

Formation of Generative Organs of Switch-Grass (*Panicum virgatum* L.) Depending on Cultivation Conditions

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ABSTRACT

The paper covers the issues of the formation of the generative organs of switch-grass plants (*Panicum Virgatum* L.) in the correlation with the varietal features and the weather conditions in an inter-phase period of “flowering-seed formation”. Switch-grass is one of the most promising perennial cereal plants to produce bio-fuel. Widespread introduction into production is held back by low seed emergence, which is due to its large dormancy state. Therefore, to study the causes of this phenomenon and the ways to reduce it is of a real relevance. In the vegetation years, the size of pollen grains ranged from 22.9 to 23.6 mkm depending on the varietal features and the weather conditions in a phase of flowering and pollen formation. A significant difference in a pollen size in a correlation with the varietal features was not recorded; the tendency towards the increase or decrease of its size was seen though. In the years under study, the sizes of pollen grains changed under the effect of weather conditions, it occurred even within one cultivar. The pollen of all the cultivar samples had the smallest size in 2018 and 2019; and in the vegetation years of 2020-2021 the average pollen sizes were much larger for all cultivars than in 2018 and 2019. Over the years under study, on the average, no significant difference in the sizes of embryo and seed, depending on the varietal features, was recorded. The growing weather conditions had a significant effect on the sizes of embryo and seeds, their length and width, and it amounted to 98-100%. The most favorable weather conditions for the processes of flowering and seed formation were created in 2019, as compared with those in 2020 and 2021, which ensured the formation of larger sizes of both embryos and seed in all the cultivars. The correlation-regression analysis of the data showed a strong linear correlation between the length and the width of embryo and the weather conditions in a phase “flowering-formation” of seed with a determination coefficient $R^2 = 0.9163$ and a correlation coefficient $R = 0.96$ for the length of embryo and $R^2 = 0.9613$ and a correlation coefficient $R = 0.98$ for the width of embryo.

Keywords: seeds, pollen, embryo, endosperm, sample cultivar.

INTRODUCTION

Nowadays the mankind faces the issue of a paramount importance: the rational use of the fuel reserves and the decrease of the effect of greenhouse gases on the environment. Scientists estimated the restraints in climate changes and how to keep it at a safe level which would prevent from the threats for the ecosystem existence; in XXI century it is advisable to use only

a quarter of all the volume of fossil fuel which is considered to be economically profitable for its consumption [Law of Ukraine, 1992]. In recent years the number of energy carriers – oil products and natural gas – is decreasing rapidly both in the world and in Ukraine. In view of the deficit of these energy carriers and a serious increase of their price, more and more attention is paid to the search and production of the alternative energy sources which can reduce the dependence of the

countries on the traditional kinds of fuel [Doronin et al., 2018], with a minimal effect on the environment and the risk of techno-genic catastrophes [Horb et al., 2017]. Alternative energy sources have a long and successful history of their consumption in several European countries (Austria, Denmark, the Netherlands, Norway, Finland and Sweden), their share ranges from 40 to 65%, and it is planned to have 100% of ecologically pure bio-energy [Taran et al., 2011].

One of the most promising perennial cereal plants to produce bio-fuel is switch-grass (*Panicum virgatum* L.). The main advantages of switch-grass, as a bio-energy crop, are its high yield capacity, a low need in water and nutrition, a reliable productivity in a wide geographical area, the reduced soil erosion, the carbon absorption and the improvement of the environment of the wild nature existence [Yogendra et al., 2012]. According to Monti [2016], it is possible to receive from 5 to 12 t of conditional fuel per hectare of this crop.

The switchgrass can be propagated by seeds and rhizomes, but the most favorable way is by seeds [Elbersen et al., 2001; Beaty et al., 1978]. A characteristic feature of seeds is a long biological dormancy period, which leads to uneven, thin emergence in the field conditions and, accordingly, to a decrease in crop productivity. The low emergence of seeds, which is caused by a large state of dormancy, is one of the main limiting factors for the widespread introduction of the switch-grass into production.

The yield capacity and quality of switch-grass seed depend on the number and quality of pollen grains, which is determined by the weather conditions during their formation and the processes of pollination and fertilization. Pollination is the transfer of pollen from the stamens to the pistil. If the pollen from the stamens is transferred to its pistil, then self-pollination occurs, if the flower of the same plant or another plant is transferred to the pistil of another plant, then cross-pollination occurs. The switchgrass cross-fertilization is usually only 0.01–0.5% and only in some cases it can reach 5% [Elbersen et al., 1978]. Pollen is mainly carried by the wind.

According to V.M. Tyshchenko and others [Tyshchenko etc., 2019], the switch-grass fertilization process is completed at an air temperature of about 22–23 °C – 2–2.5 hours after pollination, and at a temperature of about 20 °C – after 4–4.5 hours. Embryogenesis processes of the fertilized cells of the embryo sac begin

4–8 hours after fertilization. 10–12 days after pollination, the switch-grass embryo is capable of germination under the suitable conditions, although the endosperm is not formed yet. The grain filling continues from 18 to 24 days after fertilization. The period of the post-harvest ripening of switch-grass seed lasts 12–18 days.

During the growing season the weather conditions have a significant influence on the switch-grass growth and development, along with the cultivation place – the latitude of the origin, the reduction of seed dormancy and the technology elements [Bransby et al., 1997]. The hybridization efficiency largely depends on the pollen grain quality, which affects the seed productivity and is an indicator of the adaptation to the cultivation climatic conditions. The plants which produce the high-quality pollen and seeds are better adapted to new conditions [Nekrasov et al., 1982]. Therefore, it is important to investigate the peculiarities of the gametophyte formation of switchgrass plants (pollen and embryo) in the conditions of unstable moisture in the Right bank Forest steppe zone of Ukraine, depending on the cultivar features and the weather conditions during a phase of growth and development “flowering-seed formation”.

RESEARCH CONDITIONS AND METHODS

The research program implied the studies of the peculiarities of the gametophyte formation – female (embryo) and male (pollen) of switch-grass in relation to the vegetation conditions and a varietal composition. In the years of 2018–2021 the research with four cultivar samples of switch-grass of different ripening groups, different origin and ploidity was carried out at the Institute of bio-energy crops and sugar beets of NAAS: tetraploid cultivar – Morozko (Ukraine), octaploid cultivar sample Cave-in-rock (USA) and tetraploid ones: average ripening cultivar Sunburst and average-late cultivar Alamo (USA).

The pollen was taken in the period of mass flowering from 11 to 1 o'clock (AM). The analysis of the pollen was made on this very day. To determine seed and embryo sizes as well as their correlation, seeds were taken on the 28th day after the beginning of flowering. Pollen sizes were measured using the methodology/technique of H.I. Yarmoliuk and E.I. Shyriaieva [Yarmoliuk etc., 1982]. The statistical processing of the experimental data was made with help of a disperse

analysis by Fisher's method [Fisher etc., 2006] using computer software Statistica 6.0 from StatSoft [The site of the company *StatSoft*].

RESULTS AND DISCUSSION

The formation of quality seeds depends on several factors, and first of all on the processes of pollination and fertilization and the pollen quality, which is predetermined by the varietal features and the vegetation conditions in the pollination period. Sizes and viability predetermine the quality of pollen. The larger the number of viable pollen grains, the higher seed emergence is.

The pollen quality depends, to a great extent, on both internal and external factors, in particular on weather conditions of the vegetation period [Rodionenko, 1970]. In the vegetation years of 2018–2021, on the average, the size of pollen grains ranged from 22.9 to 23.6 mkm depending on the varietal features and the weather conditions in a phase of flowering and pollen formation. A significant difference in a pollen size in a correlation with the varietal features was not recorded; the tendency towards the increase or decrease of its size was seen though. Cultivar sample Sunburst (23.6 mkm) showed the largest pollen sizes, the pollen of cultivar Morozko and cultivar sample Cave-in-rock was the same – 23.4 mkm, cultivar sample Alamo showed the smallest pollen – 22.9 mkm.

In the years under study, the sizes of pollen grains changed under the effect of the weather conditions, it occurred even within one cultivar. The pollen of all the cultivar samples had the smallest size in 2018 and 2019. In these years, cultivar Morozko had the largest pollen – 17.3 and 18.0 mkm, respectively, the smallest pollen – 15.1 and 14.9 mkm – was typical for cultivar samples Sunburst and Alamo, respectively. In the vegetation years of 2020–2021 the average pollen sizes were much larger for all the cultivars than in 2018 and 2019. In 2020 the pollen sizes of cultivar Morozko and those of cultivar sample Cave-in-rock were almost the same and smaller as compared with the pollen sizes of cultivar samples Sunburst and Alamo. In 2021 the largest pollen (35 mkm) was formed in cultivar sample Sunburst, the smallest one (30.9 mkm) was formed in cultivar Morozko.

The variation of the pollen sizes by the years of vegetation is predetermined by the weather

conditions. Thus, in 2018–2020 the average daily and maximal air temperatures in the phase of mass flowering (in July) were almost the same – 22.4–25.3 and 29–30 °C, respectively; in 2021 these indicators were much higher and they amounted to 29 and 34 °C, respectively, this resulted in a faster/shorter phase of flowering which was over at the end of July.

The analysis of the factors which affected the size of pollen grains showed that the weather conditions of the vegetation period in the years when the research was carried out had the highest effect – 89.3%.

The quality of seeds – germination energy and emergence – depends on a size of embryo and the availability of nutrient substances (endosperm), required for the growth and development of embryo and sprout (Fig. 1.)

It was established that the size of a seed embryo of switch-grass changed in a correlation with the varietal features, but no significant difference by this indicator in the cultivar samples was recorded (Table 1)

In the years under study, on the average, a significant difference in the sizes of embryo and seed depending on the varietal features was not recorded. However, there was a change of these sizes depending on the cultivars. For instance, cultivar Morozko had the highest length of embryo – 48.2 mkm; cultivar sample Cave-in-rock had the shortest length, namely 44.4 mkm. The width of embryo also changed in a correlation with the cultivars: it was the largest for cultivar

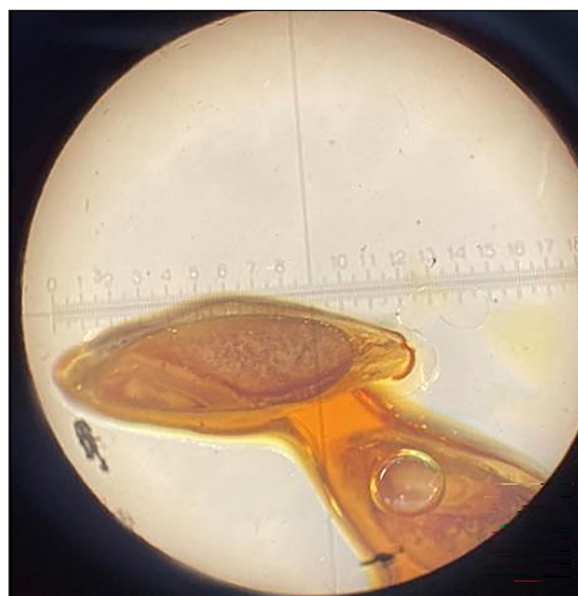


Figure 1. Section of switch-grass seed and embryo under the microscope

Table 1. Seed and embryo sizes and their correlation depending on varietal features of switch-grass (the average in 2019–2021)

Cultivar, cultivar sample	Length, mkm			Width, mkm		
	Seed	Embryo	Their correlation	Seed	Embryo	Their correlation
Cave-in-rock	90.2	44.4	2.3	33.0	21.9	1.50
Morozko	90.0	48.2	1.87	35.6	23.3	1.53
Alamo	90.4	47.0	1.92	34.3	23.1	1.48
LSD _{0.05 tot.}	7.8	6.2		9.2	5.5	
LSD _{0.05 cultivar}	4.5	3.6		5.3	3.2	

Morozko and cultivar sample Alamo – 23.3 and 23.1 mkm, respectively; the smallest width – 21.9 mkm was for Cave-in-rock. The correlation between the embryo sizes and the seed sizes showed that the embryo of the smallest sizes was formed in cultivar sample Cave-in-rock and it was almost of the same size for cultivar Morozko and cultivar sample Alamo. The weather conditions of the cultivation had the highest effect on the sizes of embryo and seed, their length and width, and it ranged from 98 to 100%.

The sizes of embryo and seed depended greatly on the weather conditions during flowering and their formation. The largest sizes (length and width) of seed and embryo were formed by all the cultivars in the vegetation year of 2019. These indicators were significantly smaller in 2020 and 2021 (Table 2).

In 2019 the weather conditions in an inter-phase period of “flowering-seed formation”, i.e., 28 days from the beginning of flowering, were favourable for the processes of fertilization and growth and the development of embryo and seed. In this period the daily air temperature was 24 °C

(Fig. 2), the moisture deficit was low (15 mm) and it was distributed evenly during 10 days (Fig. 3); all this created more favourable conditions for the processes of flowering, fertilization and seed formation than in 2020 and 2021, which contributed to the formation of larger sizes of both embryo and seed in all the cultivars.

In the vegetation year of 2020 a phase of flowering and seed formation took place in less favourable weather conditions: the average daily air temperature in a phase of flowering-seed formation was higher, namely 25.2 °C, a serious moisture deficit was recorded (41 mm), the precipitation occurred only 2 days, and 22 days were sunny, which led to the formation of the smaller sizes of embryo and seed. A significant difference in the sizes of embryo and seed depending on the varietal features was not recorded. The analysis of the correlation between the length and the width of embryo and the sizes of seed showed that in the conditions of 2020 cultivar Morozko formed the largest embryo and cultivar sample Alamo formed the smallest one.

Table 2. Seed and embryo sizes and their correlation depending on varietal features of switch-grass (2019-2021)

Year (factor A)	Cultivar sample (factor B)	Length, mkm			Width, mkm		
		Seed	Embryo	Their correlation	Seed	Embryo	Their correlation
2019	Cave-in-rock	161.2	62.5	2.58	56.7	30.9	1.83
	Morozko	162.3	72.8	2.23	63.1	32.9	1.92
	Alamo	161.7	69.6	2.32	60.1	35.5	1.69
2020	Cave-in-rock	79.6	47.2	1.69	27.5	23.3	1.18
	Morozko	78.0	47.2	1.65	29.3	26.0	1.13
	Alamo	78.7	47.5	1.66	28.5	23.4	1.22
2021	Cave-in-rock	29.8	23.6	1.26	14.8	11.6	1.28
	Morozko	29.8	24.5	1.22	14.3	11.0	1.31
	Alamo	30.7	23.8	1.29	14.2	10.4	1.37
LSD _{0.05 tot.}		7.8	6.2	-	9.2	5.5	-
LSD _{0.05 year conditions}		4.5	3.6	-	5.3	3.2	-
LSD _{0.05 cultivar}		4.5	3.6	-	5.3	3.2	-

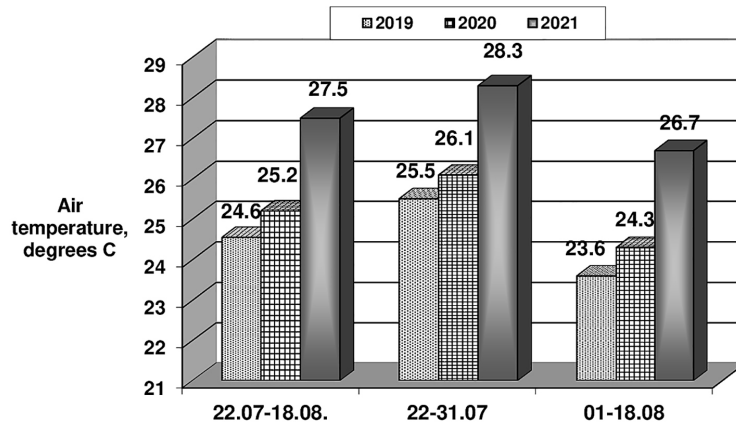


Figure 2. Air temperature in a phase of flowering-seed formation (28 days after the beginning of flowering)

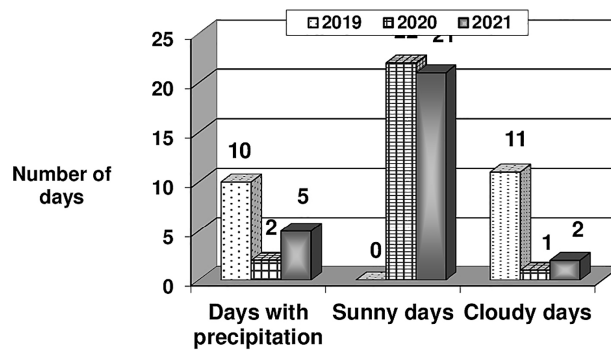


Figure 3. Weather conditions in a phase of flowering-seed formation (28 days after the beginning of flowering)

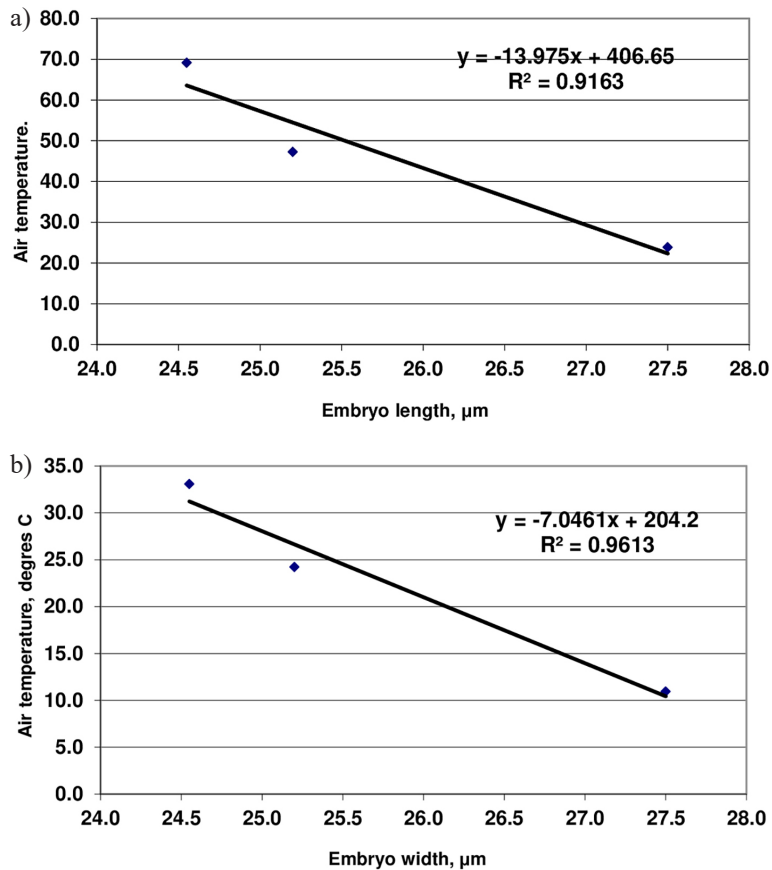


Figure 4. Dependence of embryo length and width on the weather conditions of its formation

In 2021 there were the least favourable conditions for the formation of embryo and seed. The average daily air temperature in a phase of flowering-seed formation was much higher, as compared with the previous years, and it was equal to 27.5 °C, the moisture deficit amounted to 25 mm; all this resulted in the formation of much smaller sizes of both embryo and seed in all the cultivar samples. No significant difference by these sizes depending on the varietal features was recorded.

As far as the effect of the varietal features on the formation of embryo and seed is concerned, it is to be stated that cultivar sample Cave-in-rock has had a significantly smaller length of embryo, as compared with that of cultivar Morozko and cultivar sample Alamo. The analysis of the correlation between the length and the width of embryo and the sizes of seed showed that in the conditions of 2019 cultivar sample Alamo formed the largest embryo; the smallest embryo was formed in cultivar sample Cave-in-rock.

The correlation-regression analysis of the data showed a strong linear correlation between the length and the width of embryo and the weather conditions in a phase “flowering-formation” of seed with a determination coefficient $R^2 = 0.9163$ and a correlation coefficient $R = 0.96$ for the length of embryo and $R^2 = 0.9613$ and a correlation coefficient $R = 0.98$ for the width of embryo (Fig. 4a, b).

The regression equation which describes this correlation has been written as follows: $y = 13.9756x + 406.65$ (for embryo length) and $y = 7.0461x + 204.2$ (for embryo width).

CONCLUSIONS

The sizes of pollen, embryo and seed and their ratio depended mainly on the weather conditions during flowering, pollination and their formation, the share of the effect of the “growing conditions” factor is 98-100%. No significant influence of the varietal features was recorded. There was no significant difference in the cultivars according to this indicator either.

The most favourable weather conditions for the formation of seeds and embryo in the inter-phase period “beginning of flowering-seed formation” for 28 days from the onset of flowering were at the average daily air temperature, which did not exceed 25 °C, higher humidity (10 days of slight precipitation, evenly distributed during this period) and in the absence of clear hot days.

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