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POSSIBILITIES OF SEED QUALITY IMPROVEMENT IN *MISCANTHUS SINENSIS* (ANDERSSON)

Summary

Miscanthus sinensis (Andersson) is a perennial grass producing high amounts of biomass. I It can be used for energetic purposes through burning or biogas production. A main problem in this crop cultivation consists in vegetative, expensive method of propagation. First preliminary research on seed quality improvement is carried out in order to enable propagation on a large scale. The object of presented experiment was to find out the reason for low germination ability and to search methods of seed quality improvement. Miscanthus seeds were treated with sodium hypochlorite, commercial micronutrients seed fertilizer and commercial bacteria seed treatment, ash and plasma. The ability and speed of germination and root and shoot length were measured. Tetrazolium test and microscopic observations of seeds were done. Fragility and susceptibility to damage and low vitality of embryos mean that further work is needed on the genetic characteristics and methods of seed harvesting as well as postharvest processing of this species.
***Key words**: Miscanthus sinesis (Andersson), seeds, germination ability, propagation*

MOŻLIWOŚCI POPRAWY WARTOŚCI SIEWNEJ NASION MISKANTA CHIŃSKIEGO (*MISCANTHUS SINENSIS* ANDERSSON)

Streszczenie

Miskant chiński jest wieloletnią trawą wytwarzającą duże ilości biomasy. Jest użytkowany w celach energetycznych poprzez spalanie lub produkcję biogazu. Problemem w upowszechnieniu tej uprawy jest wegetatywny, drogi sposób rozmnażania. Trwają pierwsze prace nad poprawą jakości nasion miskanta, tak aby rozmnażanie generatywne na szeroką skalę było możliwe. Celem przedstawionych badań było określenie przyczyn niskiej zdolności kiełkowania nasion oraz poszukiwanie metod poprawy ich wartości siewnej. Nasiona miskanta były traktowane chlorkiem sodu, preparatami bakteryjnymi i mikroelementami, popiołem a także plazmą. Badano zdolność i szybkość kiełkowania oraz długość korzeni zarodkowych i kiełków. Na nasionach wykonano także test tetrazolinowy oraz obserwacje mikroskopowe. Kruchość nasion i podatność na uszkodzenia oraz niska żywotność zarodków powodują, że konieczne są dalsze prace dotyczące cech genetycznych i metod zbioru i czyszczenia nasion tego gatunku.
***Słowa kluczowe**: miskant chiński, nasiona, zdolność kiełkowania, rozmnażanie*

1. Introduction

The interest in bioenergy in Europe is still rising. There is an urgent need for elaboration of cultivation technology for many species. Among them perennial grasses belong to most valuable. Genus *Miscanthus* species is one of the most promising biomass and biofuel crops. It's productivity is very high, even in cold North European conditions. The high yield and low input during the cultivation are main advantages of *Miscanthus*, but there is still a problem with propagation and plantation establishment. The major method of establishing a field of *Miscanthus* consists in planting young plants of vegetative propagation which demand intensive labour and costs. Hence the idea is close to establish a field by using seeds as it is commonly used in agriculture in many crops. Seeded *Miscanthus* would be easier to introduce on a large scale and also changing in more productive new genotypes can be quicker (Anderson et al 2014).

Thousand seed mass of *Miscanthus* seeds is very low (< 1.0 g) and the planting density (< five plants m^{-2}) is low too. For sowing purposes it seems promising to pellet the seeds to increase the volume of seeds. On the other hand, low germination ability of *Miscanthus* seeds makes seed pelleting process futile, and causes problem with gaps on the further plantation.

There is very few data about Miscanthus seeds and possibilities of their quality improvement.

Literature data show that some bacteria and fungi species can improve early growth and possibly a germination of Miscanthus seeds. One of them is *Herbaspirillum frisingense.* It enters the plant through epidermal discontinuities such as root cracks at the emerging point or root injuries. Despite the dense endophyte colonisation, no symptoms of disease were observed. (Monteiro et. al. 2012) Genome of *Herbaspirillum frisingense* was annotated and compared with related *Herbaspirillum* species. High diversity in genomics suggests that every plant species provides own metabolic niches for endophyte-plant association. (Straub et al.2013) *Pseudomonas koreensis* AGB-1 is another endophytic bacteria isolated from roots of *Miscanthus sinensis*.. It has a potential to promote plant growth and heavy metal removal from the soil. Pseudomonas was tolerant to heavy metals and exhibited plant promoting traits.(Babu et al. 2015) Mycorrhizal fungus *Gigaspora margarita* has generally been recognized - the effect of arbuscular mycorrhizal (AM) symbiosis associated with host plants is beneficial because growth and development of the plants are stimulated, drought tolerance increases and the association has a high potential for agriculture and land reclamation (Ma et al. 2007). As a result of these characteristics, arbuscular mycorrhizal fungi(AMF) have been expected to promote plant growth in degraded and denuded lands (Ma et al. 2007). But the efficiency of mycorrhizal fungus is strongly limited by the soil properties.

Tab. 1. Methods of seed treatment
Tab. 1. Metody przygotowania nasion

Method of treatment	Details (time concentration etc.)			
NaOCl	2% , 30 min.		2%, 10 min	
Miscantus ash	1 teaspoon added to germination medium			
Plasma treatment	Programme number	Time (min)	Power (%)	Presence of acrylic acid
	2	20	100	-
	3	10	50	+
	4	20	50	+
	6	10	50	-
	14	10	100	-
	19	10	100	+
	23	20	50	-
	24	40	50	-
	25	40	100	-
	26	20	100	+
	27	40	50	+
	28	40	100	+
Seed fertilizer	AKRA Saat		AKRA Saat 2	
	0.25 l + 1 l water per 100 kg of seeds.		Double concentration of fertilizer	
	Mixed for 12 h, then air dried.			
Bacteria treatment	Bacteria Mix AKRA	Rhizo Vital	FZB	
	0.1 l *Azocarus*, 0.1 l *Azotobacter*, 0.1 l MSB (Silver medium, 0.15 l water per 100 kg of seeds	0.75 l per 100 kg of seeds	0.75 l per 100 kg of seeds	
	Mixed for 12 h, then air dried.			
Combined treatment	AKRA Saat + Plasma 24		AKRA Saat +Plasma 26	

Source: own study / *Źródło:* opracowanie własne

Also a biochar can play a significant role in growth promotion. Detailed studies of biochar–root interactions are few. Biochar may affect root growth, and therefore plant performance, through two mechanisms: as a direct nutrient source and through impacts on nutrient availability (Prendergarst-Miller et al. 2013). It was found that biochar accelerates and improves early growth of another perennial grass species, *Lolium perenne* through enhancement of nutrient mobilization (Fox et al. 2014).

Literature data show that sodium hypochlorite (NaOCl) treatment can be beneficial for *Miscanthus* seeds. There are 3 ways by which NaOCl can stimulate the seed germination: scarifying seed coat that allows more water and oxygen uptake, as surface disinfecting agent and overcoming or breaking seed dormancy agent. 2% NaOCl stimulated germination of *Miscanthus sinensis* and *Miscanthus sacchariflorus* seeds especially in high germination temperature (30˚C). However NaOCl treatment damaged immature and less dried seeds resulting in lower seed germination comparing to no-treated control. (Lee et al 2012).

Low pressure (cold) plasma treatment is often used for food disinfection (Ulbin-Figlewicz et al 2015). It was also effective for disinfection of seeds of brassicaceous plants from *Rhizoktonia solani* (Nishioka et al 2014). Moreover Li et al (2015) suggested that cold plasma treatment improved oilseed rape drought tolerance by improving antioxidant enzyme activities, increasing osmotic-adjustment products, and reducing lipid peroxidation, especially in the drought-sensitive cultivar. It also promoted seed germination and seed vigour in soybean (Li et al 2014).

During seed pellet formation seeds should be dry, so not all of the presented methods can be used.

2. Material and methods

Experiments were conducted from March to May 2015 in laboratories of Hohenheim University in Stuttgart, Germany. Seeds of miscanthus were from 2014 harvest and originated from Turkey.

On the basis of a literature research on seed treatments for grass species five different treatments were chosen: (1) NaOCl treatment (2% for 30 minutes and 10 minutes), (2) *Miscanthus* ash as an additive to germination substrate, (3) low pressure plasma treatment (different combinations of time, power and presence/absence of acrylic acid) using Standard Plasma Systems Pico PCCE appliance from Diener Electronic, (4) commercial micronutrients seed fertilizer (AKRA Saat) and (5) commercial bacteria seed treatment (3 methods: Bacteria Mix – AKRA *Azocarus* + *Azotobacter* + MSB Silbermittel, Rhizo Vital and FZB – both containing *Bacillus amyloliquefaciens*). Full list of used methods is shown in table 1. Control combination were not treated, raw seeds.

Germination of seeds was conducted in climate chambers in 3 weeks cycle : 7 days of prechilling (+5°C) and 14 days of changing conditions +25°C during the day and +15°C during the night. Different combinations started to germinate in different time (but the same conditions). Due to a long germination cycle, time consuming preparation and short scholarship duration (3 months) not all combinations were examined in every way. Germination tests were conducted in 4 replications – 100 seeds each. The last germination test on separated of whole, undamaged seeds wasn't analysed statistically because of different number of replications (from 2 to 4), caused by lack of the whole seeds.

Germination ability, number of normal and abnormal seedlings, number of infected seeds, germination speed and uniformity of germination, shoot and root length were examined.

Germination speed (Pieper's coefficient,time of germination – days) (fig. 4) was measured by counting and removing germinating seeds from the seed boxes every day. Then, the results were calculated using a formula:

$$\text{Pieper's coefficient} = \frac{\sum(d_n x a_n)}{\sum a_n}$$

where:

d_n – number of the day of germination (emergence),
a_n – number of seeds germinated/seedlings emerged on this day.

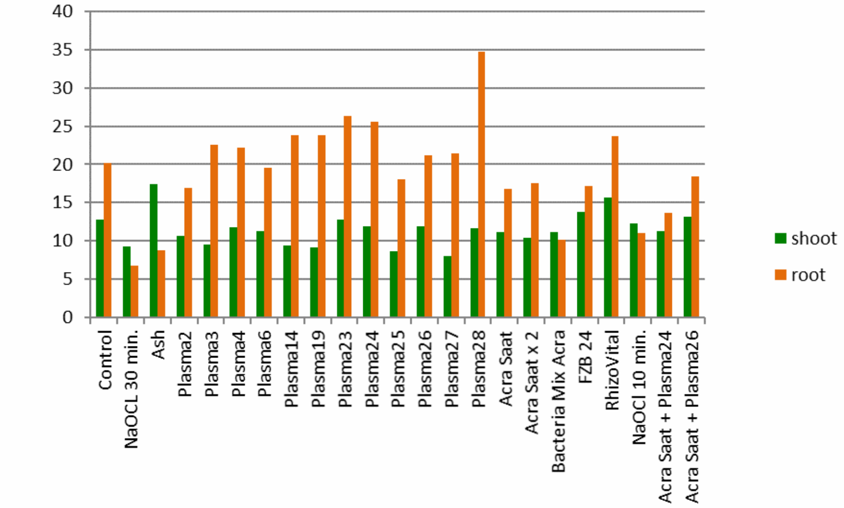
The lower is the value of Pieper's coefficient, the higher is germination speed. Some plasma treatments and treatments with commercial products containing *Bacillus amyloliquefaciens* caused a decrease in germination time by 1 day, which can be an important acceleration in field conditions. Measurements of germination speed was done only for some, chosen seed treatments.

3. Results and disscusion

Plasma treatment was the most effective treatment extending the root length in the roll test (fig. 1). Different settings (programmes) of plasma appliance resulted in different root length. The most effective ones were: 28-40 min, 100% of power, with acrylic acid, 23-20 min, 50% of power, without acrylic acid, 24-40 min, 50% of power without acrylic acid, 14-10 min, 100% of power without acrylic acid and 19-10 min, 100% of power with acrylic acid. Also soaking of seeds in commercial product containing *Bacillus amyloliquefaciens* - Rhizo Vital solution caused a visible lengthening of root and shoot in comparison to control ones. Presence of *Miscanthus sinensis* ash in the germination medium caused an elongation of shoots, but due to low germination ability this treatment wasn't considered as a promising one.

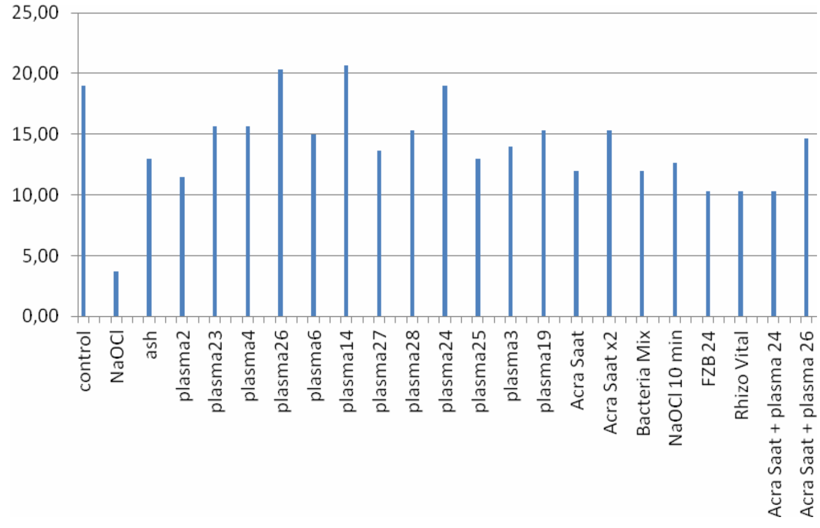
Germination ability measured according to ISTA rules of every seed bulk was low and varied from 3,7% to more than 20% (fig. 2). Germination ability of control seeds was 19%. Only 2 plasma treatments: 26-20 min, 100% of power with acrylic acid and 14-10 min, 100% of power without acrylic acid, caused an increase in germination ability comparing to untreated control.

All seed lots showed high ratio of infected and non-germinating seeds. (fig. 3) Plasma treatments didn't decrease a number of infections (as it was expected) due to high susceptibility of seeds to the mechanical damage. In later experiment, under optical microscope it was found out, that many seeds were cracked and broken. Number of normal seedling – expected to grow into healthy, vigorous plants was higher in plasma treated seed combinations and also in combinations treated with AcraSaat – commercial fertilizer containing microelements, even in doubled dose.



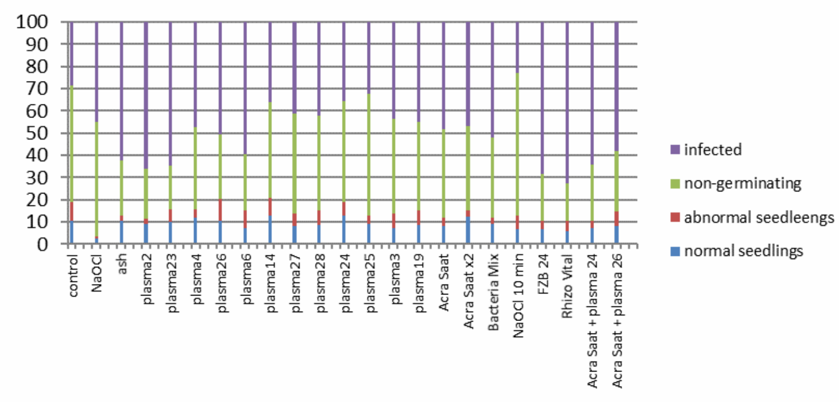
Source: own study / *Źródło:* opracowanie własne

Fig. 1. Shoot and root length [mm] after 21 days of germination, root length $LSD_{(\alpha=0,05)} = 20.2$
Rys. 1. Długość kiełków i korzeni zarodkowych [mm] po 21 dniach kiełkowania. Dla długości korzeni $NIR_{(\alpha=0,05)} = 20,2$



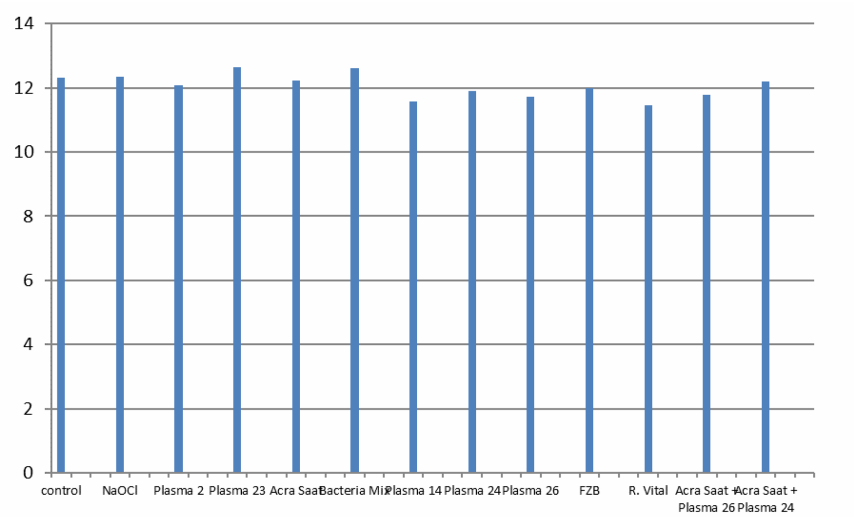
Source: own study / *Źródło:* opracowanie własne

Fig. 2 Germination ability [%], $LSD_{(\alpha=0,05)} = 11.5$
Rys. 2. Zdolność kiełkowania [%], $NIR_{(\alpha=0,05)} = 11,5$



Source: own study / *Źródło:* opracowanie własne

Fig. 3. Results of germination ability test [%]
Rys. 3. Ocena zdolności kiełkowania nasion [%]



Source: own study / *Źródło:* opracowanie własne

Fig. 4. Time of germination (days), $LSD_{(\alpha=0,05)} = 1.38$
Rys. 4. Średni czas kiełkowania (dni), $NIR_{(\alpha=0,05)} = 1,38$

Source: own study / *Źródło:* opracowanie własne

Fig. 5. Viable (bottom) and dead seeds of *Miscanthus sinensis* (Andersson) during tetrazolium test
Rys. 5. Żywe (u dołu) i martwe nasiona Miscanthus sinensis (Andersson) widoczne w teście tetrazolinowym

Source: own study / *Źródło:* opracowanie własne

Fig. 6. Not selected (left) and selected seeds of *Micanthus sinensis* (Andersson)
Rys. 6. Nie selekcjonowane (po lewej stronie) i selekcjonowane nasiona Miscanthus sinensis (Andersson)

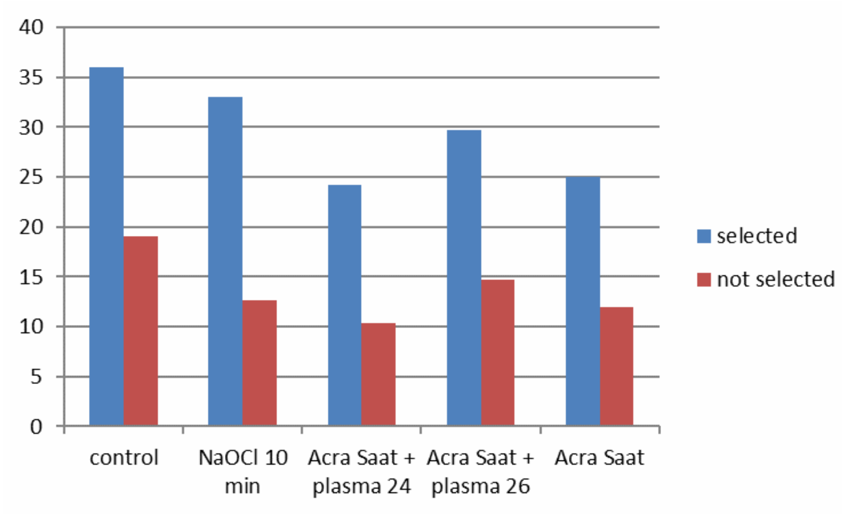


Fig. 7. Germination ability [%] of selected whole seeds in comparison to not selected ones
Rys. 7. Zdolność kiełkowania [%] wyselekcjonowanych, nieuszkodzonych nasion w porównaniu do nieselekcjonowanych

To assess the reason of low germination rate of *Miscanthus* seeds a seed-viability test using tetrazolium chloride was conducted. Seeds were imbibed, bisected longitudinally through the embryo and placed in tetrazolium chloride solution. After 5 hours live embryo tissues were colored red and dead ones stayed white. Seeds were evaluated according to *Tetrazolium Testing Hanbook* (AOSA 2010). It was found out that only 58% of embryos were alive (fig. 5). Another conclusion was that most of the tiny seeds are broken and cracked, what was visible under a microscope (fig. 6).

The last experiment was conducted on separated whole seeds (using the microscope) from chosen combinations (fig. 6). Selection of whole, not damaged seeds improved germination ability for 50% or even more (fig. 7). This part of experiment wasn't analysed statistically because of different number of replications (from 2 to 4), caused by lack of the seeds.

4. Conclusions

1. Germination ability and vigor of miscanthus seed is very low and needs to be improved in order to introduce seed propagation and seed pelleting to the practice.

2. Miscanthus seeds are very susceptible to damage which is invisible to the naked eye.

3. Only 58% of embryos of whole, not damaged seeds in examined bulk were alive.

4. Some treatments like plasma treatment slightly improve course of germination.

5. Further research on methods of seed treatment and seed selection are necessary. Although a selection of genotypes of miscanthus towards high seed vigor and seed harvesting technology improvement can bring results of better seed quality.

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Acknowledgements

The scholarship of A. Orzeszko-Rywka, at University of Hohenheim was funded by European Social Fund - Human Capital. Also special thanks to Prof. I. Lewandowski, from the division BiobasedProducts and Energy Crops, Dr S. Bopper S. and Prof. M. Cruse from the Institute of Plant Breeding, Seed Science and Population Genetics of University of Hohenheim, Stuttgart for supporting.