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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)

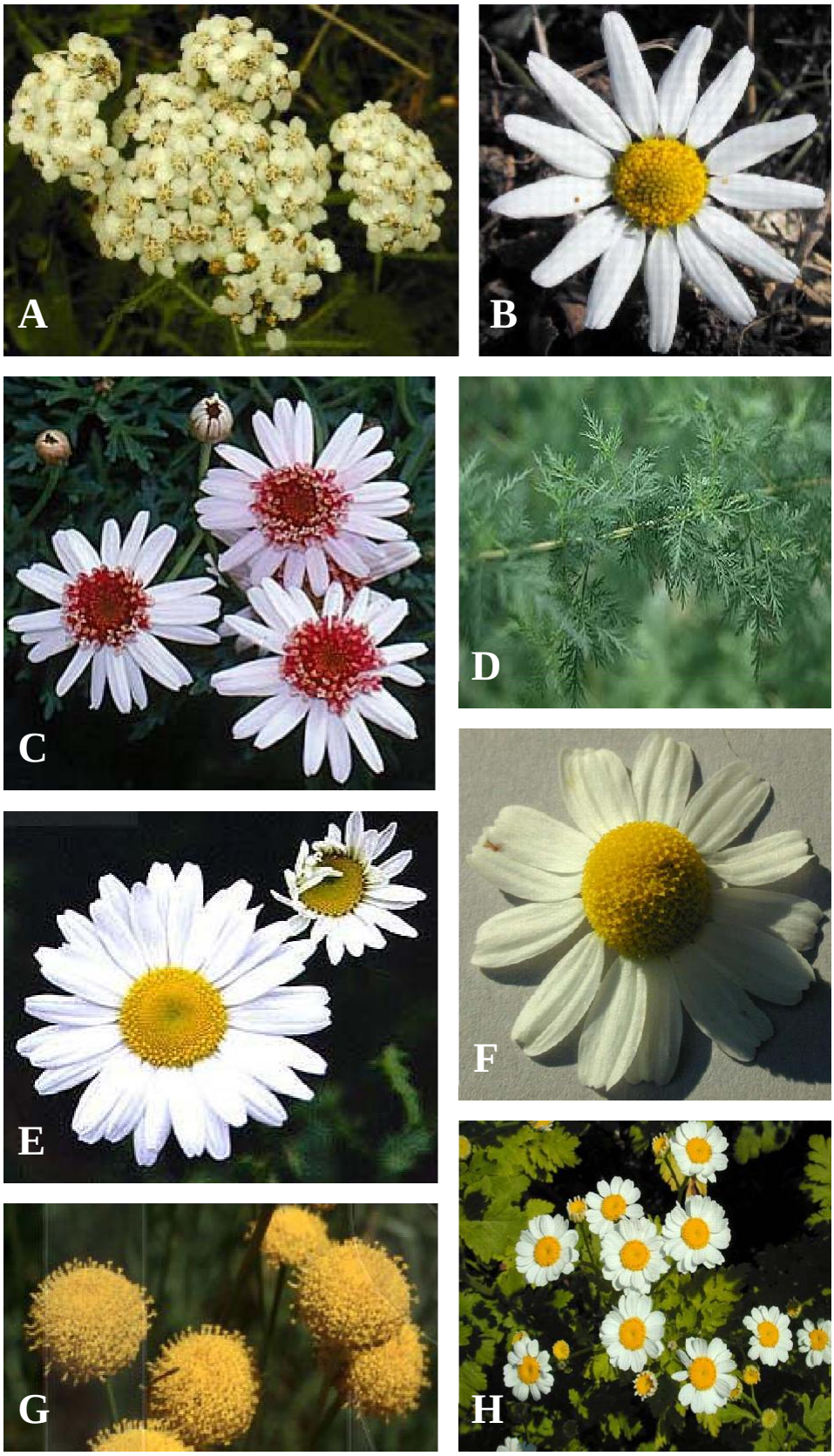


**Figure 1.** Some of the principal species within the Anthemideae. A) *Achillea millefolium*, B) *Anthemis cotula*, C) *Argyranthemum frutescens*, D) *Artemisia annua*, E) *Leucanthemum vulgare*, F) *Matricaria recutita*, G) *Santolina rosmarinifolia*, H) *Tanacetum parthenium*.

**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* | P2, P4 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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