YIELD OF LITHUANIA-GROWN SWITCHGRASS POPULATIONS DIFFERING IN PLOIDY LEVEL

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Abstract

Switchgrass is being newly adopted in Europe as alternative perennial bioenergy crop. It is an outcrossing species with significant difference in ploidy levels. The objective of this study was to compare the biomass yields of switchgrass germplasm differing in ploidy level. Field experiments were conducted in the Central Lowland of Lithuania during 2013–2015. The results suggest, differences among years of harvest were greater than differences among ploidy groups ($P<0.01$). Yield differences between ploidy groups changed from year to year and increased progressively with advancement of plant age. For example, in the third year of harvest the highest yielding group was mixed ploidy; its dry matter yield increased by 51% from the first harvest year; moreover, its dry matter was higher 28% to 9% comparing with tetraploid and octoploid groups respectively. The current study could contribute to the development of appropriate breeding approach to improve biomass yield of switchgrass.

Key words: *switchgrass, yield, ploidy, dry matter, breeding*

1. INTRODUCTION

Over the past decade, most research in European countries as well as in Lithuania has emphasized the use of lignocellulosic biomass from perennial grasses to produce biofuel (Povilaitis et al., 2016; Šiaudinis et al., 2015; Jasinskas et al., 2014; Robbins et al., 2012). Biofuels produced biochemically or thermochemically from lignocellulosic crops have high energy content; could avoid many of the environmental issues and may offer greater cost reduction potential in the long term (Jaradat, 2010). Some perennials favoured for investigation as dedicated lignocellulosic crops, such as switchgrass (*Panicum virgatum* L.), giant miscanthus (*Miscanthus* × *giganteus* J.M. Greef & Deuter ex Hodkinson & Renvoize [*sacchariflorus* × *sinensis*]), giant reed (*Arundo donax* L.), reed canary grass (*Phalaroides arundinacea* (L.) Rauschert) (Lewandowski et al., 2003; Kirchhoffs & Renquist, 2013; Zegada-Lizarazu et al., 2013). Among these, switchgrass has been identified by the United States Department of Energy as main herbaceous energy crop because of its potential for high yields, low environmental impact and low input requirements (Bouton, 2008). Switchgrass management in Europe as a bioenergy crop is relatively a new intervention. Results to date from various European studies have suggested that switchgrass is broadly adapted to many European countries. Currently, it is introduced in European countries with Mediterranean climate in the South and oceanic climate of Western Europe (Parrish et al., 2012). In more northerly climate conditions, cultivation of switchgrass will require cultivars adapted to northern latitudes. Lemežienė et al. (2015) have suggested that natural populations of switchgrass from North Dakota might have a high breeding value in the European regions with cool and moderate climates.

Authors highlighted the advantages of switchgrass (McLaughlin & Kszos, 2005; Butkutė et al., 2013; Kwit et al., 2014) and have realized this species as an ideal feedstock for second-generation biofuel systems or for direct combustion. Management of switchgrass for biofuels is gained of knowledge of the plant's biology (Parrish & Fike, 2005). Switchgrass is a warm-season C_4 type grass species. It belongs to the family *Poaceae*, subfamily *Panicoideae*, tribe *Paniceae*. The species is native to the tall grass prairie region of North America including the southern Canadian prairie (Moser & Vogel, 1995). Two distinct cytotypes of switchgrass exist that are associated with the lowland and upland ecotypes (Missaoui et al., 2006). These two main groups of ecotypes broadly are classified on the basis of morphological characteristics and habitat preference (Casler, 2012). The upland ecotypes frequently

are hexaploids (2n = 6x = 54) or octoploids (2n = 8x = 72), and the lowland ecotypes are predominantly tetraploids (2n = 4x = 36) (Sanderson et al., 1996). Zhang et al. (2011) hypothesized that such differentiation of the species may be related to ecological contrasts due to climate, day length, or habitat and perhaps account for post-glacial migrations of switchgrass. Switchgrass is an out-crossing species but apomixis may exist at low frequencies. Inbreeding and hybridization could greatly increase biomass yields of switchgrass, however hybridization between plants of different ploidy levels is very low (Hopkins et al., 1996; Taliaferro et al., 1999). Tetra- and octoploid plants are often found in the same native prairies but hexaploids are very rare in nature (Hultquist, 1997). These results indicate the existence of a strong genetic barrier that prevents interploidy crosses probably because of an endosperm imbalance (Bouton, 2007).Vogel and Mitchell (2008) found that yields could be increased by 38% though panmictic hybridization. Generalizing the grand-challenge for switchgrass biomass production is to develop high-yielding genotypes optimal for different climates with a set of desirable physical and chemical composition (Vogel et al., 2014; Casler, 2012). Biomass quality and the productivity of switchgrass can be adjusted by combining breeding methods and growing technology in the certain environmental conditions (Monti et al., 2015; Casler & Vogel, 2014; El-Nashaar et al., 2009). Even though ploidy levels in switchgrass ecotypes were identified, understanding of how ploidy contributes to productivity traits is still lacking. The objective of the present study was to test the biomass productivity of switchgrass populations differing in ploidy level and to quantify the biomass yield potential at two regimes of cutting for three years period.

2. MATERIAL AND METHODS

2.1. Plant material

Seeds of the tested germplasm were obtained from the Plant Genetic Resources Conservation Unit of the United States Department of Agriculture and Agricultural Research Service (PGRCU). The germplasm collection comprised of 29 wild ecotypes of switchgrass originated from North Dakota. The ecotype classification (upland vs. lowland) of the accessions was not reported, but the morphology was consistent with the upland designation. Investigated accessions are listed in Table 1.

Table 1. List of investigated switchgrass accessions

Lithuanian catalogue No. of sample	PGRCU code	Ploidy level	Number of plants per accession
24	PI 642310 01 SD	4x	14
25	PI 642311 01 SD	4x	14
26	PI 642312 01 SD	4x	14
30	PI 642292 01 SD	4x	14
31	PI 642293 01 SD	4x	14
33	PI 642291 01 SD	4x	14
42	PI 642286 01 SD	4x	14
47	PI 642296 01 SD	4x	14
50	PI 642299 01 SD	4x	14
57	PI 642306 01 SD	4x	13
104	PI 642232 01 SD	4x	13
105	PI 642233 01 SD	4x	14

106	PI 642234 01 SD	4x	13
107	PI 642235 01 SD	4x	14
130	PI 642262 01 SD	4x	14
134	PI 642266 01 SD	4x	13
113	PI 642242 01 SD	8x	14
114	PI 642243 01 SD	8x	13
119	PI 649249 01 SD	8x	13
129	PI 642261 01 SD	8x	14
116	PI 642245 01 SD	Mixed	14
118	PI 642247 01 SD	Mixed	11
115	PI 642244 01 SD	Mixed	14
125	PI 642257 01 SD	Mixed	14
112	PI 642240 01 SD	Mixed	14
117	PI 642246 01 SD	Mixed	14
124	PI 642256 01 SD	Mixed	14
126	PI 642258 01 SD	Mixed	14

2.2. Field trial conditions

The field trials were carried out during 2012–2015. The area where the investigations were carried out belongs to Central Lowland of Lithuania (55° 23′ N; 23° 51′ E) (Basalykas, 1958). According to the FAO-UNESCO soil classification system (FAO-UNESCO, 1997) the soil of the experimental site is classified as *Endocalcari-Epihypogleyic Cambisol* with the following characteristics of its plough layer (0-25 cm): pH_{KCl}, 6.2; humus content, 1.4%; available phosphorus (P_2O_5), 348 mg kg^{-1}; available potassium (K_2O), 215 mg kg^{-1}. The collection of switchgrass germplasm was set up using seedlings grown in a greenhouse. In the field collection each accession consisted of 28 plants spaced 0.5 m apart. The field trial was laid out in randomized complete block design with two replications. Plants were not harvested during the establishment year (i.e., 2012) to enable them to become fully established. The herbicide was applied to control weeds in the spring of each year before switchgrass started growth. Pre-planting fertilization comprised of 60 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1} and 60 kg K_2O ha^{-1}; and 60 kg N ha^{-1} was applied during each spring of the following years.

2.3. Meteorological conditions

The annual mean precipitation at the experimental site is 550–600 mm and mean annual temperature is 6.0–6.5 °C. The mean January temperature during 2012–2015 was –5.3 °C and the mean July temperature was 19.1 °C. Normally, average temperature in January, the coldest month in Lithuania, is –2.9 °C, and in the warmest month of July is 19.7 °C. However, during the 2012–2015 experimental period the weather conditions markedly differed between years. The weather data is presented in Figure 1.

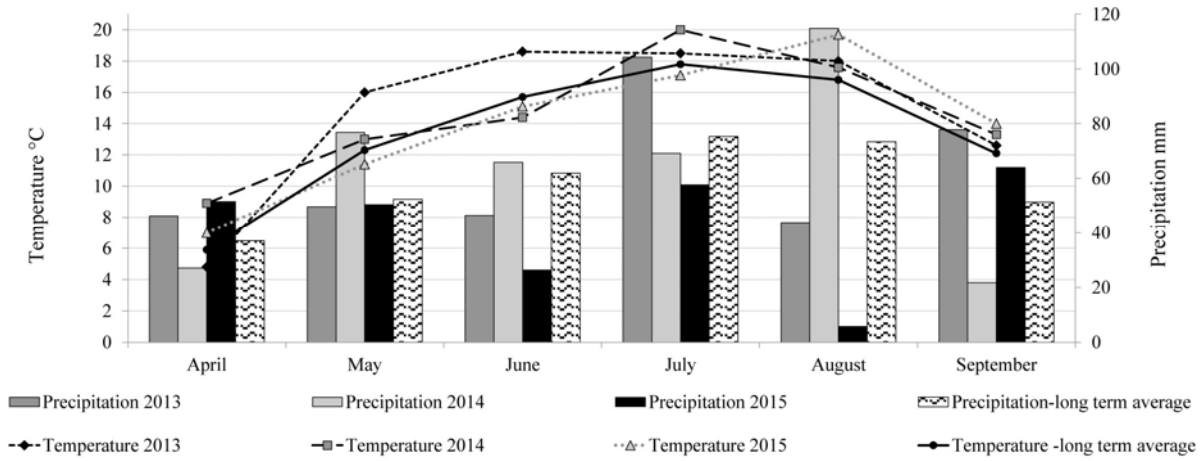


Figure 1. Monthly and long-term (1923–2013) mean temperature and precipitation during growing season, 2013–2015 at Dotnuva weather station, Lithuania

2.4. Methods of sample preparation

Ploidy levels of the accessions were estimated using a flow cytometer (Partec GmbH, Germany) (Lu et al., 1998) and performed according to our recently published procedure (Dabkevičienė et al., 2015).

Biomass yields were recorded in years 2013, 2014 and 2015. Simulating the species management under commercial cultivation conditions two levels of harvesting were planned: one and two cuts per season. For plants cut once, the first cut was at seed maturity stage. For plants cut twice, the first cut was at beginning of anthesis and the second – after re-growth of aftermath. Plants were clipped leaving 30-cm high stubbles. Dry matter yield was estimated after each cut by drying the biomass at 105˚C until a constant weight was reached. Dry matter yield was reported on a per plant basis as grams of dried biomass per plant.

2.5. Statistical analysis

Three-way ANOVAS were performed to test the effects and interaction of ploidy level, year and productivity. Fisher's protected LSD was used to compare means. Differences were considered to be significant at the 95% and 99% level. All statistical analyses were conducted using Statistica 7 (StatSoft, Inc.).

3. RESULTS AND DISCUSSION

Biomass yield is the most important factor for biofuel production and should be the primary trait for switchgrass improvement and domestication (McLaughlin & Kszos, 2005). In the current study, the results suggested that ploidy level, year of harvest and cutting regime significantly ($P<0.01$) effected fresh and dry biomass yield. Ploidy level, harvest frequency and three year interactions ($P<0.01$) were observed for all variables (Table 2). Thus, data from harvest treatments for each year were analysed separately.

Table 2. Statistical significance of switchgrass yield in 2013, 2014, and 2015

Effects	*df*	Significance of *F* ratio	
		Fresh Biomass Yield	Dry Matter Yield
Treatments	17	**	**
Ploidy level (P)	2	**	**
Cutting regime (C)	1	**	**
Year (Y)	2	**	**
Interaction P × C	2	**	**
Interaction C × Y	2	**	**
Interaction Y × P	4	**	**
Interaction Y × P × C	4	**	ns

df – degrees of freedom, **$P<0.01$; ns – not significant

The above-ground dry matter yield of switchgrass determined from three-year growth, when grass was cut once or twice per season, is shown in Figure 2. In the first treatment (when grass was cut once at seed maturity) mean dry matter yield in the first year was not high (257.7 g per plant), and as expected, significantly increased with the plant age ($P<0.01$) and provided annual increase +29% in the second (332.3 g per plant) and +8% in the third (359.1 g per plant) harvest year. Total increase in dry matter yield from the establishment year to the fourth year collection peaked +39%. Hence, our observations agree with the findings of earlier researchers that a notable increase in biomass yield in subsequent year is typical for switchgrass. For example, Elbersen et al. (2001) noted that for switchgrass yield development to reach maximum potential probably takes more than three years and further increases in average yield are likely. These findings is in agreement with Alexopoulou et al. (2008) which found that in Italy, the yield of switchgrass harvested at the end of growing season continued to increase until the third growing season while in Greece the yields were high from the second year and remained at these high levels up to the third year. Monti et al. (2015) showed visibly decrease of productivity after third year of harvest in six years annual harvesting system.

Although switchgrass has high biomass productivity, its yield is highly variable and depends on the local growing conditions. Elbersen et al. (2001) predicted that on clay soils switchgrass takes longer to reach maximum yield potential. According to the data from experiments performed in Ukraine yields of switchgrass averaged 7.7 t per ha on lower quality soils to 12 t per ha on nutrients rich soils in a delayed harvest system (Elbersen et al., 2013). Meanwhile, when switchgrass was cultivated in the drought prone sandy soils at the Northern Great Plains, annual accumulation of biomass dry matter varied from 3.5 t per ha in a drought year to 13.75 t per ha in a year with above-average precipitation (Berdahl, 2005). Together these findings suggest that there is a wide-range interaction among genotype and environment in biomass yield.

Comparing the means of dry matter yield of switchgrass when cut once or twice per season in the second treatment the yield was always lower. The biggest difference recorded in the third harvest year: the yield was two-fold (–59%) lower. This marked reduction in dry matter yield in the third harvest year can be explained by poorer growing conditions in June: low summer rainfall likely further limited greater switchgrass regrowth. These results are consistent with data obtained in earlier studies. Parish & Fike (2005) concluded that more frequent or mistimed harvests will reduce yield of switchgrass, especially over a long term. However, Liu et al. (2015) observed that yield in Virginia, USA tended to increase with more than one cut per year. Also, the author suggest that two-cut management could produce greater yields in year with sufficient rainfall, but a single harvest would be more desirable in years with normal to drier-than-normal precipitation patterns.

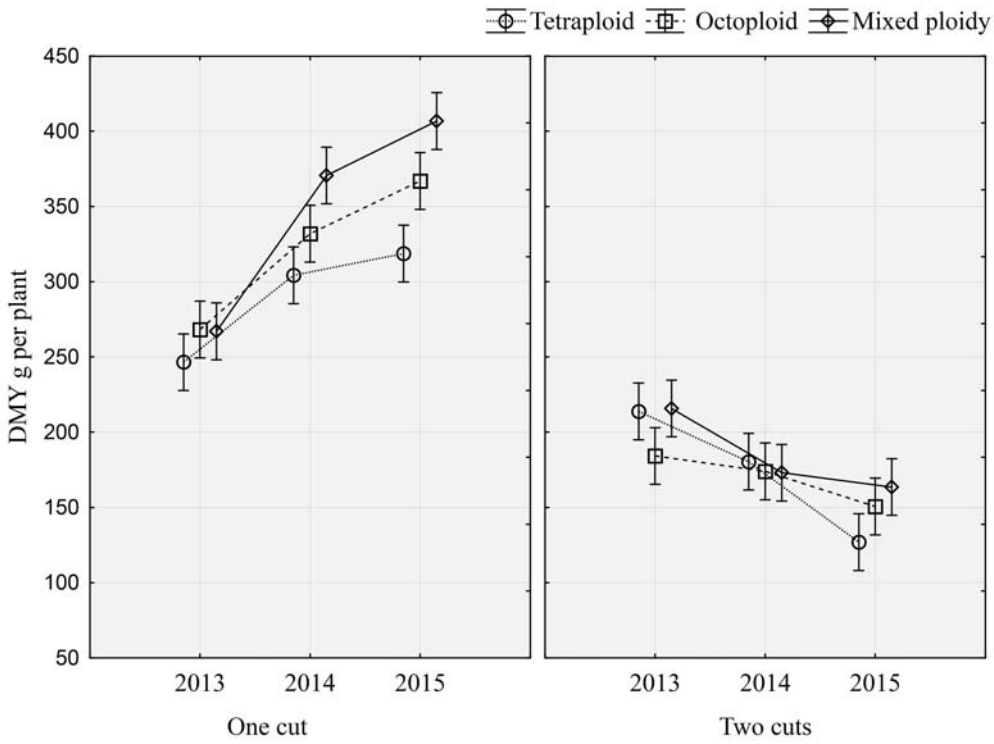


Figure 2. Dry matter yield (DMY) of switchgrass populations differing in ploidy level in 2013, 2014, and 2015 harvest years when cut once and twice per season; vertical bars denote 95% confidence intervals

The current study, comparing three years biomass partitioning among ploidy groups under single harvest system revealed that mixed ploidy populations maintained their superiority over tetraploid and octoploid populations and significantly (P<0.01) out-yielded them (Figure 2). For example, in the first harvest year when the grasses had been harvested once per season the yield of grasses was very similar: a small variation in the dry matter between the ploidy groups were seen but significant differences (P<0.05) were not. In the second harvest year the yield of mixed ploidy populations was significantly higher and increased 20% to 12% over the tetraploid and octoploid populations respectively. Moreover, in the third year its dry matter yield increased by 51% from the first harvest year and was higher 28% to 9% comparing with tetraploid and octoploid groups respectively.

Switchgrass yields among ploidy groups as affected by two cuts frequency varied by year. In the first harvest year, the yield of tetraploid and mixed ploidy populations was only slightly higher (16% to 17% respectively) than in octoploid populations. In the second harvest year, no significant differences in yields between the tested ploidy groups were observed. Despite the yield tended to significantly (P<0.01) decrease with the plant age, it is interesting to note that in the third harvest year octoploid and mixed ploidy populations produced higher annual dry matter yield comparing with tetraploids.

The greater yield response of mixed ploidy populations in 2015 may reflect the greater adaptation potential in part due to increased ploidy. Switchgrass yields typically are positively related to rainfall during the growing season (Fike et al., 2006). Precipitation during the 2015 growing season was 55 mm below historic averages for the research site. Moreover, hydrothermal coefficient accordingly to Selianinov (1928) HTK=0.1 indicated very strong drought in August. It is believed that an increase in chromosome number in polyploid plant populations enhances the level of heterozygosity and creates prerequisites for better adaptation of the species, i.e. increases its adaptability across a broad geographic range (Parisod et al., 2010). Warner et al. (1987) concluded that polyploidy can influence physiological activities such as photosynthesis. Therefore, increased scientific knowledge on how

stresses limit the productivity of different ploidy groups is needed in order to move forward and further improve the productivity and sustainability of switchgrass.

In the final part of the study, the best-performing accessions in each ploidy group based on increase over general mean yield over three harvesting years were identified. The accessions ranking changed among two harvesting treatments in each ploidy group (Table 3 and Table 4). For example, when the grasses had been harvested once per season, the highest yielding accessions in the tetraploid group were LŽI-106 and LŽI-134; their dry matter yields were 21% to 16% higher than general mean biomass yield of the entire trial during 2013–2015 (Table 3). In the second treatment, when the grasses had been harvested twice per season, the highest yielding accessions in the same ploidy group were LŽI-57 and LŽI-130; their dry matter yields were higher 25% to 19% respectively (Table 4).

Table 3. Mean dry matter yield of two high yielding accessions of switchgrass in three ploidy groups when cut once per season

Lithuanian catalogue No. of sample	PGRCU code	Dry matter yield g per plant
Tetraploid		
LŽI-106	PI 642234 01 SD	352.3**
LŽI-134	PI 642266 01 SD	336*
Average of trial, n = 17		289.8
LSD_{05}/LSD_{01}		44.8/59.7
Octoploid		
LŽI-113	PI 642242 01 SD	349.7^{ns}
LŽI-129	PI 642261 01 SD	318.7^{ns}
Average of trial, n = 4		320.6
LSD_{05}/LSD_{01}		46.2/65.2
Mixed ploidy		
LŽI-118	PI 642247 01 SD	435.3**
LŽI-117	PI 642246 01 SD	410.7*
Average of trial, n = 8		343.4
LSD_{05}/LSD_{01}		59.1/80.3

Significant at *P<0.05, **P<0.01; ns – not significant.

Table 4. Mean dry matter yield of two high yielding accessions of switchgrass in three ploidy groups when cut twice per season

Lithuanian catalogue No. of sample	PGRCU code	Dry matter yield g per plant
Tetraploid		
LŽI-57	PI 642306 01 SD	216.8**
LŽI-130	PI 642262 01 SD	207.1*
Average of trial, n = 17		173.7
LSD_{05}/LSD_{01}		30.4/40.6
Octoploid		
LŽI-113	PI 642242 01 SD	190.5^{ns}
LŽI-114	PI 642243 01 SD	184.9^{ns}
Average of trial, n = 4		169.6
LSD_{05}/LSD_{01}		26.8/37.9
Mixed ploidy		
LŽI-118	PI 642247 01 SD	232.9**
LŽI-115	PI 642244 01 SD	204.6^{ns}
Average of trial, n = 8		184.1
LSD_{05}/LSD_{01}		32/43.4

Significant at *P<0.05, **P<0.01 ns – not significant

Biomass yield is a highly complex trait and various approaches have been tested in model systems to improve this important trait. Differences between the ploidy groups demonstrated the potential to improve yield of switchgrass through selection in breeding programmes. These best-performing accessions with higher dry matter yield are acknowledged as perspective and valuable genetic source for future breeding projects.

4. CONCLUSIONS

It can be assumed that ploidy level, year of harvest and harvesting frequency had a significant effect on the dry matter yield of switchgrass. Dry matter yield increased with each harvest year when switchgrass was harvested once per season. When cut once per season, in the third year of harvest the highest yielding ploidy group was mixed ploidy comparing with tetraploid and octoploid. When cut twice per season, differences among years were greater than differences among ploidy groups. In the third harvest year, characterized by very dry conditions during summer period, mixed ploidy populations exhibited the highest dry matter production comparing with tetraploids. The higher yielding accessions during three study years were identified. Best-performing accessions can serve as a valuable genetic source for future breeding programmes. However, more research on this topic needs to be undertaken before the productivity of different ploidy groups is more clearly understood in order to improve the productivity and sustainability of switchgrass.

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