УДК 58.087:581.1 DOI 10.22314/2073-7599-2021-15-1-4-8

Determination of Plant Developmental Stability in Plant Lighting with Hyperspectral Imaging

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Abstract. The authors showed that a convenient, accurate and fast way of assessing the degree of influence of environmental factors on plants was needed to optimize photoculture. They emphasized the importance of non-destructive monitoring of crops physiological state of, for which they used phenomics technologies, for example, remote sensing using hyperspectral cameras. (*Research purpose*) To reveal the possibility of using hyperspectral imaging to determine the plant developmental stability. (*Materials and methods*) As a measure of the favorable impact of environmental factors on the growth and development of plants, their developmental stability was taken, numerically characterized by the fluctuating asymmetry value. The authors proposed to use vegetation indices determined from the leaf reflection spectra as a bilateral feature. The object of experimental research was juvenile cucumber plants. The studies were carried out in laboratory conditions. The spectral characteristics of cucumber leaves grown under different light quality of radiation were determined using a Specim IQ hyperspectral camera. Information on the spectral reflectances was extracted from the resulting data hypercube. As an example calculations were performed for Normalized Difference Vegetation Index. (*Results and discussion*) The authors revealed differences in the productivity indicators of plants grown under different light quality. They revealed a significant frequency of occurrence of Normalized Difference Vegetation Index asymmetry in two halves of the cucumber leaf surface. The fluctuating nature of this asymmetry was confirmed. They found that with a light quality providing a higher productivity of plants, lower values of fluctuating asymmetry were observed, which indicate greater stability of plant development. (*Conclusions*) The authors proposed a method for determining the plant developmental stability using a hyperspectral camera. The method was based on the assessment of the fluctuating asymmetry of vegetation indices calculated for points on the leaf surface, characterized by the same location conditions relative to the border of its left and right halves. A preliminary assessment of the possibility of determining the developmental stability by the results of phenotyping using the example of cucumber plants showed the feasibility of the method and its practical applicability.
Keywords: photoculture, fluctuating asymmetry, bilateral traits, biometrics, plant phenomics, high-performance phenotyping, hyperspectral camera, vegetation index.

For citation: Rakutko S.A., Rakutko E.N., Mishanov A.P. Opredelenie stabil'nosti razvitiya rasteniy v svetokul'ture s ispol'zovaniem giperspektral'noy s'emki [Determination of plant developmental stability in plant lighting with hyperspectral imaging]. *Sel'skokhozyaystvennye mashiny i tekhnologii*. 2021. Vol. 15. 4-8 (In English). DOI 10.22314/2073-7599-2021-15-1-4-8.

Определение стабильности развития растений в светокультуре с использованием гиперспектральной сьемки

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Реферат. Показали, что для оптимизации светокультуры необходим удобный, точный и быстрый способ оценки степени влияния факторов окружающей среды на растения. Подчеркнули важность неразрушающего мониторинга физиологического состояния сельхозкультур, для чего привлекают технологии феномики, например дистанционное зондирование при помощи гиперспектральных камер. (*Цель исследования*) Выявить возможности применения гиперспектральной сьемки для определения стабильности развития растений. (*Материалы и методы*) В качестве меры благоприятности воздействия факторов окружающей среды на рост и развитие растений приняли стабильность их развития, численно характеризуемая величиной флуктуирующей асимметрии. Предложили использовать в качестве билатерального признака вегетационные индексы, определяемые по спектрам отражения листа. Объектом исследований в лабораторных условиях стали ювенильные растения огурца. Спектральные характеристики листьев огурца, выращенных под различным спектральным составом излучения, определяли с помощью гиперспектральной камеры *Specim IQ*. Информацию о спектральных коэффициентах отражения извлекали из полученного гиперкуба данных. Для примера вычисления вели для *Normalized Difference Vegetation Index*. (*Результаты и обсуждение*) Выявили различия в показателях продуктивности растений, выращиваемых под различными спектрами. Отметили существенную частоту встречаемости асимметрии *Normalized Difference Vegetation Index* по двум половинам поверхности листа огурца. Подтвердили флуктуирующий характер этой асимметрии. Нашли, что при спектре, обеспечивающем большую продуктивность растений, наблюдаются меньшие значения величины флуктуирующей асимметрии, что свидетельствует о большей стабильности развития растений. (*Выводы*) Предложили способ определения стабильности развития растения с помощью гиперспектральной камеры. Показали, что он основан на оценке флуктуирующей асимметрии вегетационных индексов, вычисляемых для точек поверхности листа, расположенных в одинаковых условиях относительно границы его левой и правой половин. Согласно предварительной оценке возможности определения стабильности развития по результатам фенотипирования на примере растений огурца показали реализуемость способа и его практическую применимость.

Ключевые слова: светокультура, флуктуирующая асимметрия, билатеральные признаки, биометрия, феномика растений, высокопроизводительное фенотипирование, гиперспектральная камера, вегетационный индекс.

Для цитирования: Ракутько С.А., Ракутько Е.Н., Мишанов А.П. Определение стабильности развития растений в светокультуре с использованием гиперспектральной сьемки // *Сельскохозяйственные машины и технологии*. 2021. Т. 15. С. 4-8. DOI 10.22314/2073-7599-2021-15-1-4-8.

To develop optimal technologies for growing various species and varieties of plants (including in artificial conditions, in photoculture), it is necessary to study the influence of environmental conditions on biometrics, some indicators of which are diagnostic signs of the plants state [1]. Within the framework of the energy-ecological approach, the share assessment of the environmental factors influence of plant growing on the variability of biometric indicators forms the basis for optimizing the energy efficiency of photoculture and its environmental friendliness [2].

The rate of anthropogenic influence on natural systems is constantly increasing, which requires the development of methods for ecological monitoring of the environment. This problem is also relevant for artificial growing systems (greenhouses, phyto-factories etc.), where there is a need to monitor the physiological state of plants. For an adequate understanding of the state of the environment, a system of biological assessment of its quality is used – bioindication, based on taking into account the reaction of living organisms to the external factors influence. The relevance of the use of bioindication is due to its simplicity, speed and low cost. For natural conditions indicator plants are used that respond to very small deviations in environmental parameters. In artificial climatic structures, such observations are carried out directly over the cultivated plants.

To minimize the energy costs and other resources in conditions of high-intensity agricultural production, including photoculture, a convenient, accurate and fast way to assess the influence degree of environmental factors on plants, a methodology for a comprehensive assessment of the stability of their development is needed. Non-destructive monitoring of the plants pigment composition, which is closely related to their physiological state, is possible with the use of phenomics technologies, namely, remote sensing using hyperspectral cameras.

Research purpose is to identify the possibilities of using hyperspectral imaging to determine the stability of plant development.

Materials and methods. *Application of the phenomics methods to the determination the plant developmental stability.* The impact of environmental factors (temperature, illumination, humidity, etc.) on plants can be judged by their development stability. Numerically, it is expressed by the value of fluctuating asymmetry (FA), which is characterized by insignificant undirected differences in the manifestation of bilateral signs (BS) on the seemingly symmetrical sides of a biological object [3].

It is generally accepted that the minimum value of the FA corresponds to the optimal level of the influencing factor. Any deviations lead to an increase in the level of phenotypic deviations, recorded by an increase in FA.

Morphological characters are most often used as a BS in calculating the FA value. In connection with the measuring technologies and procedures development, the range of signs can be increased by including non-morphological plants signs, in particular, physiological or biochemical ones. The latter are determined by the quantitative and qualitative content of various substances in plant tissues and are directly interconnected with the physiological processes taking place in them.

The FA value is calculated from plant leaves [4]. In total, N leaves are measured, M signs are removed from each leaf. The integral asymmetry index is

$$\Phi A = \frac{1}{M \times N} \sum_{j=1}^{N} \sum_{j=1}^{M} \left[\frac{2\left|L_{ij} - R_{ij}\right|}{L_{ij} + R_{ij}} \right].$$

With manual measurements, the technique is quite laborious. The total number of measurements can be up to several hundred.

The solution to this problem is possible through the use of high-performance phenotyping methods – the procedure for assessing the plant phenotype by its size, shape, physiological and biochemical characteristics in specific environmental conditions and genome activity [5]. Modern phenotyping methods have high productivity, allow to obtain data in real time and analyze information about a whole range of parameters [6]. *Fig. 1* shows a typical set of recorded environmental factors under which plants are grown, as well as a group of parameters of biometric information determined during phenotyping.

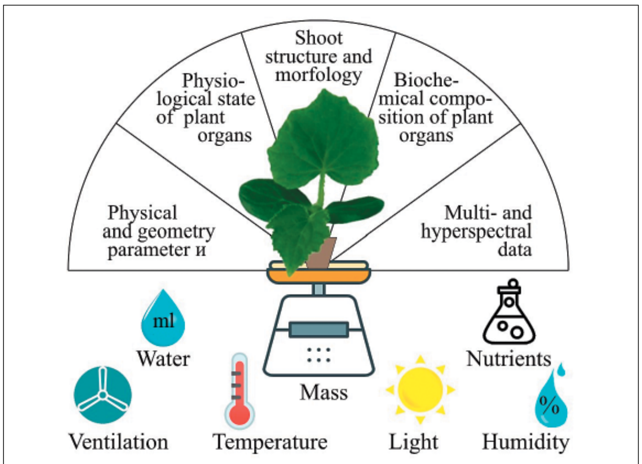

Fig. 1. Assigned factors and biometric parameters of plants measured during phenotyping

Plant phenotyping based on obtaining digital models is of value not only in basic science, but also in crop production and precision agriculture, providing a quantitative basis for describing the interaction of plants and the environment [7]. Since the potential of image analysis in the context of plant phenotyping is far from being exhausted, this technology as a scientific field is expected to be developed in the near future [8].

Phenotyping is a technology of plant phenomics as a scientific discipline, the research field of which lies at the intersection of biology, plant physiology, physics, engineering and computer technology [9]. The use of phenotyping technology in agro- and biocenoses, including in artificial bioenergy systems (greenhouses) with energy and environmental monitoring, will ensure energy efficiency and improve the environmental friendliness of products.

The developed phenotyping platforms made it possible to create a technology for assessing the efficiency of photosynthesis, describe phenotypic manifestations for a large number of genes, and link their activity with specific physiological processes, such as photosynthesis, respiration, stress resistance, and control of shoot architectonics [10]. Phenomics is a relatively young field of knowledge. Phenomic research is more focused on recording the morphometric and physiological characteristics of the aboveground parts of plants [11].

An important source of information for phenotyping is the spectral characteristics of plant leaves. The color of the leaf changes during the plant growth and development, depending on the nutritional conditions and environmental factors. NDVI (Normalized Difference Vegetation Index) is a commonly used indicator for remote assessment of plant health. This vegetation index indicates the amount of photosynthetically active biomass. The calculation of the index is based on two sections of the spectral reflection curve of vascular plants that do not depend on other factors – the maximum chlorophyll absorption (680 nm) and the maximum leaf cellular structures reflection (800 nm). High photosynthetic activity leads to less reflection in the red region of the spectrum and more in the infrared (*Fig. 2*).

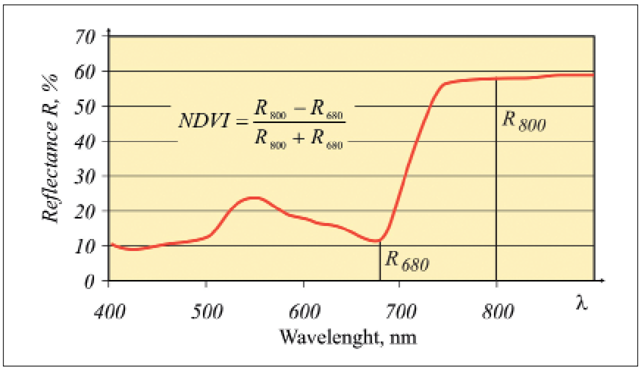

Fig. 2. Spectral reflection curve of a plant leaf

The pigment composition of a plant leaf at individual points on its surface can be determined by a non-destructive method using various instruments. The use of a hyperspectral camera allows one to study the entire surface of the leaf at once [12].

Samples of plants and their growing conditions. The objects of experimental research were cucumber plants (*Cucumis Sativus L.*) of the mid-early hybrid Safaa mix F_1. To shorten the experiment, the authors used plants in a juvenile age state, that was, from the moment of the formation of the first to the appearance of the second leaf.

The studies were carried out in laboratory conditions, in a light room, two zones of which were separated by opaque partitions. The same growth conditions were maintained, with the exception of the light quality of the radiation. In the first zone, the irradiator consisted of three-watt red and blue Star-type LEDs with an R:B ratio of 3:1 (*Spectrum 1*). In the second zone, the irradiator was based on an ELPL-VXS 50 W LED with a uniform light quality of radiation in the PAR range (*Spectrum 2*). Plant weight and central vein length of the first true leaf were used as productivity indicators. The data were processed by methods of mathematical statistics (p<0.05) using the Excel 2003 and Statistica 6.0 software packages.

Hyperspectral imaging and processing. The spectral characteristics of cucumber leaves grown under different spectral composition of radiation were determined using a Specim IQ hyperspectral camera supplied by Azimut Photonics Company LLC (Moscow). The number of pixels of the image formed by the camera was 512×512. The spectral range of measurements, divided into 240 strips was 400-1000 nm. Thus, the data hypercube had dimension 512×512×204, spectral resolution was about 2.9 nm. The camera was installed on a tripod in front of the table, on which the plants were placed at a distance of 30-40 cm. The plants were illuminated with four 100 W incandescent lamps. Visual work with hyperspectral images was carried out using the standard Specim IQ Studio software. To access the reflection coefficients from the data hypercube, a program was written in *Visual Basic*.

The information about the spectral reflection coefficients R_λ was extracted taking into account the venation pattern for paired points of the leaf surface by program. The values of the coefficients R_λ were determined at these points at wavelengths of 680 and 800 nm. *NDVI* values were calculated to the left and right of the central vein. The found values were taken as bilateral features, and the average value of the FA was calculated for all points. In this case, N×M is the total number of dots to be distinguished on the surface of the leaf. For example, *Fig. 2* shows the *NDVI* values as a bilateral indicator calculated for four paired points, to the left and right of the border between the left and right halves of the cucumber leaf.

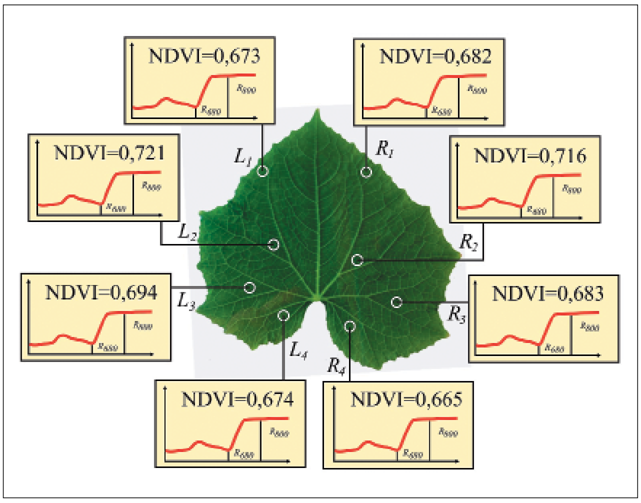

Fig. 2. NDVI as a bilateral trait

Results and discussion. In the present research, the accumulation of green mass was taken as a measure of the intensity of growth processes. Physiological, morphological and anatomical parameters of plants depended on the light quality of radiation. The high activity of the photosynthetic apparatus was associated with more efficient processes of storing light energy, which leaded to high plant productivity.

Productivity indices for *Spectrum 1*: plant raw weight M_1 = 0.66 ± 0.04 g, central vein length L_1 = 25.5 ± 2.8 mm. For *Spectrum 2*: raw weight M_2 = 0.76 ± 0.05 g, central vein length L_2 = 33.4 ± 4.3 mm. The differences in plant productivity indices were statistically significant and indicated that the second spectrum was more suitable for the growth and development of juvenile cucumber plants.

The developmental plant stability was assessed by the *FA* index of the leaf *NDVI*. A significant frequency of occurrence of *NDVI* asymmetry in two halves of the cucumber leaf surface was revealed. The fluctuating nature of this asymmetry was confirmed. *FA* values under *Spectrum 1* FA_1 = 0.0653, under *Spectrum 2* FA_2 = 0.0421. *Fig. 3* shows the relationship between leaf mass and *FA* value.

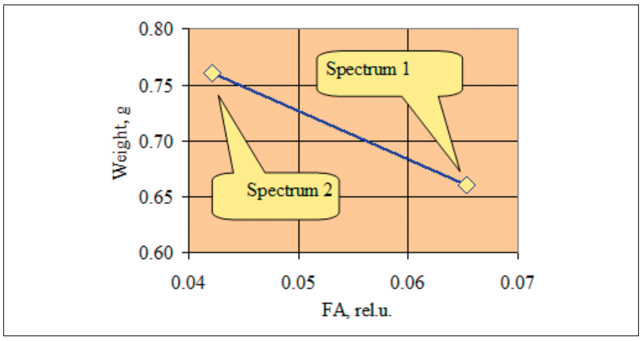

Fig. 3. Influence of the spectral radiation composition on the productivity of plants and their development stability

Lower *FA* values indicate a greater plant developmental stability under *Spectrum 2*. The spectrum of this source should be considered more favorable for plant development. With this spectrum, the best indicators of plant productivity are also observed.

Conclusions. The authors considered the phenomics methods, analyzed the possibility of their application to determine the plant development stability by high-performance phenotyping. They proposed a convenient, accurate and fast way to assess the influence degree of environmental factors on plants and their condition in general.

The authors considered the plant development stability as a measure of the impact of environmental factors. They showed that, along with morphological, the range of signs could be increased by including non-morphological properties of plants, in particular, physiological or biochemical ones.

Using the *NDVI* as an example, it was shown that the spectral characteristics of plant leaves could be used as a source of information for phenotyping.

A method for determining the plant development stability based on the results of phenotyping was proposed. A hyperspectral camera and a phenotyping procedure were used to determine the reflection coefficients required to calculate the vegetation indices. A preliminary assessment of the possibility of determining the plant development stability based on the results of phenotyping using the example of cucumber plants showed the feasibility of the method and its practical applicability.

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Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов.

Conflict of interest. The authors declare no conflict of interest.

Статья поступила в редакцию 22.01.2020
The paper was submitted to the Editorial Office on 22.01.2020

Статья принята к публикации 01.02.2021
The paper was accepted for publication on 01.02.2021