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Variability of Decorative Traits, Response to the *Aphis fabae* Attack and RAPD Diversity in Different Genotypes of *Calendula*

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Abstract

In order to identify cultivars with special decorative value and potential genitors for breeding process, 45 genotypes of *Calendula* genera were analyzed, belonging to six species: *C. officinalis*, *C. alata, C. arvensis, C. stellata, C. suffruticosa* and *C. tripterocarpa*. The average height of plants varied strongly, from 22.0 cm (*C. officinalis* cv. 'Rozovyi Sjurpriz') cm to 84.1 cm (*C. tripterocarpa* Rupr.). The lowest number of branches per plant was recorded on *C. arvensis* F. (4.6) and the largest one on *C. officinalis* LDA (16.4). The average number of flowers per plant ranged from 98.0 (*C. suffruticosa* Valh.) to 2.0 (*C. officinalis* UK). From among all genotypes, aphids (*Aphis fabae*) have attacked 19 (42.2%), and the results showed that AD% (Attack Degree) depend significantly on genotypes. RAPD analysis and phylogenetic dendrogram illustrated the relationship between genotypes and DNA polymorphism exists between the six species. Were found not only close phylogenetic links among cultivars apart of the same species, but also between different species. *C. officinalis* A., *C. alata* UK and *C. suffruticosa* formed a subgroup similar to the molecular level, but also confirming some phenotypic similarities, these species having the smallest number of petals in the corolla and the highest sensitivity to *Aphis fabae* attack. The large variability identified in *Calendula* genotypes allows the selection of potential genitors for new breeding works, with appropriate decorative characteristics and resistance to aphids attack. RAPD analyses and phenotypic study allows hypothesis regarding the success of intra- and inter-specific hybridization, thus facilitating *Calendula* breeding processes.

Keywords: *Calendula*, species, cultivar, traits, *Aphis fabae*, RAPD analysis

Introduction

The *Calendula* genera apart to the *Asteraceae* family and includes about 25 species, most common being: *Calendula officinalis, C. arvensis, C. alata, C. stellata, C. tripterocarpa, C. suffruticosa* etc. In general, the most common and cultivated species is *C. officinalis* L., while *C. arvensis* L. is less common in culture (Goncearuic, 2003; Coiciu si Racz, 1962).

Calendula officinalis L. species (pot marigold) has an extremely wide range of use, due to its properties, its well adapted to temperate climatic zones and easy to grow (Froment *et al*., 2009). It is used for setting green spaces, for interiors, as well as cut flowers in various floral arrangements (Selaru, 2007). The seeds contain fatty mixed acids (Froment *et al*., 2009; Angelini *et al*, 1997). Due to its rich content of active principles (acids, oils, pigments, flavones), *Calendula officinalis* L. is used in human medicine, veterinary medicine, nutrition, cosmetics (Racz *et al*., 1976; Dobrescu, 1981; Pintea *et al*., 2008; Barajas-Farias *et al*., 2006; Jimenez-Medina *et al*., 2006; Ukiya *et al*., 2006; European Medicines Agency - Evaluation of Medicines for Human Use, 2008).

The aim of *Calendula* breeding is to obtain genotypes with special decorative value, anthodia with large and abundant flowers, intensely coloured (yellow, orange or yellow-orange), with compact habitus, resistant to main diseases and pests, with a higher content of useful substances (Goncearuic, 2001; Baciu and Sestras, 2009).

Genotypes with abundant flowers, intensely colored, compact habitus, higher plants, are particularly suitable for decoration (Goncearuic, 2001; Baciu and Sestras, 2009). In addition, abundant flowering cultivars ensure obtaining large amounts of seeds, respectively oils per hectare (Diaconu, 1992). High seed oil content and eliminating wild characteristics (fruit dehiscence) are relatively new targets for *Calendula* breeding purposes. After Zitterl-Eglseer *et al*. (2001), the breeding work must be focused to ray florests cultivars, to improve the quality of medicinal products derived from plants.

Obtaining genotypes resistant to *Aphis fabae* attack is an important breeding goal, due directly and indirectly damaging of aphids, these pest being the main vectors of virus plants (Dixon, 1998; Oltean *et al*., 2004).

Materials and methods

The ornamental traits and the response of *Calendula* to aphids attack were analyzed in Botanical Garden of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, during 2007-2009. There were studied 45 genotypes of the *Calendula* genera, originated from 13

countries (Tab. 1). There were studied the main decorative traits of the plant: height (cm), number of branches, number of flowers, and number of petals in the corolla. Decorative plant capacity was estimated by marks, with notes from '1' (less decorative) to '10' (very decorative). Evaluation of the attack was carried out under natural infestation conditions, without using insecticide treatments to combat pests, being determined frequency of attack (F%), intensity of attack (I%) and attack degree (AD%) for each studied genotype (Baciu *et al.*, 2009). The number of plants per analysed genotype varied between 19 (variant 16) and 42 (variant 22).

Tab. 1. Origin of the analyzed *Calendula* genotypes

No.	Specie	Genotype/Cultivar	Code	Provenance/Origin
1	*C. officinalis*	'122GE2822-0002'	V35	Denmark Botanic Garden, Universitat of Copenhagen
2	*C. officinalis*	'123GEHortus Hudae'	V32	Denmark Botanic Garden, Universitat of Copenhagen
3	*C. arvensis*	'121GE2822-0001'	V8	Denmark Botanic Garden, Universitat of Copenhagen
4	*C. stellata* Cav.	'124GE2822-04'	V13	Denmark Botanic Garden, Universitat of Copenhagen
5	*C. officinalis*	*C. officinalis* L.D.a	V15	Germany (Berlin), Humblod-Universität zu Berlin, Institut für Biologie
6	*C. officinalis*	*C. officinalis* L.B	V18	Belgique (Gembloux), Faculté universitaire des sciences agronomiques
7	*C. officinalis*	*C. officinalis* F.a	V41	France Ville de Rouen, Jardin Botanique 76100 Rouen
8	*C. arvensis*	*C. arvensis* F	V22	France Ville de Rouen, Jardin Botanique 76100 Rouen
9	*C. officinalis*	*C. officinalis* SLO	V11	Slovenia Hortus Botanicus Ljubljana
10	*C. arvensis*	*C. arvensis* SLO	V30	Slovenia Hortus Botanicus Ljubljana
11	*C. officinalis*	cv. 'Prolifera Nr.215'	V37	Germany (Deutschland) Botanscher Garten der Universität, 3703 Göttingen
12	*C. officinalis*	cv. 'Prolifera Nr.214'	V27	Germany (Deutschland) Botanscher Garten der Universität, 3703 Göttingen
13	*C. officinalis*	'Bon-Bon Orange'	V16	Latvia Seed Exchange, National Botanic Garten Salasplis, LV-2169
14	*C. officinalis*	'Bon Bon Mix'	V2	Ukraine Hortus Botanicus Fominianus, Kiev
15	*C. officinalis*	*C. officinalis* UK	V39	Ukraine Hortus Botanicus Fominianus, Kiev
16	*C. officinalis*	*C. officinalis* L.D.b	V40	Germany Botanischer Garten, Universität Ulm D-89069
17	*C. officinalis*	*C. officinalis* L.F.b	V19	France Botaniquest et Zoologiques, Arboretum National de Chevreloup
18	*C. officinalis*	*C. officinalis* L.D.c	V3	Germany Universitat Bayreuth Ökolog-Botanischer Garten D-95440
19	*C. officinalis*	cv. 'Pacific-Riesen'	V6	Germany Universitat Bayreuth Ökolog-Botanischer Garten D-95440
20	*C. officinalis*	cv. 'Radio'	V42	Germany Universitat Bayreuth Ökolog-Botanischer Garten D-95440
21	*C. alata*	cv. 'Rech.f.	V31	Germany Universitat Bayreuth Ökolog-Botanischer Garten D-95440
22	*C. arvensis* L.	*C. arvensis* L.	V20	Germany Universitat Bayreuth Ökolog-Botanischer Garten D-95440
23	*C. stellata*	*C. stellata* Cav.	V29	Germany Universitat Bayreuth Ökolog-Botanischer Garten D-95440
24	*C. suffruticosa*	*C. suffruticosa* Vahl.	V28	Germany Universitat Bayreuth Ökolog-Botanischer Garten D-95440
25	*C. tripterocarpa*	*C. tripterocarpa* Rupr.	V7	Germany Universitat Bayreuth Ökolog-Botanischer Garten D-95440
26	*C. officinalis*	*C. officinalis* L.F.c	V5	France Jardin Botanique, 44094 Nantes cedex 1
27	*C. officinalis*	*C. officinalis* L.D.d	V9	Germany Botanischer Garten J.W. Goethe-Universität D-60054
28	*C. officinalis*	*C. officinalis* L.D.e	V4	Germany Botanischer Garten, Martin-Luther-Universität D-06108
29	*C. officinalis*	*C. officinalis* L.PL	V12	Poland Lublin, Hortus Farmacognosticus Academiae Medicinalis Ul. W. Chodźki 1 20-093
30	*C. officinalis*	*C. officinalis* L.D.f	V17	Germany (Chemnitz), Botanischer Garten, Grünflächenamt
31	*C. officinalis*	*C. officinalis* D.g	V36	Germany, Botanischer Garten der Cristian-Alberchts Universität Kiel, D-24098
32	*C. officinalis*	*C. officinalis* I	V38	Italy (Urbino) Instituto e Orto Botanico Universitat di Urbino, 61029
33	*C. officinalis*	*C. officinalis* D.h	V14	Germany Botanischer Garten der Cristian-Alberchts-Universität Kiel
34	*C. officinalis*	cv. 'Prycosnovjenie'	V43	Ukraine National Botanical Garden, Timirjazevska, 1, Kyiv, 01014
35	*C. officinalis*	cv. 'Pacific Beauty'	V23	Ukraine National Botanical Garden, Timirjazevska, 1, Kyiv, 01014
36	*C. officinalis*	cv. 'Gaicha Gril'	V44	Ukraine National Botanical Garden, Timirjazevska, 1, Kyiv, 01014
37	*C. officinalis*	cv. 'Fiesta Hitana'	V33	Ukraine National Botanical Garden, Timirjazevska, 1, Kyiv, 01014
38	*C. officinalis*	cv. 'Zelenoye Serdtse'	V25	Ukraine National Botanical Garden, Timirjazevska, 1, Kyiv, 01014
39	*C. officinalis*	cv. 'Rozovyi Sjurpriz'	V24	Ukraine National Botanical Garden, Timirjazevska, 1, Kyiv, 01014
40	*C. alata*	*C. alata* UK	V21	Ukraine National Botanical Garden, Timirjazevska, 1, Kyiv, 01014
41	*C. suffruticosa*	*C. suffruticosa*	V45	Ukraine National Botanical Garden, Timirjazevska, 1, Kyiv, 01014
42	*C. officinalis*	*C. officinalis* A	V26	Austria Botanischer Garten Landesregierung Klagenfurt A-902
43	*C. officinalis*	cv. 'Pacific'	V1	Czech Republic Masarykova Univerzita Brne, Lékařská faculta 66243 Brno
44	*C. officinalis*	cv. 'Plamen'	V34	Czech Republic Masarykova Univerzita Brne, Lékařská faculta 66243 Brno
45	*C. officinalis*	*C. officinalis* AZ	V10	Azerbaijan Republic Central Botanical Garden, Badamdar, AZ 1073

Tab. 2. Mean values and coefficient of variation of considered traits of *Calendula* and the response to the aphids attack and decorative value of marks

Variant (Genotype)	Height of plants		Number of branches per plant		Number of flowers per plant		Number of petals per flower		Response to aphids (*Aphis fabae*) ttack			Mark for decorative value
	(cm)	CV%	No.	CV%	No.	CV%	No.	CV%	(F%)	(I%)	(G.A.%)	
'122GE2822-0002'	33.6^{ooo}	28.5	11.4^{xxx}	23.0	12.2^{ooo}	51.6	30.3^{ooo}	13.4	2.9^{-}	30.0^{xxx}	0.9	3
'123GEHortus Hudae'	37.6^{oo}	19.0	9.3^{-}	20.5	7.1^{ooo}	45.2	35.4^{ooo}	32.7	26.6^{xxx}	21.2^{xx}	5.6^{x}	4
'121GE2822-0001'	55.0^{xxx}	16.5	10.4^{xxx}	22.9	20.6^{-}	52.2	20.4^{ooo}	18.4	17.1^{xx}	34.2^{xx}	5.8$^{x(x)}$	1
'124GE2822-04'	39.2^{oo}	28.5	4.8^{ooo}	17.8	11.7^{ooo}	27.7	21.6^{ooo}	17.4	14.8^{x}	25.5^{xxx}	3.8^{-}	2
C. officinalis L.D.a	36.1^{ooo}	31.4	16.4^{xxx}	41.0	7.4^{ooo}	66.7	28.7^{ooo}	43.7	No attack	No attack	No attack	6
C. officinalis L.B	31.1^{ooo}	48.0	3.9^{ooo}	69.9	4.1^{ooo}	106.1	25.1^{ooo}	66.4	No attack	No attack	No attack	8
C. officinalis F.a	36.2^{ooo}	28.8	10.6^{xxx}	35.5	16.5^{-}	51.0	38.2^{ooo}	8.0	No attack	No attack	No attack	6
C. arvensis F	44.4^{-}	17.0	4.6^{ooo}	19.0	32.2^{xxx}	27.5	20.1^{ooo}	13.9	No attack	No attack	No attack	1
C. officinalis SLO	42.1^{o}	18.9	9.0^{-}	20.8	16.5^{ooo}	23.1	26.9^{ooo}	7.6	7.9^{-}	31.7^{xxx}	2.4	7
C. arvensis SLO	67.7^{xxx}	13.8	7.4^{ooo}	19.8	19.8^{-}	28.7	15.9^{ooo}	7.7	12.8^{-}	25.0^{xxx}	3.2	1
cv. 'Prolifera Nr.215'	49.9^{-}	34.2	5.6^{ooo}	22.5	5.5^{ooo}	51.9	42.5$^{o(o)}$	42.4	14.7^{x}	21.0^{xx}	3.1	5
cv. 'Prolifera Nr.214'	59.6^{xxx}	35.2	13.4^{xxx}	33.8	11.0^{ooo}	53.9	115.5^{xxx}	60.5	No attack	No attack	No attack	5
'Bon-Bon Orange'	52.1^{x}	30.3	11.9^{xxx}	36.6	16.8^{ooo}	36.5	41.7^{ooo}	25.8	No attack	No attack	No attack	8
'Bon Bon Mix'	41.7	38.2	6.8^{-}	57.0	2.9^{ooo}	71.1	97.6^{xx}	54.4	No attack	No attack	No attack	10
C. officinalis UK	30.6^{ooo}	40.5	4.9^{xxx}	56.5	2.0^{ooo}	86.6	19.9^{ooo}	79.8	No attack	No attack	No attack	8
C. officinalis L.D.b	44.9^{-}	37.7	10.0^{-}	47.8	6.0^{ooo}	78.6	26.5^{ooo}	45.8	No attack	No attack	No attack	5
C. officinalis L.F.b	50.5^{-}	34.2	10.5^{xx}	40.5	16.9$^{o(o)}$	63.5	27.9^{ooo}	32.6	No attack	No attack	No attack	5
C. officinalis L.D.c	43.3^{-}	48.5	9.2^{-}	60.7	14.5$^{o(o)}$	77.0	24.4^{ooo}	52.0	14.7^{x}	25.0^{xxx}	3.7	6
cv. 'Pacific-Riesen'	41.4^{-}	36.0	8.1^{-}	38.9	10.2^{ooo}	91.2	51.3^{-}	105.5	10.8^{-}	13.7^{-}	1.5	8
cv. 'Radio'	45.9^{-}	31.0	7.9^{-}	41.9	16.1^{oo}	64.0	49.3^{-}	31.6	No attack	No attack	No attack	6
cv. 'Rech.f.'	61.2^{xxx}	29.6	9.4^{-}	34.4	32.1^{xxx}	48.1	25.6^{ooo}	4.9	7.5^{-}	30.0^{xxx}	2.2	1
C. arvensis L.	58.1^{xxx}	32.6	8.8^{-}	36.6	73.1^{xxx}	33.4	24.8^{ooo}	6.0	14.3^{-}	26.7^{xxx}	3.8	1
C. stellata Cav.	60.5^{xxx}	27.0	9.2^{-}	33.9	83.0^{xxx}	32.6	25.0^{ooo}	5.3	12.5^{-}	20.0^{xx}	2.5	1
C. suffruticosa Vahl.	65.9^{xxx}	23.0	9.3^{-}	29.4	98.0^{xxx}	26.7	25.1^{ooo}	8.1	No attack	No attack	No attack	1
C. tripterocarpa Rupr.	84.1^{xxx}	12.6	11.5^{xxx}	19.2	96.5^{xxx}	19.0	24.7^{ooo}	6.3	No attack	No attack	No attack	7
C. officinalis L.F.c	51.5^{x}	29.7	9.1^{-}	47.8	16.9^{o}	61.2	33.0^{ooo}	41.4	10.3-	18.3^{x}	1.9^{-}	8
C. officinalis L.D.d	71.2^{xxx}	14.0	9.1^{-}	31.3	17.2^{oo}	33.8	51.7^{-}	18.0	No attack	No attack	No attack	6
C. officinalis L.D.e	31.7^{ooo}	32.0	7.4^{-}	53.6	26.2^{-}	60.2	22.9^{ooo}	46.2	24.0^{xxx}	17.5^{x}	4.2^{-}	7
C. officinalis L.PL	43.8^{-}	41.4	7.4^{-}	55.7	13.8^{ooo}	65.6	176.3^{xxx}	43.8	No attack	No attack	No attack	8
C. officinalis L.D.f	46.8^{-}	35.6	9.5^{-}	49.1	23.5^{-}	65.5	31.5^{ooo}	61.0	22.6^{xxx}	15.0^{-}	3.4	6
C. officinalis D.g	47.7^{-}	38.5	7.5^{-}	54.0	17.1^{o}	52.8	31.9^{ooo}	46.5	No attack	No attack	No attack	3
C. officinalis I	35.8^{oo}	32.0	6.8^{-}	50.5	8.7^{ooo}	64.3	60.3^{-}	33.6	No attack	No attack	No attack	4
C. officinalis D.h	29.0^{oo}	49.5	5.5^{o}	67.8	3.5^{ooo}	109.5	25.3^{ooo}	50.8	No attack	No attack	No attack	4
cv. 'Prycosnovjenie'	38.9^{-}	27.2	10.7^{x}	24.2	13.7^{o}	63.1	14.0^{ooo}	62.6	No attack	No attack	No attack	10
cv. 'Pacific Beauty'	40.0^{-}	46.9	7.6^{-}	56.6	10.7^{ooo}	56.5	82.1^{x}	58.7	No attack	No attack	No attack	7
cv. 'Gaicha Gril'	43.2^{-}	34.7	9.2^{-}	44.3	12.6^{ooo}	52.2	194.0^{xxx}	41.5	No attack	No attack	No attack	10
cv. 'Fiesta Hitana'	28.8^{ooo}	34.5	6.3^{o}	62.8	3.4^{ooo}	107.1	159.6^{xx}	78.7	No attack	No attack	No attack	10
cv. 'Zelenoye Serdtse'	31.6^{o}	53.2	7.3^{-}	48.7	3.0^{ooo}	101.8	181.9$^{(x)}$	97.0	No attack	No attack	No attack	10
cv. 'Rozovyi Sjurpriz'	22.0^{ooo}	61.7	5.7^{-}	83.8	3.1^{ooo}	90.8	107.0$^{(x)}$	68.8	No attack	No attack	No attack	9
C. alata UK	62.4^{x}	17.1	9.8^{-}	19.6	54.2$^{xx(x)}$	40.4	23.2^{ooo}	3.6	20.0^{xx}	15.00^{-}	3.4	1
C. suffruticosa	60.6^{-}	27.3	8.6^{-}	39.9	58.0^{xxx}	36.2	22.8^{ooo}	3.7	40.0^{xxx}	20.00^{xx}	8.00^{xxx}	1
C. officinalis A	29.4^{ooo}	34.8	8.7^{-}	62.9	18.2^{-}	55.5	24.2^{ooo}	50.2	25.0^{xxx}	15.00^{-}	3.7^{-}	4
cv. 'Pacific'	55.9^{x}	24.9	8.0^{-}	31.6	10.8^{ooo}	58.7	60.4^{-}	36.7	No attack	No attack	No attack	5
cv. 'Plamen'	26.0^{ooo}	66.7	5.5^{ooo}	40.7	2.2^{ooo}	103.8	126.7^{xx}	88.6	25.0^{xxx}	16.67$^{(x)}$	4.2	10
C. officinalis AZ	44.1^{-}	34.2	9.9^{-}	49.4	15.9^{-}	69.0	29.9^{ooo}	19.5	No attack	No attack	No attack	3
Averrage (Control)	45.6	32.8	8.5	41.2	21.5	59.2	52.1	38.7	17.0^{-}	22.2	3.5^{-}	5.4

Symbols for differences: x, xx, xxx/o, oo, ooo; significant at P<0.05, 0.01 and 0.001 (positive, respectively negative)

The dates were processed as mean values, and analysis of variance ANOVA was performed, all of the 45 genotypes of *Calendula* being compared with the mean of the experiment, considered as control. Also, there was calculated the coefficient of variation (CV%) of analyzed traits.

For molecular analyzes, DNA was isolated from young leaves, using a protocol elaborated by Lodhi *et al.* (1994). Amplification of RAPD fragments from genomic DNA was carried out in a total reaction volume of 25 µl containing: 2 µl of genomic DNA, 5 µl buffer, 3 µl $MgCl_2$, 0,5 µl of dNTP, 1.6 µl decamer primer, and 0.2 µl *Taq* DNA polymerase. Each reaction was overlaid with sterile water. Amplifications were performed in a programmed thermocycler for 49 cycles of 40 seconds at 90°C (denaturation), 70 seconds at 48°C (fixation), 120 seconds at 72°C (elongation), and 4°C for long term keeping. It was tested 10 decamer primers with all 45 samples.

For agarose gel electrophoresis of DNA, amplification products were separated on 1.5% agarose TAE gels run at 60 V/cm for 2 hours. Gels were coloured with ethidium bromide (6 µl) and photographed under UV light. Dendogram was constructed using a Neighbor Joining Program, named Free Tree and recognized with Tree View.

Results and discussion

The results revealed large differences among analyzed genotypes for ornamental traits of plants, with significant deviations compared with the average of experience, consideration as control variant (Tab. 2).

Based on the obtained data, in the new breeding *Calendula* works it can be recommended as potential genitors: 'Rech.f.', *C. arvensis* L, *C. stellata* Cav. for a large number of flowers per plant, cv. 'Prolifera Nr. 214', *C. officinalis* L.PL, cv. 'Gaicha Gril' for a large number of petals on corolla; '121GE2822-0001', cv. 'Prolifera Nr. 214', cv. 'Rech. f.', *C. officinalis* L.D.d. for higher plant; '122GE2822-0002', *C. officinalis* L.D.a, cv. 'Prolifera Nr. 214', 'Bon-Bon Orange', *C. tripterocarpa* Rupr. for the large number of branches per plant.

A great ornamental value was registered on *C. officinalis* cultivars, six of them being scored with mark '10'. Other plant species were generally small inflorescences with crawler habitus and without decorative value, being valued with note '1'.

From among all genotypes, aphids (*Aphis fabae*) have not attacked 26 (representing 57.8%), but the results showed that aphids occur in the culture of *Calendula* and frequency, intensity and attack degree depends significant of genotypes. Some of these 26 genotypes, represented especially by cultivars presenting valuable ornamental traits, could be used as genitors for obtaining new decorative cultivars resistant to aphids attack (Baciu and Sestras, 2009).

The coefficient of variation (CV%) varied in the experiment, within genotypes and among genotypes, but generally had high values, which illustrated a large variability for principal decorative traits of plants (more than 20%).

Out of 15 decamer primers used for amplification, only five primers were amplified (Tab. 3). In all variants, RAPD primers produced a constant and reproducible banding pattern across all samples (Fig. 1).

Variation in the ability to produce RAPD fragments depended on the primer and the species / genotypes. The species polymorphism exemplified by the OPAB-18 and OPAL-20 primer represent a pattern, which has been reproduced more, or less precise, all the other three primers (OPH 20, OPA 18, OPC 15).

There were obtained 56 polymorphic bands, being performed an average of 11.2 bands per primer. The largest number of bands was generated from primer OPAL 20, which clearly illustrated the polymorphism. OPC 20 and OPH 15 primers showed the most bands in common. Number of bands generated by primer OPAB 18 was lower compared with other primers and in *C. stellata* Cav. '124GE2822-04' and *C. officinalis* D.h generated only a single band.

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

According to the dendogram (Fig. 2), genotypes were framed in four distinct groups. Group "A" included four subgroups, bringing three species (*C. arvensis*, *C. alata*, *C. officinalis*). Group "B" confirmed phenotypic similarity of origins of some *C. officinalis* provenances, respectively close phylogenetic relationship between *C. suffruticosa, C. officinalis* D.h, *C. stellata* Cav. Group "C" gathered five genotypes of *C. officinalis* with *C. suffruticosa* Vahl., and the first subset of "D" has joined two different species (*C. arvensis* si *C. alata*).

Comparing the results of the dendrogram with the peculiarities of plants, some obvious similarities at the molecular level and phenotype were observed, for example '122GE2822-0002', '123GEHortus Hudae' have short plants, small number of branches per plant and small number of petals per flower, being located in the same subgroup of "A" group; cv. 'Gaicha Gril', *C. officinalis* D.g, *C. officinalis* I, *C. officinalis* D.h, cv. 'Prycosnovjenie', cv. 'Zelenoye Serdtse', cv. 'Pacific Beauty' and cv. 'Fiesta Hitana' showed resistance to aphids attack and were classified in group "A" of the dendogram.

The obtained results and informations can be useful for new breeding works on *Calendula* by intra- and interspecific hybridization. Some infertile problems can occur when using genitors phylogenetically closed (*C. officinalis* cv. 'Pacific Beauty' si *C. officinalis* L.PL., 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.

Tab. 3. Primers used for amplification of RAPD analysis

No of entry	Primer	Nucleotidic sequence (5'-3')	Minimum and maximum no of polymorphic RAPD bands/sample
1	OPA-01	CAG GCC CTT C	Without amplification
2	OPA-18	AGG TGA CCG T	1-5
3	OPA-20	GTT GCG ATC C	Without amplification
4	OPB-10	CTG CTG GGA C	Without amplification
5	OPC-10	TGT CTG GGT G	Without amplification
6	OPC-15	GAC GGA TCA G	1-6
7	OPC-20	ACT TCG CCA C	Without amplification
8	OPH-20	GGG AGA CAT C	1-6
9	OPAB-18	CTG GCG TGT C	1-7
10	OPAL-20	AGG AGT CGG A	1-9
11	OPA-11	CAA TCG CCG T	Without amplification
12	OPB-4	GGA CTG GAG T	Without amplification
13	OPB-7	GGT GAC GCA G	Without amplification
14	OPC-8	TGG ACC GGT G	Without amplification
15	OPH-10	CCT ACG TCA G	Without amplification

Conclusions

The large variability identified in *Calendula* genotypes allows the selection of potential genitors for new breeding works, with appropriate decorative characteristics and resistance to aphids attack. Special ornamental forms deserve expanded in culture for decoration.

Due to their superior characteristics, some genotypes can be recommended as genitors in new artificial hybridization: *C. suffruticosa* Vahl., *C. tripterocarpa* Rupr. to obtain cultivars with large number of flowers per plant; *C. officinalis* L.PL and cv. 'Plamen' to the large number of petals; cultivars 'Bon Bon Mix', 'Prycosnovjenie', 'Gaicha Gril'l, 'Fiesta Hitana', 'Zelenoye Serdtse' 'Plamen' for resistance (or at least tolerance) to aphids attack.

RAPD cluster analysis represents an efficient method for determining the genetic proximities of the different species of the genera *Calendula*. Combining the information obtained in RAPD analyses with information from phenotypic study allows valuable hypothesis regarding the success of intra- and interspecific hybridization, thus facilitating *Calendula* breeding processes.

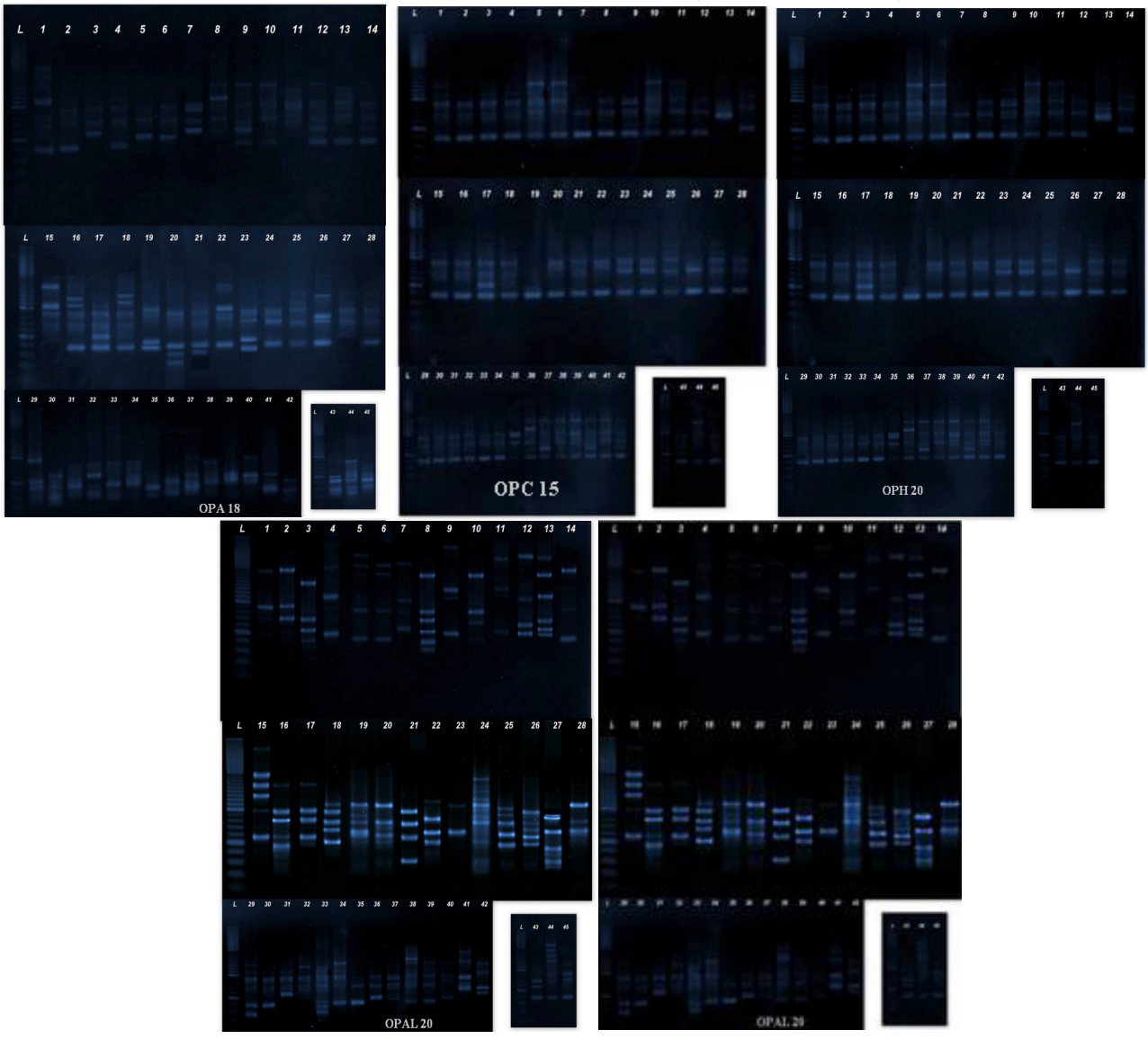

Fig. 1. DNA amplified using RAPDs primers. Lane 1 molecular weigh marker - 1000 Kb

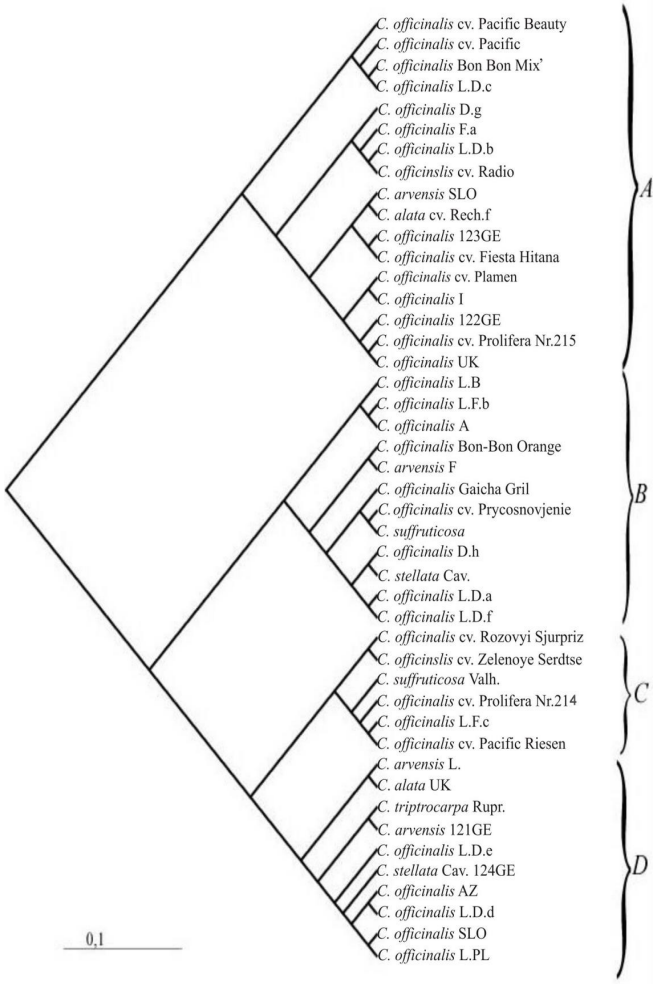

Fig. 1. Dendrogram of 45 genotypes of *Calendula*

References

Angelini, L.G., E. Moscheni, G. Colonna, P. Belloni and E. Bonari (1997). Variation in agronomic characteristics and seed oil composition of new oilseed crops in central Italy, Industrial Crops and Products 6(3-4):313-323.

Baciu, A.D. and R. Sestras (2009). Variability of seeds and decorative traits and the correlations among these at different *Calendula* genotypes. Scientific Researches Iasi, Horticulture Series, Anul II, 52.

Baciu, A.D., A. Sestras, R. Sestras and M. Buruiana (2009). The response of different genotypes of *Calendula* to *Aphis fabae* attack. Bulletin UASVM Horticulture 66(1-2):498-503.

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.

Bashir, S, K.H. Janbaz, Q. Jabeen and A.H. Gilani (2006). Studies on the spasmogenic and spasmolytic activities of *Calendula officinalis* flowers. Phytotherapy Res. 20:906-910.

Coiciu, E. and G. Racz (1962). *Calendula officinalis* L. (*Compositate*). Medicinal and aromatic plants. Academy RPR Ed. (in Romanian).

Diaconu, P., (1992). Using induced variability in *Calendula officinalis* breeding. Agronomy: Scientific researches 34:17-21 (in Romanian).

Dixon, A.F.G. (1998). Aphid ecology an optimization approach., Chapman and Hall. London.

Dobrescu, D. (1981). Pharmacotherapy. Ed. Medical, Bucharest, Romania.

European Medicines Agency, Evaluation of Medicines for Human Use, London, 6 March 2008. http://www.ema.europa.eu/pdfs/human/hmpc/calendula_officinalis_flos/26188207en.pdf

Froment, M., D. Mastebroek and K. van Gorp (2009). A Growers Manual for *Calendula officinalis* L. http://www.mbzerotill.com/files/Calendula%20Growers%20Manual.pdf

Goncearіuc, M., (2001). Variability of morphological anthodia characters of *Calendula officinalis* L. Scientific Annals of Plant Genetic Resources Centre in Moldova, Series B1:65-71 (in Romanian).

Goncearіuc, M. (2003). Contributions to *Calendula officinalis* L. Breeding. Bulletin of Moldova, Academy of Sciences Biological, Chemical and Agricultural2:101-103 (in Romanian).

Jimenez-Medina, E., A. Garcia-Lora, L. Paco, I. Algarra, A. Collado and F. Garrido (2006). A new extract of the plant *Calendula officinalis* produces a dual in vitro effect: cytotoxic anti-tumor activity and lymphocyte activation. BMC Cancer 6:119.

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

Oltean I., M. Porca and I. Ghizdavu (2004). Entomology Overview. Digital Data Ed., Cluj-Napoca (in Romanian).

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.

Selaru, E. (2007). Ornamentals. Ceres Ed., Buchurest (in Romanian).

Ukiya, M., T. Akihisa, K. Yasukawa, H. Tokuda, T. Suzuki and Y. Kimura (2006). Anti-inflammatory, anti-tumor-promoting, and cytotoxic activities of constituents of marigold (*Calendula officinalis*) flowers. J Nat Prod. 69:1692-1696.

Zitterl-Eglseer, K., G. Reznicek, J. Jurenitsch, J. Novak, W. Zitterl and C. Franz (2001). Morphogenetic variability of fa'Radio'l monoesters in marigold *Calendula officinalis* L. Phytochemical Analysis. 12(3):199-201.