



Production of Yellow Gentian (*Gentiana lutea* L.) Nursery Plants Suitable for Transplanting and Cultivation under Dry Farming Conditions in Mountain Region of Serbia

Dragoja Radanović • Tatjana Marković • Svetlana Antić Mladenović

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Summary: This paper deals with development of yellow gentian nursery plants in the open winter beds established in the mountainous region of Serbia at 1000 m a.s.l. Number, weight and size of one-, two- and three-year-old nursery plants were measured in succession for two years. The one-year-old nursery plants had an average 1.5 g fresh weight and 0.4 g dry weight, while the average length and width of the thickened root parts were 6.0 cm and 0.4 cm, respectively. The average fresh and dry root weights of the two-year-old nursery plants were 4.9 g and 1.4 g, respectively, with the length and width of the thickened root part 10.8 cm and 1.2 cm, respectively. The average root weights of the three-year-old plantlets were 15.6 g (fresh weight) and 4.7 g (dry weight), but they were too branchy and difficult for transplanting into the field. The average number of yellow gentian nursery plants per m2 of the open winter bed, following the first, second and third growing year were 714, 243 and 95, respectively. Two-year-old nursery plants proved to be the most suitable for establishing large-scale plantations for the production of yellow gentian root under dry farming conditions in the mountains of Serbia.

Keywords: cultivation, Gentiana lutea, mountain region of Serbia, nursery plants, roots, yellow gentian

Introduction

Yellow gentian (Gentiana lutea L.) is a medicinal plant widely used in folk medicine, pharmaceutical and food industries. Market demands good quality homogenized raw material, Gentianae radix, continuously grow. The content of biologically active compounds defining its quality depends on the growing conditions, plant age and ecotype, among other things (Schultze & Franz 1980), thus favouring cultivation of this endangered plant species in comparison to its collection from the wild.

Investigations regarding yellow gentian cultivation were initiated few decades ago in France and Germany (Barralis & Chadoeuf

D. Radanović • T. Marković •
Institute for Medicinal Plant Research "Dr Josif Panči

Institute for Medicinal Plant Research "Dr Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia e-mail: tmarkovic@mocbilja.rs

S. Antić Mladenović

University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade-Zemun, Serbia

1973, Franz & Fritz 1978). Very early, it also started in Romania (Heltmann 1968, 1970) and later in Italy, Finland, and the Balkans (Bezzi et al. 1986, 1996, 1997, Bezzi & Aiello 1993, Menghini et al. 1996, Galambsi 1996, Kušar & Baričević 2006, Radanović et al. 2007a, b and 2008). Although more than four decades passed since the first promotion of yellow gentian cultivation technology, its production never really reached massive expansion. The long lasting production of *G. lutea* roots, its slow growth, frequent weed control measures and full crop density maintenance in the early years are the main reasons why farmers are reluctant to cultivate this species.

After the failure of establishing yellow gentian plantations by direct sowing seeds in the field (Barralis et al. 1978), German researchers Franz & Fitz (1978) developed a successful method

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which includes breaking of the seed dormancy by stratification procedure and further production of nursery plants in the greenhouse conditions. In addition to this method, Fitz et al. (1980) presented similar results regarding the initiation of yellow gentian seed germination by the use of gibberellic acid and later planting 2- or 3-monthold plantlets in the field during the same spring season. This method gave quite good early results in Germany, France and to a certain extent in Italy and Finland (Seitz et al. 2005, Aiello & Bezzi 1998, Galambosi & Galambosi 2010). However, the authors reported problems with loss of crop density following the first winter, due to the frost which pulled out a great number of plantlets from the soil (Fritz et al. 1993, Schultze & Franz 1980, Bezzi & Aiello 1993). The quality of produced nursery plants is the key issue in achieving a good reception of nursery plants following transplantation and preserving original crop density in plantations.

Production technology for yellow gentian nursery plants in the open winter beds was developed for agro-ecological conditions of Serbian mountains and promoted six years ago (Radanović et al. 2007a). The method is based on seed stratification under the influence of winter temperatures in the natural conditions of the mountain areas and is very easy to use; it requires no special equipment and can be conducted with a small financial investment. Its use initiated the establishment of vellow gentian plantations at several localities in Serbia. However, in practice, utilization of one-yearold nursery plants for these purposes revealed some weak points; the small mass and size of such young nursery plants were the main reason for failures recorded in plantations established under dry farming conditions, common for the

majority of Serbian mountain localities above 1000 m a.s.l. Transplanted one-year-old nursery plantlets were so small and delicate, that they failed to survive the conditions of relatively low rainfall accompanied with frequent occurrence of summer temperatures above 30°C. In addition, efficient irrigation was impossible at most localities above 1000 m a.s.l. Overcoming the presented obstacles was the main issue in establishing yellow gentian large-scale cultivation suitable for dry farming conditions. The use of nursery plants older than one year has been considered a possible solution to the problem.

Therefore, the aim of this paper was to examine the characteristics of yellow gentian nursery plants when left in the open winter beds for two or three growing seasons, as the basis for recommendation of an optimal period for their transplantation under the dry farming conditions on arable land in Serbian mountain regions and similar localities in the wider Central Balkan region.

Materials and Methods

Origin of Plant Material

Seeds of *Gentiana lutea* ssp. *symphyandra* were originally collected from the natural stands of mountain Suvobor (760 m a.s.l., g. latitude N 44° 08' 10" and g. longitude E 20° 11' 07") in Serbia, and multiplied since 2002 in experimental small plantations in the forest tree nursery Kaluđerske bare of the National Park Tara in Serbia, until used for production of nursery plants in this trial.

Experimental Locality

The research was conducted in the forest tree nursery Kaluđerske bare of the National Park Tara in Serbia (1004 m a. s. l., g. latitude N 43° 53' 41" and g. longitude E 19° 33' 41"), where the

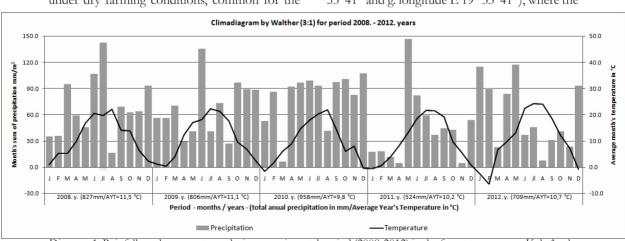


Diagram 1. Rainfalls and temperatures during experimental period (2008-2012) in the forest tree nursery Kaluđerske bare of the National Park Tara, Serbia

yellow gentian nursery plants have commercially been produced since 2007.

The main climatic conditions of the experimental locality were as follows: annual temperature was 6.1°C and precipitation was 900 mm, while average temperature for the growing season (April-September) was 11.9°C and precipitation 448 mm. Rainfalls and temperatures during the experimental period are presented in Diagram 1.

Establishing and Maintaining Open Winter Beds

The survey was conducted in open winter beds established successively every fall in 2008, 2009, 2010, and 2011 according to the technology of yellow gentian nursery plants production developed by Radanović et al. (2007a).

Open winter beds were established at the cambisol soil type. The composite soil samples were previously collected and analysed using the standard methods for soil chemical analyses (Džamić et al. 1996) in order to ensure similar soil fertility for the beds. The soil used for the open beds preparation were slightly acidic, with high humus/total nitrogen content, well supplied with the plant available potassium, and moderately supplied with plant available phosphorus (Table 1).

The open winter beds were established close to each other, prepared in the same manner, and sown each year in November. The beds were set to be one meter wide, and the seeds were sown in rows 15 cm apart from each other, applying the sowing rate of 4g/m². During the bed preparation, 6 kg/m² of FYM and 100 g/m² of NPK 15-15-15 were applied, and no additional fertilization

was performed until the end of the experimental period. Weed controls were performed manually, the beds were irrigated only in extremely dry periods, and on sunny days they were shaded with low-mounted nets (reducing insolation by 30%). During the entire experimental period there was no need for fungicide or insecticide application.

Monitoring of the Nursery Plants

Measurements regarding the growth and development of nursery plants were carried out following their first, second and third growing seasons in the open winter beds, according to the main scheme presented in Table 2.

During the growing periods in 2011 and 2012, the following periodical measurements were performed on nursery plants:

- In May: assessing the plant density by counting plants;
- In July: measuring fresh and dry weights of the aboveground and underground plant parts. Drying of fresh plant material was conducted in the oven at temperatures up to 65°C;
- In October (at the end of the growing season): assessing the plant density by counting plants; weighing fresh and dry underground plant parts; measuring the diameter of the primary root in the first centimetre of the root head; measuring the total length of root (together with root branches); measuring the length of the thickened section of the primary root and counting the number of lateral roots (only in older nursery plants).

All measurements were conducted on samples (nursery plants of different ages) randomly taken from the open beds. The samples consisted of

Table 1. Basic chemical properties of two average soil samples (0-30 cm) from the open beds

	рН		Humus	Total N	AL-P ₂ O ₅	AL-K ₂ O	
Sample	in H_2O	in KCl	%	%	mg/100g	$mg/100^{2} g$	
1	6.61	5.59	5.37	0.34	13.83	21.67	
2	6.69	5.71	5.45	0.34	12.30	18.93	

Table 2. The scheme of monitoring the growth and development of yellow gentian nursery plants in the open winter beds established in four successive years (2009-2012)

011-	Nursery plants monitoring							
Open beds establishment	Growth m	easurements	Morphological measurements					
establishment	2009	2010	2011	2012				
Fall 2008	I growing season	II growing season	III growing season	-				
Fall 2009	-	I growing season	II growing season	III growing season				
Fall 2010	-	-	I growing season	II growing season				
Fall 2011	-	-	-	I growing season				

sequences of the same row lengths, taken in four replications, from the middle of corresponding open bed; the row length for one-year-old nursery plants was 30 cm, and 80 cm for the two- and three-year-old nursery plants.

The obtained data were statistically analysed whether by the use of descriptive statistics or by ANOVA program with the implementation of the LSD-test.

Results and Discussion

Growth and Development of Nursery Plants of Different Ages

The average fresh and dry weight of the underground parts of one-, two- and three-year-old yellow gentian nursery plants observed at the end of two vegetations (2011 and 2012), as well as calculated percentage differences in the corresponding average weights (both fresh and dry) between the years are presented in Table 3.

As shown in Table 3, the highest differences between the years 2011 and 2012 in both fresh and dry average root weight were observed in oneyear-old nursery plants; in 2012 they were higher by 47.9% and 36.1%, respectively. Similar trend was observed for the root weight of the two- and three-year-old nursery plants in 2012, although the weight increase was lower (Table 3). The higher root weight of nursery plants of all ages, observed by the end of 2012, probably resulted from more favourable climatic conditions of that year in comparison to 2011 (Diagram 1). Climatic differences between the years were particularly obvious during the winter-spring period. In 2012, the amount of precipitation for the period January-April was higher by 260 mm/m², and the average temperature for March-April (beginning of growing season) was higher by 2.2°C (Diagram 1). Such conditions in 2012 positively affected the initial growth of gentian nursery plants in April which resulted in greater root weight of plantlets

of all ages observed by the end of that growing season, but most obviously in the case of one-year-olds (Table 3).

As expected, during three years of growth in open winter beds, the average weight of underground parts of gentian plantlets increased significantly. The dry root weight of one-year-old plantlets obtained by the end of the first growing period of 2011 and 2012 increased 3.0 to 3.8 times, respectively in the second vegetation, and 10.1 to 12.4 times in the third one, respectively. In addition, the average dry root weights of nursery plants at the end of third vegetation were 3.3 times higher in both years in comparison to those of the second one (Table 3).

Due to reduced spacing, nursery plants in the open beds achieved significantly lower root weight than the same age nursery plants transplanted in the field. Franz & Fritz (1978) reported that weight of fresh gentian root obtained in Bavaria ranged from 1.7 to 3.3 g for one-year-old yellow gentian plantlets, 22-43 g for two-year-olds, and 93-121 g for three-year-olds. In our previous experiments conducted in the same environmental conditions, average weight of fresh roots following the second and the third growing periods in the field were 11.8±3.9 g and 77±26.1 g, respectively (Radanović et al. 2007b). Based on these observations, it seems illogical to grow nursery plants in open beds for two years in order to achieve such low mass (Table 3), as the plantlets transplanted in the field reach several times higher weight and size in the same period of time. However, there are many evidences of one-year-old plantlets decay in the field and dramatically smaller number of surviving and persisting plants in the successive growing seasons in the mountains of the Balkans and the Apennines (Bezzi et al. 1997, Kušar & Baričević 2007). Consequently, this may justify the investment in keeping nursery plants in the open beds for two years under the dry farming

Table 3. Average weight of the underground parts of yellow gentian nursery plants of different age, recorded at the end of two vegetations (in October)

The age of		Fresh we	eight (g)	Dry weight (g)			
nursery plants	2011	2012	Ratio (%) 2012/2011	2011	2012	Ratio (%) 2012/2011	
One year old	1.21	1.79	147.93%	0.36	0.49	136.11%	
Two year old	4.69	5.09	108.53%	1.36	1.49	109.56%	
Three year old	14.28	16.95	118.70%	4.45	4.94	111.01%	
LSD 5%	1.26	1.57		0.30	0.50		
LSD 1%	1.81	2.26		0.43	0.72		

different age, ob	served at the end of to	wo growing seasons (i	n October)			
T1 C	20)11	2012			
The age of nursery plants	Root collar diameter	Primary root length*	Root collar diameter	Primary root length*		
nursery plants	(mm)	(cm)	(mm)	(cm)		

Table 4. The average length and diameter of the underground parts of yellow gentian nursery plants of

One-year old 4.18 5.58 6.33 4 48 Two-year old 10.68 10.80 13.03 10.75 Three-year old 16.00 16.30 17.40 17.48 LSD 5% 1.50 1.42 2.31 1.69 LSD 1% 2.16 2.03 3.32 2.43

Table 5. Number of yellow gentian nursery plants of different age in the open winter beds at the beginning of the growing period (mid-May) and at the end (late September) in 2012

V-11		14 May 2012		24 September 2012			
Yellow gentian nursery plants	One year old	Two year old	Three year old	One year old	Two year old	Three year old	
Average number per m ²	1616.7	386.7	138.0	714.0	243.3	95.3	
Min	1347	267	73	560	180	67	
Max	2020	567	273	900	373	147	
St. dev.	237.0	119.7	57.1	102.3	66.5	27.6	

conditions, especially if that would preserve the crop density in the following growing seasons.

The average lengths of the thickened part of the primary yellow gentian root and the root collar diameter in the first centimetre of its length given in Table 4 illustrate differences in sizes of the underground nursery plant parts of the same age between different growing seasons. Root diameters of the nursery plants of the same age were in most cases similar in both years; the biggest difference in root collar diameter between the years was 22.0% for two-year-old nursery plants, while the differences were smaller for one- and three-yearold ones, 7.2% and 8.8%, respectively. As for the length of the thickened primary root part, the biggest difference was observed in one-year-old plants (13.4%), and a bit smaller (7.2%) in threeyear-old ones, while the length of thickened root of the two-year-old nursery plants was virtually identical in both years (Table 4). The larger root collar diameter and slightly larger primary root length observed in 2012 may be associated with more favourable temperature/precipitation regime in the first part of 2012 growing season compared to those of 2011 (Diagram 1).

Root collar diameters at the end of the second and the third growing season were on average 2.8 and 3.9 times higher, respectively, than those at the end of the first growing season. The length of the thickened part of the primary root following the second growing season was approximately 1.8 times higher, and following the third growing season it was 2.8 times higher than those achieved at the end of the first growing season. In comparison to the two-year-old nursery plants, the three-year-old ones increased their root collar diameter and their root length 1.4 times and 1.6 times on average, respectively.

As it was expected, the root weight and sizes were multiplied when the one-year-old nursery plants were left to grow in the open beds for more than one growing season; the increase of weight was much higher than the increase of the lengths and the root collar diameters (Tables 3 and 4). However, these increases brought about reduction in the number of nursery plants per unit of area (Table 5).

Since yellow gentian seed germination rates prove not to be uniform each year, depending mainly on climatic factors during its formation and ripening (Radanović et al., 2005), a relatively high sowing rate (3-5 g of natural seeds per m²) has to be applied in order to provide a sufficient number of seedlings (1.616 seedlings per m²) in the open beds. Because delicate gentian plantlets are sensitive to unfavourable environmental conditions (high insolation and temperatures) in the early stages during their first growing season in the beds significant reduction in density of plantlets occurred; at the end of the first growing

^{*} Refers to the thickened primary root part

season, the number of nursery plantlets was several times lower than the number of seedlings (Table 5). Following their second and third growing seasons in the open beds, this number reduced even more by the end of the growing season (to 243.3 per m² and 95.3 per m², respectively) due to freezing in winter or competition in which plantlets with bigger habitus suppressed the less developed ones until those eventually weakened and died (Table 5). At this point it is reasonable to ask whether the lower density of plantlets in the seedlings phase would have a positive effect on the number and size of gentian nursery plants during the three-year period spent in the open beds.

Development of Nursery Plants during Three Growing Seasons in the Open Winter Beds

Depending on the weather conditions in the first growing period yellow gentian seedlings were emerging from late March and during April, while the primary radical reached of 3-7 cm in length in late April and early May. Variation range of the underground plant parts weight was very wide (Table 6), as a consequence of uneven seedlings growth and development, and their uneven density in the open bed rows. Therefore, at the end of the first growing season only 34.6% of the young nursery plants reached the fresh weight of the underground parts above 2 g (Table 6). In addition, their average root thickness was ~0.45 cm at that time and the average length of the fleshy (thickened) primary root was 6.33 cm (Table 6 and Photo 1). Consequently, plantlets of such weight and size are not always capable of surviving unfavourable conditions after being transplanted into field, thus severe drying of their mass occurs in dry farming conditions in the mountainous areas of Serbia, mostly as a consequence of insufficient soil moisture and often high temperatures during the period June-August. According to our experiences, in such circumstances only larger nursery plants were able to survive. Similar problems of losing the planted seedlings and the inability to achieve a sufficient number of plants in gentian plantations due to unfavourable climatic conditions were also observed in other countries, namely in Italy (Bezzi & Aiello 1993, Bezzi et al. 1997) and Slovenia (Kušar & Baričević 2007).

In the second growing period, up to the middle of growing season (July), nursery plants significantly increased the size and weight of their underground parts, at the same time considerably increasing the leaf rosette (Table 6 and Photo 2). Weight of their aboveground parts in this period accounted to approximately 50%

of their underground parts weight. The average total weigth of the fresh and dry plantlets were 4.44 g and 1.16 g, respectively, though interval of variation was very wide (Table 6). Unlike the open winter beds, gentian plantlets of the same age growing in the field in the middle of the second growing period achieved much higher weight and had an almost equal ratio of their fresh weight of the underground and the aboveground parts; 11.8 g root and 10.15 g leaf rosette (Radanović et al. 2007b). It is obvious that the reduced spacing between nursery plants in the open beds, compared to large scale plantation condition, considerably decreased root growth and affected the aerial plant parts in particular.

The average values of fresh and dry weights, root collar diameter, length of the thickened primary root part, and total root length reached at the end of the second season, made twoyear-old nursery plantlets much stronger in comparison to the one-year-old ones (Table 6, Photos 1 and 2). About 80% of nursery plants had fresh underground part weigth over 2 g, while 65.6% of them had dry root weight over 1 g (Table 6). Based on the obtained data, it can be assumed that 2/3 of the two-year-old nursery plants produced in the open winter beds might be suitable for establishing plantations in dry farming conditions of the mountainous areas of Serbia. The only disadvantage of their biennial growing in the open beds may be an increase in lateral branching of the primary root compared to the one-year-old nursery plants (Photos 1 and 2), though the lateral roots were quite thin and usually do not represent a great problem if appropriate planting method is applied.

In the third growing period, very wide interval of variation of the weight parameters was observed, similarly to the younger vellow gentian nursery plants (Table 6); the average fresh and dry weight of the underground parts were 17 g and 5 g, respectively. The average thickness of the root collar was 16.3 mm, and the primary root become significantly branched with 2-5 thickened lateral roots greatly differing in length (4-22 cm) (Table 6), which made them quite robust. According to their weight, almost all three-yearold nursery plants were considered acceptable for transplantation under dry farming conditions, but the vast ramification of their roots reduced their quality in terms of practical suitability in establishing plantations for yellow gentian root production. Therefore, such nursery plants might be preferentially used for returning yellow gentian to its natural habitats where this species is eradicated due to its excessive exploitation.

Table 6. Size and weight of the underground parts of one-, two- and three-year-old yellow gentian nursery plants, by the end of the growing season in October 2012

Observed properties	Mean	Min.	Max.	Std. Dev.	1 \ /		
	1 , 1	, ,,1	1.60.	1	> 2 g	1–2 g	< 1 g
One-year-old					0.0	0.0	100.0
Weight of dry underground parts (g)	0.49	0.15	0.80	0.180	0.0	0.0	100.0
Weight of fresh underground parts (g)	1.79	0.60	3.00	0.643	34.6	50.4	15.0
Root collar diameter (mm)	4.48	2.00	10.00	1.914			
Thickened primary root length (cm)	6.33	2.50	14.00	2.728			
Total root length (cm)	11.52	4.50	18.00	3.575			
	ır-old nurser						
Weight of fresh aboveground parts (g)	1.52	0.20	4.50	1.215	20.2	34.7	45.1
Weight of fresh underground parts (g)	2.92	0.55	7.40	2.114	65.1	20.2	14.7
Weight of dry aboveground parts (g)	0.35	0.04	1.00	0.280	0.0	5.3	94.7
Weight of dry underground parts (g)	0.81	0.15	2.10	0.594	10.4	30.1	59.5
Two-year-old							
Weight of fresh underground parts (g)	5.09	0.50	16.50	4.342	80.3	14.7	5.0
Weight of dry underground parts (g)	1.49	0.15	4.80	1.263	40.1	25.4	34.5
Root collar diameter (mm)	13.03	10.00	17.00	2.757			
Thickened primary root length (cm)	10.75	6.00	16.00	3.489			
Total root length (cm)	18.33	15.00	21.00	2.658			
	ar-old nurse	U 1					
Weight of fresh aboveground parts (g)	6.66	1.80	27.10	6.710	86.7	13.3	0.0
Weight of fresh underground parts (g)	15.51	4.40	52.50	12.994	100.0	0.0	0.0
Weight of dry aboveground parts (g)	1.59	0.45	6.60	1.625	20.0	33.3	46.7
Weight of dry underground parts (g)	4.24	1.20	15.00	3.668	73.3	26.7	0.0
Three-year-old							
Weight of fresh underground parts (g)	16.97	2.00	44.50	13.305	100.0	0.0	0.0
Weight of dry underground parts (g)	4.96	0.60	13.00	3.860	82.0	17.3	2.7
Root collar diameter (mm)	16.3	13,00	21,00	0.281			
Thickened primary root length (cm)	6.58	4.00	9.00	1.960			
Total root length (cm)	18.83	17.00	22.00	1.941			
Number of lateral root branches (≥ 35% of							
the primary root thickness)	4.00	2.00	5.00	1.265			

Conclusions

Based on the presented quality parameters of G. lutea nursery plants, production procedure of two-year-old nursery plants in the open winter beds might be recommended. In the mountainous regions of Serbia, at altitudes above 1000 m, approximately 195 high quality G. lutea nursery plants per square meter of the open winter beds can be produced in the two-year production period with fresh roots weight of over 2 g. The root branching depends on seedlings density in the open beds; lateral root branches, on average one or two, are thin and do not represent a problem during field transplantation. Application of such nursery plants might significantly increase percentage of their reception in the field; therefore, greater success in preserving the crop density of the established yellow gentian plantations might be achieved in the mountainous region of Serbia and the Balkans.

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Photo 1. One-year-old gentian nursery plants in October 2012



Photo 2. Two-year-old gentian nursery plants in mid-July 2012



Photo 3. Three-year-old gentian nursery plants in mid-July 2012

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Proizvodnja sadnica žute lincure (*Gentiana lutea* L.) za rasad i gajenje u uslovima suvog ratarenja u planinama Srbije

Dragoja Radanović • Tatjana Marković • Svetlana Antić Mladenović

Sažetak: U radu je prikazan razvoj sadnica žute lincure u rasadniku u otvorenim zimskim lejama zasnovanim u planinskom regionu Srbije na 1000 m nadmorske visine. Meren je broj, masa i veličina sadnica jednogodišnje, dvogodišnje i trogodišnje starosti u sukcesiji tokom dve godine. Sadnice jednogodišnje starosti imale su prosečnu masu 1,5 g (svežu) odnosno 0,4 g (suvu) i prosečnu dužinu zadebljalog dela korena 6,0 cm sa prosečnom debljinom 0,4 cm. Prosečna masa svežeg korena sadnica dvogodišnje starosti je bila 4,9 g a suvog korena 1,4 g uz dužinu zadebljalog dela korena 10,8 cm i debljinu vrata korena 1,2 cm. Prosečne mase korena trogodišnjih sadnica su iznosile 15,6 g (sveža), odnosno 4,7 g (suva), ali je koren ovih sadnica bio previše razgranat i stoga nepodesan za plantažnu sadnju. Prosečan broj dobijenih sadnica po m2 leje na kraju prve godine je iznosio 714, na kraju druge 243 i treće 95. Dvogodišnje sadnice žute lincure proizvedene u otvorenim zimskim lejama, po svojim karakteristikama se se pokazale kao najpogodnije za zasnivanju plantaža lincure za proizvodnju korena u uslovima suvog ratarenja u planinama Srbije.

Ključne reči: Gentiana lutea, koren, planinski predeo Srbije, sadnice, uzgajanje, žuta lincura