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MILOPRODUCTION OF VIABLE *MISCANTHUS GIGANTHEUS* RHIZOMES AT FERTILE AND DEGRADED SOIL

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Abstract

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Expanding supplies of home grown biomass and facilitation of the development and competitiveness of a supply chain in an sustainable manner, presents a core of government's strategy, for energy development in Republic of Serbia. Growing agroenergy corps may be one of the solutions according to analysis that were carried out. One of these corps that was found to be especially amenable due to its potentially high productivity and cultivation on degraded terrain is *Miscanthus giganteus*. The possibility of producing after mentioned plant for the biomass supply chain, at very low cost is the main aim of this study. Due to its sterility *Miscanthus giganteus* can only by propagated by vegetative division. Potential of production of viable rhizome on terrains with different rate of fertility was followed by method of experiments on the field. 6 parameters that were following growth of the rhizome and planting survival rate were monitored. According to the results, main factors that affect production of viable rhizomes are age of mother plants, and biotic effects of weed vegetation. Size of rhizomes and nursery fertilization shoved significantly smaller effect. This study concentrates on available data regarding the potential directions by which *Miscanthus* material could achieve maximum production, by high density planting.

Key words: agroenergy crops, *Miscanthus giganteus*, ecoremediation

Introduction

Miscanthus giganteus as energy crop

Lignocellulosic biomass has attracted considerable attention over the recent years as promising feedstock for the production of biofuels and value-added chemicals. *Miscanthus giganteus*, an herbaceous C4 photosynthetic perennial grass, has great potential with advantages such as little nitrogen or herbicide requirement, a long lifespan, low susceptibility to pests and diseases, as well as low moisture content at harvest (Beale and Long, 1995; Somerville et al., 2010; Long and Beale, 2011; Brosse et al., 2012).

Miscanthus giganteus is a highly productive plant species, which has been cultivated in Europe for 20 years as energy crop. The remarkable adaptability of *Miscanthus* to different environments makes this novel crop suitable for establishment and distribution under a range of European and North American climatic conditions (Lewandowski et al., 2000). As triploidit produces no seed, so it must be established vegetative by planting divided rhizome (rootstock) pieces. This process results in high establishment costs relative to crops established from seed.

There are two methods of propagation that are currently used for *Miscanthus* plants – rhizome division and micropropagation. Rhizome division is more used method because it is less expensive and generally produces more vigorous plants. To produce new planting material, two or three-year-old plants are split whilst dormant, using a rotary cultivator and the rhizome pieces collected for replanting.

A 30-40 fold increase in plants can be achieved this way over a period of 2-3 years, depending on soil conditions. Rhizome pieces must have at least 2-3 'buds' and must be kept moist before re-planting. This is best achieved by keeping rhizomes under cold-storage conditions, (<4°C) (possibly for up to a year) but they will remain viable in the field for a short period of time, if stored in a heap and covered with moist soil (Lewandowski et al., 1997).

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Energy/food

Cultivating energy crops in the existing crop land may cause a significant impact on food supply, so nonfood crops which can be cultivated in marginal land is highly desirable. The perennial grass *Miscanthus* requires only one planting activity and minimum nitrogen inputs over 10 - 20-year duration (Heaton et al., 2003).Almost all literature data refer to Miscanthus grown on arable soils in different regions of the Europe, Asia and America so the data on limited conditions of biomass production and/or materials for reproduction are scarce.

This study aims to determine the potential for production viable miscanthus rhizomes on degraded soils. Zasavica's humogley location was chosen as a mildly degraded soil, the deposol of RB Kolubara's West field was chosen as a highly degraded soil, and chernosem at the Vrsac location as a control i.e. fertile soil.

The researched issues are:

1) Is it possible to reproduce *Miscanthus* on degraded soil and what is the influence of agrotechnical measures?

2) Which of the applied measures have significant influence on the production of viable rhizomes?

3) What's the influence of the age of the mother plant and rhizome size?

Material and Methods

Locations

Field experiment was conducted at 3 locations in Republic of Serbia. Vrsac (V) 44º 6′ 18.44′′ N, 21º 18′ 7.2′′Ein Banat, at the Northeast of Vojvodina. The soil is fertile, carbonated chernozem, and previously medicinal plants were grown on this lot without application of herbicides. This location is used as a benchmark for fertile soil. Zasavica (Z) 44º 54′ 56.90′′ N, 19º34′ 10.44′′E, is located in Srem, Sremska Mitrovica municipality, near protected natural good Zasavica. The soil was a swamp, humogley type, on which the previous crop was corn. This soil was chosen as unsuitable for growing crops for food because any application of mineral fertilizers and plant protection agents could jeopardize sensitive wetland. The third location is the mullock landfill of the Mine basin Kolubara (K) east field/ 44º 28′ 16.41′′ N, 20º 14′ 59.62′′E, Lazarevac municipality. The soil is technogenic deposol and it was chosen as a highly degraded. On this lot there wasn't vegetation before this experiment i.e. the lot was created by deposition of mullock from the mine which was ended in 2010 when leveling of the lot was done.

Plant material

Plant material, rhizomes of *Miscanthus giganteus* (with a length of 10 cm and with 3 - 6 nodes), was purchased from commercial supplier and manually planted on agro-technically prepared land (plowing was conducted in the fall of the year before the planting and disking just before the planting) during 1st decade of April. Planting density was 2 rhizomes per m^2.

Investigated plates in 1st experiment were:

A - without agro technical measures;

B - agrotecnical measures applied: watering during the 1stvegetation period, once just after planting and 4 times later; fertilization (N:P:K=15:15:15 150 kg/ha just before planting) and mechanical weed control 3 times per year.

At the locations Zasavica and Vrsac there has been a monitoring of the potential of rhizome production from 3, 2 and 1 year-old plants, (planting year 2010, 2011 and 2012), and on Kolubaraonly 2 and 1 year-old (planting year 2011 and 2012). Number of shoots per tuff, tillering, and clump diameter from last year were determined as well as length of rhizome.

Rhizomes were cut by hand into pieces with 4 shoots each and they were counted. In each experiment there were a total of 40 (4 experimental plots X 10 plants) plants and the presented results are arithmetic averages of 40 calculations. Measuring was done in the first decade of April 2013. From each age category 100 pieces of rhizome were selected at random and re-planted 10cm deep by hand in previously cultivated soil at the same location. Rhizomes that have had sprouts within the 30 day period were considered to be viable.

In the second experiment, there was separate monitoring of the influence of irrigation, fertilization and weed on rhizome production from the mother plants age 1 and 3 year on the experimental field Zasavica. Rhizomes that had 2.4 or 6 buds were planted in April 2012 at planting density 5 rhizomes per m^2. Each treatment was made on 4 plots with 100 plants. In the beginning of April 2013, rhizomes were dug out and cuttings, with 3-5 sprouts, were made for counting and further planting. Other conditions were as in the previous experiment.

Data analysis

The results were statistically analyzed (ANOVA) using the Tukey's test to check for significant differences between means ($P \leq 0.05$).

Results and Discussion

Miscanthus biomass yield can be limited by poor rhizome establishment and this is linked to rhizome age and storage conditions prior to planting. To avoid poor establishment, best practice recommends field planting directly after rhizome division. Operations avoiding rhizome storage, and utilizing favorable climatic conditions at planting, may be climatologically and logistically challenging when large areas are planted at high rhizome densities (Davies et al., 2011)

Our aim is to evaluate impacts of nursery age and soil type to produce and maintain rhizome viability when planted under conditions of fertile (Vrsac) and degraded soil (Zasavica and Kolubara).

Chosen locations are different primarily by soil characteristics (humogley in Sadzak, deposol in R B Kolubara, chernozem in Vrsac). Biometric characteristics in Figure 1 show the state of growth of Miscanthus at the experimental fields in the conditions where no measures of care were applied (A) and for the field experiment in which there was fertilization directly before planting, watering right after the planting and two more times during the first summer and mechanically removing weed three times during the first year of development and once during the second year (B).The number of steams per rhizome (tillering) is an indicator aboveground biomass development in the previous vegetative period. The increase of shoots with the age of plants was recorded at all examined locations.

At Zasavica location (humogley) there was the largest number of shoots (70) with the application of agrotechnical measures and the age of 3 years, while the smallest number was found at the Kolubara location without agrotechnical measures application and age of 1 year (8). By comparing locations, it is noticeable that Miscanthus on humogley in Zasavica develops similar number of shoots as the benchmark, fertile chernozem in Vrsac.

The acquired results are consistent with the data from literature (Christian et al., 2009). Besides the height of stems, the number of shoots is the most important characteristic that determines yield of above ground Miscanthus biomass (Jezovski et al., 2011).

Clump diameter is a biometric characteristic that shows coverage of the area of land and it is important for research of the optimal planting density as well as in closing structure and weed resistance. As expected, the slowest development is found at deposol in Kolubara (24 cm) without application of agrotechnical measures, and the fastest on chernozem in Vrsac with care and protection (66 cm) in the third vegetation. Based on the examined biometric characteristics, we can notice that previous Miscanthus development was quite similar to the locations Zasavica and Vrsac, while it is significantly less at the Kolubara location (deposol). On the contrary, the effect of agrotechnical measures is the most significant at the location with the lowest quality soil, deposol.

At all three locations, the biggest production of rhizomes was from three years old nursery, and the lowest from the one from the last year, including agrotechnical measures. In the experiment without agrotechnical measures the biggest development of biomass above ground and underground was recorded in Vrsac which was expected considering soil fertility. Namely, at that location, measured content of humus was 1.71% which is significantly more than at other locations (Zasavica 0.60% and Kolubara 0.64%). With the application of agrotechnical measures parameters of Miscanthus devel-

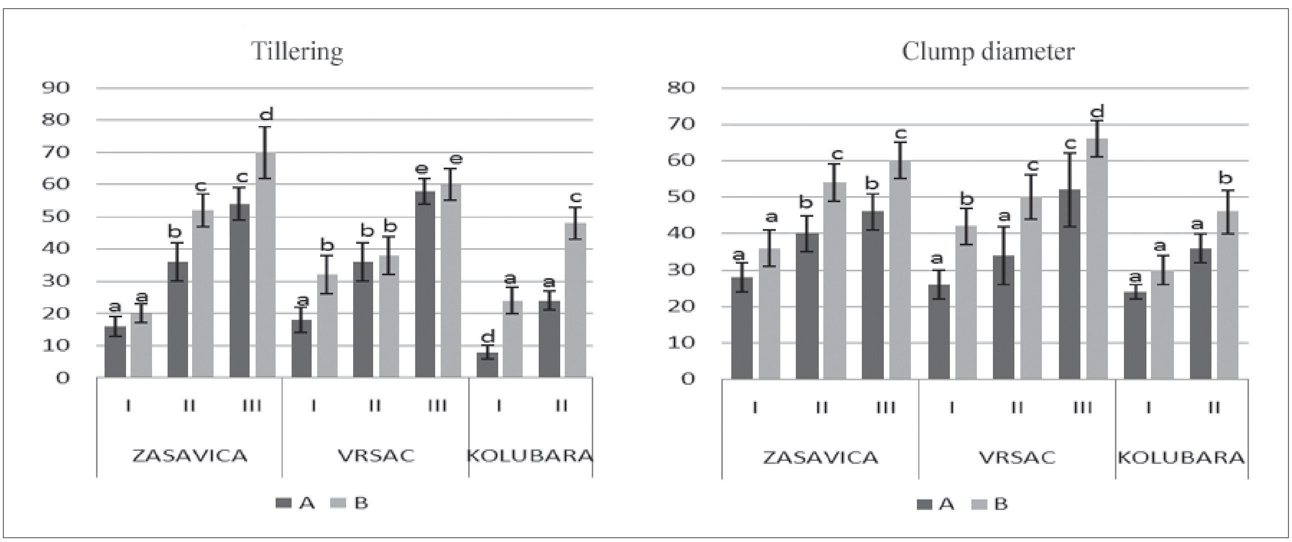

Fig. 1. Biometric characteristics of miscanthus 1) tillering (number of shoots per rhizome) and 2) clump diameter (cm) A – without any care, B – with agronomical measures, grown for 1 (I), 2 (II) and 3 (III) years at different experimental places. The results represents arithmetical means of plants ±SE. the same letter indicates no difference at level $p < 0.05$

opment at the experimental field Zasavica are getting close to the values of the experimental field in Vrsac, especially for rhizomes produced from three year plants (Figure 1).

The results of the experiment in Kolubara show that the Miscanthus development is significantly limited without agrotechnical measures even though the canopy survives in these extreme conditions. With irrigation, fertilization and weed removing, results are becoming comparable with the ones acquired in other locations. All three locations show that rhizomes are mostly viable from over 50% of rhizomes from the nursery at Kolubara to almost 95% for rhizomes produced from three year nursery at Zasavica with measures (Figure 2).

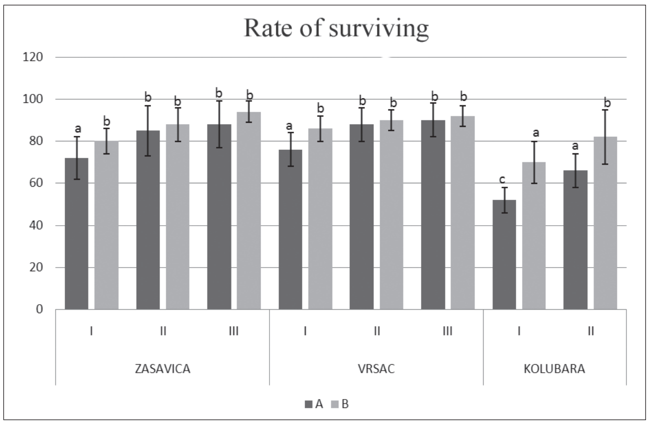

Fig. 2. Establishment rate of rhizome (%) replanted from mother plants grown for 1 (I), 2 (II) and 3 (III) years at different experimental places. The results represents arithmetical means of plants ±SE. the same letter indicates no difference at level p < 0.05

Acquired results show that on degraded, swamp soil, as the Zasavica location, yields of viable rhizomes can be almost as the ones on fertile soil (Vrsac). Similar results were also achieved for biomass yield (Drazic et al., 2012) and the possible reason is that the conditions on this site are similar to the natural conditions in which Miscanthus grows (Ji-Hoon Chung et al., 2012)

In a different experiment, monitoring was done on the influence of rhizome size, irrigation, fertilization and weed control on a production of new rhizomes (Figure 3). From mother plants that are 3 years old there can be 2-3 times more rhizome pieces then the ones that are 1 year old.

The production of rhizomes was mostly influenced by the competition with weed not considering the size of primer cutting. On the other hand, primer cuttings that had only two buds produced clumps with significantly smaller rhizomes that the ones with 4 and 6 buds.

Survival of the rhizomes planted in 2012 also depends on the age of nursery and on conditions of development from which the weed vegetation is considered to be the most important negative factor (Figure 4).

Similar results were achieved for development of biomass above ground (Drazic et al., 2010a) Watering and fertilizing have no significant impact on rhizome production and establishment rate due to soil characteristics (high level of underground water) and low demands of Miscanthus for nutrients, primarily nitrogen (Milovanovic et al., 2012).

A large amount of weed (especially at Zasavica and Kolubara experimental sites) is a consequence of the lack of weed control with total pesticide in the year before planting: in Zasavica, before Miscanthus, the soil was used for agricultural purposes with corn and in Kolubara it was a landfill, plowed before planting. The results achieved at the experi-

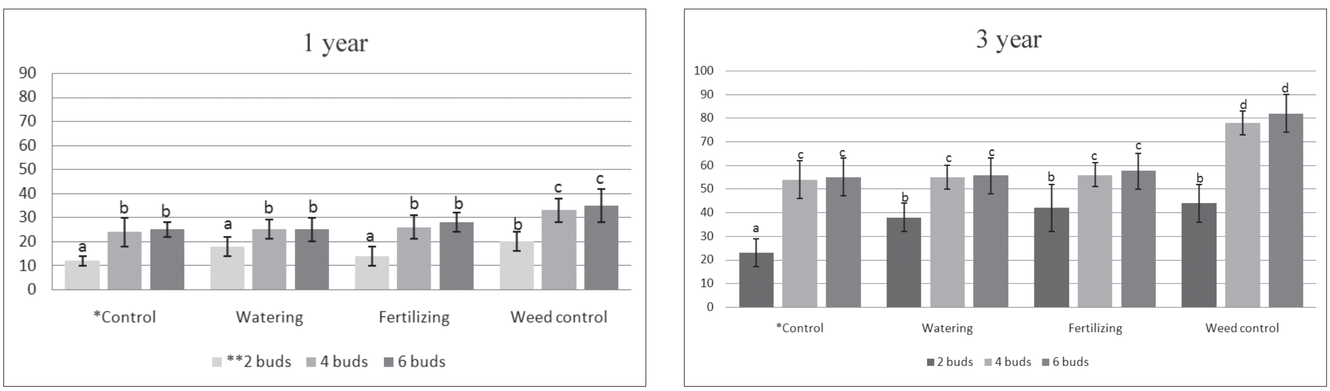

Fig. 3. Rhizome production from 1 year (A) and 3 years (B) mother plants at experimental field Zasavica

*Control plants= without any agrotechnical measures. 2, 4, 6 buds represents size of primary rhizomes. The results represent arithmetic mean from 4X10 plants. Vertical bars represents ±SE. the same letter shoves no difference at level p < 0.05

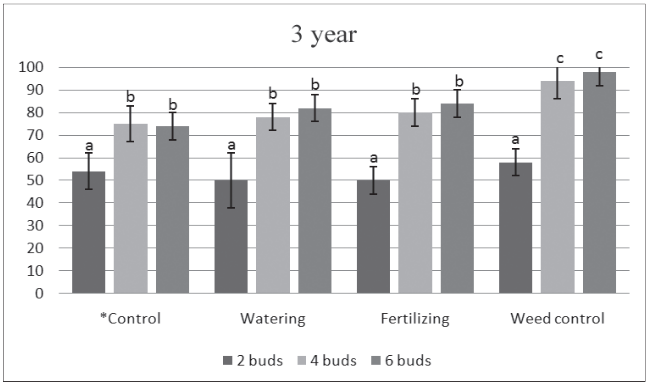

Fig. 4. Establishment rate of rhizome from 1 year (A) and 3 years (B) mother plants at experimental field Zasavica

*Control plants=without any agrotechnical measures. 2, 4, 6 buds represents velicinu primarnih rizoma. The results represent arithmetic mean from 4X10 plants. Vertical bars represents ±SE. the same letter shoves no difference at level $p < 0.05$

mental field Kolubara, even though they refer to only two vegetation show that, even in the bad conditions like these, Miscanthus development is possible, which opens possibilities for further researches for increasing production of underground as well as aboveground parts of the plant.

During the establishing of Miscanthus plantation as a base for bioenergy production chain, the biggest cost is starting plantaza, i.e. acquiring of planting material (Drazic et al., 2010b).

The results presented here point out to a possibility of establishing a nursery on relatively small surfaces, from which viable rhizomes could be produced. With a simple calculation (for example for the Sadzak site from a three-year-old nursery from one till that developed from one rhizome, one can get 75 rhizomes, and as 5 rhizomes are planted on a 1 m^2 and the survival rate is 94% one can get 350 viable rhizome cuttings per m^2. If the density of planting Miscanthus is meant for biomass production, 1 rhizome/m^2, i.e. 10 000 rhizomes/ha, it means that less than 30m^2 of nursery is enough for 1 ha of plantation.

The results of this research are completely the same as the latest publications (Atkinson, 2009). At present the establishment rate of *Miscanthusis* slow and this appears limited by economics; evidence suggests that the cost of plant propagules is one factor that constrains widespread planting. New techniques are required that simultaneously reduce unit costs of propagules and increase the speed of their availability to aid this developing industry.

Conclusion

- In agroecological conditions of the Republic of Serbia it is possible to produce viable rhizomes of Miscanthus giganteus
- Production rate depends on soil fertility and application of agrotechnical measures
- It is possible to achieve results on degraded soil close to the ones on fertile soil
- The production of planting material of Miscanthus in private nursery significantly reduces the cost of establishing a plantation and in that way the whole production chain becomes economically acceptable

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