purata peratusan percambahan benih segar adalah 47% di bawah percambahan di persekitaran tanah tinggi semulajadi dan 34% di bawah persekitaran makmal di tanah rendah.

Di Malaysia, *P. chinensis* var. *chinensis* telah digunakan secara tradisional sebagai salah satu bahan utama dalam perubatan tradisional Cina untuk merawat pelbagai jenis penyakit peparu termasuk kanser. *In vitro* sitotoksiti neutral merah berjaya digunakan untuk menyaring aktiviti sitotoksik terhadap 18 metanol mentah, diklorometana dan ekstrak air dari varieti tanah tinggi dan tanah pamah *P. chinensis* var. *chinensis* yang diperolehi daripada daun, batang dan keseluruhan tumbuhan (daun dan batang). Keputusan keseluruhan menunjukkan bahawa ekstrak metanol daripada sampel daun varieti tanah tinggi dan tanah pamah mempunyai kesan sitotoksik yang lebih tinggi terhadap sel kanser. Dua ekstrak aktif yang diperolehi daripada ekstrak metanol daun varieti tanah tinggi dan tanah rendah adalah lebih berkesan dalam ungkapan menekan sel CaSki dan sel SKOV-3. Ekstrak diklorometana dari sampel

batang varieti tanah tinggi juga menunjukkan kesan sitotoksik yang ketara ke atas sel HT-29. Memandangkan kedua-dua varieti tanah tinggi dan tanah pamah menunjukkan tanda-tanda selektiviti terhadap CaSki, SKOV-3 dan sel-sel HT-29, dengan itu penemuan ini menunjukkan bahawa *P. chinensis* var. *chinensis* perlu dikaji lebih lanjut untuk mengasingkan dan mengenalpasti sebatian aktif yang khusus dari ekstrak aktif yang menunjukkan aktiviti sitotoksik yang ketara.

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LIST OF SYMBOLS AND ABBREVIATIONS

i.e.	id est (that is)
°C	degree Celsius
<i>et al.</i>	et alia (and other)
var.	variety (botany)
syn	synonym
sp.	species
FRIM	Forest Research Institute Malaysia
KLU	The University of Malaya Herbarium
SING	The Singapore Herbarium
BKF	The Forest Herbarium, Bangkok
P/E	polar length over equatorial diameter
g	gram
%	percentage
PEG	polyethylene glycol
H ₃ CO ₃	boric acid
Ca(NO ₃) ₂ .4H ₂ O	calcium nitrate tetrahydrate
MnSO ₄ .1H ₂ O	manganese (II) sulphate monohydrate
KNO	potassium nitrate
TZ	tetrazolium
m	meter
mm	millimeter
cm	centimeter
µg	microgram
µl	microlitre
ml	milliliter
mg	milligram
SEM	scanning electron microscope
LM	light microscope
rpm	rotation per minute
cont.	continued
n	number of specimens studied
=	equals to
–	to (long hyphene)
n.a.	not available
ATCC	American Tissue Culture Collection
CO ₂	carbon dioxide
DMSO	dimethyl sulfoxide
IC ₅₀	half maximal inhibitory concentration
ELISA	enzyme-linked immunosorbent assay
OD	optical density
NR	neutral red
kg	kilogram
PBS	phosphate buffer saline
MeOH	Methanol
DCM	dichloromethane
≤	less than equal
≥	greater than equal
RPMI	Rosewell Park Memorial Institute
GPS	Global Positioning Systems

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