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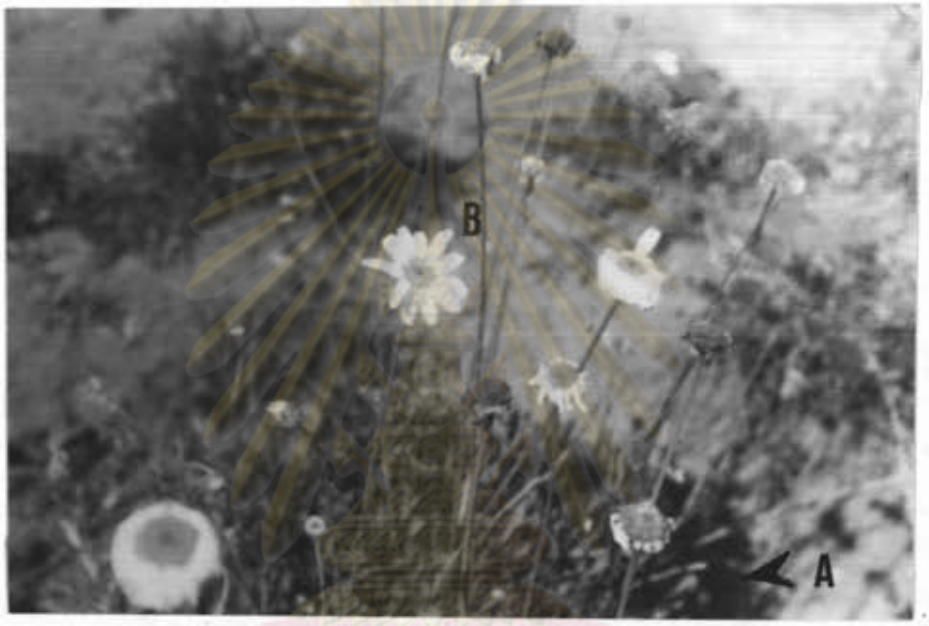
APPENDIX A



PYRETHRUM (*Chrysanthemum cinerariaefolium*)

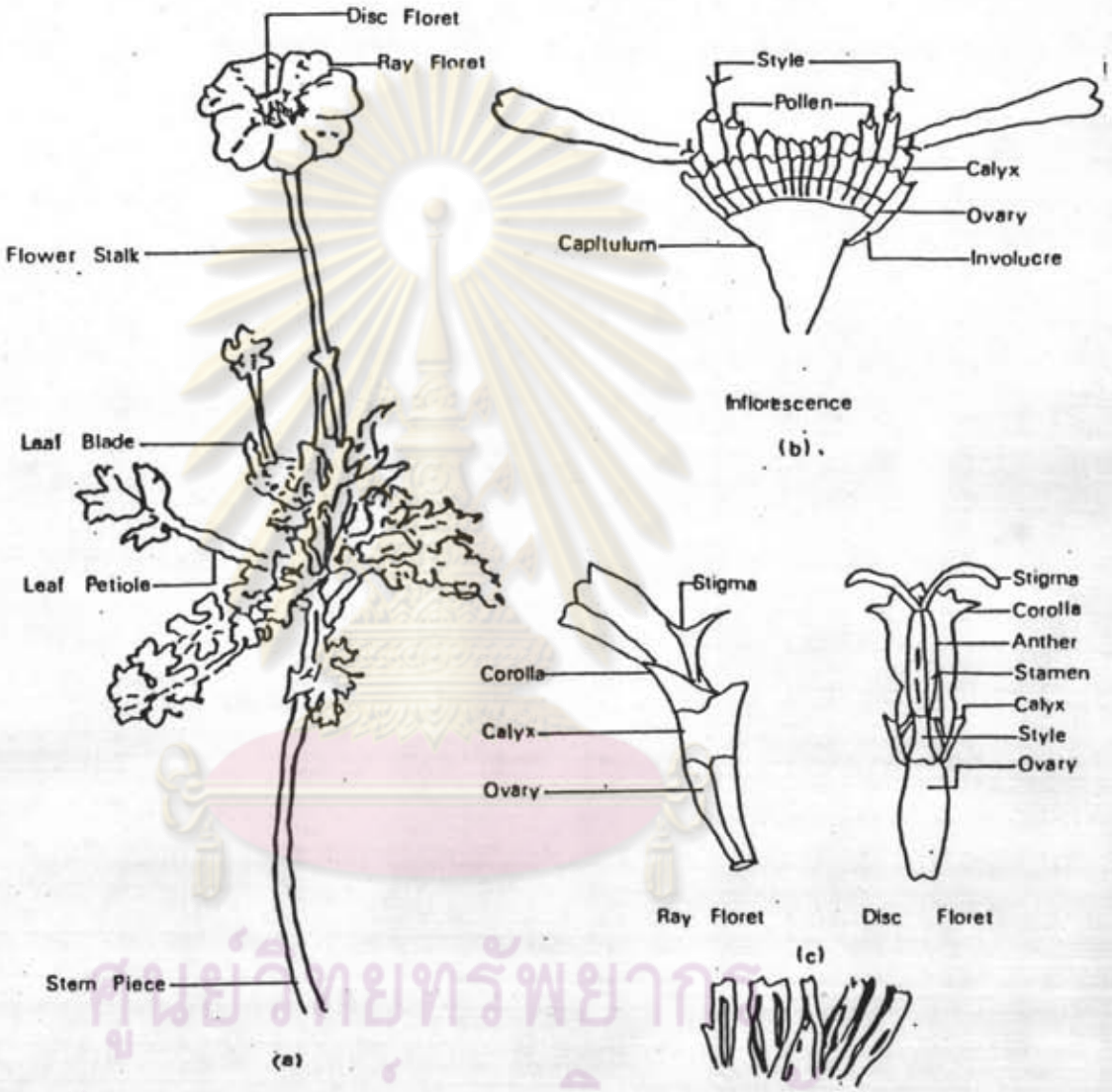
A. Natural vegetative plant portion

B. Flower head portion

APPENDIX B

SHOOT CULTURE

APPENDIX C



THE PORTION OF PYRETHRUM

(a) Whole plant organs.

(b) Flower head portion: the structure of flower head.

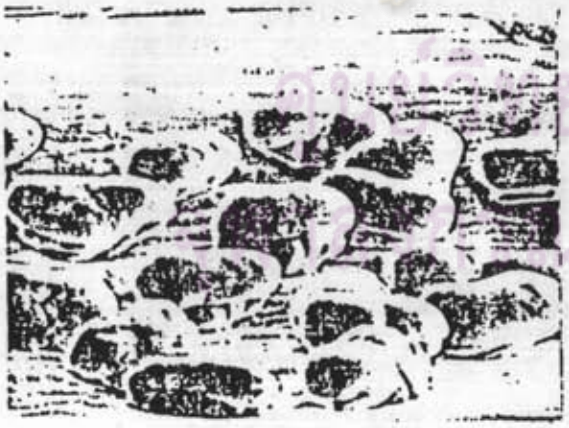
(c) Ray and disc florete.

(d) Seed illustration of pyrethrins deposited in achene wall stained with 2,4-D.

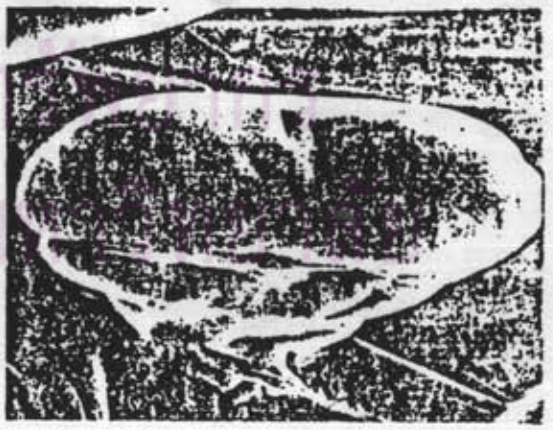
APPENDIX D

DEVELOPMENTAL STAGES OF OIL GLANDS DISTRIBUTED ON PYRETHRUM PLANT LEAF

A. Unexpanded oil glands clustered together at stage I (x400)



B. Fully expanded oil glands at stage II (x300)

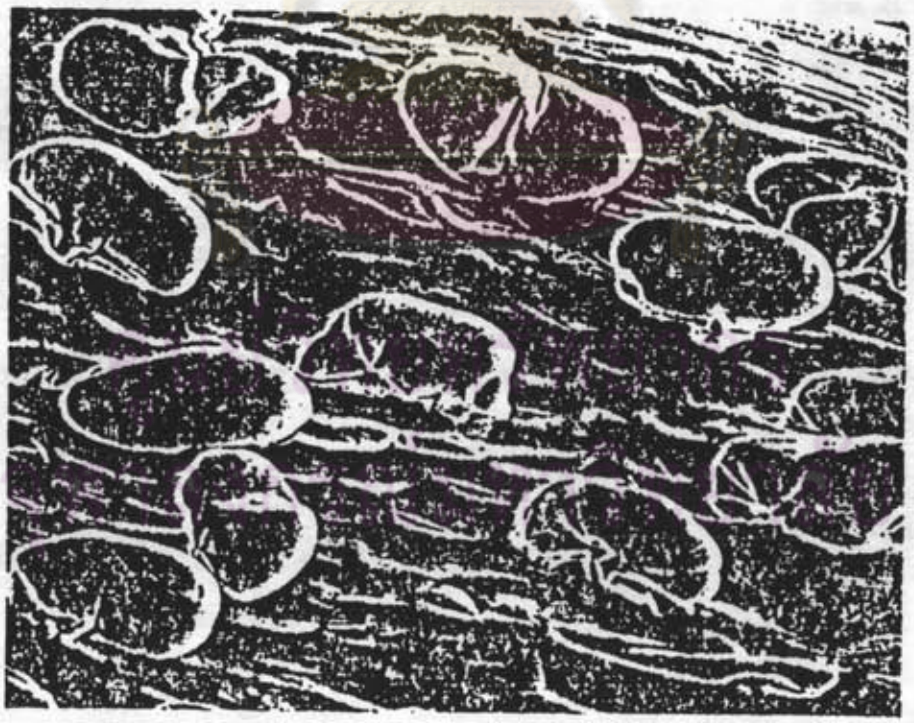


C. Magnified view of a fully developed oil gland at stage II (x800)

APPENDIX D (CONT.)

D. Collapsed oil gland at stage III (x430)

E. Magnified view of a collapsed oil gland at stage III (x1000)



F. Oil glands of a genotype showing very little collapsed in the glands at stage III (x260)

APPENDIX E

Preparation of Murashige and Skoog (MS) stock solution and MS medium (1962)

CHEMICAL AGENT	CHEMICAL FORMULA	CONCENTRATION (mg/l)	CONCENTRATION IN STOCK SOLUTION (1,000 x g/l)		
Ammonium nitrate	NH_4NO_3	1650	-		
Potassium nitrate	KNO_3	1900	-		
Calcium chloride	$CaCl_2.2H_2O$	440	-	*	
Magnesium sulphate	$MgSO_4.7H_2O$	370	-		
Potassium dihydrogen phosphate	KH_2PO_4	170	-		
Boric acid	H_3BO_3	6.2	6.2		
Potassium iodide	KI	0.83	0.83		
Sodium molypdate	$Na_2MoO_4.2H_2O$	0.25	0.25	Stock #1	1 ml
Cobalt chloride	$CoCl_2.6H_2O$	0.025	0.025		
Manganese sulphate	$MnSO_4.H_2O$	6.9	6.9		
Zinc sulphate	$ZnSO_4.H_2O$	6.14	6.14	Stock #2	1 ml
Copper sulphate	$CuSO_4.5H_2O$	0.025	0.025		
Disodium ethylenediamine tetraacetate	Na_2-EDTA	37.25	37.25		
Ferrous sulphate	$FeSO_4.7H_2O$	27.85	27.85	Stock #3	1 ml
Thiamine hydrochloride		0.1	0.1		
Nicotinic acid (Niacine)		0.5	0.5	Stock #4	1 ml
Pyridoxine hydrochloride		0.5	0.5		
Glycine		2.0	-		
Myo-inositol		100.0	-		
Sucrose		30,000	-	*	
Agar		10,000	-		

* Each agent should be separately weighted and freshly prepared before using

APPENDIX F

PREPARATION OF MURASHIGE AND SKOOG'S MEDIUM

1. The chemicals are dissolved in glass beaker with distilled water (Appendix E), the stock solutions, growth regulator solutions and 30 g sucrose added and then made to volumn.

2. pH adjusted to 5.6 agar powder (7.0 g) is added for the solid medium and made to homogenous solution.

3. The solutions, approximately 20 ml. is distributed into 50 ml. glass bottle. The bottle are stoppered with rubber lids and labelled.

4. The medium is autoclaved at 120^{o} C for 15 min. and the bottle removed for cooling at the room temperature until the medium is hard.

APPENDIX G

1. Preparation of stock solutions (dissolved chemicals in de-ionized water)

a) $NaH_2PO_4.H_2O$ = 750 mg/100 ml

Label bottle : B5 stock
$NaH_2PO_4.H_2O$
750 mg/100 ml

b) $(NH_4)_2SO_4$ = 670 mg/100 ml

Label bottle : B5 stock
$(NH_4)_2SO_4$
670 mg/100 ml

c) $MgSO_4.7H_2O$ = 1,250 mg/ml

Label bottle : B5 stock
$MgSO_4.7H_2O$
1.25 g/100 ml

d) Micronutrients

To 50 ml de-ionized water, add:

$MgSO_4.H_2O$	=	1,000 mg
H_3BO_3	=	300 mg
$ZnSO_4.7H_2O$	=	200 mg
$Na_2MoO_4.2H_2O$	=	25 mg
$CuSO_4.5H_2O$	=	2.5 mg
$CoCl_2.6H_2O$	=	2.5 mg

Add de-ionized water to final volume of 100 ml

Label bottle : B5 Micronutrients stock (100 ml)
(List chemicals and amount of each)

e) KI = 75 mg/100 ml

Label bottle : B5 stock
KI
75 mg/100 ml

f) Vitamins

To 50 ml de-ionized water add:

Nicotinic acid	=	100 mg
Thiamine.HCl	=	1,000 mg
Pyridoxine.HCl	=	100 mg
myo-Inositol	=	10,000 mg

Label bottle : B5 stock
Vitamins
(List chemicals and amounts of each)

g) 2,4-D

Dissolved 50 mg 2,4-D in 2-5 ml ethanol (heats slightly until chemical is completely dissolved). Gradually dilute to 100 ml with de-ionized water.

2. Preparation of 1-liter of B5 medium

a) To 500 ml of de-ionized water, add:

(1) $NaH_2PO_4.H_2O$ stock	=	20.0 ml
(2) $(NH_4)_2SO_4$ stock	=	20.0 ml
(3) $MgSO_4.7H_2O$ stock	=	20.0 ml
(4) Fe-EDTA (from MS stock)	=	5.0 ml
(5) KNO_3 (from MS stock)	=	26.3 ml
(6) $CaCl_2.2H_2O$ (from MS stock)	=	1.7 ml
(7) Sucrose	=	20.0 g
(8) Micronutrient stock	=	1.0 ml
(9) KI stock	=	1.0 ml
(10) Vitamins stock solution	=	1.0 ml
(11) 2,4-D stock (0.2-B5)	=	0.4 ml

b) Add de-ionized water to final volume of 1,000 ml

c) Adjust pH to 5.5-5.7

d) Disperse 600 ml to 50 ml Erlenmeyer flask (20 ml/flask)

e) Dispense 400 ml to vials (10 ml/vial)

APPENDIX H

A CHROMOSOMAL STAINING METHOD FOR ROOT TIP AND CULTURED CELLS

(Aceto-orcein, aceto-carmine Technique)

Materials and Reagents

1. Appropriate root tip and plant cultures cells, freshly subcultured leaf-derived callus and suspension culture cells, had been employed for this study.

2. Acetic-ethanol (one part of glacial acid + 3 parts of 95% ethanol)

3. Aceto-carmine or aceto-orcein staining reagent.

4. 8-Hydroxy quinoline reagent.

Pretreatment

Cut the roots ca. 5 mm starting from root tip, then rinsed twice with steriled water. For culture cells, scattered cells by the homogenizer.

Shorten the chromosome by transfering them into a small srew cap vial containing 2 ml 8-hydroxyquinoline and incubating the specimens at 1-2^{o}C for 12-24 hours.

Fixation

Added 3 ml of acetic-ethanol to the pretreated specimens. Leaved for 12-24 hours at the same temperature.

Note : The cells can be preserved in this fixative and remain in good condition at $4^{o}C$ for up to 2 weeks.

Preparation of Slides

Removed the fixed specimens into a new vial containing 70% EtOH and leaved them at the ambient temperature 1-2 hours before staining.

Dissected the root tips to piece, not longer than 1-2 mm and further placed on the clean slide. Added ca.1-2 ml of 10% HCL to the fixed cells for few min.

Exposed the slide to the fire in a few second.

Discarded the acid and washed the cells twice with steriled water.

Made the specimens dried before adding ca.2-3 ml aceto-orcein to the cluster of fixed cells and suddenly exposed to fire for a few second Repeated adding the staining reagent in the same manner, after remove some excess stain with an absorbent paper.

Finally, gently lower a cover slip into position over the drop and pressed the slides with a rubber ink roller or the thumb.

VITA

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