

A PRODUCTIVE POTENTIAL ESTIMATION OF FIVE GENOTYPES OF THE MISCANTHUS ANDERSS GENUS IN THE UKRAINIAN STEPPE ZONES CONDITIONS

ОЦІНКА ПРОДУКТИВНОГО ПОТЕНЦІАЛУ П'ЯТИ ГЕНОТИПІВ МІСКАНТУСУ ANDERSS GENUS В УМОВАХ СТЕПОВОЇ ЗОНИ УКРАЇНИ

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ABSTRACT

Five genotypes of the *Miscanthus Anderss* genus have been studied. *M. sacchariflorus* has very long rhizomes which quickly colonize the big areas. *M. Giganteus*, *M. oligostachyus* and particularly *M. sinensis* are more caespitose, their spread is slow and there is little risk of uncontrolled invasion of hedges or field, compared to the *M. sacchariflorus*. Despite that stands of *M. sacchariflorus* tend to be larger but less dense than stands of other *Miscanthus* species, *M. s. "Gracillimus"* has the highest stem density per unit of area. The biomass yield of *Miscanthus* per area in the earliest years of cultivation depends on the planting density. Under steppe conditions, optimal planting density is 14800 piece/ha for *M. sacchariflorus* and *M. Giganteus* and 20000 piece/ha for other species. Harvesting of above-ground biomass was made in the end of a growing season in October. It was revealed that during the drying process, plants lost up to 44% of water. Nevertheless, this indicator varies among species from 37.3% - *M. sacchariflorus* to 43.9% - *M. oligostachyus*. During the second year, substantial growth of productivity was observed for all species. Yield increased from 2.5 (*M. sinensis*) to 3.6 times (*M. oligostachyus*). In absolute measures, the largest values were noted for *M. Giganteus* (6.76 t/ha).

The work results show that optimum time for biomass harvesting is late autumn (November) for the solid types of fuel production or the end of the growing season (October) for the bioethanol production.

РЕЗЮМЕ

Вивчено п'ять генотипів роду *Miscanthus Anderss*. Висока вегетативна рухомість властива *M. sacchariflorus*, середня – *M. Giganteus* і *M. oligostachyus*, низька – *M. sinensis*. При визначенні щільності стояння пагонів спостерігається зворотна залежність: висока щільність – у *M. sinensis* (особливо у сорту *Gracillimus*), середня – у *M. Giganteus*, *M. oligostachyus* і низька – у *M. sacchariflorus*. Врожайність біомаси міскантуса з одиниці площі в перші роки вирощування залежить від щільності посадки рослин. В умовах степу оптимальним представляється середня щільність посадки: 14800 шт/га для *M. sacchariflorus* і *M. Giganteus* і 20000 шт/га для інших видів. Прибирання надземної біомаси проводилося в кінці вегетативного сезону в жовтні. Виявлено, що в процесі сушіння рослини втрачають до 44 % води. Тим не менш, цей показник трохи варіює між видами від 37,3% (*M. sacchariflorus*) до 43,9% (*M. oligostachyus*). Протягом другого року спостерігалось значне збільшення врожайності для всіх видів. Продуктивність збільшилася від 2,5 (*M. sinensis*) до 3,6 разів (*M. oligostachyus*). Щодо абсолютних показників, найбільші значення були зафіксовані для *M. Giganteus* 6,76 т/га. За проведеними дослідженнями оптимальний час для збирання врожаю є пізня осінь (Листопад), для виробництва біопалива або біоетанолу – кінець періоду вегетації (у Жовтні).

INTRODUCTION

Search for renewable energy sources is an integrated part of the general sustainable development of society. Today, the share of renewable energy in the world is about 13%. For example, in the European Union, the current potential of energy crops is estimated as 44-47 Mtoe/year. Until 2020, it is planned to increase biomass in gross final energy consumption to 138 Mtoe. According to recent data, in the EU the

total area under lignocellulosic energy crops (Willow, Poplar, Miscanthus, Switchgrass, Reed canary grass) is about 130-140 th. ha. At the same time the cultivation area for energy crops intended for liquid biofuels production (grain crops and rape) is much bigger and exceeds 2.5 Mha. (AEBIOM Report, 2011).

In Ukraine, the issue of sustainable bioenergy development is still at a relatively early stage of discussion, understanding and implementation. Currently, the share of biomass in the total primary energy input in the country is only 1.2% (Geletukha et al., 2014, 2016). Miscanthus is a promising crop for the production of cellulose containing biomass (Dohleman et al., 2009; Zub & Brancourt-Hulmel, 2010; Brosse et al., 2012). It is a perennial rootstock grass that originates from East Asia. Only 8-9 Miscanthus species of 16 known can grow in the temperate climate zone. They have a well-developed root system (up to 2.5 m deep), are characterized by fast growth and good resistance to low temperatures. Currently, four species of *Miscanthus* are actively studied and grown as an effective source of bioenergy raw materials. First of all, it concerns *Miscanthus Giganteus* - J.M.Greef et Deuter ex Hodk. et Renvoize. – spontaneous sterile hybrid cross between *Miscanthus sacchariflorus* (Maxim.) Hack. and *Miscanthus sinensis* Anderss. Along with other energy crops, this species takes rather large industrial areas in Western Europe and the USA. There is a lot of information about cultivation and productivity of giant miscanthus in these regions (Nixon & Bullard, 2001; Caslin et al., 2010; Williams & Douglas, 2011; Anderson et al., 2011). There are also numerous researches devoted to the study of existing and creation of new genotypes of *M. Giganteus*, *M. sacchariflorus* and *M. sinensis* for obtaining more resistant and productive varieties (Hodkinson et al., 2002; McKervey et al., 2008; Shumny et al., 2010; Nishiwaki et al., 2011; Yu et al., 2015; Cichora et al., 2015). The potential of other *Miscanthus* species is almost unknown, at a time when the possibilities of using them as an energy culture are sufficiently extensive.

The quantitative and qualitative indicators of the *Miscanthus* biomass depend not only on the species genetic component, but also on a large number of different external factors: the geographical location of the farming area, climatic conditions, water relationships, cultivation technology, availability of mineral elements etc. (Lewandowski & Schmidt, 2006; Glowacka et al., 2013; Arnoult & Brancourt-Hulmel, 2015; Matyka & Kus, 2016). It is believed that medium-dense soils with low groundwater levels are suitable for growing Miscanthus. The plant has a relatively small demand for water, corresponding to an annual rainfall of 600-700 mm. However, this demand may enhance as the amount of biomass increases. For instance, 4-5-year-old plants of *M. Giganteus*, for normal growth and development, can absorb water amount corresponding to 900 mm and more annual precipitation. Investigations of different *Miscanthus* species were carried out mainly in Western Europe and Russia in regions with sufficient water supply (Lewandowski et al., 2000; Clifton-Brown et al., 2001; Jezowski, 2008; Milovanovic et al., 2012; Kalinina et al., 2017). For example, in Germany, Denmark, Northern Ireland, England, Austria and the Centre of European Russia, the average annual precipitation varies from 550 to 700 mm, in Serbia from 850 to 900 mm, in Switzerland from 900 to 1000 mm, in Northwest Spain from 1800 to 1950 mm. In Ukraine studies of Miscanthus were carried out under the conditions of wooded district and forest-steppe, where the annual precipitation reaches 650-750 mm and 600-680 mm, respectively (Tziporenko & Rakhmetov, 2013; Rakhmetov et al., 2015; Skachok & Kvak, 2016; Kalinina et al., 2017). In the steppe zone of Ukraine, where this work was conducted, the average annual rainfall does not usually exceed 500-550 mm. Deficiency of moisture can negatively affect the yield and biomass quality. Therefore, there is a need to study and select drought-tolerant and productive genotypes of Miscanthus for economically substantiated cultivation under conditions of insufficient water supply.

MATERIALS AND METHODS

Five genotypes of the *Miscanthus* Anderss genus have been studied: *M. sacchariflorus* (Maxim.) Hack., *M. sinensis* Anderss., *M. Giganteus*, J.M. Greef et Deuter ex Hodk. et Renvoize., *M. oligostachyus* Stapf. and *M. sinensis* "Gracillimus". The planting stock for the trial was taken from the collection plants of the Oles Gonchar Dnepr National University Botanical Garden and from the Institute of Bioenergy Crops and Sugar Beet (*M. Giganteus*). The seedlings with 3-4 shoots were chosen for planting (Fig.1). Plants were set in the spring of 2015 on experimental sites according to the following scheme: for *M. sinensis*, *M. sinensis* "Gracillimus" and *M. oligostachyus*, the interval between the rows is 75 cm, the interval between the plants in the row is 60 cm (planting density is 2 pieces per m²); for *M. sacchariflorus* and *M. Giganteus* the interval between the rows is 90 cm, the interval between the plants in the row is 75 cm (planting density is 1.5 pieces per m²).

The soil of experimental plots is medium loam, low-humus black soil on loess. The physicochemical and agronomic parameters of this soil type are optimal for the normal growth and development of the *Miscanthus*. Fertilizers and irrigation were not used. The morphometric parameters and biomass productivity were subject of study.

*M. sacchariflorus**M. sinensis**M. sinensis* "Gracillimus"*M. oligostachyus**M. Giganteus*

Fig. 1 - Miscanthus saplings

Culm height from the soil surface to the collar of the flag leaf of a representative culm for each plant was measured with a measuring ruler during the second week of October. Calliper was used to determine stem diameter by clamping it on to a random plant tiller 15 cm above the ground surface. Stem number per plant was counted during the first week of October. The inflorescence length was measured by means of a ruler in August in the flowering period.

For above-ground biomass determination, the whole plants were cut by hand, using a knife, at the end of each growing season at a stable height of 10 cm and they were weighed. Then they were dried until a constant weight was achieved. These dry samples were weighed to estimate the above-ground dry matter yield. Thus fresh weight (FW) and dry weight (DW) were determined and moisture (M) was calculated as: $M(\%) = 100 \times (FW - DW) / FW$.

The soluble carbohydrates were estimated by photometric methods (Naiem and Abdelatif, 2001). Samples were quantified photometrically by measuring the change in wavelength at 660 nm. The amount of soluble carbohydrates was calculated with the help of standard curve obtained by using different concentration of standard glucose solution. The amount of sucrose was estimated as difference between total sugar content and reducing sugar content and multiplied with coefficient 0.95. Statistical analysis was conducted using the software package StatGraphics Plus5 with all tests of significance being made at a type 1 error rate of 5%.

RESULTS

The climate of the Dnepropetrovsk region is moderately continental. Over the course of a year, the temperature typically varies from -8°C to $+31^{\circ}\text{C}$. The cold season lasts from December to March with an average daily temperature -3.1°C . In winter, air temperature can sometimes fall till -20 - 25°C . The warm season lasts from May to September 15 with an average daily temperature above $+24^{\circ}\text{C}$. Often, the average day temperature in summer reaches $+30$ - 34°C , maximum $+37$ - 40°C . In recent years, there has been an increase in the average monthly temperature in comparison with the long-term data (Fig. 2a). Average annual precipitation is moderate. During the warm season, precipitation occur most often in the form of thunderstorms (63%), light rain (31%), and moderate rain (6%). The rainfall varies significantly over the course of the warm season (Fig.2b).

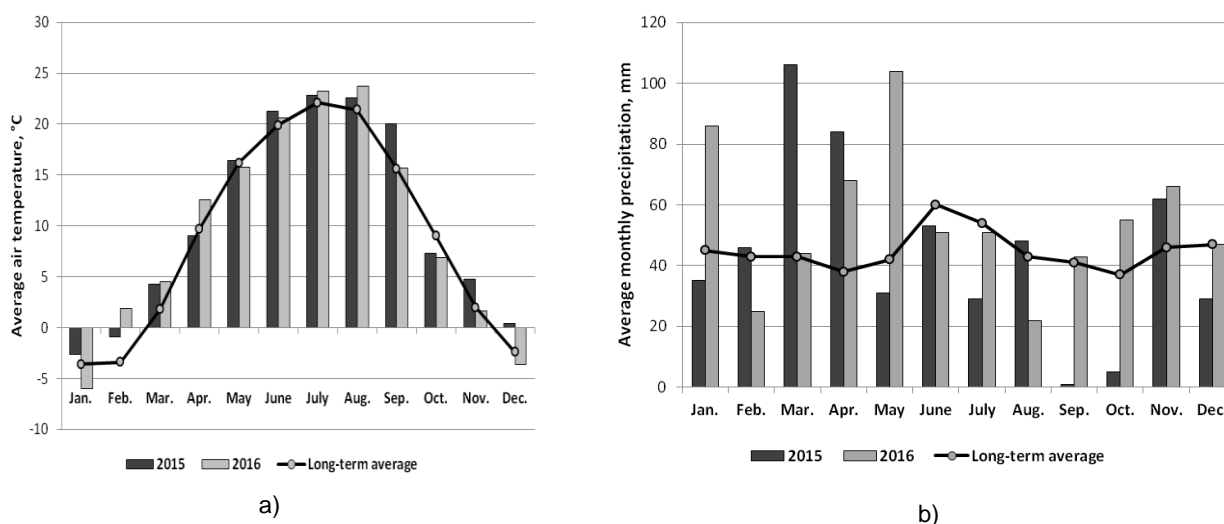


Fig. 2 - Monthly average of air temperature and precipitation in the Dnipropetrovsk region

Considering that the spring in the steppe zone is short, planting of the *Miscanthus* must be made as soon as possible so that the plants can use the soil moisture accumulated in winter and grow stronger. The optimal dates for spring planting are the first and second decades of April. Autumn planting in October is also possible. Observing the planting time, we noticed that the survival rate of saplings in the first year of cultivation is high from 80% (*M. Giganteus*) to 100% (*M. sacchariflorus*).

According to long-term phenological observations in the steppe zone of Ukraine, spring growth *Miscanthus* begins mainly at the end of April. In some years, it starts in early May. Growing season continues until the third decade of October. *Miscanthus* plants reach the greatest height in a flowering stage, which comes in August-September.

The vegetation period of *M. s. "Gracillimus"* is longer than that of other varieties. Its leaves yellowing continue during December. The flowering stage also begins later, in November, and may remain unfinished in the event of early autumn frosts.

The studied *Miscanthus* species under introduction conditions do not form seed and can only be propagated vegetatively. *Miscanthus* spreads naturally by means of underground rhizomes. *M. sacchariflorus* has very long rhizomes which quickly colonize the big areas. *M. Giganteus*, *M. oligostachyus* and particularly *M. sinensis* are more cespitose, their spread are slow and there is little risk of uncontrolled invasion of hedges or field, compared to *M. sacchariflorus*. Despite that stands of *M. sacchariflorus* tend to be larger but less dense than the stands of others *Miscanthus* species, *M. s. "Gracillimus"* has the highest stem density per unit of area.

In the first year after planting, all *Miscanthus* species actively increased aboveground and underground biomass.

Plants of *M. sinensis*, *M. Giganteus* and *M. sacchariflorus* had a stem height of about 1 m at the end of the growth season. The highest was *M. sinensis* (106.1 cm), the lowest – *M. oligostachyus* (56.5 cm). In the second year of cultivation, the most active growth was observed for *M. Giganteus* and *M. sacchariflorus*. Their height was about 2 m (Table 1). This parameter for *M. sinensis* and *M. sinensis "Gracillimus"* was less by 25-32%. The height of *M. oligostachyus* did not exceed 1 m. In the first year, the flowering phase was absent in two species: *M. Giganteus* and *M. sinensis "Gracillimus"*. The latter species did not bloom in the

second year either. Measurements of the panicle length showed that this indicator is constant, it is determined by the genotype and does not depend on the age of the plants (Table 1). Plants of *M. sacchariflorus* have the longest inflorescence, and *M. oligostachyus* - the shortest.

Table 1

	Culm height, cm		Panicle length, cm	
	First year	Second year	First year	Second year
	<i>M. sacchariflorus</i>	80.0 ± 1.23	188.8 ± 2.93	33.36 ± 0.49
<i>M. sinensis</i>	106.1 ± 2.42	142.5 ± 1.66	26.02 ± 0.40	26.88 ± 0.31
<i>M. sinensis</i> "Gracillimus"	73.1 ± 1.70	135.7 ± 2.33	–	–
<i>M. Giganteus</i>	86.7 ± 0.98	198.4 ± 2.91	–	27.08 ± 0.38
<i>M. oligostachyus</i>	56.5 ± 1.63	93.7 ± 1.86	14.52 ± 0.34	14.70 ± 0.35

The formation of new culms occurred during the entire vegetation period. During the first year of cultivation, the stem number per plant increased on average by 3-4 times (Table 2). At the end of the year, the smallest number was observed in *M. oligostachyus* plants. The clump growth intensity in the second year of cultivation was less than that in the first year and for most species it was from 95 to 120%. Plants *M. oligostachyus* showed the greatest stem number increase (by 3 times) compared to the previous year.

Measurement of the stem diameter detected the highest indicators in *M. Giganteus*, and the smallest in *M. oligostachyus* (Table 2). This parameter increases with age, but not much, from 1.4% (*M. Giganteus*) to 10.7% (*M. sinensis* "Gracillimus").

Table 2

	Stem number per plant		Stem diameter, mm	
	First year	Second year	First year	Second year
	<i>M. sacchariflorus</i>	11.97 ± 0.54	26.2 ± 0.44	4.2 ± 0.15
<i>M. sinensis</i>	15.21 ± 0.52	30.3 ± 1.25	4.22 ± 0.11	4.3 ± 0.11
<i>M. sinensis</i> "Gracillimus"	17.74 ± 0.95	34.48 ± 1.21	4.12 ± 0.10	4.56 ± 0.10
<i>M. Giganteus</i>	16.80 ± 0.64	33.12 ± 0.82	8.1 ± 0.21	8.21 ± 0.17
<i>M. oligostachyus</i>	10.22 ± 0.54	33.7 ± 1.14	3.3 ± 0.12	3.4 ± 0.10

The biomass yield of *Miscanthus* per area in the earliest years of cultivation depends on the planting density. If the planting density becomes greater than the yield also increases. However, planting more saplings can increase the cost of a plantation making from 50 to 150%. Especially as in the next years the difference between productivity on the plots differing in planting density is levelled. Usually the planting density of *Miscanthus* can vary from 10000 to 25000 pieces per hectare (Himken et al., 1997; Christian et al., 2009; Williams & Douglas, 2011; Kalinina et al., 2017). Under steppe conditions optimal planting density is 14800 piece/ha for *M. sacchariflorus* and *M. Giganteus* and 20000 piece/ha for other species. Harvesting of above-ground biomass was made in the end of a growing season in October. It is revealed that during the drying process, plants lose up to 44% of water. Nevertheless, this indicator varies among species from 37.3% - *M. sacchariflorus* to 43.9% - *M. oligostachyus*. The yield of dry biomass was moderate in the first year of cultivation. Among tall species this parameter varies from 0.79 to 2.3 t/ha. Productivity of undersized *M. oligostachyus* was the lowest, only 0.33 t/ha (Fig.3). During the second year, substantial growth of productivity was observed for all species. Yield has been increased from 2.5 (*M. sinensis*) to 3.6 times (*M. oligostachyus*). In absolute measures the largest values were noted for *M. Giganteus* (6.76 t/ha). Nevertheless, these data somewhat lower than similar indicators of *Miscanthus* productivity, received for the European and East Asian regions where there is sufficient water supply (Zub et al., 2010; Feng et al., 2015; Richter et al., 2016). In Ukraine, such comparison can be done only for *M. Giganteus* because other species practically are not studied. The comparative estimation has shown that in a steppe zone of Ukraine the productivity of *M. Giganteus* in the first years of cultivation is less by 20-50 % than in wooded district and forest-steppe (Gumentyk et al., 2013; Rakhmetov et al., 2015). Thus, there is an obvious unfavourable influence of droughty conditions on biomass yield. The aboveground biomass of *Miscanthus* can be a significant source for bioethanol production. Usually technologies of bioethanol production by *Miscanthus* biomass includes following stages: pre-treatment remove hemicellulose and get reactive cellulose; hydrolysis for fermentable sugars; fermentation for production of ethanol (Lien et al., 2005, Alvira et al, 2010, Huang et al., 2010).

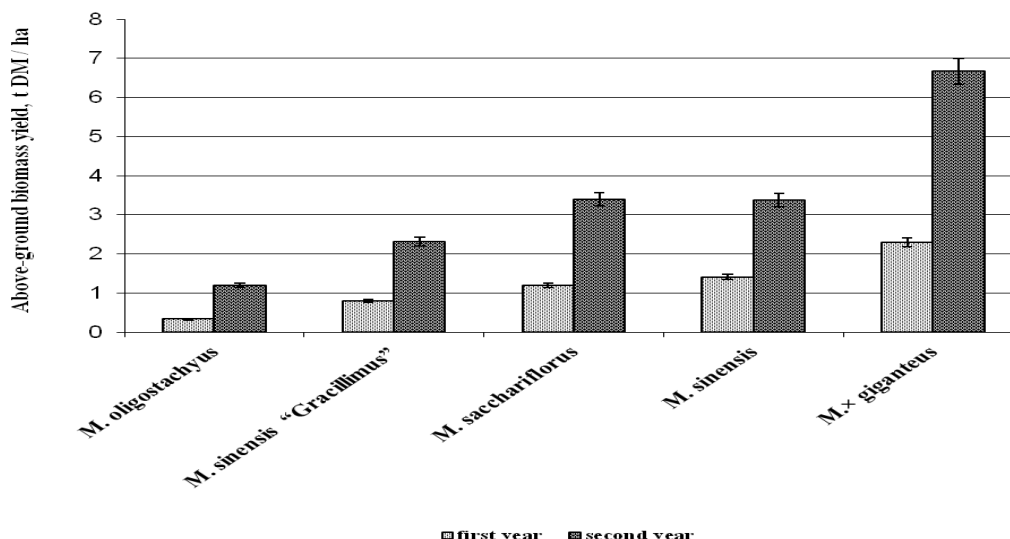


Fig. 3 - Above-ground biomass yield of *Miscanthus* under steppe zone conditions

At the same time, the biomass of *Miscanthus*, besides lignocellulosic components, contains water soluble carbohydrates and their total content is almost the same as in the case of traditional sacchariferous crops: *Sorghum*, *Eclrinochloa frumentacea*, *Setaria italica* etc (Almorades and Hadi, 2009). The soluble sugar content was estimated in the end of the growing season (Fig.4).

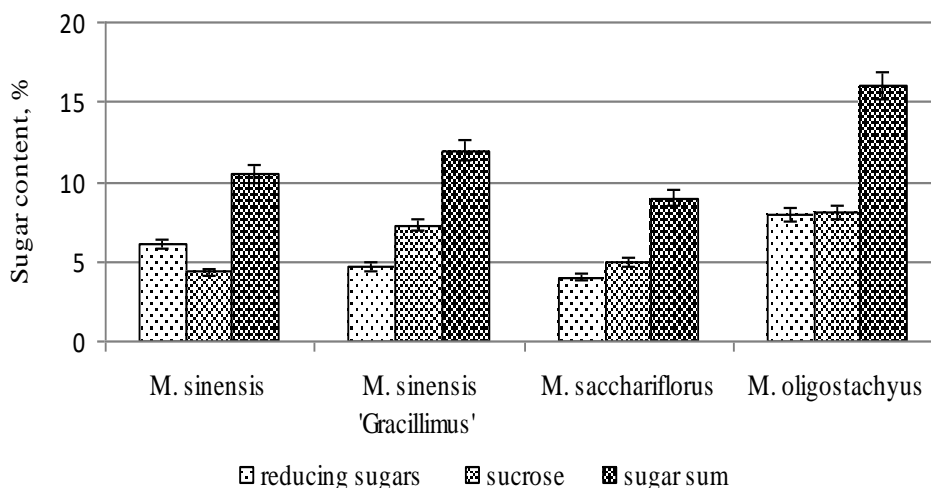


Fig. 4 - Soluble sugar content in aboveground biomass of *Miscanthus*

In this period, vegetation is still in progress. However, plants have already accumulated enough nutrients necessary for normal wintering and a cut of aboveground biomass will not damage the plant.

The sugar sum content varies in different species in range from 9.0 (*M. sacchariflorus*) to 16.05% (*M. oligostachyus*). Reducing sugars-sucrose ratio was nearly 50:50 at *M. oligostachyus*. At the *M. sinensis* reducing sugar content was greater than that of sucrose content by 40-60% and vice versa the biomass of *M. s. cv. Gracillimus* accumulates greater quantity of sucrose than reducing sugars by 56% and at *M. sacchariflorus* by 22.5%.

CONCLUSION

It is revealed that optimum terms of *Miscanthus* planting in a Ukrainian steppe zone are the first and second decade of April, and also from first to third decade of October. In the first year of cultivation, plants actively increase biomass. By the end of a vegetative season, the plants of the majority species reach heights about 1 m and stem number per plant increases at an average of three-four times. The intensity of clumps growth in the second year slightly decreases and ranges from 95 to 120%. The height of plants in the end of this period varies from 140 to 190 cm. The exception is the undersized *M. oligostachyus*, which had

stems height only over 90 cm in the second year. The stem diameter also increases with age, but not greatly (from 1.4% to 10.7%). The yield of dry biomass in the first year among tall species varies from 0.79 to 2.3 t/ha. Productivity of *M. oligostachyus* was the lowest, only 0.33 t/ha. The yield of the second year is much higher. The highest productivity was noted for *M. Giganteus* (6.76 t / ha). The average measures are characteristic for *M. sacchariflorus* and *M. sinensis*. The yield of *M. oligostachyus* does not exceed 1.2 t/ha. Arid conditions of the steppe zone have a negative impact on the biomass productivity of *Miscanthus*. A comparative assessment showed that the yield of *M. Giganteus* in the first years of cultivation is 20-50% lower than in other regions of Ukraine with a higher level of water supply.

Determination of soluble carbohydrates in *Miscanthus* leaves and stems has shown high values of reducing sugars and sucrose.

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