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Genetic and morphological diversity of Finnish tansy (*Tanacetum vulgare* L., Asteraceae)

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Abstract Morphological and genetical differences of twenty Finnish tansy (*Tanacetum vulgare)* genotypes were studied. The genotypes were distinguishable by morphology, and number of flower heads, which correlated positively with height of the plant and height of the corymb. The mean nuclear DNA content in the leaves of tissue-cultured tansy plantlets was 8.86 pg, and variation between genotypes was 27%. The genotypes were also distinguishable on the basis of their RAPD patterns. The RAPD and morphological data were subjected to analysis of principal components, according to which the genotypes were separated into two main groups. Group I included 7 genotypes from southern Finland and 2 genotypes from the eastern lake district. They started to flower later, had more flower heads and nodes per stem, and the corymb was longer than in the other genotypes that originated in the western and central part of Finland and formed group II. Our data are supported by previous studies and suggest that group I may represent native tansy populations in Finland, whereas group II may represent tansy genotypes that have been transported to other parts of Finland through agriculture and attempts of domestication.

Key words Tansy · *Tanacetum vulgare* L · Random amplified polymorphic DNA · Polymerase chain reaction (RAPD-PCR) · Flow cytometry · DNA polymorphism

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Introduction

The interest in using natural, plant-derived deterrents and pesticides instead of synthetic compounds has increased during the past few years. One of the plant species that appears to be a potential source of such compounds is tansy (*Tanacetum vulgare*; Asteraceae). Several studies have shown that essential oils or extracts of tansy contain bioactive compounds that are antifungal (Héthelyi et al. 1991), antibactericidal (Héthelyi et al. 1991; Holopainen and Kauppinen 1989; Stefanovic et al. 1988), or insect repellent (Hough-Golstein and Hahn 1992; Nottingham et al. 1991; Suomi et al. 1986; Schearer 1984). Tansy is an aromatic, herbaceous and perennial plant (Heywood 1976). It is mainly cross-pollinated, and seed production is enhanced by insect pollination (Lokki et al. 1973). Morphologically, tansy exhibits divergency: its height varies between 30 and 150 cm, the woody stem is more or less branched, leaves are pinnatisect, and the segments serrated. The inflorescence is a dense corymb which usually contains 10–70 yellow flower heads (Heywood 1976). The somatic chromosome number of tansy is $2n = 2x = 18$ (Virrankoski and Sorsa 1968). A few of the loci controlling the main monoterpene pathways for camphor and thujone were observed to be polymorphic in Finnish tansy (Lokki et al. 1973), but genetical variation within this species has not been studied further.

Tanacetum was previously considered to be a subgenus of the genus *Chrysanthemum*, which is native to several parts of Asia, China, Japan, and the Mediterranean region (Bailey and Bailey 1976). Reclassification led to the separation of the genus *Chrysanthemum* from genus *Tanacetum. Tanacetum* currently consists of about 150 species, of which fewer than 30 are utilized (Soreng and Cope 1991).

The Old World and some areas in Europe are supposed to be the native regions of *Tanacetum* spp. (Prach and Wade 1992; Soreng and Cope 1991; Heywood

1976). The ancient Greeks prepared a medicine of tansy and named the plant Athanaton (immortal), from which the name *Tanacetum* is derived (Grieve 1984). In the eighth century, tansy was grown in the gardens of central Europe from where its use spread to other parts of Europe (Mitich 1992) and America (Hussey 1974). Today, tansy is widely naturalized to north-temperate Asia, Europe (Heywood 1976) and North America (Hussey 1974; Hultèn 1968). The non-native areas of Europe such as Ireland (Heywood 1976) and northern Scandinavia (Jalas 1991) are nowadays occupied by tansy as well (Brodie 1991; Jalas 1991). In Finland tansy may be native to the coast of Finland (Hultèn 1950; Linkola and Väänänen 1940), and it is mentioned as a cultural plant (Silkkilä and Koskinen 1990) that has escaped from gardens (Hämet-Ahti et al. 1984).

Only a few of the approximately 1300 genera in the family Asteraceae (Heywood and Humphries 1977) have been studied for their genetical diversity using molecular techniques. Cultivars of *Chrysanthemum* have been identified by fingerprints produced by the restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), inter-simple-sequence-repeat (inter-SSR) polymerase chain reaction (PCR) (Wolf et al. 1995), and by DNA amplification fingerprinting (DAF) (Scott et al. 1996). Isozyme analysis was applied to differentiate 4 genera of subtribe Chrysantheminae (Fracisco-Ortega et al. 1995). The internal transcribed spacer (ITS) region of the 18S-25S nuclear ribosomal DNA repeat has been used to discriminate different populations of the aureoid *Senecio* complex (Bain and Jansen 1995), and RFLP of the chloroplast DNA has been examined to reveal the evolutionary origin of *Dendrosenecio (*Knox and Palmer 1995). The genetic relationships of domesticated and wild sunflowers have been investigated using RAPD-PCR (Arias and Rieseberg 1995). Furthermore, isozyme analysis, simple and repetitive sequences as hybridization probes, and RAPD-PCR have been applied to differentiate sunflower genotypes (Mösges and Friedt 1994). The nuclear DNA content has been investigated in only a few species of the Asteraceae family, namely *Helianthus annuus* (Arumuganathan and Earle 1991a; Michaelson et al. 1991b), *Crepis capillaris*, and *Lactuca sativa* (Arumuganathan and Earle 1991a).

We are interested in the insecticidal and other bioactive compounds of tansy. To further develop or manipulate these properties we have established tissue culture (M. Keskitalo, unpublished) and protoplast techniques (Keskitalo et al. 1995) for tansy. Knowledge of the biodiversity of tansy will permit us to select the most useful genotypes for the ongoing domestication process. Therefore, the aim of the study presented here was to examine the genetical and morphological diversity of selected Finnish tansy genotypes. The specific objectives were (1) to describe morphological diversity; (2) to study the genetic variability of tansy by the analysis of nuclear DNA content and RAPDs; (3) to test the correlation between the morphological and genetical variability and the geographical origin.

Materials and methods

Plant materials

The geographical locations of 20 tansy genotypes (*Tv* 1–*Tv* 20) collected and used in this study are listed in Table 1 and illustrated in Fig. 1. The genotypes were transplanted to an orchard of the Department of Plant Production, University of Helsinki, Finland (60°1′N) in 1991. The plants were grown 1.5 m apart in two rows without fertilization in humous coarse sand (pH 6.1). All the accessions rooted, started to grow, and flowered in the orchard. The growth of the shoots began in April or May, formation of the corymbs was observed in June, and flowering lasted from July to August or September. To avoid the dispersal of seeds, we cut the corymbs after flowering had ended in the fall.

Roots of the above-mentioned tansy genotypes were transferred to 5-l pots filled with a peat : vermiculite mixture (3 : 1) and cultured in the greenhouse at $+20^\circ \pm 5^\circ$C/$16^\circ \pm 5^\circ$C (day/night). Illumination was supplemented with fluorescent lamps to reach the photoperiod of 16 h during October – March. Plants were watered twice a week and fertilized with a 0.2% solution of NPK fertilizer (5-3-7) twice a month. They were cut down after flowering in January – February.

Shoot tips of the plants grown in the greenhouse were transferred to in vitro and cultured at $+23^\circ \pm 2^\circ$C under an illumination of 40 W and 150 μmol m^{-2} s^{-1} as previously described (Keskitalo et al. 1995). After 3 months, the tissue cultures were transferred to $+25^\circ$C and grown under a 16-h illumination of 100 μE m^{-2} s^{-1} provided by fluorescent and Gro Lux lamps.

Morphology and date of flowering

Morphology of the 20 tansy genotypes (*Tv* 1–*Tv* 20) grown in the orchard was observed for 3 years (1993–1995) at the beginning of

Table 1 Geographical origins (National Landsurvey Institute 1996) of the 20 tansy genotypes included in this study

Tansy genotype	Region	Latitude (N)	Longitude (E)
Tv 1	Iisalmi	63°33′N	27°12′E
Tv 2	Eno	62°47′N	30°08′E
Tv 3	Koli	63°06′N	29°48′E
Tv 4	Kangasniemi	61°59′N	26°39′E
Tv 5	Helsinki	60°10′N	24°57′E
Tv 6	Halikko	60°23′N	23°03′E
Tv 7	Lohjanharju	60°30′N	24°24′E
Tv 8	Tampere	61°29′N	23°48′E
Tv 9	Sääksmäki	61°11′N	24°03′E
Tv 10	Porvoo	60°23′N	25°40′E
Tv 11	Sipoo	60°22′N	25°15′E
Tv 12	Vihti	60°25′N	24°18′E
Tv 13	Hämeenlinna	60°59′N	24°27′E
Tv 14	Lemu	60°33′N	21°59′E
Tv 15	Tervakoski	60°48′N	24°38′E
Tv 16	Lieto	60°30′N	22°27′E
Tv 17	Hanko	59°49′N	23°00′E
Tv 18	Alavus	62°35′N	23°37″E
Tv 19	Hailuoto	65°00′N	24°43′E
Tv 20	Köyliö	61°06′N	22°20′E

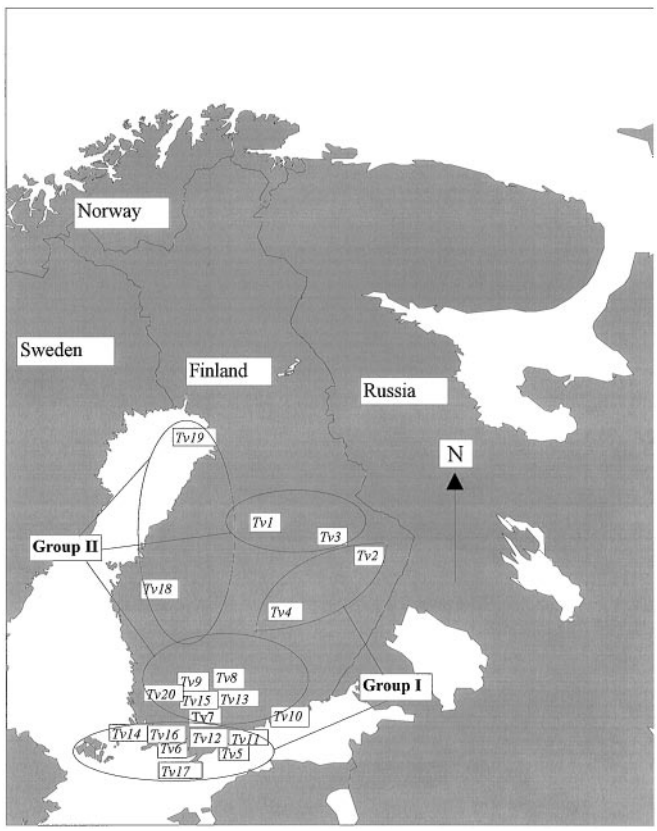

Fig. 1 The geographical origins of the 20 tansy genotypes of this study. Groups I and II are defined on the basis of a complete linkage cluster analysis of RAPDs

flowering. Height of the shoot and corymb, the number of nodes per stem, and the number of flower heads per stem were measured from ten shoots per genotype in each year. Observations on flowering were also made for 3 years (1994–1996). The start of flowering of a flower head was defined by the time point when the first row of the marginal florets had opened. Flowering of a genotype was defined to begin when 50% of the flower heads had started flowering.

Determination of nuclear DNA content

Nuclear DNA content (2C value) was determined by flow cytometry in 13 in vitro-grown tansy genotypes (*Tv* 2, 4–6, 8–12, 14, 15, 19, and 20) which had been tissue-cultured 6–8 months by subculturing them every 5 weeks. Nuclei were isolated from 30 mg of fully opened top leaves and analyzed as previously described (Arumuganathan and Earle 1991b; Valkonen 1994). Barley nuclei (cv 'Sultan'; Valkonen 1994; Bennett et al. 1982) were added to tansy nuclei as an internal standard. Relative fluorescence of the propidium iodide-stained nuclei (500 ± 250 nuclei per measurement) was measured with an EPICS Profile flow cytometer at 488 nm at the Flow Cytometry and Image Facility, Biotechnology Center, Cornell University (USA). The nuclear DNA contents in the nuclei of tansy were determined relative to the 2C values of barley. Analyses with each tansy genotype were repeated five to six times.

DNA extraction and purification

Total DNA was extracted from 20 greenhouse-grown tansy genotypes (*Tv* 1–*Tv* 20). Leaves (2.0–4.5 g) were taken from each genotype before flowering, washed with distilled water, and frozen in liquid nitrogen until the DNA extraction. The methods used for DNA extraction, purification of DNA using a $CsCl_2$-gradient, and the determination of DNA concentration have been previously described (Malkamäki et al. 1996). The plant DNA was stored in TE buffer (10 m*M* TRIS-HCl, 1 m*M* EDTA, pH 8.0) at a concentration of 50 ng/µl at $+4°C$ or at $-20°C$.

Polymerase chain reaction

The polymerase chain reaction using random 10-mer primers was carried out essentially as described by K. Koivu, U. Malkamäki, and J. P. T. Valkonen (unpublished) using the Programmable Thermal Controller (PTC-10, MJ Research). The PCR reaction mixture (25 µl final volume) contained 2.5 µl $10 \times$ PCR buffer (500 m*M* KCl, 100 m*M* TRIS-HCl pH 8.3, 1.0% Triton x-100), 200 µ*M* deoxynucleotide triphoshates (dNTPs, Promega), and 0.5 u *Taq* DNA polymerase (Promega). The concentrations of $MgCl_2$ (1, 2, 3, 4, 5, and 6 m*M*), primers (100, 200, and 400 n*M*), and template DNA (12.5, 25, 50 ng) were optimized with ten 10-mer random primers (Operon A, Alameca Calif.) (Table 2). Deionized sterile water was added to each reaction mixture to reach the volume of 25 µl. Each reaction was overlayed with mineral oil and subjected to 40 amplification cycles as follows: 15 s at 92°C for denaturation, 30 s at 36°C for annealing, and 1 min at 72°C for primer extension. Before the first cycle the samples were denaturated for 3 min at 94°C, and after the final cycle, samples were incubated for a further 4 min at 72°C to ensure completed extension of the target molecule. After amplification the PCR products were electrophoresed through 1.4% agarose gel (Promega) dyed with EtBr, run in $1 \times$ TAE (Sambrook et al. 1989) for 4 h at 80 V, and visualized by illumination with UV light. A mixture (1.3 : 1 w/w) of *Hin*dIII digested λ-DNA (Promega) and *Hae*III digested $\Phi \times 174$ DNA (Promega) or a mixture of *Eco*RI-, *Eco*RI/*Sin*I-, *Hinc*II-, and *Sin*I-digested pBR322 were used as the molecular weight standards. Analysis with each primer was carried out at least three times.

Statistical analysis

Reproducible PCR bands were scored on the basis of the presence (1) or absence (0) of a fragment. The SAS program (SAS Institute 1984) modified by Levy et al. (1991) was used to calculate the similarity and distance matrixes from RAPD-PCR profiles and to create the dendrogram from distance matrixes. The similarity and distance matrixes were calculated according the formula of Nei and Li (1979): $[S_{xy} = 2N_{xy} : (N_x + N_y)]$; where N_{xy} is the number of RAPD-PCR fragments common for the accessions x and y; and N_x and N_y are the numbers of fragments of the accessions x and y, respectively. The dendrogram was synthesized by complete linkage cluster analysis, which illustrates the genetic distance as the normalized maximum distance between clusters. The normalized maximum distance is a normalized value of the average genetic distance.

To compare the RAPD data (distance matrixes) and morphological data, we synthesized the principal components of both types of data with the SAS PRINCOMP procedure for principal component analysis (SAS Institute 1984). The principal components were graphically plotted to a scatter diagram using WINDOWS EXCEL software. The distances of principal components between groups were pair-wise tested using the *t*-test (TTEST) of SAS (SAS Institute 1984).

Statistical parameters for the results of flow cytometrical analysis and morphological data were calculated using the SAS GLM procedure of General Linear Model for Variance Analysis and Student-Neuman-Keuls (S-N-K) test (SAS Institute 1984).

Table 2 The ten random oligonucleotide primers (Operon A) used for amplification of tansy total DNA

Primer	Sequence	Total number of fragments scored	Number of polymorphic fragments between genotypes		Size range of the scored fragments (bp)
			Number	%	
A-02	5′-TGCCGAGCTG-3′	8	7	87.5	450–2000
A-03	5′-AGTCAGCCAC-3′	7	4	57.1	600–1500
A-05	5′-AGGGGTCTTG-3′	10	8	80.0	600–2300
A-07	5′-GAAACGGGTG-3′	5	4	80.0	600–1700
A-10	5′-GTGATCGCAG-3′	12	10	83.3	500–2500
A-11	5′-CAATCGCCGT-3′	10	10	100.0	500–1750
A-12	5′-TCGGCGATAG-3′	5	4	80.0	900–1750
A-13	5′-CAGCACCCAC-3′	11	7	63.6	800–2900
A-16	5′-AGCCAGCGAA-3′	11	11	100.0	700–2500
A-19	5′-CAAACGTCGG-3′	13	13	100.0	450–2800
Total/range		92	78	84.8	450–2900

Results

Morphology and date of flowering

The mean heights of tansy genotypes varied from 60.5 cm (*Tv* 19) to 115.4 cm (*Tv* 15), the mean number of nodes from 14.6 (*Tv* 14) to 26.7 (*Tv* 19), the mean number of flower heads per stem from 17.6 (*Tv* 19) to 79.8 (*Tv* 16), and the mean length of the corymb from 4.9 cm (*Tv* 19) to 24.8 cm (*Tv* 16). The date of beginning of flowering varied from the 20th of July (*Tv* 3 and *Tv* 15) to the 4th of August (*Tv* 12). Significant differences were found among the tansy genotypes in all these traits ($P = 0.0001$; for the date of flowering $P = 0.05$). Furthermore, according to the S-N-K test, the mean number of nodes per stem, the mean number of flowers, the mean length of the corymb, and the date of the flowering differed significantly between groups I and II ($P = 0.05$) (Table 3). Pearson correlation coefficients (r) between genotype height and number of nodes, number of flower heads, and height of corymbs were 0.21, 0.53, and 0.48 ($P = 0.0001$), respectively. Pearson correlation coefficients (r) between number of flower heads and number of nodes, height of corymb, and date of beginning of flowering were 0.14 ($P = 0.0005$), 0.78 ($P = 0.0001$), and 0.35 ($P = 0.1$), respectively (data not shown).

Principal components 1 and 2 of tansy genotypes based on the morphological data are illustrated in Fig. 2. The first, second, third, and fourth principal components explained 55.4%, 26.6%, 13.2%, and 4.8% of the variation, respectively. Out of 9 genotypes belonging to group I, 6 were positioned bellow the principal-1 axis, whereas 8 out of 11 genotypes of group II were above the principal-1 axis. The means of principal components 1 and 2 for group I (N = 270) and group II (N = 330) were 0.087 and -0.417, and -0.074 and 0.353, respectively. When plotted on the scatter diagram, the position of the groups was nearly symmetrical to the origo (Fig. 3). The *t*-test indicated that the means of principal components 1, 2, 3, and 4 for group I and II differed significantly from each other ($P = 0.05$), which suggested that morphology differed significantly between group I and II.

Nuclear DNA content

Analysis of tansy nuclei by flow cytometry revealed one peak of fluorescence that was close to the peak of barley nuclei used as an internal standard (2C = 10.94 pg; Valkonen 1994). The overall mean 2 C value for tansy genotypes was 8.86 pg. The means of different genotypes ranged from 7.84 pg (*Tv* 14) to 9.95 pg (*Tv* 4) (Table 4) and differed significantly ($P = 0.05$). The coefficients of variation (CV), determined as the quotient of the standard deviation of the peak and the mean peak position (channel number), were 2.6–4.7% (mean 3.3%) for different tansy genotypes.

Analysis of RAPDs

DNA purification yielded 2–10 μg total DNA per 1 g of fresh leaf tissue. The optimal dNTP, primer, $MgCl_2$, and template concentrations for different primers were 200 μl, 200 m*M*, 3–6 m*M*, and 12.5–25 ng, respectively. Ten RAPD primers were used (Table 2), and they produced a total of 92 reproducible bands (450 bp-2900 bp), of which 78 (84.8%) were polymorphic. Of the polymorphic fragments 4 were unique (i.e., occurred in only a single genotype). The total number of DNA fragments amplified with the total of ten primers was

Table 3 Morphological observations on the 20 tansy genotypes over 3 years (1993–1995). Placement in group I and group II is according to the analysis of RAPD patterns by complete linkage cluster analysis (Fig. 3). Plants were grown in an orchard at the Department of Plant Production, University of Helsinki, Finland

Group	Height of plant[a]			Number of nodes per stem[a]			Flowering								
	cm	SD		Number	SD		Date at the beginning of flowering[b]			Number of flower heads per stem[a]			Length of the corymb[a]		
							Date	SD		Number	SD		cm	SD	
I	92.8	10.3	a[c]	19.3	2.3	a	29 July	5.5	a	47.2	21.5	a	13.6	6.0	a
II	91.9	11.0	a	16.4	2.8	b	25 July	5.4	b	42.9	21.7	b	10.6	5.1	b
Mean	92.4	10.7		18.0	2.6		26 July	5.5		44.9	21.6		12.0	5.5	
Minimum	60.5	17.0		14.6	2.5		20 July	6.0		17.6	15.9		4.9	3.8	
Maximum	115.4	11.3		26.7	7.2		4 August	10.0		79.8	28.0		24.8	6.9	

[a] Observations made in 1993–1995
[b] Observations made in 1994–1996
[c] Means with the different letters indicate significant differences ($P = 0.05$) according to the S-N-K-test

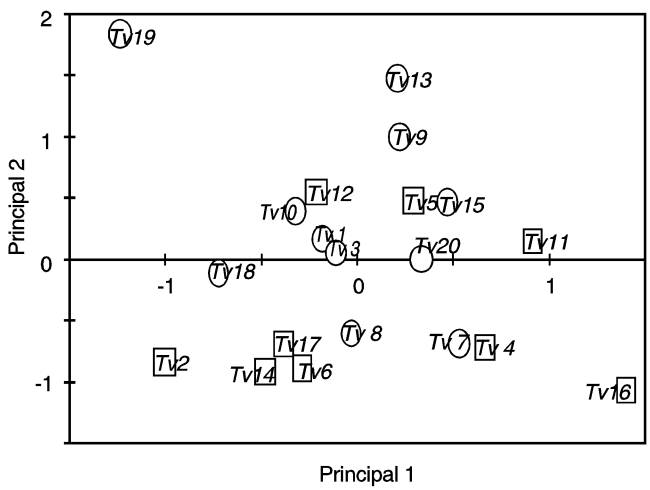

Fig. 2 Principal component analysis (Principal 1 × Principal 2) of morphological traits of the 20 tansy genotypes. The *quadrangles* indicate the genotypes of group I (Figs. 1 and 5) and the *circles* indicate the genotypes of group II (Figs. 1 and 5)

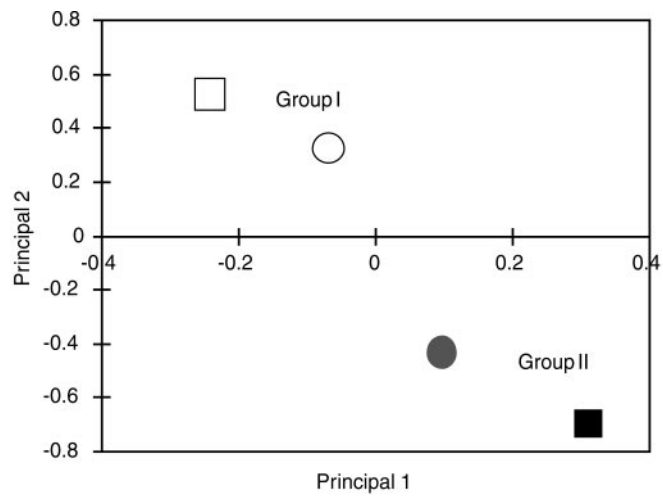

Fig. 3 Principal components of morphological data and of distance matrices calculated by complete linkage cluster analysis of RAPD-PCR amplifications of 20 tansy genotypes. The groups are created based on the results of the complete linkage cluster analysis of RAPD-PCR amplification. *White* and *black* indicate group I and group II, respectively, whereas *quadrangles* and *circles* indicate RAPD data and morphological data, respectively

38–58 per genotype. The largest numbers of polymorphic RAPDs were amplified with the primers OPA 11 (Fig. 4), 16 and 19.

The smallest genetic distance observed was 0.153, between the genotypes *Tv* 18 and *Tv* 19, whereas the widest genetic distance, 0.476, was observed between the genotypes *Tv* 8 and *Tv* 16 (Table 5). The mean genetic distance was 0.294 among all 20 tansy genotypes.

Complete linkage cluster analysis initially separated the 20 genotypes to two groups (group I and II) that were further divided to six smaller sub-groups (Fig. 5). The widest and shortest normalized maximum distances were 1.62 (0.476/0.294 = 1.62) and 0.52 (0.153/0.294 = 0.52) between group I and II, and between the genotypes *Tv* 18 and *Tv* 19, respectively. Most genotypes in the same subgroup (Fig. 5) originated in close geographical locations (Fig. 1). Seven out of 9 genotypes of group I and 10 out of 11 genotypes of group II originated south and north of latitude 60°3′N, respectively (Table 1).

The first, second, third, and fourth principal components calculated from distance matrices of RAPDs explained 18.6%, 15.4%, 13.2%, and 8.5%, respectively, of the variation of distance matrices of genotypes. The first and second principals are illustrated in Fig. 6. Most of the genotypes that belonged to group I and

Table 4 Nuclear DNA contents (2C values) in 13 tissue-cultured tansy genotypes determined by laser flow cytometry. Barley cv 'Sultan' (2C = 10.94 pg; Valkonen 1994) was used as an internal standard

Tansy genotype	Nuclear DNA content		
	pg	SD	
Tv 2	8.40	0.39	def[a]
Tv 4	9.95	0.22	a
Tv 5	9.40	0.25	b
Tv 6	9.44	0.34	b
Tv 8	8.86	0.65	bcd
Tv 9	8.74	0.40	cde
Tv 10	9.31	0.30	b
Tv 11	8.20	0.43	ef
Tv 12	8.49	0.03	de
Tv 14	7.84	0.17	f
Tv 15	8.88	0.30	bcd
Tv 19	8.38	0.18	def
Tv 20	9.19	0.25	bc
Mean	8.86	0.30	

[a] Means indicated by different letters are significanty different ($P = 0.05$)

group II were positioned below and above the principal-1 axis, respectively (Fig. 6). The means of principal components 1 and 2 of the genotypes which belonged to group I and II, respectively, were calculated and plotted to the scatter diagram (Fig. 3). Similar to morphological data, the data based on genetical differences positioned the groups nearly symmetrically to the origo: group I was below the principal-1 axis, whereas group II was above the principal-1 axis. The means of principal components 1, 2, 3, and 4 differed significantly between groups I and II ($P = 0.05$), which indicated that groups I and II differed genetically from each other.

Discussion

The first objective of this study was to analyze morphological variation of tansy. The morphology of tansy genotypes varied, as has been previously reported for Finnish tansy by Hämet-Ahti et al. (1986), and the differences were consistent over the 3 years of this study. All the variables examined distinguished at least

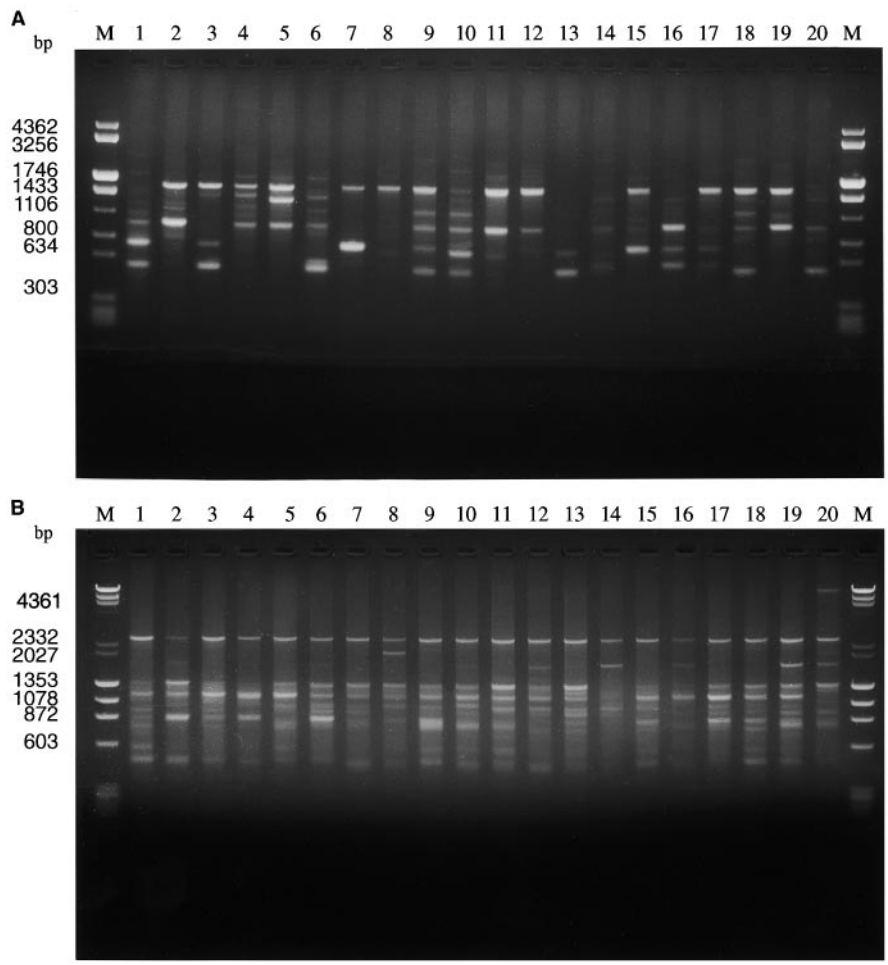

Fig. 4A, B RAPD profiles of the 20 tansy genotypes produced using the random 10-base oligonucleotide primers OPA–11 (**A**) and OPA-10 (**B**) for amplification. *Lanes 1–20* correspond to the tansy genotypes *Tv* 1–*Tv* 20, respectively. *Lane M* shows the DNA marker, for which the sizes are indicated

Table 5 Genetic distance of the 20 tansy genotypes based on RAPDs using the method of Nei and Li (1979)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Tv 1	0.000																			
Tv 2	0.320	0.000																		
Tv 3	0.225	0.333	0.000																	
Tv 4	0.262	0.258	0.259	0.000																
Tv 5	0.283	0.260	0.297	0.282	0.000															
Tv 6	0.290	0.267	0.286	0.308	0.271	0.000														
Tv 7	0.333	0.361	0.364	0.360	0.333	0.314	0.000													
Tv 8	0.415	0.316	0.388	0.410	0.325	0.405	0.333	0.000												
Tv 9	0.236	0.212	0.270	0.290	0.218	0.261	0.302	0.341	0.000											
Tv 10	0.295	0.247	0.340	0.310	0.287	0.264	0.309	0.333	0.204	0.000										
Tv 11	0.312	0.302	0.389	0.295	0.231	0.326	0.318	0.351	0.284	0.278	0.000									
Tv 12	0.307	0.263	0.358	0.245	0.248	0.275	0.278	0.280	0.314	0.235	0.163	0.000								
Tv 13	0.255	0.292	0.252	0.333	0.275	0.295	0.320	0.275	0.264	0.286	0.318	0.299	0.000							
Tv 14	0.385	0.381	0.426	0.326	0.348	0.312	0.419	0.457	0.340	0.352	0.309	0.271	0.386	0.000						
Tv 15	0.276	0.253	0.255	0.235	0.238	0.245	0.287	0.291	0.211	0.310	0.281	0.200	0.288	0.341	0.000					
Tv 16	0.327	0.269	0.304	0.271	0.364	0.243	0.327	0.476	0.263	0.348	0.326	0.295	0.377	0.319	0.284	0.000				
Tv 17	0.275	0.333	0.252	0.273	0.333	0.238	0.299	0.392	0.283	0.262	0.318	0.237	0.280	0.318	0.248	0.245	0.000			
Tv 18	0.245	0.220	0.279	0.223	0.208	0.234	0.301	0.333	0.164	0.218	0.275	0.267	0.275	0.348	0.257	0.236	0.235	0.000		
Tv 19	0.243	0.276	0.241	0.296	0.261	0.250	0.302	0.349	0.183	0.239	0.271	0.245	0.252	0.362	0.255	0.235	0.215	0.153	0.000	
Tv 20	0.340	0.340	0.333	0.320	0.260	0.320	0.368	0.375	0.250	0.247	0.279	0.326	0.286	0.365	0.293	0.385	0.327	0.240	0.238	0.000

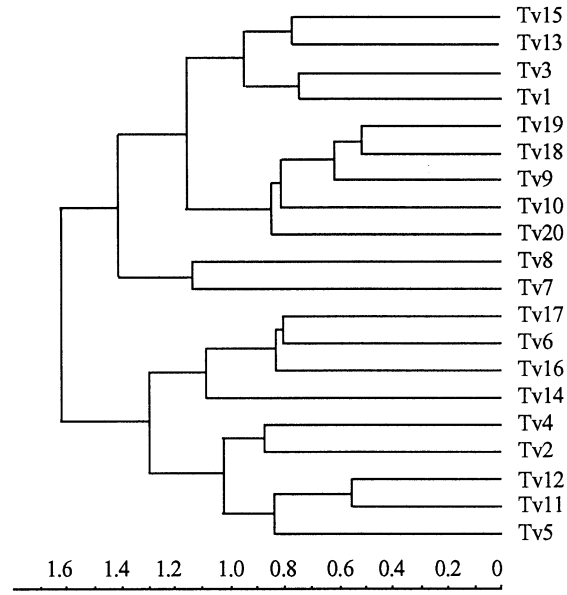

Fig. 5 Phylogenetic analysis of the 20 tansy genotypes based on the RAPDs analysis. The genetic distances between genotypes were calculated as described by Nei and Li (1979) using the complete linkage cluster analysis. The *bar* below the cluster shows the normalized maximum distance between clusters. The two main clusters correspond to group I (tansy genotypes *Tv* 2, 4, 5, 6, 11, 12, 14, 16, and 17) and group II (tansy genotypes *Tv* 1, 3, 7, 8, 9, 10, 13, 15, 18, 19, and 20) shown in Fig. 1

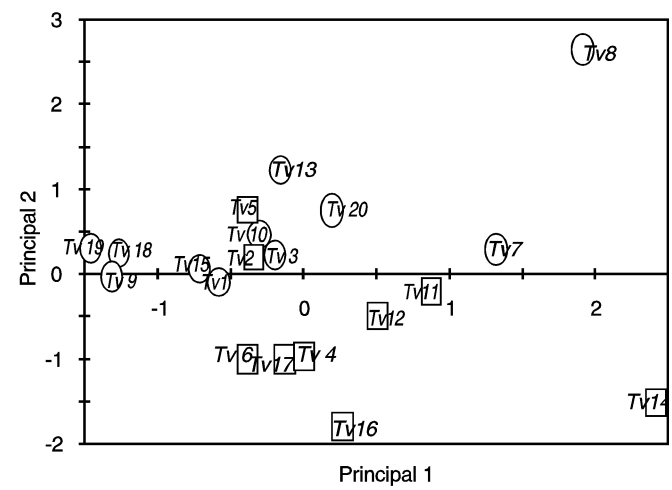

Fig. 6 Principal component analysis (Principal 1 × Principal 2) of distance matrices of RAPD polymorphism of 20 tansy genotypes. The *quadrangles* indicate the genotypes of group I (Figs. 1 and 5) and the *circles* indicate the genotypes of group II (Figs. 1 and 5)

a few tansy genotypes from other genotypes. The mean number of flower heads per stem was positively correlated with the height of the stem and corymb. Because the essential oils of tansy are usually concentrated in the flowers (Dobos et al. 1992), the selection of abundantly flowering genotypes might be an important step towards increasing the yield of essential oils. *Senecio*

interifolius is an other species of Asteraceae that also shows a positive correlation between stem height and number of florets per stem (Widén 1991). Plants of *S. interfolius* with large inflorescences start to flower earlier and have a longer flowering period than plants with smaller inflorescences (Widén 1991). Unfortunately, we could not observe the duration of flowering in our tansy genotypes because the flower heads were collected for distillation of essential oils. Tansy genotypes from southern and eastern Finland (group I) started to flower later, and had more flower heads and nodes per stem and longer corymbs than the genotypes native to the western and central parts of Finland (group II). The division of tansy genotypes into these two groups was significant according to the morphological observations subjected to multivariate analysis. Our data are consistent with those of Lokki et al. (1973) who observed that the tansy genotypes native to northern Finland started to flower earlier than the genotypes native to southern Finland. Flowering at early stage is one important character for cultured species in the northern countries. To improve this character, plant breeders should select early-flowering genotypes for breeding programs.

The second objective of our study was to analyze genetic polymorphism of tansy using the analysis of RAPDs and nuclear DNA content. The analysis of RAPDs showed that all 20 tansy genotypes examined were distinguishable. The reason for the detected, relatively high polymorphism among this species is not clear, but it may be at least partially due to the high frequency of cross pollination among many (Lokki et al. 1973) but not all (Holopainen et al. 1987) tansy geno- and chemotypes. Previous authors have observed that only 4% of the flowers produced seeds after self pollination (Lokki et al. 1973) and that the expansion of tansy occurs predominately through the dispersal of cross-pollinated seeds (Prach 1988; Lokki et al. 1973). However, cross pollination between certain tansy chemotypes can result in poor seed production, which may restrict gene flow and contribute to the different frequencies in which different tansy chemotypes occur in the nature (Holopainen et al. 1987). Other species related to *Tanacetum*, such as chrysanthemum (*Dendranthema grandiflora* Tzvelev or *Chrysanthemum morifolium* Ramat.), are also very polymorphic, probably because strong self-incompatibility mechanisms prevent gene flow between closely related genotypes (Wolf et al. 1995). The high RAPD polymorphism detected in *D. grandiflora* contrasts with the low RAPD polymorphism found among wild sunflower (*Helianthus annuus*) that can produce fertile siblings by intraspecific hybridization (Arias and Rieseberg 1995). Also, *Chrysanthemum coronarium* and *C. segetum* are genetically highly uniform (Francisco-Ortega et al. 1995). It seems that wild, cross-pollinated species without limited crossing boundaries may produce siblings that are genetically less polymorphic than those produced by species in which some restrictions in the gene flow exist.

Tansy genotypes differed in their nuclear DNA contents (2C values) by up to 27%. One explanation for this variation could be the possible occurrence of cytomictic disturbances during the cell division (Virrankoski and Sorsa 1968). Other species in the Asteraceae family have also shown intraspecific variation in their nuclear DNA contents. For example a variation of 32% has been reported among different sunflower lines (Michaelson et al. 1991b). The mean 2C value of the 20 tansy genotypes was 8.86 pg, which is higher than the 2C values reported for other species of Asteraceae (Arumuganathan and Earle 1991a; Michaelson et al. 1991a, b).

The third objective of this study was to test the possible correlation between the morphological and genetical variation examined. Morphological data and RAPD data subjected to complete linkage cluster and multivariate analysis grouped the tansy genotypes into two main groups. As the genotypes were arranged in the two groups according to the distance analysis of RAPDs and analyzed for principal components, these results also supported the division to the two groups. Thus, both the morphological and RAPD data could be used to group the 20 tansy genotypes to two similar main groups, and as explained above, this grouping was related to the geographical origin of the tansy genotypes. Vellekoop et al. (1996) observed that in *Silene latifolia* too, the morphological traits and RAPD patterns show a correlation with the geographical origin of the genotypes. Group I of this study included the 7 tansy genotypes from southern Finland that originated south of latitude 60°3′N, which coincides with the mean annual + 4°C isotherm (1961–1990) (Finnish Meteorological Institute 1997). Interestingly, the tansy genotypes *Tv* 2 and *Tv* 4 from the lake district of eastern Finland also belonged to group I. Subsequently, most of the genotypes of group II originated north of latitude 60°3′N. These results may be best explained by the different climatic conditions, such as the temperatures, south and north from the latitude 60°3′N, and the adaptation of the tansy genotype to the respective growth conditions. *S. latifolia* has been genetically and chemically grouped to western and eastern races, and this grouping coincided with the January 0°C isotherm (Vellekoop et al. 1996). Also in tansy, differences in secondary metabolites such as monoterpenes have been reported to be correlated with geographic origin. For example, genotypes containing camphor are most abundant in northern Finland, whereas genotypes containing thujone are most frequent in southern Finland (Sorsa et al. 1968). The native areas of tansy may be southern coast of Finland where it grows abundantly (Hultén 1950; Linola and Väänänen 1940). In contrast, the other parts of Finland have probably been occupied by tansy associated to human inhabitation and agriculture, where it is still less

common (Suominen and Hämet-Ahti 1993; Linkola and Väänänen 1940).

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