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Irradiation level affects fluctuating asymmetry value of bilateral traits of cucumber in juvenile phase

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Abstract. Light is an important factor of the plant's environment. The aim of research was to confirm the hypothesis on the influence of different irradiation levels on the fluctuating asymmetry (FA) value of bilateral traits of plants cultivated in the indoor plant lighting. The object of research was the plants of cucumber (*Cucumis sativus L*) as one of the main glasshouse crops. Young 14-day-old cucumber plants in the first true leaf phase were studied. Different irradiation levels (15.0, 22.5 and 30 W m^{-2}) were maintained by fluorescent lamps. An essential asymmetry of bilateral structures in plants grown under different irradiation levels was observed. It was found that statistically significant lower values of FA, that is greater stability of plant development, correspond to increased plant performance. When the irradiation level was switched from 15 to 30 W m^{-2} (by 100%), the FA index for different bilateral structures varied by different amount: in terms of cotyledons mass it decreased from 0.046 to 0.032 relative units (by 30.2%), in terms of chlorophyll content in cotyledons it increased from 0.038 to 0.073 relative units (by 88.6%). In some bilateral structures the FA index variation was rather small: in terms of the cotyledon area it was zero, in terms of the cotyledon thickness – by 1.8%. It was experimentally prove through the example of cucumber plants that FA index could be used as an indicator of plant developmental stability, characterizing the deviations of the growing environment parameters from the normal state in the indoor cultivating.

Key words: indoor plant lighting, developmental stability.

INTRODUCTION

An important scientific trend, which has been formed at the intersection of biological sciences, is the study how environment affects the individual development (ontogeny) of plants. Investigations in this area should address the issues of quantitative assessment of the relationship between the indices of plant life activity and the environmental factors in ontogeny. The level of irradiation in the range of photosynthetically active radiation (PAR) is an important environmental factor, with which the plants, being organisms with sessile mode of life, is forced to coordinate the processes of their growth and development (Smith, 1982).

In natural conditions, the ambient light changes continuously due to the changing position of the Sun above the horizon throughout the day and the year. Under the indoor plant lighting conditions, the control of irradiation modes provides an opportunity to influence both individual physiological processes in the plant and the plant developmental stability in general (Wheeler, 2008). The latter manifests itself in the interaction of random events in the plant organism and their ability to follow accurately the programme inherent in the genotype, resisting the environmental impacts in the process of development in order to form the optimal phenotype. Accordingly, the inadequate quality of the growing medium leads to developmental instability (Venâncio et al., 2016).

The most striking manifestation of the stable development of a biological object at the macro level is the phenomenon of fluctuating asymmetry (FA), consisting of minor and random deviations of the parameters of bilateral (mirror) morphological structures (Moller & Swaddle, 1997; Graham et al., 2010). Such structures are cotyledons, halves of simple leaves, opposite leaflets of compound leaves, needles of conifers in the whorl, petals of flowers, seed pod walls, and opposite leaves.

The symmetry breaking is identified by comparing the different parameters of bilateral traits (Daloso, 2014). The FA level is minimal only under optimal environmental conditions; it increases under any stress impacts (Zakharov, 1992; Palmer & Strobeck, 2003). In a number of studies it was found that the FA index is closely correlated with the quality of plant growing environment, both natural (Alados et al., 2001; Milligan et al., 2008; Veličković, 2010) and controlled (Kuznetsova et al., 2013). Therefore, it can be used as an indicator of the developmental stability of an organism, characterizing even subtle deviations in the ambient parameters, irradiation in our case, from the background state. Other studies show, however, the absence of environmental influence on FA (Bjorksten et al., 2000; Veličković & Perišić, 2006; Sandner & Matthies, 2017; Zverev et al., 2018).

Assumption that non-optimality of irradiation parameters affecting the plants is a stress factor makes it possible to use the level of FA as a plant status indicator in order to estimate the effectiveness and ecological compatibility of the indoor plant lighting, with due account for the irradiation quality (Rakutko et al., 2017).

For natural plant growing conditions, the interrelation between the level of plant FAs and the stress and quality of environment is described in numerous scientific publications (Tucić & Miljković, 2010; Silva et al., 2016; Venâncio et al., 2016), while for the indoor plant lighting more detailed research is still needed.

The aim of research was to test the hypothesis on the influence of different irradiation levels on the value of FA of bilateral traits of plants cultivated in the indoor plant lighting.

MATERIALS AND METHODS

Cucumber (*Cucumis sativus* L) was chosen for the investigations as one of the main greenhouse crops. To optimise its cultivation conditions for higher yields is a topical task for modern greenhouse complexes. Several periods are identified in the ontogenesis of cucumber, based on a set of morphological and morpho-physiological characters and histological signs. The early periods and age states are of particular interest. These are the latent period, with the age state being a seed, and the pre-generative period with two age states: (1) a sprout with heterotrophic mode of nutrition, starting from the seed planting and ending with the emergence of the folded cotyledons over the substrate surface; (2) a sprout with mesotrophic nutrition ending with the appearance of the 1st true leaf. Then comes the juvenile plant from the 1st to the 2nd true leaf; the immature plant, from the 2nd to the 4th leaf, and the virginile plant, from the 4th to the 9th leaf (Vasilevskaya, 1991).

In the pre-generative period of ontogenesis, the internal structures of a plant organism are formed; therefore, the study of growth and development processes during this period depending on environmental factors at the whole organism level is of both theoretical and practical interest.

The plants were grown on peat, the acidity of which was neutralised with dolomite meal to pH of 6.0. One kg of peat included the following mineral nutrients: K_2O – 330.2 mg, P_2O_5– 42.8 mg, CaO – 151.6 mg, mgO – 102.8 mg, N_2O_5 – 63.1 mg. The seeds of middle-early cucumber hybrid Safaa F1, were sown on February 14, 2017 to a depth of 1 cm under the 5 x 5 cm pattern. Fully sprouted cucumbers appeared on the fourth day – February 17, 2017. From February 21, 2017, the photoperiod of 16 hours was set from 07.00 to 23.00. On the ninth day, February 22, 2017, the unfolding of the first true leaf was registered. The growing was stopped on the 14th day, February 27, 2017, when the second true leaf appeared.

A comparative experiment was carried out in the three zones of the laboratory room, separated by a light-tight curtain. The air temperature of +26 °C and humidity 65–72% were maintained by an automatic control system. The moisture content of the substrate was 70%; the soil temperature was + 25–26 °C. Irradiators of the own design with fluorescent lamps L 58W / 77 Fluora OSRAM and PHILIPS MASTER TL–D Xtra 58W / 840 we used. The radiation spectrum of the light sources was characterised by approximately equal flux share in blue, green, and red bands of PAR. Different levels of irradiation (15.0, 22.5 and 30 Wm^{-2}) during the experiment in the growing zones were ensured by maintaining the required height of the irradiator suspension over the tops of the plants.

The length (L_L, R_L) and the width (L_W, R_W) of the left and right cotyledons, respectively, were used as metric parameters. The length of the secondary leaf veins from the base of the blade to their forking (first trait L_1, R_1), the distance from the forking to the leaf apex (second trait L_2, R_2) and the distance from the leaf apex to the characteristic points on the leaf edge (third trait L_3, R_3) were measured on the left and right half of the leaf, correspondingly. In addition, the chlorophyll meter CCM-200 determined the chlorophyll content index in the cotyledons (L_{cc}, R_{cc}) and on the sides of the leaf (L_{cl}, R_{cl}) in CCI relative units; mass (L_{mc}, R_{mc}), thickness (L_T, R_T) and area (L_S, R_S) of the cotyledons, as well as the mass M_L and area of the leaves S_L were measured. 40 plants from each container were used for measurements.

Linear dimensions were measured on the images obtained by the Power Five Evo camera using the XnView program. The data were processed by mathematical statistics methods ($P < 0.05$) using Excel 2003 and Statistica 6.0 software packages.

RESULTS AND DISCUSSION

Even preliminary analysis of the data obtained showed fairly high frequency of asymmetric signs in cucumber plants grown under different levels of irradiation. The frequency values of a number of traits increased monotonically with higher irradiation level (cotyledon length – from 0.73 to 0.90, chlorophyll content in individual cotyledons – from 0.80 to 0.90, chlorophyll content in the leaf halves – from 0.50 to 0.71).

The values of biometric indicators and the level of FA for individual bilateral traits are shown in Table 1. The meaning of ΔP is how much the value of the parameter P changes with a change in the irradiance level from 15.0 to 30.0 W m^{-2}.

Table 1. The values of biometric indicators and the level of FA for individual bilateral traits

Bilateral traits (indicators)	Parameters P	Irradiation, W m^{-2} 15.0	22.5	30.0	ΔP, %
1. Cotyledon width (L_W, R_W), mm	$\overline{X} \pm \sigma$	23.93 ± 1.42	24.62 ± 1.69	22.59 ± 2.43	-5.6
	FA	0.028	0.020	0.040	42.3
2. Cotyledon length (L_L, R_L), mm	$\overline{X} \pm \sigma$	41.62 ± 2.60	42.00 ± 3.11	40.04 ± 3.72	-3.8
	FA	0.024	0.027	0.033	35.5
3. Cotyledon area (L_S, R_S), cm^2	$\overline{X} \pm \sigma$	6.88 ± 0.93	7.04 ± 1.00	6.07 ± 1.11	-11.8
	FA	0.071	0.060	0.071	0.0
4. Cotyledon mass (L_{mc}, R_{mc}), g	$\overline{X} \pm \sigma$	0.36 ± 0.04	0.46 ± 0.05	0.39 ± 0.05	8.3
	FA	0.046	0.021	0.032	-30.2
5. Cotyledon thickness (L_T, R_T), mm	$\overline{X} \pm \sigma$	0.81 ± 0.05	0.80 ± 0.05	0.84 ± 0.05	3.7
	FA	0.019	0.017	0.019	1.8
6. Cotyledon CCI (L_{cc}, R_{cc}), rel.u.	$\overline{X} \pm \sigma$	85.28 ± 10.26	103.46 ± 13.53	120.47 ± 15.84	41.3
	FA	0.038	0.046	0.073	88.6
7. Leaf first trait (L_1, R_1), mm	$\overline{X} \pm \sigma$	14.00 ± 2.22	16.23 ± 2.70	18.16 ± 3.13	29.7
	FA	0.055	0.042	0.066	19.2
8. Leaf second trait (L_2, R_2), mm	$\overline{X} \pm \sigma$	26.34 ± 2.82	29.93 ± 4.46	31.95 ± 4.31	21.3
	FA	0.095	0.118	0.097	3.0
9. Leaf third trait (L_3, R_3), mm	$\overline{X} \pm \sigma$	25.36 ± 3.02	30.20 ± 4.92	32.68 ± 5.42	28.9
	FA	0.042	0.047	0.038	-9.3
10. Leaf CCI L_{cl}, R_{cl}), rel.u.	$\overline{X} \pm \sigma$	30.95 ± 2.31	37.65 ± 5.51	39.69 ± 6.65	28.2
	FA	0.069	0.062	0.059	-13.7
11. Leaf mass M_L, g	$\overline{X} \pm \sigma$	0.35 ± 0.05	0.50 ± 0.09	0.65 ± 0.12	85.7
12. Leaf area S_L, cm^2	$\overline{X} \pm \sigma$	12.12 ± 2.43	16.51 ± 4.22	20.30 ± 4.63	67.5

At the same time, other traits demonstrated the biggest values (the width of the cotyledons, their mass, area and thickness, the geometric dimensions of the leaf). Nonparametric methods of statistical analysis were applied. Most of the leaf traits had a normal distribution, except for the third trait in plants in the first experimental room zone.

The analysis revealed fluctuations in the asymmetry of traits around the zero mean that is a diagnostic sign of FA. Anti-symmetry in the analyzed traits was not observed.

Using the Spearman rank correlation coefficient, a statistically significant ($p < 0.05$) correlation was revealed between the asymmetry value of the trait and its average size for a number of traits.

Therefore, to calculate the value of FA, the normalizing formula was applied:

$$FA_i = \frac{1}{N}\sum_{i=1}^{N}\frac{|L_i - R_i|}{(L_i + R_i)} \quad (1)$$

where L_i – the value of the i-th trait for the bilateral structure on the left; R_i – the value of the i-th trait for the bilateral structure on the right; N – the number of measurements.

Significant correlation of traits between each other was revealed; therefore, FA values for individual traits were used for the analysis, without applying the complex index. Various environmental stress factors can influence different traits of FA in various ways (Roy & Stanton, 1999).

Analysis of Table 1 shows that when the irradiation level was switched from 15 to 30 W m^{-2} (by 100%), the FA index for different bilateral structures varied by different amount: in terms of cotyledons mass it decreased by 30.2%, in terms of chlorophyll content in cotyledons it increased by 88.6%.

However, in some bilateral structures, the FA level did not change: for the cotyledon area, ΔFA = 0%, for the cotyledon thickness ΔFA = 1.8%.

The above Table also presents the average values of the measured biometric indicators with the standard deviation of each indicator. Differences between the largest and smallest values of all indicators are statistically significant ($p < 0.05$).

It is of interest to identify the relationship between the FA level and the plant performance. Fig. 1 shows the dependence of the average cotyledon mass on the irradiation level. Fig. 2 shows the dependence of the FA level in terms of cotyledon mass on the irradiation level.

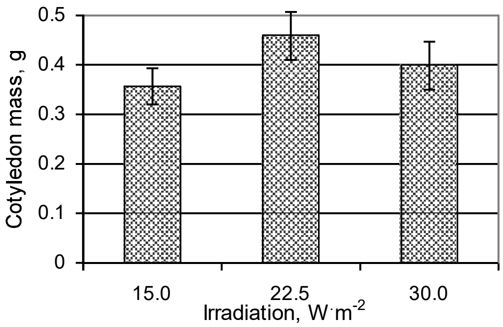

Figure 1. Dependence of the average cotyledon mass on the irradiation level.

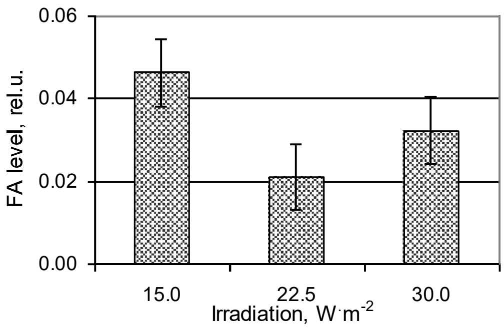

Figure 2. Dependence of the FA level in terms of cotyledon mass on the irradiation level.

It was found that statistically significant lower values of FA, that is greater stability of plant development correspond to increased plant performance in terms of raw mass of cotyledons. Plant signaling systems control their development depending on environmental conditions. Light is the most important environmental factor, the regulatory role of which is to ensure the growth and development of plants. The photosynthesis processes play a significant role in the life support system for plants. These processes are very sensitive to any changes in environmental factors, even slight ones (Darko et al., 2014).

Under heterotrophic nutrition, during the sprout stage, intensification of physiological processes under the influence of environmental factors increases the rate of metabolite efflux from the cotyledons. With the involvement of autotrophic nutrition, the processes of synthesis start along with dissimilation, and therefore the factors of the light environment are of paramount importance. When the structural and functional state of the organism becomes more complex, one of the criteria for becoming self-organised is to change the nature of the links with environmental factors from passive proportional response to differentiated perception (Belousov, 1987). In favour of the latter is the mere fact that there exist optimal values of factors, including illumination.

According to one of the approaches, the optimal parameters are those parameters of the environment under which the minimum duration of ontogeny stages is observed. Thus, for cucumber plants in juvenile and immature age states under all temperatures, the illumination of 10 kLx was found optimal by this criterion. If the accumulation rate of dry mass is taken as the growth index, then the optimal illumination is 25 kLx (Sysoeva, 1991)

In this study, the accumulation of green mass, characterized by a number of biometric indicators, was taken as a measure of the growth process intensity. Plant developmental stability was assessed by the FA index of bilateral structures. Usually this indicator is applied to the right and left halves of one leaf (Kaligarič et al., 2008).

It was assumed that the impact of environmental factors would result in greater asymmetry in the development of more distance-separated bilateral structures, which were nevertheless under the same conditions. So in this study the parameters of cotyledon were measured.

CONCLUSIONS

1. As a result of the study, a significant asymmetry of bilateral structures in juvenile cucmber plants, grown under different irradiation levels, was revealed. Frequency of asymmetry in individual structures reached 90%.

2. The asymmetry of bilateral traits was found non-directional, with the anti-symmetry not in place. This allowed classifying the observed asymmetry as fluctuating.

3. Significant correlation between individual bilateral traits was revealed. This provides an opportunity for a well-grounded choice of one specific trait or their combination to characterize the FA. The size-dependence of some traits was also revealed.

4. It was found that statistically significant lower values of FA, that is greater stability of plant development, correspond to increased plant performance in terms of raw mass of cotyledons. This confirms the original hypothesis that bigger values of FA are observed under conditions, which are less favourable for plants.

5. Using the example of cucumber plants, it was experimentally proved that FA indexes can be used to assess the quality the light environment for plant indoor cultivation.

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