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PATTERN OF PLASTICITY TO IRRADIANCE LEVELS AND GENOTYPIC CORRELATIONS BETWEEN STRUCTURAL AND PHYSIOLOGICAL LEAF TRAITS IN *IRIS PUMILA*

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Abstract – Plasticity to irradiance and genotypic correlations between structural and physiological leaf traits in *Iris pumila* were investigated in an experiment conducted at a sun-exposed dune habitat in the Deliblato Sands. A sample of six native, genetically different clones were covered with a neutral screen which transmitted ~35% of daylight, so that one clone-half of each clone experienced reduced sunlight, while the other one full sunlight. LMA, stomatal density, Ψ_{leaf}, enzymatic and non-enzymatic antioxidants and lipid peroxidation were determined in unshaded and shaded leaves of the same clone. It was found that the plasticity index, PI_v was higher for physiological than for structural traits. Genotypic correlations between trait pairs were high, but rarely significant, in contrast to the correlation matrices which were significantly different between unshaded and shaded leaves.

Key words: light intensity, phenotypic plasticity, genotypic correlations, *Iris pumila*

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INTRODUCTION

As photoautotrophic and sessile organisms, higher plants have evolved an extraordinary capacity for developmental plasticity to multiple informational signals from the radiation environments, thereby optimizing their developmental patterns in a way that maximizes light energy interception, survival and reproduction in the habitats they happen to occur (Bradshaw, 1965; Sultan, 1987; Sultan and Bazzaz, 1993; Kendrick and Kronenberg, 1994; Schlichting and Pigliucci, 1998; Tucić et al., 1998; Pigliucci, 2001; Nemhauser and Chory, 2002; Avramov et al., 2007; Niklas, 2008). Plastic responses to environmental cues are believed to support functional adjustments to the environment by allowing different genotypes to converge to a single phenotype suitable to existing environmental conditions, or by permitting a single genotype to produce different phenotypes in different environments (Sultan and Bazzaz, 1993; Tucić et al., 1998; Schmitt et al., 1999; Ackerly et al., 2000). Given that under particular environmental conditions some of the phenotypic responses increase plant function and thereby fitness more than the alternative phenotypes, such plasticity is frequently regarded as adaptive (Sultan, 1995).

In nature, light environments are very complex, both spatially and temporally so that most plants experience a mixture of light quantities and qualities. Under such circumstances, phenotypic variation expressed by individual genotypes in different light environments usually reflects both active (anticipatory) plasticity in response to specific informational cues signaling forthcoming events in the environments, and passive (inevitable) effects on the phenotype evoked by low resource level (Sultan, 2000; Pigliucci, 2001). Since light is the most important environmental factor for photoautotrophic plants, providing energy for photosynthesis and controlling their growth

and development, the ecologically important developmental responses of plants to heterogeneous light conditions include specific adjustments in all aspects of their phenotype, from morphology and anatomy, to physiology and biochemistry (Valladares and Pearcy, 1998; Evans and Poorter, 2001). However, the most visible plastic changes occur at the level of individual leaves – the photon-harvesting plant organ (Wild and Wolf, 1980; Sultan and Bazzaz, 1993; Nicotra et al., 1997; Tucić et al., 1998; Oguchi et al., 2003)

Given that plastic responses usually differ among plant traits or trait complexes, it is expected that correlations between a pair of plant traits can be altered by the environmental conditions as well (Bradshaw, 1965; Schlichting, 1989). Hence, determinations of the correlation patterns between respective traits and their sensitivity to environmental changes (plasticity of correlations) are of great importance for the magnitude and direction of selection acting on traits associated with fitness in the plant populations (Lechowicz and Blais, 1988; Schlichting, 1989; Donohue et al., 2000).

In order to reveal the influence of light quantity on plastic responses and genotypic correlations between structural and physiological leaf traits in *Iris pumila* (our model-species), a random sample of six *Iris* clones, growing naturally in an exposed site at the Deliblato Sands, were partially covered with a neutral screen that transmitted 35% of daylight, so that each clone experienced reduced and full sunlight at the same time. It has been known for some time that autochthon *Iris* genotypes express a remarkable ability to respond phenotypically to variations in ambient light conditions, such as full daylight radiation at open dune sites and reduced irradiance level in woodland understory (Tucić et al 1998; Tucić et al., 1999). The aims of this study were to elucidate (1) whether *I. pumila*, as a shade-tolerant species, displays a greater phenotypic structural plasticity of leaf traits to irradiance compared to the physiological plasticity of leaf traits, and (2) how light intensity affects the pattern of genetic correlations between structural and physiological leaf traits.

MATERIAL AND METHODS

The study species

The dwarf bearded iris, *Iris pumila*, is a rhizomatous perennial herb commonly found in the lowlands of southeastern Europe. The extant populations of the species range from southern Moravia in the north, over Austria, Hungary, Serbia, Rumania, and Bulgaria, to northern Anatolia in the south (Randolph, 1955). In Serbia, the species is very abundant in the dune system at the Deliblato Sands (44° 47' 39" N / 21° 20' 00" E to 45° 13' 10" N / 28° 26' 08" E), where it forms very large and very old circle-shaped clones (Tucić et al., 1989).

Experimental setup

From April 2007, at the peak of *I. pumila* blooming phase, to July of the same year, leaves of six large clones native to a sun-exposed population whose one half was covered with a neutral PVC screen, were grown under shaded and unshaded light conditions. In July 2007, the two fully expanded leaves were collected from the shaded and the unshaded parts of the marked clones between, 15:00 h and 16:00 h, immediately frozen in liquid nitrogen, transported to the laboratory, and stored at -70°C until preparation.

In the laboratory, the following traits were measured on the unshaded and shaded leaves: leaf mass per unit area (LMA; in g cm^{-2}), stomatal density (SD; in #mm^{-2}), activities of antioxidative enzymes (superoxide dismutase – SOD, in AU; catalase – CAT, in AU; ascorbate peroxidase – APX, in AU; glutathione peroxidase – GPX, in μmol min^{-1} mg^{-1} soluble protein, glutathione reductase - GR, in μmol min^{-1} mg^{-1} soluble protein; class III peroxidase – POD, in μmol min^{-1} mg^{-1} soluble protein; and glutathione-S-transferase – GST, in nmol min^{-1} mg^{-1} soluble protein), low molecular weight antioxidant contents (soluble phenols – Phen, in mmol g_{fw}^{-1}; and anthocyanins – Anth, in mg g_{fw}^{-1}), lipid peroxidation (MDA equivalents, in nmol g_{fw}^{-1}) and leaf water potential (Ψ_{leaf}; in MPa). A detailed description of the analytical methods are given in Vuleta et al., 2011, submitted manuscript).

The extent to which the phenotypic values of the individual traits of a genotype were changed by different environments can be quantified using various indices of phenotypic plasticity (Bradshaw, 1965; Valladares et al., 2006). In this study, light-induced plasticity in the structural and physiological traits of each *I. pumila* clone was determined by calculating the index of plasticity, PI_v (Valladares et al., 2006):

$$PI_v = |X_H - X_L| / X_H;$$

where X_H is the value of a given leaf trait from the sun-exposed clone part, while X_L is the value of the same leaf trait developed under the covered clone part. PI_v is a measure of the change in a trait from the high to the low light environment. Relationships between traits in each light environment were estimated using Pearson's correlation coefficients and the Mantel test for proportionality determination of correlation matrices.

RESULTS AND DISCUSSION

The light conditions experienced by the *I. pumila* clone markedly affected all aspects of their leaf phenotype. The average level of leaf plasticity to irradiance appeared to be strongly trait-specific (Table 1). Of all of the traits that were analyzed, anthocyanins had the highest value for the plasticity index (PI_v = 0.604), signifying their key role in photoprotection. The plasticity of the antioxidative enzymes, POD and APX, appeared to be very large (PI_v = 0.693 and 0.317, respectively) as well. The high PI_v value of APX is in accordance with its function as the main enzyme for H_2O_2 detoxification. This role of APX is also corroborated by the two-times greater activity it exhibited in the unshaded leaves compared to the shaded leaves. A high mean value estimated for POD plasticity, accompanied by a high coefficient of variation (CV %), indicates the pronounced genotype-specific responses of this enzyme that are likely due to the synergistic effects of light with several other abiotic factors occurring in exposed natural habitats. Regarding the SODs plasticity, the value of the plasticity index was greater for Cu/Zn-SOD than for Mn-SOD, suggesting that Cu/Zn-SOD plays a more important protective role in oxidative stress compared to Mn-SOD, because of its distribution in all cell compartments (Table 1). Our study provides evidence that the average leaf plasticity was strongly trait-specific, but generally higher for physiological than for structural traits. This conclusively means that when faced with variable light conditions *I. pumila* activates multiple photoprotective mechanisms, from physiological to structural ones, which allow diverse genotypes to exploit the different light environments across the Deliblato Sands.

Table 1. Mean plasticity index, PL_v (Valladares et al., 2006) and coefficient of variation (CV%) for response to light intensity within a sample of *I. pumila* clones naturally growing at an open habitat in the Deliblato Sands. The plastic responses to difference in light intensity within the open and the shaded clone halves was estimated for the antioxidative enzymes (SOD, CAT, APX POD, GR, GST and GPX), the content of low molecular antioxidants (anthocyanins and phenol), as well as for structural (SD and LMA) and physiological (MDA and Ψ_{leaf}) leaf traits. The trait acronyms are given in Text.

Trait	PL_v	CV (%)
Total SOD	0.195	43.3
Mn-SOD	0.182	55.2
Cu/Zn-SOD	0.232	23.0
CAT	0.172	38.4
APX	0.317	18.4
POD	0.693	77.8
GST	0.217	59.9
GPX	0.057	81.0
GR	0.152	36.8
Phen	0.189	65.2
Anth	0.604	8.3
MDA	0.237	82.6
LMA	0.219	77.7
SD	0.189	72.6
Ψ_{leaf}	0.232	31.3

In this study, the structural and physiological leaf traits within each *I. pumila* clone grown under different light intensities were recorded, and genotypic

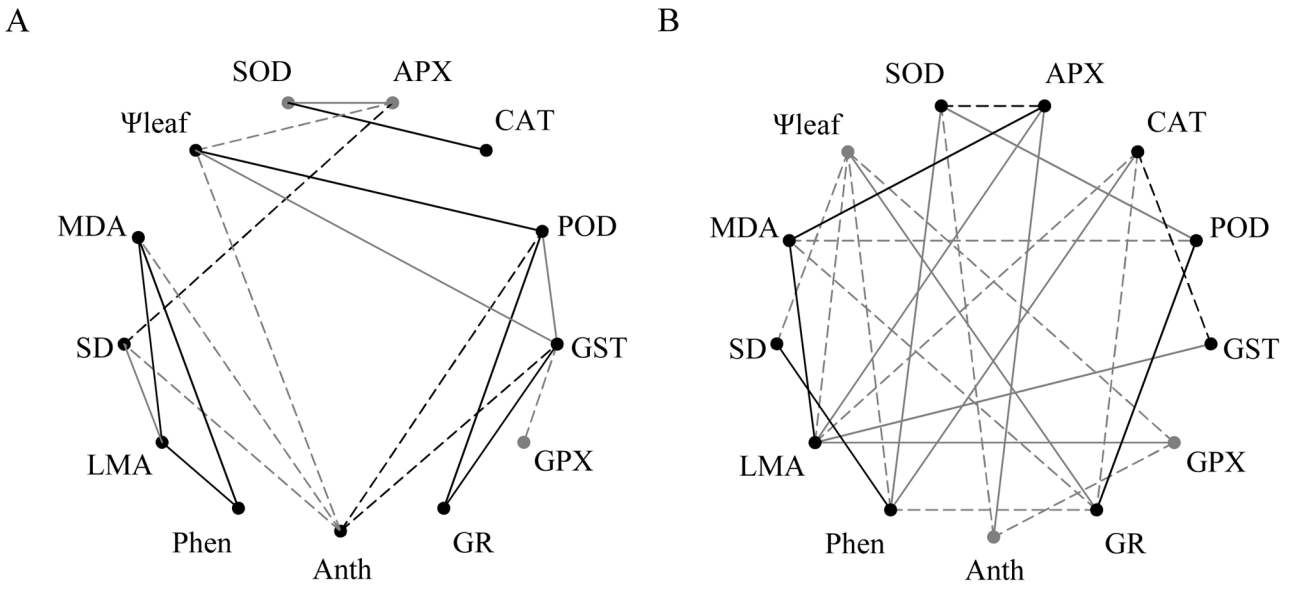

Fig. 1. Pearson's coefficients of genotypic correlations between the activity of antioxidative enzymes (SOD, CAT, APX, POD, GR, GST, and GPX), the content of low molecular antioxidants (Anth and Phen), and the value of structural (SD and LMA) and physiological (MDA and Ψ_{leaf}) leaf traits of *I. pumila* in (A) sun-exposed and (B) shaded clone halves. The trait acronyms are given in the Text.

correlations between every trait pair were calculated for each clone part. Although the magnitude of these correlations is of great interest, their significance was not tested because of the small sample size (only six data points), consequently, the extremely low resolving power of such tests (Sultan and Bazzaz, 1993). Conversely, the Mantel test detected that the correlation patterns between unshaded and shaded leaf traits were significantly different (matrix correlation r = 0.162; P = 0.095). Fig. 1 represents the correlation structure of *I. pumila* leaf traits in the two light environments. It is evident that both the magnitude as well as sign of genotypic correlation differed between the unshaded and shaded leaf traits. We assumed that the observed changes in the magnitude of correlations likely resulted from difference in the intensity of selection on each of the correlated traits. Conversely, variation in the signs of genotypic correlation may reflect changes in the direction of selection that affects each trait within the correlated pairs (Schlichting, 1989). Positive genotypic correlations between leaf traits in *I. pumila* suggest that their genetic bases partially overlap, or that there is a linkage disequilibrium between the loci encoding the given traits (Falconer and Mackay, 1996, Murren, 2002). However, negative correlations reflect a trade-off between a given trait pair, and, similar to high positive correlations, could be regarded as the genetic constraint for their independent evolution (Falconer and Mackay, 1996; Nicotra et al., 1997; Murren, 2002). A lack of correlation between a pair of traits indicates that each of them may respond independently to fluctuating environmental conditions. Consequently, both signs (positive or negative) and the magnitude of correlations among traits may have important implications for the evolution of the entire organism or for some of its modules. In our study, the total number of correlations was higher at the lower light level than at the higher (25 vs. 19, respectively). In the unshaded leaves, 11 correlations were positive and 8 were negative, while in the shaded leaves 12 correlations were positive and 13 were negative (Fig. 2). The plasticity of correlations is often recognized as a change in the number of correlated traits due to environmental variation (Pigliucci and Marlow, 2001). A greater number of correlations are thought to reflect a greater phenotypic and/or genotypic integration of a given organism's module. In *I. pumila*,

as in some other studies, a higher number of correlations revealed in leaves that were allowed to develop under a neutral screen clearly indicates that the genotypic integration of this plant module is greater in a "benign" (shaded) light environment than in a more stressful (sun-exposed) one (Schlichting, 1989; Waitt and Levin, 1993; Tucić and Avramov, 1996; Tucić et al., 1998; Avramov et al., 2007).

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