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PHENOTYPIC EPIDERMIS PLASTICITY IN POPULATION OF *RANUNCULUS ACRIS* L. *(RANUNCULACEAE)* UNDER DIFFERENT LIGHT CONDITIONS

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A total of 58 plants of one population of the *Ranunculus acris* L. subsp. *acris* species was cultivated from seed. Two groups of plants were grown in different light conditions: direct sun light and strong shade. Seven characteristics of the epidermis were analyzed before and after a one hundred day treatment. The data were processed statistically. Phenotypic plasticity was established for the epidermal cell width. Developmental variability was established for epidermal cell width, length and for the number of epidermal cells per unit of area. The population showed internal homogeneity. Comparisons with data cited displayed a strong interpopulation variability. Correlations among the characteristics are anticipated.

Key words: epidermis, phenotypic plasticity, light conditions, developmental variability, *Ranunculus acris*

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Iz sjemena jedne populacije vrste *Ranunculus acris* L. subsp. *acris* kultivirano je 58 biljaka. Jedna skupina biljaka rasla je direktno izložena sunčevu svjetlu, a druga u jakoj zasjenjenosti. Prethodno, i nakon 100 dana rastenja, analizirano je sedam osobina epiderme. Podaci su obrađeni statistički. Za širinu epidermalnih stanica ustanovljena je fenotipska plastičnost. Varijabilnost u razvitku utvrđena je za širinu i dužinu epidermalnih stanica i za broj epidermalnih stanica po jedinici površine. Uzorak pokazuje populacijsku homogenost. Usporedbe dobivenih rezultata s podacima citiranim u literaturi ukazuju na snažnu međupopulacijsku varijabilnost. Korelacije među osobinama su očekivane.

Ključne riječi: epiderma, fenotipska plastičnost, svjetlost, razvojna varijabilnost, *Ranunculus acris*

INTRODUCTION

Various species of the genus *Ranunculus* L. (*Ranunculaceae*) are among the most familiar spring wildflowers of the temperate region, with approximately 600 species worldwide (TAMURA 1967). The species *Ranunculus acris* L. is distributed in almost the whole of Europe. It is very variable, and many subspecies with intermediaries, often jointly reported as the *Ranunculus acris* complex, (TUTIN 1993, DAMBOLDT & ZIMMERMANN 1974) have been described.

The leaves of *R. acris* are of the amphistomatic type. Their anticlinal epidermal cell walls are thin and sinuously curved with an irregular size and shape. The stomata are surrounded by a few ordinary epidermal cells. Stomata of this morphology were classified as the ranunculus type by METCALFE & CHALK (1950, 1979), as the anomocytic type by CUTTER (1978), the ontogenetic anomo-mesoperigeneus type by FRYNS-CLAESSENS & VAN COTTHEMAS (1973), as the diameristic by COTTHAM (1973), as the mesoperigeneus type by PAYNE (1979), etc. In their paper, AVITA & INAMDAR (1980) gave quantitative data for epidermal characteristics for many species of the *Ranunculaceae* family, including *R. acris*.

Epidermal characteristics show taxonomic and phylogenetic significances in the family, at least on a genera level (HOOT 1991).

The phenotypic plasticity of epidermal features is well known in general. It depends on different ecological factors (DAUBENMIRE, 1959; MEIDNER & MANSFILD, 1968; METCALFE & CHALK, 1979) and other factors, for example polyploidy (STEBBINS, 1971; BRANDHAM & CUTLER, 1981). These facts make it difficult to use epidermal characteristics in diagnostics.

The aims of this paper are:

- to define quantitatively the basic epidermal characteristics and their relationships in *R. acris* L. subsp. *acris* L. from a new locality,
- to establish the phenotypic plasticity of epidermal characteristics caused by different light ratio;
- to establish the variability of epidermal characteristics caused by maturation, i. e. developmental variability.

MATERIAL AND METHODS

In total, 58 plants of *R. acris* L. subsp. *acris* (group A) were germinated from seeds in the Botanical Garden in Zagreb. All seeds originated from the same parental plant from the location of Gornja Stubica, Croatia (UTM 33TWL79). From all specimen a sample of two leaves was taken, the first time in June. After that, the plants were divided in two groups (C and D). Group C continued developing in a position with direct sun light all day, and group D continued developing in a position without direct sun light (strong shade) all day. The locations were very close, so other ecological factors were taken to be the same. After 100 days, the sampling procedure was repeated. The group that finished its development in the light is marked E, and the group which finished development in the shade is marked F (E + F = group B).

The leaves were conserved in FAA fixing fluid (JOHANSEN, 1940). Samples of the epidermis were taken from the lower surface in the middle area. Each plant specimen was measured ten times for the following characteristics:

1. stomata length (S_l, m)
2. stomata width (S_w, m)
3. epidermal cell length (E_l, m)
4. epidermal cell width (E_w, m)
5. number of stomata per mm^2 (No_s)
6. number of epidermal cells per mm^2 (No_e)
7. number of hairs per mm^2 (No_h)

The results were analysed by standard statistical tools (arithmetical mean x, standard errors S_x, standard deviation S).

The following null-hypothesis was tested:

1. Ho1: the variables have a normal distribution (Kolmogorov-Smirnov test, $\alpha = 0.05$);
2. Ho2: there are no significant differences between individuals (Kruskal-Wallis test, $\alpha = 0.01$);
3. Ho3: there are no significant differences between groups C and D, and, independently, groups E and F (Mann-Whitney test $\alpha = 0.01$; the arithmetical mean of 10 measurements was used for each specimen);
4. Ho4: there are no significant differences between groups C and E, and independently, groups D and F (Mann-Whitney test $\alpha = 0.01$; arithmetical mean of 10 measurements was used for each specimen);

The variables were correlated by Kendal-coefficient, with significance calculated at $\alpha = 0.01$ (ROHLF & SOKAL, 1969; SOKAL & ROHLF, 1981).

RESULTS

The quantitative measurements of epidermal characteristics for *Ranunculus acris* L. subsp. *acris* from Croatian area are presented in Tab. 1.

All variables for groups A and B are normally distributed (Ho1 accepted).

There are no significant differences between individuals in the particular group of plants (C, D, E, F) for all characteristics (D range: 25.48–66.10) (Ho2 accepted).

On an individual level the standard deviation shows the following span: 0.92–7.95 μm for stomata length (S_l), 1.05–5.54 μm for stomata width (S_w), 5.91–34.37 μm for epidermal cell length (E_l) and 3.37–21.31 μm for epidermal cell width (E_w).

The t_s values from Mann-Whitney test for the Ho3 hypothesis are given in Tab. 2. Before the exposure of groups C and D to different light conditions there were no significant differences in any epidermal features (Ho3 accepted). After the groups were treated with different light conditions (group E finished development in the light and group F finished development in the shade) significant differences were established for epidermal cell width (E_w) (Ho3 rejected). Thus, only this characteristic showed phenotypic plasticity.

Table 1. The basic statistical parameters for measured epidermal characteristics. Groups C and D – before treatment with different light conditions, group E contains plants that finished their development in the light (same plants as in group C after 100 days) and group F contains plants that finished their development in the shade (same plants as in group D after 100 days).

Charac.	Group	X	Sx	min	max	S
S_l (μm)	C	43.71	0.34	25.00	51.78	5.72
	D	44.07	0.29	30.00	57.50	4.95
	E	41.28	0.35	25.00	60.00	5.75
	F	42.24	0.27	25.00	55.00	4.76
S_w (μm)	C	31.30	0.19	20.00	42.50	3.27
	D	30.99	0.21	22.50	47.50	3.57
	E	31.01	0.24	22.50	45.00	3.94
	F	30.53	0.17	22.50	40.00	3.00
No_s/mm^2	C	110.69	4.89	59.17	155.32	26.33
	D	104.57	5.39	59.17	162.72	29.03
	E	106.29	7.04	51.77	184.91	36.59
	F	103.30	7.43	44.38	251.48	40.67
E_l (μm)	C	89.81	1.33	42.50	171.25	22.67
	D	91.89	1.33	45.00	180.00	22.62
	E	75.04	1.22	37.50	195.00	20.10
	F	79.58	1.09	42.50	142.50	18.91
E_w (m)	C	61.80	0.87	28.75	110.00	14.89
	D	65.26	0.86	37.50	107.50	14.66
	E	52.65	0.83	26.25	110.00	13.60
	F	56.29	0.72	25.00	95.00	12.55
No_e/mm^2	C	283.36	14.33	162.72	458.58	77.17
	D	274.94	13.09	140.53	443.79	70.53
	E	381.05	18.38	207.10	562.13	95.52
	F	332.10	12.96	244.08	517.75	70.98
No_h/mm^2	C	15.30	2.00	0.00	51.78	10.81
	D	11.47	1.99	0.00	36.98	10.75
	E	20.55	2.69	0.00	51.78	13.96
	F	17.01	1.67	0.00	36.98	9.14

The t_s values from the Mann-Whitney test for Ho4 hypothesis are given in Tab. 3. Group C was tested against group E, i. e. the same plants, but 100 days older, vs. those growing in direct sun light. Similarly, group D was tested against group F, i. e. the same plants 100 days older, but growing in strong shade. Significant differences, in both tests, were established for epidermal cell length (E_l), epidermal cell width (E_w) and the number of epidermal cells per mm^2 (No_e) (Ho4 rejected). Epidermal cell length and width decrease on average by 9.1, i. e. 13.5 μm. The number of epidermal cells per mm^2 increased on average by 78 cells per mm^2.

Fig. 2. Observed abnormalities in epidermis: (1) stomata arrested development, (2) degeneration of one guard cell, (3) degeneration of both guard cells.

During the material analysis the following abnormalities were observed and counted (Fig. 2.):

1. stomata arrested in development, 32.7 per mm^2
2. degeneration of one of guard cell, 7.05 per mm^2
3. degeneration of both guard cells, 1.9 per mm^2

DISCUSSION

As compared with epidermal characteristics of an *R. acris* sample from Berlin (AVITA & INAMDAR 1980), the samples from Croatia have a greater number of stomata ($104.72/mm^2 > 92.8 \pm 18.1/mm^2$), which are shorter and almost twice as wide (41.79 µm × 30.76 µm Gornja Stubica; 52.7 µm ± 2.7 × 16.7 µm ± 1.6 Berlin). The epidermal cells in the sample from Gornja Stubica are considerably smaller than in the sample from Berlin (77.43 µm m × 54.57 µm << 110.8 µm ± 18.2 × 71.2 µm ± 20.9), and consequently, the number of the epidermal cells in the sample from Gornja Stubica is much greater ($355.293/mm^2 > 254.4/mm^2 \pm 71.3$). Population differences are not tested but apparently exist.

The samples' epidermal characteristics before and after treatment were normally distributed about the arithmetical means (groups A and B). At the individual level there are no significant differences between plants. This fact indicates population homogenity in features measured. At the individual level, the greatest variability was observed for epidermal cell dimensions (E_l, E_w), 2–3 times greater than for stomata dimensions (S_l, S_w).

Several factors could influence the epidermal characteristics, without any connection with the genotype. For different taxa the number and the dimension of stomata depend on xeromorphous, i. e. mesomorphous climatic circumstances (MAKSIMOV 1952, ESAU 1965, FAHN 1982, WILDER 1985, LUCANSKY & CLOUGH 1986), the stomatal index depends on moisture and light intensity (CUTTER 1971, BAČIĆ 1982),

Logical negative correlations were established between number of epidermal cells and number of stomata per mm^2 (No_e, No_s) and epidermal cells and stomata dimensions, i. e. with an increase of epidermal cells and stomata dimensions, i. e. with an increase of epidermal cells and stomata dimensions their numbers per unit of area decreased. The greatest correlation was found between the length and the width of epidermal cells (Fig. 1.)

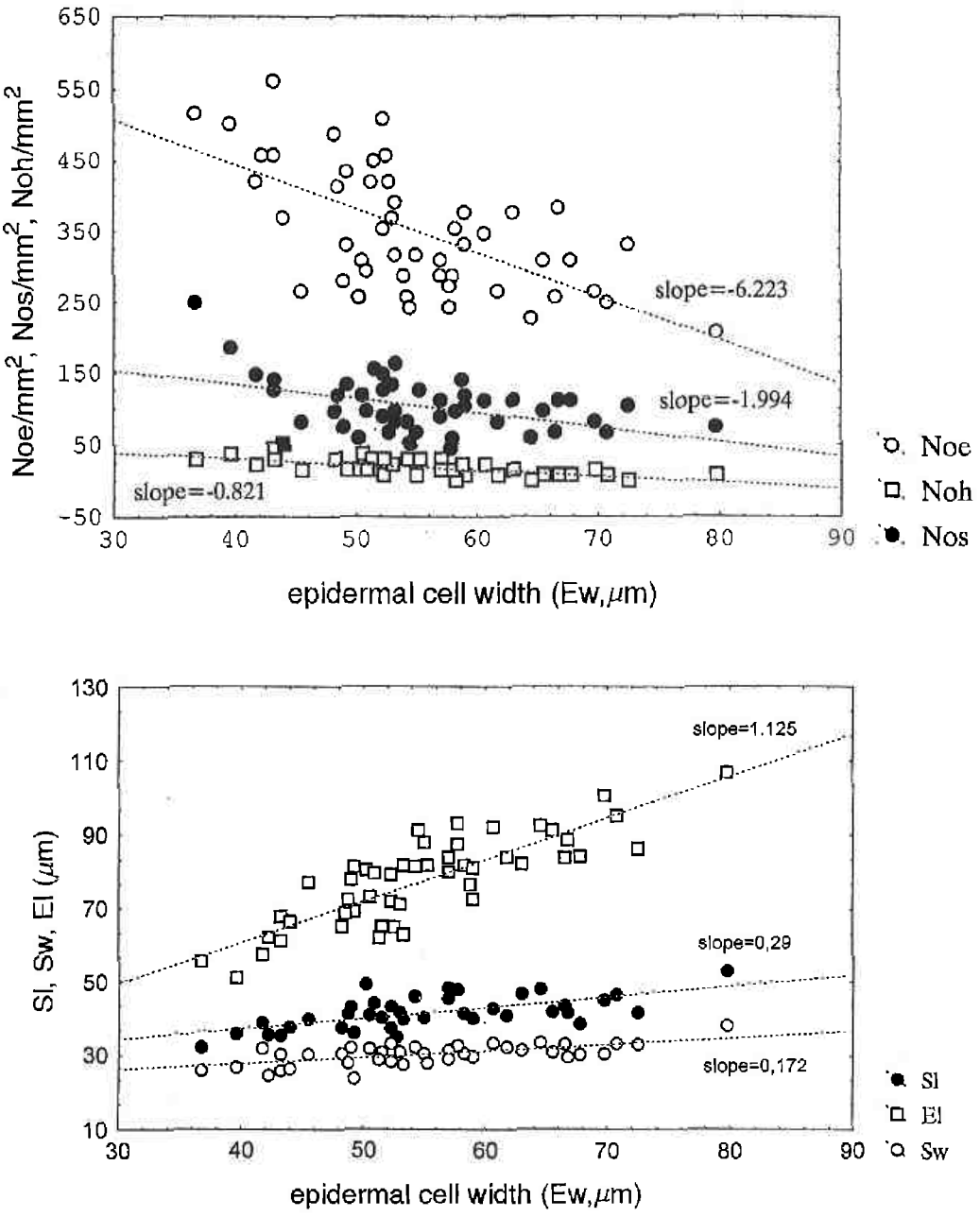

Fig. 1. Scatter diagrams showing relationships between epidermal cell widths (E_w) in group B and **(a)** – numbers of epidermal cells (No_e), stomata (No_s) and hairs (No_h) per mm^2; **(b)** – epidermal cells length (E_l), stomatal length (Sl) and stomatal width (Sw).

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the number of stomata depends on the air pollution level (SHARMA & BUTTLER 1975), etc. QI-GEN & CUTLER (1985) emphasized that because of small intraspecies variability the diagnostic value of stomata was high, but the range of particular characteristics was more dependent on ecological factors.

In this investigation, plants did not show any significant differences in epidermal characteristics before treatment with different light conditions (groups C and D). After treatment with different light intensities (groups E and F) plants showed significant differences in epidermal cell width (E_W). This indicates the phenotypic plasticity of this feature. However, the observed dissimilarity is not enough high for the clear appearance of two groups in the scatter diagram. A development of a higher number of stomata with increased light intensity and vice versa (KNECHT & O'LEARY 1972) was not observed. Also the growth in full sun light did not cause in *R. acris* the development of higher number of smaller stomata, as was cited for some other taxa (DAUBENMIRE 1959, CUTTER 1971, METCALFE & CHALK 1979).

ESAU (1965) relates that, in a given leaf, the stomata do not all arise at once but rather over a considerable period of leaf growth, and also emphasizes the importance of using leaves of comparable physiological and anatomical maturity when doing comparative studies of stomatal numbers. In this investigation, no developmental variability of stomata characteristics was observed. A significant developmental variability was observed in characteristics connected with epidermal cells: epidermal cell width, length and number/mm^2. The number of epidermal cells increased with maturity following with the decrease of the average dimensions of cells.

The sample contain both previously observed trichome types (HOOT 1991, SOLEREDER 1899, METCALFE & CHALK 1950): (1) unicellular non- glandular hairs with a raised rosette of cells surrounding the base of the trichome and (2) small, unicellular glandular hairs. The number of hairs did not show an significant dependence on other epidermal characteristics; it decreased insignificantly with the increase of epidermal cell dimensions.

Earlier cited abnormalities (AVITA & INAMDAR 1980) (arrested development, degeneration of one or both guard cells) were also observed in the sample from Croatia.

The conclusions are:

1. the populations from Gornja Stubica and from Berlin showed intested but obvious differences in epidermal structure.
2. epidermal characteristics were normally distributed.
3. differences between individuals were not significant.
4. phenotypic plasticity caused by different light conditions was observed in the width of epidermal cells.
5. developmental variability was observed only for epidermal cells characteristics: width, length and number/mm^2.
6. independent development was observed for hairs/mm^2. Other characteristics showed the expected relationships.

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SAŽETAK

Fenotipska plastičnost stanica epiderme u populaciji *Ranunculus acris* L. (*Ranunculaceae*) utjecana različitom količinom svjetlosti

T. Nikolić

Ranunculus acris L. rasprostranjen je u gotovo cijeloj Europi. Vrsta je vrlo varijabilna, opisano je mnoštvo podvrsta s međuoblicima, često skupno imenovanim kao *Ranunculus acris* kompleks. Listovi *R. acris* su amfistomatskog tipa s epidermalnim stanicama na naličju lista s tankim, sinusoidno zakrivljenim stijenkama, nepravilnog oblika i veličine. Puči na naličju lista okružene su s nekoliko stanica epiderme koje se oblikom ne razlikuju od ostalih epidermalnih stanica.

Svrha rada je ustanoviti moguću upotrebu osobina epiderme u intrapopulacijskom razgraničavanju unutar vrste i to: (1) definiranjem osnovnih karakteristika epiderme i njihovih međuodnosa s lokaliteta u Hrvatskoj, (2) ustanovljivanjem fenotipske plastičnosti epiderme zbog djelovanja različitih režima svjetlosti i (3) ustanovljivanjem fenotipske plastičnosti epiderme uzrokovane starenjem, tj. razvitkom.

Iz sjemena jedne populacije vrste *Ranunculus acris* L. subsp. *acris* kultivirano je 58 biljaka. Jedna skupina biljaka rasla je direktno izložena sunčevu svjetlu, a druga u jakoj zasjenjenosti. Analizirano je sedam osobina epiderme prije i nakon 100 dana rasta uz navedene uvijete (duljina i širina puči, duljina i širina stanica epiderme, broj puči, broj stanica epiderme i broj dlaka po jedinici površine). Podaci su obrađeni statistički.

Fenotipska plastičnost uzrokovana različitim režimom svjetlosti ustanovljena je za širinu epidermalnih stanica. Varijabilnost u razvitku, tj. promjene koje nastaju starenjem biljke, ustanovljena je za širinu i dužinu epidermalnih stanica i za broj epidermalnih stanica po jedinici površine. Uzorak pokazuje populacijsku homogenost. Usporedba dobivenih rezultata s podacima citiranim u literaturi ukazuju na snažnu međupopulacijsku varijabilnost. Korelacije među osobinama su očekivane.