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PRIVILEGE OF SELLECTED BIOTYPES OF WILD APPLES (*Malus sylvestris* Miller) FOR THE PRODUCTION OF GENERATIVE ROOTSTOCKS

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Abstract

The aim of this work is to produce the generative rootstocks for the wild apples which will be of moderate denseness but also resistant to drought. The initial material is the population of wild apple (*Malus sylvestris* Miller) in the area of Polimlje.

The study focused on few segments. Very first one included recording of the phenological traits - first flowering, full flowering, end of flowering and harvest period. The other segment comprised pomological, i.e. physical [fruit weight (g), fruit size (mm), mass of dry seed (g), and number of seed in 1 kg of the fruit]. Fruit mass and mass of 100 pieces of dry seeds were determined by measuring via the electric scale Metler 1200. The result is shown in grams with the accuracy of 0.01 g. Fruit dimensions - length and width were measured by vernier scale. Seeds from 9 selected biotypes of wild apples (*Malus sylvestris* Miller) were planted in the nursery and raised seedlings were evaluated for nursery characteristics: germination, seedling verdure, uniformity and branching. Raised seedlings were used as rootstocks for scion cultivar 'Red Delicious'. The dynamics of leaf dehydration per measured interval was determined by method of Eremeev [1].

The results of this research show that the plant height, stem diameter (corpulence), branching and uniformity of one-year old seedlings of selected biotypes of wild apple are genetic characteristics of selected biotypes of wild apple, from which rapid growth and uniformity of scions depend. Biotype 2, 8 and 9 can be suggested for the production of generative rootstocks.

The most interesting are very small fruit: Biotype 9 (8.95 g) and Biotype 8 (12.75 g). The highest water attaining capability had the leaves of selected Biotype 2 of wild apple. Over the monitored time interval (8 hours upon sample taking), leaves taken from the annual twigs of the studied selected biotypes of wild apples lost on average 32.44% of water.

Key words: *Growth, Uniformity, Generative rootstock, Selected biotypes, Resistance to drought.*

1. Introduction

The centre of diversity of the genus *Malus* is situated in East Asia. The diversity of wild and cultivated apples, as a whole, represents a great pool of traits for multiple use. This irretrievable diversity is to be preserved now and in the future by gene banks (Büttner *et al.*, [2]). The cultivated apple (*Malus x domestica* Borkh.) is not a simple taxonomic group and includes all the cultivated biotypes in the genus *Malus Mill*. In the 1920s, Vavilov [3], travelled through Central Asia and found large wild stands of M. *sieversii* in specific localities and proposed that the region could be a centre of origin of the domesticated apple. Another early study based on morphological characteristics suggested that several *Malus* species, including: *M. sylvestris* Miller, *M. prunifolia* (Willd.) Borkh., and *M. baccata* (L.) Borkh., were involved in the origin and/or domestication of the cultivated apple (Rehder [4]).

The capacity of *Rosaceae* species for interspecific hybridization, even beyond genus borders, has been exploited in breeding programmes to incorporate desirable traits of wild populations into breeding gene pools. Hybridization between fruit crops and their wild relatives has probably also occurred 'spontaneously' and individuals with intermediate phenobiotypes are known to occur throughout the European landscape. The importance of (anthropogenic induced) hybridization processes has been underestimated by conservation biologists

until recently (Allendorf *et al*., [5]). However, it is becoming more and more apparent that hybridization has led to the extinction of many populations and species and represents a severe threat especially to rare species that come into contact with other, more abundant species (Rhymer and Simberloff [6]).

Apple is the most important temperate fruit crop and has been cultivated in Europe and Asia from antiquity (Janick [7]). Apple generative rootstocks are used world-wide due to better adaption of different species of the genus *Malus* to various environmental conditions, very often to extremely harsh conditions. Generative rootstocks of pome fruits are in principle less exposed to virus attacks, since they cannot be transmitted during seed reproduction (Misic [8]). Wild apples of Montenegro have never been a subject of a comprehensive research work, neither a subject of collecting and studying processes. Nowadays, the most important issue is how to preserve it, especially since many local populations have vanished during its development or are reduced to a rather limited number of genotypes. Nevertheless, germ-plasma of wild apple in less urban areas, such as the area of Polimlje, is rather preserved.

2. Materials and Methods

The investigations were conducted continuously in the period from 2013, 2014 to 2015. They involved in situ identification, marking and careful observation of selected biotypes of wild apples (*Malus sylvestris Miller*), accessions, in the area of Polimlje.

During the fall of 2012, from the population of wild apple (*Malus syvestris Miller*) nine biotypes were selected, which were of moderate verdure in comparison to other biotypes existing in this population. Trees of 4 autochthonous apple varieties were considered as comparative parameters. The study focused on few segments. Very first one included recording of the phenological traits - first flowering, full flowering, end of flowering and harvest period. Phenological characteristics were determined as below: the beginning of flowering was recorded when at least 5% of the flowers bloomed; full flowering was accepted when at least 80% of the flowers bloomed, the end of flowering was determined when 90% of the flowers bloomed and corollas began to fall off, and harvest period was established when the seed in fruits were sufficiently collared.

Particular attention was given to the fact that they were to be located on different sites, namely different altitudes and that they needed to be healthy individual trees of full productivity with fruits having more than 8 well developed seeds. The other segment comprised pomological, i.e. physical [fruit weight (g), fruit size (mm), mass of dry seed (grams), and number of seed in 1 kg of the fruit. Fruit mass and mass of 100 pieces of dry seeds were determined by measuring via the electric scale „ Metler 1200“. The result is shown in grams with the accuracy of 0.01g. Fruit dimensions - length and width were measured by Vernier scale. The results are shown in mm. Selection of seeds is conducted according to the method of Stankovic and Jovanovic [9]. Extraction of seeds was performed manually. The method according to Misic [8], stipulates for small amounts of seeds to be extracted manually. Drying and storage of seeds were performed in shade with good air circulation. Obtained results were statistically processed by the method of variance analysis and checked by LSD tests.

Seeds from 9 selected biotypes of wild apples (*Malus sylvestris* Miller), accessions, were planted in the nursery and raised seedlings were evaluated for nursery characteristics: germination, seedling verdure, uniformity and branching. Raised seedlings were used as rootstocks for scion cultivar ‘Red Delicious’ apple. Uniformity was low (grade 1) when coefficient of variation was less than 15%, medium (grade 2) when it was from 15 to 25% and high (grade 3) when exceeding 25%.

Examples of leaves for analysis will be taken when it is dry weather: three times a year - at the end of June, the end of July and the end of August. The dynamics of leaf dehydration per measured interval was determined by method of Eremeev [1]. The loss of water due to transpiration followed by measuring the weight of leaves Slavik [10]. The dynamics of leaf dehydration was measured in order to obtain initial resistance rate of selected biotypes of wild apples (*Malus sylvestris* Miller) towards drought conditions. The dynamics of leaf dehydration of mother tree (in site) depends on the thickness of leaf cuticle and leaf average size.

3. Results and Discussion

3.1 Results

During the elaboration period of three years, Table 1 shows that the beginning (onset) of flowering was recorded in the selected 9 biotypes of wild apple in the average from May 2nd to May 10th.

By annual observation, the earliest onset flowering was recorder in the selected biotypes of wild apple ‘Biotype 9' (May 30th 2015) and the selected biotypes of wild apple 'Biotype 3 and Biotype 7' (May 13th 2014). The full flowering stage was recorded on May 8th 2015 ('Biotype 1' and ' Biotype 5'), and on May 20th 2014 ('Biotype 3' and ' Biotype 7'), and the end of flowering on May 13th 2015 ('Biotype 2' and 'Biotype 7') and May 30th 2014 ('Biotype 3' and ' Biotype 9'). Flowering lasted in the average 13 days ('Biotype 2' and ' Biotype 6') and 21 days ('Biotype 5'). The earliest end of flowering was recorded on May 13th 2015 in the selected biotypes 'Biotype 2' and 'Biotype 7' and the latest end of floren-

ing was recorded on May 30th 2014 in the selected biotypes 'Biotype 3' and 'Biotype 9'.

Concerning the autochthonous apple cultivars (control), the earliest onset of flowering was with 'Pasinka' (April 21st 2015) and the latest was with 'Sarenika' (May 3rd 2014). The earliest end of flowering occurred with the cultivar 'Pasinka' (May 9th 2015) and the latest with the cultivar 'Senabija' (May 25th 2015). During the observed period, the flowering phenophase of autochthonous cultivars lasted in average to 17 days for cultivar 'Pasinka', up to 24 days for cultivar 'Senabija'. The observation showed that the all selected biotypes of wild apple have adapted their flowering phenophase to the conditions of Polimlje. Flowering phenophase starts at the moment when the danger of late spring frosts is gone or is significantly reduced.

Table 1. Phenological characteristics of selected biotypes of wild apples (*Malus sylvestris* Miller) in Polimlje (year of 2013, 2014, 2015 and total average)

Biotype / Cultivar	location			flowering				harvest
	longit.	latitude	alt. (m)	onset	full	end	duration	date
Biotype 1	19 ° 20′ E	42° 38′N	579	7.V.2013 9.V.2014 3.V.2015 **6.V**	13.V.2013 16.V.2014 8.V.2015 **12.V**	23.V.2013 26.V.2014 16.V.2015 **21.V**	16 17 13 **15**	First ten days in November
Biotype 2	19 ° 59′ E	43° 02′N	1180	9.V.2013 11.V.2014 6.V.2015 **9.V**	13.V.2013 18.V.2014 12.V.2015 **14.V**	23.V.2013 29.V.2014 13.V.2015 **22.V**	14 18 7 **13**	First ten days in November
Biotype 3	19 ° 55′ E	42° 33′N	940	10.V.2013 13.V.2014 6.V.2015 **10.V**	16.V.2013 20.V.2014 17.V.2015 **18.V**	27.V.2013 30.V.2014 25.V.2015 **27.V**	17 17 19 **18**	First ten days in November
Biotype 4	18 ° 49′ E	42° 26′N	858	4.V.2013 6.V.2014 2.V.2015 **4.V**	10.V.2013 13.V.2014 10.V.2015 **11.V**	20.V.2013 22.V.2014 18.V.2015 **20.V**	16 16 16 **16**	End -October
Biotype 5	19 ° 55′ E	42° 33′N	940	4.V.2013 7.V.2014 1.V.2015 **4.V**	12.V.2013 15.V.2014 8.V.2015 **12.V**	25.V.2013 28.V.2014 21.V.2015 **25.V**	21 21 20 **21**	End - October
Biotype 6	19 ° 29′ E	42° 51′N	984	8.V.2013 12.V.2014 7.V.2015 **9.V**	10.V.2013 14.V.2014 9.V.2015 **11.V**	15.V.2013 18.V.2014 14.V.2015 **16.V**	14 18 7 **13**	First ten days in November
Biotype 7	19 ° 20′ E	42° 38′N	978	10.V.2013 13.V.2014 1.V.2015 **8.V**	18.V.2013 20.V.2014 9.V.2015 **16.V**	27.V.2013 29.V.2014 13.V.2015 **23.V**	17 16 12 **15**	First ten days in November
Biotype 8	19 ° 43′ E	42° 59′N	601	8.V.2013 11.V.2014 2.V.2015 **7.V**	16.V.2013 19.V.2014 10.V.2015 **15.V**	25.V.2013 29.V.2014 21.V.2015 **25.V**	17 18 19 **18**	First ten days in November
Biotype 9	19 ° 59′ E	42° 70′N	970	3.V.2013 5.V.2014 30.IV.2015 **2.V**	17.V.2013 19.V.2014 12.V.2015 **16.V**	27.V.2013 30.V.2014 21.V.2015 **26.V**	14 18 21 **18**	First ten days in November
Senabija (control)	19 ° 43′ E	42° 59′N	601	25.IV.2013 27.IV.2014 27.IV.2015 **26.IV**	9.V.2013 12.V.2014 7.V.2015 **9.V**	17.V.2013 20.V.2014 25.V.2015 **21.V**	22 23 28 **24**	End - October
Arapka (control)	19 ° 41′ E	41° 01′N	879	28.IV.2013 30.IV.2014 22.IV.2015 **30.IV**	8.V.2013 10.V.2014 4.V.2015 **7.V**	15.V.2013 18.V.2014 10.V.2015 **14.V**	17 18 18 **18**	End - October
Pasinka (control)	19 ° 29′ E	42° 50′N	974	26.IV.2013 30.IV.2014 21.IV.2015 **26.IV**	7.V.2013 10.V.2014 3.V.2015 **7.V**	13.V.2013 16.V.2014 9.V.2015 **13.V**	17 16 18 **17**	End - October
Sarenika (control)	19 ° 43′ E	42° 59′N	601	30.IV.2013 3.V.2014 24.IV.2015 **29.IV**	9.V.2013 12.V.2014 6.V.2015 **9.V**	20.V.2013 23.V.2014 13.V.2015 **19.V**	20 20 19 **20**	Mid - October
			LSD 005 LSD 001	4.58 5.84	2.45 3.54	4.13 5.11	2.15 2.95	

Pomological characteristics of fruit of selected biotypes of wild apples (*Malus sylvestris* Miller) in the area of Polimlje are given in Table 2. Fruit mass is an inherited genetic characteristic. Significant differences were noticed when comparing biotypes and controlled autochthonous varieties. The highest average fruit mass of selected biotypes of wild apples was recorded with the 'Biotype 2' (33.65 g) and the lowest with the 'Biotype 9' (8.95 g). The analysis of variance for the fruit mass parameter, where the source of variation is biotypes and varieties, recorded statistically very significant difference. This means that we can expect such fruit mass with the observed biotypes and varieties in future, with the probability of 99 %. The analysis of variance for the fruit mass parameter, where the source of variation is interaction biotype x year did not show statistically significant differences. This means that years of researches, respectively their climate characteristics do not have statistically significant or relevant impact. Therefore, the mentioned parameter is conditioned by the biotype and years and does not impact the differences between the observed biotypes or controlled varieties. During the research period, the variance coefficient (CV) for the fruit mass parameter, which was calculated on the basis of registered individual measurement indicators, was at the level of 5.29%. In terms of generative rootstock production, wild apple with generally smaller fruit mass is more commercial in comparison to the autochthonous apple varieties. The most commercial biotypes are: 'Biotype 9' (8.95 g), 'Biotype 5' (11.34 g), ' Biotype 8' (12.75 g) and the ' Biotype 3' (12.81 g).

Fruit length and width are morphological characteristics that mostly depend on genotypes. The longest average fruit length of selected biotypes of wild apples amounts to 38.19 mm ('Biotype 6') and the shortest is 22.12 mm ('Biotype 9').

Table 2. Average fruit mass and fruit length of selected biotypes of wild apples

Biotype /Cultivar	fruit mass (g)				fruit length (mm)			
	2013	2014	2015	average	2013	2014	2015	average
Biotype 1	18.04	17.52	17.83	17.79	31.72	31.64	31.71	31.68
Biotype 2	33.62	33.51	33.82	33.65	36.37	35.82	36.45	36.21
Biotype 3	13.03	12.26	13.11	12.81	27.02	26.81	26.69	26.83
Biotype 4	17.25	17.02	17.41	17.22	33.72	33.63	33.81	33.72
Biotype 5	11.31	11.02	11.69	11.34	26.15	26.13	26.31	26.21
Biotype 6	24.17	26.22	27.14	25.84	37.52	38.35	38.69	38.19
Biotype 7	29.51	27.62	30.35	29.16	35.91	35.53	37.02	36.15
Biotype 8	13.41	12.82	12.02	12.75	27.15	26.82	23.84	25.94
Biotype 9	9.05	7.78	10.02	8.95	22.15	21.73	22.49	22.12
Senabija	164.8	165.8	160.91	163.8	61.91	61.11	62.01	61.61
Arapka	116.4	121.1	119.31	118.9	55.91	57.11	57.41	56.81
Pasinka	122.9	127.7	126.21	125.6	66.81	67.51	67.91	67.41
Sarenika	148.5	150.2	151.51	150.1	77.91	79.25	80.05	79.06
LSD	0.05	0.01			LSD	0.05	0.01	
Biotype	2.78	3.69			*Biotype*	0.85	1.13	
Year	1.34	1.77			*Year*	0.41	0.54	
Biotype x Year	4.82	6.39			*Biotype x Year*	1.47	1.95	
	CV fruit mass = 5.29 %				CV fruit length = 2.17%			

Table 3 shows information for fruit width of selected biotypes of wild apples. The biggest average fruit width of selected biotypes of wild apples amounts to 42.81 mm (' Biotype 2') and the smallest is 27.78 mm (' Biotype 9').

In Table 4 are given information for mass of dry seed and number of seed in 1 kg of the fruit. Mass of dry seed (100 pieces) was 2.07 g with the ' Biotype 3' up to 3.83 g with the 'Biotype 8'. Regarding the volume of reserve material necessary for germination, the best predispositions are with the ' Biotype 8' (3.83 g) and the 'Biotype 3' (3.66 g). Variation coeffitient for the dry seed parameter amounted to 4.62 %, which indicates material homogeneity in observed biotypes. Analysis of variance for the seed mass parameter in respect to biotypes and varieties, as to the source of variation, determined a statistically significant difference. This means that we can expect such seed mass with the observed biotypes and varieties in future, with the probability of 99 %. In respect to the years, as to the source of variations, there were no statistically significant differences.

Data on the average number of seeds in 1 kg of dry seeds were obtained on the basis of the mass of 100 pieces of dry seeds and weight calculations regarding 1 kg. The average number of seeds in 1 kg of seeds fluctuates from around 22725 with the cultivar ' Senabija ' (controlled variety) to 48189 with the 'Biotype 3'.

Table 3.Average fruit width of selected biotypes of wild apples (mm)

Biotype /Cultivar	fruit width (mm)			
	2013	2014	2015	average
Biotype 1	37.24	37.17	37.21	37.21
Biotype 2	44.07	40.15	44.21	42.81
Biotype 3	31.35	31.42	31.39	31.35
Biotype 4	35.19	35.03	35.24	35.15
Biotype 5	30.61	30.39	30.11	30.69
Biotype 6	39.85	40.17	40.16	40.06
Biotype 7	44.02	42.96	43.02	43.33
Biotype 8	34.05	33.68	32.81	33.51
Biotype 9	27.91	27.35	28.09	27.78
Senabija	73.51	74.41	69.91	72.61
Arapka	48.61	50.31	49.41	49.43
Pasinka	68.51	69.41	69.01	68.97
Sarenika	81.51	81.95	81.91	81.78
LSD	0.05	0.01		
Biotype	0.82	1.09		
Year	0.39	0.52		
Biotype x Year	1.42	1.88		

CV fruit width = 1.91%

Table 4. Mass of dry seed and number of seed in 1 kg of the fruit

Biotype / Cultivar	mass of dry seed (100 piece)				number of seed in 1 kg			
	2013	2014	2015	average	2013	2014	2015	average
Biotype 1	2.715	2.783	2.811	2.769	36832	35932	35574	36113
Biotype 2	2.653	2.705	2.715	2.691	37679	36969	36832	37160
Biotype 3	2.105	2.007	2.117	2.076	47506	49826	47237	48189
Biotype 4	3.015	3.102	3.125	3.081	33167	32237	32000	32468
Biotype 5	3.076	3.104	3.117	3.099	32509	32216	32082	32269
Biotype 6	3.716	3.866	3.422	3.668	26911	25867	29223	27334
Biotype 7	3.236	3.322	3.389	3.315	30902	30102	29507	30170
Biotype 8	3.942	3.887	3.667	3.832	25368	25726	27271	26122
Biotype 9	3.219	3.261	3.342	3.274	31065	30665	29922	30551
Senabija	4.808	4.219	4.224	4.417	20799	23702	23674	22725
Arapka	3.775	3.704	3.358	3.612	26491	26998	29779	27756
Pašinka	3.607	3.629	3.885	3.707	27724	27556	25741	27007
Šarenika	4.258	4.535	4.325	4.372	23485	22051	23121	22886
LSD	0.05	0.01			LSD	0.05	0.01	
Biotype	0.26	0.36			*Biotype*	1913.31	2592.88	
Year	0.13	0.17			*Year*	919.12	1245.58	
	CV dry seed mass = 4.62 %				CV number of seed per kg = 3.68 %			

In Table 5 and Table 6 are given information for the: seed germination, plant height, stem diameter or corpulance, branching and uniformity of rootstocks. The results of this research show that the: seed germination, plant height, stem diameter, branching and uniformity of rootstocks are genetic characteristics of selected biotypes of wild apple, from which rapid growth and uniformity of scions depend.

The results depicted in the Table 5 show that the average height of seedlings of the selected biotypes of wild apples was in the range from 47.1 cm ('Biotype 9') to 66.9 cm ('Biotype 1'). The average seedling height at the control autochthonous sort was in the range from 35.2 cm ('Pasinka') up to 62.4 cm ('Senabija').

The average stem diameter (corpulence) of the selected biotypes of wild apples was in the range from 5.45 mm ('Biotype 9') up to 7.41 mm ('Biotype 3'). The average corpulence of the seedlings at the control autochthonous sort was in the range from 5.32 mm ('Pasinka') up to 6.98 mm ('Senabija').

Table 5. Seed germination and plant height of generative rootstocks

Biotype / Cultivar	Seed germination (%)				Plant height (cm)				Stem diameter (mm)			
	2013	2014	2015	Ave.	2013	2014	2015	Ave.	2013	2014	2015	Ave.
Biotype 1	77	80	88	81.7	60.5	60.5	80.2	66.9	6.35	8.35	7.52	7.41
Biotype 2	83	88	76	82.3	64.2	63.5	50.1	59.3	7.37	6.42	7.25	7.01
Biotype 3	64	69	74	69	51.5	72.2	66.4	63.4	5.98	7.91	7.35	7.08
Biotype 4	74	79	82	78.3	61.9	67.2	54.8	61.3	7.31	7.79	5.85	6.98
Biotype 5	63	67	72	67.3	69.3	58.6	68.3	65.4	6.92	7.05	6.18	6.72
Biotype 6	85	89	81	85	59.5	47.3	49.1	51.9	7.09	5.33	5.42	5.95
Biotype 7	65	68	74	69	42.8	59.5	51.6	51.3	4.98	6.81	5.89	5.89
Biotype 8	90	91	86	89	62.5	46.8	69.3	59.5	6.72	5.25	7.32	6.43
Biotype 9	85	83	80	82.7	43.3	47.5	50.6	47.1	5.01	5.53	5.82	5.45
Senabija	85	88	81	84.7	67.5	57.2	62.4	62.4	7.29	6.71	6.95	6.98
Arapka	81	84	90	85	45.7	52.5	56.5	51.6	5.37	6.25	6.85	6.15
Pašinka	73	77	82	77.3	39.5	29.1	36.8	35.2	5.71	4.62	5.63	5.32
Šarenika	75	78	85	79.3	59.8	61.9	59.2	60.3	6.67	7.01	6.81	6.83
LSD	0.05	0.01			LSD	0.05	0.01		LSD	0.05	0.01	
Biotype	0.11	0.19			*Biot.*	7.59	10.1		*Biot.*	0.72	0.96	
Year	0.01	0.17			*Year*	3.64	4.83		*Year*	0.35	0.46	
Biotype x Year	0.20	0.29			Biot. x Year	13.1	17.4		Biot. x Year	1.25	1.66	
	CV seed germination = 4.52%				CV plant height = 25.21%				CV stem diameter = 12.04%			

Table 6. Branching, verdure and uniformity of generative rootstocks

Biotype /Cultivar	branching	verdure	Uniformity of generative rootstocks		
			CV (%) Plant height	CV (%) Stem diameter	Level of uniformity
Biotype 1	4	very large	26.8	20.4	3
Biotype 2	2	medium	12.9	9.95	1
Biotype 3	2	medium	13.2	11.5	1
Biotype 4	3	medium	13.1	10.9	1
Biotype 5	3	large	18.9	16.9	2
Biotype 6	2	medium	11.9	10.2	1
Biotype 7	3	medium	13.9	11.5	1
Biotype 8	1	large	19.1	17.5	2
Biotype 9	2	lowly	8.1	7.2	1
Senabija	2	large	18.5	15.6	2
Arapka	2	lowly	9.5	7.3	1
Pasinka	1	lowly	8.9	7.9	1
Sarenika	2	medium	12.1	10.2	1

CV - coefficient of variation

While comparing verdure and uniformity of seedlings, we can observe that the most voluminous or verdurous seedlings were the ones of the Biotype 1. At the same time, these seedlings were the most ununiformed ones. Biotype 1 is situated at the altitude of 579 m above sea level. This height is suitable for growing of autochthonous apple varieties, i.e. this genotype can be pollinated with other genotypes of resident wild apples, as well as and late-flowering autochthonous and standard varieties. This wide variety of pollinators led to the fact that this genotype gives generative rootstocks with high level of heterozygosity, while the verdure level is very high. Biotype 2, 3 and Biotype 7 have the medium variability of seedlings, which can be concluded by the fact that they are late - flourishing, hence the selection of pollinators is significantly smaller. All of this caused these biotypes to pollinate with rare genotypes that flourish simultaneously as they do, resulting in their seedlings being of medium variability. Biotype 9 has low level of seedling verdure, small variability of produced seedlings, regardless of the fact it belongs to the early flourishing varieties of the wild apple. Biotype 9 is situated at an altitude of 970 m above sea level, so the assumption is that its selection of pollinators could be significantly smaller.

Table 7. Bud take, grow and uniformity

Biotype / Cultivar	Characteristics of scions								
	Bud take (%)				Grow of scions (cm)				Uniformity of scions
	2013	2014	2015	Average	2013	2014	2015	Average	
Biotype 1	90	95	93	92.7	142.7	134.5	135.5	137.6	3
Biotype 2	93	95	92	93.3	121.9	122.1	134.4	126.1	1
Biotype 3	92	90	90	90.7	119	118.2	103.5	113.6	1
Biotype 4	90	90	91	90.3	118.7	103.3	118.7	113.6	1
Biotype 5	93	93	90	92	119.6	116.1	105.1	113.6	2
Biotype 6	88	91	93	90.7	110.8	113.5	109.1	111.1	1
Biotype 7	89	88	84	87	102.7	112.8	99.8	105.1	1
Biotype 8	88	91	89	89.3	124.2	114.8	111.8	116.9	2
Biotype 9	84	90	91	88.3	96.5	113.2	102.2	103	1
Senabija	80	86	81	82.3	119.5	118	146.1	127.9	2
Senabija	78	81	82	80.3	106.4	103.1	96.8	101	1
Pasinka	91	87	83	87	90.1	99.5	94	94.5	1
Sarenika	78	76	79	87	111.4	117.5	115	114.6	1
LSD	0.05	0.01			LSD	0.05	0.01		
Biotype	0.08	0.11			*Biotype*	8.6	11.2		
Year	0.04	0.05			*Year*	4.74	5.93		
Biotype x Year	0.15	0.19			*Biotype x Year*	14.2	18.5		
	CV bud take = 5.14 %				CV grow of scion = 8.53%				

The results depicted in the Table 8 show dynamics of leaf dehydration per measured interval of the selected biotypes of wild apples.

The results of this research also showed that the water attaining capability of the leaves in one-year old seedlings of selected biotypes of wild apple as an indicator

Table 8. Dynamics of leaf dehydration per measured interval (2013, 2014, 2015 and average) %

Biotype /Cultivar		Measured interval					
		1h	2h	4h	8h	16h	24h
Biotype 1	2013	8.07	17.54	21.03	34.67	61.43	100
	2014	8.03	17.16	25.9	39.17	65.57	100
	2015	8.17	17.65	21.25	34.79	75.04	100
	average	**8.09**	**17.45**	**22.73**	**36.21**	**62.83**	**100**
Biotype 2	2013	7.83	17.19	20.32	32.07	57.2	100
	2014	7.7	17.09	20.13	31.9	56.74	100
	2015	7.95	17.47	20.75	32.76	57.79	100
	average	**7.83**	**17.25**	**20.4**	**32.44**	**57.24**	**100**
Biotype 3	2013	8.07	18.25	23.2	35.68	63.45	100
	2014	8.02	17.30	22.15	34.55	62.02	100
	2015	8.21	18.44	23.46	36.05	63.73	100
	average	**8.1**	**18**	**22.94**	**35.43**	**63.07**	**100**
Biotype 4	2013	8.17	17.81	23.18	36.3	63.79	100
	2014	8.16	17.78	22.82	35.46	62.69	100
	2015	8.31	17.85	23.22	36.35	63.92	100
	average	**8.22**	**17.82**	**23.08**	**36.04**	**63.47**	**100**
Biotype 5	2013	8.44	18.84	24.01	36.03	65.64	100
	2014	8.41	18.71	23.74	35.74	64.87	100
	2015	8.52	19.1	24.48	36.62	66.26	100
	average	**8.46**	**18.89**	**24.08**	**36.13**	**65.59**	**100**
Biotype 6	2013	8.73	19.19	24.73	37.32	65.54	100
	2014	8.67	18.54	24.02	36.61	64.95	100
	2015	8.85	19.4	25.04	35.93	64.47	100
	average	**8.75**	**19.04**	**24.59**	**36.61**	**64.98**	**100**
Biotype 7	2013	8.32	18.68	24.58	37.03	64.53	100
	2014	8.17	18.28	24.06	36.42	63.9	100
	2015	8.4	17.5	23.6	36.16	63.83	100
	average	**8.3**	**18.16**	**23.86**	**36.32**	**63.87**	**100**
Biotype 8	2013	8.92	19.07	23.58	36.22	64.6	100
	2014	8.66	18.67	23.09	34.51	61.91	100
	2015	9.1	19.6	24.33	37.19	65.79	100
	average	**8.89**	**19.11**	**23.66**	**35.97**	**64.1**	**100**
Biotype 9	2013	7.68	17.11	21.17	34.69	60.59	100
	2014	7.64	16.98	20.99	34.37	60.24	100
	2015	8.01	17.91	22.21	35.91	61.88	100
	average	**7.78**	**17.34**	**21.46**	**34.99**	**60.9**	**100**
Senabija	2013	8.57	20.01	25.38	40.4	69.15	100
	2014	8.29	19.59	24.78	39.53	68.63	100
	2015	8.82	20.41	25.92	41.13	70.54	100
	average	**8.56**	**20**	**25.36**	**40.35**	**69.44**	**100**
Arapka	2013	8.93	20.72	26.54	40.71	71.43	100
	2014	8.67	20.33	25.98	39.97	70.32	100
	2015	9.16	21.14	26.99	41.26	72.32	100
	average	**8.92**	**20.73**	**26.5**	40.64	71.35	**100**
Pasinka	2013	7.9	19.53	24.83	38.26	70.57	100
	2014	7.34	18.79	23.84	36.89	68.82	100
	2015	7.91	19.78	25.09	39.1	71.98	100
	average	**7.72**	**19.37**	**24.59**	**38.09**	**70.46**	**100**
Sarenika	2013	8.92	19.07	23.58	36.22	64.6	100
	2014	8.07	18.2	24.59	41.9	76.1	100
	2015	8.28	18.58	25.24	42.72	77.36	100
	average	**8.42**	**18.61**	**24.46**	**40.27**	**72.68**	**100**
	LSD0.05	0.15	0.21	0.82	1.42	1.75	
	LSD0.01	0.27	0.29	0.88	1.49	1.88	

of their resistance to drought was genetic characteristics of the selected biotypes. Out of the studied selected biotypes of wild apples, the highest water attaining capability had the leaves of selected Biotype 2 of wild apple. Over the monitored time interval (8 hours upon sample taking), leaves taken from the annual twigs of the studied selected biotypes of wild apple (accessions) lost on average 32.44% of water. The lowest level of the stated capability was recorded with the leaves of selected Biotype 6 of wild apple (36.61%). The dynamics of leaf dehydration was measured in order to obtain initial resistance rate of selected biotypes of wild apple (*Malus sylvestris* Miller) towards drought conditions. The dynamics of leaf dehydration of mother tree (in situ) depends on the thickness of leaf cuticle and leaf average size. With the aspect of production of generative rootstock resistant to drought, all selected biotypes of wild apple (*Malus sylvestris* Miller) have higher resistance to drought than the four autochthonous control sorts.

3.2 Discussion

Researches of Krgovic [11], show that fruit height and width are not directly proportional to the fruit mass, while, according to Brown [12], and Misic [13], the shape of fruit and its size indicate a polygenetic mode of inheritance. Rudloff and Schmidt [14], determined that there is no link between the fruit weight and number of seeds. In our study, fruit mass is an inherited genetic characteristic. The largest fruit mass of selected biotypes of wild apple was recorded with the 'Biotype 2' (33.65 g) and the smallest with the 'Biotype 9' (8.95 g). Fruit length and width are biological characteristics that depend on genotype. The longest average fruit length of selected biotypes of wild apple amounts to 38.19 mm ('Biotype 6') and the shortest is 22.12 mm ('Biotype 9'). The widest average fruit width of selected biotypes of wild apple amounts to 42.81 mm ('Biotype 2') and the narrowest is 27.78 mm ('Biotype 9'). Regarding the generative rootstock production, wild apple with the least fruit mass is more commercial than the controlled autochthonous apple varieties.

Apple seeds can be: small, medium, and large (Adamic *et al*., [15]). Stankovic [16], states that the seed quality is resembled in its morphological and biological characteristics, where the seed size is the most important morphological characteristic, but equal development of seedlings and their resistance depend on biological characteristics (potentials). Seed germination, growing of seedlings and their normal development are also influenced by the seed size, since cotyledons of larger seeds contain more reserve organic materials (Stankovic and Jovanovic, [17]). 1 kg of seeds contains 30,000 - 50,000 pieces of seeds, states Kurindi, but according to Slovic [18], such number is 30,000 - 35,000. According to Misic [19], 100 kg of medium-sized wild apple fruits give around 1.1 kg of seeds. 1 kg of seeds contains 20,000 - 40,000, or in average 30,000, seeds. Our results correspond to the average of previously quoted authors.

Having in mind the production of generative rootstocks in the Western Europe countries, Misic [8, concludes that increasing attention is being paid to fruit trees - pollinators, since only certain hybrid combinations can give at the same time the seeds and seedlings of the high quality. In our study, 'Biotype 9' has low level of seedling verdure, small variability of produced seedlings, regardless of the fact it belongs to the early flourishing varieties of the wild apple. 'Biotype 9' is situated at an altitude of 970 m above sea level, so the assumption is that its selection of pollinators could be significantly smaller.

The borders of growth, the start, the course and the duration of seedling phenophase and their variability have been primarily conditioned by genotype characteristics, which are influenced by external factors (Stampar [20]).

The results of the research conducted by Salipurovic and Dzamic [21], indicate that the mutual relation between the cultivar and the rootstock influence some vegetative parameters and apple yields. The results of our research also show that the rootstock influences the characteristics of produced scions, i.e. the height of the scion and its branching directly depend on the genetic characteristics of the used rootstocks.

The papers of Nenadovic - Mratinic [22 and 23] researched the water regime of larger number of fruit species (strawberry, walnut, peach), with the aim of determining the level of drought resistance. Likewise, by conducting researches on great number of apple cultivars (17), based on the water attaining capability parameter, the best results were for the cultivar Paramenka, and one autochthonous cultivar (II/2). The advantage was given to the apple, due to the fact it represents one of the largest consumers of moisture out of all fruit species. In our study, all selected biotypes of wild apple (*Malus sylvestris* Miller) have higher resistance to drought than the four autochthonous control sorts.

4. Conclusions

- We recommend Biotype 2 as potential apple generative rootstock due to the following characteristics: a) the highest water attaining capability had the leaves of biotype 2 of selected wild apples. The leaves were taken from the mother tree of the biotype 2, which gives fruits, and at the same time gives the seeds for the production of generative rootstocks. Over the monitored

time interval (8 hours upon sample taking), leaves taken from the annual twigs of the studied selected biotypes of wild apples lost on average 32.44% of water; b) high mass of 100 pieces of dry seeds (2.61 g), as well as very good seed germination. The average germination of seeds for this biotype of wild apple was 82.3 %; c) the rootstocks were estimated as the ones of medium verdure, while their uniformity was excellent (the level of uniformity was 1); d) the level of grafting reception with the 'Red Delicious' cultivar was the highest recorded during the experimental and production work. The average reception of this biotope was 93.3 %.

- We recommend biotype 8 as potential apple generative rootstock due to the following characteristics: a) considering the aspect of generative rootstocks production, the mass of the fruit is on a satisfactory level (12.75 g). When it comes to this small fruits, we can obtain out of their relatively small total fruit mass the largest quantity of seeds needed for the production of generative rootstocks; b) the mass of 100 pieces of dry seeds is 3.83 g, which represents the best result in regard to all elaborated biotypes of wild apple. The level of the dry matter needed for germination and the development of seedlings is very high as well (89 %); c) what can be considered as a disadvantage is the fact that this biotype's seedlings belong to the group of verdurous ones. However, what needs to be pointed out is the fact that the height of the seedlings, which average level was 59.5 cm, as well as the average seedling corpulence of 6.43 mm, classifies this biotype on the very lower border of our conditional classification. Taking all into consideration, this biotype is more than recommendable for the potential apple generative rootstock, especially in the conditions of sharp continental climate, at higher altitudes and poorer soil biotypes.

- Biotype 9 is primarily distinguished by its morphological characteristics of the fruit and seedlings when it comes to the production of generative rootstocks. The morphological characteristics are the following:

a) extremely small level of fruit mass (8.95 g);

b) low verdure and variability of the seedlings;

c) the lowest average growth of annual scions in 'Red Delicious' cultivar (103 cm) in comparison to the average growth of scions produced by grafting the generative rootstocks obtained out of other wild apple biotypes used in this elaboration and production process;

d) the level of seed germination is satisfactory (82.7 %)

e) the level of grafting reception with the cultivar 'Red Delicious' (88.3%).

- Autochthonous varieties of apples cannot be used for the generative rootstock production due to the large size of fruit (from 118.9g to 163.8g), despite the fact they have very good germination of seeds (from 77.3 % to 58%). The obtained results of the positive morphological and physiological properties of their seedlings can be valorised in terms of using autochthonous varieties of apples as initial parents for the creation of generative rootstocks.

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