Bulletin UASVM Horticulture, 69(1)/2012
Print ISSN 1843-5254; Electronic ISSN 1843-5394

Heterosis Studies for Response to *Aphis fabae* Attack in *Calendula*

Adriana-Daniela BACIU[1)], Doru PAMFIL[1)], Adriana F. SESTRAS[2)], Lucica MIHALTE[1)], Radu E. SESTRAS[1)]

[1)] University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, 3-5 Mănăştur Street, 400372, Romania; ady.baciu@yahoo.com, rsestras@yahoo.co.uk.
[2)] Fruit Research Station, 3-5 Horticultorilor Str., 400454, Cluj-Napoca, Romania

Abstract. The main objective of the present study was to estimate the heterosis in F_1 and F_2 *Calendula* hybrids for their response to *Aphis fabae* attack, as a result of crosses between genetically different parents. The aphids attack was examined as Attack Degree (AD%) on five pot marigold cultivars belonging to the *C. officinalis* L. and three different species (*C. arvensis* 121GE, *C. stellata* Cav., *C. suffruticosa*), used as genitors, as well as twenty F_1 and fourteen F_2 hybrid combinations of intra and interspecific crosses, obtained by cyclic and diallel crosses and through self-pollination. Negative heterosis calculated as mean of AD% per hybrid combination, which was desired for the trait, was identified and explained by different genetic interaction and phenomenon of heterosis (e.g. dominance, overdominance, pseudo-overdominance). Absolute heterosis values were close in most intraspecific and interspecific hybrid families in F_1 and F_2 generations. The highest desired negative value of heterosis, both in F_1 and F_2 hybrids, was resulted after self-pollination of 'Rech.f.' cultivar, the most sensitive genitor. The result was explained by the idea that partial dominance causes heterosis, because inbred descendants of this cultivars belonging to *C. alata* species become fixed for recessive or partially recessive deleterious alleles.

Keywords: aphids, hybrids, response to attack, dominance, overdominance

INTRODUCTION

Pot marigold (*Calendula officinalis*) belongs to the genus *Calendula*, in the *Asteraceae* family and it is well known and widely cultivated in temperate regions. The genus *Calendula* comprises about 25 species of annual and perennial, the best-known being: *Calendula officinalis*, *C. arvensis*, *C. alata*, *C. stellata, C. tripterocarpa*, *C. suffruticosa* etc. Due to its properties, *Calendula officinalis* L. is used in veterinary medicine and in different pharmaceutics and industries. As ornamental plant, pot marigold is used for setting green spaces, for interiors, as well as cut flowers in various floral arrangements (Şelaru, 2007). Due to its content in active ingredients (acids, oils, pigments, flavonoids) marigolds can be used both as an herb and in various nutrition diets (Dobrescu, 1981; Froment *et al.*, 2009; Pintea *et al.*, 2008).

Among the breeding goals of *Calendula*, the main objectives are focused into the following: improving decorative values of cultivars, with large anthodia and abundant flowers, intensely colored, compact habits (Gonceariuc, 2001; Kumar *et al.*, 1990), plant resistance to major specific diseases and pests (Gonceariuc, 2001; Baciu and Sestraş, 2009; Baciu *et al.* 2010).

Calendula is attractive to different pests, being in particular aphids' favourite. Aphids (*Aphis fabae*) are part of the *Homoptera* order, *Aphidida* suborder, *Aphididae* family, which also includes *Aphis pomii, Aphis gosyppii, Myzus cerasi, Brevicoryne brassicae* etc. Aphids attack cause directly damages or secondary damages through secretion of "honey dew", being the main vectors in the plant diseases transmission (Dixon, 1998; Corcău, 2011; Oltean *et al.*, 2004; Trotuş and Naie, 2008). Identify the parents and hybrids with resistance to aphids attack, is an important prerequisite for applied specific hybridization techniques in order to create artificial variability for the selection of new cultivars, tolerant or resistant to pests attack.

Furthermore, in practice plant breeders are interested in heterosis, which is subject to the interaction between genotype and environment (Acquaah, 2007). One of the objectives of this study was to estimate the heterosis in F_1 and F_2 *Calendula* hybrids for their response to *Aphis fabae* attack, as a result of crosses between genetically different parents.

MATERIALS AND METHODS

Biological material

Research on *Aphis fabae* attack on *Calendula* plants were carried out in the Agrobotanical Garden of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania. Five varieties belonging to the *C. officinalis* L. and three different species (*C. arvensis* 121GE, *C. stellata* Cav., *C. suffruticosa*) were used as genitors, following their cross resulting F_1 and F_2 hybrids. There were obtained and analyzed twenty F_1 hybrid combinations and fourteen F_2 hybrid combinations (intra and interspecific), obtained by cyclic and diallel crosses and through self-pollination (Tab. 1).

Tab. 1

Calendula genitors and hybrids analyzed for their response to *Aphis fabae* attack

Genitors	Hybrids F_1	Hybrids F_2
'Prycosnovjenie'	'Prycosnovjenie' x 'Prycosnovjenie'	'Prycosnovjenie' x 'Prycosnovjenie'
'Pacific Beauty'	'Prycosnovjenie' x 'Pacific Beauty'	'Prycosnovjenie' x 'Pacific Beauty'
'Bon Bon Mix'	'Prycosnovjenie' x 'Bon Bon Mix'	'Prycosnovjenie' x 'Bon Bon Mix'
'Bon-Bon Orange'	'Prycosnovjenie' x 'Bon-Bon Orange'	'Prycosnovjenie' x 'Bon-Bon Orange'
'Rech.f.'	'Pacific Beauty' x 'Pacific Beauty'	'Pacific Beauty' x 'Pacific Beauty'
C. arvensis 121GE	'Pacific Beauty' x 'Bon Bon Mix'	'Pacific Beauty' x 'Bon Bon Mix'
C. stellata Cav.	'Pacific Beauty' x 'Bon-Bon Orange'	'Pacific Beauty' x 'Bon-Bon Orange'
C. suffruticosa	'Bon Bon Mix' x 'Bon Bon Mix'	'Bon Bon Mix' x 'Bon Bon Mix'
	'Bon Bon Mix' x 'Bon-Bon Orange'	'Bon-Bon Orange' x 'Bon-Bon Orange'
	'Bon-Bon Orange' x 'Bon-Bon Orange'	'Bon Bon Mix' x 'Bon-Bon Orange'
	'Rech.f.' x 'Rech.f.'	'Rech.f.' x 'Rech.f.'
	'Rech.f.' x *C. arvensis* 121GE	*C. arvensis* 121GE x *C. arvensis* 121GE
	'Rech.f.' x *C. stellata* Cav.	*C. stellata* Cav. x *C. stellata* Cav.
	'Rech.f.' x *C. suffruticosa*	*C. suffruticosa* x *C. suffruticosa*
	C. arvensis 121GE x *C. arvensis* 121GE	
	C. arvensis 121GE x *C. stellata* Cav.	
	C. arvensis 121GE x *C. suffruticosa*	
	C. stellata Cav. x *C. stellata* Cav.	
	C. stellata Cav. x *C. suffruticosa*	
	C. suffruticosa x *C. suffruticosa*	

Depending on the success of hybridization, the number of hybrids analyzed in the combinations varied between three F_1 hybrids (in combination 'Pacific Beauty' x 'Bon-Bon Orange') and twenty-nine F_2 hybrids ('Prycosnovjenie' x 'Prycosnovjenie'). The results were processed as average values per genotype (for parents, on which were analyzed ten plants / cultivar or species) or hybrid combination.

Attack assessment and data processing

The *Aphis fabae* attack was examined in natural condition of infestation, in the absence of insecticide treatments, decadal, in the dynamic, in June and July. Generally, the insects affected primary shoots, upper third of them, to the base of inflorescences (antodias).

The *Aphis fabae* attack was estimated using frequency of attack (F%) and intensity of attack (I%), then was calculated the Attack Degree (AD%) (Baciu *et al.*, 2009):

$$F\% = \frac{n}{N} x100$$

n = no. of attacked plant;
N = no. of analyzed plants.

$$I\% = \frac{\Sigma(ixf)}{n}$$

i = the percentage of attacked plants;
f = no. of attacked plants at the same rate of attack;
n = no. of total plants attacked;
Σ = sum.

$$AD\% = \frac{F\%xI\%}{100}$$

F% = attack frequency;
I%= attack intensity

Because differences between genitors and F_1 hybrids combinations response to insects were identified using ANOVA 't' test (Ardelean *et al.*, 2007; Baciu *et al.*, 2009, 2010; Baciu, 2011), Duncan Multiple Range test was used in this study as a post hoc test conducted to identify which attacked variants differs from the others (among P_0, F_1 and F_2 variants).

Estimation of heterosis

Data from statistical processing of the experimental results on the aphids attack degree (AD%) of the parental forms (genitors), F_1 and F_2 hybrids ensured from crossing and self-pollination, have allowed to calculated the heterosis for the offspring of intra and interspecific combinations.

The absolute and relative values of heterosis in hybrids belonging to crosses conducted between varieties and species used as genitors and inbreeding coefficient in F_1 and F_2 hybrids resulting from self-pollination were determined.

Absolute heterosis values were determined in two ways (Ardelean, 1986; Pui and Ardelean, 2007; Baciu, 2011):

- By comparing the average AD% of F_1 hybrids with parental mean of AD%, in which case it was used the formula: $H = F_1 - (P_1 + P_2) / 2$.

- By comparing average F_1 generation with mean of trait (AD%) to the best parent, in which case there was used the formula: $H = F_1 - Pmax$.

Based on heterosis percentage calculated from Pmax / Pmin, major genetic effects involved in the development of heterosis plants response to aphids attack had highlighted, as follows (Pui and Ardelean, 2007; Pui, 2009):

a) $F_1 > Pmax$ or $F_1 < Pmin$, heterosis (probably overdominance);

b) $F_1 = Pmax$ or Pmin, complete dominance;

c) $Pmax > F_1 > P$ and $Pmin < F_1 < P$, partial dominance;

d) $F_1 = P$, absence of dominance.

To calculate relative heterosis, the formula used the average values of F_1 hybrids and genitors: $H = (\overline{F_1} - \overline{P}) / \overline{P} \times 100$; determining also the percentage of heterosis reported to the best parent (Heterobeltiosis) according to: $H = (\overline{F_1} - \overline{P_{Max}}) / \overline{P_{Max}} \times 100$; using the average of F_1 hybrids and the best genitor (with higher value).

Inbreeding coefficient in the first filial generation was calculated by dividing the average characteristic's value of F_1 hybrids obtained by self-pollination, to the average character in F_1 hybrids resulting from different parents (type A x B).

In the second generation the procedure was similar, but using the average character value (AD%) of F_2 hybrids.

Because the heterosis may be "positive" or "negative", and this is largely an artificial distinction (Acquaah, 2007), following the analyzed trait, the negative heterosis was desired.

RESULTS AND DISCUSSION

Of the analyzed variants, an adequate response to aphids attack presented twenty-two (five genitors, ten F_1 and seven F_2 hybrid combinations), all of them being registered with no attack (Tab. 2). Attacked variants were represented 53.6% of the total studied experience.

Tab. 2

Calendula genitors, F_1 and F_2 hybrids without attack of *Aphis fabae*

Genitors	F_1 Hybrids	F_2 Hybrids
'Prycosnovjenie' 'Pacific Beauty' 'Bon Bon Mix' 'Bon-Bon Orange' *C. stellata* Cav.	'Prycosnovjenie' x 'Prycosnovjenie' 'Prycosnovjenie' x 'Bon Bon Mix' 'Prycosnovjenie' x 'Bon-Bon Orange' 'Pacific Beauty' x 'Bon Bon Mix' 'Pacific Beauty' x 'Bon-Bon Orange' 'Rech.f.' x *C. stellata* Cav. 'Rech.f.' x *C. suffruticosa* *C. arvensis* 121GE x *C. stellata* Cav. *C. arvensis* 121GE x *C. suffruticosa* *C. stellata* Cav. x *C. stellata* Cav.	'Prycosnovjenie' x 'Prycosnovjenie' 'Prycosnovjenie' x 'Bon Bon Mix' 'Prycosnovjenie' x 'Bon-Bon Orange' 'Pacific Beauty' x 'Bon Bon Mix' 'Pacific Beauty' x 'Bon-Bon Orange' 'Bon Bon Mix' x 'Bon-Bon Orange' *C. stellata* Cav. x *C. stellata* Cav.

Half of no attack F_1 hybrid combinations were interspecific descendants and the other half, intraspecific progenies. Among the seven F_2 hybrid combinations, 'Prycosnovjenie' cultivar was the offspring obtained by self-pollination, and *C. stellata* Cav. species was obtained by self-pollination. Notable is the fact that in F_2, six variants without *Aphis fabae* attack were the descendants of cultivated *C. officinalis* L. species.

Tab. 3

Aphis fabae Attack Degree (AD%) of genitors, F_1 and F_2 hybrids

Generation	Genitors or hybrid combination	*Aphis fabae* Attack Degree (AD%)		
		(%)	Mean	Significance
Genitors	'Rech.f.'	22.14	15.36	P
	*C. arvensis*121GE	12.50		Q
	C. suffruticosa	11.43		Q
F_1 Hybrids	'Prycosnovjenie' x 'Pacific Beauty'	5.87	10.49	E
	'Pacific Beauty' x 'Pacific Beauty'	11.39		CD
	'Bon Bon Mix' x 'Bon Bon Mix'	6.88		E
	'Bon Bon Mix' x 'Bon-Bon Orange'	5.54		E
	'Bon-Bon Orange' x 'Bon-Bon Orange'	9.23		CDE
	'Rech.f.' x 'Rech.f.'	7.89		DE
	'Rech.f.' x *C. arvensis*121GE	20.83		A
	*C. arvensis*121GE x *C. arvensis*121GE	12.22		C
	C. stellata Cav. x *C. suffruticosa*	16.88		B
	C. suffruticosa x *C. suffruticosa*	8.18		DE
F_2 Hybrids	'Prycosnovjenie' x 'Pacific Beauty'	11.56	15.27	X
	'Pacific Beauty' x 'Pacific Beauty'	13.41		YX
	'Bon Bon Mix' x 'Bon Bon Mix'	16.00		ZYX
	'Bon-Bon Orange' x 'Bon-Bon Orange'	16.32		ZY
	'Rech.f.' x 'Rech.f.'	16.09		ZYX
	C. arvensis 121GE x *C. arvensis* 121GE	15.53		ZYX
	C. suffruticosa x *C. suffruticosa*	18.00		Z

*Averages on the same column followed by different letters between rows are statistically different, according to the Duncan test at the 5% significance level. SD5% for genitors: 9.32-9.36; for F_1 hybrids: 3.52-3.94; for F_1 hybrids: 4.51-4.76.

Among susceptible genitors to the attack of aphids, highest attack presented cv. ‘Rech.f.’ (AD% = 22.14), followed by the *C. arvensis*121GE and *C. suffruticosa* species (Tab. 3). The results indicate that the ‘Rech.f.’ cultivar, belonging to the *C. alata* species, is susceptible to the aphids attack and, in addition, transmits its sensitivity to the descendants. Thus, the progeny of ‘Rech.f.’ x *C. arvensis* 121GE cross showed the highest sensitivity to attack from all F_1 combinations (AD% = 20.83), with statistically differences compared with the rest of the variants.

On the offspring registered with the attack in F_2 hybrid combinations, the mean of AD% were relatively similar, ranging between 11.56% (‘Prycosnovjenie’ x ‘Pacific Beauty’) and 18.00% (*C. suffruticosa* x *C. suffruticosa*).

Mean values of AD% on groups of variants (P_0, F_1, F_2) were lower in F_2 hybrids (10.49%) and in parental and F_1 hybrids (15.36% and 15.27%) were larger and close related.

According to the centralized panel regarding the number and percent of parents, F_1 and F_2 hybrids, classified into different classes of aphids attack (Tab. 4), for the genitors and F_1 hybrids was obtained a normal distribution. In addition, the percentage of classes increases rather proportionally, from without attack to strong attack. A certain deviation was recorded in F_2 hybrids, because the combinations proportion with medium (middle) attack was superior to those with low attack (35.7% vs 14.3%).

Tab. 4

Number and percentage of genitors, F_1 and F_2 hybrids, enrolled in different classes, regarding attack degree of *Aphis fabae*

Attack estimation (AD%)	*Aphis fabae* Attack Degree (AD%)					
	Genitors		F_1 hybrids		F_2 hybrids	
	No.	%	No.	%	No.	%
Without attack (AD%=0)	5	62.5	10	50.0	7	50.0
Very low attack (AD%=5.51-10.0%)	-	-	6	30.0	-	-
Low attack (AD%=10.1-15.0%)	2	25.0	2	10.0	2	14.3
Middle attack (AD%=15.1-20.0)	-	-	1	5.0	5	35.7
Strong attack (AD%=20.1-25.0%)	1	12.5	1	5.0	-	-
Very strong attack (AD%=25.1-35.0%)	-	-	-	-	-	-
All genitors and F_1 hybrids	8	100.0	20	100.0	14	100.0

The values for absolute and relative heterosis in hybrids derived from artificial crosses and inbreeding coefficient in F_1 and F_2 hybrids resulted from self-pollination are shown in Tab. 5.

It should be noted that the absolute value of heterosis determined by comparing average F_1 hybrids and parental average, respectively, to the best parent, was identical, whereas one of genitors was not attacked and in the formula was introduced AD% = 0. Another note is that because of the analyzed trait, positive heterosis values are not favorable, however small values, especially negative ones, means obtaining progeny with a better response to the pest attack (possibly resistance or tolerance to the aphids attack).

The absolute heterosis values were close related in most intraspecific and interspecific hybrid families in F_1 and F_2 generations, including through heterosis direction. The only variant that there have been negative heterosis value, both in F_1 and F_2 hybrids, was ‘Rech.f.’ x ‘Rech.f.’ Otherwise, for all combinations were obtained positive heterosis values.

By comparing the average trait in F_1 generation with the best parent, heterosis effects were identified due to: overdominance (F_1> Pmax, alternative F_1 <Pmin being not possible in experience), in combinations that genitors had AD% = 0; complete dominance (F_1 = Pmax or Pmin), ex. F_1 hybrids belonging to *C. arvensis*121GE self-pollination; partial dominance

(Pmax> F_1> P and Pmin <F_1 <P), on F_1 and F_2 hybrids resulted from 'Rech.f.' self-pollination. Maybe we could accept that in this case, overdominance causes heterosis due to the superiority of heterozygotes over homozygotes at specific loci, which affect the plants' response to the pests attack (Johnson and Hutchinson, 1993).

Tab. 5

Heterozis for response to *Aphis fabae* attack (AD%) and inbreeding coefficient in F_1 and F_2 hybrids

Hybrid combination	Absolute Heterosis vs		Relative Heterosis (%) vs		Inbreeding Ratio ($F_{1or2self}/F_{1or2(AxB)}$)	
	Mean of parents	The best parent	Mean of parents	The best parent	$F_{1self}/F_{1(AxB)}$	$F_{2self}/F_{2(AxB)}$
F_1 hybrids						
'Prycosnovjenie' x 'Pacific Beauty'	5.9	5.9	-	-	-	-
'Pacific Beauty' x 'Pacific Beauty'	11.4	11.4	-	-	1.09	-
'Bon Bon Mix' x 'Bon Bon Mix'	6.9	6.9	-	-	0.66	-
'Bon Bon Mix' x 'Bon-Bon Orange'	5.5	5.5	-	-	-	-
'Bon-Bon Orange' x 'Bon-Bon Orange'	9.2	9.2	-	-	0.88	-
'Rech.f.' x 'Rech.f.'	-14.3	-14.3	-64.4	-64.4	0.75	-
'Rech.f.' x *C. arvensis*121GE	3.5	8.3	-54.4	-36.9	-	-
*C. arvensis*121GE x *C. arvensis*121GE	12.2	12.2	0.0	0.0	1.16	-
C. stellata Cav. x *C. suffruticosa*	11.2	11.2	195.1	47.7	-	-
C. suffruticosa x *C. suffruticosa*	8.2	8.2	0.0	0.0	0.78	-
F_2 hybrids						
'Prycosnovjenie' x 'Pacific Beauty'	11.6	11.6	-	-	-	-
'Pacific Beauty' x 'Pacific Beauty'	13.4	13.4	-	-	-	0.88
'Bon Bon Mix' x 'Bon Bon Mix'	16.0	16.0	-	-	-	1.05
'Bon-Bon Orange' x 'Bon-Bon Orange'	16.3	16.3	-	-	-	1.07
'Rech.f.' x 'Rech.f.'	-6.1	-6.1	-27.3	-27.3	-	1.05
C. arvensis 121GE x *C. arvensis* 121GE	15.5	15.5	24.2	24.2	-	1.02
C. suffruticosa x *C. suffruticosa*	18.0	18.0	57.5	57.5	-	1.18

Probably overdominance, as interalelic related phenomenon, due to which a heterozygous descendant (*Aa*) causes an increase or an intensification of phenotype in homozygous individuals as parental type (*Aa>AA>AA*) (Acquaah, 2007), contribute significantly to the expression of heterosis for *Calendula* hybrids response to the aphids attack.

The parental formula has a particularly importance in overdominance manifestation for plant response to attack, contributing significantly to the expression of heterosis, is supported by the high and positive value of relative heterosis of F_1 offspring of the combination *C. stellata* Cav. x *C. suffruticosa* (195.1%), but even heterobeltiozis's (heterosis relative to the best parent) of 47.7%. Similarly (obtaining descendants sensitive to *Aphis* attack), acting effects of heterosis in F2 hybrids of *C. suffruticosa* x *C. suffruticosa*.

Apart from F_1 and F_2 hybrids belonging to self-pollination of 'Rech.f.' cultivar, relatively negative heterosis was registered only on F_1 hybrids from 'Rech.f.' x *C. arvensis*121GE crosses. However, their self-pollination not assured F_2 progeny in order to permit analysis of the genetic effects in F_2 generation, also.

Inbreeding depression was not emphasized, and the differences were relatively small in F_2 compared to F_1. If in F_1 majority of inbreeding ratio had sub unitary values, the only F_2 progeny 'Pacific Beauty' x 'Pacific Beauty' achieved a nil value.

Although ‘Rech.f.’ cultivar was the most susceptible to the aphids attack and transmitted to a certain degree its sensitivity to the descendants, the registered heterosis was negative. Presumably, the hypothese that partial dominance causes heterosis is also possible, because inbred descendants become fixed for recessive or partially recessive deleterious alleles (Johnson and Hutchinson, 1993). Thus, crosses between such inbred descendents, fixed for deleterious alleles at different loci, produce genotypes, which are superior to the parents (Charlesworth and Charlesworth, 1987).

CONCLUSIONS

Whatever the theory that explains the phenomenon of heterosis - eg. dominance, overdominance or pseudo-overdominance (Birchler *et al*., 2010), the present results demonstrate that the heterosis can be exploited at *Calendula* including by increasing plant resistance to aphids attack. Therefore, the heterosis in *Calendula* may be an extremely useful method of improving both economic characteristics (Kumar *et al*., 1990) and decorative (Baciu *et al*., 2009, 2010), but also to obtain cultivars with properly response to the main diseases or pests attack (e.g. aphids). A large number of ornamental species F_1 hybrids have expanded rapidly in culture and occupy an important place in cultivars assortment and probably the same will happen on *Calendula*.

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

REFERENCES

1. Acquaah, G. (2007). Principles of Plant Genetics and Breeding. Blackwell, Oxford, UK.
2. Ardelean, M., R. Sestras and M. Cordea (2007). Experimental horticultural technique (in Romanian). Ed. AcademicPres, Cluj-Napoca.
3. Baciu, A.-D, A. Sestras, R. Sestras and M. Buruiana (2009). The response of different genotypes of *Calendula* to *Aphis fabae* attack. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture 66(1):498-503.
4. Baciu, A.-D., L. Mihalte, A. Sestras and R. Sestras (2010). Variability of decorative traits, response to the *Aphis fabae* attack and RAPD diversity in different genotypes of *Calendula*. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 38(3):265-270.
5. Baciu, A.-D. (2011). Research regarding the genetical enrichment for the *Calendula*, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. PhD Thesis. PhD Coord. Prof. R. Sestras.
6. Birchler, J.A., H. Yao, S. Chudalayandi, D. Vaiman and R.A. Veitia (2010). Heterosis. Plant Cell. 22(7):2105-2112.
7. Charlesworth, D. and B. Charlesworth (1987). Inbreeding depression and its evolutionary consequences. Annu. Rev. Ecol. Syst. 18:237-268.
8. Corcău L.D. (2011). Contributions to the study of phytophagous insects of ornamental plants, of its naturals limits (systematic biology, ecology) and the problem of reducing populations by biological and biotechnical means in order to avoid chemical pollution. PhD Thesis. Bucharest Univesrity (in Romanian).
9. Dixon, A.F.G. (1998). Aphid ecology. Chapman & Hall. London.
10. 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.
11. Dobrescu, D. (1981). Farmacoterapia. Ed. Medicala, Bucuresti.

12. Gonceariuc, M., (2001). Variability of morphological anthodia characters of *Calendula officinalis* L. (in Romanian). Analele stiintifice ale Centrului de Resurse genetice vegetale din Moldova, Seria B 1:65-71.

13. Johnson, T.E. and E.W. Hutchinson (1993). Absence of strong heterosis for life span and other life history traits in *Caenorhabditis elegans*. Genetics 134(2):465-474.

14. Kumar, S., K.G. Shanmugavelu and I. Irulappan (1990). Hybrid vigour in marigold for economic characters. South Indian Horticulture 38(3):173-174.

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

16. 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.

17. Pui, D.-A. and M. Ardelean (2007). Heterosis manifestation of several quantitative characters of ornamental interest in gillyflower (*Matthiola sp*.) breeding. Contribuţii Botanice 42:99-104.

18. Pui, D.-A. (2009). The study of intra- and interspecific variability at *Matthiola* genus and its use in breeding practice. University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. PhD Thesis. PhD Coord. Prof. M. Ardelean (in Romanian).

19. Şelaru, E. (2005). Ornamentals (in Romanian). Ed. Ceres, Bucuresti.

20. Trotuş, E. and M. Naie (2008). Research on the reduction of pathogens and specific pests of hemp crops by chemical treatment of seed. Lucrări ştiinţifice, Vol. 51/2008, seria Agronomie, USAMV Iaşi (in Romanian).