

GENETIC RESOURCES OF MILK THISTLE [SILYBUM MARIANUM (L.) GAERTN.]

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ABSTRACT

The aim of this work was to evaluate various genetic resources of milk thistle (*Silybum marianum* [L.] Gaertn.) in 2013 at two localities (Citonice and Olomouc). Genetic resources were assessed according to the approved minimal set of the descriptors. The variability of the morphological and biological characters is significantly influenced by the locality. The lowest variability had the characters of the seeds, intensity of branching and number of inflorescences on the plants.

Key words: genetic resources, Silybum marianum, minimal set descriptors

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INTRODUCTION

Milk thistle (*Silybum marianum* [L.] Gaertn.) is annual, rarely biennial medicinal plant, belonging into the family *Asteraceae*. It originates from the Mediterranean region, often wild in whole Europe, also in the mountain regions (Moudrý 2011). According to the Situational Report from 2012, the growing areas of Milk thistle still increase. Association PELEROC CZ reports that in the years 2011 and 2012, Milk thistle was cultivated on approximately 5 000 Ha with estimated yield 0.5 t.ha⁻¹. Milk thistle is produced for the content of silymarin complex in fruits (achenes). This active substance positively affects liver tissue and also is used in the therapy of some tumor diseases (Habán 2009). The risk is represented by the availability of the declared and described propagation material (seeds). Domestic and also foreign growers are highly interested in Milk thistle production. Silymarin complex is isolated from the biomass after pressing the achenes. Fatty oil is a side product and has the use in pharmaceutical cosmetology. The use of Milk thistle in feed industry is developed. All these activities influence the increase of growing area of Milk thistle directly.

MATERIAL AND METHODS

In frame of the evaluation in 2013, the characters of various genetic resources of Milk thistle (Silybum marianum [L.] Gaertn.) using the minimal set of descriptors were evaluated, approved by the Plant Genetic Resources Board of the Czech republic (12/2012). Table 1 shows the list of 15 genetic resources of Milk thistle grown on two localities, in Citonice (dist. Znojmo) and in Olomouc (Crop Research Institute, v.v.i.).

Tab. 1 List of the genetic resources of Milk thistle on two localities

| Genetic resources | Locality Citonice | Locality Olomouc |
|-------------------|----------------------|----------------------|
| 1. | A8400001 CZE | A8400001 CZE |
| 2. | A8400002 DEU | A8400002 DEU |
| 3. | A8400004 FRA | A8400004 FRA |
| 4. | A8400007 CZE 'SILYB' | A8400007 CZE 'SILYB' |
| 5. | A8400008 BEL | A8400008 BEL |
| 6. | A8400009 POL 'SILMA' | A8400009 POL 'SILMA' |
| 7. | 4067 CZE | 4067 CZE |
| 8. | 4385 HUN | 4385 HUN |
| 9. | 4386 HUN | 4386 HUN |
| 10. | 4388 HUN | 4388 HUN |
| 11. | 4389 HUN | 4389 HUN |
| 12. | 4395 DEU | 4395 DEU |
| 13. | 4398 DEU | 4398 DEU |
| 14. | 4443 SER | 4443 SER |
| 15. | 4444 AUT | 4444 AUT |

The first experimental locality is situated in the village Citonice, Znojmo region in the Southmoravian Region. The place is in altitude of 360 m.a.s.l. Climatic region is characterized as warm, mildly humid. The terrain slope is middle. The soil is deep, without the skeleton, the chernozem type (www.sowac-gis.cz, 2013). The soil was prepared in a usual way and on 18. 4. 2013, 30 seeds of each resource were planted in a density of 40×50 cm. The plant growth and development were monitored weekly. The canopy was irrigated during the vegetation period till the flowering stage of terminal bud. Three times per vegetation the manual weeding was performed.



The plants were monitored also for the attack of the pests and diseases. The pellets against Spanish slug (*Arion lusitanicus*) were applied (VANISH SLUG PELLETS, metaldehyde, 1.5 g/m², applied during the vegetation each 7 days). The aphid (*Aphis fabae*) in the stage of flowering of the terminal bud appeared. There was not performed any treatment against them, because no insecticide is registered in Milk thistle.

Second experimental locality is situated in Holice, a part of Olomouc, in Olomoucký Region. The place is located in 250 m.a.s.l. Climatic region is characterized as warm, mildly humid. The terrain slope is plane. The soil is deep, mainly without the skeleton, type of cambisol ($\underline{www.sowac-gis.cz}$, 2013). After the soil preparation, the seeds were sown into double rows (18.4.2013) and subsequently the canopy was cleaned into the density of 40×50 cm. The inter rows were distanced of 1 m, because of mechanical weeding and manual weeding and evaluation. The plants were attacked by Spanish slug (Arion lusitanicus), but the moluscocides were not applied. Also the aphids (Aphis fabae) were appeared, in the stage of terminal bud flowering. There was not performed any treatment against them, because no insecticide is registered in Milk thistle.

The morphological and biological characters according the minimal set of the descriptor are mentioned in this article.

- Morphological characters: plant (habit, height, plant width, intensity of branching, length of flower stem), leave (length, width, depth of dents, degree of marbling), inflorescence (diameter, amount, colour of flowers), achene – fruit (length, width, colour, yield, weight of 1000 seeds).
- 2) Biological characters: vegetation period (number of days from the sowing till the beginning of formation of generative organs, number of days from the sowing till the beginning of flowering the terminal bud, number of days from the sowing till the maturity of the terminal inflorescence), susceptibility to biotic stresses (*Botrytis cinerea*, Septoria silybi, Alternaria silybi, Fusarium oxysporium, Erysiphe communis).
- Yield characters: They will be evaluated in November 2013 by the chosen reference methods (silymarin complex – HPLC, oil content – Soxhlet extraction, fatty acids ratio in oil – GC).

The harvest was performed manually (by the garden scissors). Firstly the terminal inflorescences were harvested, then, the secondary. The achenes were trash out from the inflorescences manually or by stable treshing machine Haldrup LT-20. In 2014, the same 15 genotypes will be sown again in two localities (Citonice, Olomouc) to have two years results. Parallely, the perspective genotypes will be chosen for future breeding.

RESULTS AND DISCUSSION

Tab. 2 Average values of chosen characters

| Locality | Genetic | Plant | Intensit | Number | Lengt | Width | Yiled | WTS |
|----------|-----------|--------|----------|----------|-------|-------|--------|-------|
| | resources | height | y of | of | h of | of | of | (g) |
| | | (cm) | branchi | inflores | seeds | seeds | achen | |
| | | | ng | cences | (mm) | (mm) | es (g) | |
| Olomouc | A8400001 | 86.70 | 9.70 | 5.70 | 6.92 | 3.28 | 6.53 | 24.53 |
| | CZE | | | | | | | |
| | A8400002 | 77.90 | 9.40 | 3.90 | 7.12 | 3.20 | 7.93 | 24.12 |
| | DEU | | | | | | | |
| | A8400004 | 102.30 | 12.50 | 7.40 | 7.00 | 3.13 | 4.71 | 17.71 |



| | EDA | 1 | l | I | | 1 | 1 | 1 |
|----------|------------------------|--------|----------|----------|--------|-------|--------|--------|
| | FRA | 04.20 | 15.00 | 12.00 | 7.17 | 2.26 | 7.07 | 10.61 |
| | A8400007 CZE'SILYB' | 84.20 | 15.80 | 13.90 | 7.17 | 3.26 | 7.07 | 18.61 |
| | A8400008 BEL | 30.88 | 2.89 | 1.75 | 0.00 | 0.00 | 0.00 | 0.00 |
| | A8400009 | 56.80 | 12.50 | 8.50 | 7.12 | 3.40 | 2.14 | 19.35 |
| | POL 'SILMA' | | | | | | | |
| | 4067 CZE | 105.80 | 23.80 | 10.70 | 7.27 | 3.36 | 7.29 | 21.27 |
| | 4385 HUN | 90.90 | 19.30 | 8.00 | 7.06 | 3.34 | 2.49 | 16.17 |
| | 4386 HUN | 68.40 | 6.50 | 4.80 | 7.22 | 3.55 | 3.87 | 23.79 |
| | 4388 HUN | 84.40 | 11.70 | 10.20 | 6.95 | 3.46 | 9.22 | 21.48 |
| | 4389 HUN | 90.70 | 17.00 | 11.70 | 6.92 | 3.43 | 17.56 | 21.21 |
| | 4395 DEU | 91.30 | 10.10 | 6.60 | 6.89 | 3.20 | 3.69 | 17.83 |
| | 4398 DEU | 40.50 | 5.70 | 3.80 | 7.06 | 3.32 | 1.11 | 16.88 |
| | 4443 SER | 39.63 | 5.44 | 2.00 | 6.63 | 3.34 | 0.97 | 0.00 |
| | 4444 AUT | 72.50 | 10.70 | 8.30 | 7.02 | 3.33 | 8.24 | 17.61 |
| | Mean values | 74.86 | 11.54 | 7.15 | 6.56 | 3.11 | 5.52 | 17.37 |
| | SEM* | 5.4976 | 1.3044 | 0.7297 | 0.3354 | 0.16 | 1.6071 | 1.4160 |
| Citonice | Genetic | Plant | Intensit | Number | Lengt | Width | Yiled | WTS |
| | resources | height | y of | of | h of | of | of | (g) |
| | | (cm) | branchi | inflores | seeds | seeds | achen | (8) |
| | | , , | ng | cences | (mm) | (mm) | es (g) | |
| | A8400001 | 159.80 | 17.10 | 6.80 | 6.90 | 3.17 | 26.86 | 23.12 |
| | CZE | | | | | | | |
| | A8400002 | 162.90 | 14.80 | 4.50 | 7.74 | 3.38 | 14.48 | 24.86 |
| | DEU | | | | | | | |
| | A8400004 | 147.20 | 9.20 | 3.80 | 7.27 | 3.24 | 10.11 | 24.76 |
| | FRA | | | | | | | |
| | A8400007 | 148.40 | 20.10 | 5.00 | 7.37 | 3.14 | 28.60 | 23.63 |
| | CZE'SILYB' | | | | | | | |
| | A8400008 BEL | 112.80 | 15.30 | 4.10 | 7.01 | 3.08 | 19.02 | 22.15 |
| | A8400009 | 124.60 | 13.20 | 7.80 | 7.15 | 3.40 | 29.00 | 26.12 |
| | POL | 121.00 | 13.20 | 7.00 | 7.15 | 5.10 | 25.00 | 20.12 |
| | 'SILMA' | | | | | | | |
| | 4067 CZE | 133.50 | 19.80 | 6.80 | 7.49 | 3.28 | 17.07 | 22.43 |
| | 4385 HUN | 171.50 | 12.20 | 6.90 | 7.24 | 3.32 | 8.64 | 27.14 |
| | 4386 HUN | 173.00 | 23.60 | 8.80 | 7.29 | 3.59 | 34.24 | 25.86 |
| | 4388 HUN | 162.40 | 15.20 | 9.70 | 7.14 | 3.38 | 31.50 | 26.11 |
| | 4389 HUN | 123.00 | 9.30 | 8.60 | 6.97 | 3.55 | 16.98 | 24.60 |
| | 4395 DEU | 138.00 | 13.70 | 7.60 | 7.39 | 3.51 | 21.63 | 27.48 |
| | 4398 DEU | 135.20 | 23.50 | 6.60 | 7.68 | 3.42 | 20.89 | 24.17 |
| | 4443 SER | 125.90 | 14.20 | 6.80 | 7.51 | 3.34 | 16.95 | 23.55 |
| | | | | | | | | |



| 4444 AUT | 160.00 | 17.10 | 4.60 | 7.38 | 3.53 | 20.17 | 28.04 |
|-------------|--------|--------|--------|--------|------|--------|--------|
| Mean values | 145.21 | 15.89 | 6.56 | 7.30 | 3.36 | 21.08 | 24.93 |
| SEM* | 5.4976 | 1.3044 | 0.7297 | 0.3354 | 0.16 | 1.6071 | 1.4160 |

^{*}SEM = Standard Error of Mean

Table 2 shows the results of chosen morphological characters. The values were tested by one way ANOVA, for the factor locality. The plants were statistically different on these localities. The site

had significant effect on general habit, height, intensity of branching and also on yield characters. The plants in Olomouc were dry quite early, because of very dry and warm weather, as was different then in Citonice. Contrary, the plants in Citonice were massive and intensively branched.

CONCLUSIONS

The results can be concluded as follows:

- The plants in Citonice had very good growth and development from the beginning which was resulted in the characters. The plants were massive and intensively branched, and this was resulted in a higher number of inflorescences and higher yield from the plots. The plants in Olomouc grown well in the beginning of the vegetation, but they suffered by the draught and slowly died. This decreased the yield of the resources.
- 2) The aphids (Aphis fabae) and Spanish slug (Arion lusitanicus) were appeared on both localities. The plants in Citonice were attacked by some pathogens: Erisiphe communis and slightly by Septoria silybi and Alternaria silybi. In Olomouc, there were no pathogens recorded.
- 3) Preliminary, we can say, the traits with the lowest variability include the characters of the seeds, number of inflorescences and intensity of branching. But, for the detailed results, we need to continue the future research and add more data to the statistical testing.
- Yield and chemical characters will be assessed this winter at MENDELU, Opava and at UNICATT (Universitá Cattolica del Sacro Cuore, Piacenza, Italy).

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