Bulgarian Journal of Agricultural Science, 19 (No 1) 2013, 143-151
Agricultural Academy

PHENOTYPIC VARIATION AND GENETIC DIVERSITY OF *CALENDULA OFFICINALIS* (L.)

A.-D. BACIU, D. PAMFIL, L. MIHALTE*, A. F. SESTRAS and R. E. SESTRAS
University of Agricultural Sciences and Veterinary Medicine, Department of Genetics and Plant Breeding, Faculty of Horticulture, 400372 Cluj-Napoca, Romania

Abstract

BACIU, A.-D., D. PAMFIL, L. MIHALTE, A. F. SESTRAS and R. E. SESTRAS, 2013. Phenotypic variation and genetic diversity of *Calendula officinalis* (L.). *Bulg. J. Agric. Sci.,* 19: 143-151

In order to assess the genetic diversity, thirty-four genotypes of *Calendula officinalis* (L.) were evaluated by means of random amplified polymorphic DNA (RAPD) technique. The number of amplification products generated by each primer varied from 9 (OPAB-18) to 14 (OPAL-20) with an average of 11.2 bands per primer. The pairwise Nei and Li's coefficients showed relatively high similarity in *C. officinalis* SLO *vs C. officinalis* L. D. f (0.83), *C. officinalis* L. F. b *vs C. officinalis* 122GE (0.80) and the lowest similarity index was observed in *C. officinalis* L. D. b *vs C. officinalis* cv. Gaicha Gril (0.17). Fifteen morphological traits in a three-year field experiment were also evaluated. Genetic similarities (obtained from RAPD data), phenotypic similarities were used to create a cluster diagram, and the results were compared. According to this, genotypes were framed in four distinct clusters, but all studied accessions seem to appear as a monophyletic group (cluster I including groups II and III and group II also including group IV) in both dendrograms. Of all the studied genotypes twenty varieties were grouped in the same RAPD cluster and were pulled together in the same phenotypic clusters, meaning that this genotypes presented a small genetic distance and similar peculiarities. The present results illustrate the potential of phenotypic variables and RAPD markers to distinguish genetic diversity and phenotypic variation and are most needed for management in gene banks.

Key words: cluster analyses, genetic variation, genotypes, pot marigold

Introduction

The *Calendula* genus includes about 25 species, most common being *Calendula officinalis, C. arvensis, C. alata, C. stellata, C. tripterocarpa, C. suffruticosa* etc. The most current and cultivated species is *C. officinalis* L. (Goncearіuc, 2003) and it is used for setting green spaces, for interiors, as well as cut flowers in various floral arrangements (Selaru, 2007). In addition, pot marigold is used in human medicine, veterinary medicine, nutrition, cosmetics (Barajas-Farias et al., 2006; Pintea et al., 2008).

C. officinalis tolerate most soil conditions and bloom quickly from seed in bright yellow, gold, and orange flowers and present spirally arranged leaves, simple, and slightly hairy (Gilman and Howe, 1999). For examples cultivar Alpha has deep orange flower, cultivar Jane Harmony and Sun Glow has bright yellow flowers, cultivar Pink Surprise has double flower, with inner florets darker than outer florets and Variegate is a cultivar with yellow variegated leaves (Flann, 2011).

The aims of *Calendula* breeding works are focused in four main directions: to obtain genotypes with special decorative value, to obtain large and abundant flowers which will ensure obtaining large amounts of seeds, respectively oils per hectare (Diaconu, 1992), to improve the quality of medicinal products derived from plants (Zitterl-Eglseer et al., 2001), and to obtain

*Corresponding author: mihaltelucica@yahoo.com

cultivars resistant to main diseases and pests (Baciu et al., 2010).

Nowadays, many molecular methods are available for studying genetic diversity, including RFLP (Botstein et al., 1980), RAPD (Smolik et al., 2011; Williams et al., 1990), AFLP (Vos et al., 1995) and SSR (Király et al., 2012; Tautz, 1989). Therefore, molecular tools are a great support in plant breeding, genetic taxonomy, mapping and phylogeny studies (Vos et al., 1995). Of these molecular tools, RAPD (Randomly Amplified Polymorphic DNA) markers have several advantages and have been quite widely employed in genetic research. The technique is simple, rapid and only a small amount of DNA is required and most importantly, no prior knowledge of DNA sequences is required (Hadrys et al., 1992). Moreover, relativeness and distinctiveness of different genotypes can unambiguously be estimated by RAPD fingerprinting (Thomas et al., 2006).

RAPD markers have gained considerable attention particularly in genetic mapping applications, in population genetics (Haig et al., 1994), as well as in taxonomy (Chapco et al., 1992).

The present study aimed to use RAPD markers to evaluate the genetic variation within a collection of *Calendula officinalis* in order to achieve genetic relationships among the studied genotypes. In addition, different traits in several *Calendula* genotypes were considered in order to compare the genetic fingerprints with the phenotypic profiles. The obtained results may be useful in different breeding works for selection, hybridization, biodiversity assessment and conservation of diverse gene pools.

Materials and Methods

Plant materials and morphological evaluation

In order to achieve the genetic diversity of *Calendula officinalis* 34 genotypes were studied, originated from 13 countries (Table 1).

Seeds were obtained from different botanical gardens, research institutes, or universities. These genotypes were grown in cropping season of 2010 at botanical garden of the University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania (46°76' N, 23°57' E).

The plants were evaluated for different characters that describes the phenotypic aspects, such as: plant height (PlH-cm), steam diameter at 10 cm from soil (SD-cm), number of main branches (NMB), insertion angle of branches (IAB-degree), number of leaves on main branches (NLMB), leaf length (LL-cm), leaf width (LW-cm), number of buds/plant (NBP), number of flowers (NFl), number of fruits (NFr), diameter of flower (DFl), diameter of disc (DD), number of petals (NP), petal length (PL), petal width (PW) (Table 2).

DNA extraction, PCR reaction and electrophoresis

For molecular analyzes, DNA was isolated from young leaves, using a protocol elaborated by Lodhi et al. (1994) modified by Pop et al. (2004). This protocol requires only a few grams of tissue to produce total genomic DNA. RAPD fragments were amplified from genomic DNA in a total reaction volume of 25 μL containing 50 ng of genomic DNA, 2.5 mM 10 × Buffer, 2.5 mM $MgCl_2$, 200 μM of each dNTP, 0.2 μM of decameric primer, and 1 U Taq DNA polymerase (Promega). Each reaction was overlaid with sterile oil. Amplifications were performed in a thermocycler programmed for 45 cycles of 1 min at 94°C, 1 min at 38°C, 30 s at 54°C, 2 min at 72°C, and a final 15 min extension at 72°C. The amplification products were separated on 2% agarose-TAE gels run at 80 V/cm for 1 h. The gels were stained with ethidium bromide (0.5 μg/μl) and photographed under UV light.

Data analysis

The RAPD was performed on all 34 samples with 15 decametre primers (Table 3) and the RAPD bands were scored visually. Their presence was scored with 1 and absence with 0, separately for each genotype and each primer. The total number of binary RAPD product was 496 (presence/absence of the bands; Abdulla and Gamal, 2010). Estimate of genetic similarity (F) was calculated between all pairs of the genotypes according to Nei and Li (1979) based on following formula:

Similarity (F) = 2Nab/(Na + Nb)

Where Na = the total number of fragments detected in individual a; Nb = the total number of fragments shown by individual b and Nab = the number of fragments shared by individuals a and b. The resulting

Table 1
Origin of the analysed *Calendula officinalis* genotypes

No.	*Calendula officinalis* Genotype/Cultivar	Code	Provenance/Origin
1.	cv. Pacific	V1	Czech Republic Masarykova Univerzita Brne, Lékařská faculta 66243 Brno
2.	cv. Bon Bon Mix	V2	Ukraine Hortus Botanicus Fominianus, Kiev
3.	*C. officinalis* L.D.c	V3	Germany Universitat Bayreuth Ökolog-Botanischer Garten D-95440
4.	*C. officinalis* L.D.e	V4	Germany Botanischer Garten, Martin-Luther-Universität D-06108
5.	*C. officinalis* L.F.c	V5	France Jardin Botanique, 44094 Nantes cedex 1
6.	cv. Pacific-Riesen	V6	Germany Universitat Bayreuth Ökolog-Botanischer Garten D-95440
7.	*C. officinalis* L.D.d	V7	Germany Botanischer Garten J.W. Goethe-Universität D-60054
8.	*C. officinalis* AZ	V8	Azerbaijan Republic Central Botanical Garden, Badamdar, AZ 1073
9.	*C. officinalis* SLO	V9	Slovenia Hortus Botanicus Ljubljana
10.	*C. officinalis* L.PL	V10	Poland Lublin, Hortus Farmacognosticus Academiae Medicinalis Ul. W. Chodźki 120-093
11.	*C. officinalis* D.h	V11	Germany Botanischer Garten der Cristian-Alberchts-Universität Kiel
12.	*C. officinalis* L.D.a	V12	Germany (Berlin), Humblod-Universität zu Berlin, Institut für Biologie
13.	cv. Bon-Bon Orange	V13	Latvia Seed Exchange, National Botanic Garten Salasplis, LV-2169
14.	*C. officinalis* L.D.f	V14	Germany (Chemnitz), Botanischer Garten, Grünflächenamt
15.	*C. officinalis* L.B	V15	Belgique (Gembloux), Faculté universitaire des sciences agronomiques
16.	*C. officinalis* L.F.b	V16	France Botaniquest et Zoologiques, Arboretum National de Chevreloup
17.	cv. Pacific Beauty	V17	Ukraine National Botanical Garden, Timirjazevska, 1, Kyiv, 01014
18.	cv. Rozovyi Sjurpriz	V18	Ukraine National Botanical Garden, Timirjazevska, 1, Kyiv, 01014
19.	cv. Zelenoye Serdtse	V19	Ukraine National Botanical Garden, Timirjazevska, 1, Kyiv, 01014
20.	*C. officinalis* A	V20	Austria Botanischer Garten Landesregierung Klagenfurt A-902
21.	cv. Prolifera No.214	V21	Germany (Deutschland) Botanscher Garten der Universität, 3703 Göttingen
22.	123GEHortus Hudae	V22	Denmark Botanic Garden, Universitat of Copenhagen
23.	cv. Fiesta Hitana	V23	Ukraine National Botanical Garden, Timirjazevska, 1, Kyiv, 01014
24.	cv. Plamen	V24	Czech Republic Masarykova Univerzita Brne, Lékařská faculta 66243 Brno
25.	cv. 122GE	V25	Denmark Botanic Garden, Universitat of Copenhagen
26.	*C. officinalis* D.g	V26	Germany, Botanischer Garten der Cristian-Alberchts Univ. Kiel, D-24098
27.	cv. Prolifera No.215	V27	Germany (Deutschland) Botanscher Garten der Universität, 3703 Göttingen
28.	*C. officinalis* I	V28	Italy (Urbino) Instituto e Orto Botanico Universitat di Urbino, 61029
29.	*C. officinalis* UK	V29	Ukraine Hortus Botanicus Fominianus, Kiev
30.	*C. officinalis* L.D.b	V30	Germany Botanischer Garten, Universität Ulm D-89069
31.	*C. officinalis* F.a	V31	France Ville de Rouen, Jardin Botanique 76100 Rouen
32.	cv. Radio	V32	Germany Universitat Bayreuth Ökolog-Botanischer Garten D-95440
33.	cv. Prycosnovjenie	V33	Ukraine National Botanical Garden, Timirjazevska, 1, Kyiv, 01014
34.	cv. Gaicha Gril	V34	Ukraine National Botanical Garden, Timirjazevska, 1, Kyiv, 01014

similarity coefficients were used to evaluate the relationships among genotypes with a cluster analysis using an unweighted pair group method with arithmetic averages.

The program FreeTree (Hampl et al., 2001) was used for the construction of a phylogenetic tree and for the bootstrap analysis (Nei and Li distances; UPGMA method; 400 resample datasets).

Table 2
The average of main phenotypic traits of 34 *Calendula officinalis* genotype

No.	Genotype	PlH	SD	NMB	IAB	NLMB	LL	LW	NBP	NFl	NFr	DFl	DD	NP	PL	PW
1	cv. Pacific	55.91	9.65	8	60.18	19.82	11.21	4.45	24.27	10.82	8.55	4.62	2.04	60.45	2.07	0.45
2	cv. Bon Bon Mix'	41.71	7.32	6.79	49.71	16.29	10.54	3.65	9.93	2.86	1.43	5.73	1.99	97.64	2.21	0.31
3	L.D.c	43.29	8.06	9.18	57.44	23.09	10.65	2.9	23.5	14.53	20.56	3.21	1.2	24.44	1.69	0.29
4	L.D.e	31.72	6.95	7.44	52.28	19.84	11.14	3.3	43.84	26.16	21.16	3.48	0.85	22.88	1.35	0.32
5	L.F.c	51.45	7.28	9.14	48.41	22.76	11.1	2.08	29.76	16.86	20.41	3.68	1.06	33	1.71	0.31
6	cv. Pacific-Riesen	41.43	9.7	8.11	60.17	23.2	10.28	3.51	28.04	10.24	10.93	3.65	1.27	51.35	1.77	0.34
7	L.D.d	71.24	8.92	9.1	57.66	20.03	10.63	3.16	44.03	17.24	9.14	4.89	1.71	51.69	2.03	0.35
8	AZ	44.11	11.02	9.89	62.33	26.22	11.07	4.21	17.11	15.89	15.33	3.64	1.6	29.89	1.73	0.36
9	SLO	42.11	6.71	8.97	50	14.71	10.03	2.8	20.53	16.5	20.29	3.88	1.1	26.89	1.64	0.29
10	L.PL	43.85	8.11	7.4	51.25	19.5	10.96	3.82	21.45	13.85	6.8	5.57	1.19	176.3	2.5	0.32
11	D.h	29	6.67	5.5	51.5	14.83	10.3	2.98	11.83	3.5	2.5	3.4	1.13	25.33	1.57	0.4
12	L.D.a	36.13	6.73	16.38	61.15	21.77	11.35	3.68	15.77	7.43	1.51	3.03	1.01	28.66	1.6	0.54
13	cv. Bon-Bon Orange	52.12	7.76	11.95	57.07	25.34	11.95	3.64	17.71	16.83	11.44	4.3	1.4	41.66	1.97	0.41
14	L.D.f	46.87	5.56	9.52	53.23	20.68	12.27	4.33	40.55	23.52	20.97	3.07	1.11	31.52	1.88	0.27
15	L.B	31.06	6.61	3.89	40.11	13.67	6.8	3.28	8.83	4.06	1.22	3.15	0.93	25.11	1.59	0.47
16	L.F.b	50.54	9.7	10.5	60.17	23.2	10.28	3.51	28.04	16.89	10.93	3.65	1.27	27.89	1.77	0.34
17	cv. Pacific Beauty	40	9.66	7.58	52.58	19	11.43	3.91	14.17	10.75	4.08	4.43	1.49	82.08	2.08	0.38
18	cv. Rozovyi Sjumriz	22	7.46	5.71	39.43	20.71	14.26	4.54	11.29	3.14	1.57	3.46	1.8	107	1.2	0.29
19	cv. Zelenoye Serdtse	31.57	9.73	7.29	59.71	19.71	12.27	4.19	9.57	3	1.57	4.16	1.67	181.86	1.2	0.21
20	A	29.44	6.67	8.69	51.81	24.38	13.36	2.14	21.06	18.19	20.38	3.1	1.04	24.25	1.36	0.27
21	cv. Prolifera 214	59.65	8.41	13.43	57.65	23.81	12.91	3.61	21.95	11	6.49	5.07	1.36	115.51	2.2	0.28
22	123GE	37.6	8.1	9.33	60.33	12.73	11.17	3.91	12.67	7.07	1.53	5.73	1.26	35.4	2.13	0.41
23	cv. Fiesta Hitana	28.79	7.5	6.29	46.14	20.43	13.16	4.79	6.64	3.43	1.5	4.63	1.58	159.57	1.85	0.27
24	cv. Plamen	26	8.99	5.5	66.17	15.67	12.47	4.38	9.42	2.17	2.33	4.16	1.13	126.75	1.23	0.25
25	122GE	33.56	7.05	11.41	50.41	11.62	7.42	3.03	23.74	12.18	5.21	3.29	1.11	30.26	1.35	0.32
26	D.g	47.67	8.86	7.5	51.7	18.83	11.29	3.18	25.57	17.07	10.8	4.06	1.16	31.9	1.71	0.31
27	cv. Prolifera 215	49.88	6.39	5.59	47.5	15.18	10.48	3.01	11.44	5.5	3.21	5.12	1.63	42.85	1.63	0.32
28	I	35.85	8.27	6.85	55.62	16.92	10.95	3.1	16.46	8.69	6.38	3.78	1.27	60.31	1.8	0.42
29	UK	30.64	50.54	4.91	48.27	12.91	9.95	3.54	3.18	2	1.45	2.62	0.86	19.91	0.99	0.22
30	L.D.b	44.95	7.27	10	62.11	20.89	10.08	3.53	14.16	6	3	5.15	1.24	26.47	2.11	0.39
31	F.a	36.19	5.65	10.58	53.44	18.77	10.13	2.92	17.77	16.46	12.54	3.13	1.37	38.17	1.17	0.43
32	cv. Radio	45.89	8.34	7.89	59.84	20.37	13.94	3.57	33.26	16.08	23.08	4.34	1.52	49.29	1.73	0.45
33	cv. Prycosnovjenie	38.88	7.58	10.75	61.38	24.25	11.73	3.76	24	13.75	3.63	3.9	1.58	14	1.7	0.33
34	cv. Gaicha Gril	43.16	8.52	9.19	52.65	23.77	11.68	3.91	18.65	12.61	4.74	4.26	1.93	194	2.82	0.36

PlH (cm)-plant height, SD (cm)-steam diameter from 10 cm of soil, NMB-number of main branches, IAB (degree)-insertion angle of branches, NLMB-number of leaves on main branches, LL (cm)-leaf length, LW (cm)-leaf width, NBP-number of buds/plant, NFl-number of flowers, NFr-number of fruits, DFl-diameter of flower, DD-diameter of disc, NP-number of petals, PL-petal length, PW-petal width.

Table 3
The primers used for RAPD analyses at *Calendula officinalis* genotypes

No of entry	Primer	Nucleotidic sequence (5-3)	Total number of amplified bands	Number of polymorfic bands
1	OPA-18	AGG TGA CCG T	94	10
2	OPC-15	GAC GGA TCA G	83	12
3	OPH-20	GGG AGA CAT C	89	11
4	OPAB-18	CTG GCG TGT C	87	9
5	OPAL-20	AGG AGT CGG A	143	14
6	OPA-01	CAG GCC CTT C	–	
7	OPA-20	GTT GCG ATC C	–	
8	OPB-10	CTG CTG GGA C	–	
9	OPC-10	TGT CTG GGT G	–	
10	OPC-20	ACT TCG CCA C	–	
11	OPA-11	CAA TCG CCG T	–	
12	OPB-4	GGA CTG GAG T	–	
13	OPB-7	GGT GAC GCA G	–	
14	OPC-8	TGG ACC GGT G	–	
15	OPH-10	CCT ACG TCA G	–	
Total			496	56

Note: – means the absence of amplified products.

Clustering of genotypes into similarity groups was performed using the method of UPGMA (un-weighted pair-grouped method with arithmetic average). The data matrix for quantitative variables was standardized according to Corrado et al. (2009) and the analyses were conducted using PAST software (Hammer et al., 2001).

Results and Discussion

DNA amplification and similarity matrix

Out of 15 decametre primers used for amplification, only five primers were amplified (Table 3). In all variants, RAPD primers produced a constant and reproducible banding pattern across all samples. Variation in the ability to produce RAPD fragments depended on the primer and the genotypes. 56 reproducible and scorable amplification products were generated across 34 genotypes (Table 1). The number of amplification products generated by each primer varied from 9 (OPAB-18) to 14 (OPAL-20) with an average of 11.2 bands per primer. Pimers OPAL-20 and OPC-15 gave the highest percentage of polymorphic bands, while the minimum polymorphism was observed using OPAB-18 primer.

Xu et al. (2001) used the RAPD markers (9 primers) in order to discriminate light yellow-flowered and orange-flowered of *Calendula officinalis*. The total obtained number was 89 bands and the average bands of each primer were 10.

RAPD analysis fairly illustrated the genetic differences among the *Calendula officinalis* genotypes, emphasizing the phylogenetic relationship existent among them. There, can be admitted that some genotypes represents distinct genetic entities, easily recognizable at the molecular level.

Based on the proportion of shared RAPD fragments a similarity matrix was used to achieve the relatedness between the studied genotypes (Figure 1). The pairwise Nei and Li's coefficients for the analysed pot marigold genotypes were noted in the present study only for maximum and minimum genetic similarities. Relatively high similarity index was observed in *C. officinalis* SLO *vs C. officinalis* L.D.f (0.83), *C. officinalis* L.F.b *vs C. officinalis* 122GE (0.80), *C. officinalis* cv. Pacific-Riesen *vs C. officinalis* L.D.d (0.79), *C. officinalis* L.D.d *vs C. officinalis* L.D.f (0.77) genotype. The lowest similarity index were observed in *C. officinalis* L.D.b *vs C.*

officinalis cv. Gaicha Gril (0.17) followed by *C. officinalis* L.D.d *vs C. officinalis* AZ (0.20), *C. officinalis* cv. Plamen and 123GEHortus Hudae *vs C. officinalis* cv. Prycosnovjenie (Figure 1).

RAPD cluster

Genetic similarities obtained from RAPD data were used to create a cluster diagram. According to the dendogram (Figure 2) genotypes were framed in four distinct clusters. Cluster I (marked with green in diagram) consisted of the most of the *C. officinalis* genotypes provided from Ukraine National Botanical Garden (cv. Gaicha Gril, cv. Prycosnovjenie, cv. Zelenoye Serdtse, cv. Pacific Beauty) and several from Germany (*C. officinalis* D.h, *C. officinalis* L.D.a, *C. officinalis* L.D.f).

Cluster II (marked with yellow) grouped 11 genotypes indicating relatively less divergence among these as originating from closely related ancestors. Most of the accessions were provide from Germany (Botanischer Garten or Universität Ulm). Genotypes from Ukraine

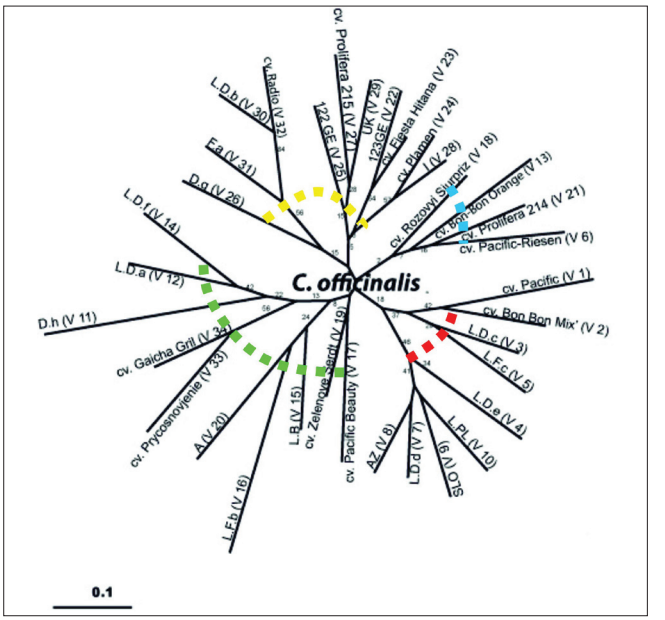

Fig. 2. RAPD cluster diagram of thirty- four genotypes of *Calendula officinalis*

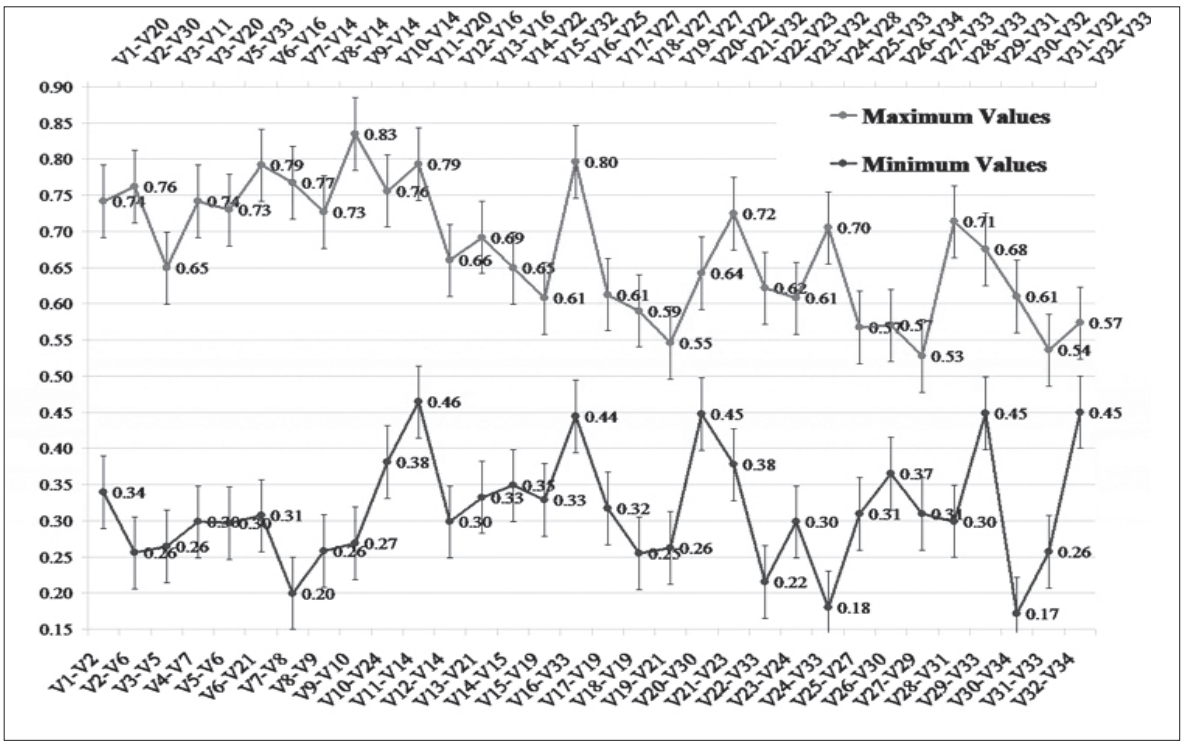

Fig. 1. Minimum and maximum values of similarity matrix for Nei and Li's coefficient for thirty-four *C. officinalis* genotypes based on bands obtained from RAPD markers (Means of V1-V34 were explain in Table 1)

National Botanical Garden (cv. Fiesta Hitana and V29) could be found in the same sub-cluster.

Cluster number III (marked with blue) joined Pacific-Riesen, Bon-Bon Orange, Rozovyi Sjurpriz and Prolifera cultivars and all of this genotypes share a common ancestor. Cultivar Bon Bon Mix and varieties from France, Slovenia, Poland and Azerbaijan Republic were grouped in cluster IV (marked with red in diagram).

Comparing the results of the dendrogram with the peculiarities of plants, some obvious similarities at the molecular level and phenotype were observed, for example 123GE, and cv. Fiesta Hitana have short plants, small number of branches per plant and small number of petals per flower, being located in the same subcluster. Cultivar Gaicha Gril, *C. officinalis* D.h, cv. Prycosnovjenie, cv. Zelenoye Serdtse, cv. Pacific Beauty showed resistance to aphids attack and were classified in diagram in the same cluster.

Soliman et al. (2008) investigated the genetic variability of *C. officinalis* correlated with seed polymorphism. Based on this study seeds morphs of balloon smooth and balloon rough as well as worm are grouped in one accession, while seeds morph curve were grouped separately in cluster.

Comparison of RAPD cluster and phenotypic cluster

Hierarchical phenotypic cluster allowed the assessment of similarity and clarified some of the relationships among *Calendula* genotypes. Cluster analysis of the selected genotypes produced a dendrogram with four groups (Figure 3). Following the dendrogram the first cluster grouped fifteen genotypes of *Calendula*, of 34 analysed, most of it provided from Ukraine and Germany. Regarding the ornamental value, 9 genotypes showed abundant flowers (with 3-5 and more than 5 rows of petals) and 6 genotypes have simple flowers (one-two rows of petals). According to Brânzilă (2007), Selaru (2007), Baciu and Sestras (2009), Baciu et al. (2010) genotypes with abundant flowers are a desired goal in breeding programme, because these varieties are reached in antioxidants (Chakraborthy, 2010).

The genotypes Zelenoye Serdtse (V19), A (V20), Prycosnovjenie (V33), D.h. (V11) and L.D.f (V14)

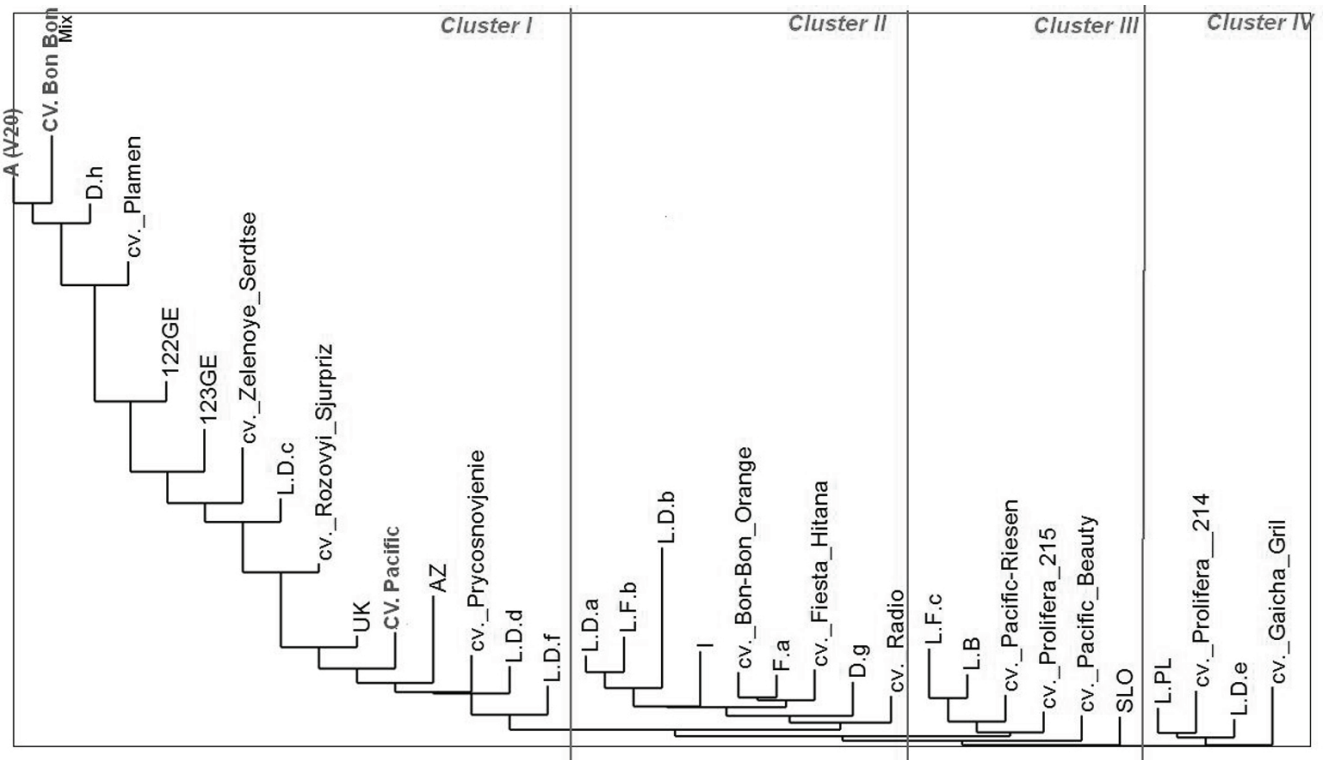

Fig. 3. Phenotypic cluster diagram of thirty-four genotypes of *Calendula officinalis*

marked with green in RAPD cluster can be noticed being grouped in the first cluster of phenotypic dendrogram. That means this genotypes presented same genetic polymorphism and similar close related traits. The genotypes Pacific (V1), Bon Bon Mix (V2), L.D.c (V3), L.D.d (V7) and AZ (V8) marked with red in RAPD cluster, in regard whit the morphological cluster presented close related traits.

The greatest similarity between RAPD and morphological cluster was notice in RAPD yellow marked cluster whit Cluster II. Based on genetic diversity the genotypes D.g (V26), F.a (V31), L.D.b (V30), Radio (V32), 122GE (V25), Prolifera 215 (V27), UK (V29), 123 GE (V22), Fiesta Hitana (V23), Plamen (V24) and I (V28) were grouped in the same RAPD cluster (yellow) and most of them were also pulled together in the phenotypic cluster. That means this varieties of *C. officinalis* presented a small genetic distance and similar peculiarities.

Vegetative development in medicinal species as well as micro climatic conditions is described in the literature as one of the factors that can interfere with the amounts of active principles and can generate polymorphism of DNA patterns (Chengqi, 2007; Nevo et al., 1998).

Most of the study regarding *Calendula officinalis* demonstrated the presence of several classes of chemical compounds as: terpenoids, flavonoids (Kurkin and Sharova, 2007), coumarines, volatile oil (Okoh et al., 2007), carotenoids and amino acids (Abajova et al., 1994), or demonstrated that *C. officinalis* has a broad range of biological effects (Della et al., 1994; Muley et al., 2009). In addition, the present study is the first report regarding the genetic variation of 34 varieties of *Calendula officinalis*.

Conclusions

The present study revealed genetic and phenotypic variation and relatedness among the 34 pot marigold varieties. The employment of RAPD markers in genetic diversity analysis facilitate grouping the genotypes, and all studied accessions seem to appear as a monophyletic group (cluster I included groups II and III and group II also included group IV).

The obtained results and information can be useful for new breeding works on *Calendula* and data on genetic diversity are most needed for management in gene banks. In breeding works several infertile problems can occur when using genitors phylogenetically closed (eg. cv. Pacific Reisen and cv. Prolifera) which appear strongly related. Maximum genetic diversity of hybrids descent will be made when genitors will belong to groups or subgroups, which are quite different and less related. Of all the studied genotypes of *Calendula officinalis* L., great decorative value presented cv. Prycosnovjenie, due to its large number of petals and due to the intense colour of ligulae flowers.

Acknowledgments

This study was financed by the POSDRU/89/1.5/S/52432 project („Organizing the National Interest Postdoctoral School of Applied Biotechnologies with Impact on Romanian Bioeconomy", Romania).

References

Abajova, R. L., S. M. Aslanov and M. E. Mamedova, 1994. Amino acids of *Calendula officinalis*. *Chem. Nat. Comp.*, **30** (15): 641-641.

Abdulla, M. and O. Gamal, 2010. Investigation on molecular phylogeny of some date palm (*Phoenix dactylifra* L.) cultivars by protein, RAPD and ISSR markers in Saudi Arabia. *Aust. J. Crop Sci.*, **4** (1): 16-22.

Baciu, A. D. and R. Sestras, 2009. Variability of seeds and decorative traits and the correlations among these at different *Calendula* genotypes. *Buletinul Lucrări Ştiinţifice Iaşi, Seria Horticultură*, **52:** 1454-7376.

Baciu, A. D., L. Mihalte, A. F. Sestras and R. E. Sestras, 2010. Variability of decorative traits, response to the *Aphis fabae* attack and RAPD diversity in different genotypes of *Calendula*. *Not Bot Horti Agrobo*, **38** (3): 265-270.

Barajas-Farias, L. M., J. I. Perez-Carreon, E. Arce-Popoca, S. Fattel-Fazenda, L. Aleman-Lazarini, S. Hernandez-Garcia, M. Salcido-Neyoy, F. G. Cruz-Jimenez, J. Camacho and S. Villa-Trevino, 2006. A dual opposite effect of *Calendula officinalis* flower extract: chemoprotector and promotor in a rat hepatocarcinogenesis model. *Planta Med.*, **72:** 217-212.

Botstein, D., R. L. White, M. Skolnick and R. W. Davis, 1980. Construction of genetic linkage map in man using restriction length polymorphisms. *Am. J. Hum. Genet.*, **32:** 314-331.

Brânzilă, I., 2007. Germination capacity of pot-marigold seeds (*Calendula officinalis* L.). *Mediul Ambient*, **1** (31): 1-2 (Ro).

Chakraborthy, G. S., 2010. Phytochemical screening of

Calendula officinalis Linn leaf extract by TLC. *Internat J Res in Ayurveda & Pharmacy*, **1** (1): 131-134.

Chapco, W., N. W. Ashton, R. K. Martel, N. Antonishishyn and W. L. Crosby, 1992. A feasibility study of the use of random amplified polymorphic DNA in the population genetics and systemic of grasshoppers. *Genome*, **35:** 569-574.

Chengqi, A., 2007. Comparative anatomy of bisexual and female florets, embryology in *Calendula officinalis* (*Asteraceae*), a naturalized horticultural plant. *Sci. Hortic.*, **114:** 214-219.

Corrado, G., M. La Mura, O. Ambrosino, G. Pugliano, P. Varricchio and R. Rao, 2009. Relationships of Campanian olive cultivars: comparative analysis of molecular and phenotypic data. *Genome*, **52:** 692-700.

Della, L. R., A. Tubaro, S. Sosa, H. Becker, S. Saar and O. Isaac, 1994. The role of triterpenoids in topical anti-inflammatory activity of *Calendula officinalis* flowers. *Planta Med.*, **60:** 516-520.

Diaconu, P., 1992. Using induced variability in *Calendula officinalis* breeding. *Agronomie: Lucrari stiintifice*, **34:** 17-21 (Ro).

Flann, C., 2011. Global Compositae Checklist. Available at: http://compositae.landcareresearch.co.nz/.

Gilman, E. F. and T. Howe, 1999. *Calendula officinalis*. Cooperative Extension Service Institue of Food and Agricultural Science, University of Florida.

Goncearіuc, M., 2003. Contributions to *Calendula officinalis* L. Breeding. *Bul Acad Stiinte a Mold, Stiinte Biol, Chim si Agric*, **2:** 101-103 (Ro).

Hadrys, H., M. Balick and B. Schierwater, 1992. Application of random amplified polymorphic DNA (RAPD) in molecular ecology. *Mol. Ecol.*, **1:** 55-63.

Haig, S. M., J. M. Rhymer and D. G. Heckel, 1994. Population differentiation in randomly amplified polymorphic DNA of red-cockaded woodpeckers *Picoides borealis*. *Mol. Ecol.*, **3:** 581-595.

Hammer, O., D. A. T. Harper and P. D. Ryan, 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica*, **4** (1) art. **4:** 9.

Hampl, V., A. Pavlicek and J. Flegr, 2001. Construction and bootstrap analysis of DNA fingerprinting-based phylogenetic trees with the freeware program FreeTree: application to *Trichomonad* parasites. *Inter. J. Syst. Evol. Microbiol.*, **51:** 731-735.

Király, I., R. Redeczki, É. Erdélyi and M. Tóth, 2012. Morphological and molecular (SSR) analysis of old apple cultivars. *Not. Bot. Horti. Agrobo.*, **40** (1): 269-275.

Kurkin, V. A. and O. V. Sharova, 2007. Flavonoids from *Calendula officinalis* flowers. *Khim. Pri. Soed.*, **2:** 179-180.

Lodhi, M. A., G. N. Ye, N. F. Weeden, B. I. Reisch, 1994. A simple and efficient method DNA extraction from grapevine caltivars and *Vitis* species. *Plant. Mol. Biol. Rep.*, **12:** 6-13.

Muley, B. P., S. S. Khadabadi and N. B. Banarase, 2009. Phytochemical Constituents and Pharmacological Activities of *Calendula officinalis* Linn (Asteraceae): A Review. *Trop. J. Pharmac. Res.*, **8** (5): 455-465.

Nevo, E, B. Baum, A. Beiles and D. N. Johnson, 1998. Ecological correlates of RAPD DNA diversity of wild barley, *Hordeum spontaneum*, in the fertile crescent. *Genet. Resour. Crop Evol.*, **45:** 151-159.

Okoh, O. O., A. A. Sadimenko and A. J. Afolayan, 2007. The effects of age on the yield and composition of the essential oils of *Calendula officinalis*. *J. Appl. Sci.*, **7** (23): 3806-3810.

Pintea, A., F. V. Dulf, C. Bele and S. Andrei, 2008. Fatty acids distribution in the lipid fractions of *Calendula officinalis* L. seeds oil. *Chemicke Listy*, **102** (15): 749-750.

Pop, R., M. Ardelean, P. Raica, I. M. Gaboreanu and D. Pamfil, 2004. Assessing the genetic polymorphism at *Vitis*, ICDVV, Valea Calugareasca, using RAPD markers. *Nat Proc ICDVV*.

Selaru, E., 2007. Ornamentals. Ed. Ceres, Bucuresti (Ro).

Smolik, M., K. Malinowska, B. Smolik and K. Pacewicz, 2011. Polymorphism in random amplified nuclear rDNA sequences assessed in certain apple (*Malus* × *domestica* Borkh.) cultivars. *Not. Bot. Horti. Agrobo.*, **39** (2): 264-270.

Soliman, M. I., R. M. H. Rizk and R. M. A. Rizk, 2008. The impact of seed polymorphism of plant genetic resources on the collection strategy of gene banks. *Global J. Biotechnol. Biochem.*, **3** (1): 47-55.

Tautz, D., 1989. Hypervariablity of simple sequences as a general source of polymorphic DNA markers. *Nucleic Acids Res.*, **17:** 6463-6471.

Thomas, G., T. Mohapatra, A. R. Rao and R. P. Sharma, 2006. Distinguishing Indian commercial wheat varieties using RAPD based DNA fingerprints. *Ind. J. Biotechnol.,* **5:** 200-206.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van de Lee, M. Hornes, A. Frifiters, J. Pot, J. Peleman, M. Kuiper and M. Xabeau, 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Res*, **23:** 4407-4414.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski and V. T. Scott, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.*, **18:** 6531-6535.

Xu, Y., J. Li and J. Ge, 2001. RAPD Analysis in the different color-flowered *Calendula officinalis. J. Shanghai Teach Univ.*, **13** (2): 200-234.

Zitterl-Eglseer, K., G. Reznicek, J. Jurenitsch, J. Novak, W. Zitterl and C. Franz, 2001. Morphogenetic variability of faradiol monoesters in marigold *Calendula officinalis* L. *Phytochem Anal.*, **12** (3): 199-201.

Received June, 2, 2012; accepted for printing January, 12, 2013.