

植物細胞〈培養／工程〉系統簡介與應用

國立高雄大學生命科學系

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§ 植物細胞培養的定義、原理與目的

§ 植物細胞的發展

A. 建立培養基模式與荷爾蒙調控模式

B. 培養基組成：無機鹽類、碳源、生長調節劑等

§ 植物細胞培養的種類

A. 癒創組織培養； B. 懸浮細胞培養； C. 體胚培養； D. 器官培養；

E. 花藥培養； F. 原生質體培養

§ 植物細胞培養的型式

A. 固態培養：試管、培養皿、三角瓶等玻璃或塑膠容器

B. 液態培養：三角瓶、生物反應器

§ 植物細胞的研發應用

A. 種原保存； B. 各種倍數體的育成； C. 基因轉殖； D. 健康種苗生產；

E. 生產有用的二次代謝產物； F. 食品工業； G. 分子農場； H. 學術研究

§ 植物細胞培養的基本配備

§ 參考資料

§ 植物細胞培養的定義、原理與目的

定義：以植物組織細胞為單位，在離體條件下，於適當的培養基與無菌及環境條件，進行培養、分化或生產有用物質。

原理：細胞全能性是指單一個活細胞具有產生完整生物個體的潛力，即植物細胞具有去分化與再分化的能力

1902年 Haberlandt提出

植物組織或細胞可以分離獨立培養，並具有分化全能性的概念

細胞全能性(*totipotency*)：

指單一個活細胞具有產生完整生物個體的潛力，即植物細胞具有去分化(*de-differentiation*)與再分化(*re-differentiation*)的能力

1930~1960年

建立培養基模式與荷爾蒙調控模式

1970年~

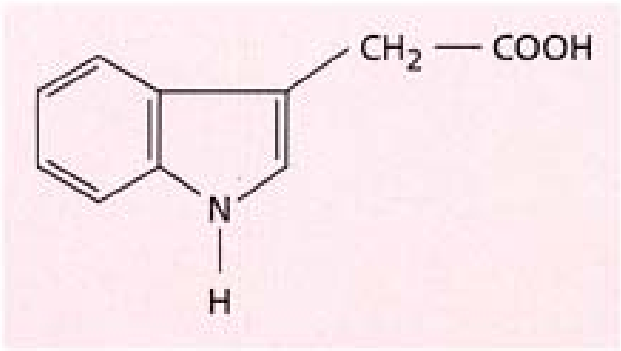
應用研究階段

目的：改善農業生產技術、保護自然資源、維護生態平衡、生物醫藥開發

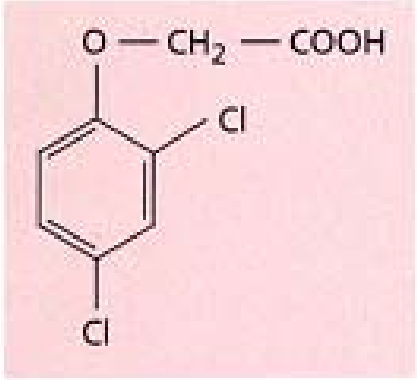
培養基組成：無機鹽類、碳源、生長調節劑等

| 一般藥劑 | |
|-----------------------|--|
| 無機鹽類 | 試藥級 |
| 蔗糖及其他糖類 | 試藥級，或台糖公司產品白砂糖（此產品不適於細胞及原生質體培養） |
| 洋菜膠 | Agar-Agar (Merck)，Bacto-agar (Difco)，或 phytigel (Merck) |
| 維生素類 | 維生素 B ₁ 、B ₂ 、B ₆ 、維生素 C 等 |
| 胺基酸類 | glycine、glutamate、glutamine、proline、methionine、cysteine 等 |
| 複合物 | yeast extract、casein hydrolysate、chacoal、coconut water |
| 其他 | inositol |
| 殺菌用藥劑 | |
| 抗生素 | ampicilin、hygromycin、kanamycin |
| 滅菌劑 | 酒精、次氯酸鈉 (NaOCl) 或漂白水、雙氧水 (H ₂ O ₂) |
| 其他 | Tween 20、清潔劑 |
| 植物生長調節劑 | |
| 生長素類 (auxin) | Indole-3-acetic acid (IAA)、indole-3-butyric acid (IBA)、 α -naphthaleneacetic acid (NAA)、2,4-dichlorophenoxyacetic acid (2,4-D) |
| 細胞分裂素類 (cytokinin) | Kinetin、benzyl adenine (BA)、2-ip |
| 激勃素 | Gibberellin (GAs)，主要使用 GA ₃ |
| 離層酸 | Abscisic acid (ABA) |
| 乙烯 | Ethrel (ethylene) |

Auxin:

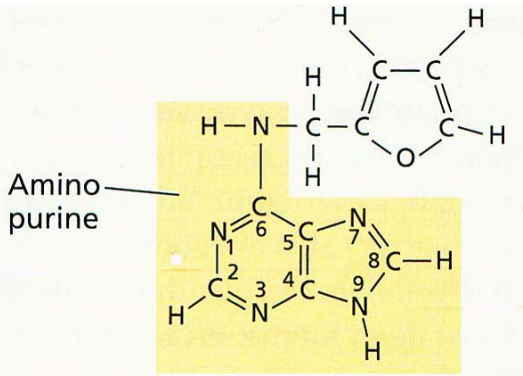


Indole-3-acetic acid (IAA)



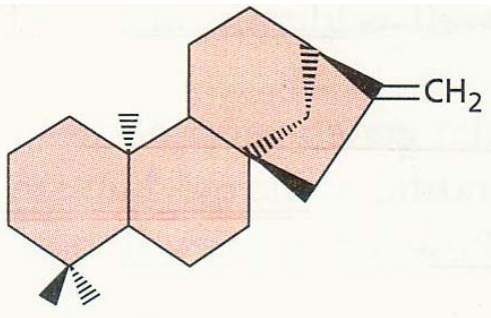
2,4-Dichlorophenoxyacetic acid (2,4-D)

Cytokinin:



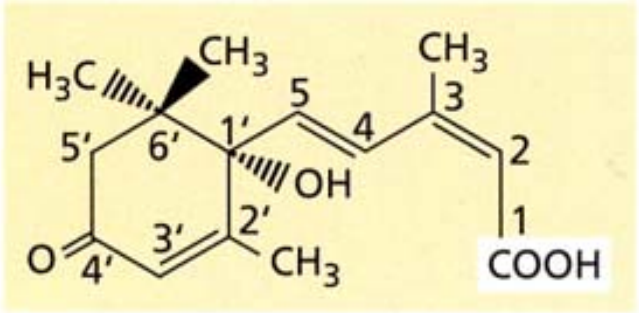
Kinetin

Gibberellin:



ent-Kaurene

Abscisic acid:



常用培养基配方

| Constituent | Concentration in culture medium (mg/ litre) | | | | |
|---|---|------------------------|----------------|----------|--------------------|
| | White's | Schenk and Hildebrandt | B ₅ | Heller's | Lismaier and Skoog |
| Ca(NO ₃) ₂ | 142 | - | - | - | - |
| KNO ₃ | 81 | 2500 | 3000 | - | 1900 |
| NaNO ₃ | - | - | - | 600 | - |
| NH ₄ NO ₃ | - | - | - | - | 1650 |
| NH ₄ H ₂ PO ₄ | - | 300 | - | - | - |
| (NH ₄) ₂ SO ₄ | - | - | 134 | - | - |
| MgSO ₄ ·7H ₂ O | 74 | 400 | 500 | 250 | 370 |
| CaCl ₂ ·2H ₂ O | - | 200 | 150 | 75 | 440 |
| KCl | 65 | - | - | 750 | - |
| KH ₂ PO ₄ | 12 | - | - | - | 170 |
| NaH ₂ PO ₄ ·H ₂ O | - | - | 150 | 125 | - |
| MnSO ₄ ·H ₂ O | - | 10 | 10 | - | - |
| MnSO ₄ ·4H ₂ O | - | - | - | 0.1 | 22.3 |
| KI | - | 1 | 0.75 | 0.01 | 0.83 |
| H ₃ BO ₃ | - | 5 | 3 | 1 | 6.2 |
| ZnSO ₄ ·7H ₂ O | - | 1 | 2 | 1 | 8.6 |
| CuSO ₄ | - | 0.2 | 0.025 | - | - |
| CuSO ₄ ·5H ₂ O | - | - | - | 0.03 | 0.025 |
| Na ₂ MoO ₄ ·2H ₂ O | - | 0.1 | 0.25 | - | 0.25 |
| CoCl ₂ ·6H ₂ O | - | 0.1 | 0.025 | - | 0.025 |
| AlCl ₃ | - | - | - | 0.03 | - |
| NiCl ₂ ·6H ₂ O | - | - | - | 0.03 | - |
| FeCl ₃ ·6H ₂ O | - | - | - | 1 | - |
| FeSO ₄ ·7H ₂ O | - | 15 | - | - | 27.86 |
| Fe ₂ (SO ₄) ₃ | 2.46 | - | - | - | - |
| Sequestrene 330 Fé | - | - | 28 | - | - |
| Na ₂ EDTA | - | 20 | - | - | 37.26 |
| Myo-Inositol | - | 1000 | 100 | - | 100 |
| Thiamine-HCl | - | 5 | 10 | - | 0.4 |
| Nicotinic acid | - | 5 | 1 | - | - |
| Pyridoxine-HCl | - | 0.5 | 1 | - | - |
| Yeast extract | 100 | - | - | - | - |
| Sucrose | 20 000 | 30 000 | 20 000 | - | 30 000 |
| pH | | 5.9 | 5.5 | | 5.8 |

**Murashige
& Skoog**

**Essential vs.
Beneficial elements**

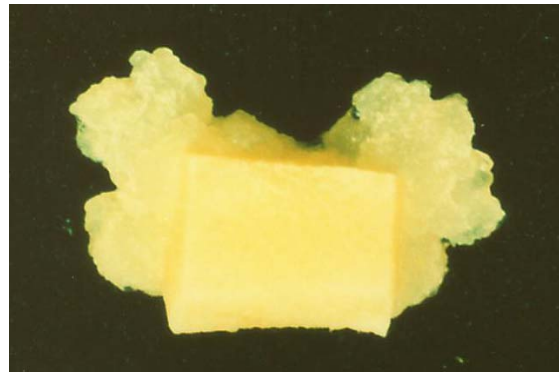
| 儲藏液編號 (No.) | 無機鹽種類 | 使用濃度 (mg/L) | 儲藏液濃度 (g/500 mL) | 倍數 |
|----------------|--|----------------|---------------------|---------|
| 1 | NH ₄ NO ₃ | 1650 | 41.25 | (50x) |
| | KNO ₃ | 1900 | 47.5 | |
| 2 | CaCl ₂ · 2H ₂ O | 440 | 11 | (50x) |
| 3 | MgSO ₄ · 7H ₂ O | 370 | 9.25 | (50x) |
| | MnSO ₄ · 4H ₂ O | 22.3 | 0.558 | |
| | ZnSO ₄ · 7H ₂ O | 8.6 | 0.215 | |
| 4 | FeEDTA | 37.5 | 0.94 | (50x) |
| 5 | KH ₂ PO ₄ | 170 | 4.25 | (50x) |
| | H ₃ BO ₃ | 6.2 | 0.155 | |
| | KI | 0.83 | 0.021 | |
| | Na ₂ MoO ₄ · 2H ₂ O | 0.25 | 0.006 | |
| 6 | | | (mg/500mL) | (1000x) |
| | CuSO ₄ · 5H ₂ O | 0.025 | 12.5 | |
| | CoCl ₂ · 6H ₂ O | 0.025 | 12.5 | |

§ 植物細胞培養的種類

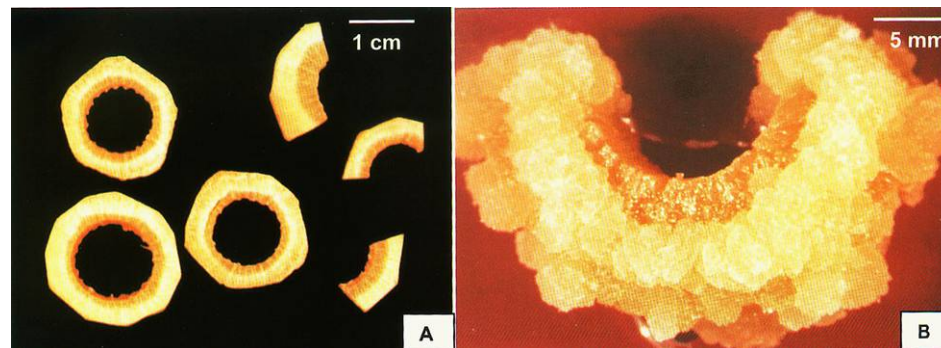
A. 癒創（合／傷）組織（**callus**）培養：

受傷的植物組織經過培養數日後會長出形狀不規則的一團細胞—癒創組織

甘藷



胡蘿蔔



§ 植物細胞培養的種類

B. 懸浮細胞 (suspension cell) 培養

細胞與液態培養基充分接觸，因此除了提供一個良好的實驗體系外，同時為植物細胞的大規模培養提供前期的技術基礎。



§ 植物細胞培養的種類

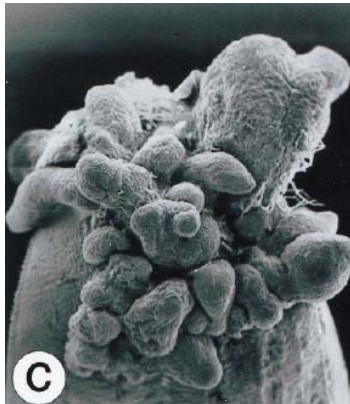
C. 體胚 (somatic embryo) 培養

在離體培養下沒有經過授精過程所形成胚的類似物—體胚或胚狀體

胡蘿蔔



文心蘭



文心蘭擬原球體

(protocorm-like body, PLBs)



§ 植物細胞培養的種類

D. 器官 (organ) 培養

生長素(auxin)與細胞分裂素(cytokinin)的比率影響癒創組織的分化



§ 植物細胞培養的種類

E. 花藥 (anther) 培養與花粉 (pollen) 培養

即孤雄生殖 (androgenesis)，可以迅速獲得純系植物

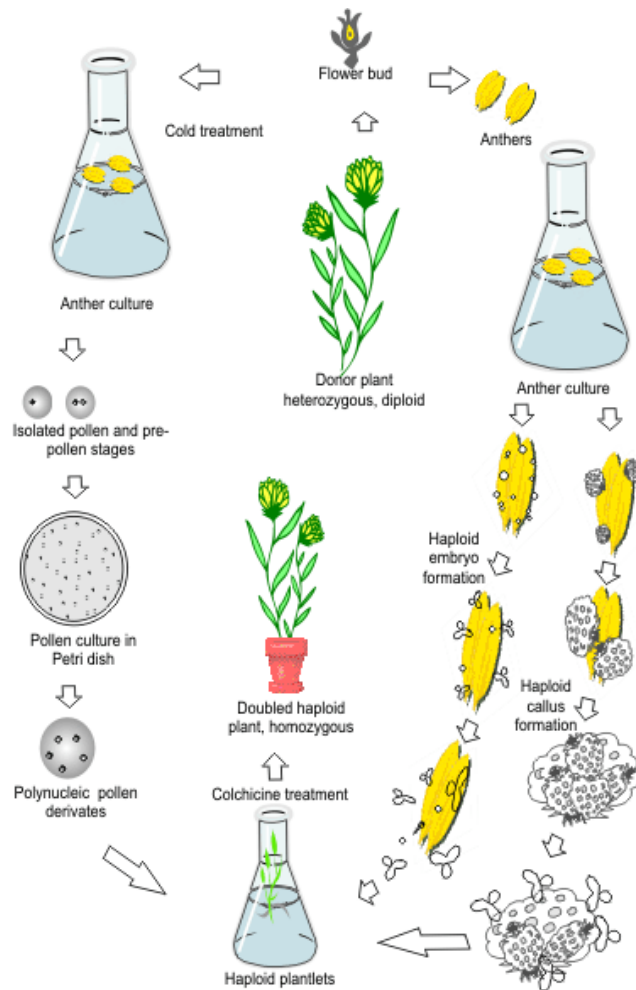


FIGURE 85: Schematic drawing of haploid production by androgenesis (anther and pollen culture)

§ 植物細胞培養的種類

F.原生質體 (protoplast) 培養

去除細胞壁的裸露球形細胞，更方便進行細胞膜生理生化及細胞壁再生機制等研究，甚至利用原生體融合得到屬間體細胞雜種 (somatic hybrid)

菸草葉片



杜鵑花瓣



利用原生質體融合獲得的屬間體細胞雜種

| 雜種組合 | 新屬 |
|--|---------------------------|
| 蘿蔔 <i>Raphanus sativus</i> ($2n = 18$) + 甘藍 <i>Brassica oleracea</i> ($2n = 18$) | <i>Raphanobrassica</i> |
| 甘藍 <i>Brassica oleracea</i> ($2n = 18$) + <i>Moricandia arvensis</i> ($2n = 27,28$) | <i>Moricandiobrassica</i> |
| 芝麻菜 <i>Eruca sativa</i> ($2n = 22$) + 甘藍型油菜 <i>Brassica napus</i> ($2n = 38$) | <i>Erucobrassica</i> |
| 二行芥 <i>Diplotaxis muralis</i> ($2n = 42$) + 甘藍型油菜 <i>Brassica napus</i> ($2n = 38$) | <i>Diplotaxobrassica</i> |
| 菸草 <i>Nicotiana tabacum</i> ($2n = 24$) + 番茄 <i>Lycopersicon esculentum</i> ($2n = 24$) | <i>Nicotipersicon</i> |
| 馬鈴薯 <i>Solanum tuberosum</i> ($2n = 24$) + 番茄 <i>Lycopersicon esculentum</i> ($2n = 24$) | <i>Solanopersicon</i> |
| 毛曼陀羅 <i>Datura innoxia</i> ($2n = 48$) + 顛茄 <i>Atropa belladonna</i> ($2n = 24$) | <i>Datirotropa</i> |
| 水稻 <i>Oryza sativa</i> ($2n = 24$) + 稻稗 <i>Echinochloa oryzicola</i> ($2n = 24$) | <i>Oryzochloa</i> |
| 阿拉伯芥 <i>Arabidopsis thaliana</i> ($2n = 10$) + 芸苔 <i>Brassica campestris</i> ($2n = 20$) | <i>Arabidobrassica</i> |

Pomato / Topato

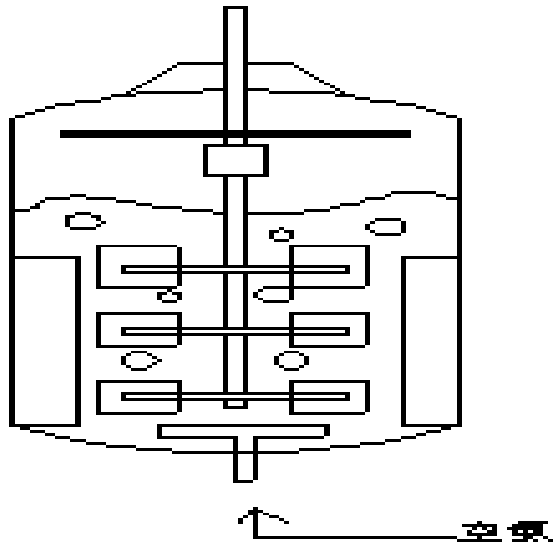
§ 植物細胞培養的型式

A. 固態培養：試管、培養皿、三角瓶等玻璃或塑膠容器

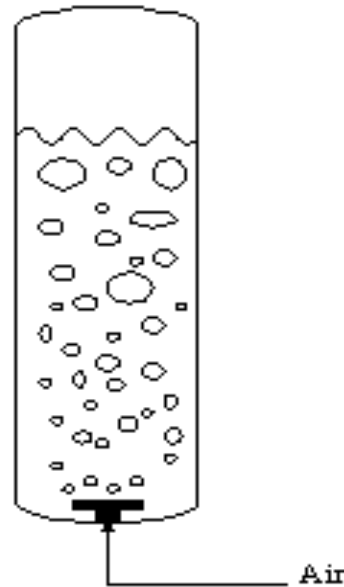


§ 植物細胞培養的型式

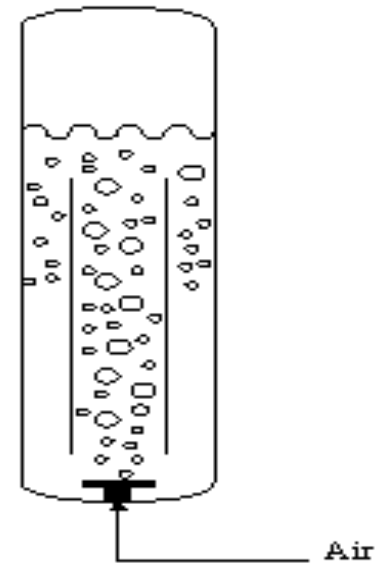
B. 液態培養：三角瓶、生物反應器



stirred tank bioreactor



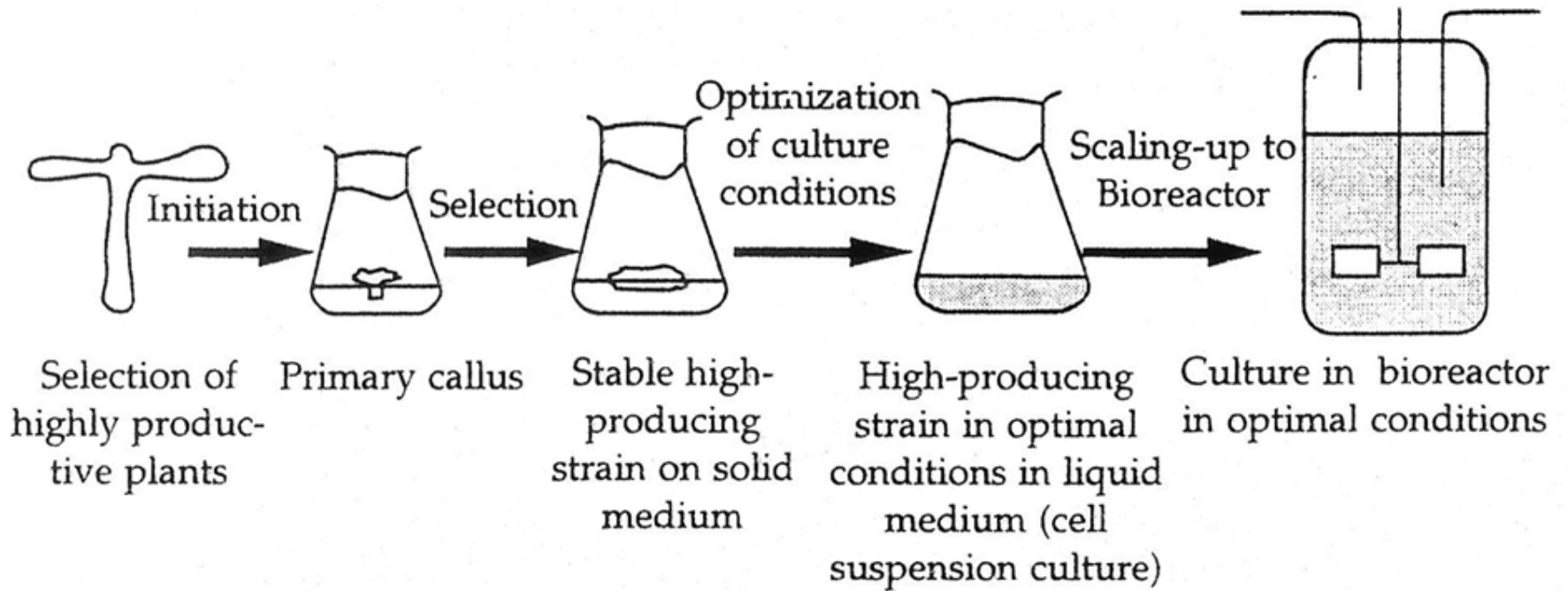
Bubble column reactor

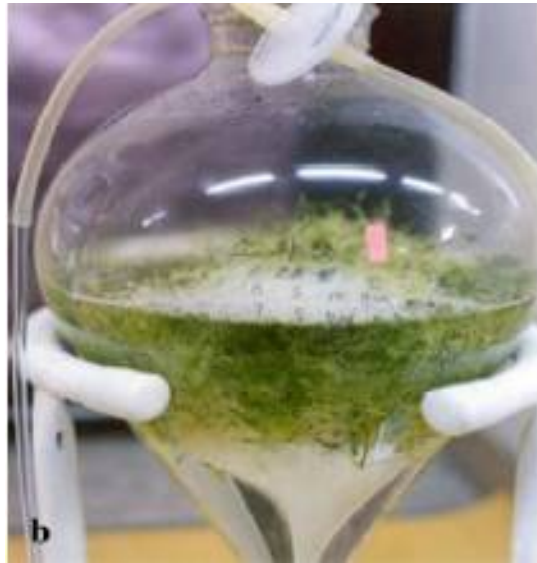
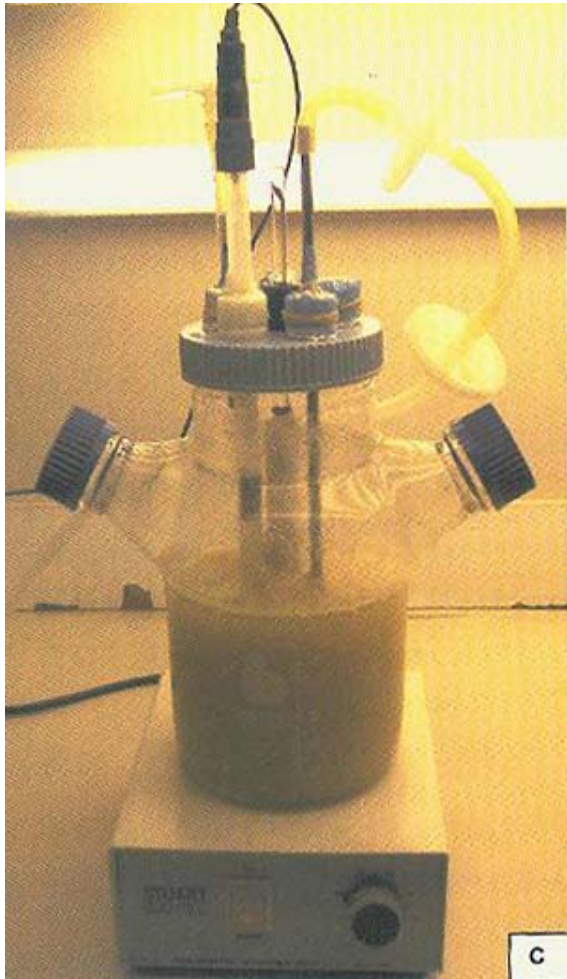


AirLift reactor

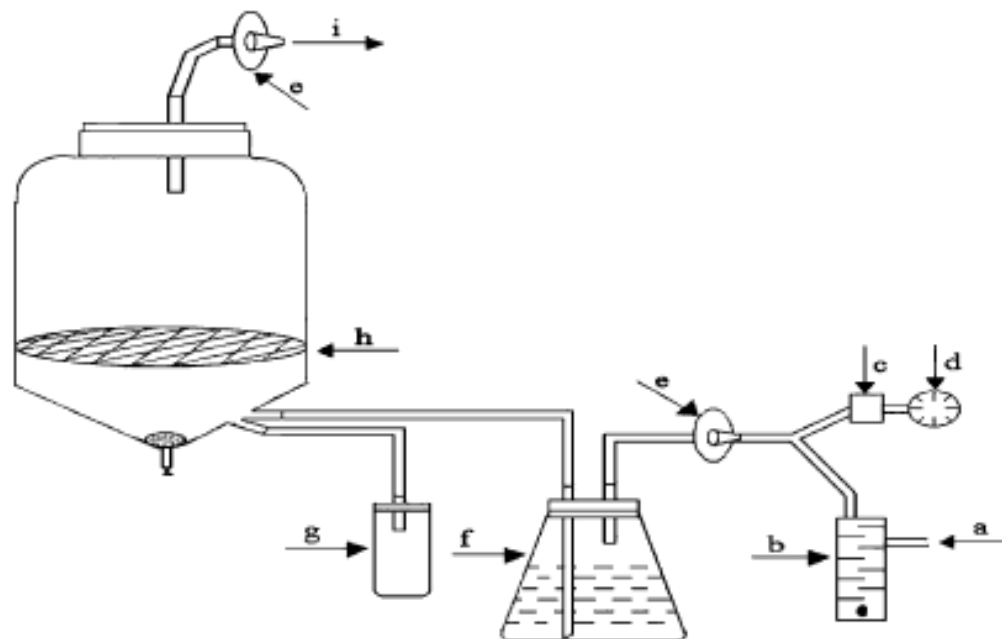
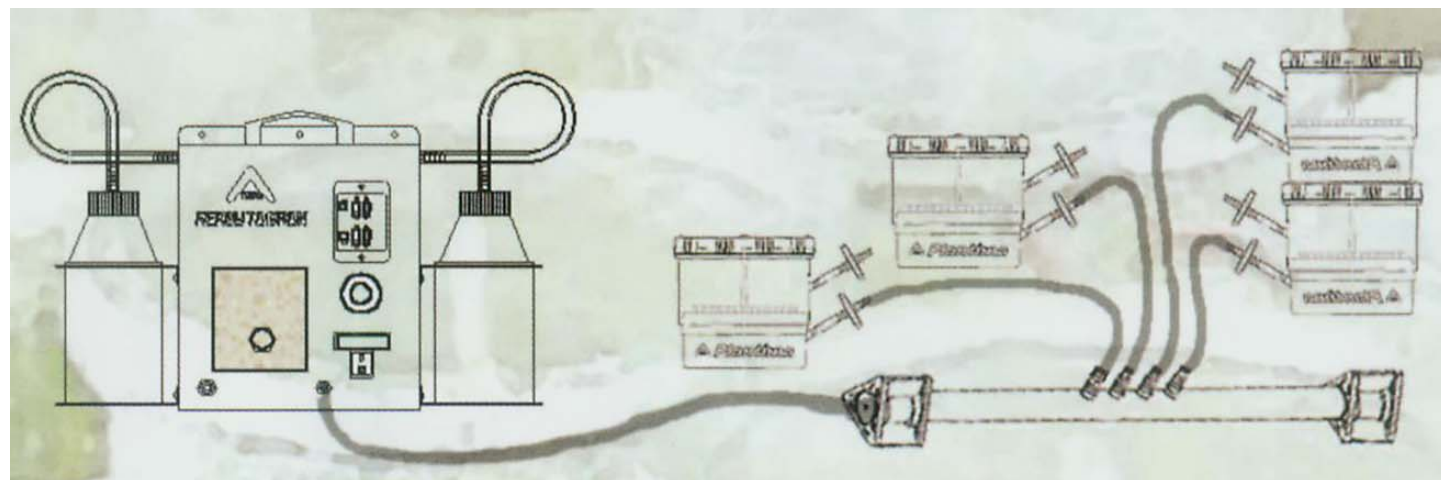
表二、植物細胞懸浮培養與微生物培養之比較

| 特 徵 | 微生物培養 | 植物細胞懸浮培養 |
|-------|-----------|---|
| 細胞大小 | 較小 1-10um | 40-200um |
| 細胞聚集 | 呈單細胞或菌絲團 | 易成團塊 |
| 生長速率 | 快 | 緩慢 |
| 細胞倍增 | 小時 | 天 |
| 接種密度 | 低 | 高 5-20% |
| 通氣量 | 高 | 低 10m moles/l/h |
| 剪力敏感度 | 不敏感 | 敏感 |
| 變異穩定性 | 穩定 | 穩定性較差 |
| 泡沫產生 | 較多 | 較少 |
| 產物形成 | 通常細胞外 | 大部份細胞液泡內 |
| | | CO₂/O₂/ethylene regulation |





淹灌式生物反應器



§ 植物細胞的研發應用

A. 植物育種：種原保存 (germplasm storage) – 超低溫保存

Table 1 Survival and growth of Salustiana sweet orange callus cultures subjected to freezing/thawing treatments

| Treatment | | Percent viable (number of cultures) | Embryo- genesis | |
|---------------------|-----------|---|--------------------|---------|
| Cryo- protection | Freezing | | | Thawing |
| + | - | - | 100 (14) | + |
| - | - | - | 100 (9) | + |
| + | Fast | Slow | 0 (20) | - |
| + | Fast | Fast | 0 (20) | - |
| + | Step-wise | Slow | 0 (30) | - |
| + | Step-wise | Fast | 0 (30) | - |
| + | Slow | Slow | 0 (32) | - |
| + | Slow | Fast | 100 (26) | + |

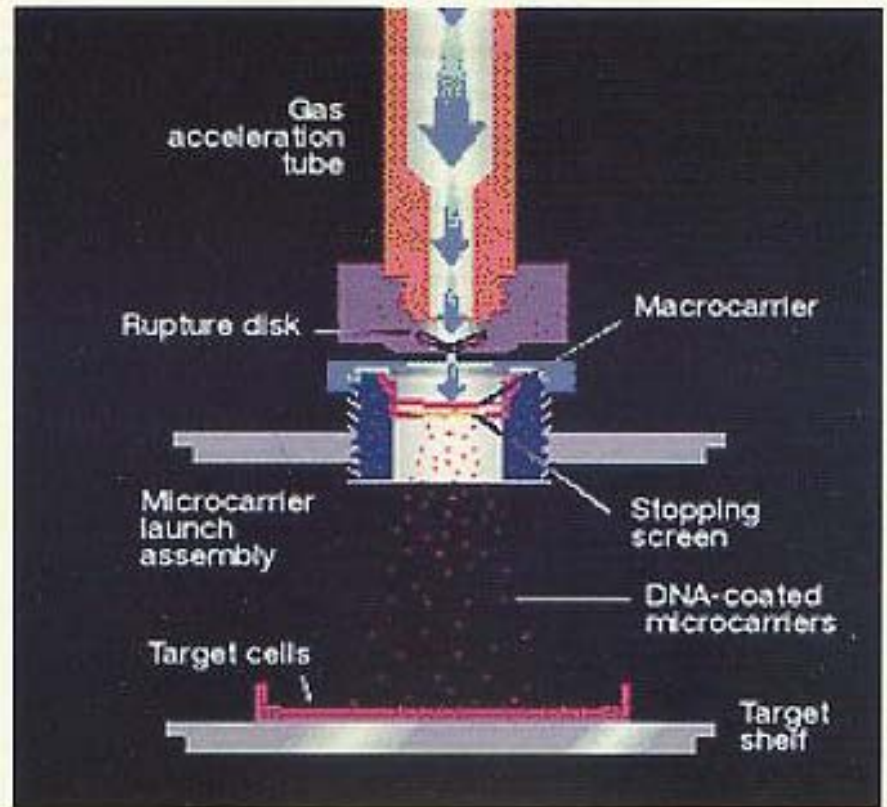
Plant Cell Report (1997) 17: 44

滲透型抗凍劑：甘油、二甲亞砜、乙二醇

非滲透型抗凍劑：葡聚糖、聚乙二醇、羥乙基澱粉、蔗糖

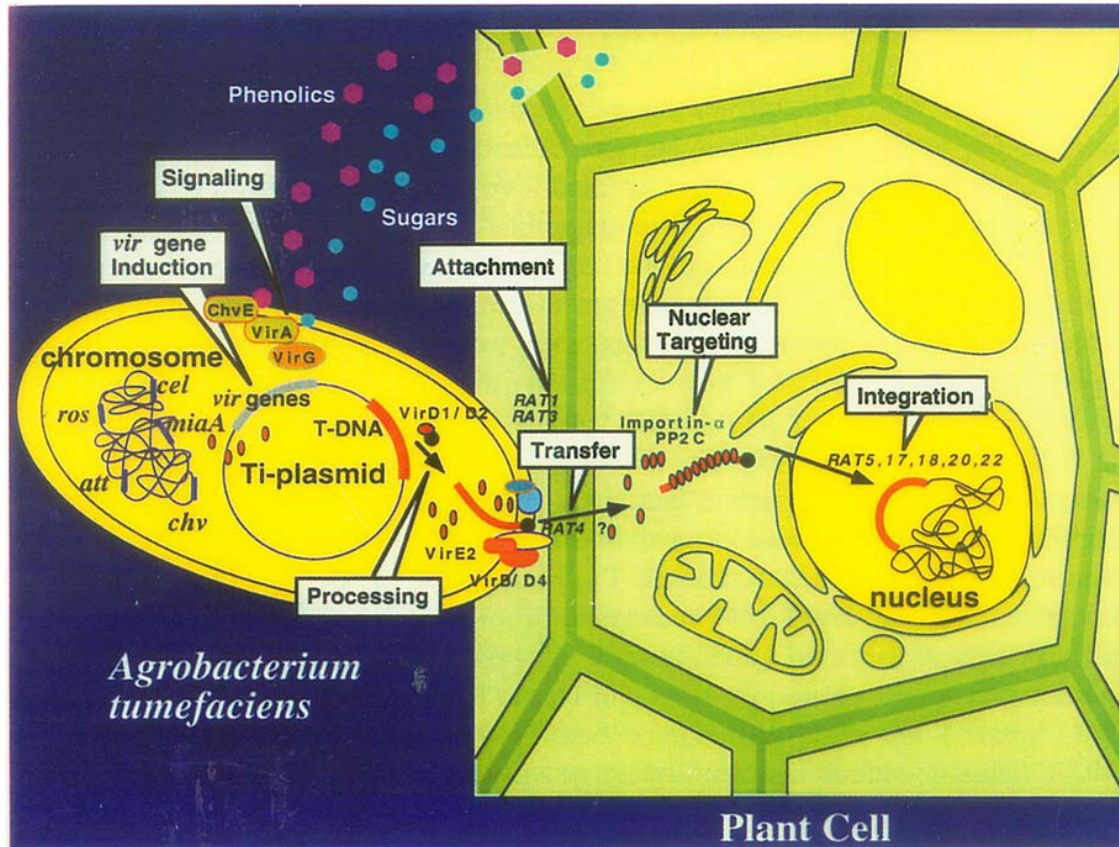
§ 植物細胞的研發應用

C. 基因轉殖：粒子槍基因轉植系統



§ 植物細胞的研發應用

C. 基因轉殖：農桿菌基因轉植系統



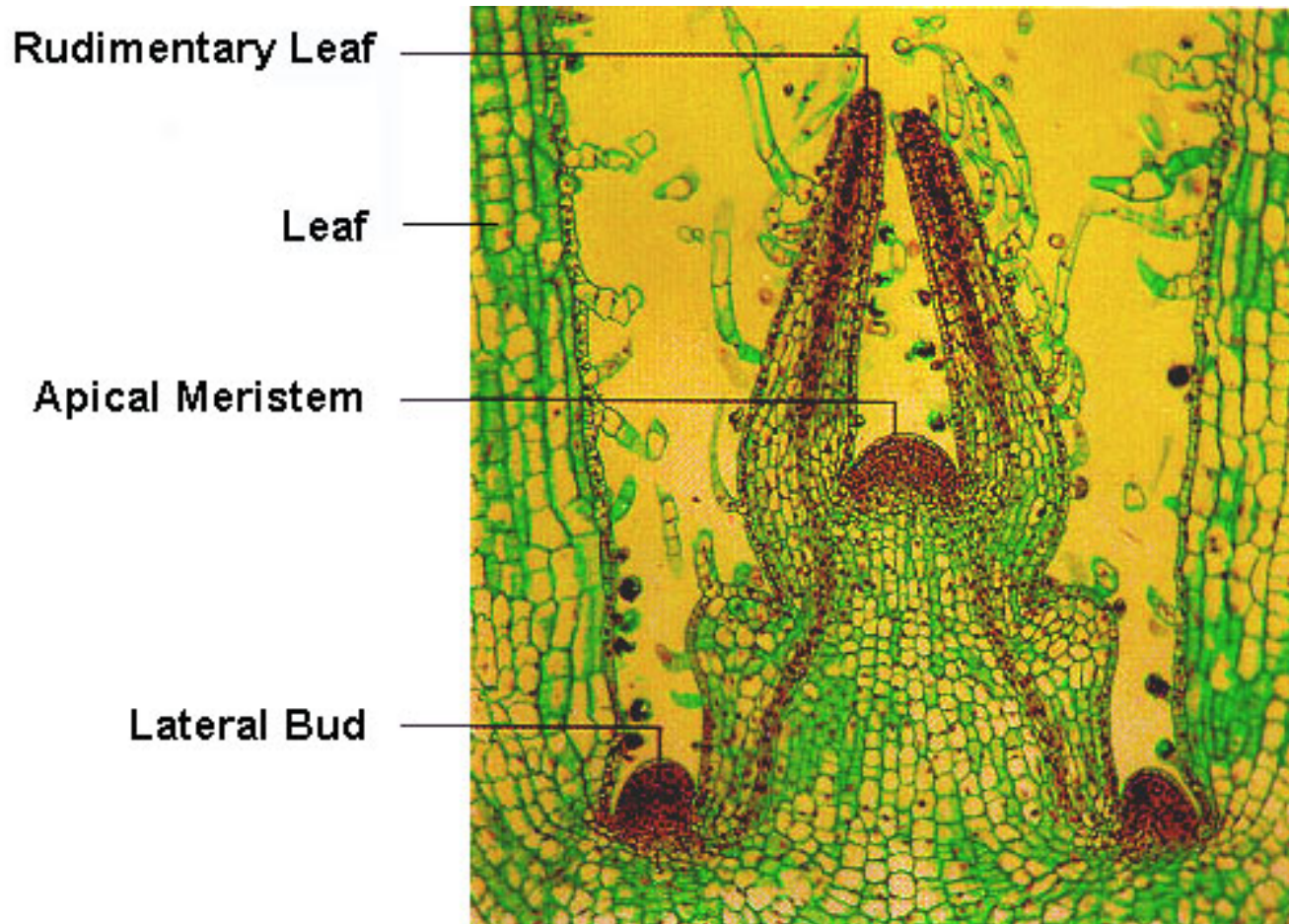
A. rhizogenes

Figure 1 Schematic diagram of the *Agrobacterium* infection process. Critical steps that occur to or within the bacterium (chemical signaling, *vir* gene induction, and T-DNA processing) and within the plant cell (bacterial attachment, T-DNA transfer, nuclear targeting, and T-DNA integration) are highlighted, along with genes and/or proteins known to mediate these events.

Annu. Rev. Plant Physiol. Mol. Biol. (2000) 51: 223

§ 植物細胞的研發應用

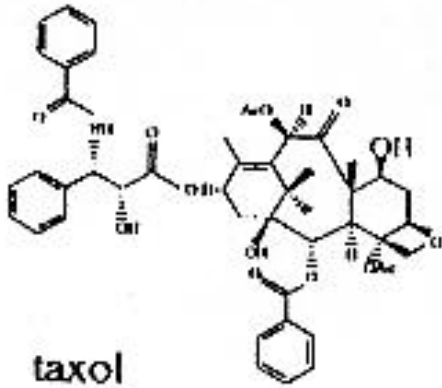
D. 健康種苗生產：利用病毒的分布有向尖端遞減的趨勢



§ 植物細胞的研發應用

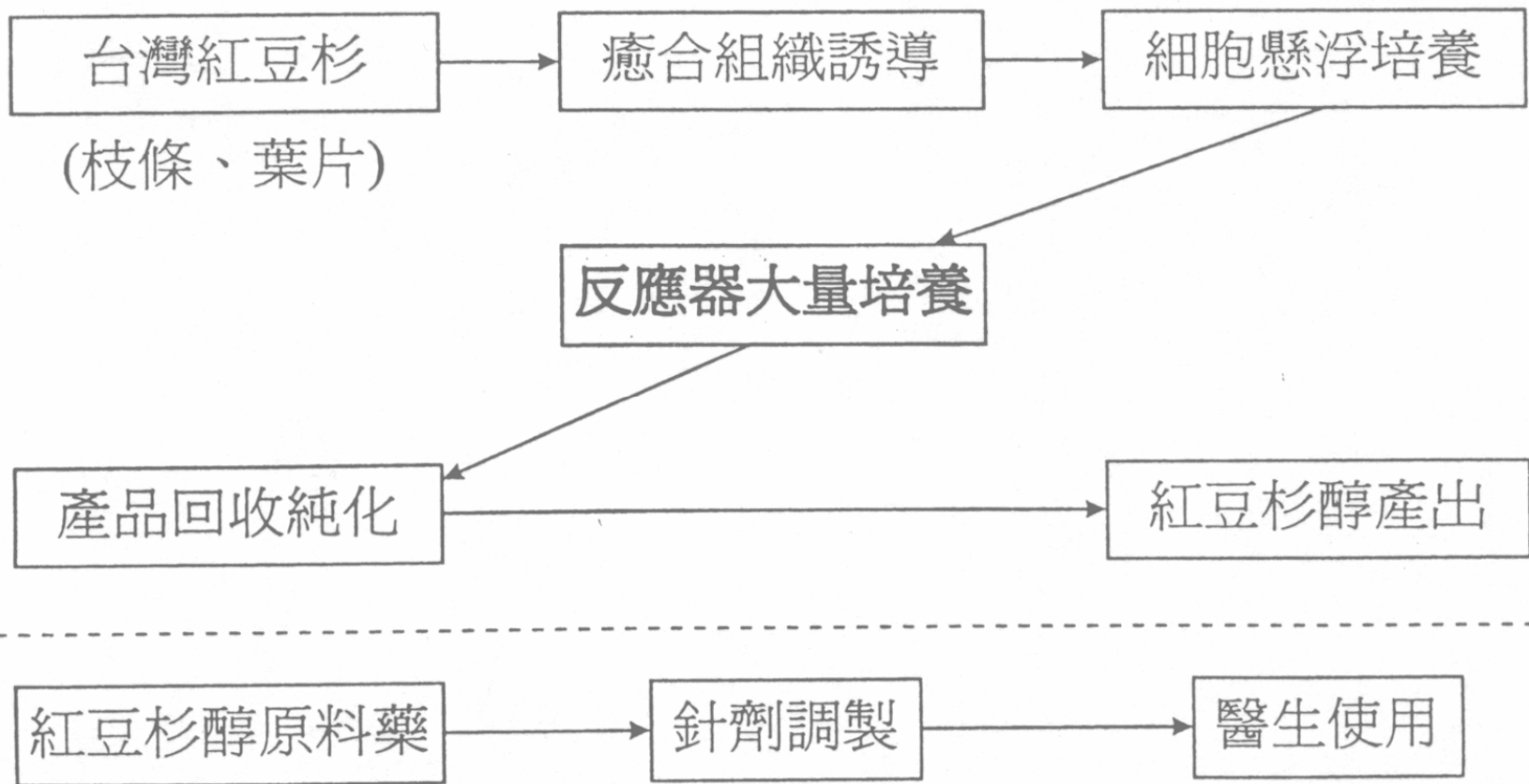
E. 生產有用的二次代謝產物：

紫杉醇 (taxol)



台灣紅豆杉的枝葉與果實





利用密植栽培台灣紅豆杉生產枝葉提煉紫杉醇



利用生物反應器培養台灣紅豆杉細胞生產紫杉醇



農業生物技術國家型科技畫

何政坤副研究員兼組長 農委會林業試驗所育林組

E. 生產有用的二次代謝產物： 紫草素 (shikonin)

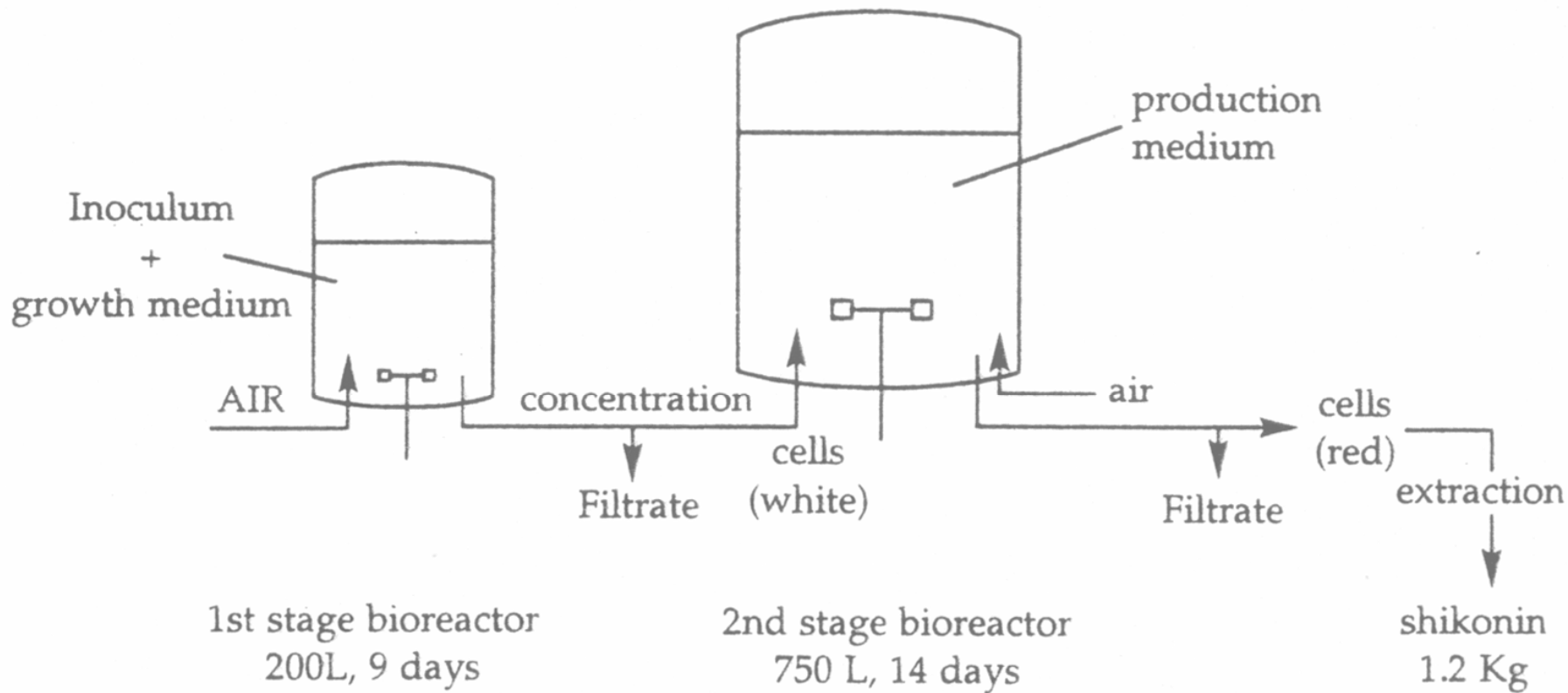


Fig. 17.3 Industrial production of shikonin by two-stage batch culture of *Lithospermum erythrorhizon* cells

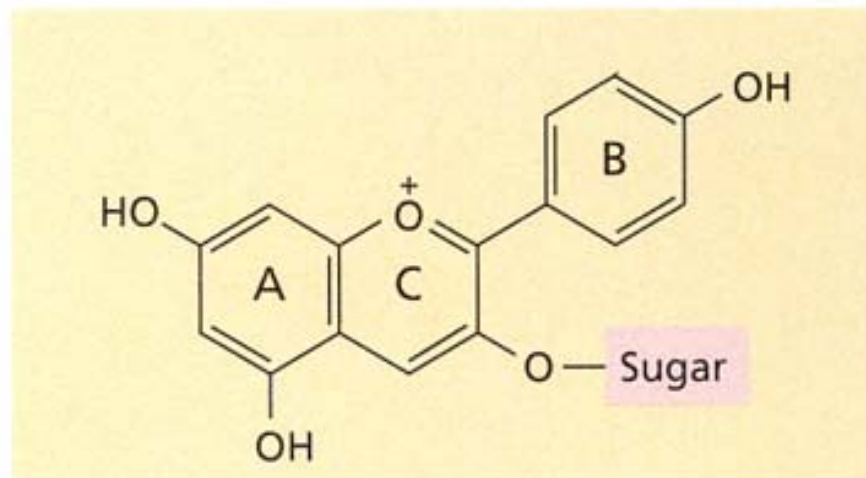
植物來源之處方藥及其化學特性

| 植物名稱 | 學名 | 成份 | 作用 |
|--------|--------------------------------|---------------------------------|--------------------------|
| 古柯 | <i>Eruthroxylum coca</i> | Novocaine 、cocaine | 麻醉 |
| 翼葉毛果芸香 | <i>Pilocarpus microphyllus</i> | Pilocarpine | 青光眼 |
| 麥角 | <i>Claviceps puipurea</i> | Ergonovine 、Ergotamine | 偏頭痛、子宮縮收、墮胎劑 |
| 墨西哥山藥 | <i>Dioscorea barbasco</i> | Dioscorine | 性荷爾蒙、更年期、骨質疏鬆 |
| 印度蛇木 | <i>Rauwolfia serpentina</i> | Resperine 、 Rescinnamine | 降血壓、鎮靜劑、精神異常 |
| 日日春 | <i>Catharanthus roseus</i> | Vinblastine Vincristine | Hodgkin's 、 兒童白血病 |
| 毛地黃 | <i>Digitalis purpurea</i> | Digitalis | 強心劑、水腫 |
| 麻黃 | <i>Ephedra sinica</i> | Ephedrine | 氣喘、感冒、水腫、腎臟病、 泌尿、生殖器 |
| 太平洋紫杉 | <i>Taxus brevifolia</i> | Taxol(paclitaxel) | 卵巢、癌乳、癌肺癌 |
| 白柳樹 | <i>Salix alba</i> | Salicylic acid | 頭痛、發燒、新血管疾病 |
| 罌粟 | <i>Papaver somniferum</i> | Morphine | 麻醉、鎮靜、下痢 |
| 奎寧 | <i>Cinchona pubescens</i> | Quinine | 瘧疾、心律不整 |
| 鬼白樹 | <i>Pedophyllum peltatum L.</i> | Etoposide 、Podofilox 、 peltatin | 抗癌、通便劑、除蟲劑、疣、 皮膚癌、睪丸癌 |
| 喜樹 | <i>Camptotheca acuminata</i> | camptothecin | 肺癌、乳癌 |

§ 植物細胞的研發應用

F. 食品工業：食品添加物

| Product type | Plant | Source |
|------------------------|--------------------------------|---|
| <i>Colours</i> | | |
| Anthocyanin | <i>Vitis vinifera</i> | Cell cultures |
| | <i>Daucus carota</i> | Cell cultures |
| | <i>Euphorbia millii</i> | Cell cultures |
| Betalaines | <i>Beta vulgaris</i> | Cell cultures and hairy root cultures |
| Crocin and Crocetin | <i>Crocus sativus</i> | Stigma proliferated in culture |
| <i>Flavours</i> | | |
| Capsicum and Capsaicin | <i>Capsicum frutescens</i> | Immobilized cell cultures |
| | <i>Capsicum annuum</i> | |
| Vanilla and Vanillin | <i>Vanilla planifolia</i> | Cell cultures |
| Safranal | <i>Crocus sativus</i> | Stigma proliferated in culture, Cell cultures |
| <i>Sweeteners</i> | | |
| Stevioside | <i>Stevia rebaudiana</i> | Cell culture by biotransformation |
| Thaumatococin | <i>Thaumatococcus danielli</i> | Transgenic cell lines |



Anthocyanin

TABLE 13.1
Effects of ring substituents on anthocyanidin color

| Anthocyanidin | Substituents | Color |
|---------------|---|---------------|
| Pelargonidin | 4'— OH | Orange red |
| Cyanidin | 3'— OH, 4'— OH | Purplish red |
| Delphinidin | 3'— OH, 4'— OH, 5'— OH | Bluish purple |
| Peonidin | 3'— OCH ₃ , 4'— OH | Rosy red |
| Petunidin | 3'— OCH ₃ , 4'— OH, 5'— OCH ₃ | Purple |

§ 植物細胞的研發應用

G. 分子農場：植物製造的藥物與功能性的蛋白質之生產

Tab. 1 Key events in the history of *Molecular Farming*.

| Year | Highlight | Reference |
|------|---|-----------|
| 1986 | First plant-derived recombinant therapeutic protein – human growth hormone in tobacco and sunflower ¹⁾ | 1 |
| 1989 | First plant-derived recombinant antibody – full-size IgG in tobacco | 2 |
| 1990 | First native human protein produced in plants – human serum albumin in tobacco and potato | 3 |
| 1992 | First plant-derived vaccine candidate – hepatitis B virus surface antigen in tobacco | 4 |
| 1992 | First plant-derived industrial enzyme – α -amylase in tobacco | 5 |
| 1995 | Secretory IgA produced in tobacco | 6 |
| 1996 | First plant-derived protein polymer – artificial elastin in tobacco | 7 |
| 1997 | First clinical trial using recombinant bacterial antigen delivered in a transgenic potato | 8 |
| 1997 | Commercial production of avidin in maize | 9 |
| 1999 | First glycan analysis of plant-produced recombinant glycoprotein | 10 |
| 2000 | Human growth hormone produced in tobacco chloroplasts | 11 |
| 2000 | Triple helix assembly and processing of human collagen produced in tobacco | 12 |
| 2001 | Highest recombinant protein accumulation achieved in plants so far – 46.1% total soluble protein for <i>Bacillus thuringiensis</i> Cry2Aa2 protein | 13 |
| 2001 | First multi-component vaccine candidate expressed in potato – cholera toxin B and A2 subunits, rotavirus enterotoxin and enterotoxigenic <i>Escherichia coli</i> fimbrial antigen fusions for protection against several enteric diseases | 14 |
| 2001 | Glycan modification of a foreign protein produced in a plant host using a human glycosyltransferase | 15 |
| 2003 | Expression and assembly of a functional antibody in algae | 16 |
| 2003 | Commercial production of bovine trypsin in maize | 17 |
| 2004 | Genetic modification of the <i>N</i> -glycosylation pathway in <i>Arabidopsis thaliana</i> resulting in complex <i>N</i> -glycans lacking β 1,2-linked xylose and core α 1,3-linked fucose | 18 |

Genomics

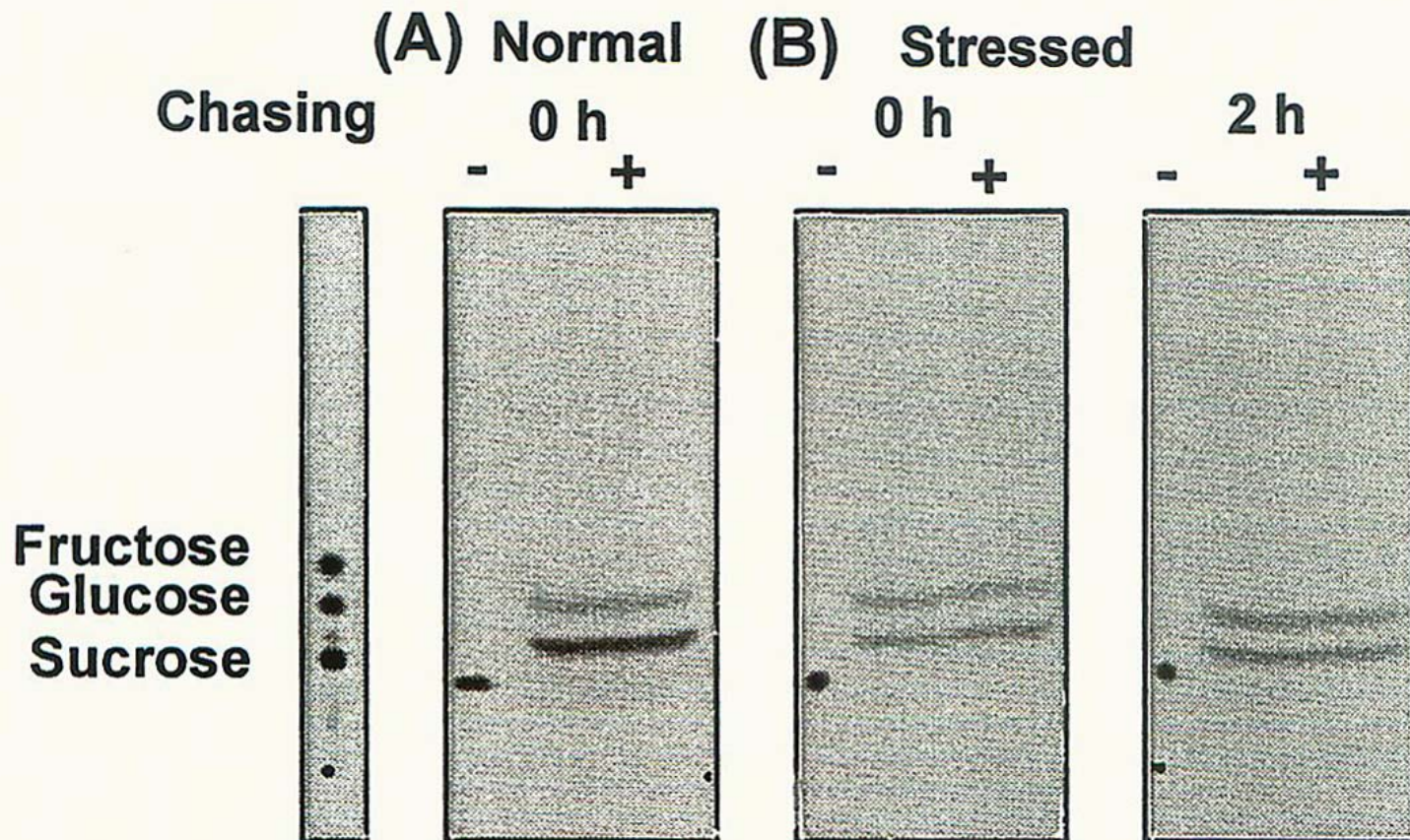
Proteomics

Metabolomics

Glycomics

§ 植物細胞的研發應用

H. 學術研究：正常與逆境處理的甘藷懸浮細胞內蔗糖組成



§ 植物細胞培養的基本配備

A. 實驗室設置：準備區，接種區，培養區

無菌操作台 (laminar flow) :

水平送風式

垂直送風式



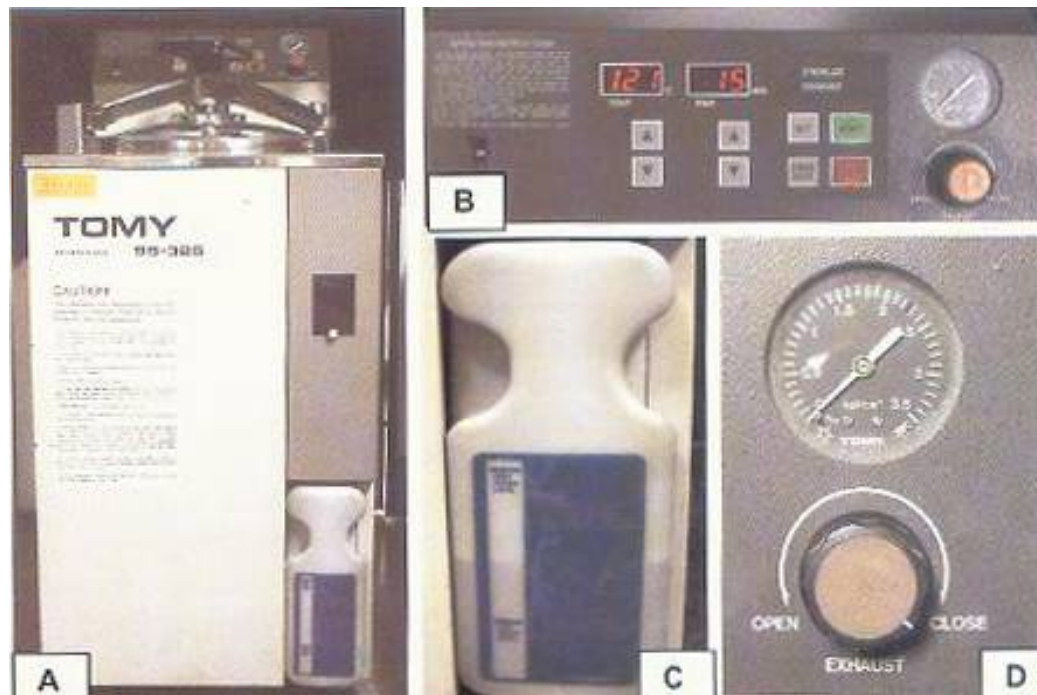
High efficiency particulate air (HEPA) filter

Prefilter

培養區：生長箱，培養室



滅菌

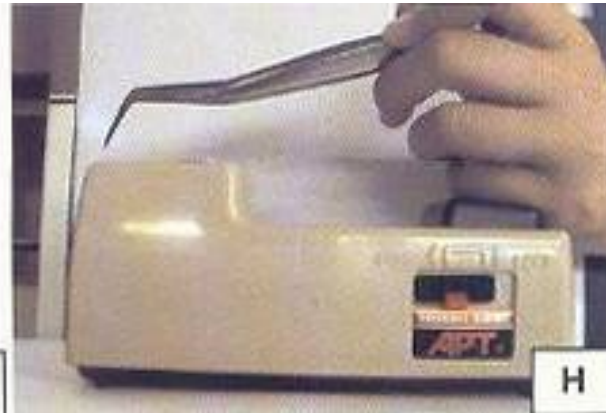


MINIMUM AUTOCLAVING TIME FOR PLANT TISSUE CULTURE MEDIA

| Volume of Medium per Vessel (ml) | Minimum Autoclaving (min) ^a | Volume of Medium per Vessel (ml) | Minimum Autoclaving (min) ^a |
|----------------------------------|--|----------------------------------|--|
| 25 | 20 | 500 | 35 |
| 50 | 25 | 1000 | 40 |
| 100 | 28 | 2000 | 48 |
| 250 | 31 | 4000 | 63 |

^aMinimum Autoclaving time includes the time required for the liquid volume to reach the sterilizing temperature (121°C) and 15 min at 121°C (Burger, 1988). Times may vary due to differences in autoclaves. Validation with your system is recommended.

| 試 藥 | 濃 度 | 滅 菌 時 間 |
|---|-----------|--------------|
| Ethanol | 70 % | <1 min |
| HgCl ₂ (mercuric chloride) | 2 % | 10 min |
| NaOCl (sodium hypochloride) | 1 % | 10 min - 2 h |
| Clorox | 10 - 30 % | 10 min - 2 h |
| H ₂ O ₂ (hydrogen peroxide) | 1 - 3 % | 10 min - 2 h |
| Antibiotics | 100 mg/L | 30 min |



C. 其它設備

冰箱、乾燥機、純水機、加熱攪拌器、pH測量器、
超音波震盪器、顯微鏡

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