Stability of Citrinae Subtribe Development in the Conditions of the Mediterranean Area

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Abstract
Fluctuating asymmetry as the value of random deviations reflects either stability or instability of development. It is quite often used by researchers to assess the level of influence of anthropogenic and climatic factors. This study is focused on the territory with favorable climate and low anthropogenic load. The object of the study includes leaf blades of woody plants *Citrus X sinensis* (Valencia), *Citrus limon* (Molla Mehmet), *Citrus reticulata* (Okitsu was C. unshiu) and *Punica granatum* (Emek) of the city of Kemer of the Republic of Turkey. The reliability of presented results is justified by the use of correct methods of study and adequate statistical processing of big data. The integrated indicator of fluctuating asymmetry was the highest for leaf blades of *Punica granatum* (0.019), *Citrus limon* (0.017), these plants are introduced species. *Citrus X sinensis* and *Citrus reticulata* were characterized by low level of destabilization of plants in the conditions of the Mediterranean Area. It is shown that the most sensitive morphological features of fluctuating asymmetry for Citrinae Citrus subtribe plants are j₄ and j₅, *Citrus limon* -- j₂ and j₅, *Citrus reticulata* -- j₅ and j₄.

1. Introduction

Both climatic conditions of the habitat and physico-chemical and biotic features of the environment have a considerable impact on growth and development of plants [1--3]. Negative influence of environmental factors on ecosystems can decrease the stability of vegetative development. The index of fluctuating asymmetry (IFA) characterizing insignificant deviation from zero difference of the right and left parts of bilaterally symmetrical feature is used to define the stability of plant development [4, 5].

The study devoted to the assessment of development stability of species within the ecosystem is quite relevant since this indicator characterizes the stability of the ecosystem in general. Biological indication performed via the index of fluctuating asymmetry provides for early diagnostics of even low disturbances in the habitat when irreversible processes in the ecosystem have not started yet.
2. Problem Statement

In recent years the bioindication method based on the assessment of the degree of fluctuating asymmetry of leaf blades of both woody and nonwoody plant species gains increasing recognition in world practice in terms of the quality of the environment [6-8]. However, according to Kozlov M.V. [9], large part of the studies is conducted at relatively low experimental level, which makes it impossible to describe the existing situation unambiguously. In fact, since the whole complex of environmental factors has an impact on plants, including abiotic and anthropogenic, it is difficult to separately identify the influence of one of these groups. We cannot but agree with the fact that without enough experimental material, in particular, data collected in the absence of anthropogenic pollution, for comparative analysis it is difficult to talk about ecological situation in the region. Besides, Russia has a certain positive experience of using this technique to assess the stability of development of woody plants and the recommended method for this assessment [10].

We also cannot neglect the fact that the use of this technique in relation to plants growing in Krasnoyarsk Krai requires special attention to abiotic and, in particular, climatic factors, which may make negative impact thus increasing the index of fluctuating asymmetry as result of its geographic location. Lack of that negative impact is possible in case the objects of the study grow in more favorable climate, in particular, in the Mediterranean Area of the Republic of Turkey.

Purpose of the study: to assess the development stability of woody plants of *Citrinae* subtribe growing in the conditions of low anthropogenic influence using the method of fluctuating asymmetry of leaf blades.

3. Materials and Methods

The object of the study includes leaf blades of woody plants *Citrus X sinensis* (Valencia), *Citrus limon* (Molla Mehmet), *Citrus reticulata* (Okitsu wase C. unshiu) and *Punica graminatum* (Emek) cultivated in a private farmstead. Figure 1 shows the place of implementation of sampling is sampling location.

Sampling was made during August 10–20, 2018 in compliance with requirements of the methodology [10, 11] from western and southern sides of the top (middle part) from five randomly selected trees of each species (remoteness from highway not less than 50 m, which confirms low level of traffic). From each woody plant we took at least 50
leaves (i = 1--0). The leaves were squeezed out between the layers of filter paper and dried under press. Table 1 shows the average size of leaf blades used in this experiment.

| Species                 | Variety          | Leaf length, mm | Leaf width, mm |
|-------------------------|------------------|-----------------|----------------|
| *Citrus X sinensis*     | Valencia         | 130.7±2.8       | 51.4±1.9       |
| *Citrus limon*          | Molla Mehmet     | 115.7±2.0       | 47.1±1.3       |
| *Citrus reticulata*     | Okitsu wase C. unshiu | 80.0±4.4 | 27.1±1.0       |
| *Púnica granátum*       | Emek             | 58.5±2.0        | 17.6±0.7       |

The prepared vegetable feedstock was scanned at the resolution of 1200 dpi. Five people took part in the experiment and measured leaf blades without mechanical damage or deformation. During study the most standard metric bilateral features were considered: $j_1$ -- width of the left and right halves of a leaf blade; $j_2$ -- distance from the basis of a leaf blade to the end of a second order vein; $j_3$ -- distance between the bases of the first and second veins of the second order; $j_4$ -- distance between the ends of the first and second veins of the second order; $j_5$ -- angle between the midrib and the second vein of the second order from the leaf basis.
Since earlier [12] it was established that the basic method of normalization of statistical data allows comparing objects of different quality, the obtained results were thus processed (Tabl. 2).

| Table 2: Formulas for calculation of fluctuating asymmetry and integral features [12]. |
|---|
| **One species by one feature** | **Sampling by one feature** | **One species by all features and integral index** |
| \( f_{a_{ij}} = (t_{L_{ij}} - t_{R_{ij}}) \) | \( f_{a_{i}} = \frac{S_{f_{a_{ij}}}}{M} \) | \( f_{a_{i}} = \frac{1}{n} \sum_{j=1}^{n} f_{a_{ij}} \) |
| \( t_{L_{ij}} = \frac{L_{ij} - M_{L_{j}}}{S_{L_{j}}} \) | \( f_{a_{i}} = \frac{S_{f_{a_{ij}}}}{M} \) | \( Fa = \frac{1}{n} \sum_{j=1}^{n} f_{a_{ij}} \) |
| \( t_{R_{ij}} = \frac{R_{ij} - M_{R_{j}}}{S_{R_{j}}} \) | \( f_{a_{i}} = \frac{S_{f_{a_{ij}}}}{M} \) | \( Fa = \frac{1}{n} \sum_{j=1}^{n} f_{a_{ij}} \) |

4. Results and Discussion

The climate of the city of Kemer of the Republic of Turkey is determined by its location in the subtropical belt and is characterized by rather mild winter and hot dry summer. Figure 2 shows the dynamics of the average monthly daytime temperatures over 2015-2018.

![Figure 2: Dynamics of daytime temperatures within a year (according to world-weather.ru).](image)

The results of dispersive analysis (Figure 3) demonstrate that as expected, the considered woody plants are characterized by relatively low index of fluctuating asymmetry. The increased values of fluctuating asymmetry of the leaves of Emek pomegranate can be explained by the introduction of this plant in the conditions of Kemer (this variety was...
cultivated and adapted for conditions of Israel). For leaf blades of Emek pomegranate in particular the greatest asymmetry was observed for the following features: distance from the basis of a leaf blade to the end of the second order vein, distance between the bases of the first and second veins of the second order and distance between the ends of the first and second veins of the second order.

The analysis of metric bilateral features of *Citrus X sinensis* (Valencia) showed the increase of asymmetry in case of such features as the distance between the ends of the first and second veins of the second order and an angle between the midrib and a vein of the second order, the second from the leaf basis. The increased values of the last feature are typical for leaf blades of *Citrus limon* (Molla Mehmet). At the same time there is also the increase of indicators related to such features as the distance from the basis of a leaf blade to the end of the second order vein. Unlike the above considered woody plants *Citrus X sinensis* the *Citrus reticulata* is characterized by considerable asymmetry of a leaf blade for the fourth (distance between the ends of the first and second veins of the second order) and the third (distances between the bases of the first and second veins of the second order) bilateral feature. For other considered metric bilateral features smaller asymmetry is recorded.

Figure 3: Indicators and integral indexes of fluctuating asymmetry: ($f_{aj}=1...5$) -- indicators of fluctuating asymmetry of corresponding bilateral features; ($Fa$) -- integral index of fluctuating asymmetry.
5. Conclusion

The study of fluctuating asymmetry of leaf blades of *Citrus X sinensis* and *Citrus reticulata* allowed revealing low destabilization of plants development in the Mediterranean Area. Most likely major failures in the development of *Citrus limon* and *Punica granatum* are connected with their introduction.

It is shown that the most sensitive morphological feature of fluctuating asymmetry for *Citrinae* subtribe *Citrus X sinensis* are the fourth and the fifth bilateral features, *Citrus limon* -- the second and the fifth, *Citrus reticulata* -- the third and the fourth.

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