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Seed Characteristics of Wild Pear Genotypes from the *in situ* Population of the Banja Luka Region

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Abstract

Wild pear (*Pyrus communis* var. *piraster* (L.) Ehrh.) is widely used as a generative rootstock for pear grafting. Seeds for the production of this rootstock should be taken from the registered mother plants. The aim of this research was to select mother plants in wild pear population from three localities (Banja Luka, Manjača, and Potkozarje). Fruit bearing regularity of selected trees was observed from 2012-2016, and the number of seeds per fruit was analysed in 2013, 2015 and 2016. Seed germinability and vitality analysis was carried out on 14 selected tress (genotypes). Seed germinability was tested according to the ISTA guideline. Seed vitality was determined by the tetrazolium test (TT). Accessions from Banja Luka locality expressed the highest fruit production. The highest number of seeds was determined in the genotypes marked as No. 6 and No. 21, amounting to 7.53 and 7.21 seeds, respectively. Seeds of the tested wild pears have shown good vitality. The highest seed vitality and germination of 92% was obtained from the accession No. 20. On the basis of the results, accessions marked with numbers: '6', '8', '20', '25', '31' and '32' can be used as mother plants for pear rootstock production.

Key words: mother plant, fruit bearing regularity, germinability, vitality.

Introduction

Wild pear (*Pyrus communis* var. *pyraster* (L.) Ehrh.) is a multiple valuable and useful wild fruit tree because of the value of wood, as well as for the nutritional value of its fruits. This species contributes to the diversity and stability of forest ecosystems.

Wild pear is also used as rootstock seedlings in the production of young pear plants. Unlike quince, which requires light soils with low lime content, wild pear as a rootstock seedling has advantages in stressful environments (drought, cold winter, high lime content in the soil, high groundwater level, and heavy soil). Strong growth and lack of uniformity of seedlings restrict their use in intensive orchards with high density (Hrotko, 2008). Seeds for the production of rootstock seedlings are collected from the mother plants which are selected by their regular fruit productivity, the number of seeds per fruit, vitality and seed germination. The rootstock should regulate the lushness of the variety, the early yielding and regular fruiting, fruits of uniform size, and shape. In the Banja Luka region, the populations of wild pears *in situ* are comprised of accessions with different morphological characteristics, which indicates large diversity of populations in the examined area. Research in the area of the Forest Park Starčevica has shown a high degree of polymorphism between the examined trees (Antic et al, 2016).

The seeds of fruit species, especially fruit trees belonging to the *Rosaceae* family, are characterized by the physiological form of dormancy. The conditions for interruptions of dormancy are different among particular plant species (Čmelik and Perica, 2007). Seed stratification is one of the ways for overcoming dormancy and, according to the ISTA (International Rules for Seed Testing) guideline (2011), for the genus *Pyrus* spp., it is achieved by cold stratification during the period of 3 months at temperatures of 3-5°C. Due to the pronounced dormancy of pear seed, it is recommended that the vitality of seeds be determined using some of the biochemical tests.

Biochemical tests are an indirect way of evaluating seed vitality (Milošević et al., 2010), and most often a tetrazolium test is used in order to obtain information about seed vitality quickly.

Vital seed should exhibit tissue coloration that is important for normal germination development. The germination of wild pear seeds is predominantly conditioned by the genotype and agro-ecological conditions during seed development and maturation. The values of germination of wild pear seeds are different and range from 2% to 94% (Kulina, 2001; Milutinović et al., 2005; Orešković et al., 2006; Zavišić et al., 2014).

The aim of this research was to select the trees, out of the total number of accessions collected from the wild pear populations located in the Banja Luka region that meet the requirements to be mother plants for seed production of generative rootstocks.

Material and Methods

The study that included 33 trees of *in situ* wild pear populations was carried out during the five-year period (2012-2016) in three locations of the Banja Luka region: Banja Luka, Manjača and Potkozarje (Gradiška). As part of the study of the genetic diversity of the wild pear population (*Pyrus communis* var. *pyraster*), several individual trees were isolated in several localities in the Banja Luka region. The trees were separated with the aim of separating the accessions from the natural population with certain positive characteristics as the starting material for the variety selection program and the pear rootstocks.

The number of studied accessions differs by locality, and the review of marks or the accession number with the name of the site and the mapped data is given in Table 1.

The fruit bearing regularity of the selected trees was followed during the five-year period (2012-2016), and the number of seeds in the fruit during the three years (2013, 2015 and 2016) depending on when the accessions were fruit-bearing. The average number of seeds per fruit was determined on a sample of 50 fruits collected from different positions in the crown. The seed germination and vitality analysis was carried out in 2015 with 14 accessions which were fruit-bearing that year. The examination of the germination of the stratified seed was carried out in the laboratory using a sand germination test at an alternating temperature of 20-30°C (ISTA, 2011). During the seed germination, the energy of seed germination was determined 7 days after in laboratory conditions, and total germinability was recorded after 21 days. Seed vitality was determined by the tetrazolium test (TT), where pre-prepared seeds were immersed in 1% solution of colourless 2,3,3-triphenyltetrazolium bromide (Patil and Dadlani, 2009) for 18 hours, and after that the degree of colouring of cotyledons and of hypocotyls was determined. In these studies, four repetitions with 50 seeds were performed for each genotype.

The obtained data were biometrically processed, classified and compared. The fruiting regularity of wild pears by localities is presented graphically. Average values and standard errors in regards with the number of seeds in the fruit were determined.

Tab. 1. Geographical position and marks of genotypes selected from the wild pear population

Географски положај и ознаке одабраних принова дивље крушке

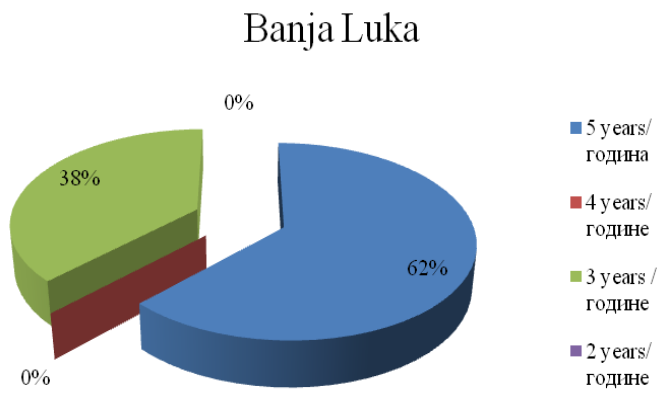
Locality *Локалитет*	Accession number *Принова*	Site *Потес*	Longitude (° ' ") *Географска дужина*	Latitude (° ' ") *Географска ширина*	Altitude (m) *Надморска висина*
Banja Luka	1	Motike	17 7 51.0E	44 49 15.9N	300
	2	Motike	17 7 33.5E	44 49 34.6N	330
	3	Motike	17 7 32.7E	44 49 35.1N	326
	4	Motike	17 7 56.9E	44 49 22.7N	268
	5	Motike	17 7 56.9E	44 49 22.7N	268
	6	Priječani	17 13 21.0E	44 49 20.8N	160
	7	Saračica	17 5 55.7E	44 48 44.3N	363
	8	Saračica	17 5 55.7E	44 48 44.3N	363
Manjača	9	Rekavice	17 7 41.6E	44 42 34.0N	504
	10	Kola	17 5 43.0E	44 40 37.9N	485
	11	Kola	17 5 46.5E	44 40 36.7N	482
	12	Kola	17 5 48.1E	44 40 35.0N	484
	13	Kola	17 5 53.6E	44 40 34.8N	475
	14	Kola	17 5 49.0E	44 40 32.2N	480
	15	Kola	17 5 51.3E	44 40 28.7N	465
	16	Kola	17 5 52.9E	44 40 26.4N	464
	17	Kola	17 5 54.2E	44 40 24.2N	473
	18	Kola	17 4 59.1E	44 40 57.8N	483
	19	Dedić Lokva	17 4 39.8E	44 41 55.8N	496
	20	Dedić Lokva	17 3 50.9E	44 42 37.1N	495
	21	Dedić Lokva	17 1 59.9E	44 43 43.1N	441
	22	Dedić Lokva	17 1 59.9E	44 43 43.1N	441
	23	Dedić Lokva	17 1 45.1E	44 43 46.9N	450
	24	Bukvalek	17 4 46.3E	44 44 55.4N	377
	25	Bukvalek	17 6 41.0E	44 44 58.6N	431
	26	Šljivno	17 0 24.3E	44 39 18.1N	627
	27	Šljivno	17 0 12.4E	44 39 20.9N	595
	28	Šljivno	17 0 12.4E	44 39 20.9N	595
	29	Šljivno	17 0 13.3E	44 39 25.6N	600
	30	Šljivno	17 0 7.4E	44 39 24.5N	602
Potkozarje	31	Berek	17 12 30.7E	45 1 57.6N	144
	32	Berek	17 14 1.6E	45 2 33.4N	114
	33	Jazovac	17 11 54.4E	45 1 25.5N	164

Results and Discussion

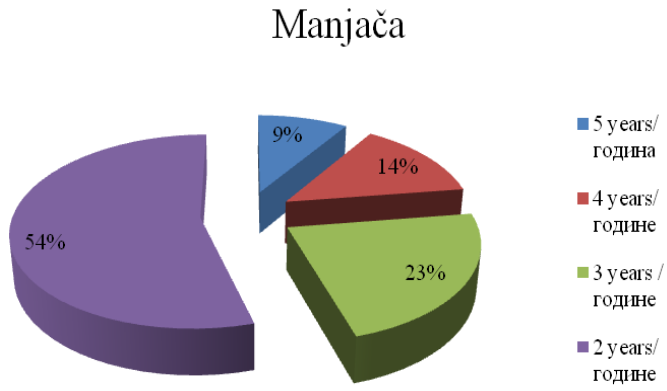
During the five-year period (2012-2016), the fruit-bearing regularity of the selected wild pear trees was followed at the examined localities and for each locality the observations are presented graphically. All the selected accessions were fruit-bearing in 2012 and 2013, and the tree genotype was different, even in the same locality. The most frequent fruit-bearing during five years was in the Banja Luka locality (Graph 1). Out of the total number of accessions, 62% were fruit-bearing in all five years, while the remaining accessions were bearing fruit for three years.

At the Manjača locality most accessions were fruit-bearing during two years (Graph 2), while only 9% of accessions were fruit-bearing during all five years.

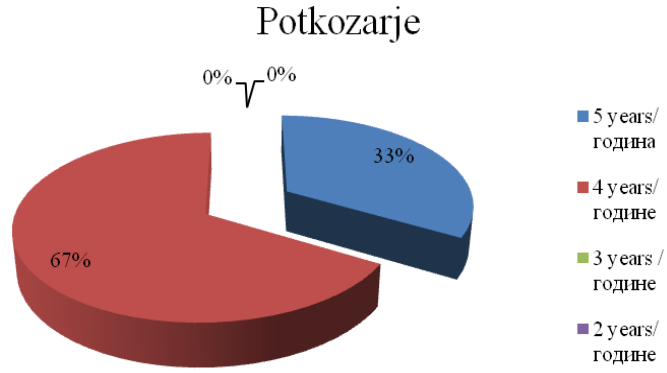
According to the fruit-bearing regularity, the Potkozarje locality stands out (Graph 3), where the trees were fruit-bearing for all five or four years. The difference in fruiting by localities is due to the influence of ecological factors, and above all the occurrence of frosty days during March and April. The occurrence of frosty days was more frequent in Banja Luka and Manjača in comparison with the Gradiška locality.



Graph 1. Fruit-bearing of selected pear trees at the Banja Luka locality
Плодоношење одабраних стабала дивље крушке на локалитету Бања Лука



Graf. 2. Fruit-bearing of selected pear trees at the Manjača locality
Плодоношење одабраних стабала дивље крушке на локалитету Мањача



Graf. 3. Fruit-bearing of selected pear trees at the Potkozarje locality
Плодоношење одабраних стабала дивље крушке на локалитету Поткозарје

In order to select mother plants for obtaining the seeds, one of the key parameters was the number of developed seeds in the fruit. The average number of seeds per fruit which was observed in mother plants, grown in different localities, is shown in Table 2.

Tab. 2. Number of seeds in the wild pear fruit (mean value and standard error)
Број сјеменки у плоду дивље крушке (просјечна вриједност и стандардна грешка)

Locality *Локалитет*	Accession *Принова*	Year *Година*	Number of seeds *Број сјеменки*		Average *Просјек*	
			$\bar{x}$	$\pm S_{\bar{x}}$	Accession *Принова*	Locality *Локалитет*
Banja Luka	1	2015	3.68	±1.32	3.08	4.09
		2016	2.48	±1.23		
	2	2013	1.60	±0.95	1.73	
		2015	1.86	±0.70		
	3	2013	3.18	±2.11	2.90	
		2015	2.62	±0.97		
	4	2015	3.52	±1.74	3.76	
		2016	4.00	±1.46		
	6	2015	6.74	±2.01	7.53	
		2016	8.32	±1.25		
	7	2015	4.56	±1.83	4.73	
		2016	4.90	±1.93		
	8	2015	4.32	±1.95	4.73	
		2016	5.14	±2.01		
Manjača	9	2015	2.06	±1.54	3.04	4.96
		2016	4.02	±2.22		
	13	2013	5.50	±1.84	5.91	
		2015	6.32	±1.62		
	19	2015	4.34	±1.99	3.88	
		2016	3.42	±1.47		
	20	2013	4.16	±1.90	4.41	
		2015	4.66	±2.26		
	21	2013	8.34	±1.76	7.21	
		2015	6.08	±2.00		
	23	2013	6.88	±1.79	6.06	
		2016	5.24	±1.66		
	24	2013	5.26	±1.29	5.25	
		2016	5.24	±1.92		
	25	2015	3.22	±1.11	4.38	
		2016	5.54	±2.08		
	27	2015	4.22	±2.54	3.06	
		2016	1.90	±1.16		
	28	2013	4.78	±2.09	4.82	
		2015	4.86	±1.25		
Potkozarje	31	2013	3.94	±2.06	4.04	4.38
		2015	4.14	±1.92		
	32	2015	4.10	±1.40	4.62	
		2016	5.14	±1.65		
	33	2015	5.96	±1.59	4.48	
		2016	3.00	±1.32		

To select mother plants for seeds, it is desirable that the number of seeds in the fruit be as high as possible. Of all the examined wild pear accessions, the accession 6 (Banja Luka locality) with average of 7.53 and the accession 21 (Manjača locality) with 7.21 seeds in the fruit are distinguished. The accessions 13, 23, and 24 had more than five developed fruit seeds, all of them from the Manjača locality. Mratinić (2000) states that the number of normally developed seeds in the wild pear fruit varied from 1.2 to 10.1. In the studies of wild pear genotypes from indigenous populations of Serbia, the pear fruit had 3.89 seeds on average (Paunović et al., 2012), and in the research at the territory of Bosnia and Herzegovina, the Trebević locality, the wild pear fruit had 5.36 seeds on average (Kulina, 2001).

The seeds were collected from 14 trees which were fruit-bearing 4 or 5 years in the fall of 2015. The results of the vitality analysis by the tetrazolium test (TT), as well as the germination analysis, are shown in Table 3.

Tab. 3. Seed vitality and germinability of the selected wild pear trees (2015)
Виталност и клијавост сјемена odabrabih stabala дивље крушке

Accession *Принова*	Vitality (TT*) (%) *Виталност*	Germinability (%) *Клијавост*	
		7^{th} day of seed germination *7. дан наклијавања*	21^{st} day of seed germination *21. дан наклијавања*
1	78	21	41
4	70	13	32
6	66	11	35
7	70	13	28
8	74	28	50
9	78	13	40
13	80	14	33
19	86	0	15
20	94	69	92
25	74	13	43
27	80	0	9
31	74	21	48
32	72	24	50
33	52	9	31

Note: TT - tetrazolium test / тетразолиум тест

According to the TT results, wild pear seed showed good vitality, and in most cases good vitality is followed by good germination.

Exceptions are the accessions 27 and 19, whose seeds showed good vitality of 80% and 86%, respectively, and germination was 9% and 15%, respectively. During the preparation of seeds for the TT, the removal of seed coats for accessions 27 and 19 was more difficult compared to other accessions, especially at the tip of the seeds where the germ is. On the basis of this, it can be concluded that a longer period of moistening is required for the seeds of the mentioned accessions in order to break the seed coat and to germinate.

Seed germination determines germination energy and total germinability. The germination energy is the physiological characteristic of the seed to quickly and evenly germinate and sprout and to withstand the adverse factors of the environment. In this study, the best germinability of 81% was obtained by the accession 20, and besides this, the accessions with more than 50% of germinability were obtained by the accessions 1, 8 and 32. The germinability of wild pear seeds from the population of western Serbia (Milutinović et al., 2005) were 13.45%, which is lower than the results obtained in this research. The studied populations of wild pears in Montenegro (Šebek et al., 2007) and Trebević (Kulina, 2001) showed high percentage of seed germinability, 84% and 93%, respectfully. In the studies performed in Delnice and Karlovac regions of Croatia, the germinability of wild pear seeds was very low, only 1-2% (Orešković et al., 2006).

Seed germinability can be influenced by several factors, above all the characteristics of the mother plant as well as the ecological conditions during the year of fruit-bearing, while the accessions with later-maturing fruits have better germinability (Zavišić et al., 2014). The studied accessions mostly mature during the second half of September and the beginning of October, except for the accession 7, which matures in the middle of August and has shown the lowest germinability.

Conclusion

Selection of mother plants' seed for production of generative rootstocks is done on the basis of the fruit quality, fruit-bearing regularity, the number of seeds per fruit and seed germinability. The accessions from Banja Luka and Potkozarje localities stand out for their fruit-bearing regularity. More than seven seeds in the fruit have had quality characteristics of an accession for a mother plant, and in this study, the accessions 6 and 21 are distinguished. Vitality and seed germinability are important seed characteristics in the production of generative rootstocks. Several accessions showed relatively good seed germinability, more than 40%, particularly the accession 20 with germinability of 81%.

Good seed vitality does not have to mean good germinability. Based on the results of this research the accessions 6, 8, 20, 25, 31 and 32 can be selected to be used as mother plants for seeds.

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Карактеристике сјемена одабраних стабала дивље крушке из *in situ* популације бањалучке регије

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Сажетак

Дивља крушка (*Pyrus communis* var. *piraster* (L.) Ehrh.) се користи као подлога за калемљење гајене крушке. Сјеме за производњу сијанаца се скупља са матичних стабала. Циљ овог рада је да се из *in situ* популације дивље крушке на три локалитета (Бања Лука, Мањача и Поткозарје) издвоје матична стабла. Редовност родности стабала је праћена током петогодишњег периода (2012-2016), а број сјеменки у плоду је анализиран 2013, 2015. и 2016. године. Код 14 принова је вршена анализа клијавости и виталности сјемена. Клијавост сјемена је испитивана у лабораторији методом у складу са ISTA Правилником, а виталност сјемена је утврђена помоћу тетразолиум теста. Принове са локалитета Бања Лука су имале највеће плодоношење. По броју сјеменки у плоду издвајају се принове 6 и 21 са 7,53 и 7,21 сјеменки. Плодови осталих принова су имали просјечно 3-4 сјеменке. По резулатима тетразолијум теста сјеме испитиваних принова дивље крушке показује добру виталност. Добром виталношћу сјемена и клијавошћу од 92% издваја се принова 20. На основу добијених резултата за матична стабла се могу издвојити принове означене бројевима: 6, 8, 20, 25, 31 и 32.

Кључне ријечи: матично стабло, редовно плодоношење, клијавост, виталност.

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