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Short communications

DIRECTIONAL ASYMMETRY ESTIMATES DEVELOPMENTAL INSTABILITY IN PLANTS: A CASE REPORT IN *PLANTAGO MAJOR* L. LEAVES

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In the present study, an additional approach was used to evaluate the potential effects of the industrial development on natural population of the common plantain (*Plantago major* L.), previously estimated using s_i^2 as an index of fluctuation asymmetry (FA) together with the coefficient of variation (CV). In this study, directional asymmetry (DA), a form of asymmetry in bilaterally symmetric traits, was used in order to estimate its rule as a potential indicator of developmental instability in leaf traits. Obtained results shows significantly higher DA values for the vein distances within a leaf (VD) on environmentally stressed area than in the control site. Reporting significantly higher values of DA for VD on the environmentally stressed sample and a positive relationship between FA and CV values obtained for the same trait within the same site, in a previous study, the study corroborates the potential of DA as an indicator of developmental perturbations.

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Developmental stability (DS) or homeostasis refers to the ability of an individual to product a consistent phenotype in a given environment (GRAHAM *et al.* 1993). However, developmental instability (DI), inability of bilateral organ or organism to buffer its development against disturbances and to produce predetermined phenotype MØLLER and SWADDLE (1997), can result from a wide variety of environmentally (or genetically) induced perturbations (VALENTINE and SOULÉ, 1973; VALENTINE *et al.*, 1973; SIEGEL and DOYLE, 1975; SIEGEL *et al.*, 1992; YABLOKOV, 1986; CLARKE, 1992; 1993).

Fluctuating asymmetry (FA), antysymmetry (AS) and directional asymmetry (DA) are the three recognized types of asymmetry in morphological traits (PALMER and STROBECK, 1992) and they are dynamically interrelated (GRAHAM *et al.*, 1998). Each type of asymmetry is characterized by a different combination of the mean and variance of the distribution of (R-L) differences (VAN VALEN, 1962; PALMER and STROBECK, 1986; 1992), where R and L represent measurement of the right and left sides of bilaterally symmetrical traits, respectively.

FA is the random deviation from perfect bilateral symmetry and is characterized by normally distributed (R-L) differences about a mean of zero (VAN VALEN, 1962; PALMER and STROBECK, 1986; LEARY and ALLENDORF, 1989; PARSONS, 1990). AS is associated with a bimodal distribution of (R-L) about a mean of zero or more subtly as a broad peaked unimodal (platykurtic) distribution of (R-L), PALMER and STROBECK (1992). DA is characterized by a normally distributed (R-L) where the mean departs significantly from zero.

Traditionally, the most commonly used index of developmental stability is FA (LUDWIG, 1932). Concerning the DA as an index of developmental perturbations in animals and plants, there are opposite opinions. DA, a form of adaptive asymmetry, is thought to be genetically determined and thus should not be considered as an indicator of DS. However, MCKENZIE and CLARKE (1988) as well as LEARY and ALLENDORF (1989) have suggested that adaptive asymmetries (DA and AS) could also be used as indicators of developmental stability, as they are a form of developmental invariance. Besides, LEAMY *et al.* (1998) convincingly hypothesized that a higher DA may result from stress.

The main aims of the study were: 1) to clarify DA responses in leaf traits of *Plantago major* L. (Plantaginaceae) to environmental stressors and 2) to evaluate whether DA can be used as an indicator of developmental perturbations in plants or not.

Two bilateral, linear dimensions on each leaf: (1) leaf width (LW, which is the distance from the midrib to the right and left margins at a leaf's widest point), and (2) vein distances within a leaf (VD, which is the distance between veins in the left and right side of a leaf, measured at a leaf's widest point) were analyzed. This set of morphometric traits has recently shown to be suitable in environmental

quality monitoring study on *P. major* (VELIČKOV and PERIŠIĆ, in press). The traits were measured with a digital caliper (0.01 mm accuracy). Each measurement of the left and right sides for each leaf were performed twice during two independent sessions and all measurements were performed by the same person (V. M.) to detect the feature confounding asymmetry analysis such as the measurement error.

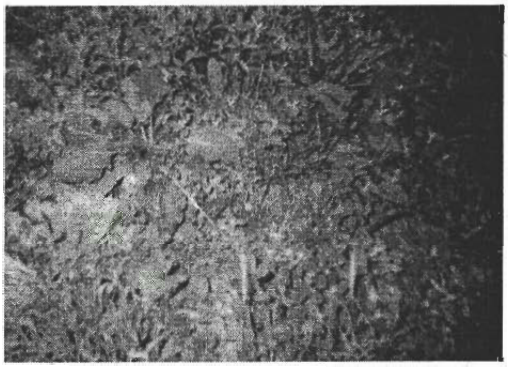

Fig. 1. - *Plantago major* in its natural habitat in Zemun (G. G.) site (arrow)

Thirty-two fully developed leaves per site (1 leaf per plant; each leaf with five major veins) were sampled in an area of 1 ha, during June of 2004, at two sites in northern Serbia. The samples were the same used by VELIČKOVIĆ (2005). The Karaburma site is located near the Pančevo site, which is the site of a large petrochemical complex and fuel storage site in Serbia and includes an ammonia plant ("Azotara", founded in 1962), a factory for chemical fertilizers ("HIP Petrohemija", founded in 1975), and a crude oil refinery ("Rafinerija Pančevo", founded in 1968). The data obtained from a long-term monitoring study of the Pančevo site, based on the UNMIX Version 2.4/MATLAB Version 6.5 (HENRY, 2001), at three measured locations (Vojlovica, Starčevo and Vatrogasni Dom) showed that in the Pančevo site were detected extremely high concentrations of toxic compounds (ICTMB, 2004). For this kind of investigation, however, it is important to point at certain chemical compounds, their metabolites and unwanted by-products because they represent the most dangerous chemical pollutants with long-term negative effects on environment, human health and living organisms. Results showed that the main contaminants released are: NH_3, NH_4^+, benzene, xilene, hydrocarbons from oil and oil derivatives, S and HS. (ICTMB,2004). Interestingly, up to 4.5% in oil derivatives is represented by sulphur. Also, in natural gas, HS is presented in range of 0.86-5.40 mg/l. Investigation performed in "HIP Petrohemija" showed that the "bio-filter" is one of the most dangerous contaminants of the air (with emissions of benzene of about 64kg h^{-1} and of xilene of about 34kg h^{-1}). Moreover, "Rafinerija Pančevo" is the dominate source of environmental contaminants like volatile organic compounds, CO, SO_x, No_x, polycyclic aromatic compounds (their metabolic transformations by aquatic and terrestrial organisms are into carcinogenic and mutagenic metabolites). The highest concentrations of benzene were detected lately in the night and early in the

morning that is positively correlated with the most intensive time of traffic flow. Average value for the amount of released benzene in atmosphere in 2005 was 5μg m^{-3} (ICTMB, 2004). The Zemun (Gornji Grad) site is a forested area, far from any known contamination. *P. major* plant in its natural habitat in Zemun (G. G.) site (Fig. 1). The two sites are approximately 150 km apart.

Before proceeding with the asymmetry analysis the Pearson correlation coefficient (r^2), between original and repeated measurements, for each side of each trait, is a calculated ($0.998< r^2>0.999$) indicating measurements reliable. Descriptive statistics for (R-L) values are calculated for each leaf trait, separately by site (Table 1.).

Table 1. - Descriptive statistics calculated for (R-L) values for leaf width (LW) and vein distances within a leaf (VD) in two analyzed populations of the common plantain

Site	trait	N	Min.	Max.	KS	Variance	SD	Skew±SE	Kurtosis±SE
Karaburma	LW	32	-0.30	0.40	0.143 (ns)	0.035	0.188	0.293±0.214	-0.459±0.409
	VD	31	-0.35	0.78	0.179 (ns)	0.037	0.191	1.667±0.421**	6.790±0.421**
Zemun	LW	32	-0.40	0.35	0.146 (ns)	0.031	0.176	0.376±0.215	0.098±0.409
(G. Grad)	VD	32	-0.20	0.25	0.191 (ns)	0.014	0.119	0.419±0.414	-0.717±0.409

N = sample size; Min. = minimum; Max. = maximum; KS = Kolmogorov Smirnov test of normality; SD = Standard Deviation;
SE = Standard Error; ns = statistically non significant; **= significant at $p< 0.001$ (t-test results for $df= \infty$, PALMER (1994)).

According to PALMER (1994) the level of DA for each trait, separately by site, was calculated as the mean square of the side factor (MS_{side}) of the two way-mixed model ANOVAs (in which sides represent fixed factor and individuals represent random factor).

Because the mean squares of the ANOVAs results are variances ($0.002< MS_{side} x10^2 >0.508$ the differences between samples were analyzed by comparing the heterogeneous of variances, therefore F-test (LEHMANN, 1959) was performed. Obtained results showed significantly higher DA values on environmentally stressed sample than in the reference one for VD: $F=1.897$; $df_{(1,2)}= 30,31$; $a=0.05$. Reporting significantly higher values of DA for VD on the environmentally stressed sample and a positive relationship between FA and CV values obtained for the same trait within the same site (VELIČKOVIĆ, 2005) the study corroborates the potential of DA as an indicator of developmental perturbations.

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DIREKCIONA ASIMETRIJA PROCENJUJE RAZVOJNU NESTABILNOST BILJAKA: SLUČAJ LISTOVA BOKVICE (*PLANTAGO MAJOR* L.)

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I z v o d

U ovom radu, korišćen je dodatni pristup u proceni potencijalnih efekata industrijskog razvitka na prirodne populacije obične bokvice (*Plantago major* L.) prethodno procenjenih korišćenjem (σ_i^2) indeksa fluktuirajuće asimetrije (FA) i koeficijenta varijabilnosti (CV). U ovoj studiji procenjena je uloga direkcione asimetrije (DA), oblik simetrije bilateralno simetričnih karaktera, kao mogućeg indikatora razvojne nestabilnosti karaktera lista. Dobijeni rezultati pokazuju značajno veću vrednost DA za karakter: rastojanje među venama unutar lista (VD) na stresnom području u odnosnu na kontrolni lokalitet. Ukazujući na značajno veće vrednosti DA za VD za uzorak sa stresnog lokaliteta i pozitivan odnos između vrednosti FA i CV za VD u prethodnoj studiji, ova studija ide u prilog korišćenju DA kao indikatora sredinskih poremećaja.

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