

## PECULIARITIES FOR AGRICULTURAL TECHNOLOGY OF *GENTIANA LUTEA*

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### Abstract

*The increased demand for the pharmaceutical market puts pressure on the wild species which on the other hand need to be protected. Collecting the wild plant species for pharmaceutical purpose may become a threat for the conservation of rare species such as Gentiana lutea which was declared as protected species for Romania since 1977. The purpose of this article was to develop a technology for the cultivation of this species as a crop plant. The starting material may be either seeds either seedlings, each of them involving different negative or positive aspects. The produced rhizomes morphometrically characterized and production were calculated. The entire technological chain is described and it may be considered that Gentiana lutea may become a valuable crop species for producers as well as for the pharmaceutical industry.*

**Key words:** crops, field cultivation, *Gentiana lutea*, pharmaceuticals, Romania

### INTRODUCTION

Among the species of *Gentiana* genus, *Gentiana lutea* L. (i.e. popular name *rifling*) is known as one of the most valuable medicinal plant. It is part of Romania wild flora and it can be today harvested only in nature. After 1971, this species was declared as nature monument species by the Romanian Academy, with clear restrictions for being collected from the wild flora in our country. This was the momentum when research started for initiating the cultivation of this species. The biology of the species was studied by Heltmann [4],[5],[6] and natural dissemination gave some clues for its use as crop species.

Attempts to introduce *Gentiana lutea* in culture were reported in various countries such as: Russia (in the southwest region of the Altai Mountains), in the Baltic Republics, near Kiev [2], [3], [7], [8], [9], [10] and [11]. Following recent concerns related to food security and climate change [1] for the use of this species it was considered that the variability of the species is low in terms of differences among different populations in Europe. Given that in our country meets at

high altitudes on rocky places, through meadows, sunny slopes in culture this species was zoned in wet and cool-Carpathian regions or counties perimeter areas such as Brasov, Sibiu and Neamț [5]. The scope of this article is to present relevant parts on the entire flow of cultivation technology of the species.

### MATERIALS AND METHODS

The technology of *Gentiana lutea* is presented in line with the current specification known for the crop plants.

Seeds are originating from the nature, Brașov county, the Postăvaru Mountains and morphological traits of the rhizomes were determined such as: length, diameter, weight. The experimental field was conducted in Brașov area at an altitude of 500 m, 200 m less compared to the natural habitat.

### RESULTS AND DISCUSSIONS

#### *Rotation plot*

*Gentiana lutea* L. is a perennial plant, and as such are grown outside the crop rotation. Making culture is mainly by planting seedlings and for this reason it is

recommended that prior working the land, cleaning the field of weeds. It was observed that the cultivation of *Gentiana* is followed by the invasiveness of the weedy species which are removing, for the first stages the installation of the seedlings. In case of crops rotation we are recommending crops for green fodder legume species with a short period of vegetation (peas, beans pods) and grain crops are then able to follow this species (Photo 1).



Photo 1. *Gentiana lutea* L. in its natural habitat

#### Fertilization

The research that has been done so far has yet to determine fertilizer needs of the plant. Following our results we can appreciate that phosphorus is making a significant contribution to the formation of the root system and increase plant vigour. Nitrogen and potassium in combination with other nutrients, creates favourable balance harmonious growth and development of plants. Rifling requires calcium supplements which is also due to the calcareous substrate it is growing in the wild and which is highly contributing to the healthy status of the plant. In organic culture a beneficial contribution has the application of manure as a source of nutrients and the possibility of improving the physical properties of soil condition and approaching those prevailing culture in spontaneous flora.

In terms of the ratio of macronutrients (NPK), we recommend a ratio of 1: 1.5 :1. Nitrogen may be applied in doses of 120-150 kg/ha active ingredient of which 1/3 spring planting, and 2/3 years springs, for the 2<sup>nd</sup> and 3<sup>rd</sup> of

culture. Phosphorus and potassium may be supplied in an amount of 150-180 kg/ha, respectively, 120-150 kg/ha active, half of which fall in basic work to prepare the ground and the other half, the two "equally in autumn for the 1<sup>st</sup> and 2<sup>nd</sup> culture.

Autumn, with deep ploughing is given and calcium (4-5 t / ha) and manure in the amount of 25-30 t / ha.

Fertilizers applied during the growing season during performing incorporating mechanical hoeing.

Well fermented manure has a favourable action on the production of roots, the recommended dose of 20-40 t / ha should be administered prior plant.

#### Soil preparation

Abolition of previous culture is a compulsory and therefore the destruction by chopping crop residues remaining from previous crop field will be needed. Along with shredding plant debris, the land must undergo basic work. It is impossible to start a culture without the profound working of the soil. The seedlings are very sensitive in the first period of acclimation.

The requirements for quality and time of soil working are greater if *Gentian* is seeded directly in the field. Thus, the acclimation will be faster in case you use seeds instead of seedlings.

We recommend in the first stage a land plowing at 28-32 cm depth using 2 or 3 reversible plows furrows in aggregate harrow stars. If the soil is dry, it first executes a work surface plow or disc harrow, following that deep plowing to make optimum soil moisture. It has to be at least similar to the moisture from the natural conditions into the mountains.

Prepare seedbed for crop establishment of grooves requires attention. The land must be loose and cut to a depth of 18-20 cm to ensure optimum water storage and root development. Seedbed preparation is done by combiner or in aggregate with disc harrow teeth and adjustable to achieve a small and uniform surface layer. Soil loosening must be made from a crossing unit without reversing the layers to soil water is lost through evaporation process.

In cultures established by direct seeding field is mandatory roller work layer before and after seeding with smooth rollers.

When planting the seedling, the demands on seedbed preparation are not as high.

#### *Propagation*

Gentian is multiplied by seed or in vegetative stage. However the generative propagation is the most easy to be used even the genetic variability may be expressed.

Sowing directly in the field or use seedlings are methods for taking into account local conditions and possibilities and weighing the economic aspects as well as the soil conservation issues.

Planting of seedlings is a propagating safer but more expensive and requires the existence of a labour force. On contrary sowing directly in the field is conditioned by the lack of weeds in a crop land for rifling.



Photo 2. *Gentiana lutea* as a crop species in the field experiment

#### *Culture establishment*

It is a highly important link in the technology and culture of this species requiring the preparation in advance by taking into account all factors: biological, physical and chemicals [7]. Regardless of the method used (direct seeding or seedling field), the chosen seed shall correspond to qualitative indicators included within limits to ensure a uniform chain with vigorous plants able to highlight as much their own genetic potential in production terms (Photo 2).

For direct seeded crops, the best time for sowing is on the brink of winter in August or September. Also at this age seeding and seedling production will be performed in layers. Following studies undertaken

recommendation is to sow fresh seed in the fall. Emergence is difficult to occur next spring, quite late for Romania conditions (April-May).

The plants remain in layer until the fall or next spring when planting is definitely the place to plot.

#### *Technical parameters – planting distance and sowing depth*

The distance between rows, whether directly sown or planted seedling is 50 cm. In the case of the seedling planting, the distance between the plants in billons ranges between 15 and 20 cm. The same size range between plants in the billon is left by thinning when culture is achieved by sowing in billon further away. The density of the culture may be 10 to 14 plants / m<sup>2</sup>.

In cold layers, the distance between billons is 12.5 to 15 cm. The recovered seedlings may be used in filling the gaps.

The distance between plants in the billon will be higher (40-50 cm) on seed lots that are held separately from the surface to produce roots.

Seeding depth is between 0.5 and 1.5 cm. Special care is given to planting depth when executing this work to the era of the fall. Plants are buried in soil at 1.5-2 cm more than the depth at which they grew up in layers.

In the case of standard seed or seedling for a hectare of *Gentiana lutea* are used 4-6 kg of seeds which must have physical purity between 80 and 85% and a germination of at least 50-40%.

Given the long period interval is recommended seed germination of indicator plants (lettuce, mustard) to carry out blind hoeing between billons.

On an area of 350-400 m<sup>2</sup> of layers is necessary to obtain seedlings for planting one hectare of crop. The amount of 0.4 to -0.5 kg seed is sufficient for seeding the surface layers.

For planting one hectare of seedlings 120000-150000 of exemplars are required.

For direct sowing in the field may be used Drills SUP SUP-21 and-29 or any similar technology fitted with small seeds and limiting distribution for the coulter.

#### *Crop culture maintenance*

Weed control and thinning are the major working maintenance in culture of this species. The crops grown by sowing directly in the field weed risk is particularly high because rifling seeds spring up pretty hard. Therefore, in late spring when the land was weeded it can be performed a control by treatment with Gramoxone or Reglone doses of 3-5 t/ha, applied before the advent of rifling plants at the soil surface. In organic farming this is much expensive and it needs labour work.

Although it was significant in combating chemical and agronomic methods are part of the integrated control system. In integrated weed control, biological control techniques, physical, chemical, mechanical and their integration into a sustainable strategy the primary aim.

Other maintenance, mechanical weeding, hand weeding, may begin immediately after plant emergence indicators. They are carried out during the growing season, whenever needed. The crops grown by seedling immediately after planting and mechanical and manual weeding are running. In layers outside of weed removal, thinning, bedding, watering at times to execute when necessary.

The springs for the 2<sup>nd</sup> and 3<sup>rd</sup> years shall apply doses of nitrogen, and the autumns for the 1<sup>st</sup> and 2<sup>nd</sup> years the phosphorus and potassium, have to be incorporated by mechanical hoeing.

#### *Diseases and pests control*

The scientific literature does not indicate the presence of pests and diseases in this species. However, studies undertaken by us have made observations on the fruit with seeds by a ladybug yet unidentified. This is particularly important seed crops and would probably solve through a program of insecticide treatments.

As for diseases, relatively small surfaces on which *Gentiana* is cultivated, the few diseases that met did not produce significant damage.

#### *Harvesting*

Gentian roots can be harvested when the plants have at least 3 years old. In the literature there are recommendations how that culture is harvested when plants of Gentian of 4-5 years old (Heltmann and Silva, 1970;

Claus et al., 1977). It should however be stressed that there is a risk that some of the active principles of roots, as *amarogentina*, to reduce the weight of plants with increasing age.

The time period for harvesting gentiana is set for the fall during October or later during long autumns. During this period the processes of biological activity ceased foliage and roots are slowed down.

Until the completion of studies on the dynamics of accumulation of active principles that could set a different time of harvest, can be considered optimal harvest age remains autumn.

Harvesting seeds from seed lots is when 60-75% of the grains became yellow-brown.

Harvesting module consists of small surfaces in manual removal of roots, with spade and generally used on larger areas plows beet or sloppy plows which detaches soil.

After deployment, the earth shook and roots are gathered in piles. Follow continue to work to remove dry portions, injured and with the package, remnants of leaves.

Production that can be obtained from a hectare is about 6000-8000 kg fresh roots.



Photo 3. Rhizomes of *Gentiana lutea* – production in the field experiment

If fruit seed lots rods cut with a sickle in the last whorl fruit, bring on the premises where under sheds on concrete platforms in small stacks allow them to dry. When all the seeds were given specific colour move to threshing. This can be done, depending on the amount of material with smoothing tools, combine or by tapping and shaking. Debris removal sticks, leaves, fruit is done using specific technology.

#### *Raw material processing*

Immediately after harvest occurs, the conditioning of the roots by shaping and



transport in areas for processing as fast as possible. Thus, roots are following washing, small roots removal and then leave a few days to boil at a specific temperature. The thick roots are spliced in two, and the long cut into segments of 10-15 cm.

The next step is drying, which can be achieved in natural conditions in barns, warehouses, bridges, clean, well ventilated, or in the sun on concrete platforms.

In drying, hot air drying is faster with their quality wins. Here, the optimum drying temperature is between 50° C and 40°C.

From 3.5-4.5 kg fresh roots it can be obtained 1 kg of dried roots. It is estimated that the entire production of dried root may be 1500-2000 kg / ha.

Once the roots have reached the maximum 13% humidity, they will be packaged in bags, bale and store in clean rooms, dry and airy.

#### Biological examination

Gentian root and conditioned obtained by the above-mentioned technology, must contain at least 33% soluble solids material.

In a macroscopic analysis, we proposed the establishment of characters seen with the naked eye or with a magnifying glass, and those which can be determined by sensing the smell and taste.

Thus the examination of fresh roots and rhizomes fragments found that they are of cylindrical shape, 51 cm to 98 cm in length, 2.8 cm - 7.9 cm in diameter (Table 1). Please note that determining the dimensions (length, thickness, etc.) was carried out in the most developed underground organ, with a ruler.

The colour is generally yellow-brown on the outside and yellow inside. The colour could be seen on a dry, both outside and inside, on fresh fracture. If this character was not different between genotypes selected.

Root surface shows longitudinal ribs and transverse striations surface rhizomes. Fracture is smooth and non fibrous and non soft.

The smell is weak and very bitter taste, which indicates the possible presence of alkaloids, glycosides and certain principles or assets. If this character was not different between selected genotypes.

From these studies it can be said that the it is formed a well developed rhizome, which starts numerous roots.

The number, size and vigour leaf spring is occurring in close correlation with the package diameter and root length of the plant went into winter.

The main morphological features of the underground organs for the 4 families identified of *Gentiana lutea* L., are presented in Table 1.

Table 1. Results concerning the morphological characteristics of the underground organs of the 10 studied families of *Gentiana lutea* L.

| No | Genotype       | Rhizomes      |             |             |            |
|----|----------------|---------------|-------------|-------------|------------|
|    |                | Diameter (cm) | Length (cm) | Branches no | Weight (g) |
| 1. | G <sub>1</sub> | 6.2           | 91          | 4           | 1,210      |
| 2. | G <sub>2</sub> | 7.0           | 98          | 3           | 1,410      |
| 3. | G <sub>3</sub> | 7.1           | 93          | 4           | 1,320      |
| 4. | G <sub>4</sub> | 7.9           | 72          | 4           | 680        |

Source: Own calculation.

The analysis of measurements made in the package diameter showed a high variability, which is confirmed by the value obtained to calculate the coefficient of variation S= 34.2% (Table 2).

Although a population with a complex well represented rhizome, however there is great variation among the four families studied. Rhizome length variability had values ranging between 51 and 98 cm. These values have generated a high coefficient of variation S= 21.51% (Table 2). It should be however noted that these values depend very much on rainfall and their distribution among the vegetation period.

Table 2. General characteristics for rhizomes, morphometrical parameters – direct (variant, standard deviation) and derivatives (variability coefficient) for selected *Gentiana lutea* L

| Measurements      | Variant s <sup>2</sup> | Standard deviation | Average | Variability Coefficient |
|-------------------|------------------------|--------------------|---------|-------------------------|
| Diameter (cm)     | 3.46                   | ±1.86              | 5.44    | 34.20                   |
| Length (cm)       | 265.73                 | ±16.30             | 75.8    | 21.51                   |
| Brench (no)       | 265.73                 | ±0.699             | 3.6     | 19.42                   |
| Fresh weighth (g) | 185,139                | ±430.28            | 785     | 54.81                   |

If the number of branches of underground organs variability was medium, in some cultures showing interest forms with a high degree of branching high, assuming that the ramifications of failing to accumulate a higher content of active ingredients compared to the main rhizome.

The total mass of underground organs had the largest range of variation, the values being between 680 g to 1,410 g (Table 1). Thus the coefficient of variation of  $s\% = 54.81$  (Table 2), had a high value. However, we can assume that a subjective question this variability, where nature and unevenness can be discussed in terms of age, because the species is perennial and plants that were performed measurements were derived from wild.

The data presented in Table 1 column 4, it can be concluded that in terms of the diameter of the package, the study population presented a variability that allows selection of works on desired genotypes.

Rhizome length per plant has a value of coefficient of variation large enough to make the selection in the direction of the character to be effective.

Number of branches of underground bodies had a middle value of the coefficient of variation. This character is of interest to the extent that would allow the successful selection of works in terms of increasing the number of branches

For the total mass of underground organs, the coefficient of variability presented a high value. This feature is particularly important because it is primarily responsible for the accumulation of active principles.

Looking at all of the obtained data, it is clear that the biological material examined and selected of Gentian 4 families were characterized by an increased genetic polymorphism. Using the selection of this material one may enable the future phenotypic and genetic differentiation, both quantitatively and qualitatively for valuable families.

## CONCLUSIONS

The agricultural potential use for *Gentiana lutea* proved that this species may become a crop species of valuable importance from a

pharmaceutical point of view. Therefore, for the future it might be possible to exploit more the agricultural peculiarities of the species in the benefit of the farmers.

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