

## SUSTAINABLE PROPAGATION OF HORSERADISH (*Armoracia rusticana*)

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### SUMMARY

Despite good agro-ecological conditions for its production and various possibilities of use in Serbia, horseradish is grown on relatively small areas. Due to good market prices and high yields that can be achieved by growing horseradish, a large number of farmers are interested in its production. Unfortunately they must manage production of horseradish planting material on their own. A field trial was set up on the sandy loam at the experimental site in the village Polatna (Municipality of Žabari), during the two consecutive growing seasons, 2019 and 2020, in order to determine the most efficient way of producing horseradish planting cuttings. Healthy plants were collected from the field where horseradish was grown in the previous period. After preliminary separation of first and second market class intended for selling, plants of approximately the same size appropriate for further reproduction were selected. Crown cuttings (upper part of roots – roots head) were cut off the remain roots and the cuttings were divided in three groups: a) crown cuttings (CC), b) whole root cuttings (WRC), and c) half root cuttings (HRC), i.e. the treatments in our study. The average number of cuttings per plant during the experiment was 2.29 and the average weight of cuttings was 21.98 g. The highest number of cuttings per plant was recorded in the treatment of CC in both years of research, while cuttings propagated by WRC and HRC had a higher average weight compared to cuttings propagated by CC. When the goal of cultivation is intensive multiplication of planting material in order to increase the production quantity of cuttings for planting, it is most efficient to use CC. In that case, a significantly smaller share of marketable yields are expected. The usage of WRC as a planting

material provides producers with the highest yields and the highest share of first class roots in the total yield, yet with a sustainable amount of planting cuttings.

**KEYWORDS:** cuttings, horseradish, planting material, propagation

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### INTRODUCTION

Horseradish (*Armoracia rusticana* Gaertn., Mey., & Scherb. syn. *Armoracia lapathifolia*) is a perennial species belonging to the Brassicaceae family. Its roots contain significant amounts of biologically active and beneficial phytochemicals. Its distinctive pungent flavor derives from the high content of glucosinolates and breakdown sulfur products such as isothiocyanates. Horseradish is a highly valued raw material for its chemical properties which make it useful for different purposes (Gafrikova et al., 2014; Kim et al., 2015; Choi et al., 2017; Petrović et al., 2017; Manuguerra et al., 2020). In Serbia, young horseradish leaves are used for some local and traditional dishes. Cleaned and finely grated roots are used for preparation of different salads as a pungent condiment. As a long lasting tradition in some regions of Serbia, larger pieces of horseradish roots are placed in jars and barrels together with pickled vegetables or sauerkraut. This is done in order to preserve the freshness of the vegetables during the several month long storage period,

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as well as to prevent and slow down unsuitable microbiological activities. According to many sources it has a long history in ethnomedicine (Tucakov, 1984; Miladinović et al., 1997; Marzocco et al., 2015). Its use in the chemical industry is also important, where it serves as a raw material for the production of certain semi-finalized products (peroxidases), necessary for the production of some pharmaceutical products (Lavery et al., 2010). Therefore, horseradish can be considered as a vegetable crop (leaves and roots), as an aromatic and spice crop, as well as an industrial crop.

However, despite good agro-ecological conditions for its production and various possibilities of use in Serbia, it is grown on relatively small areas. Moreover, some elementary horseradish products are imported.

The electronically available FAO statistical files do not show data on the area under horseradish crops, its average yield or total annual production, both at the global level and at the level of continents or individual countries (FAOSTAT DATA, 2020).

Worldwide, horseradish is propagated asexually, mostly via root or crown cuttings. After harvest, the main root can be processed, and the side roots are retained for planting the following year. Planting material can be provided also via tissue culture, meristem tip culture *in vitro* and using transgenic material (Miladinović et al., 1997; Soudek et al., 2005; Uchanski, 2007).

The Statistical Office of the Republic of Serbia also does not present data related to areas, average yields or total production of horseradish (RZS RS, 2020). The data concerning the average prices and offers on the Serbian market during the last 15 years are available thanks to Serbian Agricultural Information Market System (STIPS Database). According to STIPS Database, the average prices of horseradish on the Belgrade's

Wholesale Market, during the past decade varied from 2.25 to 5 dollars per kilogram and the offer was mostly below average (Republic of Serbia, 2020a). Due to good market prices and high yields that can be achieved by growing horseradish, a large number of farmers are interested in its production.

However, there are no registered varieties of horseradish on the Serbian list of accepted varieties of agricultural plants (Republic of Serbia 2020b). Considering that companies and institutes that deal with vegetable seeds do not offer horseradish planting material (IFVC NS, 2020; IVC SP, 2020) Serbian producers must manage this issue on their own.

The aim of this research was to determine the most efficient way of producing horseradish cuttings in order to help interested farmers in the production of their own planting material and to strengthen the production of horseradish in central Serbia.

## MATERIAL AND METHODS

A field trial was set up during a two year period (2019-2020) at the experimental location in the hilly village Polatna near the Great Morava river (Municipality of Žabari). The soil type on the experimental site (44.41912° north latitude, 21.25908° east longitude, 211.2 m above sea level) was sandy loam of good chemical properties, but without calcium carbonate (Table 1).

In mid-November of 2018, well established healthy horseradish plants were collected from the field where horseradish was grown in the previous period. After preliminary separation of first and second market class intended for market, plants of approximately the same size appropriate for further reproduction were selected. Whole plants are brushed and cleaned from large pieces of soil and then washed with tap water. After drying, prepared plants were

Table 1. Soil chemical properties at the experimental location

| pH (KCl) | CaCO <sub>3</sub> (%) | Humus (%) | Total N (%) | Available P (ppm) | Available K (ppm) |
|----------|-----------------------|-----------|-------------|-------------------|-------------------|
| 5.15     | 0.0                   | 2.30      | 0.115       | 196.3             | 283.0             |

put into plastic bags and some sand was added in order to minimize the loss of moisture. In the late winter, the cuttings were prepared for planting. Vineyard shears were used for cutting. Before cutting, the shears' blade was cleaned with a dry cloth and dipped into the ethanol solution (70%) for 20 seconds. This was repeated before processing of each subsequent plant in order to avoid potential spread of infectious diseases. All leaf petioles were shortened to 2 cm above the root head. Crown cuttings (upper part of roots - roots' head) were cut off the remaining roots and the cuttings were divided in three groups: a) crown cuttings (CC), b) whole root cuttings (WRC), and c) half root cuttings (HRC). These were also the treatments in our study. The average WRC were more than 1 cm in diameter in mid-point and 15 to 20 cm long, obliquely cut at the top. HRC were up to 1 cm in diameter in mid-point and various lengths (about 15 cm). Initial CC were less homogeneous material but experimental CC of approximately the same volume were selected by an experienced technician.

The previous crops were garden peas grown as subsequent crops. After harvesting the peas,

the major nutrients were applied in the amount of 72 kg of nitrogen, phosphorus and potassium per hectare (commercial mineral fertilizer NPK 16:16:16). The harvest remains from peas were chopped and incorporated into the surface layer of the soil using a disc harrow and a rotary tiller. Basic land cultivation and planting of root cuttings (WRC and HRC) were performed during late March in rows, created with a triple plow with a special device (approx. spacing 70 cm between rows and approx. 25 cm within a row) at depth of about 15 cm. Planting of CC was done on cultivated land by stabbing CC into soil by hand to a depth of about 5 cm. There were 18 elementary plots, distributed over a completely randomized system (in three repetitions), with size of 2.8 x 5.2 m, consisting of 4 rows each and surface area of 14.56 m<sup>2</sup>.

After planting, classic harrow was used for soil surface alignment (treatments WRC and HRC). After shoot emerging, spaces between the rows were cultivated several times after appearance of seasonal weeds and additional mineral fertilizer was added in amounts of 25 kg N ha<sup>-1</sup> (Calceous NH<sub>4</sub>NO<sub>3</sub>). Weed control within rows was performed manually. The presence of some pests, predominantly flea beetles

Table 2. Mean daily temperature (T) and sum of precipitation (P) for horseradish crop during the trial (2018-2020), with long-term averages

| Months | 2018/2019 |               | 2019/2020 |               | 1981-2010 |        |
|--------|-----------|---------------|-----------|---------------|-----------|--------|
|        | T (°C)    | P (mm)*       | T (°C)    | P (mm)*       | T (°C)    | P (mm) |
| Dec    | 1.6       | 58.6          | 4.7       | 55.5          | -         | -      |
| Jan    | -0.3      | 86.4          | 1.1       | 17.0          | 0.1       | 45.0   |
| Feb    | 3.6       | 23.3          | 6.2       | 71.0          | 1.5       | 42.2   |
| Mar    | 9.5       | 5.3           | 8.6       | 50.0          | 6.2       | 41.5   |
| Apr    | 13.2      | 62.6          | 12.2      | 2.0           | 11.8      | 57.2   |
| May    | 14.9      | 139.0         | 15.8      | 93.0          | 17.0      | 59.8   |
| Jun    | 22.9      | 95.1 (75.0)   | 20.4      | 89.6 (78.0)   | 19.9      | 81.6   |
| Jul    | 22.7      | 54.0 (32.0)   | 22.0      | 110.6 (89.0)  | 21.9      | 61.4   |
| Aug    | 23.8      | 27.9 (12.0)   | 23.0      | 62.0 (43.0)   | 21.5      | 55.9   |
| Sep    | 18.9      | 25.6 (0.0)    | 19.5      | 30.3 (37.0)   | 16.8      | 57.5   |
| Oct    | 14.3      | 9.8 (4.0)     | 12.9      | 101.8 (86.0)  | 11.7      | 51.8   |
| Nov    | 11.7      | 43.9 (28.0)   | 6.4       | 15.7 (12.0)   | 6.0       | 48.4   |
| Dec    | -         | -             | -         | -             | 1.4       | 50.7   |
| Sum    | -         | 631.5 (526.2) | -         | 698.5 (633.5) | -         | 653.0  |

\*values within parentheses are related to the seasonal precipitation correction for the experimental site in the village Polatna

(*Phyllotreta* spp.) and damaged leaves were noticed during the late spring and summer. All measures applied except the mineral fertilization (farmyard of acceptable quality was not available) were in accordance to the Law on Organic Production and the accompanying regulations.

The horseradish crop was harvested in mid-November (11/13/2019). Samples of 10 plants per treatment replication were taken (inner rows were used for sampling in order to avoid border effects). The number of root cuttings (productive) per plant was provided by counting and the average mass of cuttings was taken using technical scale. The same above described procedure was repeated once again in the second year of the study. Flea beetles appeared in a smaller number during the second year, and the harvest and sampling were organized during the last three days of October.

Data on climate parameters for the nearest meteorological station (Veliko Gradište) were provided using the internet portal of the Republic Hydrometeorological Institute of the Republic of Serbia (RHMZRS, 2020). However, due to the specifically modified weather conditions at the experimental field site, during some summer and autumn months, precipitation was measured with the help of a round vessel with a graded scale, and the results are shown in parentheses (Table 2). Two-way ANOVA and further LSD-testing were performed using Statistica 7 software package for Microsoft Windows.

## RESULTS AND DISCUSSION

Since horseradish is a plant with moderate heat requirements, it is considered that average temperatures during the experiment were not a limiting factor for achieving good yields. On the other hand, the unbalanced distribution of precipitation and the amounts that were significantly below the multi-year average in certain summer months, during the first year of trial, had a negative effect on the horseradish crop grown without additional irrigation and negatively hit the yields and observed parameters (Table 2).

Based on the analysis of variance, significant differences of number of horseradish cuttings per plant in regard to the type of cuttings and years were noted (F values 178.29\*\* and 31.34\*\*, respectively,  $p < 0.01$ ). Significant effect ( $p < 0.05$ ) of the type of cuttings on average mass of reproductive cuttings was also recorded (F = 6.08\*). Oppositely, the interactions of the type of cuttings and years were not statistically significant for the investigated parameters with F values of 3.68 and 0.24, respectively (Table 3).

The average number of cuttings per plant during the experiment was 2.29 and the average weight of cuttings was 21.98 g. The highest number of cuttings per plant was recorded in the treatment of CC in both years of research. The difference was statistically significant compared to other treatments. A

Table 3. Analysis of variance for tested parameters

| Effect                       | SS      | df | MS      | F                  | p      |
|------------------------------|---------|----|---------|--------------------|--------|
| Number of cuttings per plant |         |    |         |                    |        |
| Intercept                    | 95.68   | 1  | 95.68   | 2919.07            | 0.0000 |
| Type of cuttings             | 11.69   | 2  | 5.84    | 178.29**           | 0.0000 |
| Year                         | 1.03    | 1  | 1.03    | 31.34**            | 0.0001 |
| TC x Y                       | 0.24    | 2  | 0.12    | 3.68 <sup>ns</sup> | 0.0568 |
| Error                        | 0.39    | 12 | 0.03    |                    |        |
| Average mass of cuttings (g) |         |    |         |                    |        |
| Intercept                    | 8698.29 | 1  | 8698.29 | 972.02             | 0.0000 |
| Type of cuttings             | 108.78  | 2  | 54.39   | 6.08*              | 0.0150 |
| Year                         | 17.65   | 1  | 17.65   | 1.97 <sup>ns</sup> | 0.1855 |
| TC x Y                       | 4.29    | 2  | 2.14    | 0.24 <sup>ns</sup> | 0.7906 |
| Error                        | 107.38  | 12 | 8.95    |                    |        |

Table 4. The effects of different types of cuttings (CC – crown cuttings; WRC – whole root cuttings; HRC – half root cuttings) on the number of cuttings per plant and average mass of cuttings during the trial (2019–2020)

| Year | Type of cuttings | Horseradish parameters    |                              |
|------|------------------|---------------------------|------------------------------|
|      |                  | No. of cuttings per plant | Average mass of cuttings (g) |
| 2019 | CC               | 3.03 <sup>b</sup>         | 17.24 <sup>c</sup>           |
|      | WRC              | 1.70 <sup>d</sup>         | 23.72 <sup>ab</sup>          |
|      | HRC              | 1.46 <sup>d</sup>         | 22.02 <sup>abc</sup>         |
| 2020 | CC               | 3.83 <sup>a</sup>         | 20.35 <sup>bc</sup>          |
|      | WRC              | 2.07 <sup>c</sup>         | 25.83 <sup>a</sup>           |
|      | HRC              | 1.73 <sup>d</sup>         | 22.75 <sup>ab</sup>          |

<sup>a-d</sup> Values within the same column, followed by different letters are significantly different according to LSD test ( $p < 0.05$ )

Table 5. The share of first and second class and planting cuttings in the total yield depending on the type of cuttings used for planting (CC – crown cuttings; WRC – whole root cuttings; HRC – half root cuttings)

| Type of cuttings | Share of total yield (%) |              |                   |
|------------------|--------------------------|--------------|-------------------|
|                  | First class              | Second class | Planting cuttings |
| CC               | 15.7                     | 28.1         | 56.2              |
| WRC              | 44.6                     | 29.9         | 25.5              |
| HRC              | 34.2                     | 39.5         | 26.3              |

smaller number of cuttings per plant compared to the average was recorded in the treatment of WRC and HRC, between which there was generally no statistically significant difference, except for the treatment of WRC in the second year of the study (Table 4). The production of main and lateral roots is probably related to the genetic traits of the used horseradish population, yet Uozumi et al. (1992) found that naphthaleneacetic acid (NAA) or indole-3-butyric acid treatment stimulated branch emergence and shoot formation on the roots.

When it comes to the average mass of cuttings, significantly lower were those obtained from CC, while cuttings propagated by WRC and HRC had a higher average weight compared to cuttings propagated by CC. In general, higher values of this parameter were achieved in the second year of investigation but without statistical significance, probably due to the high heterogeneity of the material.

Except when the WRC were used, the average yields of fresh horseradish root per hectare were significantly below the values

achieved by Perlaki & Djurovka (2009), as well as Rivelli et al. (2016), but different short term cultivation goals should be kept in mind (data not shown). However, a significant difference was observed in the percentage of first and second class yields and planting cuttings in relation to the total root yield (Table 5).

## CONCLUSIONS

When the goal of cultivation is intensive multiplication of planting material in order to increase the production quantity of cuttings for planting, it is most efficient to use CC. However, in case of the use of CC as planting material, a significantly smaller share of marketable yields i.e. first and second class roots in the total yield must be taken into account. Farmers should use CC only as an initial material and give priority to WRC or HRC respectively. The usage of WRC as a planting material provides producers with the highest yields and the highest share of the first class roots in the total yield, yet with a sustainable amount of planting cuttings.

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## SAŽETAK

### ODRŽIVO UMNOŽAVANJE RENA (*Armoracia rusticana*)

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Uprkos dobrim agroekološkim uslovima za proizvodnju i različitim mogućnostima upotrebe, u Srbiji se ren gaji na relativno malim površinama. Zbog dobrih tržišnih cena i visokih prinosa koji se mogu postići gajenjem rena, veliki broj poljoprivrednika zainteresovan je za njegovu proizvodnju. Veliko je ograničenje to što sami moraju proizvesti sadni materijal. Poljski ogled je postavljen na peskovitoj ilovači na eksperimentalnoj lokaciji u selu Polatna (Opština Žabari), tokom dve uzastopne sezone (2019. i 2020. godine) kako bi se utvrdio najefikasniji način proizvodnje reznica za sadnju rena. Zdrave biljke sakupljane su sa polja na kojem se u prethodnom periodu gajio ren. Nakon preliminarnog razdvajanja prve i druge tržišne klase namenjene prodaji, odabrane su biljke približno iste veličine pogodne za dalje razmnožavanje. Matični kalemi (gornji deo korena – glava korena) odsečeni su od preostalih korena a sav materijal podeljen je u tri grupe: a) vršne reznice (CC), b) cele korenove reznice (WRC) i c) manje korenove reznice (HRC). Ujedno su ovo bili i tretmani u toku izvođenja ogleda. Prosečan broj reznica po biljci tokom eksperimenta bio je 2,29, a prosečna masa reznica bila je 21,98 g. Najveći broj reznica po biljci zabeležen je pri upotrebi CC tokom obe godine istraživanja, dok su reznice koje su dobijene umnožavanjem WRC i HRC imale veću prosečnu masu u poređenju sa reznicama koje se razmnožene iz CC. Kada je cilj gajenja intenzivno umnožavanje sadnog materijala kako bi se povećala količina proizvedenih reznica za sadnju, najefikasnije je koristiti CC. U tom slučaju očekuje se znatno manji udeo tržišnog prinosa. Korišćenje WRC kao sadnog materijala pruža proizvođačima najviše prinose i najveći udeo prvoklasnog korena u ukupnom prinosu, ali uz održivu količinu reznica za sadnju.

**KLJUČNE REČI:** ren, reznice, sadni materijal, umnožavanje