SOME PHYSIOLOGICAL FEATURES AND THE PRODUCTIVITY OF THE ENERGY CROPS *MISCANTHUS X GIGANTEUS* AND *SORGHUM ALMUM* UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

This article presents the results of scientific research on some physiological processes in the energy crops *Miscathus x giganteus* 'TITAN' and *Sorghum almum* 'ARGENTINA', grown under the conditions of the central area of the Republic of Moldova, the dry biomass yield and quality indices of the obtained solid biofuels. The obtained results show the establishment of the physiological-biochemical activity in the species *M. x giganteus* by the values of the indices of photosynthesis intensity *(*3.39-8.68*^{2}µmol m$^-$ s^{-1}),* in correlation with the intensity of transpiration (0.59-2.02 mmol m^{-2} s^{-1}*)*, respiration (0.02 mol m^{-2} s^{-1}) and the efficiency of photosynthetically active radiation (PAR) (320-385. µmol m^{-2} s^{-1}). Comparative values of the resulting indices were established simultaneously for the species *S. almum*, with the values estimated in the process of photosynthesis (15.77-17.18 µmol m^{-2} s^{-1}), the intensity of transpiration (1.26-1.84 mmol m^{-2} s^{-1}) and the efficiency of photosynthetically active radiation (PAR) 1061-1565 µmol m^{-2} s^{-1}. These results show that the values of photosynthesis and PAR indices are more active and higher in *M. x giganteus* as compared with the values of the indices of *S. almum*. It was established that the stem dry biomass yield in the second season reached 1.18-1.89 kg/m^2, with a content of 45.23-45.64% carbon, 5.76-5.91% hydrogen, 0.25-0.40% nitrogen, 0.05-0.06% sulphur, 1.25-4.40% ash, 18.99-19.20 MJ/kg gross calorific values and 17.30-17.45 MJ/kg net calorific values. The specific density of briquettes reached 770-850 kg/m^3 and the specific density of pellets 970-1070 kg/m^3. The local cultivars of *M. x giganteus* and *S. almum* may serve as feedstock for renewable energy production

Key words: *Miscathus x giganteus* 'Titan', *Sorghum almum* 'Argentina' physiological parameters, productivity

The mobilization and domestication of new plant species should be able to take advantage of the growing understanding of the process of adaption, the knowledge of biological peculiarities, productivity, chemical composition and economic value of these plants in new soil and climatic conditions. Domesticating new plant species specifically for energy production may allow access to species that are better suited to energy production and avoid diversion of food crops.

The *Poaceae* C_4-plants, having a more effective photosynthetic pathway, possess such features as resistance to aridity, high photosynthetic yield and a high rate of CO_2 capture when compared with C_3 plants (Lewandowski I. *et al*, 2000). Based on their physiological properties, they have great potential of biomass production, frequently higher than the productivity of trees. Promising grasses, C_4 photosynthetic pathway, belong to the genus *Miscanthus* Andersson which includes 16 accepted species names and the genus *Sorghum* Moench with 31 species (www.theplantlist.org/1.1/browse/A/Poaceae).

Perennial *Poaceae* – C_4 plant species play an important role as a CO_2 sink, significantly increasing the content of soil carbon; their dry organic matter possesses many beneficial features making them high-potential energy crops, therefore, there has been increasing interest in their use for this purpose since the mid-1980s.

The natural hybrid between *Miscanthus sinensis* x *Miscanthus sacchariflorus*, a sterile triploid plant, was developed in Japan, introduced as ornamental plant in Denmark in 1935 and distributed to some European countries towards the end of 1970s. But its exact taxonomic position had not been examined in detail for a long time, and the plant was called *Miscanthus sinensis giganteus*, until Greef and Deuter (1993) conducted the syntaxonomy and nominated it as *Miscanthus×giganteus* Greef et Deu., and included it in genus *Miscanthus*, section *Triarrhena*, family

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Poaceae (Xi, Jezowski, 2004). The natural hybrid *Miscanthus* × *giganteus* is characterized by erect culm, growing 2.5-3.5 m tall, sometimes it can reach even 5 m in height, with 9-12 nodes, without hairs, sometimes shoots and aerial shoots are produced from the lower nodes. The leaves are 50-60 cm long; the ligule is truncate, with hairs, 2-3 mm long. The inflorescence is a 30-55 cm long panicle, with 15-21 cm long ramifications. It blooms at the end of September, but does not produce seeds. It propagates vegetatively by pieces of rhizomes or plantlets obtained by tissue culture.

The exceptionally vigorous growth and remarkable adaptability of *Miscanthus* × *giganteus* to different environments make this novel crop suitable for cultivation and distribution under a range of European and North American climatic conditions *Miscanthus* is a high-yielding lignocellulosic crop providing up to 40 t/ha/year of dry matter (Kiesel H., Lewandowski I., 2016; Farrar *et al*, 2018).

Sorghum almum Parodi, also called Columbus grass or almum sorghum is native to South America; it was first developed in Argentina in 1936 as natural hybrid between *Sorghum bicolor* and *Sorghum halepense*, confirmed as species in 1943 by Parodi L.R. It is a robust, tussocky, short-lived perennial plant. It has numerous tillers and thick short rhizomes, reproduces by seed and rhizomes. The culms are thick and solid, with pith inside, with 6-13 nodes, up to 1 cm thick at the base and 300 cm tall. The leaves are green with the central veins light yellow, 1.3-3.8 cm wide and 45.7-81.3 cm long, glabrous. The inflorescence is a spreading pyramidal panicle, branched, 15-61 cm long and 8-25 cm wide, with secondary and tertiary branches, resembling the shape of a tail. It blooms in July-August, develops fruits in September-October. Caryopsis is light brown or reddish brown, 2.5-4.0 mm long and 2-2.3 mm wide, dorsally compressed, with round hilum. The weight of 1000 seeds is 7-9 g. In the soil, it develops a fibrous root system with short curved rhizomes on which new vigorous shoots grow. *S. almum* tolerates a wide range of soil types and temperatures and is drought resistant. It reproduces by seed and rhizomes. The species *S. almum* is studied in scientific centres and universities in different regions of the world (Popescu V., Albu M., 1970 Rakhmetov and Rakhmetova, 2008; Heuzé et al., 2015). The green mass productivity of Columbus grass under the conditions of Uzbekistan reached 211 t/ha (Avutkhonov *et al*, 2016). In Ukraine, it reached 20 t/ha dry mass (Rakhmetov and Rakhmetova, 2008), the maximum yield, 49.5 t/ha, in was attested in Gaudiano di Lavello, southern Italy (Corleto *et al*, 2009).

The goal of the morpho-physiological research on the above-mentioned species is to contribute to the process of increasing the productivity of plants by making use of their entire biological potential. Abiotic stress factors (long droughts, diurnal variations of temperature and humidity) are one of the main causes of the decrease in the yields of cereals and technical plants (Petcu C. *et al*, 2007), which can cause decreases in productivity that may sometimes reach 70%. These aspects of impact with mixed (abiotic-biotic) environmental factors in the Republic of Moldova motivated us to investigate some peculiarities of the morpho-physiological indices and aspects of phytosanitary records, on experimental sectors, in terms of the plant-environmental factor system. The purpose of the research was based on its topicality and it was focused on the morpho-physiological processes as essential indices in highlighting the physiological-biochemical activity in plants of *Miscanthus x giganteus* and *Sorghum almum*, as well as their dry biomass yield and quality indices of the obtained solid biofuels.

MATERIAL AND METHOD

The local cultivars of perennial energy crops: 'Titan' *Miscanthus giganteus* and 'Argentina' *Sorghum almum* created in the "Alexandru Ciubotaru" National Botanical Garden (Institute), registered in the Catalogue of Plant Varieties and patented by the State Agency on Intellectual Property (AGEPI) in of the Republic of Moldova, maintained in monoculture in the experimental plot of the NBGI Chişinău, N 46°58′25.7″ latitude and E 28°52′57.8″ longitude, served as subjects of the research.

The investigations were carried out in the 2nd year of vegetation. Surveys were made to determine the phenological stages under the climatic conditions of the Republic of Moldova, focused on the foliage in full process of vegetation (Бейдеман И., 1974). The activity of the photosynthetic indices, the PAR efficiency, the stomatal conductance, the transpiration in connection with the ostiolar oscillations, compared in time, between the leaves of the *M. x giganteus* and *S. almum* plants were determined with the help of the specific equipment – the portable gas analyser, *ADC BioScientific Ltd. model LCA*.

Applying the above-mentioned equipment, the activity of the physiological processes was analysed in the morning hours (7.00-11.00 hours). The portable device used in the field can indicate, through photochemical sensitizing reactions on the photosynthetic apparatus, values based on the volume of CO_2/O_2 exchange in the assimilating tissue. It measures the intensity of transpiration and ostiolar oscillations in leaves, determine some reactions related to the intensity of photosynthetically active radiation (PAR), the temperature of the subostiolar chamber, by methods of determining the temperature inside the leaf [https://www.adc.co.uk/].

The dry biomass of *M. x giganteus* and *S. almum* was harvested manually in February, then it was chopped and milled in a beater mill equipped with a sieve with diameter of openings of 10 mm (for briquettes) and 6 mm (for pellets). Scientific research on the biomass for the production of solid biofuel was carried out. The contents of C, H, N, S and Cl were analysed using the elemental analyser Vario Macro Cube CHNS & Cl, the moisture content of the plant material was determined by CEN/TS 15414 in an automatic hot air oven MEMMERT100-800; the content of ash was determined at 550ºC in a muffle furnace HT40AL according to CEN/TS 15403; automatic calorimeter LAGET MS-10A with accessories was used for the determination of the calorific value, according to CEN/TS 15400; the briquetting was carried out by hydraulic piston briquetting press BrikStar model 50-12 and pelleting - by the equipment developed in the Institute of Agricultural Technique „Mecagro"; the mean compressed (specific) density of the briquettes and pellets was determined immediately after removal from the mould as a ratio of measured mass over calculated volume.

Figure 1 ***M. x giganteus*: A – intense vegetative growth development; B – the end of the growing season**

RESULTS AND DISCUSSIONS

The identification of the efficiency values of the morpho-physiological processes in the system of the realized experimental entity includes a set of conditions and processes correlated with the environmental factors, which maintain the ontogenetic susceptibility (diurnal, seasonal, annual) of the plants as a whole, dependent on the biotope and the environmental factors. The study carried out has been intended to play a part in the complex research on the peculiarities of ecological-biological and homeostatic adaptation of the species of *M. x giganteus* in comparison with *S. almum (figure 2*) under the environmental conditions of the experimental sector.

M. x giganteus is a perennial plant, adapted to the environmental conditions, which comes out of dormancy in spring, in the last days of April. During this period, starting from the second year of vegetation, shoots with leaves twisted into a tube, brown at the base, emerge at the soil surface. In the next 8-10 days, the plants only grow in height (15-25 cm), and then the leaves acquire their normal morphological appearance – linear leaf blade. In the vegetative stages of development, the plants grow fast. They accumulate green mass throughout the growing season, which gives the plant high potential due to the extensive leaf surface (*figure 1 A*). At the end of May, they can reach 100-120 cm in height, with 6-9 long leaves. Under the climatic conditions of our country, the plants start the generative phase but are not able to complete it. They develop inflorescences from the end of September, but do not produce seeds. The growing season ends when the leaves and stems turn brown and dehydrate, thus, the senescence stage occurs in November (*figure 1 B*).

Figure 2 **Plants of *S. almum* in the collection of NBGI: A – vegetative phases; B – generative phases**

Physiological-biochemical processes are facilitated by the factors responsible for photosynthesis and the water regime in the investigated species (phenological stage, age, leaf position and surface) and are mostly determined by the leaf surface, the amount of effective assimilating pigments, the mobility of physiological water forms, stomatal oscillations, the presence of nutritional elements and their mobility, the activity of enzyme systems in impact with the abiotic environmental conditions, all these factors contribute to the value of the metabolic potential that also determines the productive potential of plants.

The intensity of this process of nutrition with carbon varies mostly depending on the intensity of active photosynthetic radiation within the following limits: values of 231-1783 µmol/$m^{-2}s^{-1}$ were found in 2-year-old plants, established at the average temperature of the leaf of 30.6-31.7°C and the temperature in the chamber of the device for establishing the physiological parameters of 28.5-31.4 °C, at the same time and under equal conditions.

Thus, the values of the intensity of the photosynthetic process obtained during the morning hours are about 0.38-8.68 µmol/$m^{-2}s^{-1}$ in the species *M. x giganteus* as compared with the species *S. almum* (1.19-17.18 µmol m^{-2} s^{-1}), which indicates the high vigour of the plants and the amount of assimilatory pigments in the leaf mesophyll (*Table 1*).

Table 1

Results of establishing the comparative values of the physiological indices of *M. x giganteus* and *S. almum*

Species / Time		Intensity of the photosynthesis (A) $\mu mol\ m^{-2}\ s^{-1}$	Intensity of transpiration (E) $mmol\ m^{-2}\ s^{-1}$	Stomatal conductance to CO_2 (g) $mol\ m^{-2}\ s^{-1}$	Intensity of photosynthetically active radiation (PAR) $\mu mol\ m^{-2}\ s^{-1}$
***M. giganteus* (year 2)**	7:00-9:00	0.38-0.39	0.58-0.83	0.01-0.02	231-257
	9:00-10:00	0.94-0.97	0.57-0.59	0.02	320-385
	10:00-11:00	3.39-8.68	2.02	0.02	1728-1783
***S. almum* (year 2)**	7:00-9:00	1.19-4.64	0.71-1.01	0.02	124-201
	9:00-10:00	4.21-4.69	1.04-1.14	0.01-0.02	480-483
	10:00-11:00	15.77-17.18	1.26-1.84	0.02-0.03	1061-1565

The comparative values of the photosynthetic intensity of the species *M. x giganteus* with those of sorghum plants, show that they are relatively equivalent, because, taking into account the fact that no agrotechnical maintenance procedures were undertaken, the plants grew spontaneously under natural conditions, with no irrigation and no additional fertilizers, but greater deviations were noticed in the sorghum crop (1.19-17.18 µmol/$m^{-2}s^{-1}$), which explained the high tolerance through the more xeromorphic structure of sorghum leaves, with mechanisms of adaptation, established in time and space, to adverse environmental conditions and stress factors (drought and heat), recorded also during the investigation period (*figure 3*).

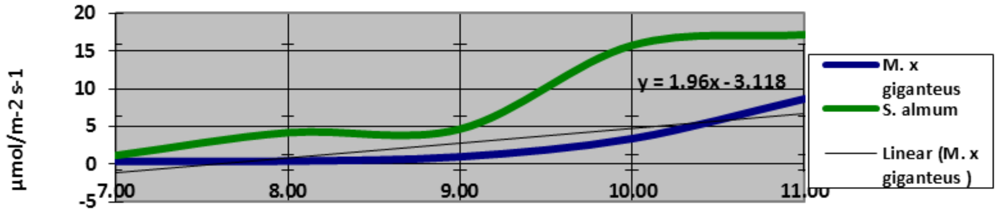

Figure 3 Graphical estimation of comparative values of photosynthesis intensity in plants of *M. x giganteus* and S. *almum* depending on the environmental factors and age (2 years)

The values of the intensity of transpiration under equivalent conditions of 28-32°C, relative air humidity 80-86%, analysed between 7.00 and 11.00 a.m., also varied in the species *M. x giganteus* from 0.58 to 2.02 mmol/$m^{-2}s^{-1}$ (*figure 4*).

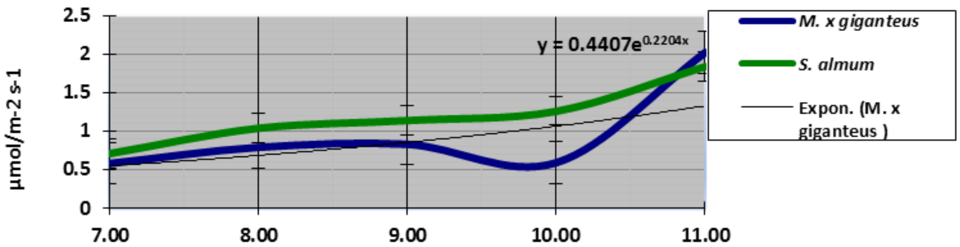

Figure 4 **Graphical estimation of the values of the intensity of the transpiration process in *M. giganteus* and *S. almum* plants depending on environmental factors and age (2 years)**

Other important values established at the same time refer to the indices of ostiolar oscillations of stomatal cells related to the volume of CO_2 mol/$m^{-2}s^{-1}$ exchange, they also oscillated within the limits of 0.01-0.03, where the access of CO_2 in equal volumes was stable regardless of the intensity of transpiration and photosynthesis. These processes are favoured mostly by the intensity of efficient photophosphorylation reaction of C-4 type, which determines the quality of the effective spectrum of light captured and converted, which varied within the limits of 231-1783 µmol/$m^{-2}s^{-1}$ in 2-year-old plants of *M. giganteus*, as compared with *S. almum*, which achieved values of 124-1565 µmol/$m^{-2}s^{-1}$, between 7:00 and 11:00 a.m., motivated by individual mechanisms of the species in starting photoactive and hydroactive reactions to regulate the processes of photosynthesis and transpiration.

The physiological processes of various crops are of interest to specialists, from a bioeconomic point of view. Sandoval Pedroza A. et al., 2017, described these processes in maize, the intensity of photosynthesis being of 27.40-30.00 µmol CO_2 /$m^{-2}s^{-1}$, transpiration 21.78-24.04 mmol $H_2O/m^{-2}s^{-1}$. Other authors mentioned the rate of assimilation in sunflower (26.4 µmol CO_2 /$m^{-2}s^{-1}$) and globe artichoke (23.9 µmol CO_2 /$m^{-2}s^{-1}$) (Archontoulis S. et al., 2012).

It was found that in the second year, the growth and development rates, yield, moisture and leaf share in the harvested biomass of the studied *Poaceae* species differed. The dry mass yield *M. x giganteus* '*Titan'* reached 1.89 kg/m^2 with a 13.2 % content of leaf and panicles, but dry mass yield of *S. almum* 'Argentina' was 1.18 kg/m^2 with a 19.8% leaves and panicles in the harvested biomass. The quality indices of biomass and the obtained solid biofuels are shown in Table 2. The investigation showed that *M. x giganteus* biomass had high gross calorific values (19.20 MJ/kg) and low ash content (1.25%) as compared with *S. almum*. We found that the harvested biomass of the studied *Poaceae* is characterized by an optimal content of carbon (45.23-45.64%), hydrogen (5.76-5.91%) and sulphur (0.05-0.06%), and low content of nitrogen (0.25-0.40 %). The specific density of the obtained solid biofuels from *M. x giganteus* biomass reached 850-1070 kg/m^3 and from *S. almum* biomass 770-970 kg/m^3, respectively, perhaps because of the morpho-anatomical structure of this plant.

Table 2

The dry biomass yield and quality indices of biomass and the obtained solid biofuels

Indices	*Miscanthus giganteus* '*Titan'*	*Sorghum almum* 'Argentina'
Dry biomass yield, kg/m^2	1.89	1.18
Carbon, % DM	45.64	45.23
Hydrogen, % DM	5.91	5.76
Nitrogen, % DM	0.40	0.25
Sulphur, % DM	0.06	0.05
Ash, % DM	1.25	4.40
Gross calorific values, MJ/ kg DM	19.20	18.99
Net calorific values, MJ/ kg DM	17.45	17.30
Specific density of briquettes, kg/m^3	850	770
Specific density of pellets, kg/m^3	1070	970

Some authors mentioned various findings about the energy biomass quality of the *Miscanthus* and *Sorghum* species. Stolarski M.J. et al. (2014) stated that the *M. x giganteus* harvested in April contained 23.23% moisture, 1.90% ash, 50.43 % C, 5.84 % H, 0.028% S, 19.18 MJ/kg HHV, 14.16MJ/kg LHV, but *M. sinensis* respectivly 18.87% moisture, 2.26% ash, 48.59 % C, 5.83 % H, 0.034% S, 19.14 MJ/kg HHV, 15.05 MJ/kg LHV. Heuze V. et al. (2015) noted that the gross calorific value of *S. almum* forage reached 17.8 MJ/kg. Ivanova T. et al. (2018) mentioned that the sweet sorghum biomass contained 7.7% moisture, 3.9% ash, 70.8% volatile materials, 43.1 % C, 5.27 % H, 0.61% N, 0.04% S, 0.09% Cl, 37.3% O, 18.9 MJ/kg HHV, 17.7MJ/kg LHV, the briquettes had an average density of 617.5 kg/m^3 and 90.5% mechanical durability. Țîței V., Roșca I. (2021) reported that *S. almum* milled biomass had bulk density 89-133 kg/m^3, ash content 3.71% and gross calorific value 18.6 MJ/kg, but *M. x giganteus* milled biomass: 138-167kg/m^3, 1.90-2.51% and 18.7-20.0 MJ/kg, respectively. Voca N. et al. (2021) reported that *M. x giganteus* biomass contained 2.01% ash, 12.14% coke, 10.14% fixed carbon, 81.6% volatile matters, 50.20% lignocellulose, 24.87% hemicelulose, 13.89 % lignin, 51.52 %C, 6.09% H, 0.18% N, 0.07 % S, 42.14%O, 17.64MJ/kg HHV and 16.31MJ/kg LHV. Daraduda N., Marian G. (2022) remarked that harvested in spring *M. sinensis* contained 81.0% DM, 2.21% ash, 83.48% volatile matters, 49.01 %C, 5.23% H, 0.27% N, 0.03 % S, 0.03% Cl, 43.02% O, 19.29MJ/kg HHV and 18.14MJ/kg LHV, but *M. x giganteus* biomass: 81.6% DM, 1.19% ash, 83.65% volatile matters, 49.07 %C, 5.86% H, 0.47% N, 0.04 % S, 0.04% Cl, 43.38% O, 19.69MJ/kg HHV and 18.41MJ/kg LHV.

CONCLUSIONS

The results of the investigations carried out to establish the activity and intensity indices of the photosynthetic process in correlation with the intensity of transpiration, respiration and the intensity of photosynthetically active radiation (PAR), determined comparatively in two-year-old plants of the species *Miscanthus x giganteus* and *Sorgum almum*, revealed significant values and their efficiency in periods of maximum vegetative activity with biomass accumulation and estimated productivity. The analyses made by us revealed the efficiency of the conversion of light energy, in the researched species, compared to the intensity of sunlight: it reached values of 231-1783 µmol/m^{-2}s^{-1} in 2-year-old plants of *M. x giganteus* and 124-1565 µmol/m^{-2}s^{-1} in *S. almum*, under equal conditions. The indices of photosynthesis intensity of *M. x giganteus* (3.39-8.68^{2} µmol m^{-} s^{-1}) varied in correlation with the intensity of transpiration (0.59-2.02 µmol m^{-2} s^{-1}), respiration (0.02 mol m^{-2} s^{-1}) and the efficiency of photosynthetically active radiation (PAR) (320-385. µmol m^{-2} s^{-1}). Comparative values of the resulting indices were established simultaneously for the species *S. almum*, with the values estimated in the process of photosynthesis (15.77-17.18 µmol m^{-2} s^{-1}), the intensity of transpiration (1.26-1.84 µmol m^{-2} s^{-1}). Both species possess mechanisms of adaptation to pedological drought and resistance to some abiotic stress factors, under the environmental conditions of the Republic of Moldova.

The biochemical research has demonstrated that the stem dry biomass yield in the second growing season reached 1.18-1.89 kg/m^2, with a content of 45.23-45.64% carbon, 5.76-5.91% hydrogen, 0.25-0.40% nitrogen, 0.05-0.06% sulphur, 1.25-4.40% ash, 18.99-19.20 MJ/kg gross calorific value and 17.30-17.45 MJ/kg net calorific value. The specific density of briquettes reached 770-850 kg/m^3 and the specific density of pellets 970-1070 kg/m^3. The local cultivars of *M. x giganteus* and *S. almum* may serve as feedstock for renewable energy production.

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