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*Ivana M. Vico*[1], *Dragica B. Janković*[2], *Branka B. Krstić*[1], *Aleksandra R. Bulajić*[1], *Nataša D. Dukić*[1]

[1] Faculty of Agriculture, Sv. Dimitrije 22
11000 Belgrade—Zemun, Serbia and Montenegro
[2] Agricultural Institute "Dr Petar Drezgić", Nemanjina 6
22000 Sremska Mitrovica, Serbia and Montenegro

# MULTINUCLEATE *RHIZOCTONIA* SP. — PATHOGEN OF SUGAR BEET AND SUSCEPTIBILITY OF CULTIVARS UNDER FIELD CONDITIONS*

ABSTRACT: Sugar beet root rot has severely occurred in our country recently, especially in localities of Pazova, Pećinci, Ruma, Sremska Mitrovica and Šid. From diseased roots as well as from soil collected from the localities where decay occurred, fungal isolates were obtained by bait plant method. Based on their characteristics, they were identified as multinucleate *Rhizoctonia* sp.

During the year of 2004 in Mitrosrem trial field T-11, where the presence of multinucleate *Rhizoctonia* sp. was confirmed, an experiment under the coordination of Committee for Acknowledgement and Registration of New Cultivars in our country was conducted in order to determine cultivars' tolerance, i.e. their susceptibility and possibility for growing on infested fields. Six cultivars of sugar beet, Laetitia (as standard) and five new ones were included in the investigation. The trial was conducted in accordance with the established and accepted method (Ministry of Agriculture, Forestry and Water Resources, Republic of Serbia). Susceptibility of investigated cultivars was evaluated according to significant production characteristics: root yield, sugar content, corrected sugar content, thick juice Q, molasses sugar, content of K, Na and amino-N, polarized sugar yield and white sugar yield, as it was recommended by the method.

Conducted investigations have revealed that tested sugar beet cultivars showed different reactions to natural infection with multinucleate *Rhizoctonia* sp. Concerning root yield as the most important agricultural characteristic, statistically significantly higher yield was obtained with the cultivar under code mark 5 (61.120 kg/ha), whereas the cultivar marked under code 6 had significantly lower yield comparing to the standard (38.100 kg/ha).

KEY WORDS: multinucleate *Rhizoctonia* sp., natural infection, tolerant cultivars, yield

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## INTRODUCTION

*Rhizoctonia solani* is the most studied species of *Rhizoctonia* genus and was described for the first time on potato in 1858 (S n e h et al., 1991). Based on the number of nuclei in their cells, fungi belonging to *Rhizoctonia* spp. can be divided in two groups: binucleate and multinucleate. Species described as *Rh. solani* has more than three nuclei in its cells, thus this species belongs to the group of multinucleate *Rhizoctonia* sp. According to new criteria in the taxonomy of *Rhizoctonia*, it is recommended to describe *Rh. solani* as multinucleate *Rhizoctonia* sp. (S n e h et al., 1996).

Multinucleate *Rhizoctonia* sp. is economically important for growing on numerous plant hosts worldwide and in our country it is common on potato, beans, alfalfa (V i c o et al., 1996), tomato, cabbage (I v a n o v i ć and I v a n o v i ć, 2001) and ornamentals (V i c o et al., 2005). Besides mentioned hosts, in several localities in our country, this fungus is proved to be causing sugar beet root rot (V i c o et al., 2004).

In localities of Pazova, Pećinci, Ruma, Sremska Mitrovica and Šid, massive decay of sugar beet has been observed. From diseased roots, as well as from the soil originating in these localities, fungus was isolated using plant bait method and was identified as multinucleate *Rhizoctonia* sp., based on some of its characteristics (V i c o et al., 2004). This fungus can survive as sclerotia or mycelia on plant debris in infested soil for a very long period (S c h e i d e r and W h i t n e y, 1986). Since it is polifagous, attacking numerous plant hosts, its inoculum accumulates in the soil although crop rotation is employed. In these conditions, after multinucleate *Rhizoctonia* sp. is registered in a certain locality, the only economically justified and available control measure is growing resistant or tolerant cultivars (H a r v e s o n, 2003).

The aim of described investigations was to examine reactions of some sugar beet cultivars to natural infection in order to evaluate their capacity for growing on soil infested by multinucleate *Rhizoctonia* sp.

## MATERIAL AND METHODS

During 2003, on Mitrosrem field in Glac locality, near Sremska Mitrovica, where sugar beet rot had been noticed in previous years, five soil samples were collected. These samples, each consisting of 1 kg of soil, were investigated for the presence of plant pathogenic fungi using bait method (F o x, 1993). Soil was placed in appropriate pots and sowed with sugar beet, cv. Dana. After seedlings had emerged in greenhouse conditions, symptoms were observed each day. Greenhouse and phytopatological investigations were conducted in the facilities of Department of Phytopathology, Faculty of Agriculture, Belgrade, Zemun.

From bait plants-seedlings with disease symptoms, isolation by usual methods was performed on potato-dextrose agar (PDA). Previously superficially disinfected (1 min, 2% Natriumhipohlorit, NaClO) root and crown fragments were placed or immersed in PDA media. Incubation was at 23°C, in dark. After fungi were developed around the plant fragments, a colony fragment was transferred, first on fresh PDA and afterwards on water agar (WA) where hyphal tip isolates were obtained.

In order to identify the obtained fungal isolates, macroscopic and microscopic morphological, as well as some biological characteristics were investigated. Colony appearance and characteristics like shape, dimensions and characteristics of spores and mycelia, as well as the number of nuclei in cells were studied. On seven days old fungal colonies on PDA, incubated at 24°C in dark, macroscopic properties of isolates were studied, following the criteria proposed by Muntanola-Cvetković (1987). Studied properties were growth rate, colony appearance, colony edge characteristics, colour, the presence of fructifying bodies, exudation, scent and pigmentation. Seven days old fungal colonies on PDA and WA were used for observing and studying microscopic characteristics. Studied microscopic properties were mycelia appearance, hyphal appearance, branching and septation, characteristics of septae, sporulation, as well as the appearance of myceliar bodies. Hyphal width and the length from the branching point to the first septae and dimensions of spores and myceliar bodies were measured. The number of nuclei per cell in obtained isolates was investigated applying two methods: shafranin O staining (Bandoni, 1979) and staining with aniline blue in lactophenol (0,5% solution) (Burpee et al., 1978). The number of nuclei per cell was determined in 100 randomly selected hyphal or moniliformous cells in seven days old cultures of investigated isolates, grown on PDA and WA media.

During 2004 in the Mitrosrem field, in Glac locality, where multinucleate *Rhizoctonia* sp. presence was proved, a field experiment was set up, under the guidance of Committee for Acknowledgement and Registration of New Cultivars. Six sugar beet cultivars, Laetitia (as standard) as well as five new ones were included in the investigation. The experiment was set up in accordance with the recommended and accepted methodology of Cultivar Committee (Ministry of Agriculture, Forestry and Water Resources, Republic of Serbia). It was designed in five replications according to random block system. Sugar beet was planted at the beginning of April 2004, in the rows, 50 cm apart with 20 cm distance between plants in one row. During vegetation, usual agricultural and disease control measures were applied. Root harvesting was manual, at the beginning of October. The number of plants per experimental plot was calculated and expressed in 000/ha. Yield was measured and roots were then transported to Laboratory of Sugar Technology Department, Faculty of Food Technology, Novi Sad. Evaluated tolerance of investigated sugar beet cultivars was based on significant productional characteristics such as the number of harvested plants, root yield, sugar content, corrected sugar content, thick juice Q, molasses sugar, K, Na, and amino-N content, polarized sugar yield and white sugar yield, as it is recommended by the method. Obtained results were statistically analysed as separate monofactorial trials according to random block system. Pair evaluations were calculated using LSD test at 0,05 and 0,01 levels of significance.

## RESULTS

### *Symptoms on bait plants and identification of fungi*

On diseased sugar beet seedlings, 7—10 days after emerging, damping off occurred as the result of root and crown necrosis (Figure 1). From diseased bait plants with symptoms of necrosis, fungus was isolated on PDA. After obtaining hyphal tip cultures, the identification was carried out.

Isolates expressed following macroscopic characteristics: beige to brown colonies, rich and well developed showing rapid growth (average 24,55 mm per day); edge uninterrupted and smooth; mycelium developed on the glass; sclerotia (numerous, thick, spherical, light to dark brownish, usually in groups, 0,5—3 mm in diameter) were formed superficially in the colonies, 5—6 days after transferring to PDA and usually distributed near colony edge (Figure 2).

Microscopic properties of investigated isolates were: mycelia wavy and multicelullar; young hyphae branching under almost right angle; at the branching point, where lateral hyphae began to grow, there was a characteristic narrow point near which septae could be found on the lateral branch (Figure 3); moniliformous cells appeared in long chains (Figure 4). Hyphal width was from 5,5—7,5 μm, hyphal length from the branching point to the first septae was 4,7—7,5 μm, hyphal width at the narrow point was 4,5 μm on average.

### *Symptoms during vegetation*

During the field trial, complete plant decay was found in some sugar beet cultivars. On a few of the remaining plants there were symptoms of rot which could be seen during harvesting, at the end of the experiment. Symptoms on mature plants were dark brown necrosis and superficial root splitting (Figure 6). Necrosis and rot were spreading towards root core. Plant decay and root rot were observed in different intensity on investigated sugar beet cultivars. During root harvesting, a similar number of 1—4 rotted roots per replicate was established with cultivars under codes 2, 4 and 5, while the cultivar under code 6 had 17—20 roots with symptoms. Nevertheless, all investigated sugar beet cultivars expressed similar capability of finishing vegetation in the soil infested with multinucleate *Rhizoctonia* sp. (Figure 5). The number of harvested plants was not statistically different comparing to the standard. The only exception was the cultivar under code 5 where statistically significantlly higher number of plants completed the vegetation (Table 1).

### *Productional properties of investigated sugar beet cultivars*

In the conditions of natural infection with multinucleate *Rhizoctonia* sp., sugar beet cultivars expressed different productional properties and the results were summarised in Table 1.

As it can be seen in Table 1, the highest yield was recorded for the cultivar under code 5 (61,120 kg/ha) and this was statistically significantlly higher comparing to the standard, cv. Laetitia, which was under code 1. The lowest yield was recorded for the cultivar under code 6 (38100 kg/ha) and this was statistically significantlly lower than the standard. The highest sugar content was found in the cultivar under code 3 (15,58%), followed by the cultivar under code 5 (15,40%). Both were statistically significantlly higher comparing to the standard. Remaining cultivars, 2, 4 and 6 also had statistically significantly higher sugar content comparing to Laetitia (14,17%). Concerning plant number per ha, the only statisticaly significant difference comparing to the standard was with the cultivar under code 5 where the higher number of plants managed to complete vegetation (95400 plants/ha).

In Table 2, the remaining productional characteristics were summarized. Among these, the important ones are polarized sugar yield and white sugar yield which were the highest with the cultivar under code 5 (5418 and 7904 kg/ha, respectively) and the lowest with the cultivar under code 6 (5759 and 4830 kg/ha, respectively).

## DISCUSSION

Obtained fungal isolates were identified as multinucleate *Rhizoctonia* sp. based on disease symptoms on bait plants, i.e. sugar beet seedlings, isolation and morphological properties as well as on the number of nuclei in the cells (Sneh et al., 1991; Ceresini, 1991). The presence of plant pathogenic fungi *Rhizoctonia* sp. was confirmed in the soil of Mitrosrem T-11 field, which made it appropriate for setting the cultivar experiment.

During vegetation, with all investigated sugar beet cultivars, necrosis and decay of smaller number of plants were established. Not all the plants that had been planted and then emerged, managed to complete vegetation. Multinucleate *Rhizoctonia* sp. is the causal agent of necrosis and decay of sugar beet, causing yield reduction by 2—30% (Scheider and Whitney, 1986) and even up to 50% (Galliann, 1998). This yield reduction is greatly due to plant decay before harvesting. In the conditions of this experiment, with all investigated sugar beet cultivars, a large number of plants was able to finish vegetation, so all investigated cultivars posses a certain level of tolerance towards *Rhizoctonia* sp. and the capacity to be cultured in infested soil.

For evaluating the capability of sugar beet cultivars to grow in infested soil, productional properties such as yield and sugar content proved to be appropriate. These two parameters separated investigated cultivars and emphasised differences between them on the level of significance LSD 0.05 and 0.01. The cultivar under code 5 had statistically significantly higher yield in the conditions of this experiment and statistically very significantly higher sugar content comparing to the cultivar Laetitia which represented the standard as an acknowledged tolerant sugar beet cultivar. The selection of new sugar beet cultivars tolerant against multinucleate *Rhizoctonia* sp. and their introduction into practice represent an intensive research field in the world. Leonard and H a n s o n (2003) recommend yield measurement and disease intensity assessment as tools for selecting tolerant sugar beet genotypes in the conditions of natural infection. L e o n a r d (2003) examined the tolerance of sugar beet cultivars against multinucleate *Rhizoctonia* sp., but cultivar reaction was compared to a susceptible standard. In that way, the reaction of tolerant cultivars was more obvious. Based on the results obtained in this investigation and comparing them to literature data, sugar beet cultivar under code 5 could be recommended for further evaluations, because it showed statistically significantly higher yield and statistically very significantly higher sugar content than the standard.

Results obtained in conducted investigations showed that tolerant sugar beet cultivars are available and able to give satisfactory yield in the conditions of soil infestation with multinucleate *Rhizoctonia* sp. in our climate. Since the growing of tolerant sugar beet genotypes on infested soil is the only economically justified control measure of sugar beet root rot caused by multinucleate *Rhizoctonia* sp., results obtained in presented investigations are significant for scientific as well as for practical aspects of sugar beet production.

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Table 1. Major productional characteristics of investigated sugar beet cultivars naturally infected with multinucleate *Rhizoctonia sp.* during 2004.

| Sugar beet cultivars | Root yield (t/ha) | Plant No (000/ha) | Sugar content (%) |
|---|---|---|---|
| 1 | 50,80 | 83,00 | 14,1667 |
| 2 | 59,64 | 92,60 | 14,9500* |
| 3 | 50,28 | 87,00 | 15,5833** |
| 4 | 53,52 | 93,00 | 14,8833* |
| 5 | 61,12* | 95,40* | 15,4000** |
| 6 | 38,00* | 79,40 | 15,1000* |
| LSD 0.05 | 10,08044 | 9,959461 | 0,672487 |
| LSD 0.01 | 13,75012 | 13,5851 | 0,956532 |
| CV | 14,65922% | 8,588364 | 2,462034 |

Note:
 * Statistically significant difference comparing to standard with LSD 0,05.
** Statistically significant difference comparing to standard with LSD 0,01.

Table 2. Other measured productional characteristics of investigated sugar beet cultivars naturally infected with multinucleate *Rhizoctonia* sp. during 2004.

| Sugar-beet cultivars | Corrected sugar content % | Thick juice Q | Molasses Sugar % | K | Na | Amino N | Polarized sugar yield 000 kg/ha | White sugar yield 000 kg/ha |
|---|---|---|---|---|---|---|---|---|
| | | | | mmol/100°S | | | | |
| 1 | 11,28 | 90,68 | 2,29 | 30,05 | 20,66 | 10,14 | 7,120 | 5,668 |
| 2 | 11,66 | 92,54 | 1,69 | 25,09 | 10,72 | 11,95 | 8,915 | 7,548 |
| 3 | 13,46 | 93,63 | 1,52 | 23,11 | 8,85 | 8,22 | 7,833 | 6,769 |
| 4 | 12,44 | 92,42 | 1,84 | 24,41 | 15,15 | 9,07 | 7,963 | 6,657 |
| 5 | 12,92 | 92,51 | 1,88 | 24,36 | 14,74 | 8,90 | 9,418 | 7,904 |
| 6 | 12,68 | 92,44 | 1,82 | 24,41 | 14,11 | 9,96 | 5,759 | 4,830 |

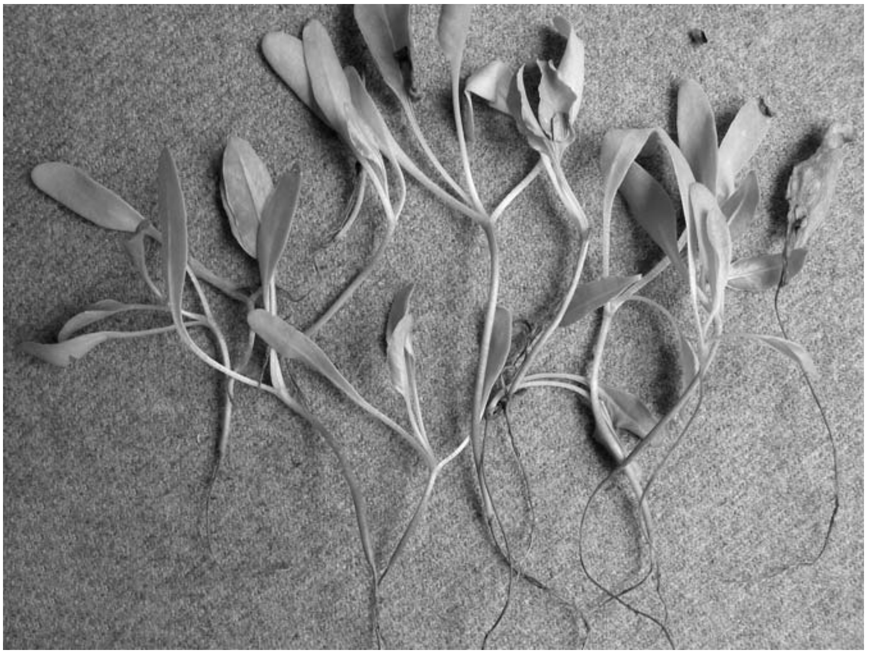

Figure 1. Multinucleate *Rhizoctonia* sp.: Root necrosis of bait plants

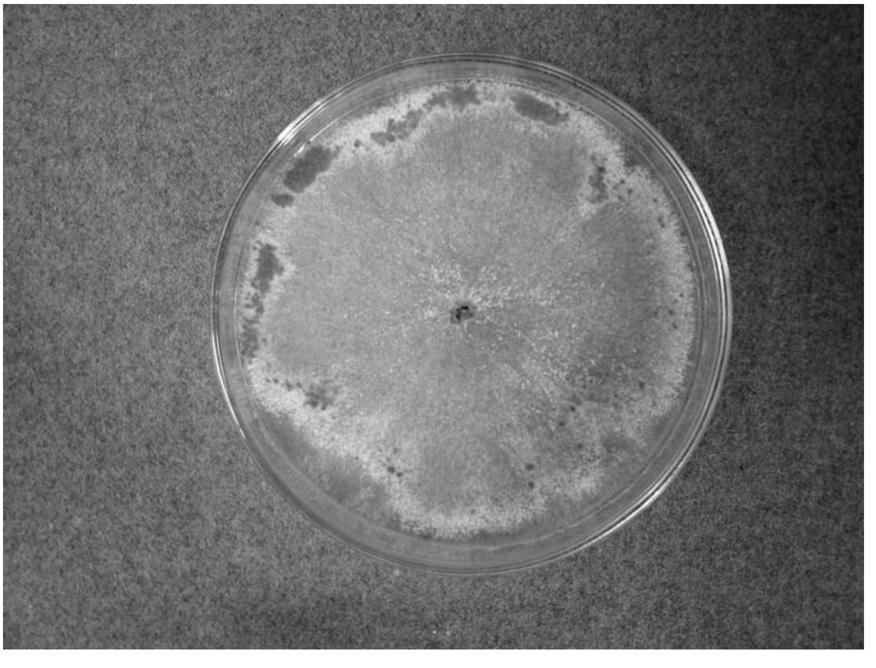

Figure 2. Multinucleate *Rhizoctonia* sp.: Sclerotia on colony edge on PDA

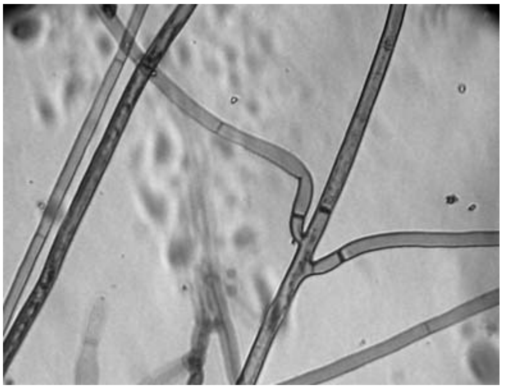

Figure 3. Multinucleate *Rhizoctonia* sp.:
Junction point of branching hyphae

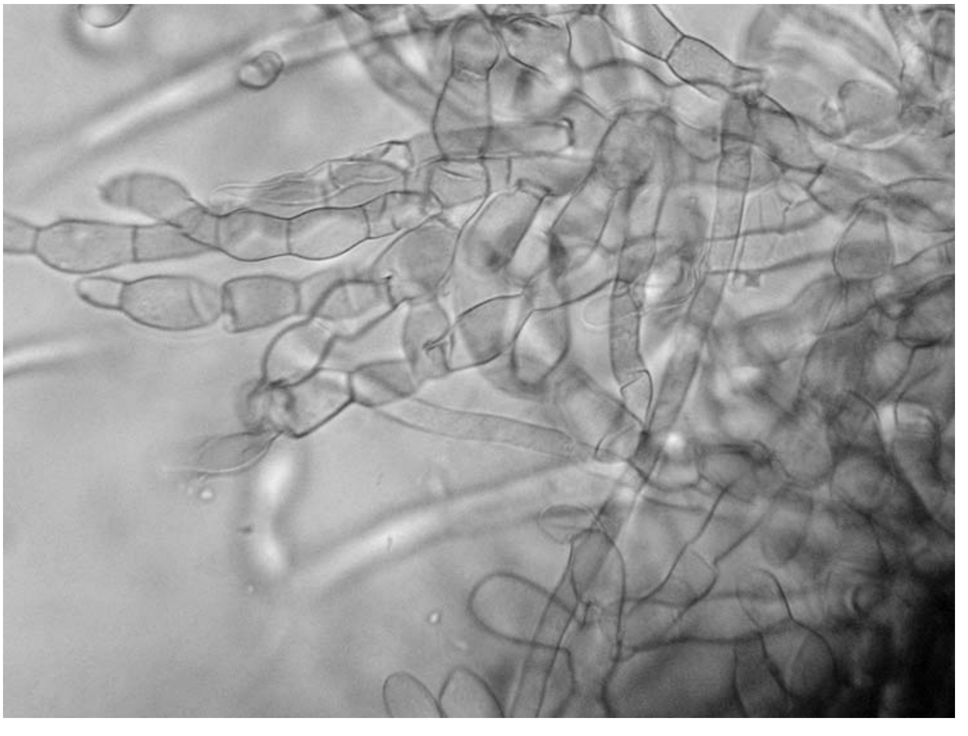

Figure 4. Multinucleate *Rhizoctonia* sp.: Moniliformous cells, detail

Figure 5. Multinucleate *Rhizoctonia* sp.: Multiple nuclei in the hyphal cells

Figure 6. Multinucleate *Rhizoctonia* sp.: Necrosis and splitting on sugar beet roots

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# ВИШЕЈЕДАРНА *RHIZOCTONIA* SP. — ПАТОГЕН ШЕЋЕРНЕ РЕПЕ И ОСЕТЉИВОСТ СОРТИ У ПОЉУ

Ивана М. Вицо[1], Драгица Б. Јанковић[2], Бранка Б. Крстић[1],
Александра Р. Булајић[1], Наташа Д. Дукић[1]
[1] Пољопривредни факултет, Немањина 6,
11000 Београд—Земун, Србија и Црна Гора
[2] Пољопривредни институт „Др Петар Дрезгић", Св. Димитрија 22,
22000 Сремска Митровица, Србија и Црна Гора

Резиме

Последњих година уочено је масовно пропадање шећерне репе у нашој земљи и то у локалитетима Пазове, Пећинаца, Руме, Сремске Митровице и Шида. Из оболелих коренова и из земљишта прикупљеног са терена где је пропадање уочено, методом мамака изолована је гљива која је по својим особинама идентификована као вишеједарна *Rhizoctonia* sp.

У току 2004. године на парцели Митросрема Т-11, где је изолацијом доказано присуство вишеједарне *Rhizoctonia* sp., постављен је оглед у оквиру сортне комисије за признавање и регистрацију нових сорти у нашој земљи са циљем утврђивања толерантности, односно осетљивости појединих сорти и тиме њихове погодности за гајење на инфестираном земљишту. У испитивања је укључено 6 сорти шећерне репе: *Laetitia* (као стандард) и још пет нових сорти. Оглед је посејан по утврђеној и прихваћеној методи сортне комисије (Министарство пољопривреде, шумарства и водопривреде Републике Србије). Осетљивост испитиваних сорти оцењивана је на основу значајних производних особина: принос корена, поларизација, кориговани садржај шећера, Q густог сока, садржаја сећера у меласи, садржаја K, Na и амино N, принос поларизационог шећера и принос кристалног шећера, како то метода и захтева.

На основу обављених истраживања установљено је да се испитиване сорте шећерне репе различито понашају у условима природне заразе вишеједарном *Rhizoctonia* sp. У погледу приноса, као најважније производне карактеристике, статистички значајно виши принос од стандарда испољила је сорта која се води под шифром 5 (61.120 kg/ha), док је сорта која се води под шифром 6 имала статистички значајно нижи принос у поређењу са стандардом (38.100 kg/ha).