PRELIMINARY COMMUNICATION

Screening of presence of the chosen anthocyanin colorants in the *Limniris* group Irises

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Abstract

This article is focused on monitoring of six chosen anthocyanins - cyanidin-3-galaktoside, cyanidin-3-glukoside, peonidin-3-glukoside, pelargonidin-3-glukoside, malvidin-3-glukoside, and delphinidin-3-glukoside in 69 Limniris group Irises. Flowers from these plants were collected, lyophilised and we prepared methanol extract from them. This extracts was investigated using HPLC-DAD (λ = 520 nm). Obtained results were compared with above-mentioned standards. Obtained results show minimal correspondence to available standards, malvidin-3-glukoside and delphinidin-3-glukoside was detected in cultivar *Iris sibirica* ´Elfelde´ and cyanidin-3-galaktoside in sample of pure botanical *Iris ensata*.

Key words: *Limniris*, Anthocyanin, HPLC-DAD method, *Iris sibirica, Iris ensata*

Introduction

Iris is divided into six subgenera and 12 sections with section *Limniris* further divided into 16 series (Wilson, 2004). These can be found growing throughout the Northern Hemisphere in forests, on the sides of mountains, along coast lines, in swamps and wet meadows, and in dry, scrubby regions (Austin, 2005).

Early botanists earmark the Limniris group on the basis of the missing „beard“, sepals and on presence of rhizomatous roots. Lawrence put this group under the *Spathula* section and a Mathew made subgenus *Limniris*. Even before them Dykes species that belongs to the Limniris group divided into two sections. First one was *Evansia*, which species are nowadays marked as group *Limniris* section *Lophiris*, and second section was *Apogon*, species with narrow sepals which are now mentioned group *Limniris* section *Limniris* (Dykes, 1974).

To the *Limniris* group nowadays belong around eighty species which don't have the same evolution history. The presence of the rhizomes and absence of the „beard“ are just the signs within the genus *Iris* and are not defined as an monophyletic signs of the group. But the phylogenetic group within the *Limniris* group show some geographical cohesiveness (similarity) as is for example geographical distribution of species. First group are species from Asia and North America and their representatives are irises from the series *Sibiricae*, *Laevigatae* and *Californicae*. Second group are European species and species from central part of Asia and their representatives are only one monophyletic group, series *Spuriae*.

Most of the growers divided Irises from the *Limniris* group on the basics of morphological characteristics to the groups with similar morphology, occurrence and growing conditions on the Siberian irises, Japanese irises, Louisiana irises, Laevigata irises and Pacific coast irises.

Siberian irises, to this group belongs eleven species. The first and most typical is *Iris sibirica*. It shares many characteristics with the other ten species that form the Siberian group, most importantly their preference for seasonal moisture in the garden. (Speichert and Speichert, 2004). According to Tillie et al. are species from the series *Sibiricae* split into two monophyletic groups, since Mathew (1981) has noted that in some classifications the *Sibiricae* contain only *I. sanguinea* Hornem. ex Donn, *I. sibirica* L. and *I. typhifolia*

Kitagawa, and the rest (including *I. chrysographes* Dykes and some other species) are placed in *Chrysographes*. This division had previously been proposed by Werckmeister (1967) and others, and cytological research shows differences between these groups (Tillie et al., 2000). Siberian irises can be divided into two groups. The first group, consisting of the most common and easiest-to-grow species, includes the traditional blue-flowered types that are derived from *Iris sanguinea* and *I. sibirica*. In the wild *I. sanguinea* grows in damp areas and along rivers that stretch from Russia (including Siberia) to northern China, into Korea, and Japan. *Iris sibirica*, the plant from which this group inherits its name, does not actually grow in Siberia. Its natural range starts in northern Italy and spreads to Turkey, then up into south-eastern Russia. Many Siberian irises presently in cultivation are diploids, but due to the pioneering work of Currier McEwen an increasing number of tetraploids are being introduced. Diploid sibs have delicate stems and narrow foliage, whereas tetraploid sibs have more hefty stems and larger flowers with greater substance. The second group of Siberian irises comes from China and the Himalayas where the plants grow in marshy areas and wet meadows. All members of this group, *Iris chrysographes* is an good example, have 40 chromosomes, unlike irises derived from *I. sanguinea* and *I. sibirica*, which have only 28 chromosomes (Austin, 2005). Siberian iris cultivars are excellent for the bog garden, for the edge of the stream, or for a seasonal wet spot in the backyard. In colder climates, they prefer wet soils in the spring and summer, but generally require drier conditions in the fall and winter. If they are placed in the pond in the spring, they should be removed before winter frost arrives, and mulched in the perennial border. In warmer climes, where the temperatures do not drop below -7 °C, they do not need mulch. When first offered Siberian irises were limited in colour to blue and white. Now, the range has been greatly expanded to include deep purple-reds to light lavender-pinks. Flowers are anywhere from 5 to 10 cm wide, depending upon the selection. Since 1970, hybridizers have been cross-pollinating the various species in the Siberian group with *I. sibirica*, creating hybrids whose parentage is now so complicated that the cultivars are no longer listed with a species name. Several hundred Siberian iris cultivars are registered with the American Iris Society (Speichert and Speichert, 2004).

Japanese irises, still sometimes referred to as Kaempferi irises, are usually thought of as water dwellers, which they are not. The wild species of Japanese irises can be found growing in damp meadows where the soil is slightly acid. (Austin, 2005).

Louisiana irises these are moisture-loving species bred originally from three species of the southeaster United States: *Iris brevicaulis*, *I. fulva*, and *I. nelsonii* (Kingsbury, 2011). Austin is adding to the group two more species *I. giganticaerulea* and *I. hexagon (*Austin 2005). Speichert and Speichert proclame that Louisiana irises are hybrids of the eight *Iris* species in the series *Hexagonae* (Speichert and Speichert, 2004). They are interesting in that they are amongst the very few ornamental plant genera whose breeding history is entirely American, with no Old-World input (Kingsbury, 2011). Louisiana's will grow from drought conditions to water as deep as 10–15 cm, and they tolerate seasonal flooding. They do well in the garden with supplemental watering. They need no special care overwinter. They produce more running rhizomes than the other irises, with some growing 60 cm or more in a season. Plant height varies from 40 to 90 cm. The seed tend to be very large, corky things and much less abundant than those produced by the other species. (Speichetr and Speichert, 2004). The Louisiana irises provide a striking example of an introgressive swarm. Two parental species, *Iris fulva* and *I. hexagona*, have produced numerous hybrid populations in southern Louisiana. The hybrid individuals found in the hybrid zones are not true F1 hybrids but are the progeny resulting from numerous backcrosses to the parental species. A mixture of phenotypes is present in the hybrid swarm, with differing levels of similarity to the parental species among hybrid offspring

(Ness, 2003). Hybridization can also give rise to new species with the same ploidy as the parental species. Molecular studies have confirmed the natural occurrence of homoploid hybrid speciation in *Iris, Stephanomeria, Helianthus, Pinus, Paeonia* and *Penstemon* (Henry, 2005). But the new made Louisiana iris hybrids mustn't be so viable as the parental plants. The survivorship frequencies for *I. brevicaulis, I. fulva* and introgressed genotypes of these two species were tested in research made in 2010. Survivorship estimates were derived after a severe (water depth of several feet) and extended (ca. four month) natural flooding event. Results of this experiment show that the survivorship was highest in *I. fulva* (0,273% plants survive) > interspecies *I. fulva* (0,092% plants survive) > interspecies *I. brevicaulis* (0,055% plants survive) > *I. brevicaulis* (0% plants survive). This pattern of survivorship is consistent with previous observations suggesting greater tolerance to root/rhizome submersion by *I. fulva* relative to *I. brevicaulis* (Arnold et al. 2010).

Laevigata group is group of irises, which in the wild all grow in wet conditions, including shallow, standing water. The group includes *Iris ensata, I. pseudacorus, I .versicolor* and *I. virginica* (Austin, 2005).

Pacific coast irises are natives of the western coast of North America produce flowers in a combination of colours that are not available among most other irises. To this group belongs *I. bracteata, I. douglasiana, I. hartwegii, I. innominata, I. munzii, I. purdyi, I. tenax, I. tenuissima* (Austin, 2005).

Tillie unlike divided the *Limniris* group just to three parts *Sibiricae* (see above), *Californicae* a *Spuriae*. Species of series *Californicae* form one monophyletic group and come together with the two groups of *Sibiricae*. There are hybrids between the *Californicae* and *Sibiricae* species, the so called "Cal-Sibe hybrids". The species of series *Spuriae* examined here form a monophyletic group, apart from *I. graminea* L., which previously has been placed in other groups. *Iris graminea* and *I. foetidissima* L. form a sister pair. The authors also mentioned that the Rodonienko placed these two species in his subgenus *Xyridion* and also mentioned that *I. graminea* and *I. foetidissima* were put in the *Spathula* group by Tausch in 1823. These species show some similarities; for example, both are evergreen in the climatic conditions of USA (Tillie et al., 2000).

Material and methods

In the experiment were used eleven Iris species (*I. versicolor, I. sintenisii, I. spuria, I. sibirica, I. lactea, I. ensata, I. setosa, I. crocea, I. pseudacorus, I. orientalis, I. tectorum*) in sixty nine samples made from the flowers of the pure botanical species and also from the hybrids of these species.

Plants from which the flowers were obtained were grown by the 4th year on the grounds of Horticulture Faculty in Lednice. Plants grown in the free soil, on the sunny spot, in rows oriented in the east-west direction. Plants grow in the conditions of loamy soils, in the direct sun in rows with spacing between plants 70 cm and between rows 50 cm. The spring irrigation is introduced on the experimental ground and it run each 3 days in summer to provide enough water (3 l for plant) for the plants to grow. In summer the plants also get 50 grams amount of the classical NPK (15-15-15) fertilizer on each square meter of the experimental field. From each taxon were taken 80 grams of sepals and petals. They were homogenized and used for the preparation of the extract. Extraction was conducted in the following manner: 3 grams of the sample were put into the methanol acidified by the hydrochloric acid (1:50, Penta, Czech Republic) and homogenized for 5 minutes (VORTEX Genius 3, IKA, Deutschland). The obtained homogenized mass was centrifuged (10 min, 16.000 g, Eppendorf 5430R, Czech Republic). For the measurement itself was used optimized HPLC with DAD detector, detection goes on by λ=520 nm. Retention time

of the measured samples together with the spectra was compared to the spectra and retention times of the six most common anthocyanin in the flowers (cyanidin-3-galaktoside, cyanidin-3-glukoside, peonidin-3-glukoside, pelargonidin-3-glukoside, malvidin-3-glukoside, and delphinidin-3-glukoside). According to the wave length 520 nm, which is specific for anthocyanin we just try to detected these colorants.

Results and discussion

Due to most authors the colours range of Iris flowers is from the whitest whites to the deepest blues, with purple and lavender hues, and reds, yellows, browns and greens, essentially every colour in the rainbow (Speichert and Speichert, 2004). Austin is mentioning also brown and black colour and even green and red tones (Austin, 2005). Some species have flowers deeply veined or heavily marked with a yellow glow toward the centre of the petals (Speichert and Speichert, 2004). In some cases the falls may have a totally different contrasting colour from the standards (Beutler, 2007).

Table 1. Measured values for the retent time and peak area

Sample	Retent time	Peak area
cyanidin-3-glykozide	9,596	13053,2
peonidin-3-glykozide	10,287	81,8
pelargonidin -3-glykozide	10,443	14058,6
malvidin-3-glykozide	11,455	618,4
Iris sibirica ´Elfelde´	11,49	127,6
delphinidin-3-glykozide	12,332	122,5
Iris sibirica ´Elfelde´	12,334	3712
cyanidin-3-galaktozide	14,01	274,4
Iris ensata	14,01	540,2

From all the sixty nine samples used in the experiment twenty samples was without any colorants so their graphs results have no peaks during the whole retention time. In these taxa should be some other fenolic compounds but these were not detected with the used wave length. These samples were mostly from taxa with the white and yellow flowers. Other forty nine samples have one to five peaks in various retent times so we can assume that these samples contain anthocyanins. Used HPLC method doesn't allow us to define the structure of these anthocyanins. We were able positively identify, due to the relative compliance in retent time with the retent time of the anthocyanins standards just two samples. The *Iris sibirica* ´Elfelde´ have similar retent time as delphinidin-3-glukoside standard and as malvidin-3-glukoside standard and sample of botanical *Iris ensata* have the same retent time as cyanidin-3-galaktoside standard.

The results from chemicall analysis of the colourants in Iris flower are not published so often but research from 2004 prove that the often co-pigment for blue colour of the Iris flowers is flavone C-glycoside (Davies, 2004). The anthocyanins were proved in floral tissues of *Iridaceae* in 2000. Ninety five pigments have been characterised from about thirty plant species, so that there is considerable variation with possible taxonomic interest. The genus *Iris* is relatively consistent with delphanin, delphinidin 3-*p*coumarylrutinoside-5-glucoside. The malvidin analogue to this pigment has been reported from *Iris ensata* (Harborne and Williams, 2000). The content of the delphinidin was proved also with our experiment.

Conclusions

Our research find out some interesting facts conected with the anthocyanin colours included in the flowers of the *Iris* species from *Limniris* group. Altough there was visual conformity in the flower colours, or even colour conformity prooved with the colour charts, we dont find out the compliance on the molecular level. Due to the wide spectrum of the possible anthocyan colourants the experiments should be repeeted wit another colour standards.

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