NARDI FUNDULEA, ROMANIA
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ROMANIAN AGRICULTURAL RESEARCH, NO. 38, 2021
First Online: January, 2021. DII 2067-5720 RAR 2021-17

RESULTS REGARDING THE STAGE OF SOME GROWTH AND REPRODUCTIVE ELEMENTS IN *Primula officinalis* Hill.

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ABSTRACT

This paper aims to study aspects of biology and cultivation technology for introduction into the culture of the species *Primula officinalis* Hill., medicinal valuable plant both from a phytotherapeutic and economic point of view. The species *Primula officinalis* Hill. is popularly known as cuckoo's beak, aglic, aglicel or cuckoo's boot

The research was performed on plants harvested from spontaneous flora (Rucăr-Bran area). Then the plants were acclimatized in the greenhouses of NIRDSPB Braşov, Technology laboratory. After acclimatizing the material, a rigorous selection was made, choosing the most uniform plants in terms of number of leaves, height and health.

The experimental factors were factor A - distance between rows with graduations: 25 cm, 50 cm, 75 cm and factor B - distance between plants in a row with graduations: 10 cm, 25 cm, 50 cm.

Our results shown that the variant with a large nutrition space (75/25 cm), with an average number of seven plants grown in a row, has an average of eight stems with flowers on each plant, being clearly superior to the other variants. The production of fresh herba (kg/ha) for the species *Primula officinalis* L. was influenced by both factors (A and B).

By variance analysis regarding the two factors studied on the average number of flowering stems it is found that the number of flowering stems depends, largely, on the nutrition space.

Keywords: *Primula officinalis*, biology, distance between rows, distance between plants in a row, flowering stems, fresh herba.

INTRODUCTION

Medicinal plants have played an important role in the human evolution.

Wanting to heal his wounds, both physical and emotional, the man embarks on an initiatory journey, to discover the remedies to alleviate his suffering.

Today we cannot explain by what mechanism of human intelligence, in the distant epochs of the first civilizations, peoples from different continents used very special plants, but which contained, as it results from current research, chemically related active principles and therefore, with similar action (Constantinescu and Bojor, 1969).

Thus, the Greek philosopher and historian Herodotus mentioned the Dacian skill in using using herbs to heal wounds and relieve pain. After the Roman conquest, the pharmaceutical and therapeutic knowledge of the Greeks and Latins completed the Dacian cultural treasure (Muntean et al., 2007). Săvulescu (1952) appreciated that: “the popular botany in our country was born with the people, evolved with it and in it are reflected the history, the occupations, the sufferings and it joys”.

In recent years, for the protection of the spontaneous medicinal flora in our country, experiments have been experimented to maintain and increase its economic potential. Encouraging results have been obtained in this direction by reseeding or replanting the exploited species from a certain area (Alexan et al., 1983).

For medicinal and aromatic purposes, from our spontaneous flora are currently systematically obtained over 2640 products

Received 2 July 2020; accepted 6 January 2021.

from plant parts: roots, rhizomes, rind, buds, stems, shoots, leaves, flowers, stigmas, fruits, seeds (source CTPMA-MADR).

The researches that are the subject of the current paper appeared as a necessity, because in many European countries, the species *Primula officinalis* Hill. synonymous with *Primula veris* L. is on the red list of endangered plants, due to irrational harvesting of spontaneous flora, herbicides of alpine and subalpine pastures, uncontrolled deforestation of forests.

This species is under partial legal protection in Poland; the plant can be harvested from areas where the species is widespread, in low hill areas and in lower mountain areas (Zajac and Zajac, 2001; Mirek et al., 2002).

Primula officinalis Hill., popularly known as the “primrose”, is a herbaceous species belonging to the *Primulaceae* family, being one of the 400 species of the genus *Primula*. The primrose is a small plant, usually found in calcareous pastures, poor in nutrients, meadows or coastal dunes. The plant grows in warm, sunny, dry habitats, most often on meadows and pastures, but also in open deciduous forests (Hegi, 1965; Valentin and Kress, 1972). Some of its natural sites are endangered as a result of massive deforestation, by cultivating land or by intensive grazing. It can also be found along forest edges and in mixed forests of oak and beech (Brys and Jacquemyn, 2009).

In the pharmaceutical industry are used *Primula officinalis* flowers that contain about 2% triterpene saponins, phenolic glycosides and flavonoids: 3', 4', 5'-trimethoxyflavone, quercetin and its derivatives, kaempferol and 3-glucoside limocitrin (Hegnauer, 1990; Harbone and Baxter, 1993; Huck et al., 1999; Stecher et al., 2003). Numerous secondary metabolites produced in the underground organs of primrose can be obtained only from cultivated plants, while flowers can also be harvested from natural habitats (Draxler et al., 2002).

Starting from Racoviță's (1934) statements that “the vegetal resources of the spontaneous flora should be exploited rationally, care should be taken that the access to reserves does not lead to an exploitation that exceeds the limits that a resource can bear without prejudice” (Ștefuleac, 1976), instead of excessive harvests, it is recommended to introduce in the culture some valuable species from a phytotherapeutic point of view, maintaining a sustainable ecosystem, thus avoiding their extinction.

MATERIAL AND METHODS

The research was performed on the species *Primula officinalis* harvested in the spring of 2015 from spontaneous flora (Rucăr-Bran area). Then the plants were acclimatized in the greenhouses of NIRDSPB Braşov, Technology laboratory. After acclimatizing the material, a rigorous selection was made, choosing the most uniform plants in terms of number of leaves, height and health.

The experience established in autumn 2016 (date of planting: October, 20), aimed to establish the plants optimal nutrition space.

A two-factor experiment was designed, located according to the method of subdivided plots, with three repetitions of type 3 x 3 x 3, the length of a variant being 2 m, pathways with a width of 1 m and 9 rows of plants per plot.

Factor A - distance between rows with graduations: 25 cm, 50 cm, 75 cm;

Factor B - distance between plants in a row with graduations: 10 cm, 25 cm, 50 cm;

The interaction with the density of 25/10 is considered the control of the experience:

- the surface of the plots in graduation a1 (25 cm) = 4.5 m^2;
- the surface of the plots in graduation a2 (50 cm) = 9 m^2;
- the surface of the plots in graduation a3 (75 cm) = 13.5 m^2;
- the total experimental surface including the pathways (27 m^2 * 3 + 13.5 m^2 * 2) = 108 m^2;
- number of plants on plots - b1; (200 cm / 10 cm) = 20 * 9 = 180 * 3 = 540;
- number of plants on plots - b2; (200 cm / 25 cm) = 8 * 9 = 72 * 3 = 216;
- number of plants on plots - b3; (200 cm / 50 cm) = 4 * 9 = 36 * 3 = 108;
- total number of plants per experiment: 540 + 216 + 108 = 864.

The date of emergence was noted in dynamics, from the emergence of the first plants to the end of the emergence of all plants/variant. The start date of flowering was noted in dynamics, from the emission of flowering stems, to full flowering, when the results obtained were processed graphically.

The harvest for herba was done when 90% of the plants were in bloom.

RESULTS AND DISCUSSION

The experience followed the dynamics of seedlings in the first year. It is noted that on 10.03.2017 (Figure 1), the 25/50 cm interaction recorded the best grip with a percentage of 69%, and the weakest emergence was presented by the 75/50 cm variant with 58%. The other experimental variants were between the two values, with an average of 62%.

The second dynamic took place on 21.03.2017, when most of the plants started growing. An almost uniform development of the plants is observed, the variants a2b1 and a3b1 coming very close to a1b3, which registered the best grip at the first dynamics. Variant a3b3 still shows a weaker increase, but the difference from the average of the other variants is not significant (Figure 2).

The plants start in vegetation of about 2-3 weeks earlier than in the spontaneous flora, from where the mother plants were harvested. After the first winter in the experimental field, it was observed that the plants adapted very well to the conditions of NIRDPSB Braşov.

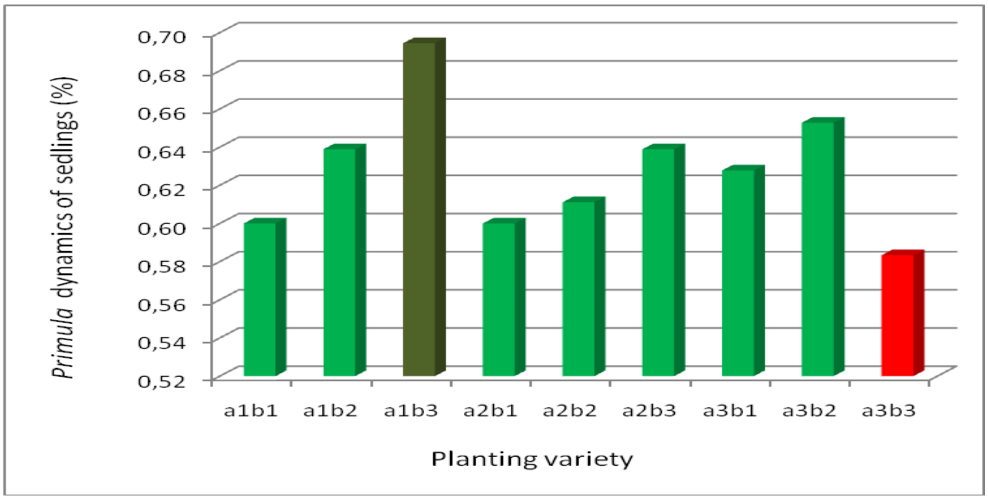

Figure 1. Primula officinalis - plants by seedlings in 10.03.2017

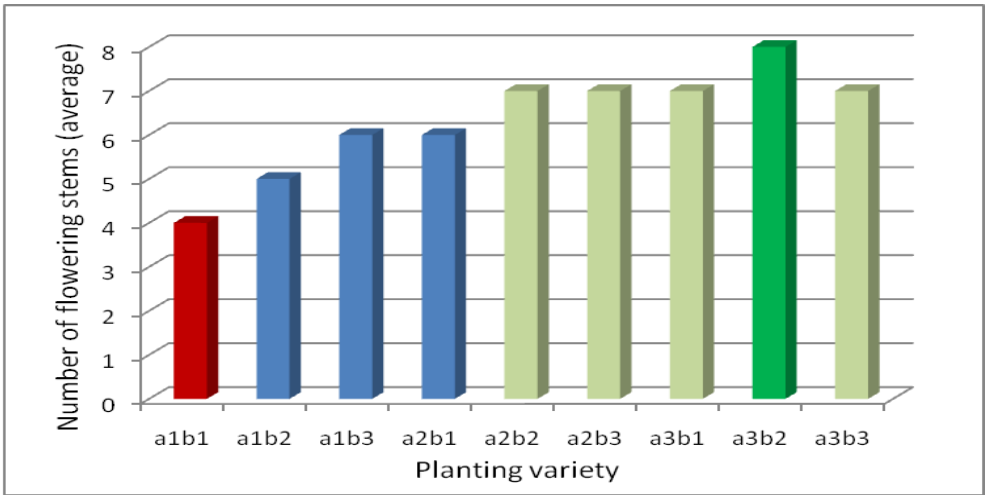

Figure 2. Primula officinalis - emerged and developed plants on 21.03.2017

The number of flowering stems was noted when all the experimental plants reached full bloom. Figure 3 shows graphically the differences in the number of flowering stems in the experiment with the nutrition space. In the first year after planting, the combination of planting distances 25/10 cm, having the smallest nutrition space and the highest plant density in the whole experience, presents the fewest flowering stems. The variant with a large nutrition space (75/25 cm), with an average number of 7 plants grown in a row, has an average of 8 flowering stems on each plant, being clearly superior to the other variants.

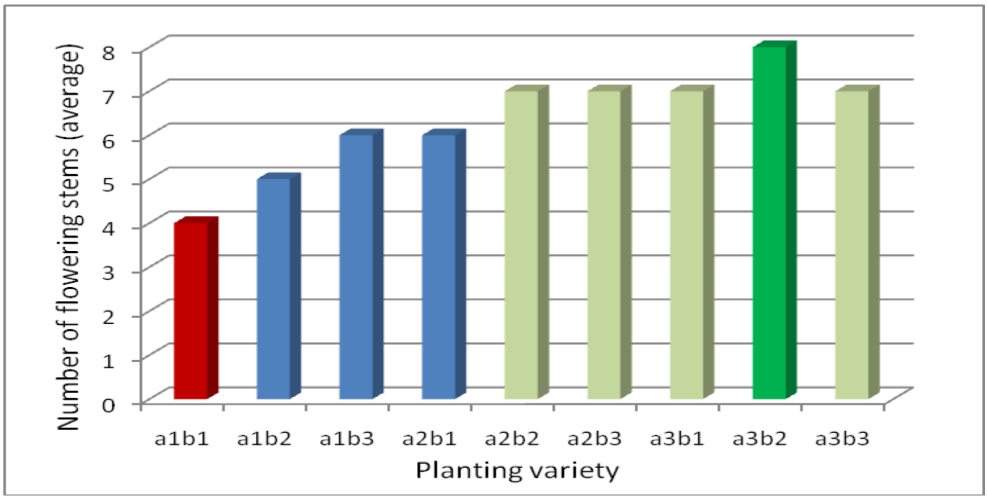

Figure 3. Average number of flowering stems at the bifactorial experience in 2017

The study of the influence of factor A (distance between rows) and factor B (distance between plants per row) on the height of *Primula* plants revealed differences of the two factors on plant height, with a decisive role on the production per unit of studied area.

Factor A, the distance between rows, acted favorably on the average height of the plants, reported to the distance of 25 cm, taken as a control. Plants grown at longer distances between rows recorded significant positive differences in height (Table 1).

Factor B (distance between plants in a row) ensures very significant differences from the distance of 10 cm between plants in a row (taken as control), with an average difference of 7.78 cm at the distance of 25 cm and 6.44 cm at 50 cm.

Table 1. Influence of distance between rows and and between plants on rows on average plant height

Experimental year 2017											
A factor influence						B factor influence					
Symb.	Dist. between rows (cm)	Average (cm)	%	Diff. (cm)	Sign.	Symb.	Dist. between rows (cm)	Average (cm)	%	Diff. (cm)	Sign.
A1	25	32.78	100.0	0.00	Mt.	B1	10	31.11	100.0	0.00	Mt.
A2	50	36.78	112.2	4.00	*	B2	25	38.89	125.0	7.78	***
A3	75	38.00	115.9	5.22	*	B3	50	37.56	120.7	6.44	***
DL (p 5%)				3.38		DL (p 5%)				2.74	
DL (p 1%)				5.59		DL (p 1%)				3.85	
DL (p 0.1%)				10.47		DL (p 0.1%)				5.44	

The influence of the interaction of the distance between plants per row (factor B) at the same distance between rows (factor A) on the average height of plants in 2017 is shown in Table 2. The interaction B2A1 (25/25 cm) with very significant differences compared to control (10/25 cm), reaching an average height of 38.67 cm. Equally large differences from the control (10/50 cm) are registered to the variants planted at distances 25/50 and 50/50, with an average height of 40.67 cm and 39.67 cm, respectively. From the last interaction analyzed, where the variant is 10/75 cm, it appears that the planting distances 25/75 cm and 50/75 cm do not lead to significant differences in plant height. In the experimental conditions of 2017, the lowest height was recorded at a density of 10/25 cm. This difference is due to the low nutrition space compared to the other variants.

Table 2. The influence of the interaction between plants distance in a row (B) and the distance between rows (A) on the height of *Primula officinalis* Hill. plants

Experimental year 2017					
Interaction	Distance (cm)	Average (cm)	%	Difference (cm)	Significance
B1A1	10/25	25.33	100.0	0.00	Mt.
B2A1	25/25	38.67	152.6	13.33	***
B3A1	50/25	34.33	135.5	9.00	**
B1A2	10/50	30.00	100.0	0.00	Mt.
B2A2	25/50	40.67	135.6	10.67	***
B3A2	50/50	39.67	132.2	9.67	***
B1A3	10/75	38.00	100.0	0.00	Mt.
B2A3	25/75	37.33	98.2	-0.67	-
B3A3	50/75	38.67	101.8	0.67	-
DL (p 5%)				4.75	
DL (p 1%)				6.67	
DL (p 0.1%)				9.42	

From the interaction of factor A (distance between rows) on factor B (distance between plants per row) on the average height of plants (Table 3) there are very significant positive and significant positive differences from the control at planting distances 75/10 cm and 50/50 cm, with an average height of 38.0 cm and 39.67 cm, respectively. The other distances combinations analyzed did not show statistically assured differences from the distance of 25 cm between rows.

Table 3. The influence of the interaction between distance from rows (A) and the distance between plants in a row (B) on the average height of *Primula officinalis* Hill. plants

Experimental year 2017					
Interaction	Distance (cm)	Average (cm)	%	Difference (cm)	Significance
A1B1	25/10	25.33	100.0	0.00	Mt.
A2B1	50/10	30.00	118.4	4.67	-
A3B1	75/10	38.00	150.0	12.67	***
A1B2	25/25	38.67	100.0	0.00	Mt.
A2B2	50/25	40.67	105.2	2.00	-
A3B2	75/25	37.33	96.6	-1.33	-
A1B3	25/50	34.33	100.0	0.00	Mt.
A2B3	50/50	39.67	115.5	5.33	*
A3B3	75/50	38.67	112.6	4.33	-
DL (p 5%)				5.11	
DL (p 1%)				7.65	
DL (p 0.1%)				12.25	

By variance analysis regarding the two factors studied on the average number of flowering stems, it is found that the number of flowering stems depends, largely, on the nutrition space (Table 4). Analyzing the influence of the interaction of the two factors on the average number of flowering stems it was observed that there were significant differences in the number of flowering steams depending on plants distance in a row, at the same distance between rows.

Table 4. The influence of factors A and B on the average number of flowering stems in *Primula officinalis*

Experimental year 2017											
A factor influence						B factor influence					
Symb.	Dist. between rows (cm)	Average (no.)	%	Diff. (no.)	Sign.	Symb.	Dist. between rows (cm)	Average (no.)	%	Diff. (no.)	Sign.
A1	25	5.22	100.0	0.00	Mt.	B1	10	5.78	100.0	0.00	Mt.
A2	50	6.56	125.5	1.33	-	B2	25	6.78	117.3	1.00	*
A3	75	7.44	142.6	2.22	*	B3	50	6.67	115.4	0.89	*
DL (p 5%)				1.77		DL (p 5%)				0.80	
DL (p 1%)				2.92		DL (p 1%)				1.13	
DL (p 0.1%)				5.47		DL (p 0.1%)				1.59	

The influence of the interaction between the distance between rows (A) and the distance between plants per row (B) on the average number of flowering stems (Table 5), shows significant differences in two variants (V7 and V8), compared to the control variant. The other variants analyzed did not register significant influences.

Table 5. The influence of the interaction between the density of plants in a row (B) and the distance between rows (A) on the average number of flowering stems

Experimental year 2017					
Symbol	Variant (cm/cm)	Average (no.)	%	Difference (no.)	Significance
B1A1	10/25	4.33	100.0	0.00	Mt.
B2A1	25/25	5.33	123.1	1.00	-
B3A1	50/25	6.00	138.5	1.67	*
B1A2	10/50	6.00	100.0	0.00	Mt.
B2A2	25/50	6.67	111.1	0.67	-
B3A2	50/50	7.00	116.7	1.00	-
B1A3	10/75	7.00	100.0	0.00	Mt.
B2A3	25/75	8.33	119.0	1.33	-
B3A3	50/75	7.00	100.0	0.00	-
DL (p 5%)				1.39	
DL (p 1%)				1.95	
DL (p 0.1%)				2.76	

The influence of interaction between rows distance (A) and the distance between plants on row (B) regarding the average number of flowering stems (Table 6) present significantly difference in two variants (V7 and V8) compared to the control variant. The other analysed variants did not have significant influences.

Table 6. The influence of the interaction between the distance between rows (A) and the distance between plants per row (B) on the average number of flowering stems in 2017

Experimental year 2017					
Interaction	Distance (cm)	Average (cm)	%	Difference (cm)	Significance
A1B1	25/10	Media	100.0	0.00	Mt.
A2B1	50/10	4.33	100.0	0.00	Mt.
A3B1	75/10	6.00	138.5	1.67	-
A1B2	25/25	7.00	161.5	2.67	*
A2B2	50/25	5.33	100.0	0.00	Mt.
A3B2	75/25	6.67	125.0	1.33	-
A1B3	25/50	8.33	156.3	3.00	*
A2B3	50/50	6.00	100.0	0.00	Mt.
A3B3	75/50	7.00	116.7	1.00	-
DL (p 5%)				2.09	
DL (p 1%)				3.27	
DL (p 0.1%)				5.65	

Analysis of variance for fresh herba production is shown in Table 7, where was analyzed the variance of the distance between rows and between plants per row on the production of fresh herba (kg/ha). The calculated F value is higher than the table value (F5% and F1%), which shows that the effects of the variants on the production of fresh herba (kg/ha), are real, true and are not the result of experimental errors.

Table 7. Analysis of variance regarding the influence of the distance between rows (A) and between plants per row (B) on the production of fresh herba (kg/ha)

Source	The sum of the squares	Degrees of freedom	Average square	F factor
A	167512300	2	83756140	97.468**
B	252298500	2	126149200	228.083**
AB	31600180	4	7900045	14.284**
R	982156	2	491078	
AR	3437289	4	859322	
BR	2714978	4	678744	
ABR	3922044	8	490256	
Er. A	3437289	4	859322	
Er. B	6637022	12	553085	
Total	462467400	26		

The production of fresh herba (kg/ha) for the species *Primula officinalis* L. in the experimental year 2017 was influenced by both factors (A and B), the production being very distinctly significantly negative in relation to the control variant, both A and B planting variant.

The comparative study of the influence of factors A and B on the production of fresh herba (/plant), in the three experimental years, showed that the optimal distance of plants per row is 25 cm between plants, and between rows, the distance of 50 cm gives to the plant enough nutritional space to ensure significantly higher yields, compared to the control (Table 8).

Table 8. The influence of factors A and B on the average production of fresh grass (g/plant)

Experimental year 2017											
A factor influence						B factor influence					
Symb.	Dist. between rows (cm)	Average (g)	%	Diff. (g)	Sign.	Sym.	Dist. between rows (cm)	Average (g)	%	Diff. (g)	Sign.
A1	25	31.89	100.0	0.00	Mt.	B1	10	31.11	100.0	0.00	Mt.
A2	50	36.56	114.6	4.67	*	B2	25	38.33	123.2	7.22	***
A3	75	38.78	121.6	6.89	*	B3	50	37.78	121.4	6.67	**
DL (p 5%)				4.36		DL (p 5%)				3.47	
DL (p 1%)				7.22		DL (p 1%)				4.87	
DL (p 0.1%)				13.52		DL (p 0.1%)				6.87	

Following the comparisons of the interaction from factor B to factor A and from A to B (Table 9), a distinctly significant positive influence is found in factor B toward control, highlighting the distances 25/50 cm and 50/50 cm with distances of 25 cm, respectively 50 cm between plants in a row.

Table 9. The influence of the interaction regarding the distance between plants in a row (B) and the distance between rows (A) on the production of fresh herba (g/plant)

Experimental year 2017					
Symbol	Variant (cm/cm)	Average (g)	%	Difference (g)	Significance
B1A1	10/25	27.67	100.0	0.00	Mt.
B2A1	25/25	33.00	119.3	5.33	-
B3A1	50/25	35.00	126.5	7.33	*
B1A2	10/50	29.33	100.0	0.00	Mt.
B2A2	25/50	40.33	137.5	11.00	**
B3A2	50/50	40.00	136.4	10.67	**
B1A3	10/75	36.33	100.0	0.00	Mt.
B2A3	25/75	41.67	114.4	5.33	-
B3A3	50/75	38.33	105.5	2.00	-
DL (p 5%)				6.01	
DL (p 1%)				8.43	
DL (p 0.1%)				11.90	

CONCLUSIONS

Plants of *Primula officinalis* species were harvest from spontaneous flora and then acclimatized in the greenhouses. After acclimatizing the material, a rigorous selection was made, choosing the most uniform plants in terms of number of leaves, height and health. The experience established in autumn 2016 aimed to establish the plants optimal nutrition space.

The obtained results showed that *Primula officinalis* brought from spontaneous flora and planted in greenhouse conditions formed a rich rosette of leaves (7-10 leaves/plant).

During flowering, when the herba was harvested, the flowers represented 13.81% of the weight of the plant, the leaves 57.57% and the root 28.62%.

If a high production of flowers is pursued, a minimum distance between rows of 50 cm ensures an optimal nutrition space.

For the production of roots (radix), the distance of 75 cm between rows is favorable for the development of the root system.

The herba production (g/plant) was very good for the variants planted at a distance of 50 cm between rows.

In the case of large areas, where the works are carried out mechanized, the option of planting at a distance of 50 cm between rows and 10 cm between plants per row ensures high yields of herba (8800 kg/ha).

ACKNOWLEDGEMENTS

Some data are part of research PhD programme of the first author. The first author is thankful to NIRDPSB Braşov for support during PhD programme.

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CTPMA-MADR, Comitetul Tehnic Plante Medicinale și Aromatice - Ministerul Agriculturii și Dezvoltării Rurale.