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INFLUENCE OF RHIZOME MASS ON THE CROP ESTABLISHMENT AND DRY MATTER YIELD OF *MISCANTHUS×GIGANTEUS* OVER TEN SEASONS

Željko S. Dželetović[1], Gordana Z. Andrejić[1], Aleksandar S. Simić[2*] and Hakan Geren[3]

[1]University of Belgrade, INEP - Institute for the Application of Nuclear Energy,
Department for Radioecology and Agricultural Chemistry,
Banatska 31-b, 11080 Belgrade, Serbia
[2]University of Belgrade, Faculty of Agriculture,
Nemanjina 6, 11080 Belgrade – Zemun, Serbia
[3]University of Ege, Faculty of Agriculture,
Department of Field Crops, Izmir, Turkey

Abstract: The aim of the present investigation was to assess the influence of rhizome mass on the success of plantation establishment and biomass yield of the bioenergy crop *M.* × *giganteus* during 10 years of cultivation. The experiment included three treatments with different rhizome masses: 10–20 g (very low); 25–35 g (low), and 40–60 g (medium mass). Planting density was 2 rhizomes m^{-2}. The plants were harvested by mowing of the whole above-ground biomass each year in February. Out of the total number of planted rhizomes, the lowest emergence was noticed in very low mass rhizomes. In the first season, the greatest number of stems and crop height were encountered under the treatment with the highest rhizome mass. In the second season, crop heights were almost equal in all treatments. During the first two seasons, the highest biomass yields were recorded under the treatments with the highest rhizome masses. Although the analyzed parameters were highest with the rhizomes of 40–60g during the crop establishing stage, starting from the third season of cultivation, high yields of above-ground biomass may be obtained also with lower mass rhizomes. Having the highest biomass yield (25.85±7.36 Mg DM ha^{-1}), the crop established with rhizomes of 25–35 g clearly stood out.

Key words: biomass yield, crop establishment, *M.*× *giganteus*, rhizome mass.

*Corresponding author: e-mail: alsimic@agrif.bg.ac.rs

Introduction

Miscanthus × *giganteus* Greef et Deu. is a perennial biomass crop particularly suitable for substitution of fossil fuels in energy production. It is a non-invasive perennial grass crop, which is a naturally sterile allotriploid, 2n = 3x = 57 (Nishiwaki et al., 2011), originating from East Asia. Regarding biomass production, it may be cultivated continuously for 15–20 seasons. *M.*× *giganteus* is a C_4 grass with highly efficient utilization of light and water (Lewandowski et al., 2000; Cosentino et al., 2007). The biofuel potential of miscanthus is highly valued (Perić et al., 2018a, b). Thanks to efficient biomass production, which has very good combustion quality (Cvetković et al., 2016; Bilandžija et al., 2017), miscanthus should give a significant contribution to sustainable agricultural production of combustible biomass in the near future (Dohleman et al., 2012; Mishra et al., 2013; Perić et al., 2018a). The high yield of miscanthus, together with its relatively low macronutrient concentrations and intermediate removal rates, indicates its advantages over other grasses as a biomass crop choice (Oliveira et al., 2017). Along with profitability, it is estimated that the energy production based on miscanthus cultivation will also contribute to the multi-functionality of agriculture (Daraban et al., 2015).

Contrary to the majority of other agricultural crops, miscanthus is a plant with an extensive root system, which remains dormant during winter, but it may respond quickly to increased plant requirement for assimilates at the start of growing season in the spring (Himken et al., 1997). Rhizome mass comprises approximately 2/3 of root biomass (Dželetović and Glamočlija, 2015). It has a shallow root system, with almost 90% of root biomass concentrated in the surface soil layer (0–35 cm) (Monti and Zatta, 2009). However, its roots reach the depth of 2 m, thus enabling absorption of soil moisture during dry summer months (Neukirchen et al., 1999). This depth surpasses rooting depths of most annual cultivated crops. Deeply rooted crops, like miscanthus, are more tolerant to drought, because they have access to more humid soil layers (Chaves et al., 2002).

Rhizomes have the key role in nutrient translocation in miscanthus. From the end of summer to winter, most nutrients are efficiently translocated from above-ground biomass to rhizomes, where they are stored until the next season, when they mobilize for the purpose of growth and development of new shoots (Masters et al., 2016; Nassi o Di Nasso et al., 2011). At the end of vegetation season, a part of nutrients is returned to soil through discarded leaves. Thanks to nutrient cycling, their concentrations in above-ground biomass are low during winter (Masters et al., 2016; Nassi o Di Nasso et al., 2011; Singh et al., 2015), which makes the biomass a very good raw material for combustion (Bilandžija et al., 2014; Singh et al., 2015).

M.× *giganteus* crop may be established by planting rhizomes or by micro-propagation of plants in April or May. Currently, most *M.*×*giganteus* crops are established by rhizome planting (Atkinson, 2009). Plants propagated from rhizomes develop shoots faster and display a lower number of above-ground parts (stems), but the stems are stronger and denser than above-ground parts of micro-propagated plants which develop their shoots slower (Lewandowski, 1998). Good miscanthus crop establishment by rhizome propagation depends on three components (Hocking et al., 2011): 1) vigor, potential energy of sprouting from rhizome fragments; 2) land management and cultivation systems that enable appropriate density of planting of unimpaired rhizome fragments; 3) soil conditions during and after the planting. All these components should be optimal in order to obtain the best possible initial sprouting, which should be accompanied by further good stimulative management (Hocking et al., 2011).

The influence of rhizome mass on biomass yield of miscanthus was investigated only in the initial stage of the crop development (the first two seasons of cultivation). It was found that the initial planting density did not influence the maximal yield value, but it exerted an influence on the rate of achieving the maximal biomasses (Miguez et al., 2008). According to Pyter et al. (2010), rhizomes of 50–60 g should possess sufficient metabolic supplies to enable their survival during storage as long as 4 months, from digging up the rhizomes until their planting on new plots or in new areas of cultivation. According to the results of Humentyk et al. (2013), the optimal density is 15,000 plants ha^{-1}, with the rhizomes of 30–60 g. As for the crop establishment, rhizome purchase represents absolutely the greatest expense (Jain et al., 2010). Because of this, the crop producers, as a rule, insist on purchasing rhizomes with the highest mass. The aim of our investigation is to assess the influence of the planted rhizome mass on the success of culture establishment and on the biomass yield during the first 10 seasons of cultivation.

Materials and Methods

The field experiment was carried out on the experimental plot of INEP (Institute for the Application of Nuclear Energy), Zemun, Serbia (44°51´ N, 20°22´E, 82 m a.s.l.; Figure 1), on non-carbonate chernozem (pH in water: 6.7; pH in 1M KCl: 5.5; total organic C: 1.71%; total N: 0.14%; available P_2O_5: 6.0 mg 100 g^{-1}; available K_2O: 17.8 mg 100 g^{-1}).

Serbian climate is mostly moderate continental. Average July (the hottest month of the year) temperature is ≥22°C, and average January (the coldest month of the year) temperature values vary mostly between 0 and -2°C (Dželetović et al., 2013). In Serbia, annual precipitation curve displays the two maxima: in the late spring and in the late autumn, while winters and summers are mostly dry periods.

Experimental treatments included 3 different rhizome masses: (1) rhizomes of very low mass: 10–20 g, which are not regularly used for planting; (2) rhizomes of low mass: 25–35 g; and (3) rhizomes of medium mass: 40–60 g, which are considered appropriate for planting. The experimental plots of 20 m^2 (5m×4m) each were positioned in three replications in a randomized complete block design. The rhizomes were planted on previously prepared soil surface, at 10 cm depth, on April 19, 2008. The planting was performed with the fragments of 3-year-old rhizomes, which had been stored for 30 days, between digging up and planting. This is the storage period most frequently encountered in Serbia: rhizomes are dug out in March, and the planting is usually performed at the end of April. In regard to the one-year-old rhizome, three-year-old fragments show the earliest sprouting and the highest number and mass of stems (Khan et al., 2011).

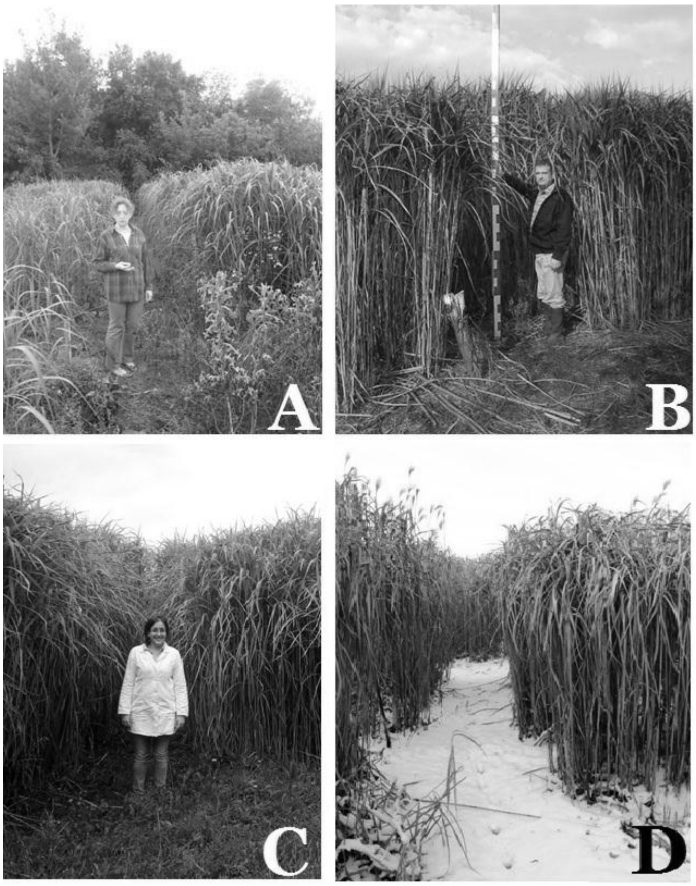

Figure 1. The authors of the study in the experimental field of INEP, Zemun (the 2016/2017 season): A) June (average crop height of 1.7–1.8 m); B) September (3.2 m); C) December (3.0 m); D) February (2.7 m). A decrease in biomass height was due to lodging caused by the snow cover.

Planting density was 2 rhizomes m^{-2}. Only in the first cultivation season (2008), weed elimination and irrigation were applied, when necessary, in order to provide optimal conditions for growth and development of the planted crop. The fertilization was performed each year by applying 667 kg NPK 15:15:15 ha^{-1} mineral fertilizers (100 kg N ha^{-1} + 100 kg P_2O_5 ha^{-1} + 100 kg K_2O ha^{-1}) immediately before crop sprouting (between April 1 and 10). Harvest was performed each year in February by mowing. Biomass yield was weighed immediately after harvesting (fresh mass). Samples of above-ground biomass from each treatment were collected for determination of average water content. After 72 h of drying at 60–70°C and subsequent cooling, the dry mass was weighed and water content and dry mass were determined.

The experiment lasted for 10 years. In the first season, the following parameters were monitored: sprouting, crop height, number of shoots (stems) and crop overwintering (rhizome freezing). In the second season, crop height was measured. Values presented in tables and figures represent arithmetic means of three replications for each treatment, with standard deviation (SD). The results were statistically analyzed (ANOVA) using the Tukey's test to check for significant differences between means ($P \leq 0.05$). Variation in yield between individual treatments (%) and the coefficient of variation (CV) of biomass yield between seasons were determined.

Results and Discussion

The first above-ground shoots were detected on May 2, 2008. However, sprouting was very irregular in the first season. It is well known that, after planting, some rhizomes will not sprout and develop, and that the initial sprouting may vary significantly. Former experiments showed that miscanthus responded favorably to fertilization, especially with nitrogen (Capecchi et al., 2013; Soare et al., 2017; Stępień et al., 2014; Xu et al., 2017; Živanović et al., 2014).

One month later, our results show comparatively high percentage of sprouting of the planted rhizomes – ≥75% (Figure 2). By the end of June 2008 (60 days after sprouting initiation) the sprouting had been completed. It amounted to 80% for 10–20 g rhizomes, while for those of 25–35 g and 40–60 g, it reached ≥92.5%. Similar results were obtained by Huisman and Kortleve (1994), who report on sprouting rates of 70–95% for rhizome fragments planted immediately after harvesting from maternal plants and 50–60% for rhizome fragments stored before planting. In the Netherlands, the researches show that the rhizomes with mass higher than 50 g, planted short time after harvest, successfully sprouted with the rates of 91–98% (Christian and Haase, 2001). Pyter et al. (2009), however, reported a sprouting rate of 60–70%.

In the second season, overall and uniform sprouting began on April 7, 2009. From the third until the ninth season, the date of sprouting outset depended on climate and weather conditions, but the sprouting mostly began around April 12 each year. This is somewhat earlier than in West Europe, where sprouting begins in the second half of April (Himken et al., 1997; Lewandowski et al., 2000).

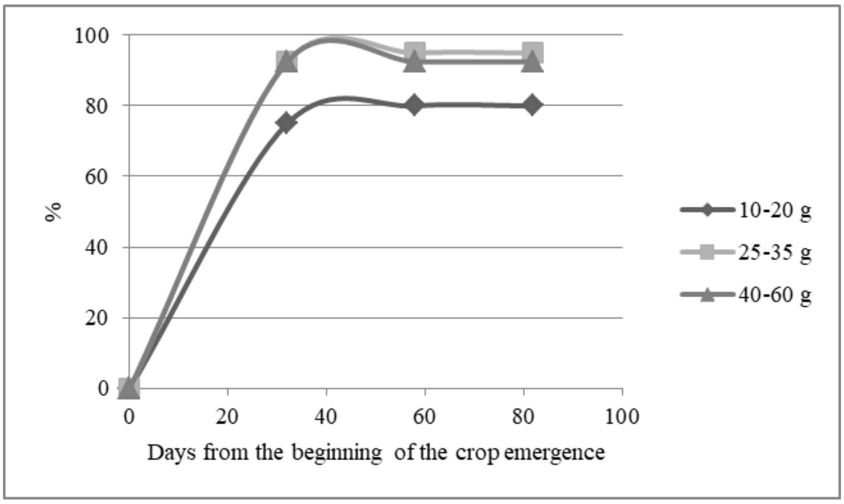

Figure 2. The percent of sprouted rhizomes in the first season (different curves represent rhizomes with different masses, $P \leq 0.05$).

Although *M.× giganteus* is a C_4 plant recognized as tolerant towards cold (Fonteyne et al., 2016), the main problem in *M.× giganteus* production is poor overwintering of rhizomes in the first season after planting. This problem is also encountered in Serbia to a lesser degree (Dželetović et al., 2013). During the winter, snow cover efficiently protects rhizomes against freezing. Lack of the snow cover, accompanied by strong frost, may cause freezing of the rhizomes in the first season. We found a comparatively high level of sprouted rhizomes of the experimental crop which had survived over the winter in the first season (Table 1). There were only 3% of frozen sprouted rhizomes among those of 10–20 g and 40–60 g during the first winter (2008/2009). An investigation conducted in Germany (Christian and Haase, 2001) revealed that, for successful crop establishment, rhizome fragments should be 200 mm long and planted at the depth of 200 mm. Good viability rates were also noticed when rhizomes of uniform size were planted at 100 mm, but overwintering rates were lower. In a Danish experiment, *M.× giganteus* rhizomes were separated into two groups, according to size. Overwinter survival of small (length <10 cm) and larger rhizomes (length >10 cm) was 34% and 82%, respectively (Christian and Haase, 2001).

Table 1. Frozen rhizome ratio during the first winter after planting.

Mass of the planted rhizomes	Number of sprouted rhizomes (±S.D.)	% of sprouted rhizomes	Number of frozen rhizomes (±S.D.)	% of frozen rhizomes
10–20 g	32.0±2.6	80.0 [b*]	1.0±1.0	3.1 [b]
25–35 g	38.0±2.0	95.0 [a]	0.3±0.6	0.8 [a]
40–60 g	37.0±2.6	92.5 [b]	1.0±1.0	2.7 [b]

*Different small letters within the same row indicate significance at the 0.05 level.

In the first season, the average stem number (Figure 3) increased most rapidly in the plots with the highest mass rhizomes. According to Khan et al. (2011), this is to be expected considering that there is a positive linear correlation between rhizome fragment mass on the one hand and stem number and fresh mass on the other. However, in an investigation conducted by Easson et al. (2010) in the Northern Ireland, where rhizome fragments weighing 26, 76 and 204 g on average were planted at the beginning of May, at the depth of 7.5 cm, it was found that the number of stems increased with the decrease of the mass of the planted rhizomes from 204 g to 26 g in the first season. Similarly, the same tendency continued in the second and third seasons. Besides, Easson et al. (2010) found that the increase of stem number resulted in a significant increase of dry matter yield regardless of the time of harvest.

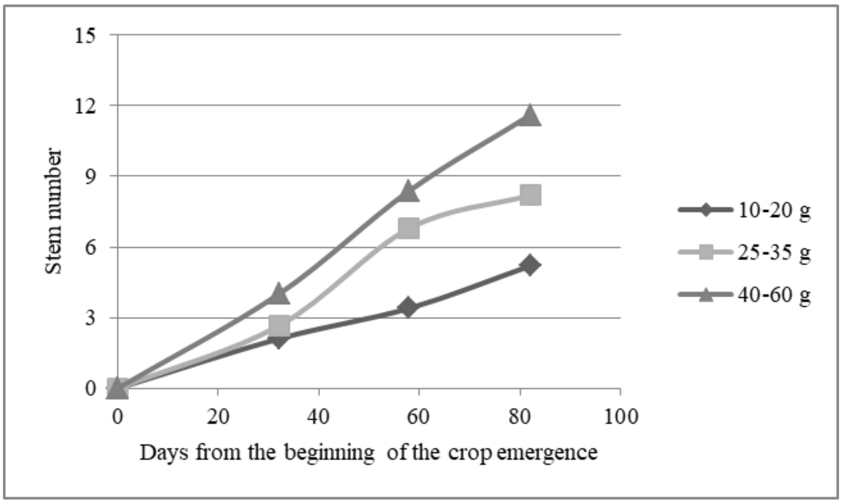

Figure 3. The average stem number per m^2 in the first season (different curves represent rhizomes with different masses, $P \leq 0.05$).

In the first season of this study, rhizomes with masses of 25–35 g and 40–60 g produced the crop of the approximately same height (Figure 4). Rhizomes with the very low mass produced plants lower by 10–20 cm. This tendency continued in the second season (Figure 5), but the difference was less pronounced. *M.× giganteus* is characterized by the crop establishment stage during which the yield is increased each year. This is followed by the maximal yield stage with variable duration. Establishing stage duration depends strongly on the method of the establishment (Lesur et al., 2013) and it can last 2–5 years (Price et al., 2004). Based on results presented in Table 2, maximum yields were obtained in the fourth and the ninth season. Obtaining high yields started from the third season. On the basis of the results of Clifton-Brown et al. (2004), the region of the West Balkans (South-East Europe) is considered to be favorable for obtaining high biomass yields of miscanthus (20–40 Mg dry matter per hectare).

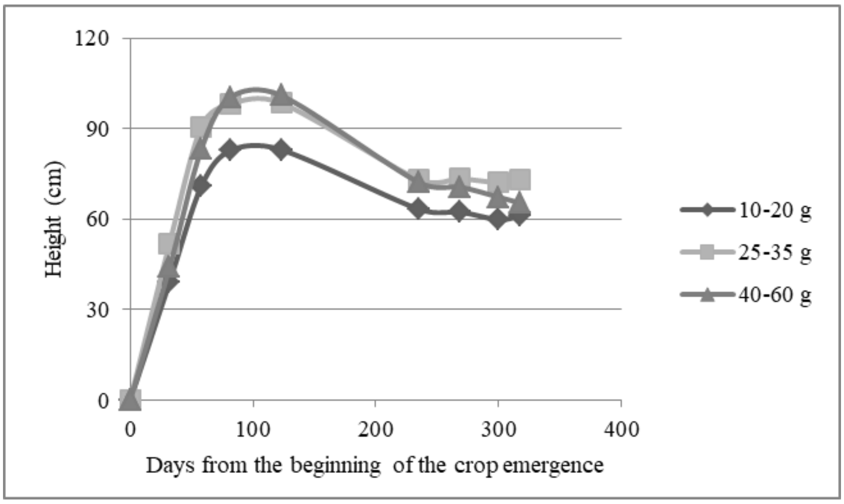

Figure 4. Average heights of the experimental crops in the first season (different curves represent rhizomes with different masses, $P \leq 0.05$).

In the first season, very low above-ground mass yields were achieved, about 1 Mg ha^{-1}. This is mainly ascribed to undeveloped root system of miscanthus (Dželetović et al., 2013). During the first season, miscanthus develops a loose root system, with the original rhizome in the center. In the second season, a dense system of lateral roots is formed together with a great number of new rhizomes (Dželetović and Glamočlija, 2015). Humentyk et al. (2013) found that, in the first season when the mass of the planted rhizomes was 20–30 g, at the end of the vegetation season, the root system mass amounted to 471 g (the 18-fold increase), while with the rhizomes of 90–120 g, the total root system mass amounted to 664 g (the 6-fold increase). On the other hand, Pyter et al. (2010) did not find a significant influence of rhizome size on the production of above-ground biomass. The initial rhizome mass of 60–75 g produced approximately 33% higher above-ground biomass than other rhizomes, which suggests that the optimal rhizome mass is within this range (Pyter et al., 2010). In our experiment, biomass yield in the second year of growth, with rhizomes of different masses, ranged from 5.5 to 8.1 Mg ha^{-1}.

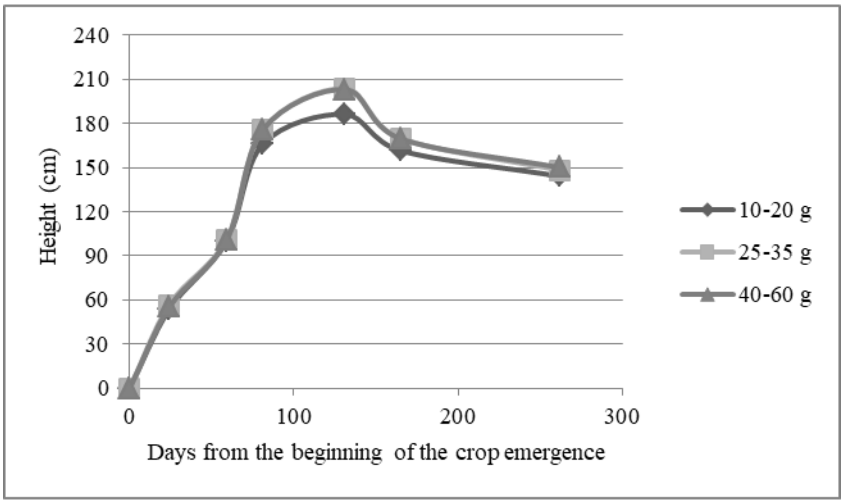

Figure 5. Average heights of the experimental crops in the second season (different curves represent rhizomes with different masses, $P \leq 0.05$).

The stage of maximal yields (stage of mature plantation, seasons 3–10) was characterized by significant seasonal differences in above-ground biomass yields, from 14.24 to 37.38 Mg DM ha^{-1} (Table 2). Variations as big as those, with some deviations from mean values for the seasons 3–10 achieving ±32%, occurred most probably due to varying weather conditions in individual seasons. Namely, at appropriate nitrogen supply, a maximal yield increase is achieved when water is not a limiting factor (Cosentino et al., 2007). Drought can cause a significant reduction in the yield of miscanthus crop (Kørup et al., 2018). Water supply from atmospheric precipitations exerts a powerful influence on above-ground biomass yield in agro-ecologic conditions characteristic for the wider Belgrade region (Dželetović et al., 2014).

Average above-ground mass yield of 23.02–25.85 Mg DM ha^{-1}, which was obtained in the seasons 3–10 (Table 2) falls within the range recorded in the West Balkans: 18.23–29.13 Mg DM ha^{-1} after November harvests at 3 locations in Croatia (Bilandžija et al., 2016); and 11.26–28.29 Mg DM ha^{-1} after February harvests at two locations in the wider region of Belgrade, Serbia (Dželetović et al., 2014). On eroded claypan soils, economically marginal for grain crops, Yost et al. (2017) obtained winter biomass yield from 13.3 to 23.8 Mg ha^{-1}, which was comparable to more productive soils. In a 12-year field trial that was conducted in southwest Germany, with N fertilization, *M.*× *giganteus* produced 18.3 Mg ha^{-1} yr^{-1}, and 13.6 Mg ha^{-1}yr^{-1} without N fertilization (Xu et al., 2017). Besides, our results show that the coefficient of variation for a season (CV≤0.05) decreased with the aging of the plantation. Variability among the yields under different treatments also changed with the age. During the establishing stage, the lowest standard deviation was found with the rhizomes of 40–60 g, while during the mature plantation stage (seasons 3–9) the same rhizomes actually displayed the highest standard deviation of the yields (Table 2).

Table 2. Mean yield of above-ground biomass of miscanthus established by planting rhizomes of different masses (Mg ha^{-1} DM ± S.D.).

Season	Rhizome mass		
	10–20 g	25–35 g	40–60 g
1	0.63±0.06^{b*}	0.96±0.08^{b}	1.05±0.05^{b}
2	5.53±0.44^{b}	7.60±0.46^{b}	8.12±0.32^{a}
3	24.77±1.49^{b}	27.48±1.38^{b}	25.11±1.01^{a}
4	31.77±1.62^{b}	31.99±1.64^{b}	26.85±1.64^{b}
5	18.45±0.72^{a}	28.66±0.74^{a}	16.50±0.86^{b}
6	18.27±0.55^{a}	19.10±0.56^{a}	16.18±0.48^{a}
7	31.24±1.22^{a}	32.19±1.28^{a}	28.74±1.40^{a}
8	16.56±0.37^{a}	19.46±0.42^{a}	17.93±0.38^{a}
9	31.54±1.06^{a}	37.38±1.28^{a}	35.29±1.21^{a}
10	14.24±1.06^{b}	20.58±1.18^{b}	17.60±0.99^{b}
Mean yield of above-ground biomass in the seasons 3–10	23.35±7.38	25.85±7.36	23.02±7.04
Yield index of base rhizome mass of 40–60 g (seasons 3–10)	101.4%	112.3%	100.0%
Statistical dispersion (variability) of the yields among different treatments in the seasons 1–10 (%)	162.0	123.3	100.0
Statistical dispersion of the yields among different treatments for the seasons 3–10 (%)	120.6	91.2	100.0

[*]The coefficient of variation (CV) within a season: a) CV≤0.05; b) 0.05<CV≤0.10.

The existing cropping procedure for planting rhizomes requires the use of modified potato planters (Dželetović, 2012; MAFF, 2001). Miscanthus rhizomes that are heavier are generally of irregular shape, thus cannot be inserted into standard holes of potato planters. It is not uncommon for rhizomes to get stuck in the holes thus slowing down the planting process even when the wider holes (existing holes are either replaced or adapted) in planters are used.

Our results justify the use of low mass rhizomes in the establishment of *M.* × *giganteus* crops. Technically and practically, the use of smaller rhizomes (25–35 grams) significantly simplifies the process of establishing this crop. Furthermore, by using low mass rhizomes, the planting process will be faster, which also contributes to lowering the cost of crop establishment.

Conclusion

Plantation establishment lasted for two years. Out of the total number of planted rhizomes, the least successful plantlet emergence was noticed with very low mass rhizomes, while the emergence with low mass and medium mass rhizomes was better. During the plantation establishing stage, number of stems, crop height and above-ground biomass yield of *M.× giganteus* were optimal with the rhizomes with the highest mass (40–60 g).

However, in mature plantation, high yields of above-ground biomass can also be achieved with the rhizomes with lower mass. With the highest biomass yield (yield of above-ground biomass), the crop established with rhizomes of 25–35 g clearly stood out. Our results support the use of rhizomes that are of relatively lower mass (25–35 grams) for establishing miscanthus crops.

Acknowledgments

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UTICAJ MASE RIZOMA KORIŠĆENIH PRI ZASNIVANJU USEVA NA PRINOS BIOMASE *MISCANTHUS* × *GIGANTEUS*

Željko S. Dželetović[1], Gordana Z. Andrejić[1], Aleksandar S. Simić[2*] i Hakan Geren[3]

[1]Univerzitet u Beogradu, INEP - Institut za primenu nuklearne energije, Departman za radiologiju i poljoprivrednu hemiju, Banatska 31-b, 11080 Beograd, Srbija
[2]Univerzitet u Beogradu, Poljoprivredni fakultet, Nemanjina 6, 11080 Beograd – Zemun, Srbija
[3]Egejski univerzitet, Poljoprivredni fakultet, Departman za ratarstvo, Izmir, Turska

R e z i m e

Cilj našeg istraživanja je bio da procenimo uticaj mase rizoma bioenergetskog useva *Miscanthus* × *giganteus* na uspešnost zasnivanja i prinos biomase tokom prvih 10 godina gajenja. Ogledni tretmani su obuhvatili 3 mase rizoma: (1) 10–20 g (veoma male); (2) 25–35 g (male); i (3) 40–60 g (srednje mase). Gustina sadnje je bila 2 rizoma m^{-2}. Žetva košenjem celokupne nadzemne biomase izvođena je svake godine u februaru. Od ukupnog broja posađenih rizoma najslabije nicanje zabeleženo je kod rizoma najmanje mase. U prvoj godini gajenja najveći broj stabala i najveću visinu useva zabeležili smo u tretmanima sa najvećom masom rizoma. Međutim, u drugoj godini gajenja visina useva sva tri tretmana bila je približno ista. U prve dve godine gajenja najveću biomasu prinosa ustanovili smo u tretmanima sa najvećom masom rizoma. Iako su u fazi zasnivanja useva analizirani parametri bili najbolji sa rizomima mase 40–60 g, visoki prinosi nadzemne biomase od treće godine gajenja mogu se dobiti korišćenjem rizoma manjih masa. Jasno se ističe usev zasnovan sa rizomima mase 25–35 g sa najvećim prinosom biomase (25,85±7,36 Mg SM ha^{-1}).

Ključne reči: prinos biomase, zasnivanje useva, *M.* × *giganteus*, masa rizoma.

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*Autor za kontakt: e-mail: alsimic@agrif.bg.ac.rs