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Characteristics of reproductive organs of *Miscanthus sinensis* anderss., *Miscanthus sacchariflorus* (Maxim.) Hack, *Miscanthus x giganteus* j.m.greef denter ex hodk., renvoize

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Abstract
The morphometric parameters of the panicle of the initial breeding material of three species of the genus Miscanthus were determined. Panicle length of *Miscanthus sacchariflorus* and *Miscanthus sinensis* is 18-20 cm, width is 8-11 cm, and for *Miscanthus x giganteus* it is 20-23 cm and 14-16 cm, respectively. Miscanthus is a monoecious plant; its flower bears both the pistil and the stamen. The color of anthers varies from yellow to pinkish-yellow. Anther tissues are composed of elongated cells 70-100 µm long. The pistil has an ovary with two styles, which bear long feathery stigmas. The stigma length is 2.0-2.8 mm. The stigma varies in color from white to bright pink. The shape of stigma feathery is moderately branched. The number of small branches is 10-15. The location is alternate. The length of the pistil feathers is 160-300 microns; the width is 20-30 microns. The pollen of various Miscanthus species differs in qualitative and quantitative characteristics as size, homo- or heterogeneity. Thus, the pollen of *Miscanthus sacchariflorus* and *Miscanthus sinensis* is characterized by a rounded shape, evenness and uniformity, 43-48 µm in diameter, while the pollen of *Miscanthus x giganteus* is more heterogeneous in size, its diameter is 23-45 µm. The pollen has one rounded ornamented pore with a diameter of 2.7-4.0 microns.

Keywords: stigma of pistil, pollen, cytology, anther tissues

Introduction

Miscanthus is a genus of highly productive perennial creeping stem grasses, belongs to the “plants the founders” of the world biofuel industry, as an unconventional renewable energy source (Hastings *et al.* (2009) [8], Baybakova (2015), Clifton-Brown *et al.* (2015) [2], Clifton-Brown *et al.* (2017) [3], Robson *et al.* (2020) [18]. Today, miscanthus is also considered from the standpoint of global ecology as a culture that inhibits soil erosion, as well as an effective absorbent of carbon dioxide, which reduces the greenhouse effect. For the chemical industry, it is a promising source of plant raw materials for the production of lignin-cellulosic biomass and composite materials, substitutes for wood and plastic (Shumnyiy *et al.* (2010a) [21], Shumnyiy *et al.* (2010b), Gismatulina *et al.* (2017) [5], Clifton-Brown *et al.* (2019) [14]. Miscanthus is also considered as a promising bio-economic crop due to combination of its high productivity and environmental performance, multi-vector use of biomass and possibility of growing on economically marginal lands, which are usually characterized by abiotic stresses (drought, floods, rockiness, steep slope, wind influence), insufficient amount of nutrients and soils contaminated with heavy metals and other pollutants (Tóth *et al.* (2016) [25]. Usually these lands are not suitable for food production, what allows to use a large global resource of underutilized marginal lands to massively increase the production and use of biomass and remove carbon dioxide from the atmosphere (Lewandowski *et al.* (2016) [14], Wagner *et al.* (2019) [28]. Miscanthus is a representative of the tribe Andropogoneae (grasses and sedges), distributed in countries of East Asia, in particular, Japan, China, India, West Africa, Australia, South America and Eastern Russia. Giant miscanthus (*Miscanthus giganthus* J.M.Greef Denter ex Hodkinson Renvoise *(M. Giganthus))*, a natural allotriploid (2n = 3x = 57), hybrid between Amur silvergrass (*Miscanthus sacchariflor* (Maxim.) Hack (*M. Sacchariflorus*)) (2n = 4x = 76) tetraploid and Chinese miscanthus (*Miscanthus sinensis* Anderss. *(M. Sinensis))* (2n = 2x = 38) diploid considered to be the most promising among a large number of miscanthus species. Miscanthus is a perennial grass with C_4 photosynthesis that provides the highest efficiency in the transformation of solar energy into biomass. Furthermore, these plants differ advantageously from the most other C4 plants in their tolerance to cold and thermolability of the key enzymes of photosynthesis-Rubisco and Pyruvate orthophosphate dikinase, which allows maintaining a high level of photosynthesis with a decrease in temperature, in contrast to other C4 plants, in particular corn, in which the enzyme activity decreases with temperature reduction (Syvash (2019). Commercial Giant miscanthus is a sterile triploid hybrid; its seeds were collected from sympatric populations of Amur silvergrass and Chinese miscanthus on the island of Hokkaido (Japan). Only one Japanese clone of Giant miscanthus, which is propagated by rhizome division and *in vitro* - has become widespread in the world. (Slusarkiewicz-Jarzina *et al.* (2017) [23], Ings *et al.* (2013) [12]. The expansion of this clone is limited by low reproduction rate, sensitivity to drought and other stressors (Hastings *et al.* (2017) [9], high production costs for starting plantations for *M. Giganteus* (Hastings *et al.* (2019) [10]. UK breeders believe these limitations can be overcome by producing more robust seed-propagated miscanthus hybrids; and natural diversity of plants found in various environments throughout East Asia could provide breeders with the genetic resources to increase productivity and resilience across a wide range of agro-climatic conditions and unfavorable abiotic loads (Hodkinson *et al.* (2016) [11].

In the UK, Germany and other European countries, Miscanthus breeding strategy focuses on the use and selection of wild types, parental forms and offspring to create promising new hybrids *M. sinensis* × *M. Sacchariflorus* with high yields, resistance to cold, drought and other stress factors and production seeds (Malinowska *et al.* (2017) [15], Clifton-Brown *et al.* (2017) [3], Clifton-Brown *et al.* (2019) [14]. To solve these problems, studies on adaptive breeding are being carried out; in particular, experiments with phenomics on drought tolerance were carried out on wild and improved germplasm (van der Weijde *et al.* (2017) [26], Vergun *et al.* (2017) [27]. It is known that one of the reproductive barriers within sympatric populations of plants of the Miscanthus genus, as well as a significant obstacle to hybridization, is the asynchrony of the flowering periods of these components, the lack or absence of information in the world scientific literature on the morphology, cytology of pollen, pistils, features of Miscanthus pollination and obtaining hybrid seeds. Possession of this knowledge will make it more likely to determine the genetic value of the breeding material, improve its fertility and successfully hybridize the components. The aim of the research is to analyze the morphometric characteristics of reproductive organs (anthers, pollen, pistils) of the initial breeding material of representatives of the genus Miscanthus obtained *in vitro.*

Materials and Methods

The experiments were carried out at the Institute of Institute of Bioenergy Crops and Sugar Beet, NAAS of Ukraine during 2014-2019. In the studies the reproductive organs - anthers, pollen, pistils of *M. Sinensis, M. Sacchariflorus, M. Giganteus* were used. Plants of these miscanthus species were obtained and propagated *in vitro.* We used seeds of *M. Sinensis* from the "Jelitto" firm collected in 2012, *M. Sacchariflorus* seeds of Russian breeding, *M. Sacchariflorus* clones from Belgium and rhizomes of *M. Giganteus*. For plant propagation, sterile seeds of *M. Sinensis*, *M. Sacchariflorus* and buds from *M. Giganteus* rhizomes were planted *in vitro* on a modified Murashige-Skoog medium. Plants of *M. Sinensis* and *M. Sacchariflorus* were obtained *in vitro* by indirect morphogenesis (Gontarenko and Lashuk (2017a) [6], *M. Giganteus* - by micropropagation. Shoots from morphogenic calli of *M. Sinensis*, *M. Sacchariflorus* and clones of *M. Giganteus* 2–3 cm in height were separated and transplanted into a medium for propagation and rhizome growth stimulation. After the formation of 10–15 cm long rhizomes, microplants were planted in open ground using plastic flasks for adaptation, which were removed 6–8 days after plant transferring from *in vitro* to *ex vitro* (Gontarenko and Lashuk (2017b) [7]. Plant material – reproductive organs (anthers, pollen, pistils) of *M. Sinensis*, *M. Sacchariflorus*, *M. Giganteus* were selected in the flowering phase in the 4th–5th year of growing in open ground. To carry out cytological studies using light microscopy, temporary preparations of the pistils, unfertilized ovule, anthers and pollen, unstained or stained with 2% carmine solution in 45% acetic acid or methylene blue solution, were used (Pausheva (1988) [16]. For all miscanthus species, measurements were made and the amount of pollen of various diameters was counted in tenfold repetition.

Results

According to the research results, the beginning of the growing season of miscanthus in the conditions of the Kiev region occurs at the end of April - beginning of May. The flowering phase (panicle appearance phase) in representatives of different species of the Miscanthus family occurs 100-135 days after the beginning of the growing season - late July-early August (Lashuk (2019) [13], Rakhmetov *et al.* (2015) [17]. According to observations, *M. Sacchariflorus* starts to bloom first (last decade of July). *M. Sinensis* blooms in the second decade of August., *M. Giganteus* starts to bloom (early October) later than the rest species, however, in some years (unfavorable climatic conditions), the giant miscanthus may not form the panicle at all. The panicle in plants of the *Miscanthus* family differs significantly in shape and morphometric parameters: in length, width, number of branches in panicles and their length. The panicle can be spindle-shaped, conical or elliptical (Zinchenko *et al.* (2016) [29], Roik *et al.* (2016a) [19], Roik *et al.* (2016b) [20]. The panicle length varies from 15 to 30 cm, the width is 6-15 cm. The flower consists of two lodicules (floral scales or membranes), a sitting ovary with two racemose stigmas on long columns and three stamens. There are long silky hairs at the base of the lodicule. The lower lodicule is distinguished by a geniculate awn up to 1.5 cm long. According to the results of morphometric studies, the panicle length of Amur silvergrass and Chinese miscanthus was within 18-20 cm, and for Giant miscanthus it varied from 20 to 23 cm. The width of Chinese miscanthus can reach 8-9 cm, Amur silvergrass – 9-11 cm, giant miscanthus – 14-16 cm (Figure 1).

Fig 1: Inflorescences of various miscanthus species

1). Fan-shaped panicles of *M. Sacchariflorus* from *in vitro*; 2). Fan-shaped panicles of *M. Sinensis* from *in vitro*; 3). Fan-shaped panicles of *M. Giganteus* from *in vitro*; 4). Fan of *M. Giganteus* from rhizomes The miscanthus flower bears both stamens and the pistil. The stamens have long filaments and oblong anthers (Figure 2, 3). The anthers of Chinese miscanthus and Giant miscanthus are light yellow, yellow or pinky-yellow.

The color of the anther tissue of Amur silvergrass is predominantly pink-yellow. Anther tissues are composed of elongated cells (Figure 4), their length is about 70-100 µm in Amur silvergrass.

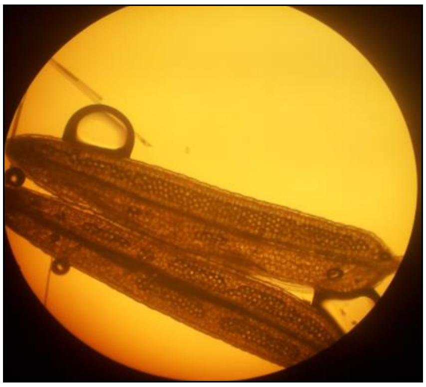

Fig 2: The anther of *M. sacchariflorus*

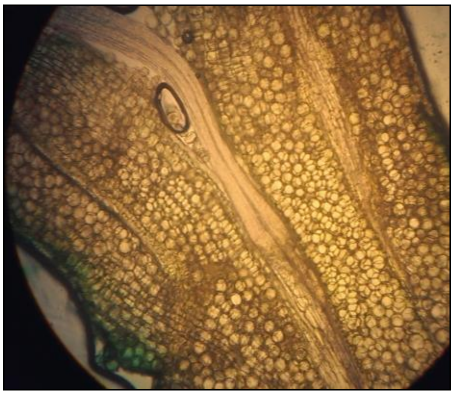

Fig 3: The anther of miscanthus with pollen

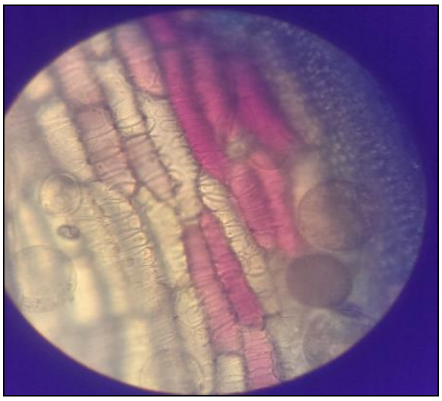

Fig 4: Anther tissues of *M. sacchariflorus*

Miscanthus pistil consists of an ovary with two columns, which bear long branched feathery stigmas. The stigma length is 2.0-2.8 mm. In Giant miscanthus and Chinese miscanthus, stigma color varies from white to pink (Figure 5-8), while in Amur silvergrass stigmas are bright pink.

Fig 5: *M. sacchariflorus* pistil (general view)

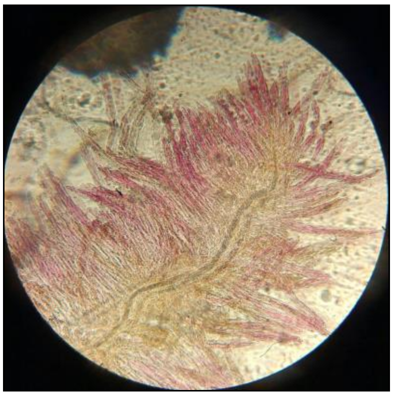

Fig 6: Immature stigma of *M. giganteus* pistil

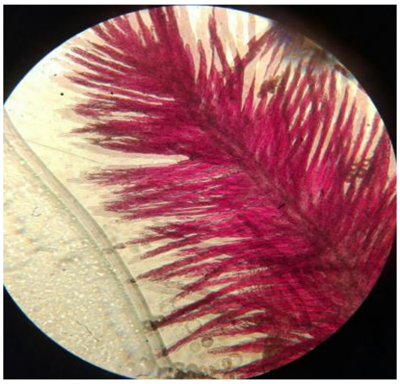

Fig 7: The stigma of the *M. sacchariflorus pistil*

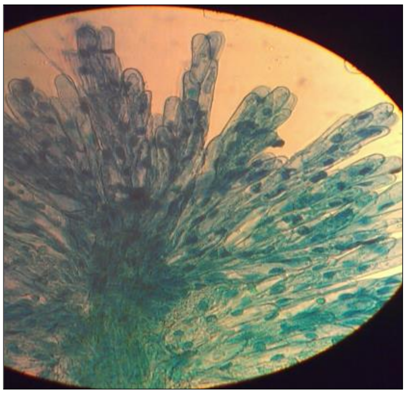

Fig 8: The top of the pistil of *M. Giganteus* stigma

A similar shape of the stigma and the variation in pigmentation of mature stigmas of *M. Sinensis* from pink to purple was observed in studies of gametophyte by American scientists. The feathers are moderately branched. The number of small branches can be up to 10-15 pcs. The arrangement is alternate (Figure 9, 10).

Fig 9: Feather of the stigma of *M. sinensis* pistil

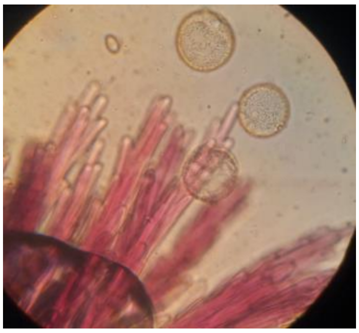

Fig 10: Pollination of *M. sacchariflorus*

The length of the pistil feather varies depending on their location. At the end of the pistil, the length is 160-200 microns, in the middle and at the base - 270-300 microns, its width - 20-30 microns. According to the data of cytological analysis of pollen, it was found that pollen of various miscanthus species differs in qualitative and quantitative characteristics (size, homo- or heterogeneity). So, the pollen of Amur silvergrass and Chinese miscanthus is characterized by rounded shapes, evenness and almost uniformity of sizes - 43-48 microns in diameter (Figure 11, 12), while the pollen of Giant miscanthus is more heterogeneous, varies in size, the diameter is 23-45 microns, but the number of small microspores is small – 5-10% of the total in the field of view (Figure 13).

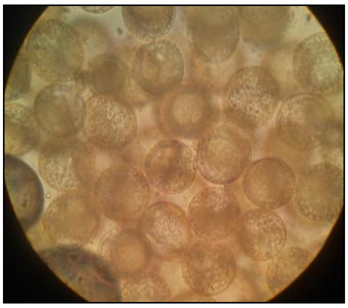

Fig 11: Pollen of *M. sinensis*

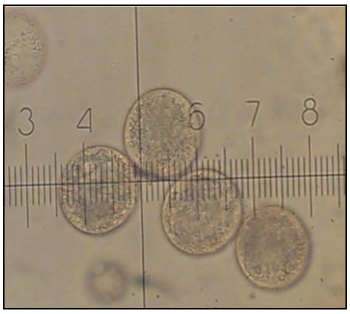

Fig 12: Pollen of *M. Sacchariflorus*

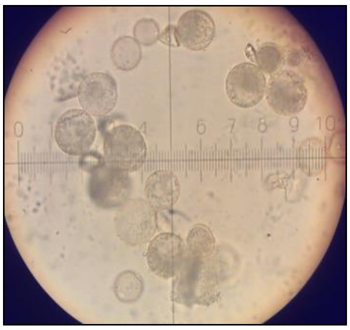

Fig 13: Pollen of *M. Giganteus*

According to literature sources, a wide range of sizes of Giant miscanthus pollen is associated with different levels of its ploidy (Żur *et al.* (2013) [30] (Figure 14). The pollen grain has one rounded ornamented pore with an inner diameter of 2.7-4.0 µm.

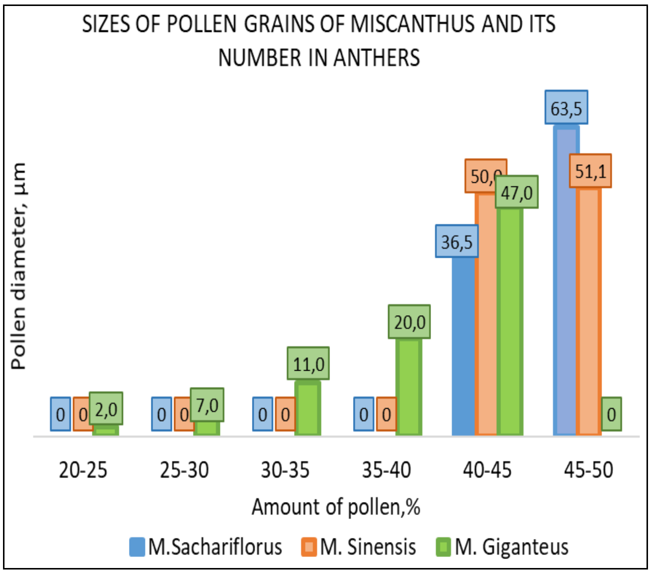

Fig 14: Sizes of pollen grains of *M. sinensis, M. saccharifloru*s, *M. giganteus*, µm

Morpho-biometric features of miscanthus reproductive organs – pollen and stigma – are important biological characteristics of the species, variety, breeding material. The effectiveness of pollination of the flower and formation of high-quality seeds depend on the quality of pollen and its quantity. The obtained data make it possible to assess the morphometric parameters of the reproductive organs of three miscanthus species *M. Sinensis*, *M. Sacchariflorus*, *M. Giganteus* - the size and branching of the stigma of the pistil, the size and heterogeneity of pollen, which should be taken into account in miscanthus breeding. The study of reproductive organs is of considerable interest not only from the perspective of fundamental biology, but also from an applied aspect - in the practical selection of such a highly important energy and technical culture as miscanthus.

Conclusions

Based on the results of cytological studies, morphological and cytological characteristics of the reproductive organs – pistils, anthers, pollen – of the breeding material of three species *Miscanthus sinensis Andersson*, *Miscanthus sacchariflorus (Maxim.) Hack*, *Miscanthus x giganteus J.M.Greef Denter ex Hodk.*, *Renvoize* propagated *in vitro* have been given. The results of these studies should be taken into account in further breeding work to obtain diploid and triploid miscanthus hybrids.

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