

## Review

# Anthemideae: advances in tissue culture, genetics and transgenic biotechnology

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Members of the Anthemideae include important floricultural (cut-flower) and ornamental (pot and garden) crops, as well as plants of medicinal and ethno-pharmacological interest. Despite the use of many of these plants (over 1400 species) in the extraction of important secondary metabolites and essential oils, the greatest emphasis has been on their *in vitro* tissue culture and micropropagation. Few studies have been conducted on genetic transformation, with those primarily focused on increasing yield of compounds in plants. This review, the first and only available for plants within this Family, highlights all the available literature that exists on Anthemideae (excluding ornamental chrysanthemums) *in vitro* cell, tissue and organ culture, micropropagation and transformation.

**Key words:** *Achillea*, *Anthemis*, *Artemisia*, *Matricaria*, *Santolina*, *Tanacetum*.

## INTRODUCTION

Members of the Anthemideae top over 1400 species (the most common known by different names globally, Table 1) and consist of one of the most important global cut flower and pot plants, *Dendranthema grandiflora*, as well as important medicinal and aromatic plants from which many important secondary metabolites and essential oils are extracted. Despite this, the number of studies conducted on the tissue culture and micropropagation of its members are few, focusing only on one or two individual species that produce compounds of high economic value. Furthermore, in these same species the scarce genetic transformation studies have been primarily conducted to increase yields of those compounds or oils. A summary of these research findings is presented in this review.

Members of the Anthemideae have occupied an important place in the cultural practices the world over. A review on ornamental chrysanthemum biotechnology is

discussed elsewhere (Teixeira da Silva, 2003). Garland chrysanthemum, *Chrysanthemum coronarium* and *C. segetum* are widely distributed in the Mediterranean, western Africa and Asia. *C. coronarium*, cultivated in Japan, China and Southeast Asia, is closely related to lettuce, and is a valuable edible species (Oka et al., 1999). *C. coronarium* var. *coronarium* is an ornamental, often found as a common weed, while *C. coronarium* var. *spatiosum* is used as a Chinese vegetable (chop-suey). Green leaves and stems of *C. segetum* are also consumed as vegetables. Chrysanthemum is a source of various valuable metabolites (Schwinn et al., 1994).

The *Chrysanthemum*-complex is a group that includes *Achillea*, *Ajania*, *Anthemis*, *Arctanthemum*, *Argyranthemum*, *Artemisia*, *Balsamita*, *Chrysanthemum*, *Dendranthema*, *Heteranthemis*, *Hymenostemma*, *Ismelia*, *Leucanthemella*, *Leucanthemum*, *Matricaria*, *Nipponanthemum*, *Pyrethrum*, *Tagetes*, *Tanacetum* and

**Table 1.** Some common names of main species within the Anthemideae.

Species	Name(s) (LANGUAGE)
<i>Achillea millefolium</i>	Hazanbal (A-Egypt); hazanbul, milfoil, millefeuille, nosebleed, soldier's woundwort, staunchweed, thousand leaf/seal/weed, woundwort, yarrow (E); siankärsämö (Fi); bauchweh-/blutstill-/garben-/grillen-kraut, bibhenderlkraut, gerreworzel, mausleiter, schafrippe, schafzunge, tausendblatt, schafgarbe, tausendblättchen (G); milefoglio (montano) (I); seiyonokogirisou (J); aquiléa, erva-de-carpinteiro, mil-folhas, milefólio (P); colchon de pobre, milenrama (S); röllika (Sw); vândiêp, đurongky (V); biranjasif, cickafarkkoro, civanpercemi, duizendblad, rojmari, rolleka, rollike, tlalquequetzal (O)
<i>Anthemis nobilis</i>	Roman/sweet chamomile (E); camomila-romana (P); manzanilla de Castilla (S)
<i>Artemisia absinthium</i>	Absinthium, common wormwood, wormwood, old woman (E); absinthe, feuilles ameres (F); koiruoho (Fi); wermut (G); assenzio vero (I); niga-yomogi (J); losna, absinto, erva-dos-vermes (P); ajenjo official, ajenjo (S); äkta malört (Sw); pelin, madderwort, shih (O)
<i>Artemisia annua</i>	Quinghao, ch'ou hao, huang hua hao, ts'ao hao (C); (sweet/annual) wormwood, sweet Annie (E); assenzio annuale (I); kuso-ninjin (J); thanhcao, thâocao, chênôï (V)
<i>Artemisia dracunculul</i>	Dragon sagewort/wormwood, false tarragon, French tarragon, little dragon, mugwort, (true) tarragon (E); rakuuna (Fi); Estragon (F); Estragão (P); Tagantes (S); thanhcao rông (V)
<i>Matricaria chamomilla</i>	Babung (A-Egypt); German/Hungarian/single/wild chamomile (E); camomile (F); echte kamomille (G); camomilla commune (I); kamitsure (J); camomila (P); manzanilla dulce (S); papatya (T); amerale, babunnej, bayboon, matricaria (O)
<i>Tanacetum parthenium</i>	Feverfew, bachelor's buttons, featherfew, featherfoil, flirtwort (E); reunuspäivänkakkara (Fi); grande camomille (F); mutterkraut (G); erba-amara vera, matricale (I); natsu-shiro-giku (J); catinga-de-mulata (P); mattram (Sw)
<i>Tanacetum vulgare</i>	Common tansy, tansy, (gold) buttons (E); pietaryrtti (Fi); tansaisie (F); rainfarn (G); tanaceto, erba-amara selvatica (I); yomogi-giku (J); atanásia, tanaceto (P); renfana (Sw); solucanotu (O)

A = Arabic, C = Chinese, E = English, F = French, Fi = Finnish, G = German, I = Italian, J = Japanese, O = others, P = Portuguese, S = Spanish, Sw = Swedish, T = Thai, V = Vietnamese.

*Tripleuspermum*, among others (Figure 1; Khallouki et al., 2000). Numerous species contain medicinally and cosmetically important compounds and essential oils, some of which (e.g. flavonoids) have been used to differentiate members of the Asteraceae-Anthemideae including genera *Achillea*, *Artemisia* and *Tanacetum* (Williams et al., 1999).

## REGENERATION

### Manipulation of morphogenesis

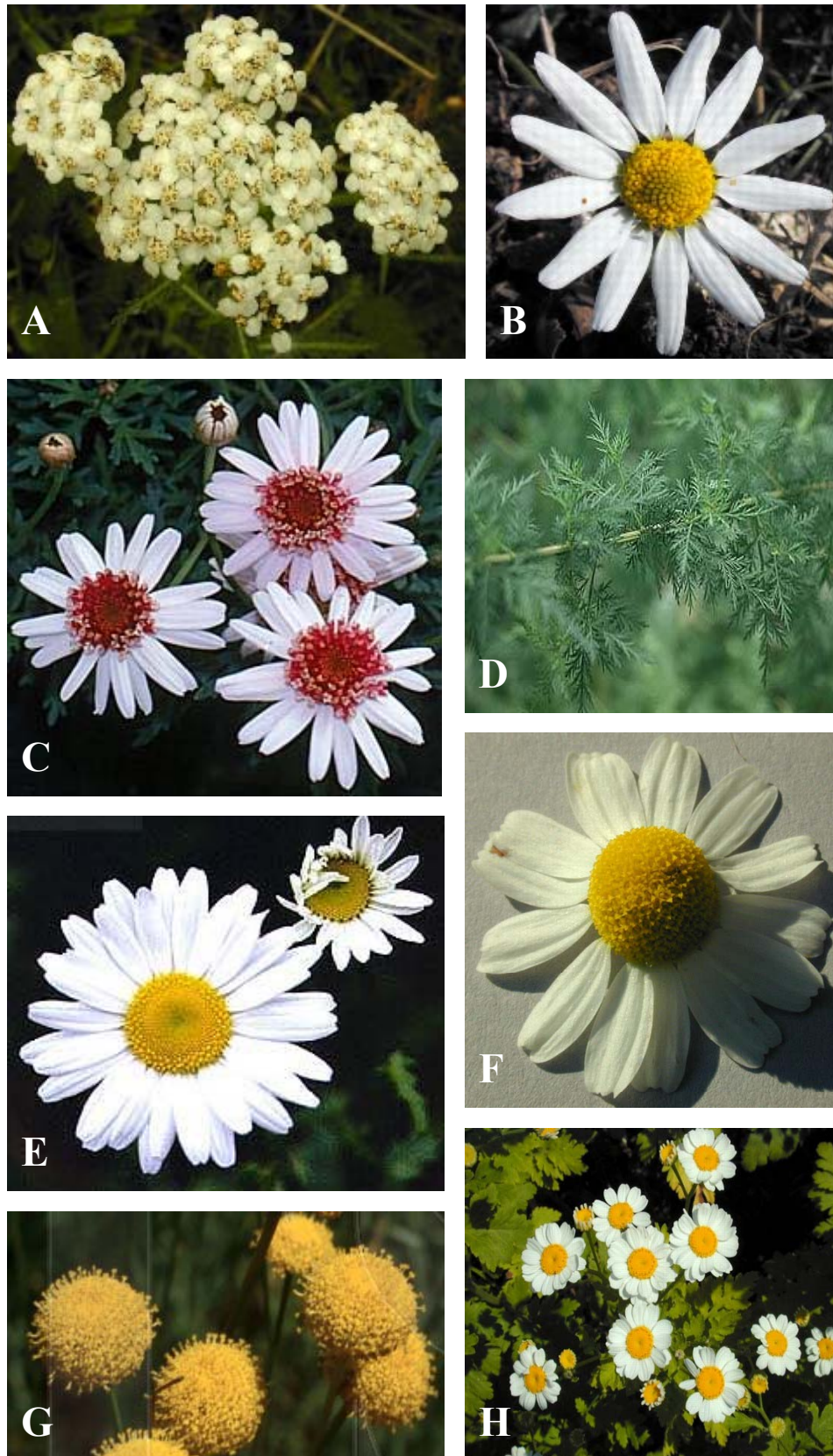
*In vitro* induction of roots has been achieved in *Chrysanthemum*-complex species. In *Achillea millefolium*, hairy root cultures (*Agrobacterium rhizogenes*-induced root production) were established for the biosynthetic production of terpenes in a bioreactor system (Lourenço et al., 1999). Hairy root cultures and cell suspension cultures of *A. millefolium* have been established to biotransform terpenes and to produce essential oils in a controlled environment, the biggest drawback being the low yield (Figueiredo et al., 1995). The A4-Y strain of *A. rhizogenes* induced hairy roots in *Matricaria recutita* (Máday et al., 1999) while in adventitious root cultures of *Anthemis nobilis*, geranyl isovalerate was accumulated (Omoto et al., 1998).

### Effect of additives and other factors on morphogenesis

A number of tissue culture studies have been conducted with the aim of inducing various target organs from a number of explant sources. This has been achieved in many primary species of the Anthemideae (Table 2).

Numerous studies have recently been completed on the effect of a number of factors and media additives on chrysanthemum thin cell layer (TCL) morphogenesis. To further enhance the medium-dependence of explants, TCLs were used in the experiments. TCLs, derived from cells, tissues or organs, are of a small size, excised either a) longitudinally (ITCL), being thus composed of a few tissue types or b) transversally (tTCL), thus composed of several tissue types, but which are normally too small to separate, as in the case of chrysanthemum. In the TCL system, the morphogenic and developmental pathways of specific organs may be clearly directed and controlled (Nhut et al., 2003).

Most aminoglycoside antibiotics, frequently used in Anthemideae transformation (Table 3), negatively affect *in vitro* growth and morphogenesis (shoot and root formation) of chrysanthemum tTCLs (Teixeira da Silva, 2002). In tansy (*Tanacetum vulgare*), cefotaxime, rifampicin and gentamycin (antibiotics commonly used to eliminate Gram-negative bacteria in *in vitro* shoot cultures)



**Figure 1.** Some of the principal species within the Anthemideae. A) *Achillea millefolium*, B) *Anthemis cotula*, C) *Argyranthemum frutescens*, D) *Artemisia annua*, E) *Leucanthemum vulgare*, F) *Matricaria recutita*, G) *Santolina rosmarinifolia*, H) *Tanacetum parthenium*.

Table 2. Regeneration studies of species within the Anthemideae.

Genus and species	Principal cultivar + others	Explant source ▲	TCL	GD	Organ	№ O/E*	Medium PGR composition*1 (MS basal)	Reference
<i>T. vulgare</i>	n.s.	Stem	No	-	Callus	n.s.	WG + 2,2,4-D 10% CM	Banthorpe & Wirz-Justice, 1972
<i>T. cinerariaefolium</i>	Indianapolis White Giant #4	Shoot tip	No	-	Shoot	5	2 K 0.02 NAA	Roest & Bokelmann, 1973
<i>C. cinerariaefolium</i>	n.s.	Shoot, root	No	-	Callus, root	n.s.	0.05 2,4-D	Chumsri & Staba, 1975
<i>T. cinerariaefolium</i>	n.s.	Stem	No	-	Callus	n.s.	1 NAA or 1 2,4-D 0.2 K + different irradiation	Aoki et al., 1976
<i>T. cinerariaefolium</i>	n.s.	Seedling	No	-	R, S, callus	n.s.	0.5 2,4-D 0.75 K or 1 NAA or 1 IAA	Cashyap et al., 1978
<i>C. cinerariaefolium</i>	n.s.	Shoot tip	No	-	Shoot	n.s.	1 NAA 1 BA	Grewal & Sharma, 1978
<i>M. chamomilla</i>	n.s.	Flower, leaf, shoot	No	-	Callus	n.s.	1.5 2,4-D, 1.2 K, 10% CM	Szöke et al., 1979
<i>C. cinerariaefolium</i>	Clone 4331	Shoot tip	No	-	Shoot	2.8-21.5	0.02-2 NAA 0.2/2 BA 0.02 IAA 1-5 K	Wambugu & Rangan, 1981
<i>M. chamomilla</i>	n.s.	Leaf	No	-	Crown gall	n.s.	PGR-free + activated charcoal; 12hr light	Beiderbeck, 1982
<i>M. chamomilla</i>	<i>M. inodora</i> , <i>A. nobilis</i> <i>A. ptarmica</i>	Callus	No	-	C, R, shoot	n.s.	0.5 NAA 0.1/0.5 K 1 2,4-D	Čellárová et al., 1982
<i>M. chamomilla</i>	n.s.	Shoot	No	-	Suspension	n.s.	PGR-free + high light intensity; 5 NAA 2.5 K	Bisson et al., 1983
<i>C. cinerariaefolium</i>	+ <i>C. coccineum</i> Golden Mass	Leaf, stem, floret ▲	No/yes	-	Callus	n.s.	0.5 2,4-D 0.5 BA	Zieg et al., 1983
<i>C. cinerariaefolium</i>	n.s.	Shoot tip	No	-	Shoot	n.s.	0.1 2,4-D 3 BA	Zito et al., 1983
<i>M. chamomilla</i>	n.s.	Leaf, stem	No	-	Callus	n.s.	0.5/5 NAA 1/2.5 K	Reichling et al., 1984
<i>C. cinerariaefolium</i>	Ecuadorian cv. n.s.	n.s.	No	-	Shoot	1.1-3.5	20 BA + varying effects of light/darkness	Staba et al., 1984
<i>Art. annua</i>	From the wild	Stem	No	-	R, S, callus	n.s.	0.05-2 IBA/NAA (C); 0.05 NAA 0.2 BAP (S)	Nair et al., 1986
<i>C. cinerariaefolium</i>	HSL 801 (lines C5, C9, C10)	Leaf	No	Yes	C, shoot	n.s.	PGR-free / 1 IBA 1 NAA; pyrethrum leaf>stem	Paul et al., 1988
<i>T. vulgare</i>	+ <i>T. parthenium</i> , <i>A. vulgare</i>	Leaf, stem	No	Yes	Callus	n.s.	10% CW, 6 2,4-D or 0.5 NAA 0.1 K	Banthorpe & Brown, 1989
<i>C. cinerariaefolium</i>	n.s. (high pyrethrin lines)	Leaf	No	-	Callus	100%	5 K 2 2,4-D	Ravishankar et al., 1989
<i>Art. annua</i>	n.s.	Seedling, callus	No	-	Callus	n.s.	1 2,4-D 0.1 K (liquid suspension)	Tawfiq et al., 1989
<i>M. recutita</i>	n.s.	Leaf	No	-	Callus	100%	LS + 0.01 IAA or 3 2,4-D	Čellárová et al., 1990
<i>Art. pallens</i>	Bangalore vars.	Seedling, callus	No	-	C, shoot	n.s.	4 2,4-D 4 BA	Benjamin et al., 1990
<i>T. coccineum</i>	n.s.	Achene ▲, petal ▲	Yes	-	Callus, shoot	0-89%	0.2 NAA/2,4-D 2 BA	Fujii & Shimizu, 1990
<i>Art. douglasiana</i>	Dihydroleucodin lines	Leaf, stem, root	No	-	Callus	n.s.	4 (IAA/IBA/NAA/2,4-D/2,4,5-T) 1 GA3	Pestchanker et al., 1990
<i>C. cinerariaefolium</i>	n.s. 3-yr old plants	Axillary bud	No	-	Shoot	n.s.	½RT + 1 2,4-D	Zito & Tio, 1990
<i>Art. dracunculul</i>	French stocks	Leaf, stem	No	-	Callus	n.s.	2 NAA 0.5 BAP	Cotton et al., 1991
<i>M. chamomilla</i>	n.s.	Shoot primordia	No	-	Shoot	n.s.	2 NAA 2 BAP (auxin induces oil bodies)	Takano et al., 1991
<i>A. millefolium</i>	ssp. <i>millefolium</i>	Hypocotyl	No	-	Callus	n.s.	B5 + 1.5 2,4-D 0.1 K (darkness); 100 mglyol	Figueiredo & Pais, 1991; et al., 1995
<i>Art. princeps</i>	var. <i>orientalis</i>	Leaf, hypocotyl	No	-	Callus	n.s.	1 2,4-D, 2 NAA, 1 K	Kil et al., 1992
<i>Art. annua</i>	<i>A. rhizogenes</i> transformed	Root	No	-	Callus, gall	100%	0.05 pcPA 0.05 BAP/2iP	Kim et al., 1992
<i>Art. annua</i>	n.s.	Leaf	No	-	Callus	100%	MS/B5 + 0.5-2 2,4-D 0.025-0.1 BA 0.5-2 NAA	Basile et al., 1993
<i>C. cinerariaefolium</i>	HSL 801, SL 821	Shoot tip	No	-	Callus	100%	0.5 2,4-D 0.5 BA	Dhar & Pal, 1993
<i>An. nobilis</i>	n.s. <i>A. tumefaciens</i> C-58 galls	Flower bud	No	-	Galls, shoot	n.s.	B5 + 0.05 2,4-D 0.4 NAA 1 BA	Fauconnier et al., 1993
<i>Art. annua</i>	Vietnamese origin (seeds)	Leaf, stem	n.s.	-	Shoot	n.s.	0.2 BAP 0.05 NAA	Woerdenbag et al., 1993
<i>Art. annua</i>	n.s.	Hypocotyl	No	-	Callus	n.s.	1 2,4-D; 0.5 NAA 0.5/2.5 BAP; 2.5 NAA	Brown, 1994
<i>Art. annua</i>	From the wild	Hypocotyl, leaf, root	No	-	Callus, shoot	n.s.	2 Z 1 NAA 2 BA (C); 3 BA 0.2 NAA (S)	Panigo & Giulietti, 1994
<i>A. asplenifolia</i>	n.s.	Nodal culture	No	-	Shoot	n.s.	1 BAP 0.1 IAA 0.025 GA3 → PGR-free	Wawrosch et al., 1994
<i>T. vulgare</i>	n.s.	Leaf, petiole, protoplast	No	-	C, protoplast	0-89%	0.2 2,4-D 1-2 NAA 0.5-4 BAP 0.25 K 0.1 GA3	Keskitalo et al., 1995
<i>An. nobilis</i>	n.s.	Young shoot	No	-	Shoot	1.8-6.5	No PGRs; liquid media	Asai et al., 1995
<i>Art. annua</i>	n.s.	Stem	No	-	Shoot	1.1-9.8	0.5 K 0.2 IAA + cotton fiber	Moraes-Cerdeira et al., 1995
<i>Art. annua</i>	<i>A. rhizogenes</i> transformed	Root	No	-	Hairy root	n.s.	25-35 BA	Mukherjee et al., 1995
<i>C. cinerariaefolium</i>	HY C,D SY A,B	Leaf, stem, flower ▲	No/yes	-	Callus	n.s.	4 NAA 0.4 BAP → ½MS + 4 NAA 0.4 BAP	Barthomeuf et al., 1996
<i>T. parthenium</i>	n.s.	Leaf	No	-	Shoot	n.s.	4.5 NAA 4.5 BAP	Brown et al., 1996
<i>Art. annua</i>	P <sub>2</sub> , P <sub>4</sub> high artemisinin lines	Leaf	No	-	Callus, shoot	3.1	0.67 2,4-D 0.5 BA 0.35 GA3 (C); 0.5/5 BA (S)	Ferreira & Janick, 1996
<i>Art. annua</i>	n.s.	Leaf, stem	No	-	Shoot	Many	1 NAA 3 BAP 0.1 GA3	Gulati et al., 1996
<i>T. vulgare</i>	( <i>L. maximum</i> ) Moon max	Leaf, stem, shoot tip	No	-	Shoot	48-74	0.2 NAA 0.25 BA	Kumar et al., 1996
<i>Art. absinthium</i>	n.s.	Shoot tip	No	-	Callus, shoot	1.5	0.15 IAA 0.2 BA	Nin et al., 1996
<i>Art. annua</i>	West Virginia/Yugoslavia	Leaf, stem, root	No	-	R,S, callus	100%	0.05 NAA 0.5 BA	Vergauwe et al., 1996b
<i>Art. sphaerocephala</i>	Wild desert plant (seeds)	Callus protoplast	No	-	Callus	11%	KM8P + 0.01-0.67 2,4-D 0.1-0.5 K	Xu & Jia, 1996
<i>Art. absinthium</i>	5 lines	Shoot tip	No	-	Shoot	n.s.	0.2 BA 0.05 NAA	Nin et al., 1997
<i>Art. pallens</i>	n.s. (encapsulated shoot tip)	Seedling	No	-	Shoot	100%	1 NAA 0.3 BAP 1 biotin	Sharief et al., 1997
<i>Art. annua</i>	<i>A. rhizogenes</i> transformed	Root	No	-	Hairy root	100%	0.01 GA3	Smith et al., 1997

Table 2 contd.

<i>T. parthenium</i>	n.s.	Stem node	No	-	S, hairy root	100%	1 BA 0.1 NAA (shoot); LBA 9402 (hairy root)	Stojakowska & Kisiel, 1997
<i>A. ceretanica</i>	#10222/1 (tetraploid)	Stem node	No	-	Shoot	0.7-2.8	0.7-6 BAP 1 K 0.001-0.2 IAA	Wawrosch et al., 1997
<i>Art. annua</i>	<i>A. rhizogenes</i> transformant	Root	No	-	Hairy root	n.s.	PGR-free + various nitrate, phosphate levels	Weathers et al., 1997
<i>C. cinerariaefolium</i>	n.s.	Axillary bud	No	-	Shoot	n.s.	No PGR	Chen et al., 1998
<i>Art. annua</i>	n.s.	Cell culture (bioreactor)	No	-	Cell culture	n.s.	0.5 BA 0.05 NAA (biotransformation)	Liu et al., 1998,1999
<i>An. nobilis</i>	n.s.	Root	No	-	Hairy root	n.s.	1 IAA 0.5 NAA/IBA	Omoto et al., 1998
<i>Art. annua</i>	n.s.	Seedling	No	-	Shoot	n.s.	1 BAP → 2 IAA	Usha & Swamy, 1998
<b>C. cinerariaefolium</b>	High pyrethrum lines	Flower head ▲	Yes	-	Callus	100%	1 NAA 1 BAP (static culture)	George et al., 1999
<b>C. cinerariaefolium</b>	High pyrethrum lines	Flower head ▲	Yes	-	Callus	60-95%	0.2-4 NAA 0.2-41 BAP	Hitmi et al., 1999b,2001
Hybrid	<i>T. vulgare</i> x <i>T. cinerariaefolium</i>	Leaf protoplast	No	-	Callus	n.s.	6.4 BAP 0.8 NAA	Keskitalo et al., 1999
<i>T. parthenium</i>	+ <i>A. annuum</i>	Seedling	No	-	Callus	n.s.	0.5 NAA/BAP or 1 2,4-D	Sy & Brown, 1999
<i>Art. annua</i>	001, 025 high artimisinin	Leaf	No	-	Shoot	n.s.	0.05 NAA 2 BAP	Chen et al., 2000; Sa et al., 2001; Liu et al., 2002, 2003
<i>An. nobilis</i>	Flore Pleno	Leaf	No	-	Shoot	<9.6	0.2 NAA 0.2-1 BA → 0.1 IBA	Echeverrigaray et al., 2000
<i>M. recutita</i>	BK-2, Degumil	Shoot tip	No	Yes	R,S,hairy root	n.s.	No PGR + A4-Y, R-1601, 15834 <i>A. rhizogenes</i>	Máday et al., 2000
<i>Arg. frutescens</i>	Butterfly	Shoot tip	No	-	Shoot	n.s.	0.1-2.5 BAP/2IP/K	Seyring & Vogt, 2000
<i>S. canescens</i>	Lagasca	Shoot tip	No	-	Shoot	1.3-11.9	0.01-1 BA 1.3 linoleic acid	Casado et al., 2000
<i>Art. annua</i>	<i>A. rhizogenes</i> transformant	Root	No	-	Hairy root	n.s.	PGR-free	Xie et al., 2000
<i>Art. annua</i>	A201, A202	Leaf	No	Yes	Callus, shoot	n.s.	1 NAA 0.5 BA	Chenshu et al., 2003

*A. Achillea*, *An. Anthemis*, *Arg. Argyranthemum*, *Art. Artemisia*, *L. Leucanthemum*, *M. Matricaria*, *S. Santolina*, *T.(syn. C.) Tanacetum* (syn. *Chrysanthemum*). All media MS (Murashige and Skoog) except for WG = Williams and Goodwin basal medium; ‡ = Linsmaier and Skoog basal medium. pcPA = p-chlorophenoxyacetic acid; 2IP = N-isopentenylaminopurine; 2,4,5-T = 2,4,5-trichlorophenoxyacetic acid; DMSO = dimethyl sulphoxide. TCL = thin cell layer, ▲ = single cell type (syn. TCL). GD = genotype dependence; O/E = organ per explant; \* = percentages represent the number of explants forming organs; \*1 = PGR values in mg/l. K = kinetin; BA = 6-benzyladenine; BAP = (N3)6-benzylaminopurine; 2IP = 6-(dimethylallylamino)-purine; 2,4-D = 2,4-dichlorophenoxyacetic acid; IBA = Indole-3-butyric acid; IAA = 3-indole acetic acid; NAA = α-naphthalene acetic acid; Z = Zeatin; CW/CM = coconut water/milk; PPM = Plant Preservative Mixture™; C = callus, R= root, S = shoot, SE = somatic embryo. n.s. = not specified.

Table 3. Antibiotics (aminoglycoside and *Agrobacterium*-eliminating) used in Anthemideae genetic transformation studies.

Principal cultivar(s) + others	Source	AA	Selection	Initial Selection‡	Regeneration‡	Reference
<i>Anthemis nobilis</i>	Flower	CF	Early	1000	1000	Fauconnier et al., 1993
<i>Artemisia absinthium</i>	Hairy root	A	Early	500	500	Kennedy et al., 1993
<i>Artemisia annua</i>	Hairy root	None	None	-	-	Qin et al., 1994
<i>Artemisia annua</i>	Hairy root	None	None	-	-	Mukherjee et al., 1995
<i>Artemisia annua</i> 001,025	Hairy root	None	None	-	-	Paniego & Giulietti, 1996* <sup>1</sup>
<i>Artemisia annua</i>	Leaf	K	Late (3w)	K20	K20	Vergauwe et al., 1996a,b, 1998
<i>Artemisia annua</i>	Hairy root	None	None	-	-	Bannerjee et al., 1997
<i>Artemisia annua</i>	Galls	None	None	-	-	Ghosh et al., 1997* <sup>1</sup>
<i>Artemisia absinthium</i>	Leaf	KAR	Early	K50 A500 R10	K50 A500 R10	Nin et al., 1997
<i>Tanacetum parthenium</i>	Leaf	None	None	-	-	Stojakowska and Kisiel, 1997
<i>Achillea millefolium</i>	Roots	A,CF	Early	A500, 250 CA+CF	A500, 250 A+CF	Lourenço et al., 1999
<i>Matricaria recutita</i>	Roots	CA	Early	800	800	Máday et al., 1999
<i>Artemisia annua</i> 001, 025	Leaf	K	Early	15	20	Chen et al., 2000
<i>Matricaria recutita</i> BK-2 +1	Hairy root	A,CF	Early	CF 800	CF250, A1000	Máday et al., 2000
<i>Artemisia annua</i>	Hairy root	K	Early	100	100	Xie et al., 2000
<i>Artemisia annua</i>	Hairy root	n.s.	n.s.	n.s.	n.s.	Liu et al., 2002

‡ = mg/l; \*<sup>1</sup> = artemisinin production. Antibiotics used: A = ampicillin, CA = carbenicillin, CF = cefotaxime, K=kanamycin, R=rifampicin. Selection: early (0-3d), late (>3d); n.s. = not specified.



all had pronounced negative effects on shoot growth and development (Keskitalo et al., 1998). In both studies, the effect of the antibiotic concentration on plant morphogenesis and explant survival depended on the size of the explant, the choice of explant source, the timing of infection by *A. tumefaciens* and selection pressure in genetic transformation. In separate experiments on the effect of other antibiotics on shoot regeneration, a gradient of phytotoxicity has been shown: bialaphos<sup>®</sup>>chloramphenicol>rifampicin>streptomycin>minomycin>ampicillin>penicillin G = penicillin V (Teixeira da Silva et al., 2003). Another study (Teixeira da Silva and Fukai, 2001) showed the importance that *Agrobacterium* selective agent (carbenicillin, cefotaxime or vancomycin) has on maximizing chrysanthemum shoot regeneration capacity, while minimizing phytotoxicity and explant mortality, in one case (cefotaxime up to 250 mg/l) stimulating shoot formation. Contrasting results were found in *in vitro* cultures of *Argyranthemum frutescens*, where aureomycin, vancomycin, cefotaxime, carbenicillin and augmentin all inhibited root and shoot formation  $\geq 40$  mg/l, differentially controlling *Agrobacterium* growth (Seyring, 1999).

### CONVENTIONAL BREEDING

Nature has played a role in inducing polyploidy in chrysanthemum through evolution, giving rise to tetra-, hexa-, octa- and decaploids, but humans too have contributed, through artificial interference, to changes in chrysanthemum. Many techniques are still employed by chrysanthemum breeders to improve varieties such as chromosome-doubling. GISH (genomic *in situ* hybridization) was used to confirm the successful intergeneric hybrid between *Dendranthema lavandulifolia* and *Ajania remotipinna* (El-Twab et al., 1999) and the use of FISH (fluorescence *in situ* hybridization) and GISH to confirm hybrids between *Leucanthemella* and *Nipponanthemum* (Ogura and Kondo, 1998; El-Twab and Kondo, 2001).

nrDNA internal transcriber spacer (ITS) and cpDNA *trnL/trnF* intergenic spacers were used to analyze the phylogeny of the Anthemideae (Oberprieler, 2002). ITS of nuclear ribosomal DNA were sequenced, and morphological cladistic analyses, cytology and isozyme analysis were conducted to differentiate 52 species from 32 genera and 8 subtribes of the Anthemideae (Francisco-Ortega et al., 1997). In separate studies, oligonucleotide fingerprinting and RAPD analysis were used as markers of stability during *Achillea* spp. micropropagation (Wallner et al., 1996).

Chromosome studies still continue to be important in separating chrysanthemum species (Kondo et al., 1998) while, due to high ploidy, isozymes/allozymes are effective in differentiating cultivars (Roxas et al., 1993). In the case of the Chrysantheminae, the geographic origin

of genera and species within it could be deciphered by the use of PAGE (polyacrylamide gel electrophoresis) for a number of enzyme systems (Francisco-Ortega et al., 1995). Allele frequency data for polymorphic loci could be obtained when nine allozyme profiles were used to differentiate different populations of *Achillea* (Purdy and Bayer, 1996).

### TRANSFORMATION

Few transformation studies have been conducted on members of the Anthemideae (Tables 3-5). The use of *A. rhizogenes* in the production of transgenic hairy roots allows for the mass production of secondary metabolites through a bioreactor system. In addition the use of *A. tumefaciens*, biolistics or any other gene transfer technique would confer the ability to transform economically important medicinal and aromatic varieties to modify characteristics such as compound yield, plant shape, height and growth morphology, longevity, horticultural traits, insect and disease resistance, and resistance to environmental stresses. In the Anthemideae, the main focus has been the use of hairy roots in bioreactor systems to improve the yield of economically and pharmacologically important compounds such as artemisinin (100 mg = approx. 70 USD) in *Artemisia annua*, parthenolide (100 mg = approx. 50 USD) in *Tanacetum parthenium* or pyrethrins in *Tanacetum* (syn. *Chrysanthemum*) *cinerariaefolium*. Artemisinin is one of the most important commercial antimalarials, and this compound also shows antitumor and antiviral activities, among others, while parthenolide is primarily used in pesticides but also shows many biological activities, including antibacterial, anticancer, anti-inflammatory and fungicidal activities (USDA-ARS-NGRL, 2003). In the case of *Artemisia absinthium*, genetic modification of the plant was done to increase essential oil yield (Table 5). No genetic transformants have been obtained by biolistics.

### CRYOPRESERVATION AND GERMLASM PRESERVATION

Cryopreservation, an important method for the conservation of plant genetic resources (Engelmann, 2000) uses freeze preservation in liquid N<sub>2</sub> to immobilize metabolic activity, thus suspending changes that may arise in the plant cell genome. Storage of ornamental chrysanthemum (and to a limited extent other members of the Anthemideae) genetic resources has been achieved through cryopreservation, low temperature preservation, and room temperature preservation (Fukai, 1995) in which the successful cryopreservation of shoot tips involves the ability to regenerate thawed shoots as well as maintaining their genetic composition.

**Table 4.** Studies involving transformation (primarily *Agrobacterium*-mediated) of Anthemideae members.

Principal cultivar(s) + others	Source	Strain	CCP (d)	L/D	№ O/E	OD (λ)	Antibiotic	Concentration‡	Reference
<i>Artemis annua</i>	Leaf, stem	EHA101,C58	2-2.5	L/D	<27%	3-58%	K	20	Vergauwe et al., 1996a,b, 1998
<i>Artemisia absinthium</i> (5 lines)	Leaf	AR1855,LBA9402	∞	L/D	0-100%	n.s.	K,A,R	K50 A500 R10	Nin et al., 1997
<i>Artemis annua</i> lines 001,025	Leaf	LBA4404	1.5-2	L/D	n.s.	10x dilution	CA	500→100	Chen et al., 2000

CCP = co-culture period in the light (L) or dark (D); OD = optical density (λ = wavelength of spectrophotometer). Antibiotics used: A = ampicillin, CA = carbenicillin, K = kanamycin, R = rifampicin. ‡ = µg/ml X→Y, X=initial concentration early in selection, Y=final concentration later in selection. n.s. = not specified.

**Table 5.** Details of Anthemideae transformation studies.

Principal cultivar(s) + others	Transgene(s)	Promoter	TrE%*	LtTEX	LsTEX	PCR	Southern	Others	Change(s)	Reference
<i>Artemis annua</i>	<i>nptII,GUS,SOD,BAT</i>	CaMV35S	0-27%	Callus	Plant	Yes	No	Assays	Artemisinin levels	Vergauwe et al., 1996a,b, 1998
<i>Artemisia absinthium</i> (5 lines)	<i>nptII,opines</i>	CaMV35S	0-100%	Hairy root	Plant	Yes	Yes	No	Essential oil	Nin et al., 1997
<i>Artemis annua</i> lines 001,025	<i>nptII,FDS</i> cDNA	CaMV35S	5-29	n.s.	Plant	Yes	Yes	Northern	TLC	Chen et al., 2000

TrE = transformation efficiency, either as \* № positive shoots or explants or № explants x 100 (i.e. %). TEX = transgene expression; LtTEX = localization of transient TEX, LsTEX = localization of stable TEX; n.s. = not specified.

Cryopreservation of *C. cinerariaefolium*, or Dalmatian pyrethrum, was achieved by a 3 day pre-culture period in sucrose-enriched medium, and using a 7.5% DMSO cryoprotectant, with an average cryopreservability rate at 62% (Hitmi et al., 1998a, 1998b, 1999a). Cryopreservation, however did not affect the biosynthetic properties, the composition or the amount of pyrethrins (Hitmi et al., 2000a). Sucrose was shown to be an effective cryoprotectant to confer freezing tolerance to pyrethrin cell cultures (Hitmi et al., 1999a, 1999c, 2000b).

Root tips of *A. rhizogenes*-transformed hairy roots in *Artemisia annua* resulted in a 65% regrowth rate following liquid N<sub>2</sub> immersion (Teoh et al., 1996). *A. annua* callus could be cryopreserved in a cryoprotectant containing 15% ethylene glycol, 15% dimethyl sulfoxide, 30% glycerol and 13.6% sucrose, a simplified and effective method for long-term storage of callus without an effect on regeneration (Chenshu et al., 2003). *Artemisia pallens* encapsulated shoot buds could regenerate well, especially in the presence of ABA (Sharief et al., 1997).

## POSTHARVEST BIOTECHNOLOGY

Few Anthemideae plants (excluding the ornamental chrysanthemums) are used as cut flowers. For cut *Achillea filipendulina* flowers, 8-HQC (8-hydroxyquinoline citrate) or exogenous ethylene decreased vase life whereas STS (silver thiosulphate) with or without 0-8% sucrose increased it (Redman et al., 2002).

## CONCLUSIONS

Members of the Anthemideae comprise a large number of species, many of which have economic medicinal and aromatic value, which can be increased with the exploration of *in vitro* culture techniques (tissue culture, cryopreservation) to increase yield and standardize quality, and molecular methodologies to improve growth characteristics and maximize yield.

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## REFERENCES

- Aoki S, Kaneto K, Hashimoto S, Oogai H (1976). Production of pyrethrin by tissue culture. Chem. Abstr. 89:20589q.
- Asai I, Yoshihira K, Omoto T, Shimomura K (1995). Growth and essential oil production in shoot culture and regenerates of *Anthemis nobilis* L. Plant Tiss. Cult. Lett. 12:303.
- Bannerjee S, Zehra M, Gupta MM, Kumar S (1997). *Agrobacterium rhizogenes*-mediated transformation of *Artemisia annua*: production of transgenic plants. Planta Med. 63:467-469.
- Banthorpe DV, Brown GD (1989). Two unexpected coumarin derivatives from tissue cultures of Compositae species. Phytochemistry 28:3003-3007.
- Banthorpe DV, Wirz-Justice A (1972). Terpene biosynthesis. Part 1. Preliminary tracer studies on terpenoid and chlorophyll of *Tanacetum vulgare* L. J. Chem. Soc. Perkin Transact. 1:1769-1772.
- Barthomeuf C, Hitmi A, Veisseire P, Coudret A (1996). Identification and assay of pyrethrins in *Chrysanthemum cinerariaefolium* calli. Biotechnol. Tech. 10:639-642.
- Basile DV, Akhtari N, Durand Y, Nair MSR (1993). Toward the production of artemisinin through tissue culture: determining nutrient-hormone combinations suitable for cell suspension cultures. In Vitro Cell. Dev. Biol. – Plant 29:143-147.
- Beiderbeck R (1982). Two-phase culture: one way to isolate lipophilic substances from plant cell suspension cultures. Zeids. Pflanzenphysiol. 108:27-30.
- Benjamin BD, Sipahimalani AT, Heble MR (1990). Tissue cultures of *Artemisia pallens*: organogenesis, terpenoid production. Plant Cell, Tiss. Org. Cult. 21:159-164.
- Bisson W, Beiderbeck R, Reichling J (1983). The production of essential oil of chamomile cell suspension cultures in various two-phase systems. Planta Med. 47:164-168.
- Brown AMG, Lowe KC, Davey MR, Power JB (1996). Feverfew (*Tanacetum parthenium* L.): tissue culture and parthenolide synthesis. Plant Sci. 116:223-232.
- Brown GD (1994). Secondary metabolism in tissue culture of *Artemisia annua*. J. Natl. Prod. 57:975-977.
- Casado JP, Navarro MC, Utrilla MP, Martínez A, Jiménez J (2000). Micropropagation of *Santolina canescens* Lagasca and *in vitro* volatiles production by shoot explants. Plant Cell, Tiss. Org. Cult. 69:147-153.
- Cashyap MM, Kueh JSH, MacKenzie IA, Pattenden G (1978). *In vitro* synthesis of pyrethrins from tissue cultures of *Tanacetum cinerariaefolium*. Phytochemistry 17:544-555.
- Čellarova E, Greláková K, Repčák M, Hončariv R (1982). Morphogenesis in callus tissue cultures of some *Matricaria* and *Achillea* species. Biol. Plant. 24:430-433.
- Čellarova E, Rychlova M, Seidelova A, Hončariv R (1990). Comparison of mitotic activity and growth in two long term callus cultures of *Matricaria recutita* L. Acta Biotechnol. 10:245-251.
- Chen DH, Ye HC, Li GF (2000). Expression of a chimeric farnesyl diphosphate synthase gene in *Artemisia annua* L. transgenic plants via *Agrobacterium tumefaciens*-mediated transformation. Plant Sci. 155:179-185.
- Chen ZL, Hou SW, Yu HY (1998). Tissue culture of *Pyrethrum cinerariaefolium*. Acta Bot. Yunn. 20:351-354.
- Chenshu A, Wang X, Yuan X, Zhao B, Wang Y (2003). Optimization of cryopreservation of *Artemisia annua* L. callus. Biotechnol. Lett. 25:35-38.
- Chumsri P, Staba EJ (1975). Pyrethrins: content and larvicidal activity of *Chrysanthemum* plants and tissue cultures. Acad. Pharm. Sci. Abs. 5:169.
- Cotton CM, Gramshaw JW, Evans LV (1991). The effect of  $\alpha$ -naphthalene acetic acid (NAA) and benzylaminopurine (BAP) on the accumulation of volatile oil components in cell cultures of tarragon (*Artemisia dracunculus*). J. Exp. Bot. 42:377-386.
- Dhar K, Pal A (1993). Factors influencing efficient pyrethrin production in undifferentiated cultures of *Chrysanthemum cinerariaefolium*. Fitoterapia 64:336-340.
- Echeverrigaray S, Fracaro F, Andrade LB, Biasio S, Atti-Serafini L (2000). *In vitro* shoot regeneration from leaf explants of Roman Chamomile. Plant Cell, Tiss. Org. Cult. 60:1-4.
- Ei-Twab MHA, Kondo K, Hong D (1999). Isolation of a particular chromosome of *Ajania remotipinna* in a chromosome complement of an artificial F1 hybrid of *Dendranthema lavandulifolia* X *Ajania remotipinna* by use of genomic *in situ* hybridization. Chrom. Sci. 3:21-28.
- Ei-Twab MHA, Kondo K (2001). Molecular cytogenetic identification of the parental genomes in the intergeneric hybrid between *Leucanthemella linearis* and *Nipponanthemum nipponicum* during meiosis and mitosis. Caryologia 54:109-114.
- Engelmann F (2000). Importance of cryopreservation for the conservation of plant genetic resources. In: Engelmann F, Takagi H (eds.) Cryopreservation of tropical plant germplasm: current research progress and application. JIRCAS, Tsukuba, Japan/IPGRI, Rome,



- Italy, pp. 1-5.
- Fauconnier ML, Jaziri M, Marlier M, Roggemans J, Wathelet JP, Lognay G, Severin M, Homes J, Shimomura K (1993). Essential oil production by *Anthemis nobilis* L. tissue culture. *J. Plant Physiol.* 141:759-761.
- Ferreira JFS, Janick J (1996). Roots as an enhancing factor for the production of artemisinin in shoot cultures of *Artemisia annua*. *Plant Cell, Tiss. Org. Cult.* 44:211-217.
- Figueiredo AC, Pais MSS (1991). *Achillea millefolium* cell suspension cultures: establishment and growth conditions. *Biotechnol. Lett.* 13:63-68.
- Figueiredo AC, Pais MSS, Scheffer JJC (1995). Composition of the essential oil from cell suspension cultures of *Achillea millefolium* ssp. *millefolium*. *Plant Cell, Tiss. Org. Cult.* 40:113-118.
- Francisco-Ortega J, Crawford DJ, Santos-Guerra A, Sá-Fontinha S (1995). Genetic divergence among Mediterranean and Macronesian genera of the subtribe Chrysantheminae (Asteraceae). *Amer. J. Bot.* 82:1321-1328.
- Francisco-Ortega J, Santos-Guerra A, Hines A, Jansen RK (1997). Molecular evidence for a Mediterranean origin of the Macronesian endemic genus *Argyranthemum* (Asteraceae). *Amer. J. Bot.* 84:1595-1613.
- Fujii Y, K Shimizu (1990). Regeneration of plants from achenes and petals of *Chrysanthemum coccineum*. *Plant Cell Rep.* 8:625-627.
- Fukai S (1995). Cryopreservation of germplasm of chrysanthemums. In: Bajaj YPS (ed.) *Biotechnology in Agriculture and Forestry*, vol. 32. *Cryopreservation of Plant Germplasm I*. Springer-Verlag, Berlin, pp. 447-457.
- George J, Rajasekaran T, Ravishankar GA (1999). A modified culture vessel for improved callus growth and pyrethrins content of pyrethrum (*Chrysanthemum cinerariaefolium* Vis.). *Pyrethrum Post* 20:49-54.
- Ghosh B, Mukherjee S, Jha S (1997). Genetic transformation of *Artemisia annua* by *Agrobacterium tumefaciens* and artemisinin synthesis in transformed cultures. *Plant Sci.* 122:193-199.
- Grewal S, Sharma K (1978). Pyrethrum plant (*Chrysanthemum cinerariaefolium* vis.) regeneration from shoot tip culture. *Indian J. Exp. Biol.* 16:1119-1121.
- Gulati A, Bharel S, Jain SK, Abdin MZ, Srivastava PS (1996). *In vitro* micropropagation and flowering in *Artemisia annua*. *J. Plant Biochem. Biotechnol.* 5:31-35.
- Hitmi A, Bartheuf C, Coudret A (1998a). Production of pyrethrins with *Chrysanthemum cinerariaefolium* Vis. callus culture: improvement of callus growth and pyrethrin synthesis. *J. Plant Physiol.* 153:233-236.
- Hitmi A, Bartheuf C, Coudret A, Sallanon H (1998b). Retention strength evolution of intracellular water during cryoconservation of *Chrysanthemum cinerariaefolium* Vis. cell cultures. *Comp. Rend. Sci. Soc. Biol. Fil.* 192:719-724.
- Hitmi A, Bartheuf C, Sallanon H (1999a). Cryopreservation of *Chrysanthemum cinerariaefolium* shoot tips. Effects of pretreatment conditions and retention of biosynthetic capacity. *Cryo Letters* 20:109-120.
- Hitmi A, Bartheuf C, Sallanon H (1999b). Rapid mass propagation of *Chrysanthemum cinerariaefolium* Vis. by callus culture and ability to synthesize pyrethrins. *Plant Cell Rep.* 19:156-160.
- Hitmi A, Coudret A, Bartheuf C, Sallanon H (1999c). The role of sucrose in freezing tolerance in *Chrysanthemum cinerariaefolium* L. cell cultures. *Cryo Letters* 20:45-54.
- Hitmi A, Bartheuf C, Sallanon H (2000a). Cryopreservation of *Chrysanthemum cinerariaefolium* shoot tips. *J. Plant Physiol.* 156:408-412.
- Hitmi A, Bartheuf C, Sallanon H (2000b). Role of intracellular water retention strength in freezing tolerance of *Chrysanthemum cinerariaefolium* Vis. cell cultures. *J. Plant Physiol.* 157:47-53.
- Hitmi A, Bartheuf C, Sallanon H (2001). Effects of plant growth regulators on the growth and pyrethrin production by cell cultures of *Chrysanthemum cinerariaefolium*. *Austr. J. Bot.* 49:81-88.
- Kennedy AI, Deans SG, Svoboda KP, Gray AI, Waterman PG (1993). Volatile oils from normal and transformed root of *Artemisia absinthium*. *Phytochemistry* 32:1449-1451.
- Keskitalo M, Kanerva T, Pehu E (1995). Development of *in vitro* procedures for regeneration of petiole and leaf explants and production of protoplast-derived callus in *Tanacetum vulgare* L. (*Tansy*). *Plant Cell Rep.* 14:261-266.
- Keskitalo M, Angers P, Earle E, Pehu E (1999). Chemical and genetic characterization of calli derived from somatic hybridization between tansy (*Tanacetum vulgare* L.) and pyrethrum (*Tanacetum cinerariaefolium* (Trevir.) Schultz-Bip.). *Theor. Appl. Genet.* 98:1335-1343.
- Keskitalo M, Arja P, Savela ML, Valkonen JPT, Simon J, Pehu E (1998). Alterations in growth of tissue-cultured tansy (*Tanacetum vulgare* L.) treated with antibiotics. *Ann. Appl. Biol.* 133:281-296.
- Khallouki F, Hmamouchi M, Younos C, Soulimani R, Bessi re JM, Essassi EM (2000). Antibacterial and molluscicidal activities of the essential oil of *Chrysanthemum viscidifolium*. *Fitoterapia* 71:544-546.
- Kil BS, Yun KW, Lee SY (1992). Influence of *Artemisia princeps* var. *orientalis* components on callus induction and growth. *J. Chem. Ecol.* 18:1455-1462.
- Kim NC, Kim JG, Lim HJ, Hahn TR, Kim SU (1992). Production of secondary metabolites by tissue culture of *Artemisia annua* L. *Agric. Chem. Biotechnol.* 35:99-105.
- Kondo K, Tanaka R, Hizume M, Kokubugata G, Hong D, Ge S, Yang Q (1998). Cytogenetic studies on wild *Chrysanthemum sensu lato* in China. VI. Karyomorphological characters of five species of *Ajania* and each one species of *Branchanthemum*, *Dendranthema*, *Elachanthemum*, *Phaestigma* and *Tanacetum* in highlands of Gansu, Qinghai and Sichuan provinces. *J. Jap. Bot.* 73:128-136.
- Kumar A, Kumar VA, Nautiyal MC (1996). High frequency *in vitro* regeneration of 'Moon' max daisy (*Leucanthemum maximum*). *Indian J. Agric. Sci.* 66:20-24.
- Liu CZ, Wang YC, Guo C, Ouyang F, Ye HC, Li GF (1998). Production of artemisinin by shoot cultures of *Artemisia annua* L. in a modified inner-loop mist bioreactor. *Plant Sci.* 135:211-217.
- Liu CZ, Wang YC, Zhao B, Guo C, Ouyang F, Ye HC, Li GF (1999). Development of a nutrient mist bioreactor for growth of hairy roots. *In Vitro Cell. Dev. Biol. - Plant* 35:271-274.
- Liu CZ, Guo C, Wang YC, Ouyang F (2002). Effect of light irradiation on hairy root growth and artemisinin biosynthesis of *Artemisia annua* L. *Process Biochem.* 38:581-585.
- Liu CZ, Guo C, Wang YC, Ouyang F (2003). Comparison of various bioreactors on growth and artemisinin biosynthesis of *Artemisia annua* L. shoot cultures. *Process Biochem.* (in press).
- Lourenço PML, Figueiredo AC, Barroso JG, Pedro LG, Oliverira MM, Deans SG, Scheffer JJC (1999). Essential oils from hairy root cultures and from plant roots of *Achillea millefolium*. *Phytochemistry* 51:637-642.
- M day E, Sz ke  , Muskath Z, Lemberkovi s E (1999). A study of the production of essential oils in chamomile hairy root cultures. *Europ. J. Drug Metab. Pharmacokin.* 24:303-308.
- M day E, Tjih k E, Sz ke   (2000). Occurrence of formaldehyde in intact plants, micropropagated plants and hairy root cultures of chamomile (*Matricaria recutita* L.). *Plant Growth Reg.* 30:105-110.
- Moraes-Cerdeira RM, Krans JV, McChesney JD, Pereira AMS, Franca SC (1995). Cotton fiber as a substitute for agar support in tissue culture. *HortScience* 30:1082-1083.
- Mukherjee S, Ghosh B, Jha TB, Jha S (1995). Genetic transformation of *Artemisia annua* by *Agrobacterium rhizogenes*. *Indian J. Exp. Biol.* 33:868-871.
- Nair MSR, Acton N, Klayman DL (1986). Production of artemisinin in tissue cultures of *Artemisia annua*. *J. Nat. Prod.* 49:504-507.
- Nhut DT, Teixeira da Silva JA, Aswath CR (2003). The importance of the explant on regeneration in thin cell layer technology. *In Vitro Cell. Dev. Biol. - Plant* 39:266-276.
- Nin S, Morosi E, Schiff S, Bennici A (1996). Callus cultures of *Artemisia absinthium* L.: initiation, growth optimization and organogenesis. *Plant Cell, Tiss. Org. Cult.* 45:67-72.
- Nin S, Bennici A, Roselli G, Mariotti D, Schiff S (1997). *Agrobacterium*-mediated transformation of *Artemisia absinthium* L. (wormwood) and production of secondary metabolites. *Plant Cell Rep.* 16:725-730.
- Oberprieler C (2002). A phylogenetic analysis of *Chamaemelum* Mill. (Compositae: Anthemidae) and related genera based upon nrDNA and cpDNA *trnL/trnF* IGS sequence variation. *Bot. J. Linn. Soc.* 138:255-273.

- Ogura H, Kondo K (1998). Application of genomic *in situ* hybridization to the chromosome complement of the intergeneric hybrid between *Leucanthermella linearis* (Matsum.) Tzuvelev and *Nipponantherum nipponicum* (Franch. et Maxim.) Kitamura. *Chrom. Sci.* 2:91-93.
- Oka S, Muraoka O, Abe T, Nakajima S (1999). Adventitious bud and embryoid formation in garland chrysanthemum leaf culture. *J. Jap. Soc. Hort. Sci.* 68:70-72.
- Omoto T, Asai I, Ishimaru K, Shimomura K (1998). Geranyl isovalerate accumulation in adventitious root culture of *Anthemis nobilis*. *Phytochemistry* 48:971-974.
- Panigo NB, Giulietti AM (1994). *Artemisia annua* L.: dedifferentiated and differentiated cultures. *Plant Cell, Tiss. Org. Cult.* 36:163-168.
- Panigo NB, Giulietti AM (1996). Artemisinin production by *Artemisia annua* L.-transformed organ cultures. *Enzyme Microb. Toxicol.* 18:526-530.
- Paul A, Dhar K, Pal A (1988). Organogenesis from selected culture lines of pyrethrum *Chrysanthemum cinerariaefolium*. *Pyrethrum Post* 17:17-20.
- Pestchanker LJ, Giulietti AM, Pestchanker MJ, Guerreiro E, Giordano OS (1990). The sesquiterpene lactone dihydroleucodin in tissue culture from *Artemisia douglasiana*. *Phytochemistry* 29:1853-1854.
- Purdy BG, Bayer RJ (1996). Genetic variation in populations of the endemic *Achillea millefolium* ssp. *megacephala* from the Athabasca sand dunes and the widespread ssp. *lanulosa* in western North America. *Can. J. Bot.* 74:1138-1146.
- Qin MB, Li GZ, Yun Y, Ye HC, Li GF (1994). Induction of hairy root from *Artemisia annua* with *Agrobacterium rhizogenes* and its culture *in vitro*. *Acta Bot. Sin.* 36:165-170.
- Ravishankar GA, Rajasekaran T, Sarma KS, Venkataraman LV (1989). Production of pyrethrins in cultured tissues of pyrethrum *Chrysanthemum cinerariaefolium* Vis. *Pyrethrum Post* 17:66-69.
- Redman PB, Dole JM, Maness NO, Anderson JA (2002). Postharvest handling of nine specialty cut flower species. *Sci. Hort.* 92:293-303.
- Reichling J, Bisson W, Becker H (1984). Various factors affecting the accumulation of essential oils in intact plants and in callus cultures of *Matricaria chamomilla*. *Planta Med.* 50:334-337.
- Roest S, Bokelmann GS (1973). Vegetative propagation of *Chrysanthemum cinerariaefolium in vitro*. *Sci. Hort.* 1:120-122.
- Roxas NJL, Tashiro Y, Miyazaki S, Takeshita A, Oshima T (1993). Isozyme analysis in Higo chrysanthemum (*Dendranthema grandiflora* Tzuvelev). *J. Jap Soc Hort Sci* 61:919-924.
- Sa G, Mi M, Ye HC, Liu BY, Li GF, Chong K (2001). Effects of *ipt* gene expression on the physiological and chemical characteristics of *Artemisia annua* L. *Plant Sci.* 160:691-698.
- Schwinn KE, Markham KR, Given NK (1994). Floral flavonoids and their potential for pelargonidin biosynthesis in commercial chrysanthemum cultivars. *Phytochemistry* 35:145-150.
- Seyring M (1999). Effect of antibiotics on elimination of *Agrobacterium tumefaciens* and the growth of shoot cultures of *Argyranthemum frutescens in vitro* and *in vivo*. *Gartenbauwissenschaft* 64:177-182.
- Seyring M, Vogt G (2000). Influencing growth of endophytic bacteria and quality of shoots in plant tissue cultures of *Argyranthemum frutescens*. *Gartenbauwissenschaft* 65:115-120.
- Sharief MU, Jagadishchandra KS, Johnson TS, Ravishankar GA (1997). Propagation of *Artemisia pallens* by encapsulated *in vitro* grown shoot buds. *J. Med. Arom. Plant Sci.* 19:712-716.
- Smith TC, Weathers PJ, Cheetham RC (1997). Effects of gibberellic acid on hairy root cultures of *Artemisia annua*: growth and artemisinin production. *In Vitro Cell. Dev. Biol. – Plant* 33:75-79.
- Staba EJ, Nygaard BG, Zito SW (1984). Light effects on pyrethrum shoot cultures. *Plant Cell, Tiss. Org. Cult.* 3:211-214.
- Stojakowska A, Kisiel W (1997). Production of parthenolide in organ cultures of feverfew. *Plant Cell, Tiss. Org. Cult.* 47:159-162.
- Sy LK, Brown GD (1999). Coniferaldehyde derivatives from tissue culture of *Artemisia annua* and *Tanacetum parthenium*. *Phytochemistry* 50:781-785.
- Szöke E, Shavarda AL, Verzar-Petri G, Kuzovkina IN (1979). Effects of growth regulators and light on essential oil synthesis in chamomile tissue cultures. *Herba Hung.* 18:7-19.
- Takano H, Hirano M, Taniguchi K, Tanaka R, Kondo K (1991). Rapid clonal propagation of *Matricaria chamomilla* by tissue cultured shoot primordia. *Jap. J. Breed.* 41:421.
- Tawfiq NK, Anderson LA, Roberts MF, Phillipson JD, Bray DH, Warhurst DC (1989). Antiplasmodial activity of *Artemisia annua* plant cell cultures. *Plant Cell Rep.* 8:425-428.
- Teixeira da Silva JA (2002). The role of antibiotics in plant tissue culture infection and genetic transformation of plants. *Newslett. Intl. Soc. Chemother.* 6:13.
- Teixeira da Silva JA (2003). Tissue culture and cryopreservation of chrysanthemum: a review. *Biotechnol. Adv.* 21:715-766.
- Teixeira da Silva JA, Fukai S (2001). The impact of carbenicillin, cefotaxime and vancomycin on chrysanthemum and tobacco TCL morphogenesis and *Agrobacterium* growth. *J. Appl. Hort.* 3:18-27.
- Teixeira da Silva JA, Nhut DT, Tanaka M, Fukai S (2003). The effect of antibiotics on the *in vitro* growth response of chrysanthemum and tobacco stem transverse thin cell layers (tTCLs). *Sci. Hort.* 97:397-410.
- Teoh KH, Weathers PJ, Cheetham RD, Walcerz DB (1996). Cryopreservation of transformed (hairy) roots of *Artemisia annua*. *Cryobiology* 33:106-117.
- USDA-ARS-NGRL (2003). Dr. Duke's phytochemical and ethnobotanical databases. <http://www.ars-grin.gov/duke/chem-activities.html>.
- Usha R, Swamy PM (1998). *In vitro* micropropagation of sweet wormwood (*Artemisia annua* L.). *Phytomorphology* 48:149-154.
- Vergauwe A, van Geldre E, Inzé D, van Montagu M, van den Eeckhout E (1996a). The use of amoxicillin and ticarcillin in combination with a  $\beta$ -lactamase inhibitor as decontaminating agents in the *Agrobacterium tumefaciens*-mediated transformation of *Artemisia annua* L. *J. Biotechnol.* 52:89-95.
- Vergauwe A, Cammaert R, Vandenberghe D, Genetello C, Inzé D, van Montagu M, van den Eeckhout E (1996b). *Agrobacterium tumefaciens*-mediated transformation of *Artemisia annua* L. and regeneration of transgenic plants. *Plant Cell Rep.* 15:929-933.
- Vergauwe A, van Geldre E, Inzé D, van Montagu M, van den Eeckhout E (1998). Factors influencing *Agrobacterium tumefaciens*-mediated transformation of *Artemisia annua* L. *Plant Cell Rep.* 18:105-110.
- Wallner E, Weising K, Rompf R, Kahl G, Kopp B (1996). Oligonucleotide fingerprinting and RAPD analysis of *Achillea* species: characterization and long-term monitoring of micropropagated clones. *Plant Cell Rep.* 15:647-652.
- Wambugu FM, Rangan TS (1981). *In vitro* clonal multiplication of pyrethrum (*Chrysanthemum cinerariaefolium* Vis.) by micropropagation. *Plant Sci. Lett.* 22:219-226.
- Wawrosch C, Kopp B, Kubelka W (1994). *In vitro* propagation of *Achillea asplenifolia* Vent. through multiple shoot regeneration. *Plant Cell Rep.* 14:161-164.
- Wawrosch C, Kopp B, Kubelka W (1997). *In vitro* propagation of tetraploid *Achillea ceretanica* Sennen. *Pharmac. Pharm. Lett.* 7:116-118.
- Weathers PJ, Hemmavanh DD, Walcerz DB, Cheetham RD, Smith TC (1997). Interactive effects of nitrate and phosphate salts, sucrose, and inoculum culture age on growth and sesquiterpene production in *Artemisia annua* hairy root cultures. *In Vitro Cell. Dev. Biol. – Plant* 33:306-312.
- Williams CA, Harbourne JB, Geiger H, Hoult JRS (1999). Variations in lipophilic and polar flavonoids in the genus *Tanacetum*. *Phytochemistry* 52:1301-1306.
- Woerdenbag HJ, Lüers JFJ, van Uden W, Pras N, Malingré TM, Alfermann AW (1993). Production of the new antimalarial drug artemisinin in shoot cultures of *Artemisia annua* L. *Plant Cell, Tiss. Org. Cult.* 32:247-257.
- Xie D, Wang L, Ye H, Li G (2000). Isolation and production of artemisinin and stigmaterol in hairy root cultures of *Artemisia annua*. *Plant Cell, Tiss. Org. Cult.* 63:161-166.
- Xu ZQ, Jia JF (1996). Callus formation from protoplasts of *Artemisia sphaerocephala* Krasch and some factors influencing protoplast division. *Plant Cell, Tiss. Org. Cult.* 44:129-134.
- Zieg RG, Zito SW, Staba EJ (1983). Selection of high pyrethrin producing tissue cultures. *Planta Med.* 48:88-91.
- Zito SW, Zieg RG, Staba EJ (1983). Distribution of pyrethrins in oil glands and leaf tissue of *Chrysanthemum cinerariaefolium*. *Planta Med.* 47:205-207.
- Zito SW, Tio CD (1990). Constituents of *Chrysanthemum cinerariaefolium* in leaves, regenerated plantlets and callus. *Phytochemistry* 29:2533-2534.