Thesis

Data on the biology and natural viral infection of millet species

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1. BACKGROUND AND OBJECTIVES OF THE RESEARCH

1.1. Background

The genus Millet (*Panicum*) is one of the largest and extremely species-rich groups of grasses, with more than five hundred species in the world. Their spread and becoming difficult to control weeds have been induced by intensive plant protection, the widespread and long-term use of pesticides, especially aminotriazines, and climate change. Due to its wild forms, they are now known as a crop-weed species complex in herbological practice. According to the results of the National Field Weed Surveys, only two species (*P. miliaceum* ssp. *miliaceum*, *P. capillare*) were previously included in the weed flora of Hungary, but in recent decades several new adventitious species have appeared and become dangerous weeds. Control of millet is hampered by the fact that new *Panicum* species will continue the infection and spread in the near future. The appearance of these species in the maize weed flora is another challenge for control both for the producer and for plant protection professionals, as their germination biology and thus the timing of proper control are different. After Csiky et al. (2004), in 2006 Pál and Pinke reported a wider appearance and weeding of late millet (*P. dichotomiflorum*) in maize. An additional species was identified for the first time in 2009 in Zala County, the *Panicum riparium*.

Separation of millet species and subspecies within *P. miliaceum* is difficult and therefore requires serious expertise, so plant protection faces a new challenge, as in addition to species and subspecies segregation, plant protection technologies also need to be optimized to achieve the desired effect. In order for all this to happen, we must first clarify the biology of the species found in Hungary. We need to know how different the germination biology of millet species and subspecies is, what kind of competition they have, whether they contain allelochemicals, whether they have allelopathy, and how they influence the germination and growth of maize.

1.2. Objectives

The aims of our research were:

1. To study the germination biology of cultivated millet, weedy millet and *P. riparium* and to explore the differences between them, to study whether species and subspecies have dormancy, and to study the effect of climate change on the germination biology of the species.
2. To study the strength of the competition of the listed species in a greenhouse pot experiment.
3. Do they have allelopathy and is there a difference between them in terms of the strength of allelopathy.
4. To assess the natural grain virus infection of millet species and their viral epidemiological role.

2. MATERIALS AND METHODS

2.1 Germination biological tests

We studied the germination of two different seed samples of these three millet species as a function of temperature. The experiment was performed at the Institute of Plant Protection of the Georgikon Faculty of the University of Pannonia in 2014 under laboratory conditions.

The two *Panicum miliaceum* subsp. *miliaceum* sample was collected in 1985 from Keszthely and one sample from Tarján in 2013, both *Panicum riparium* seed samples were collected in 2010 from Mérk and Nagykálló, and two *Panicum miliaceum* subsp. *ruderale* sample from Keszthely and Pusztadobos. During the study, 50-50 seeds were placed in a 20 cm diameter Petri dish on a double layer of filter paper in 4 replicates per species and temperature range. The germination temperatures were 20, 25, 30 and 35 oC, respectively. Samples were placed in a heatable thermostat and then evaluated at two time points. On the third day, the germination percentage was examined, and on the seventh day, the shoot and root lengths of the seedlings were measured. Statistical evaluation was a one-way analysis of variance performed with Microsoft Excel software.

2.2 Investigation of allelopathy of millet species

2.2.1. Laboratory experiments

In the 2015 study, we examined the allelopathic effects of cultivated millet (*Panicum miliaceum* subsp. *miliaceum*), weedy millet (*Panicum miliaceum* subsp. *ruderale*) and the *Panicum riparium* species on maize germination and seedling growth in a bioassay experiment. Green plant samples of the weeds included in the study were collected in the summers of 2015 and 2016. Samples of cultivated millet from grain storages around Keszthely. *Panicum riparium* and *Panicum miliaceum* subsp. *ruderale* was collected at Lesencetomaj. Hybrid seeds of MV Koppány were used in the experiment.

In the experiment, an aqueous plant extract with a dilution series of 3: 2.5%, 5%, 7.5 w / v% was prepared by soaking for 24 hours and treated with corn kernels. 25 corn kernels were placed in double-layer filter paper in petri dishes and treated with 15 ml of extract (distilled water for the control).

The Petri dishes were then placed in a germination incubator at 20 ° C for 7 days. Moisture lost during incubation was replaced with water spray.

When evaluating the experiment, the germination percentage was determined on day 7, then the grains in germination were examined individually and the number of healthy germs was determined.

Finally, the length of the primary root and shoot were measured. Data were recorded in an Excel spreadsheet and then evaluated by one-way analysis of variance.

2.2.2. Pot experiments

In the second part of the series of experiments, we performed a pot experiment in 2017 with cultivated millet (*Panicum miliaceum* subsp. *miliaceum*), weed millet (*Panicum miliaceum* subsp. *ruderale*) and *Panicum riparium* species. Plant samples were identical to those tested in the laboratory experiment. In the 2-liter culture vessels, a plant sample corresponding to the concentrations of 2.5%, 5%, 7.5 m / V% was incorporated into the soil.

For each concentration, 4 replicates were planned and 5 grains of feed maize (MV Koppány) were sown to a depth of 3 cm per pot. Only potting soil and corn were added to the control pots.

The pots were placed in a BINDER brand growth chamber at 20 ° C for 30 days. Constant humidity was maintained by irrigation throughout the experiment.

Evaluation was performed after 30 days. The percentage of germination per treatment was determined, the length of the shoot and root per plant was measured, and the dry weight of the root and shoot per culture pot was measured. Data were recorded in an Excel spreadsheet and then evaluated by one-way analysis of variance.

2.3 Competition experiment

The aim of the experiment was to control *P. miliaceum* subsp. *miliaceum*, *P. miliaceum* subsp. *ruderale* and *P. riparium* were examined for their ability to compete with maize by an additive method. The experiment was performed in 2014 and then in 2015 with the *P. miliaceum* subsp. *miliaceum* and *P. miliaceum* subsp. *ruderale* species were repeated.

The site of the study was the greenhouse of the Institute of Plant Protection of the Georgikon Faculty of the University of Pannonia. 2 kg of dried, sieved soil was used per culture vessel with a surface area of 450 cm2. 5 MV Koppány maize hybrid and 0, 5, 10 and 20 millet plants were grown per culture dish, all in 4 replicates. The time and depth of sowing were the same for maize and millet. The samples examined were: *Panicum miliaceum* subsp. *miliaceum* from Keszthely (1985) and Tarján (2013). *Panicum miliaceum* subsp. *ruderale* from Keszthely (2010) and

Pusztadobos (2012), and two *Panicum riparium* samples from Mérk and Nagykálló (2010). Samples were stored at -20 ° C until use.

The experiment was evaluated one month after germination, during which the length of maize and millet shoots and roots, fresh and air-dry shoot weight and fresh and air-dry root weight were measured. Statistical evaluation of the study was performed using SPSS using a three-way analysis of variance.

2.4 Weed virological experiments

During the study, 45 millet (*Panicum miliaceum*) leaf samples were collected in September 2014 and 2015. 35 samples were taken from the cereal growing areas on the border of Lesencefalu and 10 from the areas near the Fenyves allé in Keszthely. When selecting the samples, we collected the leaves of the plant that showed the symptoms of viral infection. Until the laboratory tests were performed, the samples were individually stored frozen in polyethylene bags.

In plant virology, the most commonly used so-called a double antibody sandwich (DAS ELISA) method was used to detect viral infections.

For testing, LOEWE Biochemica ryegrass mosaic virus (BMV), ryegrass striped mosaic virus (BstMV), barley stripe mosaic virus (BSMV), barley yellow dwarf virus (BYDV), Wheat streak mosaic virus (WSMV), Wheat dwarf virus (WDV) reagents were used.

The next step was to examine the viral infection of millet (P. *miliaceum* subsp. *miliaceum*) samples collected from two sites using an objective diagnostic method using small RNA (sRNA) HTS to detect any viral infection.

Samples were taken from two locations in Hungary in August 2019. In both the “Büdös-árok” (BA) and “Újmajor-susnyás” (US) samples, we randomly sampled 10 plants that showed different symptoms. During sample collection, whole plants showing symptoms were collected. RNA was extracted from the samples by the phenol-chloroform method (White and Kaper, 1989). The resulting nucleic acid extracts were stored at -70 ° C until use.

RNA pools of individuals were prepared by mixing the same amount of RNA from different organs of the same plant from different individuals and representing all plants in the sample from a given collection site. This pooling strategy allowed the detection of viral infection in each of the plants in the sample. These kits were used to generate the sRNA library (2 libraries in total). The FASTQ files of the sequenced directories have been deposited in the GEO and are accessible with serial number GSE147185.

Bioinformatics analysis of HTS results for virus diagnosis was performed using the CLC Genomic Workbench (Qiagen). After quality control of the cut and scans, the QC report was generated using the CLC Genomic Workbench embedded protocols. MEGA7 was used for phylogenetic analysis of viral sequences (Kumar et al, 2016). The evolutionary story was subtracted by the “neighbor-joining” method (Saitou and Nei, 1987). Evolutionary distances were calculated by the Jukes-Cantor method (Jukes and Cantor, 1969)

3. RESULTS

Our laboratory experiment demonstrated that the millets tested were stable to germination from 20 degrees Celsius to 35 degrees Celsius, and the germs were not damaged at elevated temperatures, nor did we experience any decline in their development. *Panicum riparium* samples showed a slight decrease in germination, but this was not statistically significant. No significant difference was detected between the number of seeds germinated at the studied temperatures.

Among the species studied, *P. miliaceum* subsp. *miliaceum* proved to be the strongest competitor, similarly to *P. miliaceum* subsp. *ruderale* subspecies, however, the *P. riparium* species has a much weaker growth and therefore did not prove to be a strong competitor in the experiment. Between the two *P. miliaceum* subsp. *ruderale* were significant differences The development of maize was influenced differently, so our result confirms the effect of environmental factors in competition.

The two *P. miliaceum* subsp. *miliaceum* sample showed a significant difference in both study years. In the case of the Keszthely sample, the fresh shoot weight decreased continuously, in the culture vessel containing most millet the shoot weight of maize was 62% lower than the control. In the case of the Tarján sample, by increasing the number of individuals, the fresh shoot weight was 48% higher in the pots containing 10 millets than in those containing 5 millets. This is due to the phenomenon of intraspecific competition.

Between the two *P. miliaceum* subsp. *ruderale* significant differences were also found. In the Keszthely sample, the fresh shoot weight increased with the change of the number of individuals, but decreased in the 20 millet cultivars, while in the Pusztadobos sample it decreased sharply compared to the control (1.98 g compared to 10.87 g of the control, 20 0.59 g of fresh shoot weight). A similar trend was observed when measuring air-dry shoot mass.

In summary, *P. miliaceum* subsp. *miliaceum* and *P. miliaceum* subsp. *ruderale* has no negative allelopathy to maize based on our studies. The germination % in the control population was 87%. With the 2.5% *Panicum miliaceum* subsp. *miliaceum* leaf extract 90% of the seeds were germinated, 90% of seeds treated with 5% extract, and 94% of seeds treated with 7.5% extract germinated. Cultivated millet stimulated maize germination at all three concentrations, although this stimulatory effect was not statistically significant. *Panicum miliaceum* subsp. *ruderale* leaf stem extracts had a positive effect on the germination of maize seeds. For the 2.5% and 7.5% extracts, the increase was small, but for the 5% extract a significant increase of 6.5% was observed. The shoot length of the corn seedling was slightly affected, this is negligible. It promoted root development at low concentrations, while at higher concentrations it inhibited development. All concentrations of weed leaf stem extract had a positive effect on germination. The 7.5% extract increased germination the least. *P. riparium* has negative allelopathy based on our studies. From a practical point of view, this is an extremely important achievement, as research on the danger of P. riparium is a relatively “new” species in Hungary (Király et al, 2009), only from the last few years. The 2.5% leaf stem extract of *Panicum riparium* significantly inhibited the shoot formation of maize seedlings.

In terms of root length, the 2.5% extract did not cause a significant change, a decrease of 0.2% was observed compared to the control. The average root length of the seeds treated with the 5% extract was slightly higher than that of the control plants. This concentration resulted in a 4.1% increase in root length. The average root length of the seeds treated with 7.5% extract was 68.78 mm, which was 3.3% smaller on average than in the control plants. In my opinion, further investigation of *P. riparium* is definitely warranted.

In a pot experiment, *Panicum miliaceum* subsp. *miliaceum* plant parts fell short of the germination percentage from the control group. In this case, germination decreased the least in the 5% soil-plant residue treatment, and in the Petri cup experiment, the 5% solution had the greatest stimulating effect.

A *Panicum miliaceum* subsp. *miliaceum* had a negative effect on both root mass and shoot mass. Treatment alone at 7.5% yielded higher shoot weight than control plants, however, the difference was not statistically significant.

In the samples we collected (using the DAS ELISA serological method), infections by WSMV, WDV, and BSMV were more significant compared to BYDV and BstMV viruses. The presence of BMV could not be confirmed from the samples examined.

Our small RNA study points out that millet has a viral reservoir role, which is why it is important to control it in our fields. We were able to confirm the presence of AWSMV at both sampling sites, and we were the first in Hungary to detect the presence of BYSMV (Barley yelloy striate mosaic virus) and BGV (Barley virus G).

4. NEW SCIENTIFIC RESULTS

Based on the results of laboratory in vitro and pot experiments performed during the four experimental years, I conclude the following new scientific results:

1) The germination of *P. miliaceum* subsp. *miliaceum,* and *P. miliaceum* subsp. *ruderale* is not affected negatively by the temperature range of 20-35 C, rising temperatures do not affect the germination of the two subspecies. Germination of *P. riparium* is not significantly reduced by rising temperature.

2) Based on our pot competition experiments, *P. miliaceum* subsp. *miliaceum* and *P. miliaceum* subsp. *ruderale* species tested under controlled conditions in a pot experiment proved to be a strong competitor to maize, showing both plant growth and incorporated dry matter content.

   The growth and development of maize could not be significantly affected by *P. riparium*.

3) We have demonstrated by laboratory allelopathy experiment that *P. miliaceum* subsp. *miliaceum*, and *P. miliaceum* subsp. *ruderale* shoot extract does not have negative allelopathy to maize germination and initial growth, during the initial development we observed a shoot growth stimulating effect.

   P. riparium has an anti-germination effect and significantly inhibits the shoot and root growth of maize.

   In the allopathic pot experiment, *P. miliaceum* subsp. *miliaceum* significantly inhibited maize germination as well as shoot and root growth.

4) In the virological examination of P. miliaceum (DAS ELISA) we proved that several grain viruses can infect the species. Of the 45 samples tested,

19 were found to be infected. In the samples we collected, infections by WSMV (10), WDV (6), and BSMV (6) were significant compared to BYDV (2) and BstMV (1) viruses. We also diagnosed complex infections.

5) The small RNA studies proved that *Panicum miliaceum* appears as a new host plant of WSMV in Hungary.

6) We managed to detect different strains of BYSMV (Barley yellow striate mosaic virus), which have not been diagnosed in Hungary yet. Furthermore, we managed to detect Barley virus G, which has not been described in Hungary so far.

5. SCIENTIFIC PUBLICATIONS IN THE TOPIC OF THE DISSERTATION

5.1. Journal article in English-language relate to the subject of the dissertation

Pásztor Gy., Nádasy E. (2016): Effect of temperature on germination of different panic weed species. GEORGIKON FOR AGRICULTURE: A MULTIDISCIPLINARY JOURNAL IN AGRICULTURAL SCIENCES 20. (1) 163-170.

Pásztor Gy., Szabó R., Béres I., Takács A., Nádasy E. (2016): Common millet (*Panicum miliaceum*)-a new weed problem in Hungary. Review Part 1 and 2. NÖVÉNYTERMELÉS 65. Suppl. 215-222.

Pásztor Gy, Szabó R., Takács A., Henézi Á., Nádasy E. (2017): The natural viral infections of the weedy *Panicum miliaceum* (L.). COLUMELLA: JOURNAL OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES 4. Suppl 1. 35-38.

Pásztor Gy., Nádasy E., Takács A. (2018): The implications of *Panicum miliaceum* in the viral epidemiology of cereals. In: Henning, Nordmeyer; Lena, Ulber (szerk.) 28. Deutsche Arbeitsbesprechung über Fragen der Unkrautbiologie und -bekämpfung : 28th German Conference on Weed Biology and Weed Control. Braunschweig, Németország : Julius Kühn-Institut. 469-472.

Pásztor Gy., Galbacs Zs. N., Kossuth T., Demian E., Nádasy E., Takács A. P., Varallyai E. (2020): Millet could be both a Weed and serve as a Virus Reservoir in Crop Fields. Plants, Special Issue “New findings in plant virology toward guiding rational control strategies. **(IF: 2.762)**

5.2. Journal article in Hungarian-language relate to the subject of the dissertation

Pásztor Gy., Nádasy E. (2015): Magyarországi kölesfajok kompetíciós képességének vizsgálata kukoricában. GEORGIKON FOR AGRICULTURE: A MULTIDISCIPLINARY JOURNAL IN AGRICULTURAL SCIENCES 19. (1) 146-153.

Pásztor Gy., Henézi Á., Grúz A., Kormos É., Szabó R., Gáborjányi R., Kazinczi G., Nádasy E., Takács A. P. (2016): A köles (*Panicum miliaceum* L.) mint gyomnövény természetes vírusfertőzöttségének vizsgálata. NÖVÉNYVÉDELEM 52 (1). 44-47.

Pásztor Gy., Nádasy E. (2016): A köles fajok (*Panicum* spp.) hazai elterjedése, biológiája és a védekezés lehetőségei. MAGYAR GYOMKUTATÁS ÉS TECHNOLÓGIA 17. (2). 3-14.

Pásztor Gy., Nádasy E. (2016): A kukorica és a kölesfajok kompetíciós képességének tanulmányozása tenyészedényes kísérletben. GEORGIKON FOR AGRICULTURE: A MULTIDISCIPLINARY JOURNAL IN AGRICULTURAL SCIENCES 20. (1) 113-119.

Szabó R., Fábián G., Menyhárt L., Pásztor Gy., Cseh E. (2017):A termesztett köles, mint gyomnövény (*Panicum miliaceum* L.) csírázásának gátlása növényi kivonatokkal. GEORGIKON FOR AGRICULTURE: A MULTIDISCIPLINARY JOURNAL IN AGRICULTURAL SCIENCES 21. (1) 145-152.

Henézi Á., Pásztor Gy., Takács A. (2017): A köles (*Panicum miliaceum* L.) mint gyomnövény szerepe a gabonavírusok epidemiológiájában. GEORGIKON FOR AGRICULTURE: A MULTIDISCIPLINARY JOURNAL IN AGRICULTURAL SCIENCES 21. (1) 114-118.

Pásztor Gy., Lőrincz D., Szabó R., Nádasy E. (2019): Különböző köles fajok kompetíciós hatása kukoricában, GEORGIKON FOR AGRICULTURE: A MULTIDISCIPLINARY JOURNAL IN AGRICULTURAL SCIENCES 23. (1), 62-69.

5.3. Oral presentations, posters in English-language (abstract)

Pásztor Gy., Szabó R., Béres I., Takács A., Nádasy E. (2014): Common millet (*Panicum miliaceum*)-a new weed problem in maize in Hungary. In: Ahmet, Uludag; Ayse, Yazlik; Khawar, Jabran; Süleyman, Türkseven; Uwe, Starfinger (szerk.) 8th International Conference on Biological Invasions from understanding to action : Proceedings Canakkale, Törökország : Canakkale Onsekiz Mart University, 221.

Szabó R., Pásztor Gy. (2018): Study on the allelopathic effect of *Amaranthus retroflexus, Datura stramonium* and *Panicum miliaceum* on the germination of maize. In: Dusan, Kovacevic (szerk.) Agrosym 2018: Book of proceedings: 9th International Scientific Agriculture Symposium, East Sarajevo, Bosznia-Hercegovina: Faculty of Agriculture East Sarajevo, 889-894

Pásztor Gy., Szabó R., Henézi Á, Nádasy E., Takács A. (2018): The role of the invasive weed *Panicum miliaceum* in the epidemiology of cereal viruses. In: NEOBIOTA 2018: 10th International Conference on Biological Invasions: New Directions in Invasion Biology: Programme and book of abstracts

Pásztor Gy., Nádasy E., Szabó R. (2019): The competitive ability of different millet species on maize under field conditions. In: Kende, Z.; Bálint, Cs.; Kunos, V. (szerk.) 18th Alps-Adria Scientific Workshop: Alimentation and Agri-environment : Abstract book. Gödöllő, Magyarország : Szent István Egyetem Egyetemi Kiadó, 126-127.

5.4. Oral presentations, posters in Huungarian-language

Pásztor Gy., Nádasy E. (2016): Különböző kölesfajok allelopatikus hatásának vizsgálata kukoricában. In: Horváth, J.; Haltrich, A.; Molnár, J. (szerk.) 62. Növényvédelmi Tudományos Napok. Budapest, Magyar Növényvédelmi Társaság, 66-66.

Pásztor Gy., Nádasy E., Takács A.P (2019).: Egy Zala megyei kölespopuláció természetes gabonavírus-fertőzöttségének vizsgálata. In: Haltrich, A.; Varga, Á. (szerk.) 65. Növényvédelmi Tudományos Napok. Budapest, Magyar Növényvédelmi Társaság Elnöksége, 56.

5.5. Other scientific articles in English-language

Pásztor Gy., Szabó R., Nádasy E. (2017): Investigation of the phytotoxic effect of herbicide 2,4-D with hormonal function on winter wheat. COLUMELLA: JOURNAL OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES 4. Suppl 1. 163-168.

Nádasy E., Pásztor Gy., Béres I., Szilágyi G. (2018): Allelopathic effects of *Abutilon theophrasti, Asclepias syriaca* and *Panicum ruderale* on maize. In: Henning, Nordmeyer; Lena, Ulber (szerk.) 28. Deutsche Arbeitsbesprechung über Fragen der Unkrautbiologie und -bekämpfung: 28th German Conference on Weed Biology and Weed Control. Braunschweig, Németország, Julius Kühn-Institut, 454-458.

5.6. Other scientific articles in Hungarian-language

Szabó R., Fábián G., Menyhárt L., Nádasy E., Cseh E., Pásztor Gy. (2016): Gyógy- és fűszernövény kivonatok hatása a termesztett köles, mint gyomnövény (*Panicum miliaceum* L. subsp. miliaceum) csírázására. MAGYAR GYOMKUTATÁS ÉS TECHNOLÓGIA 17. (2) 25-34.

Pásztor Gy., Nádasy E., Cserpes M., Takács A. P. (2019): A *Solanum nigrum* szerepe a burgonyavírusok terjedésében GEORGIKON FOR AGRICULTURE: A MULTIDISCIPLINARY JOURNAL IN AGRICULTURAL SCIENCES 23. (1) 36-40.