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EVALUATION OF THE GROWTH DYNAMICS AND MORPHOLOGICAL CHARACTERISTICS OF GENETIC SOURCES OF *SILYBUM MARIANUM* (L.) GAERTN.

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Abstract

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The aim of this work was to evaluate the growth dynamics and selected morphological characteristics of genetic sources of milk thistle (*Silybum marianum* L. Gaertn.) for the further development of the minimal set of descriptors. Milk thistle is grown in the Czech Republic for its achenes; however, the quality of achenes can be reduced by many factors, by the occurrence of fungal pathogens mainly. The growth dynamics and morphological characteristics of milk thistle during the vegetation period in the years 2010–2013 at two localities were evaluated. The cluster analysis of the data showed the similarity for some of the accessions and confirmed the dependence of the data value to the climatic conditions. Source from Serbia, Slovakia, Romanian variety 'De Prahova', German accessions SIL 2 and SIL 8, Hungarian accesion RCAT 040360 DDR and Czech variety 'Silyb' seem to be promising genetic sources from the viewpoint of growth and development in the Czech Republic.

Keywords: genetic source, *Silybum marianum*, growth dynamics, morphological characteristics

INTRODUCTION

Milk thistle (*Silybum marianum* L. Gaertn.), an important medicinal plant from the *Asteraceae* family, is native to the Mediterranean region, gradually spread to the warm sunny areas of many countries. While in some states of North- and Central America, Africa, Australasia and in the Middle and Near East the milk thistle is considered a problematic invasive weed (Holm *et al.*, 1997; Montemurro *et al.*, 2007). In Central and East European countries, Austria, Germany, Egypt, China and Argentina it is commercially cultivated as a medicinal plant. The anthodia provide the seeds with a valuable number of flavonolignans (silymarin), consists of several compounds such as silybin, silychristin, silydianin. These active ingredients are strong antioxidants and play an important role in the therapy of liver diseases, intoxications, acute and chronic viral hepatitis, diabetes and have some oncology indications (Habán *et al.*, 2009; Bhattacharya, 2011). In addition, the seeds contain relatively high levels of fatty oil with prevailing unsaturated fatty acids, linoleic and oleic mainly (El-Mallah *et al.*, 2003; Ghavami and Ramin, 2008). During the years 2010–2013, milk thistle was cultivated on 4 500–5 000 ha in the Czech Republic.

For a detailed description of genetic sources, genus- or species-specific classifiers are created that determine the rules for the evaluation of descriptive characteristic expressions. In the case of plants for which the international classifiers haven't been worked out yet, the curator of that respective plant collection is mandated to develop minimal sets of descriptors in cooperation with the administrator of the database Evidence of Plant Genetic Resources in the Czech Republic (Faberová, 2011). Currently, a minimal set of descriptors was approved for milk thistle in the Czech Republic and it will be further developed over the next few years. The aim of this work was to evaluate the growth dynamics and selected morphological characteristics of genetic sources of milk thistle.

MATERIAL AND METHODS

During the years 2010–2013, the growth dynamics was evaluated (number of plants emerging over 4 periods); morphological characteristics (plant height in full flowering, the number of harvestable anthodia per plant). Selected flower buds were isolated before flowering for the autogamy level verification. The design of the experimental plots describes the Tab. II.

As for the experiment during the year 2011, the growth was ploughed for technical reasons in the phase of 10 true leaves. Thus, there isn't any relevant data for the year 2011. To analyze how the genotypes are clustered according to their characteristics, the hierarchical clustering method was applied. Since there are plenty of missing values in our dataset, the regression imputation was used in the first step. It is based on an iterative procedure where in each step one variable is used as a response variable and the remaining variables serve as the regressors. In the next, due to different units for measuring plant height (cm) and the number of anthodia (pcs), the dataset was mean-centered and scaled. Afterwards, the hierarchical clustering was applied and the result is displayed using cluster dendrogram. All the computation was done using R-software.

I: *Genetic sources of milk thistle evaluated in individual years*

No.	Genetic source	2010	2012	2013
1	Serbia, regional variety (source: Institut za proučavanje lekovitog bilja „Dr Josif Pančić“, Beograd, Serbia)	+	+	+
2	Slovakia (Research Institute of Plant Production, Piešťany)	+		
3	Bulgaria, regional variety (source: Institute of Plant Genetic Resources, K. Malkovo, Bulgaria)	+	+	
4	Austria (BVAL – 901047)	+		
5	Austria (BVAL – 901578 'De Prahova') – (variety, Romania, source: Österreichische Agentur für Gesundheit und Ernährungssicherheit, Austria)	+	+	+
6	Germany (SIL 1)	+		
7	Germany (SIL 2)	+		+
8	Germany (SIL 4)	+		
9	Germany (SIL 10)	+		
10	Hungary (RCAT 040358 HUN)	+		+
11	Hungary (RCAT 040360 DDR)	+		+
12	Hungary (RCAT 074546 ESP)	+		+
13	Hungary (RCAT 077005 DEU)	+		
14	Czech Rep., 'Silyb' (registered variety, IREL, s. r. o., CZE)	+	+	+
15	Germany (SIL 8)	+		+

+ The genetic source was sown in a given year

II: *Characterization of the experiments (2010–2013)*

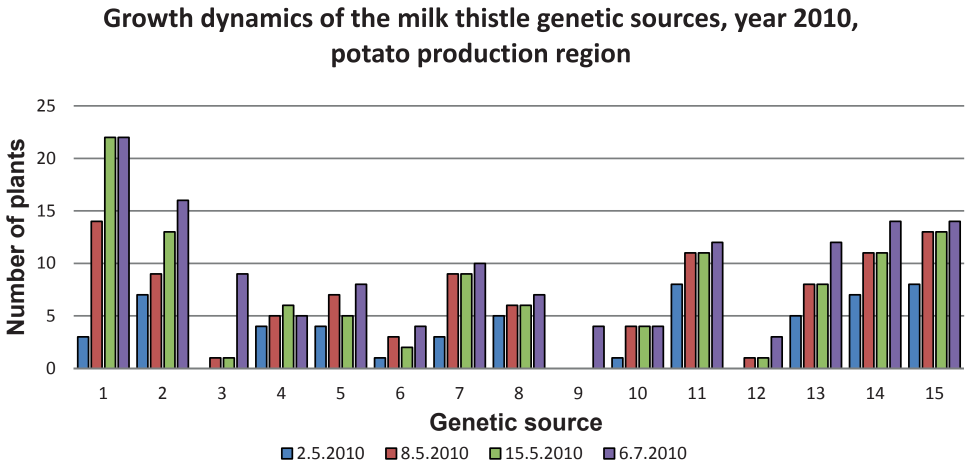
Year	Production region	Date of sowing/number of seeds sown/row spacing	Treatment
2010	Potato production region, mildly warm region, 450 m a.s.l.	18. 4. 2010 25 seeds 0.50×0.25 m	manual hoe-work, no irrigation no fungicides, no herbicides
2012	Maize production region, mildly dry region, 360 m a.s.l.	14. 4. 2012/ 'De Prahova' 21. 4. 2012 20 seeds 0.50×0.25 m	manual hoe-work, regular irrigation pirimicarb – aphids (1.5 g/3 l) metaldehyde – moluscocide (12 g per 8 m^2)
2013	Maize production region, mildly dry region, 360 m a.s.l.	18. 4. 2013 30 seeds 0.50×0.40 m	manual hoe-work, regular irrigation metaldehyde – moluscocide (1.5 g/m^2)

RESULTS AND DISCUSSION

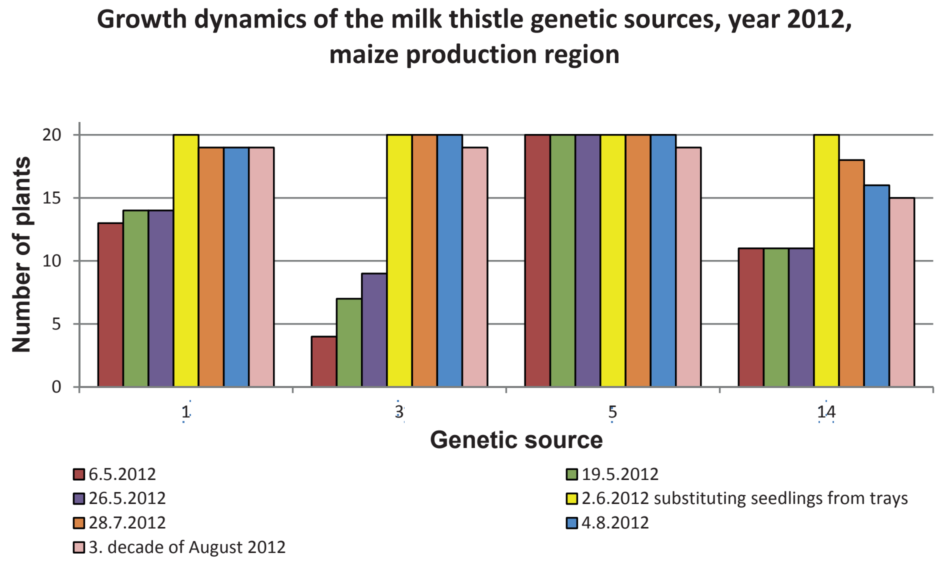
Evaluation of the Emerging Dynamics

Very uneven emerging of the milk thistle genetic sources was observed at the locality PPR in 2010. 14 days after sowing the emerging varied in the range of 0–32%; the genotypes 3, 9 and 12 didn't emerge in the first term (Fig. 1). In the second evaluation term, i.e. after 20 days, significant onset of emerging was observed for all genotypes except the accession 9. The quickest emerging accessions were 1, 7 and 15. One month after the seeding (the third term) the highest emerging observed was 88% for the accession 1. For most genotypes the emerging varied around 50%. In the last term of evaluation the highest amount of emerged plants (88%) were observed for the accession 1 and 64% for the accession 2. The accessions 4, 6, 9, 10 and 12 showed a very bad level of emerging, total number of plants were < 16%. The total number of emerged plants didn't achieve 100% for even one accession. The accessions 3 and 9 started to emerge in July. These are probably later genetic sources that require a higher soil and air temperature, which wouldn't guarantee the required yield in our conditions.

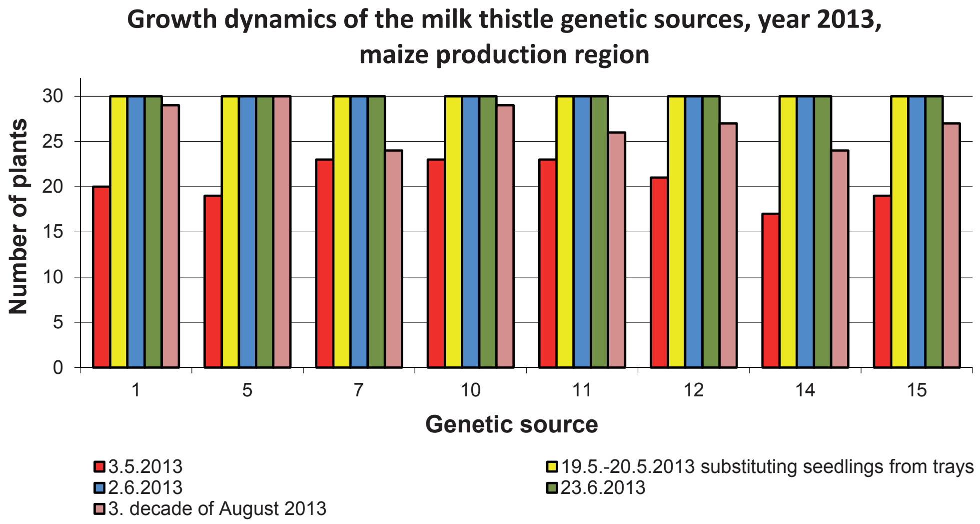
Growth dynamics of 4 selected genotypes: the accessions 1, 3, 5 and 14 sown at the locality MPR in 2012 is shown in the Fig. 2. The quickest emerging plants were the Serbian source and variety 'De Prahova', for which the 100% emerging was observed only 15 days after the sowing. Due to the very low emerging of the genetic sources and because of the need of plants for the descriptor evaluation, some pre-planted seedlings were planted at the plot on 2. 7. 2012. In July the plants were attacked by mildew and started to dry. Variety 'Silyb' (14) showed a very sensitive reaction where there were only 15 harvestable plants at the time of



1: *The rate of emerging – growth dynamics of the milk thistle genetic sources, year 2010, potato production region (PPR)*



2: *The rate of emerging – growth dynamics of selected milk thistle genetic sources, year 2012, maize production region (MPR)*



3: *The rate of emerging – growth dynamics of selected milk thistle genetic sources, year 2013, maize production region (MPR)*

harvest (i.e. 75% of all plants). Variety 'De Prahova' was relatively resistant against the mildew attack.

The best emerging genetic sources sown in 2013 were 7, 10 and 11 with 23 emerged plants (i.e. 76.7%). The accession 14 showed the lowest amount of emerged plants, 17 (i.e. 56.7%). In spite of the fact that pre-planted seedlings were planted instead of seeds that didn't rise well in the previous year (19.–20. 5. 2013), only the genetic source 5 had 100% of the plants at the time of harvest. The lowest amount of harvestable plants (24 plants, i.e. 80%) were found for the accession 7.

Evaluation of Milk Thistle Morphological Characteristics

The average height of the milk thistle plants in 2010 was 61–129 cm, the plants were higher and branched out less, while at the other locality in 2012 the height was 71–98 cm and the plants had a more compact, sessile habitus. The highest plants from all three years were observed in 2013 where the height of plants varied in the range of 126–173 cm. The influence of the environment and the year is evident.

Apart from the total plant height, the number of fully developed harvestable anthodia with ripe, developed and typically dark brown brindled achenes was evaluated at both localities. Pinkish beige achenes are not developed, usually infested with fungi, have bad germination and don't contain needed amount of requested active compounds.

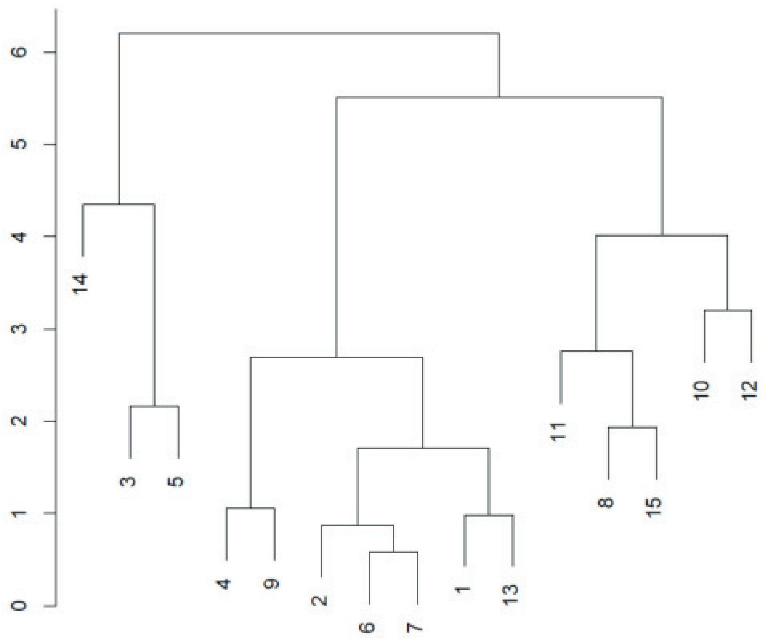
The number of harvestable anthodia showed a positive correlation with the plant height in all three years (Tab. III). Also Andrzejewska and Sadowska (2008), Ottai and Abdel-Moniem (2006) and Gresta *et al.* (2006) have reported that seed yield per plant, number of anthodia per plant and plant height showed a high positive correlation. Generally, at both localities and in all years, the highest number of harvestable anthodia and thus the assumption of highest yield of achenes was shown by the German genetic source SIL8, Czech variety Silyb, Hungarian RCAT 040360 DDR, 074546 ESP and Serbian landrace. The lowest number of harvestable anthodia per plant on average, was found for Austrian BVAL – 901047, Hungarian RCAT 077005 DEU, Romanian variety 'De Prahova'.

Fig. 4 shows the dendrogram, a classification of studied accessions of milk thistle. We can see three basic groups. The first cluster, separated from two others, includes the genetic resources from Bulgaria, Romania ('De Prahova') and Czech variety 'Silyb', while the last one differs from the other two southern European sources. The second cluster contains 7 very similar genetic sources at the same distance, German, Austrian accessions, Serbian landrace and Slovakian accession. There is one interesting fact: There was a reason to suppose that Slovakian accession 2 is also the variety 'Silyb', although its origin was not mentioned, but the results from the dendrogram did not confirm that. The third cluster includes 5 accessions grouped into two small clusters. Three accessions came from Germany and two sources from Hungary.

The cluster analysis shows a considerable variation among the studied genetic resources, based on some chosen characteristics. This phenotypic variation is due to the effects of different localities for growing,

III: *Correlation coefficient of two morphological characteristics of milk thistle accessions in individual years*

Morphological characteristic	Number of anthodia (pcs)		
	2010	2012	2013
Plant height (cm)	0.75	0.44	0.24



4: *Classification of milk thistle accessions by the cluster analysis*

e.g. the climatic conditions and site characterization and also on the base of different genetic structures (Ram *et al.*, 2005; Shokrpour *et al.*, 2011). Gresta *et al.* (2006) confirmed that agronomic features are affected less by the environmental factors than the genetic. Milk thistle has a good adaptation to various environments. However, as it can be seen from our cluster organising, the accessions from similar localities are together in the clusters, e. g. German, Balkan, Hungarian together with German and Slovakian. The highest genotypic variation, the highest heritability and genetic advance was reported for seed yield per plant and number of anthodia (heads) per plant (Ram *et al.*, 2005).

With the spreading of areas sown with medicinal plants in the Czech Republic it is necessary to solve the problem of protection against known or newly identified pathogens and pests. Milk thistle is susceptible mainly to fungi (*Septoria silybi*, *Alternaria* ssp.) and to aphids (Moscow and Lindow, 1989; Kavallieratos *et al.*, 2007; Koláčková, 2011). Their occurrence can be influenced by agrotechnical measures to some extent, but under favourable conditions these measures are insufficient and chemical protection will be necessary.

CONCLUSION

The limiting factors for the growth and development of milk thistle is not only the altitude (up to 500 m above the sea level), but also the weather course during the emerging and anthodia ripening. The genetic sources 1 (Serbia), 2 (Slovakia), 5 (Austria BVAL – 901578 'De Prahova'), 7 (Germany SIL 2), 11 (Hungary RCAT 040360 DDR), 14 (Czech Republic 'Silyb') and 15 (Germany SIL 8) seem to be promising genetic sources from the viewpoint of growth and development.

Currently, the methodology of integrated production of milk thistle in the Czech Republic is being developed. Many problems of the agro-technology and breeding aims were not worked out, such as synchronous maturity, achene shedding, reducing the spines, question of varietal technology or locality-technology. Also because the number of the new candivars applied into the state evaluation has increased recently (6), there is a necessity to develop the set of descriptors for the evaluation of distinctiveness, stability, and uniformity (DUS tests) of new varieties. This descriptor under UPOV does not exist yet.

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REFERENCES

ANDRZEJEWSKA, J., SADOWSKA, K. 2008. Effect of cultivation conditions on the variability and interrelation of yield and raw material quality in milk thistle. *Acta Sci. Pol. Agric*., 7(3): 3–11.

BHATTACHARYA, S. 2011. Phytotherapeutic properties of milk thistle seeds: an overview. *J. Adv. Pharm. Educ. Res*., 1: 69–79.

EL-MALLAH, M. H., EL-SHAMI, S. M., HASSANEIN, M. M. 2003. Detailed studies on some lipids of *Silybum marianum* (L.) seed oil. *Grasas Aceites*, 54(4): 397–402.

GHAVAMI, N., RAMIN, A. A. 2008. Grain yield and active substance of milk thistle as afected by soil salinity. *Commun. Soil. Sci. Plant. Anal*., 39(17–18): 2608–2618.

GRESTA, F., AVOLA, G., GUARNACCIA, P. 2006. Agronomic characterization of some spontaneous genotypes of milk thstle (*Silybum marianum* L. Gaertn.) in Mediterranean environment. *J. Herbs Spices Med. Plants*, 12(4): 51–60.

HABÁN, M., OTEPKA, P., KOSIBA, L., HABÁNOVÁ, M. 2009. Production and quality of milk thistle (*Silybum marianum* L. Gaertn.) cultivated in cultural conditions of warm agro-climatic macroregion. *Horti. Sci*., 36: 25–30.

HOLM, L. G., DOLL, J., HOLM, E., PANCHO, J., HERBERGER, J. 1997. *World Weeds. Natural Histories and Distribution*. NewYork: Wiley.

KAVALLIERATOS, N. G., TOMANOVIĆ, Z., SARLIS, G. P., VAYIAS, B. J., ŽIKIĆ, V., EMMANOUEL, N. E. 2007. Aphids (Hemiptera: Aphidoidea) on cultivated and self-sown plants in Greece. *Biologia*, 62: 335–344.

KOLÁČKOVÁ, P. 2011. *Kvalitativní parametry zdravých a napadených rostlin ze skupiny LAKR*. Diplomová práce. Brno: Mendelova univerzita v Brně, 1–96.

MONTEMURRO, P., FRACCHIOLLA, M., LONIGRO, A. 2007. Effects of some environmental factors on seed germination and spreading potential of *Silybum marianum* Gaertner. *Ital. J. Agron*., 3: 315–320.

MOSCOW, D., LINDOW, S. E. 1989. Infection of milk thistle (*Silybum marianum*) leaves by *Septoria silybi*. *Phytopathol*., 79: 1085–1090.

OTTAI, M. E. S., ABDEL-MONIEM, A. S. H. 2006. Genetic parameter variations among milk thistle, *Silybum marianum* varieties and varietal sensitivity to infestation with seed-head weevil, *Larinus latus* Herbst. *Int J. Agric Biol*., 8: 862–866.

RAM, G., BHAN, M. K., GUPTA, K. K., THAKER, B., JAMWAL, U., PAL, S. 2005. Variability pattern and correlation studies in milk thistle (*Silybum marianum* Gaertn.). *Fitoter*., 76(2): 143–147.

SHOKRPOUR, M., TORABI GIGLOO, M., ASGHARI, A., BAHRAMPOUR, S. 2011. Study of some agronomic attributes in milk thistle *Silybum marianum* Gaertn. Ecotypes from Iran. *J. Med. Plants Res*., 5(11): 2169–2174.

FABEROVÁ I. 2011. Oral communications.

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