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Study of several parameters in *Rosa canina L.* genotypes from native habitats in Romania and the *in vitro* response of this species

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Abstract: The variability of some fruit parameters for 68 dog rose genotypes (belonging to 43 native and one cultivated populations from 38 locations of Romania) was investigated during 2008-2009. Considering the values of the anlyzed indices and especially of the fruit fresh biomass and their content in vitamin C, it was ascertained that the genotypes belonging to populations from Cuejdi, Cuejdi-Ponor, Bicaz Chei and Gheorghieni, and the genotypes P2, P10 and P11 from the population of Pietricica Mountain are of perspective for the amelioration of the dog rose. There were isolated some genotypes with a particular plant architecture, different fruit shapes and sizes, fewer prickles on their branches, with the hips uniquely attached to the branches (not in clusters) etc, forms that may be useful in the amelioration program of this species.
The initiation of *in vitro* cultures of dog rose may be achieved using axillary and apical shoot tips, harvested during the summer and inoculated on the hormone-free medium Murashige-Skoog (1962) or on its variants enriched with BAP or with kinetin and NAA. Our preliminary data evinced that the most intense morphogenetic response of the shoots was on the MS medium enriched with BAP (1 mg l^{-1}) and IBA (0.5 mg l^{-1}), a medium variant that enhanced the multiple shooting, the formation of callus at the shoot base, the indirect caulogenesis (via callus) and the rhyzogenesis (seldom). The multiple shooting was also stimulated on the hormone-free MS and on the hormone-enriched variants KN, BG, BDG, BGN and BGZ. A critical period for the dog rose micropropagation seems to be the acclimatization of neoplantlets to the septic conditions.

Key words: dog rose, biodiversity, vitamine C, *in vitro* culture

Introduction

Rosa canina L. (the dog rose) is a shrub that is common along the roads, slopes, stubble fields, at the limit of the forests etc., from the sea level to the altitude of

[*] Anthropological-Biocosmological-Informational Reflections

1200 m. This pentaploid (2n = 35), xeromesophytic species is spread in Europe, northeast Africa and western Asia, reaches the height of 2-5 m; its stems and branches are covered with prickles, (Pârvu, 2000; Tiţă, 2003; Petrova et al., 2007; Kiliçgun and Dehen, 2009). The dog rose fruit (Cynosbati fructus) are well-known and used as an important source of biologically active compounds: sugars, organic acids, pectins, flavonoids, tannins, carotenoids, fatty acids, vitamins (mainly vitamin C, along with vitamins B_1, B_2, K, PP, E), macro- and micro-elements etc, (Pârvu, 2000; Demir şi Ozcan, 2001; Tiţă, 2003; Stănescu et al., 2004; Arsenescu, 2008; Orhan et al., 2009). It was ascertained that the dog rose seeds may be of great interest as they contain oil and mineral substances. The dog rose oil is mainly comprised by the acids: linoleic, oleic, linolenic, palmitic, stearic and arachidonic, (Ozcan, 2002). The chemical compounds from the hips confer the fruit the following properties: vitaminisant, astringent, colagogue, choleretic, diuretic, antidiarrhoeic, antioxidant etc, (Pârvu, 2000; Tiţă, 2003; Arsenescu, 2008). Recent data showed that the dog rose fruit display the following effects: anti-inflammatory, antioxidant, and anti-mutagene (Kiliçgun and Dehen, 2009), or that they have anti-diabetic properties, probably due to their content in mono- and oligosaccharides and pectins (Orhan et al., 2009).

The dog rose may be a plant of great perspective, considering its pharmaceutical and alimentary importance, the fact that it is not very demanding to the soil texture and may be hence cultivated on degraded or unsuitable land for ordinary crops. The cultivation of this species implicitly raises the issue of promoting some valuable forms (productive and with top quality fruits), or forms with fewer prickles on stems and branches. The most facile solution in this case is to identify and take over some genotypes of perspective from the spontaneous flora. In this view, a thorough research on local populations of *Rosa canina* L. was accomplished during 2008-2011 in order to evince such genotypes. The main characters that led to the choice of genotypes were: the shape, the size and the color of the mature fruit, the density of hips/plant and of prickles/ stems and branches. Our investigations were effected on populations of dog rose from 38 locations of 9 Romanian counties. We studied the variability of the same parameters in dog rose genotypes from the same population. Considering the fact that the references mention that higher-altitude dog rose shrubs comprise a higher amount of vitamin C in the hips, we tested several genotypes belonging to populations that lie at various altitudes, from the plain area up to the submontain and mountain regions.

In order to initiate crops of *R. canina,* it is not enough to isolate some valuable genotypes from the spontaneous flora, but it requires a technique of multiplication and providing the planting material, as well. This fact led to some research to initiate *in vitro* cultures of dog rose and the study of the morphogenetic response of some explants, in view of elaborating an *in vitro* multiplication technique for this species. The references only mention few data on this topic. Studying the

micropropagation of four species belonging to the *Rosa* genus (*R. canina* „Inermis, *R. indica* „Major", *R. mannetti* and *R. multiflora*), Kucharska et al., (2006) noticed that, depending on the species, a series of factors such as: the gelifying agent, the level of BAP, the silver nitrate, the initial length of the shoots, the removal of the shoot tips etc, stimulated the number and quality of the provided shoots. The rhyzogenesis of the shoots was ameliorated by the adding of vitamin B2 in the nutritive medium enriched with auxins, or on the auxin-free medium variant supplemented with active charcoal. Running the tests for the rapid *in vitro* micropropagation of *Rosa canina* and *R. damascena*, Abbas (2010) noticed that the best shoot proliferation (3.17 micro-shoots/subculture each 4 weeks) appeared on the MS medium supplemented with BA (4.44 μM) + IBA (0.49 μM) + GA3 (0.58 μM). The percentage of the shoots' enrooting reached 100% on half-strength MS, supplemented with 4.9 μM IBA. The induction of callus of *Rosa canina* was accomplished by Eşitken and Ercişli (2001) by the cultivation of nodal fragments (of 0.4-0.5 cm) on the MS medium enriched with high doses of NAA and BA; the best callus proliferation was induced by supplementing the nutritive medium with 6 mg l^{-1} NAA + 2 mg l^{-1} BA.

Material and methods

In order to evince some characters' variations for the dog rose hips and also to observe the plants' architecture, the presence or absence of prickles on the branches, the density of the hips on branches etc, during 2008-2011 (from mid - September to mid - October) there were studied 68 genotypes of 43 native populations and one cultivated population, situated in 38 locations from 9 Romanian counties. To choose the proper genotypes to be tested, the main investigated parameters were (as it is mentioned above): the architecture of the plants, the shape, size and color of the fruit, the density of the hips/plant etc. The next parameters were analyzed on the fruit samples (of 100 hips) from each dog rose genotype: maximum length (height) and width (diameter), fresh biomass/hip and the amount of vitamin C in the fresh matter. The biometrical data were processed and statistically interpreted. The amount of vitamin C from the hips was dosed using the method described by the Romanian Pharmacopoeia (1998).

The explants used to initiate the *in vitro* culture of dog rose were: apical and axillary shoot tips, harvested in several stages from the cultivated individuals of S.C. „Fructex" Bacău (during May-July). The possibility of initiating the cultures during the unsuitable season was investigated as well. In this view, during the cold season of 2011 there were cut off some dog rose branches of 60-80 cm from a Piatra Neamţ population, that were maintained in water in laboratory conditions to induce their vegetation and provide shoots to initiate new *in vitro* cultures. We thought such a strategy may reduce the explants' disinfection time, that favoured a higher survival rate after the sterilization previous to the inoculation on the nutritious

medium, fact that was not confirmed. The shoot tips (of about 2 cm in length) were disinfested by successive treatments with $HgCl_2$ (0.1%) and chloramine-T (5%), for different periods of time, so that would ensure the both the disinfection and survival of explants. The disinfested explants were inoculated on the hormone-free Murashige-Skoog (1962) or on other variants of MS. The nutritive medium comprised succhrose as a charbon source (30 g/l) and was solidified (8.5 g/l). The best results during the *in vitro* culture initiation at the dog rose were provided using the shoot tips harvested during the month of July; at that moment, the percentage of their survival post-disinfection was about 50%. The shoots that grew on the initiation medium variants were subsequently used as a source of explants to test the morphogenetic reaction on several variants of MS (enriched with growth regulators).

The medium variants that were used during our investigations are displayed by Table 2. Some of the results revealed by these observations and tests were presented in a previous paper (Ghiorghiţă et al. 2012b). Our present contribution is a synthesis of all the past investigations meant to draw some conclusions regarding the biodiversity of this species and its *in vitro* response. The results of this synthetic approach are presented by the Tables 1 and 2, and by the Figures 1 and 2. There are certain comparisons among these results and some data displayed by a study on *Rosa canina* L. we effected during the 1970s (Băra, Ghiorghiţă et al., 1976/1977).

Results and discussions

Our investigations on *Rosa canina* L. during 2008-2011 comprised 38 locations in Romania, and 43 native populations and one cultivated population and were accomplished on 68 genotypes. While our previous studies (Băra, Ghiorghiţă et al., 1976/1977) aimed to evince the variations of some morpho-physiological parameters of the fruit on average samples harvested from various local populations of Romania, these investigations were meant to isolate some genotypes of dog rose from native populations, to correspond to the amelioration goals: the architecture of the shrubs, the density of prickles/plant, the density of hips/plant, the size, shape and colour of the fruits, the amount of vitamin C within the hips etc. The data displayed by Table 1 showed that the fruit length for these genotypes ranged between 16.49 (the Chiriţeni genotype/Neamţ county) and 26.22 mm (genotype P9 from the Pietricica population, Piatra Neamţ), the maximum fruit diameter ranged between 10.92 mm (the Almaş-1 genotype/NT) and 16.80 mm (the genotype from Gheorghieni/HG), the ratio fruit length/fruit diameter (that offers data on the fruit shape) - between 1.09 (the genotype from Cheile Bicazului/NT) and 1.96 (genotype P9, Pietricica population), the individual average biomass of the hips - from 1.0943g (the genotype from Almaş-1/NT) to 3.2309g (the genotype P10 - the Pietricica Mountain), and the amount of vitamin C within the fresh fruit - from 74 mg% fresh matter (the genotype P16 on the Pietricica Mountain) to 1324.2 (the

genotype from Chiriţeni/NT). To be mentioned that the longest dog rose hips (27.49 mm) were found within the cultivated population (the genotype Sărata-3, that registered the highest ratio between fruit length/fruit diameter, of 2,26).

Table 1 – The values of some morpho-physiological and biochemical parameters of the fruits from *Rosa canina* genotypes investigated during 2008-2011

No.	Population/ Genotype/ County	Fruit length (mm)	Fruit diameter (mm)	Fruit length/Fruit diameter	Fruit biomass (g)	Vit. C (mg/ 100g fresh matter)
C	Control*	17.81	11.65	1.53	1.4817	547.5
Genotypes investigated in 2008						
1	Cândeşti-1/VN	18.23	12.54	1.45	1.4552	464.0
2	Cândeşti-2/VN	16.77	13.43	1.25	1.7029	424.4
3	Cândeşti-3/VN	22.04	12.75	1.73	1.8600	418.2
4	Cândeşti-4/VN	19.62	13.52	1.45	1.9100	478.6
5	Bâtca D-nei-1/NT	19.69	13.24	1.49	1.8687	589.8
6	Bâtca D-nei-2/NT	18.70	14.80	1.26	2.1747	661.6
7	Bâtca D-nei-3/NT	21.27	12.54	1.70	1.6831	721.2
8	Almaş-1/NT	18.35	10.92	1.68	1.0943	552.4
9	Almaş-2/NT	19.65	15.03	1.31	2.4400	515.3
10	P.Neamţ	18.96	12.25	1.55	1.4344	543.3
11	Negreşti-1/NT	22.43	13.86	1.62	2.2000	527.4
12	Negreşti-2/NT	19.96	14.13	1.41	2.0590	555.9
13	Negreşti-3/NT	18.49	14.42	1.28	2.0318	451.0
Genotypes investigated in 2009						
14	Sărata/BC	23.00	12.09	1.90	1.6867	1069.2
15	Sărata-1/BC **	22.22	13.84	1.60	2.1674	743.3
16	Sărata-2/BC **	24.54	15.43	1.59	2.8644	-
17	Sărata-3/BC **	27.49	12.17	2.26	2.0940	-
18	Bârzuleşti/BC	22.60	13.27	1.70	1.9944	1120.8
19	Măgura-1/BC	20.40	11.03	1.85	1.3128	1058.7
20	Măgura-2/BC	19.71	12.90	1.53	1.7043	1137.1
21	Bicaz/NT	22.05	13.55	1.63	2.0051	951.4
22	Bicaz Ardeal/NT	17.92	15.60	1.15	2.3184	961.1
23	Bicaz Chei/NT	22.30	15.36	1.45	2.8219	1150.0
24	Ceahlău/NT	22.10	14.49	1.52	2.1099	1200.5
25	Cheile Bicazului/NT	17.87	16.40	1.09	2.4105	951.6
26	Chiriţeni/NT	16.49	14.76	1.12	1.9716	1324.2
27	Cuejdi/NT	22.95	15.94	1.44	3.0063	1074.0
28	Cuejdi-Ponor/NT	23.32	15.10	1.54	2.7006	945.3
29	Izv. Muntelui-NT	20.51	14.86	1.38	2.4012	769.4
30	Neagra/NT	24.02	12.80	1.88	2.1334	992.3
31	Neguleşti/NT	25.83	13.40	1.93	2.3603	1230.5
32	Piatra Şoimului/NT	21.04	13.18	1.60	1.8831	1070.2
33	Potoci/NT	18.06	13.17	1.37	1.7229	765.3
34	Ruginești/NT	23.90	12.77	1.87	2.0611	1188.1
35	Taşca/NT	20.82	14.88	1.40	2.0788	1179.0

36	Cândeşti-5/VN	24.76	14.37	1.72	2.7621	-
37	Dumitreşti/VN	21.14	16.17	1.31	2.7248	-
38	Jitia-Cerbu/VN	22.24	11.82	1.88	1.6200	-
39	Vintileasca/VN	19.61	15.48	1.27	2.2281	-
Genotypes analyzed in 2010						
40	Strunga/IS	24.31	15.21	1.60	2.8765	130
41	Borzont/HG	22.32	14.89	1.50	2.3790	100
42	Gheorghieni/HG	19.53	16.80	1.16	2.7140	300
43	Praid/MS	20.44	15.97	1.28	2.5591	170
44	Trei Sate/MS	24.75	14.40	1.72	2.5264	190
45	Vâlcele/CJ	21.53	16.09	1.34	2.8713	120
46	Mija/DB	18.51	11.86	1.56	1.3663	220
Genotypes investigated in 2011						
47	Dorna Arini/SV	19.58	15.03	1.30	2.4551	100
48	Satu Mare/SV	22.95	14.31	1.60	2.0854	390
49	Broşteni/SV	20.31	12.93	1.57	1.6683	240
50	Borca/NT	23.46	14.54	1.61	2.2809	400
Analyzed genotypes from the population on the Pietricica Mountain - Piatra Neamț (2010-2011)						
51	P1 (Pietricica Mt)	23.05	15.13	1.52	2.8493	210
52	P2	19.80	16.41	1.21	2.9209	220
53	P3	26.00	13.92	1.87	2.7670	240
54	P4	21.69	13.92	1.56	2.2440	230
55	P5	23.53	14.86	1.58	2.8478	210
56	P6	19.26	11.95	1.61	1.3333	170
57	P7	21.26	11.92	1.78	1.8106	270
58	P8	20.53	15.04	1.36	1.9135	290
59	P9	26.22	13.36	1.96	2.4208	280
60	P10	25.59	15.79	1.62	3.2309	310
61	P11	18.78	16.01	1.17	2.7821	300
62	P12	20.95	13.78	1.52	2.1568	190
63	P13	23.00	12.83	1.79	2.1240	180
64	P14	25.81	14.51	1.78	2.2544	150
65	P15	22.95	13.22	1.74	2.1801	240
66	P16	18.46	12.40	1.49	1.6347	74
67	P17	20.92	13.33	1.57	2.0396	340
68	P18	21.35	11.60	1.84	1.7201	120
Total average values		**21.44**	**13.97**	**1.53**	**2.1775**	**523.1**

*The average values calculated on our previous observations (Băra, Ghiorghiță et al., 1976/1977) of 32 local populations of dog rose (15,815hips) from 15 Romanian counties;
** Cultivated genotypes.

In order to compare our present results to the previous ones (Băra, Ghiorghiță et al., 1976/1977) we calculated an average of the values obtained more than three decades ago in the study of the same parameters in 32 native populations of dog rose (relevant data, as the number of analyzed fruits was 15,815); we may report to those values (control values) in this present study. It was ascertained that of all

the 68 genotypes that were tested during 2008-2011 only 2 genotypes (Cândeşti-1/VN and Chiriţeni/NT) display a lower average length of the fruit, 3 genotypes (Almaş-1/NT, Măgura-1/BC and P18 from the Pietricica Mountain/NT) have a lower average diameter and 5 genotypes (Almaş-1/NT, Piatra Neamţ, Măgura-1/BC, Mija/DB and P6 - Pietricica Mountain) are characterized by a lower average biomass/fruit, (Table 1). Compared to the previous study on dog rose, study that evinced an average fruit length of 17.81 mm, an average fruit diameter of 11.65 mm and an average fruit biomass of 1.4817 g, this present research revealed superior average values of these parameters, of 21.44 mm, 13.97 mm and 2.1775 g/fruit, respectively. The results are obvious, considering that during the actual investigations, the fruits belonged to a sole genotype (and were not the average value of all the genotypes from a population, as it was the case of the previous tests). On the other hand, a factor was the selection of these genotypes on certain criteria, including the fruit size. The ratio maximum length/diamater (L/D) offers data on the fruit shape. Some of the genotypes provided elongated hips, with an L/D higher than 1.7; among all these, the most representative were Sărata, Sărata-1/BC, Neguleşti/NT and P9, with an L/D ratio exceeding 1.9. On the contrary, there were certain genotypes that provided almost round hips, with an L/D close to 1.0; this was the case of the genotypes from Cheile Bicazului, Chiriţeni, Bicaz Ardeal, and P11 (the county of Neamţ) and Gheorghieni (the county of Harghita), with an L/D ratio from 1.09 and 1.17. To note the fact that, among the genotypes with elongated fruit, some were tronconic (with the fruit basis either in the inferior half, or in the superior half of the hip), other were spindle – shaped. Regarding the fresh biomass of the fruit, a number of 9 genotypes (Sărata-2/BC, Bicaz Chei, Cuejdi, P1, P2, P5 and P10/NT, Strunga/IS and Vâlcele/CJ) provided hips with an average biomass exceeding 2.8 g (2.82 and 3.23 g), (Table 1). We were not able to depict a firm correlation between the fruit length or diameter and their biomass, although the larger fruit obviously present a higher biomass. To note the fact that 45 of all the 68 investigated genotypes presented an average biomass higher than 2 grams/fruit (from this viewpoint, there is a large basis of selection).

The study of the variability of the analyzed parameters displayed that the"fruit length" is a parameter with a lower to medium variability. The variation coefficient (s%) ranged between 5.00 (the genotype P10, from the Pietricica/NT population) in 2010 to 17.37 (the genotype Ceahlău/NT), during the year 2008. The most of the studied genotypes registered values of s% lower than 10. To the amelioration of this species, it would be best that the investigated parameters should be as stable as possible. The genotypes from Bicazu Ardelean, Bâtca Doamnei-2 and -3, and P2, P8, P10 (belonging to the Pietricica population), displayed a very low variability of the „fruit length", with an s% lower than 7.0. The highest values of the variation coefficient were noticed in the genotypes

Ceahlău/NT and Gheorghieni/HG (with an s% of 17.37 and 14.37, respectively), (Ghiorghiţă et al., 2012a; 2012c). The fruit diameter in the *R. canina* genotypes was a parameter with a generally low variability, even lower than for the fruit length. The value of s% for this parameter ranged from 4.68 (the Praid genotype) and 17.19 (the Cheile Bicazului genotype). This parameter was very stable in case of the genotypes from Praid, Dorna Arini, Trei Sate, Bârzuleşti, and P13 (from the Pietricica population), that registered values of s% under 6.0. A rather higher variability of this character (though medium) was encountered in the genotypes: Bâtca Doamnei-1, P3 and P15 (the Pietricica population), with an s% between 10.0 and 17.19 (Ghiorghita et al., 2012a; 2012c). On the contrary, the individual fruit biomass is a parameter with a generally high variability, the most analyzed genotypes registered values of the variation coefficient (s%) over 20. The value of s% for the „fruit biomass" ranged from 8.14 (the genotype P18 from the Pietricica population) to 29.58 (the genotype from Cheile Bicazului). A low to medium variability (s% from 8.14 and 17.02) was noticed in the genotypes: P7, P8, P18 (from the Pietricica population), Bâtca Doamnei-3, Praid, Bicazu Ardelean, Broşteni, Izvoru Muntelui, Jitia-Cerbu, Rugineşti, Trei Sate. The opposite situation was observed for the genotypes: Cheile Bicazului, Gheorghieni, Almaş-2, Satu Mare, P6 and P14 (of the Pietricica population), in which the variation coefficient of the fruit biomass displayed values between 24.59 and 29.58 (Ghiorghiţă et al.,2012a; 2012c).

Regarding the amount of vitamin C, the previous investigations on *R. canina* L. (since 1976) revealed an average level of 547.5 mg% fresh matter. The present tests evinced an average level of vitamin C of 532.5 mg% during 2008, of 1060 mg% fresh matter in 2009, of 218.4 mg% fresh matter in 2010 and of 223.4 mg% fresh matter in 2011; as an overall conclusion, the average amount of vitamin C, during this present investigations (2008-2011), was of 523,1 mg% fresh matter.

Compared to the average values of the analyzed parameters, some of the dog rose genotypes investigated during 2008-2011 appear to be of great perspective in the amelioration of this species by the size and individual fruit biomass and by the content in vitamin C from the fresh fruit. This is the case of the genotypes: Cuejdi/NT, Bicaz Chei/NT, Gheorgheni/HG, and P10, P11 from the population on the Pietricica mountain (the fruit aspect is displayed by the Figure 1).

a) The genotype from Cuejdi (NT)

b) The genotype from Bicaz Chei (NT)

c) The genotype from Gheorghieni (HG)

d) The genotype from Cuejdi-Ponor (NT)

e) The genotype P10 (Pietricica Mountain)

f) The genotype P11 (Pietricica Mountain)

Figure 1 – The aspect of the hips belonging to several valuable genotypes of dog rose

The observations effected at the moment of harvesting the hips from various local populations proved that some of the investigated genotypes present fewer prickles (Bicaz Chei/NT) or are almost deprived of prickles (Dumitreşti/VN genotype), that some of the selected individuals display a higher density of hips/branches (those that belong to the populations Rugineşti/NT, Cândeşti-5/VN, P9, P10/NT), by hips with an uniquely disposed on branches (not in clusters)(P10, P11/NT), that there is a large array of fruit color (when the hips are mature) – from orange-reddish (Chirițeni/NT, Ceahlău/NT, Dumitreşti/VN, Gheorgheni/HG, Rugineşti/NT, P8 and P17/NT etc) to dark-cherry (Măgura-2/BC, P4, P14, P15/NT). These are genotypes and characters that may be useful in the amelioration of dog rose.

As we mentioned during the chapter „Material and methods", one of the purposes of our investigations was the study of the morpho-physiological parameters, that were analyzed within the same population of dog rose (the study of various genotypes). A population of dog rose from the North-eastern peak of Pietricica mountain (Piatra Neamț) was chosen. There were analyzed 18 genotypes of dog rose that are spread on the whole area of this mountain peak (from west to east, from the bottom to the top), that were different by the same characters that were previously mentioned. The data from Table 1 show that these genotypes displayed the following: the fruit length between 1.46 mm (P16) and 26.22 mm (P9), the maximum fruit diameter - from 11.60 mm (P18) to 16.41 mm (P2), the average fresh biomass/fruit - from 1.3333 g (P6) to 3.2309 (P10), and the amount of vitamin C - from 74 mg%g fresh matter (P16) to 340 mg% fresh matter (P17). The ratio fruit length/fruit diameter oscillated from 1.17 (P11 – genotype with round-shaped hips) and 1.96 (P9-genotype with elongated fruts).

The variability of the analyzed parameters fitted to some more reduced limits than the one previously mentioned. Therefore, s% for „the fruit length" ranged between 5.0 (genotype P10) and 11.84 (genotype P6), revealing a generally low variability of this character. Only 4 out of 18 genotypes presented a variation coefficient higher than 10. A similar case was registered for „the fruit diameter", s% ranged from 5.84 (genotype P13) to 12.93 (genotype P3); only 2 out of 18 genotypes displayed values of s% over 10, which indicates a higher stability of this parameter. For „the fruit biomass" the values of s% ranged from 8.14 (genotype P18) to 26.25 (genotype P6); 7 out of 18 studied genotypes displayed an s% over 20 (high variability), the majority displayed an average variability of this parameter, (Ghiorghiță et al., 2012c). It may be assessed that the variability of the analyzed characters is lower within the same population of dog rose, at the level of its various genotypes, compared to the case in which the genotypes belong to different populations.

The presented data lead to the existence of remarkable variations from one genotype to another (within the same population), regarding the size, shape, fruit

biomass and their amount of vitamin C. These results justify the requirement to analyze as many as possible genotypes from various populations of dog rose, to isolate some forms that are valuable for their productivity and quality, that would be cultivated and used in the amelioration of dog rose. Of all the 18 dog rose genotypes investigated within the Pietricica population (Piatra Neamț), 6 (P1, P2, P3, P5, P10, P11) provided fruit with an average biomass exceeding 2.7 g/hip. The most important genotypes regarding the fruit biomass and the amount of vitamin C in the fruit were: P10 (3.2330 g/fruit, and 310 mg/100 g fresh matter), and P11 (2.7821 g/fruit, and 300 mg/100 g fresh matter), (Table 1).

Another purpose of our investigations was the identification of a clonal multiplication method, to assure the multiplication of some valuable forms. To reach this goal, we considered that one possible way would be the *in vitro* micropropagation of this species. In this view, an *in vitro* culture of dog rose was initiated to test the response of some explants, depending on the type of explant, the growth regulators from the nutritive medium, the culture incubation details etc., and based on these observation, to elaborate a technology to provide planting material. The first *in vitro* culture initiation experiences began in the spring of 2010.We noticed that this stage was more difficult than expected. The tips of apical and axillary shoots (more often used during the tests) are very sensitive to the chemical disinfectants. During 2010 and 2011, throughout the vegetative season and apart from it, the *in vitro* culture initiation was resumed to establish the best duration and moment of explant disinfection (that would ensure aseptic shoots, and also their survival). The medium variants (starting with the basal Murashige-Skoog (1962) medium) that were used during the *in vitro* tests in dog rose are displayed by Table 2 and consisted either of the MS medium enriched with one growth regulator (cytokinin or auxin), or in combinations of cytokinins+auxins, cytokinins+auxins+giberellic acid.

The *in vitro* culture initiation was accomplished on several medium variants presented in the Table 2, and the results obtained by present showed that only the hormone-free MS and B1 medium assured the survival of about 10 - 50% of the inoculated shoots, depending on the culture initiation time period. The best results were obtained using the explants harvested at the beginning of July. The low percentage of survival required an optimal solution to diminish the microbial load of explants and reduce the time of disinfection. In this view, during the cold season of 2011, stem fragments (seedlings) of dog rose were gathered in two phases (mostly the distal part of the shoots provided on the roots during the latest vegetative season), that were kept in a room, placed in water pots for 2 weeks (meanwhile the stem buds provided shoots). It was ascertained that, though the disinfection periods (with mercury chloride and chloramine-T) were reduced considerably, the shoots tips did not survive and were affected by necrosis after the *in vitro* inoculation. Therefore, the shoot tips harvested during the summer

had a better response during the *in vitro* initiation, even if the disinfection time periods were longer, the plants' resistance to chemical agents was higher.

Table 2 – Variants of MS to test the *in vitro* morphogenetic response of some *Rosa canina* L. explants

Var.	Regulatorii de creştere prezenţi în mediul MS (mg/l)							
	BAP	2.4-D	GA	IAA	IBA	Kin	NAA	Zt
MS	-	-	-	-	-	-	-	-
A				2.0				
AZ				0.5				1.0
B1	1.0							
B2	2.0							
BA	1.0			0.5				
BB	1.0				0.5			
BD	1.0	0.5						
BG	1.0		0.5					
BK	0.5					0.5		
BN1	1.0						0.5	
BN2	2.0						0.5	
BN3	2.0						4.0	
BBG	2.0		0.05		0.5			
BGN1	1.0		0.1				0.04	
D		2.0						
KN						1.0	0.5	
N							2.0	
BDG*	2.0	0.5	0.1					
BGN2*	3.0		0.1				1.5	
BGZ*			0.1		1.0			2.0

*Variants of MS that contain ½ of the macro- and micro-elements, and a double amount of glycine

Ten days after the inoculation, the shoot tips that were resistant to the disinfection resumed their growth and generated rosettes of multiple shoots. A month later, the shoots were separated and then transferred on other medium variants. On the most medium variants displayed by the Table 2, the response of the shoots was poor. They registered a good response during the transfers from MS or B1, to BA, then on KN or BB. It was ascertained that the BB medium variant stimulated their development, the formation of new rosette-shaped multiple shoots and a callus ring surrounding the stem base. The callus was hard, globulous or horizontally-proliferative, green, average proliferation. The callus frequently provided about 4-5 new shoots/explant. Sporadically, some shoots generated roots on the BB medium variant, (Figure 2). The leaves that were in contact with the nutritive medium provided (especially at the petiole level) a similar callus to the one generated by the stem. This callus was slowly-proliferative and did not generate

shoots. The leaf fragments inoculated on the D medium variant (2 mg l^{-1} 2.4-D) thickened and produced short roots covered by many absorbant hairs.
On the nutritive medium supplemented with kinetine and NAA, the inoculated shoots either displayed a similar response to the one on BB (even poorer), or provided only callus at the contact with the nutritive medium, and the shoot disappeared. To note that the multiple shooting was present on the medium variants comprising 3 growth regulators (BDG, BGN2 and BGZ), and the mineral compounds from the MS medium were halved; the shoots were rosette-shaped, and the callus was not generated at the shoot base, as it was the case of the shoots transferred on BB, (Figure 2).
The stem callus provided on BB was passed on BB and other medium variants, to induce either its more intense proliferation, or its differentiation. Transfered on the same medium variant (BB), the callus kept its initial properties, its proliferation was average, and it sporadically grew shoots. On the BG (BAP + GA_3) medium variant, the callus had a similar response, its proliferation speed was slower than on BB; in time, the callus became shiny and turned brown, proving that it degenerates faster than on BB. The callus' transfer on the same hormonic variant (BG), but on the basal medium for woody plants (WP), induced its proliferation (poorly represented), some of its fragments provided small reddish shoots, (Figure 2). On BN (BAP + NAA) medium, the callus kept its consistency, was green or light green, with a good proliferation, it grew horizontally, but did not differentiate shoots or roots. The transfer of callus on some medium variants enriched only with auxins (2 mg l^{-1} IAA, NAA or 2.4-D) did not improve its proliferation or its differentiation. The callus was sub-cultivated on some medium variants comprising cytokinins (1-2 mg l^{-1} BAP; 0.5 mg l^{-1} BAP + 0.5 mg l^{-1} Kin) favoured a slight intensification of callus cell multiplication, but it did not induce the caulogenesis.
The shoots obtained on BB, BG, BGN, BGZ variants etc., were passed on the hormone-free MS to induce the rhyzogenesis. The shoots' enrooting was not very efficient (about 50%) and was accompanied by callus at the shoot base in some cases. The shoot transfer on KN (Kin + NAA) sporadically induced the rhyzogenesis, and the presence of callus at the stem base became a rule. During 2011, the nutritive medium was altered, the mineral elements was halved, with no improvement of the shoot enrooting. The composition and consistency of the nutritive medium need to be changed for the future experiments. Other medium types (beside MS and WP) should be used, in order to improve the *in vitro* rhyzogenesis of the dog rose shoots.

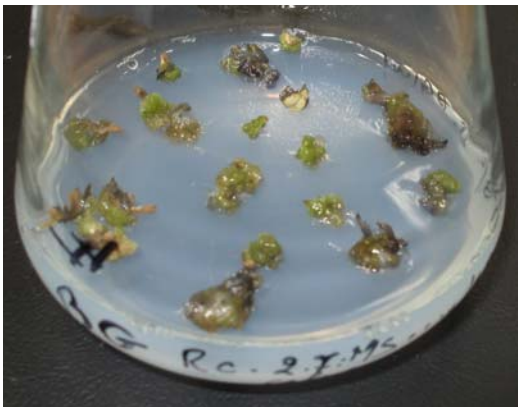
a. *In vitro* culture initiation of dog rose

b. Multiple shooting and callus on BB variant

c. Callus and shoots generated on BB variant

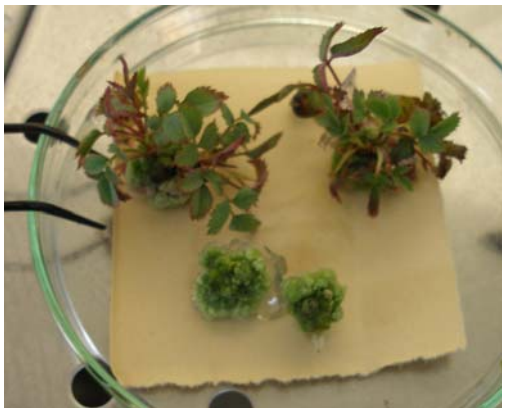
d. Shoots generated on KN variant

e) Callus and shoots (subcultivation on BG)

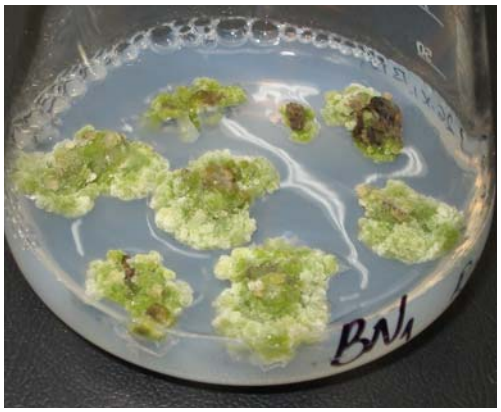


f. Callus subcultivated on WP medium, BG variant

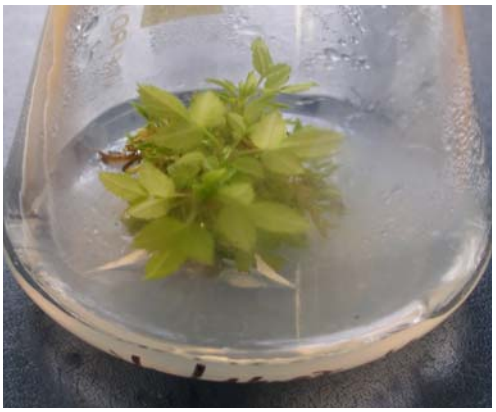
g) Globulous callus and shoot rosettes on BB variant



h) Callus and shoots obtained on BB variant



i) The aspect of callus transfered from BB on BN variant



j) Multiple shoots on BBG variant

k) Shoots and neoplantlets on KN variant

l) Multiple shooting on BGZ variant

m) Multiple shooting on BDG variant

n) Shoot rosettes on BB variant

o) Neoplantlet regenerated on hormone free MS medium

p) Neoplantlet obtained on BB variant

q) The aspect of neoplantlet's roots generated on hormone free MS medium

r) Accomodation of the *in vitro* regenerants of dog rose

Figure 2 – The aspects of the *in vitro* morphogenetic reaction of *Rosa canina* L. explants

The dog rose regenerants provided *in vitro* were acclimatized to the septic environment in a hydroponic system (Figure 2), and the acclimatization was difficult and slow. The shoots easily loose water by perspiration and about 25% of the regenerants did not survive to this process. Future investigations are required to identify some methods to improve the rate of survival for the dog rose regenerants at the moment of their transfer to the *ex vitro* environment.

Conclusions

In view of isolating some valuable genotypes of dog rose that are useful for this species' amelioration, some observations were made to investigate several morpho-physiological and biochemical parameters of the hips of 68 genotypes belonging to 43 native and one cultivated populations from 38 locations of Romania (during 2008-2011). At the same time, *in vitro* cultures of *Rosa canina* L. were started, the morphogenetic response of some explants was tested, in view of elaborating a micropropagation method for this species. The results displayed the following:

1. The average length of the mature hips of the 68 dog rose genotypes ranged between 16.49 and 26.22 mm, the average width (diameter) – between 10.92 and 16.80 mm, the ratio fruit length-width (that offers details about their shape) – from 1.09 (roundish hips) to 2.26 (elongated hips), the average biomass per fruit ranged from 1.3333 to 3.2309 grams, and the amount of vitamin C from the fresh hips – from 74 to 1324.4 mg/100 g;

2. Within the same population of dog rose (that lies on the Pietricica Mountain, Piatra Neamt), the average length of the mature hips ranged from 18.46 to 26.00 mm, the average width of the hips – from 11.60 to 16.41 mm, the average biomass/hip – from 1.3333 to 3.2309, the ratio fruit length/diameter – from 1.17 to 1.96, and the fruit amount of vitamin C: between 74-340 mg/100 g fresh matter. This data prove the need to investigate the various genotypes that form a population in order to isolate some genotypes that are valuable both for their productivity and quality, useful for this species amelioration;

3. The statistics applied to the biometrical data evinced that the fruit length and diameter display a generally low (sometimes average) variability, while the fruit biomass presents an average to high variability;

4. Considering the values of the analyzed parameters, first of all by the fruit fresh matter and their content in vitamin C, it was ascertained that of all the 68 investigated genotypes, there are a few of perspective in the amelioration of the dog rose: the genotypes from the population of Cuejdi, Cuejdi-Ponor, Bicaz Chei and Gheorghieni, and the genotypes P2, P10 and P11 belonging to the population from the Pietricica Mountain;

5. The investigations effected on the local populations of dog-rose evinced some genotypes with a particular plant architecture, with different fruit shapes and sizes, fewer prickles on their branches, with the hips uniquely attached to the branches (not in clusters) etc, forms that may be useful in the amelioration program of this species;

6. The investigations on the *in vitro* response of the dog rose showed that the initiation of *in vitro* cultures of this species may be achieved using axillary and apical shoot tips, harvested during the summer and inoculated on the hormone-free medium Murashige-Skoog (1962) or on its variants enriched with BAP or with kinetin and NAA;

7. After the initiation of the *in vitro* cultures, the surviving shoots displayed the most intense morphogenetic response by their transfer on the MS medium enriched with BAP (1 mg l^{-1}) and IBA (0.5 mg l^{-1}), a medium variant that enhanced the multiple shooting, the formation of callus at the shoot base, the indirect caulogenesis (via callus) and the rhyzogenesis (seldom). The multiple shooting was stimulated on the hormone-free MS and on other variants of MS, as well (KN, BG, BDG, BGN and BGZ);

8. The stem callus provided on BB or on KN (at the stem base) was compact, green and average-proliferative. The callus evolved well by its subculture on BB, and on other variants as well (BK, BN, D), variants that did not enhance its differentiation (neither caulogenesis nor rhyzogenesis appeared);

9. The acclimatization of the dog rose *in vitro* regenerants to the *ex vitro* environment takes a longer period of time compared to other species (about 3 weeks) and a high air humidity. The loss of regenerants during acclimatization may rise considerably if this condition is disregarded;

10. In view of micropropagating the dog rose, there are needed more thorough investigations, some tests of new medium variants to stimulate the direct caulogenesis (and avoid the formation of callus), and the enrooting of shoots, finding new efficient solutions for the acclimatization of the regenerants to the *ex vitro* conditions; these observations will represent solid arguments to establish an efficient technology for the micropropagation of the dog rose.

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