

## ENZYMATIC VARIABILITY OF SOME *Achillea* GENOTYPES WITH DIFFERENT POLYPLOIDY LEVEL AND FROM DIFFERENT GEOGRAPHICAL REGIONS

G.C. CORNEANU\*, ELENA GLODEANU\*, V. HANESCU\*,  
GH.G. POPESCU\*, MIHAELA CORNEANU\*

**Key words:** genotypes, *Achillea* species, polyploidy level

**Abstract:** The content in peroxidase isozymes from leaves and inflorescence at four *Achillea* species with different level of ploidy, native from different geographical regions (from mountain to field) was analysed. Some aspects of peroxidase implication in species adaptation at environmental and organogenesis processes are discussed.

*Achillea* genus presents over 100 herbaceous species, perennial, rarely biannual, spreading in temperate regions from Europe, Asia, North America and north of Africa. In Romania exist over 26 species and hybrids (Prodan and Nyárády, 1964), in different geographical regions. Some species present a therapeutically importance, the volatile oil extracts from inflorescence and leaves being used in different affections (Ciulei et al., 1993, Lawless, 1993, a/o). The species present a great variability, regarding morphological features, chromosome numbers, content in active substances (azulene, proazulene, amides, a/o), as well as the content in peroxidase isozymes (Corneanu et al., 1995). In this paper is discussed the content in peroxidase isozymes from four species harvested from different geographical zones.

### Material and methods

The peroxidase isozymes content from leaves and inflorescence was performed at four *Achillea* species (mature plants) from different geographical regions.

*Achillea distans* W. et K. is meet in forest, at skirt forest and in rocks places from mountain to alpine region. The mature plants of 25-40 cm height, were harvested from the skirt forest, on the border read, near Râncea Station, Parâng Mountains (Gorj district), from an altitude of 1600 m.

---

\*University of Craiova, Biology Dept., 1100 - Craiova

*Achillea crithmifolia* W. et K., vegetate on steep slope from field until mountain region. The plants of 35-50 cm height, were harvested near Novaci town, in a submountain region.

*Achillea millefolium* L., frequently in all country, vegetates in meadows, clearings, at skirt forest, sometime in shady places from the hilly region. Was analysed a population from Cruset village (Gorj district), from an altitude of 300 m, with mature plants of 60-110 cm height.

*Achillea setacea* W. et K., common in all country, spread in dry hayfield, on arid hilly, abundant in sandy soil or loessial soil, even in semialkaline places. Is common in hayfield and pasture ground, on uncultivated sandy places. Mature plants, with a height of 60-80 cm, were harvested from a full of precipes region (Bucovăț, Palilula vale) near Craiova town, at an altitude of 125 m.

The chromosome number was establish from the metaphase from radicles top (Feulgen stain, squash type preparation).

The inflorescence and leaves content in peroxidase isozymes was determined in all species, from the mature plants (at flowering), through electrophoresis in agarose gel thin layer (Cardy et al., 1980). From electrophoregrames was establish the fractions number of peroxidase isozymes and their activity in densitometric units.

## Results and discussions

In the somatic cells, was recorded a chromosome number characteristic for these species: *A. distans*  $2n=6x=54$ , *A. crithmifolia*  $2n=2x=18$  and *A. setacea*  $2n=2x=18$ , similarly with the values from specialised literature (Bolkhovskikh et al., 1963). The chromosomes are short, with a length of 1.8-4.0  $\mu\text{m}$ , having the specie features (Corneanu, 1981).

The values recording for the peroxidase isozymes are presented in Table 1. The content in total densitometric units of the peroxidase isozymes, recorded different values, in generally being superior at the populations from the hilly and field regions (Table 1). In inflorescence were recorded, in generally, higher values, in comparison with the ones from leaves. Number of cathodic fractions is lower in comparison with the anodic fractions, both in leaves and in inflorescence. This finding, underlined their implication in the synthesis and organogenesis processes. On other hand, the values of the cathodic fractions, in percentile densitometric units, from leaves, recorded superior values in the mountain and submountain regions, being of 34.36% in *A. distans* (mountain region), 31,34% in *A. crithmifolia* (submountain region), 29.19% in *A. millefolium* (hilly region) and 11.47% in *A. setacea* (field region). The enhanced values of the cathodic fractions of the peroxidase isozymes in the mountain and submountain regions (respectively in polyploidy species), in comparison with the hilly and field regions (Table 2), suggest their implication in plant resistance at unfavourable temperatures, a characteristic for

polyploidy species. In *A. distans* leaves, the number of cathodic and anodic fractions is similar (4 : 4), but their percentile values are very different (34.36% : 65.15%), being similar to the ratio from other analysed species. The value of anodic fractions from leaves, in densitometric units, present inferior values at species from mountain and submountain regions (5.26 in *A. distans* and 7.47 in *A. crithmifolia*), and superior values at species from hilly and field regions (10.94 in *A. millefolium* and 14.77 in *A. setacea*). Similarly, the anodic fractions from inflorescence present, in generally, superior values at species from field and hilly regions, in comparison with species from submountain and mountain region (Table 2).

The anodic fractions analysis of peroxidase isozymes from inflorescence (number fractions, their value in densitometric units or percentile), recorded values characteristic for each genotype (Table 2). The anodic fractions values (in densitometric values) from inflorescence present, in generally, superior values at species from field (*A. setacea*) and hilly regions (*A. millefolium*), in comparison with species from submountain (*A. crithmifolia*) and mountain region (*A. distans*). On other hand, the value in percentile densitometric units of anodic fractions from inflorescence, presented superior values in *A. millefolium* and *A. distans*, in comparison with *A. crithmifolia* and *A. setacea* (Table 2). These findings are, probably, in correlation with the plant biosynthesis processes.

Were establish some correlation between the content in peroxidase isozymes in leaves and inflorescence (together) on a hand and other features of the four analysed *Achillea* species on other hand. The positive correlation exist between: the all four analysed species on a hand and total densitometric units ( $r = +0.8883, **$ ); number cathodic fraction ( $r = +0.7229, *$ ), anodic fractions and the all four species ( $r = +0.9415, ***$ ) on other hand, a/o. Also, negative correlation exist between: altitude of species origin on a hand and total densitometric units ( $r = -0.6996, 0$ ), cathodic fractions number ( $r = -0.7612, 0$ ), anodic fractions in DU ( $r = -0.7737, 0$ ) on other hand, a/o (Fig. 1).

## Conclusions

The leaves and inflorescence content in peroxidase isozymes at *Achillea distans* (Rânca, mountain region), *A. crithmifolia* (Novaci, submountain region), *A. millefolium* (Cruset, hilly region) and *A. setacea* (Craiova, field region) was recorded and analysed.

1. The content in total densitometric units of the peroxidase isozymes, recorded, in generally superior values at the populations from the field regions, as well as in inflorescence in generally, in comparison with the ones from leaves.

2. The number of anodic fractions, both in leaves and in inflorescence, is higher in comparison with cathodic fractions. This suggests their implication in the synthesis and organogenesis processes.

3. In the mountain region with severe temperature, the value percentage of the cathodic fractions from leaves, recorded upper values in comparison with the value

recorded in field region. This suggests, the implication of the cathodic fraction in plant resistance at cold, in the mountain and submountain regions being spreader the two polyploid species: *A. distans* and *A. crithmifolia*.

4. Between the four analysed species or there origin place on a hand and some features of peroxidase isozymes on other hand, were establish some correlation, with an importance regarding the species degree adaptation at environmental and their synthesis and organogenic activity.

## References

1. Bolkhovskikh Z., Grif V., Matvejeva T., Zacharyeva G., 1969 – Chromosome numbers of flowering plants. Izd. Nauka, Leningrad
2. Ciulei I., Grigorescu Em., Stănescu Ursula, 1993 – *Plante medicinale, fitochimie și fitoterapie*, vol. 2, Edit. Medicală, București
3. Corneanu G.C., Hănescu V., Corneanu Mihaela, 1995 – Genetics and biochemical features of some *Achillea* sp. genotypes from different zones. *Acta Phytotherapica Romanica*, II (2): 33-34
4. Corneanu G.C., 1981 – Studii cariologice asupra speciilor genului *Achillea* L. (Fam. *Compositae*) din România (Nota 1). *Studii și Cercetări, SOMN, Craiova*, p.: 62-66
5. Cardy B.J., Struber C.W., Coodman M.M., 1980 – Tehniques for agarose gel electrophoresis of enzymes. North Carolina State University
6. Lawless Julia, 1993 – *Enciclopedia degli oli essenziali*. Tecniche Nuove, Milano. 1993
7. Prodan I., Nyárády E.I., 1964 – Genul *Achillea* L. in: *Flora României*, vol. IX (Eds. T. Săvulescu, E.I. Nyárády), Edit. Academiei Române, București, p.: 362-409

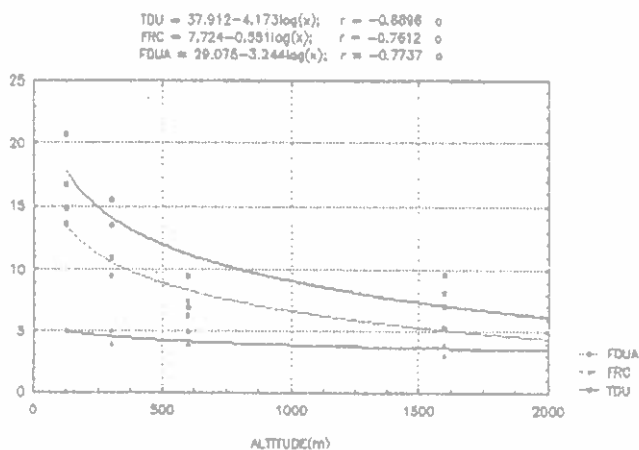


Fig. 1 – Correlation between altitude and peroxidase isozyme indices in *Achillea* sp.

TDU = total densiometric units

FRC = cathodic fractions number

FDUA = percentile densiometric units of anodic fractions

Table 1.  
Peroxidase isozymes from leaves and inflorescence of some *Achillea* species

Specie, origin, organ	TDU	Fraction number	Cathodic fractions		Anodic fractions	
			FDU	FDU%	FDU	FDU%
1	2	3	4	5	6	7
<i>A. distans</i> Rânca leaves	8.07	8	0.90	11.15	3.30	40.88
			1.26	15.16	0.56	6.93
			0.10	1.24	0.70	8.67
			0.55	6.81	0.70	8.67
<i>A. distans</i> Rânca inflorescence	9.64	11	0.48	4.97	1.70	17.62
			0.68	7.05	1.80	18.66
			1.44	14.93	0.39	4.04
					0.80	8.29
					1.10	11.40
					0.35	3.62
					0.48	4.97
		0.42	4.35			
<i>Acrithmifolia</i> Novaci leaves	6.95	14	0.15	2.16	0.90	12.94
			0.48	6.90	0.33	4.74
			0.35	5.03	0.30	4.32
			0.36	5.17	0.33	4.74
			0.84	12.08	0.72	10.35
					0.56	8.05
					0.72	10.35
					0.49	7.04
		0.42	6.03			
<i>Acrithmifolia</i> Novaci inflorescence	9.46	11	0.39	4.12	3.08	32.55
			0.52	5.49	0.60	6.34
			0.72	7.61	1.43	15.11
			1.56	16.49	0.49	5.18
					0.25	2.64
					0.12	1.27
					0.30	3.17
<i>A. millefolium</i> Cruset leaves	15.46	15	0.25	1.61	3.50	22.61
			0.36	2.32	0.67	4.32
			0.56	3.62	1.26	8.14
			1.35	8.72	0.90	5.81
			2.00	12.92	1.65	10.66
					0.60	3.87
					0.50	3.23
					0.36	2.32
					1.30	8.39
		0.20	1.29			

1	2	3	4	5	6	7
<i>A. millefolium</i> Cruset leaves	13.49	12	0.14	1.04	3.70	27.41
			0.84	6.22	0.76	5.63
			2.25	16.67	0.90	6.67
			0.75	5.55	0.52	3.85
					1.00	7.41
					0.50	3.70
					0.70	5.18
		1.43	10.59			
<i>A. setacea</i> Craiova leaves	16.69	12	0.30	1.79	4.80	28.75
			0.66	3.95	1.10	6.59
			0.42	2.51	4.50	26.95
			0.18	1.07	0.88	5.27
			0.36	2.15	0.64	3.83
					0.75	4.49
		2.10	12.57			
<i>A. setacea</i> Craiova inflorescence	20.68	12	0.25	1.20	7.00	33.81
			1.35	6.52	1.90	9.17
			0.60	2.89	1.75	8.50
			4.10	19.80	0.54	2.60
			0.75	3.62	0.75	3.62
					0.60	2.89
		1.08	5.21			

TDU = total densitometric units

FDS = densitometric units per fraction

FDS% = percentual densitometric units per fraction

Table 2.

Cathodic and anodic fractions values of the peroxidase isozymes at some *Achillea* species

Specie	Organ	Cathodic fractions			Anodic fractions		
		Number	FDU	FDU %	Number	FDU	FDU %
<i>A. distans</i>	leaves	4	2.81	34.36	4	5.26	65.15
<i>A. distans</i>	inflores	3	2.50	26.95	8	7.04	72.85
<i>A. crithmifolia</i>	leaves	5	2.18	31.34	9	7.47	68.56
<i>A. crithmifolia</i>	inflores	4	3.19	31.71	7	6.27	66.26
<i>A. millefolium</i>	leaves	5	4.52	29.19	10	10.94	70.59
<i>A. millefolium</i>	inflores	4	3.98	29.48	8	9.51	70.44
<i>A. setacea</i>	leaves	5	1.92	11.47	7	14.77	88.45
<i>A. setacea</i>	inflores	5	7.05	34.04	7	13.62	65.80