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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)
Achillea millefolium	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); seiyou-nokogirisou (J); aquiléa, erva-de-carpinteiro, mil-folhas, milefólio (P); colchon de pobre, milenrama (S); röllika (Sw); vạndiệp, dươngky (V); biranjasif, cickafarkkoro, civanpercemi, duizendblad, rojmari, rolleka, rollike, tlalquequetzal (O)
Anthemis nobilis	Roman/sweet chamomile (E); camomila-romana (P); manzanilla de Castilla (S)
Artemisia absinthium	Absinthium, common wormwood, wormswood, 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)
Artemisia annua	Quinghao, ch'ou hao, huang hua hao, ts'ao hao (C); (sweet/annual) wormwood, sweet Annie (E); assenzio annuale (I); kuso-ninjin (J); thanhcao, thảocao, chènội (V)
Artemisia dracunculus	Dragon sagewort/wormwood, false tarragon, French tarragon, little dragon, mugwort, (true) tarragon (E); rakuuna (Fi); Estragon (F); Estragão (P); Tagantes (S); thanhcao rồng (V)
Matricaria chamomilla	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)
Tanacetum parthenium	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)
Tanacetum vulgare	Common tansy, tansy, (gold) buttons (E); pietaryrtti (Fi); tanaisie (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 (lTCL), 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
T. vulgare	n.s.	Stem	No	-	Callus	n.s.	WG + 2 2,4-D 10% CM	Banthorpe & Wirz-Justice, 1972
T. cinerariaefolium	Indianapolis White Giant #4	Shoot tip	No	-	Shoot	5	2 K 0.02 NAA	Roest & Bokelmann, 1973
C. cinerariaefolium	n.s.	Shoot, root	No	-	Callus, root	n.s.	0.05 2,4-D	Chumsri & Staba, 1975
T. cinerariaefolium	n.s.	Stem	No	-	Callus	n.s.	1 NAA or 1 2,4-D 0.2 K + different irradiation	Aoki et al., 1976
T. cinerariaefolium	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
C. cinerariaefolium	n.s.	Shoot tip	No	-	Shoot	n.s.	1 NAA 1 BA	Grewal & Sharma, 1978
M. chamomilla	n.s.	Flower, leaf, shoot	No	-	Callus	n.s.	1.5 2,4-D, 1.2 K, 10% CM	Szöke et al., 1979
C. cinerariaefolium	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
M. chamomilla	n.s.	Leaf	No	-	Crown gall	n.s.	PGR-free + activated charcoal; 12hr light	Beiderbeck, 1982
M.chamomilla	*M. inodora, A. nobilis A. ptarmica*	Callus	No	-	C, R, shoot	n.s.	0.5 NAA 0.1/0.5 K 1 2,4-D	Čellárová et al., 1982
M. chamomilla	n.s.	Shoot	No	-	Suspension	n.s.	PGR-free + high light intensity; 5 NAA 2.5 K	Bisson et al., 1983
C. cinerariaefolium	+ *C. coccineum* Golden Mass	Leaf,stem,floret▲	No/yes	-	Callus	n.s.	0.5 2,4-D 0.5 BA	Zieg et al., 1983
C. cinerariaefolium	n.s.	Shoot tip	No	-	Shoot	n.s.	0.1 2,4-D 3 BA	Zito et al., 1983
M. chamomilla	n.s.	Leaf, stem	No	-	Callus	n.s.	0.5/5 NAA 1/2.5 K	Reichling et al., 1984
C. cinerariaefolium	Ecuadorian cv. n.s.	n.s.	No	-	Shoot	1.1-3.5	20 BA + varying effects of light/darkness	Staba et al., 1984
Art. annua	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
C. cinerariaefolium	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
T. vulgare	+*T. parthenium*, *A. vulgaris*	Leaf, stem	No	Yes	Callus	n.s.	10% CW, 6 2,4-D or 0.5 NAA 0.1 K	Banthorpe & Brown, 1989
C. cinerariaefolium	n.s. (high pyrethrin lines)	Leaf	No	-	Callus	100%	5 K 2 2,4-D	Ravishankar et al., 1989
Art. annua	n.s.	Seedling, callus	No	-	Callus	n.s.	1 2,4-D 0.1 K (liquid suspension)	Tawfiq et al., 1989
M. recutita	n.s.	Leaf	No	-	Callus	100%	LS + 0.01 IAA or 3 2,4-D	Čellárová et al., 1990
Art. pallens	Bangalore vars.	Seedling, callus	No	-	C, shoot	n.s.	4 2,4-D 4 BA	Benjamin et al., 1990
T. coccineum	n.s.	Achene▲, petal▲	Yes	-	Callus, shoot	0-89%	0.2 NAA/2,4-D 2 BA	Fujii & Shimizu, 1990
Art. douglasiana	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
C. cinerariaefolium	n.s. 3-yr old plants	Axillary bud	No	-	Shoot	n.s.	½RT + 1 2,4-D	Zito & Tio, 1990
Art. dracunculus	French stocks	Leaf, stem	No	-	Callus	n.s.	2 NAA 0.5 BAP	Cotton et al., 1991
M. chamomilla	n.s.	Shoot primordia	No	-	Shoot	n.s.	2 NAA 2 BAP (auxin induces oil bodies)	Takano et al., 1991
A. millefolium	ssp. *millefolium*	Hypocotyl	No	-	Callus	n.s.	B5 + 1.5 2,4-D 0.1 K (darkness); 100 miglyol	Figueiredo & Pais, 1991; et al., 1995
Art. princeps	var. *orientalis*	Leaf, hypocotyl	No	-	Callus	n.s.	1 2,4-D, 2 NAA, 1 K	Kil et al., 1992
Art. annua	*A. rhizogenes* transformed	Root	No	-	Callus, gall	100%	0.05 pcPA 0.05 BAP/2iP	Kim et al., 1992
Art. annua	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
C. cinerariaefolium	HSL 801, SL 821	Shoot tip	No	-	Callus	100%	0.5 2,4-D 0.5 BA	Dhar & Pal, 1993
An. nobilis	n.s. *A.tumefaciens* 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
Art. annua	Vietnamese origin (seeds)	Leaf, stem	n.s.	-	Shoot	n.s.	0.2 BAP 0.05 NAA	Woerdenbag et al., 1993
Art. annua	n.s.	Hypocotyl	No	-	Callus	n.s.	1 2,4-D; 0.5 NAA 0.5/2.5 BAP; 2.5 NAA	Brown, 1994
Art. annua	From the wild	Hypocotyl, leaf, root	No	-	Callus, shoot	n.s.	2 Z 1 NAA 2 BA (C); 3 BA 0.2 NAA (S)	Paniego & Giulietti, 1994
A. asplenifolia	n.s.	Nodal culture	No	-	Shoot	n.s.	1 BAP 0.1 IAA 0.025 GA3 → PGR-free	Wawrosch et al., 1994
T. vulgare	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
An. nobilis	n.s.	Young shoot	No	-	Shoot	1.8-6.5	No PGRs; liquid media	Asai et al., 1995
Art. annua	n.s.	Stem	No	-	Shoot	1.1-9.8	0.5 K 0.2 IAA + cotton fiber	Moraes-Cerdeira et al., 1995
Art. annua	*A. rhizogenes* transformed	Root	No	-	Hairy root	n.s.	25-35 BA	Mukherjee et al., 1995
C. cinerariaefolium	HY C,D SY A,B	Leaf, stem, flower▲	No/yes	-	Callus		4 NAA 0.4 BAP → ½MS + 4 NAA 0.4 BAP	Barthomeuf et al., 1996
T. parthenium	n.s.	Leaf	No	-	Shoot	n.s.	4.5 NAA 4.5 BAP	Brown et al., 1996
Art. annua	P_2, P_4 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
Art. annua	n.s.	Leaf, stem	No	-	Shoot	Many	1 NAA 3 BAP 0.1 GA3	Gulati et al., 1996
T. vulgare	(*L. maximum*) Moon max	Leaf, stem, shoot tip	No	-	Shoot	48-74	0.2 NAA 0.25 BA	Kumar et al., 1996
Art. absinthium	n.s.	Shoot tip	No	-	Callus, shoot	1.5	0.15 IAA 0.2 BA	Nin et al., 1996
Art. annua	West Virginia/Yugoslavia	Leaf, stem, root	No	-	R,S,callus	100%	0.05 NAA 0.5 BA	Vergauwe et al., 1996b
Art. sphaerocephala	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
Art. absinthium	5 lines	Shoot tip	No	-	Shoot	n.s.	0.2 BA 0.05 NAA	Nin et al., 1997
Art. pallens	n.s. (encapsulated shoot tip)	Seedling	No	-	Shoot	100%	1 NAA 0.3 BAP 1 biotin	Sharief et al., 1997
Art. annua	*A. rhizogenes* transformed	Root	No	-	Hairy root	100%	0.01 GA3	Smith et al., 1997

Table 2 contd.

T. parthenium	n.s.	Stem node	No	-	S, hairy root	100%	1 BA 0.1 NAA (shoot); LBA 9402 (hairy root)	Stojakowska & Kisiel, 1997
A. ceretanica	#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
Art. annua	*A. rhizogenes* transformant	Root	No	-	Hairy root	n.s.	PGR-free + various nitrate, phosphate levels	Weathers et al., 1997
C. cinerariaefolium	n.s.	Axillary bud	No	-	Shoot	n.s.	No PGR	Chen et al., 1998
Art. annua	n.s.	Cell culture (bioreactor)	No	-	Cell culture	n.s.	0.5 BA 0.05 NAA (biotransformation)	Liu et al., 1998,1999
An. nobilis	n.s.	Root	No	-	Hairy root	n.s.	1 IAA 0.5 NAA/IBA	Omoto et al., 1998
Art. annua	n.s.	Seedling	No	-	Shoot	n.s.	1 BAP → 2 IAA	Usha & Swamy, 1998
C. cinerariaefolium	High pyrethrum lines	Flower head▲	Yes	-	Callus	100%	1 NAA 1 BAP (static culture)	George et al., 1999
C. cinerariaefolium	High pyrethrum lines	Flower head▲	Yes	-	Callus	60-95%	0.2-4 NAA 0.2-41 BAP	Hitmi et al., 1999b,2001
Hybrid	*T. vulgare* x *T cinerariaefolium*	Leaf protoplast	No	-	Callus	n.s.	6.4 BAP 0.8 NAA	Keskitalo et al., 1999
T. parthenium	*+A. annuum*	Seedling	No	-	Callus	n.s.	0.5 NAA/BAP or 1 2,4-D	Sy & Brown, 1999
Art. annua	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
An. nobilis	Flore Pleno	Leaf	No	-	Shoot	<9.6	0.2 NAA 0.2-1 BA → 0.1 IBA	Echeverrigaray et al., 2000
M. recutita	BK-2, Degumil	Shoot tip	No	Yes	R,S,hairy root	n.s.	No PGR + A4-Y, R-1601, 15834 *A. rhizogenes*	Máday et al., 2000
Arg. frutescens	Butterfly	Shoot tip	No	-	Shoot	n.s.	0.1-2.5 BAP/2iP/K	Seyring & Vogt, 2000
S. canescens	Lagasca	Shoot tip	No	-	Shoot	1.3-11.9	0.01-1 BA 1.3 linoleic acid	Casado et al., 2000
Art. annua	*A. rhizogenes* transformant	Root	No	-	Hairy root	n.s.	PGR-free	Xie et al., 2000
Art. annua	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
Anthemis nobilis	Flower	CF	Early	1000	1000	Fauconnier et al., 1993
Artemisia absinthium	Hairy root	A	Early	500	500	Kennedy et al., 1993
Artemisia annua	Hairy root	None	None	-	-	Qin et al., 1994
Artemisia annua	Hairy root	None	None	-	-	Mukherjee et al., 1995
Artemisia annua 001,025	Hairy root	None	None	-	-	Paniego & Giulietti, 1996*[1]
Artemisia annua	Leaf	K	Late (3w)	K20	K20	Vergauwe et al., 1996a,b, 1998
Artemisia annua	Hairy root	None	None	-	-	Bannerjee et al., 1997
Artemisia annua	Galls	None	None	-	-	Ghosh et al., 1997*[1]
Artemisia absinthium	Leaf	KAR	Early	K50 A500 R10	K50 A500 R10	Nin et al., 1997
Tanacetum parthenium	Leaf	None	None	-	-	Stojakowska and Kisiel, 1997
Achillea millefolium	Roots	A,CF	Early	A500, 250 CA+CF	A500, 250 A+CF	Lourenço et al., 1999
Matricaria recutita	Roots	CA	Early	800	800	Máday et al., 1999
Artemisia annua 001, 025	Leaf	K	Early	15	20	Chen et al., 2000
Matricaria recutita BK-2 +1	Hairy root	A,CF	Early	CF 800	CF250, A1000	Máday et al., 2000
Artemisia annua	Hairy root	K	Early	100	100	Xie et al., 2000
Artemisia annua	Hairy root	n.s.	n.s.	n.s.	n.s.	Liu et al., 2002

‡ = mg/l; *[1] = 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®>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 ≥40 mg/l, differentially controlling *Agrobacterium* growth (Seyring, 1999).

CONVENTIONAL BREEDING

Nature has played a role in inducing popyploidy 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 desciphered 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 antivial 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 GERMPLASM PRESERVATION

Cryopreservation, an important method for the conservation of plant genetic resources (Engelmann, 2000) uses freeze preservation in liquid N_2 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
Artemis annua	Leaf, stem	EHA101,C58	2-2.5	L/D	<27%	3-58%	K	20	Vergauwe et al., 1996a,b, 1998
Artemisia absinthium (5 lines)	Leaf	AR1855,LBA9402	∞	L/D	0-100%	n.s.	K,A,R	K50 A500 R10	Nin et al., 1997
Artemis annua 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
Artemis annua	*nptII*,*GUS*,*SOD*,*BAT*	CaMV35S	0-27%	Callus	Plant	Yes	No	Assays	Artemisinin levels	Vergauwe et al., 1996a,b, 1998
Artemisia absinthium (5 lines)	*nptII*,*opines*	CaMV35S	0-100%	Hairy root	Plant	Yes	Yes	No	Essential oil	Nin et al., 1997
Artemis annua lines 001,025	*nptII*,*FDS* 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_2 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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