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EFFECT OF PLANTING DENSITY AND SUPPLEMENTAL NITROGEN NUTRITION ON THE PRODUCTIVITY OF MISCANTHUS

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ABSTRACT

Variability of Miscanthus morphological traits were the subject of two-year study (2010 and 2011). Plant height, stem number and number of developed leaves per plant was studied in dependence of the applied rhizomes planting density (2 and 3 per m^2), as well as of applied nitrogen amount used for supplemental plant nutrition.

The results showed that planting density had an impact on the investigated morphological characteristics of Miscanthus, while the affect of nitrogen was dependent on the weather conditions and distribution of rainfall, and was stronger during the first year at a maximum rate of this nutrient. In the second year, rhizomes planting density showed a stronger effect on the number of developed leaves per plant. Variation in number of stems per rhizome was evident in the planting year or in the second year, as well as among treatments.

The number of leaves was increased by increasing nitrogen amounts, so in both years it was the largest at the maximum applied nitrogen amount of 100 kg ha^{-1}. Usage of 100 kg ha^{-1} of nitrogen in the first year, produced plants with significantlly higher outgrowths number per rhizome compared to other applied variants. In the second year rhizomes planting density had a strong affect on the outgrowths number.

Key words: Miscanthus, planting density, nitrogen, morphological characteristics, rhizome, productivity.

INTRODUCTION

The great interest world wilde on biofuels can be explained by their potential to reduce a country's dependence on petroleum products importing, to reduce CO_2 emissions, and contribute to economical development of rural areas (Oljača et al., 2007). The growth rate of alternative fuels usage increases significantly, about 15% annually. Field crops are in the usage for the biofuels production. Analysts assert the inevitable increase in food prices and social problems in developing countries. These problems could be resolved by further improvement of ethanol producing technology from cellulose. High-quality energy crop should have adequate adapting capacity and affordable conversion of accessible solar energy into biomass with maximum efficiency, minimal inputs and favorable impact on the ecosystem (Heaton, 2004).

Miscanthus - Chinese sedge (*Miscanthus x giganteus* Greef et Deu) is a perennial plant, a representative of the grass family (*Poaceae*), native to eastern Asia. Genus Miscanthus has many species in the wider area of Asia. Energy cane Miscanthus (Chinese cane or Chinese reed grass or elephant ear) is a plant that refines the wastewater during which neutral sludge deposition remains, excellent as fertilizer. It is capable to process even heavy metals. It does not require special care and can bee grown on contaminated soils, which eventually turn into fertile. It is excellent raw material for biomass and for construction industry. It has similar characteristic as the energy willow. It purifies sewage. Natural triploid *Miscanthus x giganteus* genotype Greef et Deu, which is used for organic biomass production, was obtained by crossing *Miscanthus sacchariflorus* and *Miscanthus sinensis.* This interspecific triploid hybrid develops panicle inflorescence, but the

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flowers in them are sterile. As the plant does not form seed, its extension over some area is slow and cannot spread unchecked to surrounding areas. Its reproduction is vegetative with underground organs – rhizomes. Rhizomes parts with at least two buds are used for the plantation. Miscanthus develops perennial underground stems – rhizomes, from which aboveground yearling steams emerge during the April. At the end of August, during the first planting year, new born stems grow up to 1.5 m, during the second year up to 2.5 m, and in the third year of production up to 3-4 m. Stems are strong, straight and do not lodge.

Miscanthus biomass should enable successful substitution of fossil fuels with new renewable sources (Dzeletovic et al., 2007). Compared with other ligno-cellulosic plants, as well as with crop residues (for example, cereal straw), Miscanthus biomass has a higher combustion ratio what Lewandowski and Kicherer (1997), Lewandowski and Heinz (2003) emphasize. With better biomass combustion which is similar in quality with the wooden mass, there would be less carbon dioxide emited in the atmosphere, what significantly reduces the greenhouse effect. Besides use for energy purposes, Miscanthus recently become interesting and important as a raw material in the paper industry.

Production increasing from renewable energy sources is set as a goal to be reached in most developed countries, especially after the Kyoto Protocol (1997) adoption about climate changes, gas emissions reducing and the greenhouse effect. Biomass which produce energy, is one way that can realize Kyoto protocol's commitments. Energy-crops represent a new type of husbandry crops, as response to the need of reducing atmospheric carbon dioxide amount.

Biomass represent a very broad term in the energy production, which among other things, covers all forms of organic matter such as wood, herbaceous plants organic matter, agricultural crops, agricultural residues, aquatic vegetation, animal originated manure and municipal solid waste (Ikanović et al., 2011).

There is a huge number of plants that have the ability of solar energy conversion into biomass with high efficiency, including herbaceous crops, short rotation woody crops, forage crops (alfalfa, clover, switchgrass and Miscanthus), sugar crops (sugar cane, sugar beet, sweet and fiber sorghum), grains (corn, barley and wheat) and oilseeds (soybeans, rapes, palm, sunflower, safflower, canola and cotton) (Glamočlija et al., 2011 and Ikanović et al., 2010).

The annual production per hectare is equivalent to the value of 400 GJ for C4 crops, 250 GJ for grain, and 70 GJ for oilseeds (biodiesel). Dželetović et al. (2007, 2009) asserts that C_4 perennial crops represent suitable bioenergy-crops because they efficiently use the available resources, retain carbon in the soil, have a high degree of efficiency in water usage for bioproduction, they are not invasive species and have modest requirements on plant nutrition.

Best suited energy crops for combustion in power plants, with regard to our climatic and soil conditions, would be thick poplar tree plantings, baled soybean straw and Miscanthus.

Miscanthus production success is largely dependent on the weather conditions during the crop establishment year (Drazić et al., 2010), as well as on the main plant nutrition elements repletion (N, P, K). Grass family planted crops significantly react on the increased nitrogen nutrition, especially in regions with low fertility soils (Booker et al., 2007, Ikanović et al., 2011).

The main objective of this research is a preliminary study of the adaptation possibilities to agro-ecological conditions of South-eastern Europe of this perspective new plant introduction to our agricultural production. Field trials were set to determine the optimum amount of nitrogen and planting density for the plants development during the year of crops establishment.

MATERIAL AND METHODS

Two-year (2010-2011) studies were carried out in the Agricultural University on Radmilovac experimental field, Belgrade.

Field experiments were set on brown forest soil (by FAO soil classification eutric cambisol).

Miscanthus rhizomes of Austria's Johannes Furtlehner Company from Hofamt Priela were used.

The research included two planting densities - 2 rhizomes per m^2 (G_1) and 3 rhizomes per m^2 (G_2) and two variants with nitrogen usage of 60 kg ha^{-1} (N_1), 100 kg ha^{-1} (N_2). The variant without nitrogen was control one (N_0). N fertilizers were applied with the soil preparation for planting and with appearance of aboveground shoots during the second year. The experiment was conducted in a randomized block system with 10 replications. Basic plot size was 25 m^2 (5 m x 5 m).

Miscanthus preceding crop was winter wheat, after which shallow crop residues plowing was performed during July and deep plowing in October. Rhizomes of 10-15 cm long with 2-5 nodes that had been previously moistened with warm water were used for planting. Planting was done by hand in the third decade of April into a depth up to 10 cm. During the plant growing season in the first and second year, weeds were pulled by hand hoeing only, without usage of herbicides.

Meteorological conditions are variable and unpredictable (Popović et al., 2011, 2013). Weather conditions, especially water regime in the planting year were not favorable because the spring months, April and May had only 40 mm of rainfall, what resulted a delayed germination of plants (Figure 1).

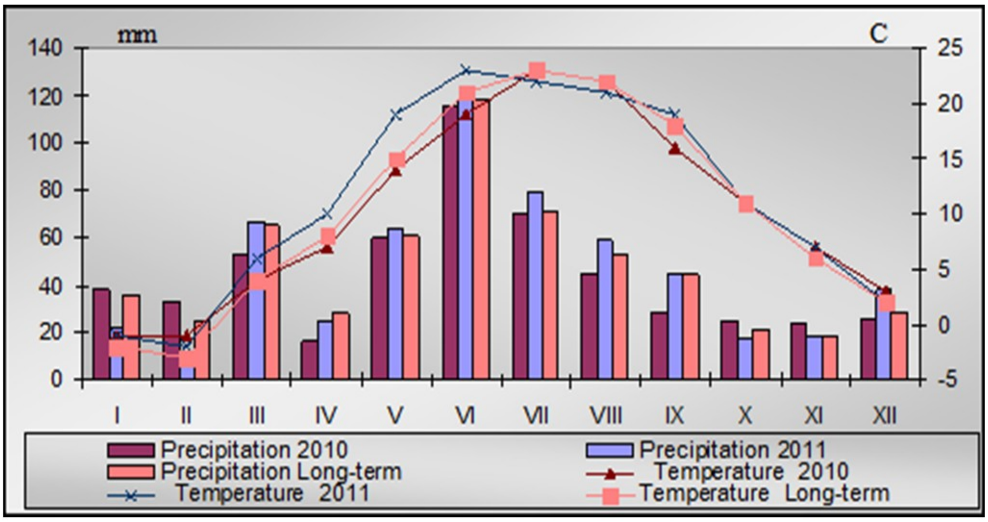


Figure 1. Rainfall (mm) and daily mean temperature (^{0}C) for growing period (Hydrometeorological station, Belgrade, Serbia)

Heavy rainfall in June and later in the summer had very favorable effect on the plants development during the first year. The second year was more favorable regard amount and distribution of rainfall. Thermal conditions were favorable for plant growth in both years. During the winter of 2010/11. there was no strong frost so no winterkilled rhizomes were recorded.

Measurements of morphological traits (plant height, number of mature stems and developed leaves per stem) were carried out during the period of maximum plants growth. In the first year it was early September, and the second in early October. Stem height was measured from ground level to the peak node. Number of stems per rhizome included all shoots above 10 cm height, and number of leaves per stem included all developed leaves on aboveground shoots.

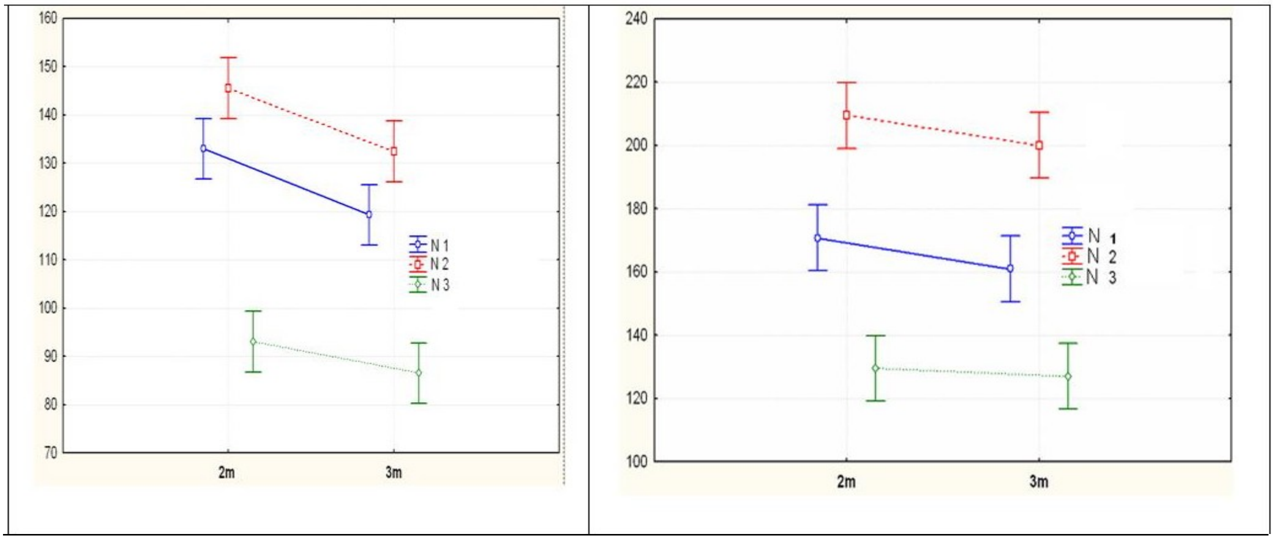
The analysis of the experimental data was performed by using of analytical statistics, with statistical software STATISTICA 10 for Windows (StatSoft). Examination of the differences among the Miscanthus rhizomes planting density and used nitrogen amounts as well as their interaction was performed by variance analysis method for double-factorial experiment (ANOVA), followed by LSD test for significance level of 0.05% and 0.01%.

RESULTS AND DISCUSSION

Miscanthus × giganteus a sterile triploid hybrid developed by crossing the species *Miscanthus sinensis* and *Miscanthus sacchariflorus*, can grows up to 3.5 m height. 3-6 years are needed for the full plantations establishment under Miscanthus and the maximum possible yield. Examined morphological characteristics showed great dependence on planting density and plant nitrogen nutrition intensity (Figures 2-4).

Plant height

The amount of nitrogen fertilizers had a significant affect on the growth of Miscanthus plants (Figure 2). In the crop establishment year the average height was 118.3 cm. The smallest plants were in control variant, and the highest in the variant with 100 kg ha^{-1}. Plants were higher in variants with nitrogen by 41% and 54%. These differences were significant compared to the control.



2 010 2011

Figure 2. Plants height variation at different nitrogen amounts and different rhizomes densities, 2010 - 2011

During the second year, nitrogen was found to increase plant height by 29% (N_1 variant) and 60% (N_1 variant).

Statistical analysis of variation showed that the differences among the plants height at all levels of nitrogen plants nutrition were statistically highly significant in both years, while the variation in plant height at the different planting densities was statistically highly significant in the first year (P<0.01). In the second year (2010) the differences between plant heights at different planting densities were not statistically significant (P>0.05) (Table 1).

The findings, obtained by Dželetović et al. (2009) showed that the average height of Miscanthus plants depended on the intensity of nitrogen nutrition. On chernozem soil type in the planting year it ranged from 80 cm up to 130 cm, and in the second year from 130 cm up to 172 cm.

Jorgensen et al. (2003) concluded that varieties respond differently to the N increase in the first year of life. There is great importance of nitrogen nutrition for plants during the initial development at most species of the *Poaceae* family, as pointed out by Marsalis et al. (2010).

Table 1. Observed traits differences statistical significance (F test and LSD test) and Partial Eta Squared of value

Traits	Test	2010			2011		
		N	Crop density	Interaction	N	Crop density	Interaction
Plant height	F test	***	***	NS	***	***	NS
	LSD 5 %	6.1576	5.0277	8.7082	10.1656	8.3002	14.3763
	1 %	8.1055	6.6181	11.4629	13.3812	10.9257	18.9239
	Partial Eta Squared of value	0.8304	0.2563	0.0287	0.8009	0.0516	0.0116
Number of stems per rhizome	F test	***	***	NS	NS	***	NS
	LSD 5 %	0.5004	0.4086	0.7077	2.6881	2.1949	3.8016
	1 %	6587	0.5378	0.9316	3.5384	2.8891	5.0041
	Partial Eta Squared of value	0.1776	0.4061	0.0047	0.0118	0.5142	0.0068
Number of leaves per stem	F test	***	***	***	***	***	NS
	LSD 5 %	0.6556	0.5353	0.9272	0.6186	0.505	0.8748
	1 %	0.863	0.7047	1.2205	0.8142	0.6648	1.1515
	Partial Eta Squared of value	0.288	0.2203	0.1301	0.1585	0.6028	0.0025

^{ns}- not significant, P>0.05 ***- significant at P<0.01

Planting density

Planting density also affected the plant height (Figure 2). In the first year of the G_1 variant plants were higher by 9.8%, and during the second year by 4%. The observed factor had statistically highly significant effect on Miscanthus plants height, but nitrogen had a greater effect on plant height compared to the rhizomes planting density.

Planting density significantly affected the number of outgrowths in both years (Table 1). Higher number of outgrowths was in the denser crop planting (G_2).

In 2010 the partial eta squared coefficients indicate that the impact of both factors (nitrogen and rhizomes planting density) on the number of outgrowths was statistically significant.

Number of stems per rhizome

Variations in the number of stems per rhizome were evident in the planting and the second year, as well as among treatments. By using 100 kg ha^{-1} of nitrogen during the first year substantially greater number of outgrowths per rhizome was formed, compared with other variants. At the N_1 variant (60 kg ha^{-1}) the number of outgrowths compared to control was not increased (Table 1, Figure 4).

Nitrogen did not affect the number of outgrowths during the second year. Rhizome planting density had a stronger affect (η^2=0.406) compared to the nitrogen nutrition variation effect (η^2=0.177). Interaction of factors had very small impact (η^2=0.004).

In the second year, 2010, only the rhizome planting density had a strong effect on the number of outgrowths (η^2=0.514). The effect of nitrogen and of the interaction of investigated factors was very low, P>0.05. That shows that the treatments acted independently (Table 4).

Miscanthus emerged as one of the best candidates in the lignocellulosic energy crop group that thrives in a temperate continental climate areas. As C_4 photosynthetic path species, it is characterized by high photosynthesis efficiency, and high rates of CO_2 fixation.

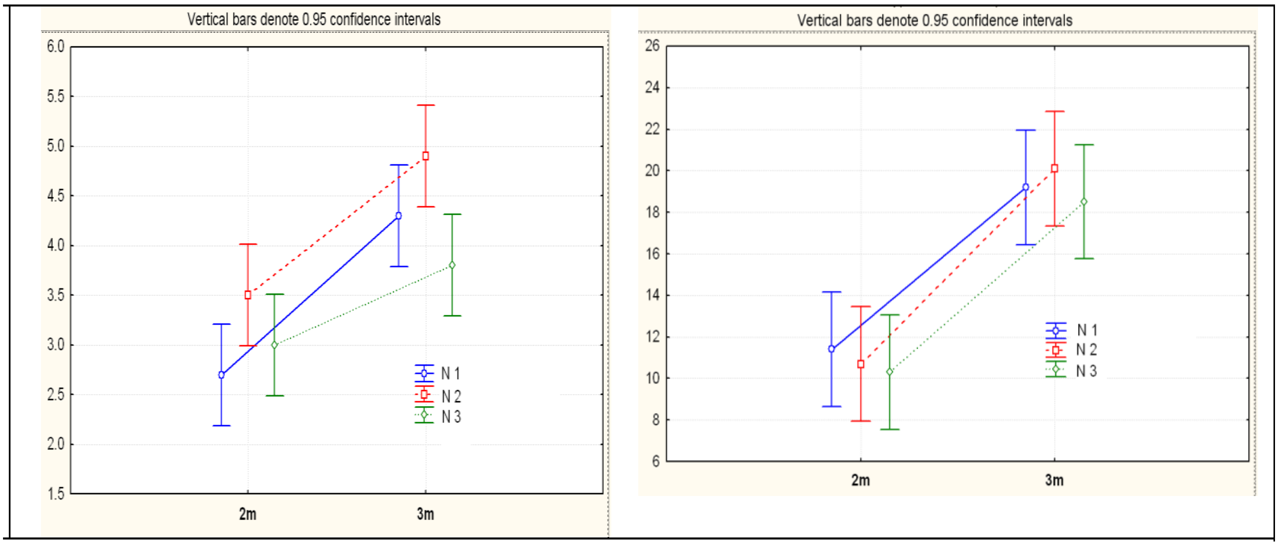


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Figure 3. Number of stems per rhizome at different amounts of nitrogen and rhizomes planting densities, 2010-2011

Due to the ability to perform translocation of nitrogen and other compounds in the rhizomes in the fall, *Miscanthus* × *giganteus* very effectively uses nutrients for bio-production, and can produce a consistently high yield over 15 years, without nutrients addition or with a minimal supplemental fertilization.

Number of leaves per stem

Statistically significant effect of both studied factors on the number of leaves per stem was recorded during the planting year.

With increasing of nitrogen amounts, the number of leaves was increased at the N_2 variant and it was significantly higher compared to other variants ($P<0.01$). Number of leaves per stem in the variant with 60 kg $ha^{-1}$of nitrogen and the control was not significantly different (Table 1). By using of 100 kg of nitrogen per ha^{-1} during the second year, the number of leaves was significantly increased compared with the variants N_0 and N_1, while between them there was no difference ($P<0.05$) (Table 1).

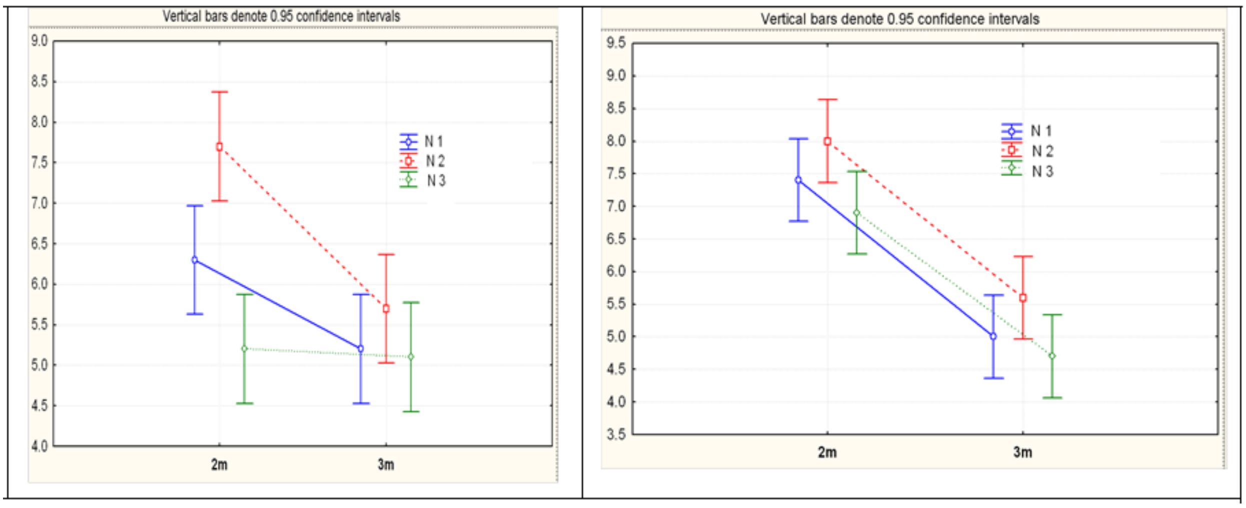

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Figure 4. Number of leaves per stem at different amounts of nitrogen and rhizomes planting densities, 2010-2011

Larger number of leaves per stem was in the variant G_1 (2 rhizomes per m^2), so the planting density significantly affected the number of leaves per plant in both years ($P<0.01$). The effect of the evaluated factors, measured with partial eta squared coefficients in both investigation years indicate not only statistically significant, but also a very large effect on the number of developed leaves per stem.

In 2010, the effect of nitrogen was stronger ($\eta^2=0.288$), while in the second year of planting rhizomes density showed a stronger affect ($\eta^2=0.603$) on the number of leaves per stem. Interactions between studied factors showed statistically significance only during the first trial year. Pearson's correlation coefficient indicated that the number of leaves per stem and Miscanthus plant height are in positive and statistically very strong correlation, while the number of leaves and number of outgrowths are in statistically highly significant and negative ($P<0.01$) correlation. Dependence of Miscanthus plant height and outgrowts number had not statistical significance ($P>0.05$), (Table 2).

Table 2. Observed traits correlation matrix, 2010-2011

Parameter	Plant height	Outgrowths number per rhizome	Developed leaves per stem number
Plant height	-	0.02^{ns}	0.44**
Outgrowths number per rhizome	-	-	-0.53**

^{ns}- not significant at $P>0.05$; **- significant at $P<0.01$

The results of previous researches about increased nitrogen nutrition in C_4 plants of the grass family have confirmed their huge dependence on nitrogen, which was manifested as statistically significant increase in the total leaves mass in total aboveground biomass (Ikanović et al., 2011 and Glamočlija et al., 2011). Under favorable water regime, Miscanthus has better germination during the first year, gives greater leaf mass, as well as number of leaves, what is confirmed by scientifical results (Ercoli et al., 1999; Dražić et al., 2009).

As pointed out by Christian et al. (2008) and Babović et al. (2010) regarding the ability of nutrients and minerals translocation from the aerial parts to the rhizomes from September to March, Miscanthus × giganteus is characterized by high efficiency of nutrient usage and can produce large amount of biomass over 15 years without adding nutrients, thus significantly reducing the financial inputs, compared with the cultivation of annual energy crops such as maize.

CONCLUSIONS

Results of Miscanthus morphological characteristics in terms of nitrogen nutrition intensification showed that the effect of this element utilization depends on the water regime. The lowest plant height had the control variant, and the highest the variant with 100 kg ha^{-1}. During the second year nitrogen also influenced plant height increasing.

Planting density also had influence on plant height. Variations in the number of stems per rhizome were evident in the planting year and in the second year, as well among treatments. With nitrogen amounts increasing the number of leaves increased, so in both years the largest variations were at the maximum amount of applied nitrogen of 100 kg ha^{-1}. During the first year, a stronger effect of nitrogen ($\eta^2=0.288$) was observed, while in the second year rhizomes planting density showed a stronger effect ($\eta^2=0.603$) on the number of developed leaves per stem. Variations in the number of stems per rhizome were evident in the planting and second year, as well among treatments. Optimal biomass increment was obtained with 100 kg per ha^{-1} of nitrogen under favorable water regime.

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