

DOES PHYSIOLOGICAL INTEGRATION AMONG INTRACLONAL RAMETS OF *IRIS PUMILA* ENHANCE STRESS TOLERANCE IN HETEROGENEOUS ENVIRONMENTS?

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Abstract - We tested the hypothesis that sharing essential resources through interconnected ramets increases stress tolerance of a clonal plant in patchy environments. A number of circle-shaped clones of *Iris pumila* naturally growing in a sun-exposed habitat were selected and cut into two equal halves with different integration status: one with intact and the other with disconnected rhizomes. One-half of each clone was then shaded with a neutral screen to provide 50% of ambient irradiance, so that one-half of both clones consisted of connected and disconnected halves (referred to as "clone quarters"). Leaves collected from each clone quarter were analyzed for malondialdehyde (MDA) content (a stress indicator) and the values of three functional traits, specific leaf area (SLA), leaf dry matter content (LDMC) and leaf water content (LWC). MDA content was greater in unshaded (more stressed) than in shaded (less stressed) leaves. All three morphological traits changed with light gradient, but only SLA was impacted by the ramet integration status. SLA and MDA tended to be inversely related in each clone quarter, indicating a (compensatory) mechanism utilized by *I. pumila* plants in coping with environmental stress. SLA and LDMC were highly negatively correlated in general, but less strongly in interconnected ramets compared to those with a disrupted connection. The results suggest that intracolonial physiological integration confers a fitness benefit to *I. pumila* plants in heterogeneous environments, likely by balancing two fundamental plant activities – rapid biomass production and nutrient conservation.

Key words: Physiological integration, functional leaf traits, stress tolerance, *Iris pumila*

INTRODUCTION

The clonal habit in plants results from the production of genetically identical and physiologically semi-dependent units – ramets, which are physically connected by either overground or underground lateral stems (Hartnett and Bazzaz, 1983). Physical connections between ramets allow the physiological exchange of essential environmental resources including water, nutrients and photosynthates (Hartnett and Bazzaz, 1983; Stuefer and Huber, 1998). Within natural habitats, the quantities of resources required for plant growth and development are not

uniformly distributed in space and time. Moreover, even within the same clone, there appears to be a trade-off between the accessibility of different abiotic factors (e.g., light intensity or temperature vs. soil water content) (Hutchings and Wijesinghe, 2008). The capability for reciprocal translocation of necessary nutrients from ramets with high to ramets with low nutrient supply is expected to increase the overall performance of a clone in heterogeneous environments (Alpert, 1999). Namely, by integrating local heterogeneity in resource availability, the resource sharing among ramets can buffer individual clones against a patch-specific natural selection.

Such a physiological interconnection among ramets has been recognized as the basic strategy of clonal plants to cope effectively with variable resources, with resulting fitness benefit (de Kroon et al., 2005).

Apart from resource translocation, the physiological interconnection among ramets may affect cellular metabolism within a clone, especially under stressful ambient conditions that frequently occur in the wild. The exposure of plants to stressful environmental conditions (strong light intensity, temperature extremes, drought, wounding, and pathogens) can cause the disruption of the balance between generation and elimination of the reactive oxygen species (ROS), leading to oxidative stress (OS; Mittler, 2002). One of the consequences of OS is peroxidation of polyunsaturated fatty acids in cellular membranes, and the formation of toxic aldehydes such as malondialdehyde (MDA) (Manoharan et al., 2005). The elevated content of MDA in plant cells or tissues has been extensively used as an indicator of the ROS-induced oxidative damage to macromolecules such as proteins, lipids and DNA (Taulavuori et al., 2001).

Ecophysiological leaf traits that signify various aspects of resource uptake and utilization (e.g. leaf size, SLA, photosynthetic rate) are thought to be closely related to plant fitness (Ackerly et al., 2000). The observed variation in their phenotypic expression reflects different adaptive strategies that plants use to cope with fluctuating environmental conditions (Vendramini et al., 2002). Specific leaf area (SLA) is the major determinant of a plant's ecological function that reflects the expected return of previously captured environmental resources (Westoby, 1998). The value of SLA depends strongly on irradiance level and water availability, decreasing notably with reduction in light and/or water supply (Sellin, 2001; Cunningham et al., 1999). A lower SLA is associated with a long leaf-life span, nutrient maintenance and drought resistance (Ackerly et al., 2002). Leaf dry matter content (LDMC) has also been accepted as an important functional trait. LDMC is associated with the content of leaf proteins and the volume of cytoplasm, reflecting the structure of leaf

cells (Wilson et al., 1999). Leaf water content (LWC) is a trait strongly correlated with leaf tissue density and leaf thickness (Garnier and Laurent, 1994). Since LWC is associated with leaf nitrogen content and leaf assimilatory capacity, it is commonly used as a predictor of plant performance (Wilson et al., 1999).

This experiment was designed to elucidate the physiological consequences of physical integration between intraclonal ramets in a rhizomatous herb, *Iris pumila* L. We assumed the following: (i) if an *Iris* genet functions as a physiologically integrated system, then the experimental disconnection of a group of ramets from the parental clone will impact metabolic processes within the detached clone part, and consequently the phenotypic expression of leaf functional traits, SLA, LDMC and LWC; (ii) if shaded ramets acquire insufficient nutrition through a physical interconnection with their illuminated counterparts, they will exhibit better leaf performance relative to the disconnected ramets, experiencing similar light conditions.

MATERIALS AND METHODS

The study species

The dwarf bearded iris, *Iris pumila*, is a perennial rhizomatous species that is very abundant in the dune system at Deliblato Sands (44° 47' 39" N / 21° 20' 00" E to 45° 13' 10" N / 28° 26' 08" E) – a sandy area situated about 50 km northeast of Belgrade, Serbia. Within its natural habitats, the species forms round-shaped clones composed of tightly packed rhizome segments, spreading from the center of a clone toward its periphery (Fig. 1). Clonal genotypes of *I. pumila* exhibit a remarkable flower color polymorphism due to the segregation of several gene loci. Hence, each of the floral color variants can be considered as a unique clonal genotype (Tucić et al., 1988).

Experimental setup

For this study, we selected a population of *I. pumila* that occupied an exposed site at the top and on the south-facing slope of a dune. In early spring, ten

circle-shaped clonal genotypes were cut into two equal parts with different integration status: one with physically connected rhizome segments (C) and the other with disconnected rhizomes (D). One-half of each clone was then shaded with a neutral screen (transmitted 50% of ambient irradiance) so that both C and D parts consisted of a fully illuminated (E) and a shaded quarter (S) (Fig. 1).

Leaf sample collection

During the most stressful season (summer), four fully expanded leaves were harvested from each quarter of 10 clonal genotypes, one for morphological and one for MDA content analysis. Before sampling, both the air temperature near the chosen clone and the lamina temperature of each collected leaf were recorded using an infrared laser non-contact thermometer (Crop TRAK, Spectrum Technologies, Inc., Plainfield, USA). Light intensity (PAR, photosynthetically active radiation; in $\mu\text{mol m}^{-2} \text{s}^{-1}$) was recorded at the sampling date between 12:00 and 15:00 h under clear sky conditions using a point quantum sensor (LI-190SA, LI-COR, Inc., Lincoln, USA). A digital plant moisture system (SKPM1400, Skye Instruments Ltd, United Kingdom) was used for measuring leaf water potential (Ψ_{leaf} , MPa) in the last fully developed leaves at midday (12:00-14:00 h).

Lipid peroxidation

The level of lipid peroxidation, in terms of malondialdehyde (MDA) content, was measured spectrophotometrically using a modified TBA-MDA method (Hodges et al., 1999).

Specific leaf area (SLA), leaf water content (LWC) and leaf dry matter content (LDMC)

To estimate specific leaf area (projected leaf area per unit dry mass in $\text{cm}^2 \text{g}^{-1}$) (Wilson et al., 1999), leaf water content [(fresh leaf biomass – dry leaf biomass) / fresh leaf biomass] (Vendramini et al., 2002) and leaf dry matter content (the ratio of leaf dry mass to saturated fresh mass) (Li et al., 2005), the fresh and dry leaf weight, and fresh leaf area were measured

The digital image of leaf area was recorded using an optical scanner (HP ScanJet 3800).

Statistical analysis

To test for differences in the mean values of functional leaf traits as well as the average MDA content between the distinct integration type and among the contrasting irradiation treatments, two-way ANOVA was applied using the GLM procedure from the SAS package (SAS Institute 2003). The ANOVA model included the following sources of variation: connection (differences between the connected and disconnected clonal parts), light (differences between the contrasting light treatments) and connection-by-light interaction (differences between connected and disconnected clonal parts for the trait plasticity within light treatments). Given that the experimental design was balanced, the F-tests were computed using the Type I expected mean squares (MS). To test for differences between the clonal quarters in the mean of each analyzed trait, a one-way ANOVA was used. Prior to each ANOVA, the data were checked for normality and homogeneity of variance. Since all traits exhibited normal distribution, they were used untransformed. Relationships between the trait pairs within distinct integration regimes were estimated using Pearson's correlation analysis (proc CORR from the SAS).

RESULTS AND DISCUSSION

Naturally growing *I. pumila* clones are tightly physiologically and physiologically integrated, which permits the intraclonal support of wounded and stressed ramets (Stuefer and Huber, 1998). Numerous studies have documented that clonal integration influences leaf features such as lamina area (Liao et al., 2003; Chu et al., 2006), water potential (Alpert, 1999), as well as carbohydrate, nitrogen and chlorophyll content (Saarinen and Haansuu, 2000).

Since at the Deliblato Sands, particularly during summer, naturally growing *I. pumila* plants are simultaneously exposed to high irradiance levels, elevated air temperature and soil water scarcity, we

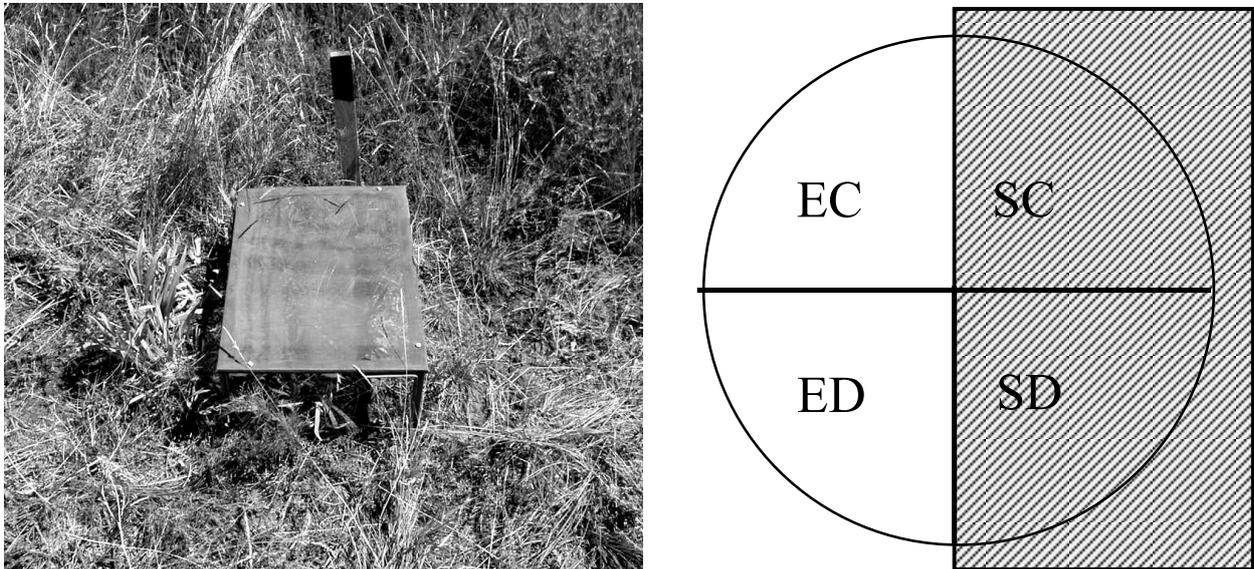


Fig. 1. Experiment setting; a) in the field; b) schematic illustration of rhizome integration status: SD - shaded disconnected, SC - shaded connected; EC - exposed connected; ED - exposed disconnected.

expect that these plants experience severe environmental stress (Manitašević et al., 2007; Vuleta et al., 2010). Physical parameters illustrating local environmental conditions prevailing within sampling site are shown in Table 1. As shown in Table 1, the leaf physiological parameters – leaf surface temperature (LT) and water potential (Ψ) – differed considerably between the clonal quarters. For example, ramets belonging to the sun-exposed clone quarter had up to 4°C higher leaf surface temperature compared to their shaded counterparts. However, the minimum of leaf water potential was recorded in ramets from the shaded and disconnected clone part, and the maximum in the exposed and disconnected quarter of the clone. The results conclusively indicate that the sun-exposed ramets encountered the stronger abiotic stresses relative to those growing in the shade.

The changes in cellular biochemistry were found to be the first response of an organism to environmental stresses (Miller-Morey and Van Dolah, 2004). The content of malondialdehyde, a product of lipid peroxidation, has been considered as a reliable indicator of OS, occurring within the cell (Tomma-

sino et al., 2012). To examine alterations in the level of lipid peroxidation in *I. pumila* genotypes ascribed to different types' clonal integration as well as the contrasting light microenvironments, the content of MDA in leaves from detached vs. interconnected ramets and shaded vs. sun-exposed ones was analyzed. We found that the amount of MDA changed with irradiation level and ramet-integration status of the clone part (Table 2). Regardless of the integration status of the ramets, the content of MDA was higher in sun-exposed leaves than in those developing in the shade. The results suggest that ramets of the same clone exposed to full sunlight experienced a stronger oxidative stress than those in the shade. The lowest concentration of MDA was determined in ramets from the shaded and disconnected clone part. In leaves of all other ramets, the amount of MDA was nearly 50% higher than in the disconnected ramet part from the shade (Table 2). Moreover, comparison of the MDA content in leaves from the two shaded clonal parts revealed that the shaded ramets connected with illuminated ones contained 25% more MDA than the shaded ramets without the physical connection, indicating a physiological expense that the connected ramets have to incur due to

Table 1 Physical and physiological parameters (mean±SE) describing habitat and leaf characteristics, respectively, of *Iris pumila*. Light quantity (photosynthetically active radiation, PAR), leaf temperature (LT) and leaf water potential (Ψ) were measured within each of the four clonal quarters: ED - exposed disconnected, EC - exposed connected, SC - shaded connected, SD - shaded disconnected.

	Clonal quarter			
	ED	EC	SC	SD
Habitat characteristics characteristics				
PAR ($\mu\text{mol}^{-2}\text{s}^{-1}$)	1326 ± 59	1326 ± 59	479 ± 36	479 ± 36
Soil moisture (%)	2.69 ± 0.23	2.40 ± 0.11	2.48 ± 0.24	2.40 ± 0.13
Leaf characteristics				
Leaf temperature (°C)	40.0 ± 0.7	40.7 ± 0.4	36.1 ± 0.4	36.1 ± 0.6
Ψ (MPa)	-0.79 ± 0.05	-0.98 ± 0.07	-0.89 ± 0.12	-1.15 ± 0.11

Table 2 Biochemical and morphological traits expressed in leaves of *I. pumila* plants belonging to different clone parts. The presented values refer to the mean ± SE of ten leaf samples. *P* denotes significance levels from an ANOVA of observed differences between the four clonal quarters: ED - exposed disconnected, EC - exposed connected, SC - shaded connected, SD - shaded disconnected.

Trait	Clonal part				P
	ED	EC	SC	SD	
Biochemical					
MDA (nmol g ⁻¹)	51.95 ± 6.08	51.45 ± 3.04	46.71 ± 8.38	36.60 ± 6.02	0.31
Morphological					
SLA (cm ² g ⁻¹)	134.5 ± 3.2	128.7 ± 1.6	152.6 ± 4.1	162.6 ± 4.9	<0.0001
LWC (gg ⁻¹)	0.801 ± 0.003	0.803 ± 0.003	0.811 ± 0.006	0.817 ± 0.002	0.030
LDMC (gg ⁻¹)	0.183 ± 0.003	0.181 ± 0.004	0.167 ± 0.004	0.164 ± 0.003	0.002

Table 3 *F*-values of the two-way ANOVAs for the effects of connection (C), light (L) and their interaction (C x L) on the biochemical (malondialdehyde content, MDA), physiological (leaf water content, LWC; leaf dry matter content, LDMC) and morphological (specific leaf area, SLA) leaf traits of *Iris pumila*.

Trait	df	Source					
		Connection (C)		Light (L)		C x L	
		F	P	F	P	F	P
Biochemical							
MDA	1	0.71	0.41	2.38	0.13	0.64	0.43
Morphological							
SLA	1	4.66	0.04	51.05	<.0001	0.33	0.57
LWC	1	0.13	0.76	9.12	0.0040	1.00	0.32
LDMC	1	0.01	0.93	18.38	0.0001	0.30	0.59

the association with the ramets from the illuminated clone part. The obtained results are in accordance with the findings of Caraco and Kelly (1991) who showed that the translocation of water and nutrients to resource-deficient ramets reduced the performance of the supplying ramets.

Because leaf ecophysiological traits are linked to resource uptake and utilization, they are expected to influence plant fitness (Ackerly et al., 2000). The two most important leaf traits related to carbon gain, SLA and LDMC, were found to be reliable indicators of resource-use strategies in the sand dune environments

(Garnier, 1992). It has been documented that SLA can be extremely sensitive to environmental changes, including reduction of water and/or nutrient availability, irradiance and temperature elevation (Vendramini et al., 2002, Cunningham et al., 1999, McDonald et al., 2003; Meziane and Shipley, 2001). For example, SLA tends to correlate positively with leaf nitrogen per unit dry mass, but negatively with leaf lifespan and assimilation rates (Ackerly et al., 2002). Given that leaves with high SLA are more productive than those with low SLA, high-SLA phenotypes are expected to be ecologically advantageous in resource-rich environments (Poorter and de Jong, 1998).

Our results show the mean values of SLA varied significantly in different parts of an *Iris* clone (Table 2). The shaded ramets exhibited a higher (above 20%) SLA compared to the sun-exposed ramets (Table 2). The two-way ANOVA which was used to compare the impacts of integration and light regime on SLA expression, revealed that both of the two factors significantly influenced the phenotypic values of SLA (Table 3). In the shaded treatment, ramets with the intact connections displayed a lower SLA compared to those with the detached rhizomes. The reduction in SLA was found to be associated with stress tolerance (Fernandez-Going et al., 2012). Leaves with a smaller SLA are capable of recovering their photosynthetic machinery after high-temperature stress more effectively than leaves with a greater SLA. In addition, during equal heat stress, species with the small SLA value accumulated a higher amount of chloroplast sHsp compared to those with the greater SLA (Knight and Ackerly, 2001). A statistically significant effect of the connection on SLA, obtained in a two-way ANOVA (Table 3), corroborated our hypothesis that between-ramet connections enhance ramet performance, and ultimately fitness.

The variation patterns of SLA values and MDA contents tended to be inversely related, irrespective of the clonal integration and irradiation status. However, the Pearson's correlation coefficients between MDA and SLA were higher in leaves of intact ramets compared to those with discarded connections ($r = -0.417$, $P = 0.1$ and $r = -0.092$, $P = 0.72$, respec-

tively). Given that SLA correlates negatively with leaf life span (Ackerly et al., 2002), the observed trade-off between SLA value and MDA content might be a compensatory mechanism in sun-exposed ramets to cope efficiently with oxidative stress. However, to make more valid conclusions, analyses that are more comprehensive need to be performed.

As we expected, the leaves of shaded ramets displayed higher water contents than their exposed counterparts (Table 1). The highest LWC value was recorded in the shaded and disconnected ramets. The two-way ANOVAs testing the effects of integration, light, and their interaction on LWC and LDMC, revealed only a significant effect of light on the mean values of these two traits (Table 3). As has already been mentioned, LDMC can be an indicator of plant resource use, as well as a predictor of the relative growth rates of a plant (Pontes et al., 2007). In our experiment, LDMC and SLA changed inversely with the variation of ambient light conditions (Table 2). Since a fast plant growth rate can be promoted by the co-occurrence of high SLA and low LDMC, with efficient nutrient conservation through a positive relationship between low-SLA and a high-LDMC (Poorter and de Jong, 1999), our results indicate that ramets of the same *I. pumila* clone will produce leaves with unique physiological and morphological characteristics, depending on their integration status and light conditions they happen to encounter.

Correlation analyses revealed that a highly negative association between SLA and LDMC appeared to be weaker in the physically interconnected ramets compared to those that were physically disconnected ($r = -0.456$, $P = 0.04$, and $r = -0.628$, $P \leq 0.01$ respectively). Thus, it seems reasonable to assume that the physiological communication between ramets promotes a rapid biomass production in all associated ramets, in spite of an unequal availability of the essential resources caused by the micro-environmental heterogeneity.

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