Flower colour morphs of *Iris pumila* differ in the amounts of Hsp90 and phenolic compounds

Branka Tucić, Sanja Manitašević Jovanović and Ana Vuleta

Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia

Introduction

The intraspecific flower-colour polymorphism is a rare phenomenon in the angiosperm species. It mostly appears in insect-pollinated temperate herbs, whose flowers contain red, purple or blue anthocyanin pigments. The main ecological function of anthocyanin-based flower colouration is to attract animal pollination vectors (Menzel and Shmida 1993). Accumulating evidence suggests, however, that intraspecific variation of floral traits may result not only from pollinator-mediated selection but also from selection pressures related to environmental heterogeneity and stress tolerance (Warren and Mackenzie 2001). A large number of ecophysiologists support the hypothesis that anthocyanins serve to protect plant cells against a multitude of abiotic stresses, including strong light, UV radiation, temperature extremes, and water deficit (Neill and Gould 2003; Hueghes et al. 2005). In plant tissues predisposed to photoinhibition anthocyanins may act as light attenuators and/or strong antioxidants (Neill and Gould 2003; Hueghes et al. 2005). Phenols are the plant secondary metabolites that also have the protective function. Plants mainly produce phenols for defense against various abiotic and biotic stresses, such as UV radiation and many microorganisms.

The heat shock proteins 90 (Hsp 90s) are molecular chaperones essential for the proper folding of many proteins, some of which are involved in signal transduction. They also participate in the protein refolding under conditions of denaturing stress. Because of their dual role in signal transduction and stress response, Hsp90s are hypothesized to "link developmental program to environmental contingency" (Rutherford and Lindquist, 1998).

The aim of this study was to elucidate the mechanism(s) underlying the intraspecific anthocyanin-based flower-colour polymorphism in an entomophilous perennial herb, *Iris pumila*.

Material and Methods

The experimental plants used in this study were grown in an experimental garden under relatively uniform environmental conditions. They originate from a natural population of *Iris pumila* that experienced full-sunlight within its natural habitat.

Iris pumila L. (Iridaceae) is a rhizomatous perennial herb strikingly polymorphic for flower color. Because flower-colour polymorphism in *I. pumila* is genetically based, each of the flower color morphs existing in a population can be considered as a unique clonal genotype (Tucić et al. 1988).

Here we analyzed the amounts of three biochemical compounds, anthocyanins, phenols and Hsp 90s, contained in the perianth tissue of distinctively pigmented floral phenotypes. Anthocyanins were extracted as described in Petrini et al. (2002). The content of total soluble non-structural phenols was determined by the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). A SDS-PAGE and Western blot analysis was used to assess the amount of Hsp 90 A and Hsp 90B proteins (Manitašević at al. 2007).

To test for differences between the mean values of each biochemical compounds a Kruskal-Wallis test from the SAS package was used. Analyses of the within-population variation and the relationships between floral biochemical traits were performed using a PCA-based factor procedure from the SAS package.

Results

A total of 100 *I. pumila* clones stemming from a sun-exposed natural population were classified into distinct flower colour morphs, one, hypopigmented (W/Y = white/yellow), and six, pigmented (LB = light blue, B = blue, DB = dark blue, LV = light violet, V = violet, and DV = dark violet).

The amounts of anthocyanins, total phenols and two Hsp90 proteins (inducible Hsp 90A and constiutive Hsp90B) were quantified in the perianth tissue of each of these flower colour classes. Table 1 shows that the mean values of all but one plant compouds, Hsp 90A, were significantly different between distinct flower-colour morphs.

The mean anthocyanin content was found to be extremely low in the white/yellow flowered morphs (Fig. 1). But it gradually increased from light- to dark-coloured classes. As a rule, the violet-pigmented flowers contained more anthocyanins in the perianth compared to their blue counterpars (Fig. 1).

Conversely, the amount of total phenols appeared to be the highest in the white/yellow colour morphs (Fig. 1). In all classes of the blue-pigmented flowers their content was simiraly large, but relatively greater than that recorded in the violet-coloured flowers. In violet floral classes, the amount of total phenols increased progressively from light- to dark-coloured morphs.

The wight/yellow flowers contained comparatively more Hsp 90B than the anthocyanin-pigmented ones (Fif. 1). The content of Hsp 90B protein decresed gradually with the increase of flower colour intensity, and appeared to be greater in the blue flower morphs compared to their violet counterparts.

A factor analysis applied on four perianth compounds (anthocyanins, phenols, and two Hsp 90s) showed no clear separation of these molecules among the flower colour morphs analysed (Table 2). Of these four compounds, Hsp 90B appeared to load positively onto PC 1 in all but two colour classes, DB and LV, which could signify the importance of Hsp 90B for perianth cells functioning. The two antioxidants, antocyanins and phenols loaded positively onto PC 1 or PC 2, either jointly (B and DB morphs, respectively) or individually (LB, LV, V, and LB, V, respectively), depending on the flower pigmentation.

Table 1

Results of the Kruskal-Wallis test

Variable	χ^2	DF	*P*
Anthocyanins	70.99	6	<.0001
Phenols	19.18	6	0.0039
Hsp 90A	7.02	6	0.3193
Hsp 90B	13.05	6	0.0423

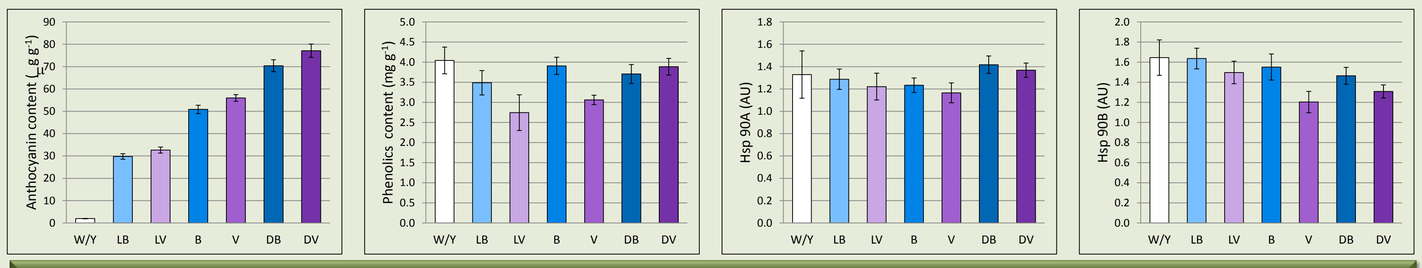

Figure 1

The mean (±SE) amounts of anthocyanins, phenols and two Hsp 90 forms, Hsp 90A and Hsp 90B, recorded in perianth tissue of distinct *I. pumila* flower morphs developed under similar environmental conditions in a common- garden.

Table 2

Results of a factor analysis on distinct flower colour morphs in *I. pumila*. ● = factor loadings > + 0.60; ○= factor loadings < -0.60

	W/Y	Light Blue		Blue		Dark Blue		Light Violet		Violet		Dark Violet		
Variable	PC1	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC3
Anthocyanins		●		●			●	●			●	○		
Phenols	○		●	●			●	○		●				●
Hsp 90A	●				●	●					●		●	
Hsp 90B	●	●		●		○			●	●		●		

Conclusions

(1) Flower-colour genotypes of *I. pumila* produce unique combinations of stress-protective molecules (anthicyanins, phenols and Hsp90s) in order to achieve cellular homeostasis in the perianth tissue under fluctuating temperature conditions, which regularly occur within their natural habitats during the blooming phenophase.

(2) Anthocyanin-based flower colour polymorphism in *I. pumila* populations might be maintained not only by pollinator preferences, but also by environmental heterogeneity, meaning that the ability to synthesize anthocyanins would be selectively advantageous under stressed environmental conditions but disadvantageous (costly) under non-stressed ones.

Literature

Hueghes N M, Neufeld H S, Burkey K O (2005) New Phytol 168: 575-583.

Manitašević S, Dunđerski J, Matić G, Tucić B (2007) Plant Cell Environ 30: 1-11.

Menzel R, Shmida A (1993) Biol Rev 68: 81-120.

Neill S O, Gould K S (2003) Funct Plant Biol 30: 865-873.

Petrini F, Iannelli M A, Massacci A (2002) Plant Cell Environ 25: 1251-1259.

Rutherford S L, Lindquist S (1998) Nature 396: 336-342.

Singleton V L, Rossi J A (1965) Am J Enol Vitic 16, 144-158.

Tucić B, Miljković S, Vujčić S, Tarasjev A. (1988) Acta Oecol/ Oecol PL 9: 211-219.

Warren J, Mackenzie S (2001) New Phytol 151: 237-241.

Acknowledgments - This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 173007).

Branka Tucić

E-mail: btucic@ibiss.bg.ac.rs

Department of Evolutionary Biology

Institute for Biological Research "Siniša Stanković"

University of Belgrade

142 Despot Stefan Blvd

11060 Belgrade, Serbia