ISSN 1062-3604, Russian Journal of Developmental Biology, 2012, Vol. 43, No. 5, pp. 259–263. © Pleiades Publishing, Inc., 2012.
Original Russian Text © E.A. Erofeeva, 2012, published in Ontogenez, 2012, Vol. 43, No. 5, pp. 320–324.

Developmental Stability of a Leaf of *Pisum sativum* L. under the Influence of Formaldehyde in a Wide Range of Doses

E. A. Erofeeva
Lobachevskii State University of Nizhni Novgorod, Nizhni Novgorod, 603950 Russia
e-mail: ele77785674@yandex.ru

Received March 28, 2011; in final form, May 4, 2011

Abstract—The influence of formaldehyde in a wide range of doses on the stability of development of the third leaf of pea (*Pisum sativum* L.) was studied. The developmental stability of the leaf was assessed by the change in the value of the directional asymmetry of the right and left leaflets caused by the fluctuating asymmetry of these morphological structures. When subjected to a toxic agent, the studied parameter exhibited a paradoxical effect. In minimum studied concentrations, formaldehyde disturbed stability of leaf development, which was manifested in an increase in the asymmetry of the right and left leaflets. At medium concentrations of the toxicant, the asymmetry was less than the control level, which indicated an increase in the developmental stability of the pea leaf. Maximum studied concentrations of formaldehyde, close to sublethal, again reduced the stability of development of the pea leaf and led to an increase in the asymmetry of its leaflets compared with the controls.

Keywords: *Pisum sativum* L., leaf, asymmetry, formaldehyde, paradoxical effect

DOI: 10.1134/S1062360412050025

INTRODUCTION

Stability of development characterizes the ability of an organism to maintain the trajectory of development within certain bounds (Zakharov, 1987). Fluctuating asymmetry is widely used as a means of stabilizing the development of bilateral morphological structures of plants. It is understood as random insignificant deviations from the symmetrical state (Zakharov et al., 2001) owing to the stochastic nature of molecular processes that provide the expression of genes (ontogenetic noise) (Leamy and Klingeberg, 2005). Fluctuating asymmetry of a nonhereditary nature is also observed against the background of hereditary types of asymmetry, such as antisymmetry and directional asymmetry (Zakharov, 1987). It is known that fluctuating asymmetry increases under the influence of any environmental stress factors (Zakharov et al., 2001; Hoffman and Woods, 2003). Due to this, the fluctuating asymmetry of the leaf of various plant species is widely used to estimate the level of pollution of the environment (Gelashvili et al., 2004). At the same time, more evidence has been accumulated in toxicology that, apart from the classical monotone dose–effect dependences (S-shaped, exponential), nonmonotone responses, which include hormesis (Kefford et al., 2008; Calabrese and Blain, 2009) and paradoxical effects (Batyan et al., 2009), are also found rather often. The question of whether such phenomena may be observed under the influence of various priority pollutants on the fluctuating asymmetry of the plant leaf remains open. At present, one of the priority pollutants of the atmospheric air and soil in large cities is formaldehyde, which is caused by the rapid growth in the number of cars (Belkina, 2008). In the conditions of ecosystems of urbanized territories, a whole range of pollutants exert an impact on a plant, which hampers analysis of the nature of influence of separate substances. Experimental models are more adequate when studying this issue compared with the conditions of urban ecosystems.

Due to this, the aim of the paper is to study the stability of development of the pea leaf (*Pisum sativum* L.) in the conditions of the experiment through the change in the directional asymmetry of its leaves produced by the fluctuating asymmetry of these morphological structures under the influence of formaldehyde in a wide range of doses.

EXPERIMENTAL

The seeds of the pea (*Pisum sativum* L.) of the Al'bumen sort were grown for five days in distilled water. After the appearance of the root, the seedlings of the control group were placed into containers with Knop's nutrient solution (1 mM of $Ca(NO_3)_2$, 0.25 mM of KH_2PO_4, 0.125 mM of $MgSO_4$, and 0.25 mM of KNO_3) with the addition of microelements (Hogland). In nine experimental groups, apart from the components mentioned above, the nutrient solution contained formaldehyde in a concentration of 0.063×10^{-2} to 0.16 g/L. Neighboring concentrations dif-

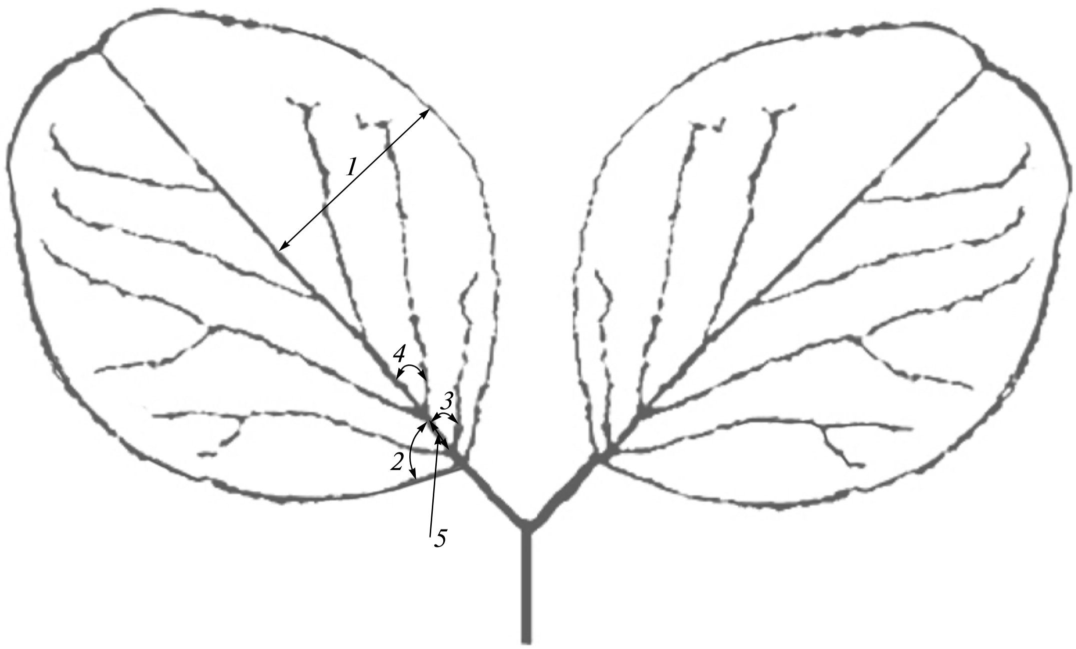

Fig. 1. Measurements of the right and left leaflets of the third leaf of *P. sativum*: (*1*) 1/2 of the width of the leaf in the area of 1/2 of the length of the central vein; (*2*) angle between the central vein and the lower edge of the leaf; (*3*) angle between the central vein and the first from the bottom vein of the second order; (*4*) angle between the central vein and the second from the bottom vein of the second order; (*5*) distance between the bases of the first and second from the bottom veins of the second order.

fered twofold. The range of concentrations was selected in previous experiments so that maximum used concentrations of formaldehyde were close to sublethal, i.e., had a considerable toxic effect on the growth of the seedling but did not cause the death of plants.

In each group, *P. sativum* was grown in four containers 250 mL in volume (6 plants per each container; $n = 24$). To prevent volatilization of formaldehyde, containers were closed with lids with small apertures, through which the roots of the seedlings were placed into the solution. The plants from the control and experimental groups were grown on the solutions described above for 16 days at 20–22°C and 17-hour-long daylight hours. Every four days, the solutions were replaced with fresh ones.

On day 17, the asymmetry of leaflets of the fully-formed third pea leaf was determined. The *P. sativum* leaf is complex, and in the Al'bumen sort, it consists of two leaflets. Immediately after collection, the leaves were scanned and the asymmetry of the right and left leaflets was determined. It was established in preliminary experiments that directional asymmetry is characteristic of the *P. sativum* leaflets: the inner side of each leaflet (relative to the plane of the leaf symmetry) is always smaller than the outer one. It is known that change in the magnitude of inherited types of asymmetry (directional asymmetry and antiasymmetry) of the bilateral morphological structures in populations of one species are determined by the fluctuating asymmetry (Zakharov, 1987). In addition, five relatively independent characteristics of the *P. sativum* leaf were selected in preliminary experiments. The correlation between the characteristics was either absent or weak (the Spearman's coefficient did not exceed 0.20 at $p < 0.05$). These parameters were used to estimate the magnitude of the asymmetry of *P. sativum* leaflets under the influence of formaldehyde (Fig. 1).

The parameters of electronic pictures of pea leaves were measured using Adobe Photoshop CS3. The integral indicator of the leaf asymmetry was calculated using the algorithm of normalized differences:

$$\bar{A} = \frac{1}{mn}\sum_{i=1}^{m}\sum_{j=1}^{n}\frac{|L_{ij} - R_{ij}|}{(L_{ij} + R_{ij})},$$

where L_{ij} and R_{ij} are *j*th parameter in *i*th leaflet, respectively, to the left and right of the symmetry plane (Zakharov et al., 2000).

The magnitude of the fluctuating leaf asymmetry observed against the background of directional asymmetry was not defined. Due to this, the term leaf asymmetry is used throughout the paper. This means that the differences in the level of directional asymmetry between the right (or left) leaflets of the third leaf of plants from different groups are determined by the magnitude of their fluctuating asymmetry.

The results of studies were statistically processed using the BIOSTATISTICA 4.03 and STATISTICA 6.0 programs. Nonparametric tests of Kruskal–Wallis, Newman–Keuls, and Wilcoxon were used for statistical

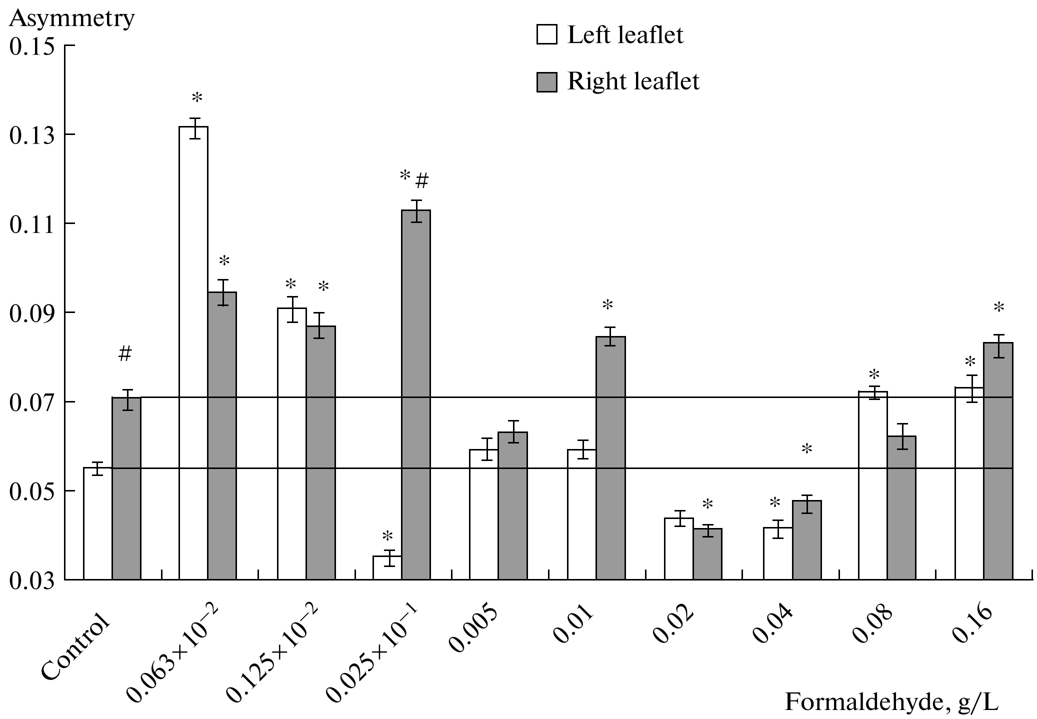

Fig. 2. Influence of formaldehyde in a wide range of doses on the asymmetry of the right and left leaflets of the third leaf of *P. sativum* (Me ± S_{Me}). Symbols indicate that differences are significant ($p < 0.05$) relative to (*) the asymmetry of the analogous leaflet in the control group or (#) the asymmetry of left leaflet in the same group.

analysis, since the Shapiro–Wilk criterion showed that the sampling distribution in a number of groups differed from the norm. The diagrams give the medians and their errors. The nonparametric Spearman's correlation coefficient was used in the correlation analysis.

RESULTS

The lowest studied formaldehyde concentrations (0.063×10^{-2}–0.125×10^{-2} g/L) caused an increase in the asymmetry of the right and left leaflets of *P. sativum* compared with the control level (Fig. 2). However, with the subsequent increase in the concentration of the toxicant up to 0.02–0.04 g/L, instead of the expected worsening in the stability of leaf development, a decrease in the asymmetry of the right and left leaflets by 30–40% was observed compared with the analogous parameter in the control group (Fig. 2). And finally, when applied in the highest studied concentrations (0.08–0.016 g/L), formaldehyde again increased the asymmetry first in the left and then in the right leaflets (Fig. 2).

The Spearman's correlation analysis helped us to establish that a strong positive correlation ($r = 0.81$; $p = 0.010$) exists between the asymmetry of the right and left leaflets. At the same time, in the control and one of the experimental groups (0.005 g/:), the asymmetry of the right and left leaflets differed considerably statistically.

DISCUSSION

The results of the study showed that with an increase in the formaldehyde concentration in the solution, the asymmetry of the right and left leaflets of the third leaf of *P. sativum* changed nonmonotonously. Development of the so-called paradoxical effect was observed. It is known that the manifestation of paradoxical effects consists in the following: as the dose or concentration of the toxic agent is reduced, its toxicity increases, and vice versa: with an increase in the dose, its effect is reduced (Batyan et al., 2009). Paradoxical effects look like depressions on the dose–effect curves (Bulatov et al., 2002). In our case, formaldehyde had a toxic effect and lead to an increase in the asymmetry of *P. sativum* leaflets in small doses; in the range of medium doses, the toxicity did not manifest itself and the developmental stability of the morphological structures of the *P. sativum* leaf in the experimental groups exceeded the control level. Under the influence of the highest studied doses of formaldehyde, a toxic effect was again observed, which was expressed in the increase in the asymmetry of the right and left *P. sativum* leaflets. Thus, depending on the dose, formaldehyde could lead to both a decrease in the developmental stability of morphological leaf structures and an increase in this parameter or not cause any effect at all.

The data obtained by us agree with the results of some authors, which indicate that pollution of the environment does not always lead to impaired stability in the development of various organisms. Absence of increase in the fluctuating asymmetry or even decrease in this parameter was demonstrated under the influence of a chemical pollution on plants (Ambro-Rappe et al., 2007; Erofeeva et al., 2009), mammals (Gileva and Kosareva, 1994), and insects (Leamy et al., 1999), as well as under the influence of anthropogenic pressure on amphibians (Vershinin et al., 2007). It is possible that one of the reasons for the facts cited above could be the nonmonotonous nature of the dose–effect dependences, one of the manifestations of which is the paradoxical effect.

To explain the absence of impaired developmental stability under the influence of anthropogenic pressure, suggestions were made about the influence of stabilizing selection, which severs marginal variants of individual development under significant technogenic pollution, as a result of which a decrease in the dispersion of characteristics and the mean value of the fluctuating asymmetry in the population could be observed (Gileva and Kosareva, 1994). Under the influence of formaldehyde on the developmental stability of the *P. sativum* leaf in experimental conditions, the influence of selection is excluded, since all plants from experimental and control groups survived. Thus, it is logical to assume that the decrease in the asymmetry is determined by the processes of phenotypic adaptation in *P. sativum*.

At present, the mechanisms underlying the nonmonotonous dose–effect dependences under the influence of various toxicants are not known (Batyan et al., 2009). It is possible that a decrease in the asymmetry of the *P. sativum* leaf in the area of the medium studied formaldehyde concentrations is determined by an increase in the activity of defensive systems of the plant up to the level that does not only compensate the toxic effect of the pollutant but also increases the stability of the morphogenesis of the leaf. Minimum studied concentrations of the toxicant are not able to produce such an activation, whereas in the area of the maximum studied concentrations close to sublethal, a breakdown of adaptation capabilities of the plant and disruption in the stability of the leaf development occur.

It is known that the development of the shape of a plant leaf, including its symmetry, is controlled by a number of genes that are studied primarily in *Arabidopsis thaliana* (L.) Heynh. They include such genes as *AS1, AS2, CP3, AN2*, etc. (Semiarti et al., 2001; Kirpicheva and Sokolov, 2009). An increase in the developmental stability of the *P. sativum* leaf under the influence of formaldehyde may be caused by a decrease in the fluctuations of molecular processes underlying the expression of similar genes. The possible mechanisms of this effect may be the increase in the activity of the enzymes of the formaldehyde detoxication, such as gluthathione-dependent formaldehyde dehydrogenase, which provides a binding of the toxicant to gluthathion (Díaz et al., 2003), as well as the antioxidant system that hampers the damage of proteins by active forms of oxygen (Polesskaya, 2007).

Thus, based on the facts stated above, a conclusion can be drawn that in a wide range of doses formaldehyde caused a nonmonotonous change in the developmental stability of the third *P. sativum* leaf, which was assessed by the change in the magnitude of the directional asymmetry of the right and left leaflets caused by the fluctuating asymmetry of these morphological structures. A paradoxical effect was revealed under the influence of the toxicant on the studied parameter. In minimum studied concentrations, formaldehyde disrupted the stability of leaf development, which was manifested in the increase in the asymmetry of the right and left leaflets. In the area of medium concentrations of the toxicant, the asymmetry was lower than the control level, which indicated an increase in the developmental stability of the pea leaf. Maximum, close to sublethal, studied concentrations of the formaldehyde decreased the developmental stability of the pea leaf again and led to an increase in the asymmetry of its leaflets compared with the controls.

REFERENCES

Ambo-Rappe, R., Lajus, D.L., and Schreider, M.J., Translational Fluctuating Asymmetry and Leaf Dimension in Seagrass, *Zostera capricorni* Aschers in a Gradient of Heavy Metals, *Environ. Bioindicators*, 2007, vol. 2, no. 2, pp. 99–116.

Batyan, A.N., Frumin, G.T., and Bazylev, V.N., *Osnovy obshchei i ekologicheskoi toksikologii* (Fundamentals of General and Environmental Toxicology), St. Petersburg: SpetsLit, 2009.

Belkina, T.D., *Sostoyanie gorodov Rossii 2008* (The State of Russian Cities in 2008), Moscow: Gorod-Region-Sem'ya, 2008.

Bulatov, V.V., Khakhaev, T.Kh., Dikii, V.V., et al., The Problem of Low and Ultra-Low Doses in Toxicology. Fundamental and Applied Aspects, *Ross. Khim. Zh.*, 2002, vol. 46, no. 6, pp. 58–62.

Calabrese, E.J. and Blain, R.B., Hormesis and Plant Biology, *Environ. Pollut.*, 2009, vol. 157, pp. 42–48.

Díaz, M., Achkor, H., Titarenko, E., and Martinez, M.C., The Gene Encoding Glutathione-Dependent Formaldehyde Dehydrogenase/GSNO Reductase Is Responsive to Wounding, Jasmonic Acid and Salicylic Acid, *FEBS Letts.*, 2003, vol. 543, nos. 1–3, pp. 136–139.

Erofeeva, E.A., Sukhov, V.S., and Naumova, M.M., Two-Phase Dependence of Some Ecological–Morphological and Biochemical Parameters of Silver Birch (*Betula pendula*) Leaf on the Level of Road Pollution, *Povolzh. Ekol. Zh.*, 2009, no. 4, pp. 50–57.

Gelashvili, D.B., Chuprunov, E.V., and Iudin, D.I., Structural and Bioindicational Aspects of Fluctuating Asymmetry of Bilaterally Symmetrical Organisms, *Zh. Obshch. Biol.*, 2004, vol. 65, no. 5, pp. 433–441.

Gileva, E.A. and Kosareva, N.L., Reduction of Fluctuating Asymmetry among House Mice in Territories Polluted with Chemical and Radioactive Mutagens, *Ekologiya*, 1994, no. 3, pp. 94–97.

Hoffmann, A.A. and Woods, R.E., Associating Environmental Stress with Developmental Stability: Problems and Patterns, *See Polak*, 2003, pp. 387–401.

Kefford, B.J., Zalizniak, L., Warne, M.St.J., and Nugegoda, D., Is the Integration of Hormesis and Essentiality into Ecotoxicology Now Opening Pandora's Box?, *Environ. Pollut.*, 2008, vol. 151, no. 3, pp. 516–523.

Kirpicheva, I.V. and Sokolov, I.D., Expression of *AN1, AN3, AS1*, and *CP3* Genes in Leaves of *Arabidopsis thaliana* (L.) Heynh., *Nauk. Visnik LNAU*, 2009, no. 8, pp. 26–32.

Larry J., Leamy I., and Klingenberg, C.P., The Genetics and Evolution of Fluctuation Asymmetry, *Annu. Rev. Ecol. Evol. Syst.*, 2005, vol. 36, pp. 1–21.

Leamy, L.J., Doster, M.J., and Huet-Hudson, Y.M., Effects of Methoxychlor on Directional and Fluctuating Asymmetry of Mandible Characters in Mice, *Ecotoxicology,* 1999, vol. 8, pp. 63–71.

Polesskaya, O.G., *Rastitel'naya kletka i aktivnye formy kisloroda* (Plant Cell and Reactive Oxygen Species), Moscow: KDU, 2007.

Semiarti, E., Ueno, Y., Tsukaya, H., Iwakawa, H., Machida, C., and Machida, Y., The *ASYMMETRIC LEAVES2* Gene of *Arabidopsis thaliana* Regulates Formation of a Symmetric Lamina, Establishment of Venation and Repression of Meristem-Related Homeobox Genes in Leaves, *Development*, 2001, vol. 128, pp. 1771–1783.

Vershinin, V.L., Gileva, E.A., and Glotov, N.V., Fluctuating Asymmetry of Measurable Parameters in *Rana arvalis*: Methodology, *Russ. J. Ecol.,* 2007, vol. 38, no. 1, pp. 72–74.

Zakharov, V.M., *Asimmetriya zhivotnykh* (The Asymmetry of Animals), Moscow: Nauka, 1987.

Zakharov, V.M., Chubinishvili, A.T., Dmitriev, S.G., et al., *Zdorov'e sredy: Praktika otsenki* (Environmental Health: The Practice of Assessment), Moscow: Tsentr ekologicheskoi politiki Rossii, 2000.

Zakharov, V.M., Zhdanova, N.P., Kirik, E.F., et al., Ontogenesis and Population: Evaluation of Developmental Stability in Natural Populations, *Russ. J. Dev. Biol.*, 2001, vol. 32, no. 6, pp. 336–351.