The effect of soil and climatic conditions on the distribution of nutrients in *Actinidia arguta* leaves

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The patterns of the distribution of nutrients in kiwiberry (*Actinidia arguta* (Siebold & Zucc.) Planch. ex Miq.), family Actinidiaceae (Gilg & Werderm), leaves growing under different soil and climatic conditions (Ukraine and China) were studied. Using scanning electron microscopy, significant differences were shown in the distribution of assimilates and mineral nutrients in the leaves of kiwiberry cultivated under different climate and soil conditions (Kyiv city, Ukraine and Jiamusi, China). The leaves of plants grown in China have higher concentration of all of the studied nutrients exception for silicon. The differences found in the content of macro- and microelements in plant tissues are consistent with their total content in the soil, and depend on the synthesis of low molecular weight organic compounds, namely, hydroxybenzoic, benzoic and triterpene acids. An increase in the silicon content in the leaves of kiwiberry plants grown in Ukraine indicates the moisture deficit in the soil. This conclusion is confirmed by the anatomical differences viz. the presence of additional interlaminary formations and fewer stomata number per 1 mm² of leaf surface. The specific feature of 'Perlyna sadu' cultivar was high concentrations of sodium in the leaves of kiwiberry cultivated under different climate and soil conditions (Kyiv city, Ukraine and Jiamusi, China). The leaves of plants grown in China have higher concentration of all of the studied nutrients except for silicon. The differences found in the content of macro- and microelements in plant tissues are consistent with their total content in the soil, and depend on the synthesis of low molecular weight organic compounds, namely, hydroxybenzoic, benzoic and triterpene acids.

**Keywords**: fruit vines; climatic edaphic factor; assimilates; mineral nutrients; remobilization.

**Introduction**

Presently kiwiberry (*Actinidia arguta* (Siebold & Zucc.) Planch. ex Miq.) is becoming increasingly popular among consumers and manufacturers in many countries around the world. The kiwiberry fruits are used both fresh and as a raw material for the production of dried fruits, compotes, juices, jams, wines. Numerous studies have shown a high content of biologically active substances, vitamins (C, E, K), polysaccharides, polyphenols, triterpenoids, alkaloids in fruits. They are characterized by analgesic, antibacterial, antiradiant, antitumour and other pharmacological activities (Niu et al., 2019; Qing et al., 2019). As a commercially cultivated plant kiwiberry is the most popular in New Zealand, USA, Japan, Belgium, Germany, Italy and Poland. A significant contribution to the popularization and selection of kiwiberry in Ukraine was made by researchers of M. Grishko National Botanical Garden of the National Academy of Sciences of Ukraine (NBG), who created 20 high-yield cultivars promising for industrial and household cultivation (Skrypchenko & Latocha, 2017).

The study of the ecology of nutrients is now becoming increasingly important, since it is known that in different soils mineral compounds are almost never found in such quantities and in such balanced proportions that would be optimal for growth and development of plants (Zaimenko, 2008; Jankowski et al., 2018a, 2018b; Loza et al., 2018; Lykholat et al., 2018). The latter always compensate for the negative effects of the environmental stress factors by triggering adaptive changes in the plant nutrition and other physiological processes (Zaimenko, 2019). Soils, in turn, are characterized by varied nutrient content, which determine the chemical composition of plants. At the same time, the effect of mineral compounds on the growth and development of plants depends on the physical, chemical, and biological parameters of the soil, as well as on the external conditions and physiological adaptation of organisms (Sing et al., 2017).

The main prerequisite of adaptation is the internal processes that occur in the biosystem and ensure the stability of its external functions relative to various environmental parameters (Voloshin & Subbotin, 1987). There are two types of adaptations: those supported by all structural elements of a certain system level, and compensation. Stable adaptive mechanisms ensure the adaptation of the plant organism to certain environmental conditions, which remain constant for a lasting period of time. The labile adaptive mechanisms make it possible to adapt to irregular relatively short-term changes in the environmental conditions (Kornienko et al., 1965). The combined functioning of these two groups of adaptive mechanisms ensures the maximum adaptation of plants to specific conditions with minimal loss of energy. Moreover, the ability of plants to regulate the uptake and assimilation of nutrients and the possibility of using them to control metabolic processes and the

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synthesis of biologically active compounds is of particular importance. The nutrient content in soil and plants is one of the most important components of the macro- and microelement circulation in the ecosystem. Plant growth and development depend on environmental conditions, so the availability of nutrients is determined not only by their concentration in soil solution, but also by their combined effect on the physiological processes in plants. Differences in the content of macro- and microelements significantly affect the photochemical processes, and therefore, they are crucial for plant development. Nevertheless, the mechanisms involved in the accumulation and remobilization of elements of mineral nutrition in the tissues and organs of plants remain still unclear.

Only fragmentary data are available, for example, regarding differences in the distribution of mineral nutrition elements in the foliar tissues affected by phytopathogens (Belan et al., 2015). The studies using x-ray microanalysis showed that out of the thirty three mineral elements found in the leaf tissue only distribution of potassium and calcium changed along the perimeter of the lesion. The highest potassium content was found in asymmetrical leaf tissues, decreasing in the direction of the transition zone and reaching minimal values in symmetrical tissues. And, on the contrary, the highest level of calcium was recorded in symmetrical tissues, decreasing in the direction of the transition zone and reaching minimal values in asymmetrical tissues surrounding the foci of lesions caused by phytopathogens.

More recent studies using microprobe x-ray fluorescence spectrometry (µ-XRF), revealed the accumulation of selenium, calcium, potassium, copper and manganese in necrotic areas of leaves affected by phytopathogens (Silva et al., 2018). In contrast, sodium was evenly distributed in leaf tissues. In addition, the fluorescent method turned out to be quite informative in the case of a hierarchical assessment of differences in the content of nutrients by chlorophyll indices (Kalajli et al., 2018). The results of the analysis of the distribution of macro- and microelements in tissues during aging and leaf modulation are also noteworthy (Maillard et al., 2015). It was found that, regardless of the plant species, calcium and manganese, unlike other elements, are mainly mobilized from roots to shoots. A number of authors identified proteins involved in the regulation of plant nutrition by zinc, manganese and phosphorus, ensuring their remobilization between leaves and shoots (Dong et al., 2018). It was proved that the transport and distribution of zinc and cadmium in tissues clearly differed between legumes and cereal plant species, although the effect of high zinc concentrations on cadmium transport was less pronounced (Tümä et al., 2019). At the same time, a high concentration of zinc in the nutrient medium negatively affected the remobilization of this element from the root to the shoot and from the oldest to the youngest leaves. Jamaí & Ma (2014) identified four different strategies in the remobilization of mineral nutrients: xylem-switch, phloem-tropic, phloem-kickback and minimum-shift, based on specific molecular transport processes. Interesting results were obtained by Wang et al. (2019) when studying the effect of phenolics on the remobilization of macronutrients in wound tissues. In particular, the low synthesis of phenols contributed to an increase in the level of nitrogen in plants and a decrease in the supply of phosphorus and potassium. The application of Fe3O4 nanoparticles positively affected the accumulation of phosphorus, potassium, calcium, manganese, and iron in the roots, stems, and leaves of plants (Souza et al., 2019).

Since the concentration of macro- and micronutrients in plant tissues is the most reliable indicator of the chemical state of plants, which provides for the control of a large number of other factors, we studied the distribution of biogenic elements in the leaves of the woody vine of kiwiberry. It should be noted that the quantitative analysis of chemical elements is also a reliable criterion for assessing the imbalance in species of various ecomorphotypes, in particular, the Ca:Mg ratio is much higher in woody vines.

Materials and methods

The object of the study was generatively mature plants of kiwiberry (Actinidia arguta) ‘Sentyabrskaya’, ‘Krasunya’, ‘Kievskaya krupnoplodnaya’ and ‘Perlina sadu’ cultivars, grown under different soil and climatic conditions, namely: in Ukraine (Kyiv city, NBG) on grey forest soil and in China (Jiamusi University, Heilongjiang Province) on black earth. Leaf and soil samples were collected during fruit ripening stage. The distribution of nutrients in leaf tissues was determined using scanning electron microscopy. Dehydrated samples were placed on aluminum or graphite plates, coated by gold film 5 ± 2 μm thick. Then, the samples were placed on titanium plates and analyzed using a JED-2300 X-ray spectrometer integrated with a JSM 6060 LA scanning electron microscope.

The morphometric measurements were carried out using electron microscopic images. The surface of stomatal cells and their number per unit of leaf area was evaluated using UTHSCA Image 3.0 by electron microscopic negatives scale.

The total content of mineral elements in the soil was analyzed using an iCAP 6300 Duo optical emission JCP spectrometer. The soil organic compounds were extracted by maceration in acetone, and afterwards, identified by Agilent 1100 DAD-HPLC + C chromatographic system using an Agilent Zorbax Eclipse Plus CL8 column.

The photosynthetic pigments (chlorophyll a, b and carotenoids) were extracted by dimethylsulfoxide (DMSO): 200 mg of sample leaf tissue was poured into 5 mL of DMSO, kept in a thermostat for 12 hours. Their content was determined spectrophotometrically (Wellburn, 1994) by Specord 2000 (Analytic Jena), at wavelengths: 480 nm for carotenoids, 649 and 665 nm for chlorophyll b and a, respectively. Anthocyanins were extracted from the leaves by 0.1 N HCl. Their content was determined spectrophotometrically at the wavelength of 490 nm (Pisarev et al., 2010).

Bioelectric potential (BEP) of plants was determined using redox electrodes by potentiometric and conductometric analytic techniques (Martynenko, 1988). The BEP was measured by the surface abstraction method with silver chloride electrodes of the EVL-1M3 grade and an electromagnetic transducer. This method allows one, firstly, to study the native electrical characteristics of the whole plant, and, secondly, it doesn’t have drawbacks characteristic to contact methods such as intrusion into the thermal and gas regime of the leaves.

The groups of values were compared by U-criterion Mann-Whitney. This is a statistical criterion used to assess differences between two independent samples, allows us to identify differences in the parameter value between small samples for P < 0.05; P < 0.01, P < 0.001. Quantitative indicators of the content of mineral nutrients in leaves and soil are given as arithmetic mean with standard deviation.

Results

The significant differences in the distribution of nutrients in the leaves of A. arguta plants, developed in the soil and climatic conditions of Kyiv and Jiamusi, were shown with the use of scanning electron microscopy. The leaves of plants of the Chinese plot were characterized by a higher concentration of mineral elements, except silicon. When studying the distribution of nutrients in leaves along the stem, the presence of three zones was revealed for the first time – the lower one saturates the roots, the upper one – saturates the leaves in the phase of active growth and the middle one, in which the assimilates are distributed in two directions. A directly proportional relationship was found between the biosynthesis of photosynthetic pigments and electrophysiological activity, especially for the leaves of the lower tier. With the presence of zones where there is a change in the polarity of bioelectric potentials and the distribution of assimilates, we can suppose there are different functional roles of individual parts of plants. In particular, the leaves of the lower tier perform a storage function. The middle part is less conservative and characterized by a high sensitivity to external factors. We marked it as a synthesis zone. The upper part is a zone of active growth. The results of a comparative analysis of the indicators of the number of chloroplasts in the leaf cell proved that the obtained dependence can be used as a diagnostic criterion for assessing the predicted plant productivity at an early stage of their development.

Currently, insufficient information is available about the relationship between the leaf and root nutrition of woody vines, including kiwiberry. The lack of information regarding the metabolic independence of various plant organs in the mineral nutrition system impedes the development of optimal technology for their cultivation, taking into account environmental factors.
A comparative analysis of the distribution of nutrients in the leaves of kiwiberry grown under different climatic and soil conditions viz. in Kyiv city (Ukraine) on grey forest soil and in Jiamusi (China) on black earth revealed significant differences in their concentration (Table 1). Figures 1 and 2 present information on the distribution of chemical elements in plant tissues of kiwiberry obtained by scanning microscopy.

**Fig. 1.** Distribution of biogenic elements in the leaves of *A. arguta* which grew in the conditions of Kyiv: scanning electron microscopy was used

**Fig. 2.** Distribution of biogenic elements in leaves of *A. arguta* which grew in conditions of Jiamusi (Scanning electron microscopy was used)

The leaves of kiwiberry, especially of those from China, were characterized by a rather higher concentration of chlorine. In general, the tissues of the leaves of plants that grew under the conditions of Jiamusi were characterized by a higher content of nutrients, which is explained by the higher level of organic matter in the soil. In particular, the humus content in the experimental plots in the grey forest soil was 2.8%, and in the black earth – 3.9%. It is known that the leading role in the structure formation of humus belongs to pro-humic substances, primarily phenolic compounds. The results of our studies showed a higher concentration of low molecular weight organic compounds in the soil samples from China, especially terpenoids, the content of which was 10 times higher in comparison with samples of grey forest soil from Ukraine (Fig. 3).
The obtained dependence testifies to the active development of soil microorganisms that enrich the rhizosphere of Actinidia plants with biologically active compounds and basic elements of mineral nutrition. In addition, the presence in the soil of benzoic, oxybenzoic and triterpenoid acids indicates the high biological activity of plants A. arguta, whose rhizosphere is enriched by these compounds under the influence of extracts secreted by the roots. Unfortunately, almost nothing is known about the mechanisms of the influence of the rhizosphere microorganisms on the absorption of nutrients by the root system of plants. The resulting dependence indicates the active development of soil microorganisms that enrich the kiwiberry rhizosphere with biologically active compounds and nutrients. In addition, the presence of benzoic, hydroxybenzoic and triterpenoid acids in the soil indicates the high allelopathic potential of kiwiberry. The revealed tendency in the distribution of nutrients in the kiwiberry leaves is consistent with the results obtained by the analysis of the total content of mineral elements in soil samples from experimental sites (Table 2).

Table 1
Distribution of nutrient elements in A. arguta leaves (% wet mass)

| Element | Soil and climatic conditions | Kyiv | Jiamusi |
|---------|-----------------------------|------|--------|
| C       | 62.00 ± 2.83                | 60.12 ± 2.11 |
| O       | 36.63 ± 1.25                | 36.33 ± 1.58 |
| Na      | 0.10 ± 0.01                 | 0.73 ± 0.04** |
| Mg      | 0.24 ± 0.03                 | 0.51 ± 0.05** |
| Al      | 0.34 ± 0.04                 | 0.73 ± 0.09* |
| Si      | 0.34 ± 0.05                 | 0.16 ± 0.02* |
| P       | 0.03 ± 0.004                | 0.25 ± 0.04* |
| S       | 0.06 ± 0.005                | 0.30 ± 0.04* |
| Cl      | 0.08 ± 0.007                | 0.22 ± 0.03* |
| K       | 0.04 ± 0.003                | 0.21 ± 0.04* |
| Ca      | 0.13 ± 0.02                 | 0.21 ± 0.05* |
| Fe      | 0.01 ± 0.001                | 0.03 ± 0.002* |

Note: n = 5; * – P < 0.05, ** – P < 0.01.

High concentrations of sodium and aluminum in the kiwiberry leaves were revealed, regardless of the place of plant growth. The results obtained regarding the barrier and barrier-free entry of nutrients into plant tissues require a more detailed study.

Back in the 1960s, the localization of assimilate distribution among various organs of a plants was first proved using the carbon label technique. It was expressed in the predominance of assimilate intake from leaves located in different layer along the kiwiberry stem revealed the following three functional layers, namely: the lower one, providing the supply of assimilates to roots; the upper one containing leaves in the phase of active growth; the middle one, redistributing the assimilates in the both directions (Table 3).

Table 2
Gross content (mg/kg) of mineral elements in soils of test areas

| Exposition area | Al  | Ca  | Fe  | K   | Mg  | Na  | P   | Si |
|-----------------|-----|-----|-----|-----|-----|-----|-----|----|
| Kyiv, grey forest soil | 2997 | 5072 | 2930 | 4574 | 3608 | 3172 | 276 | 455 |
| Jiamusi, black earth | 11700 | 6468 | 8963 | 2354 | 1900 | 2028 | 219 | 421 |

Note: n = 5; * – P < 0.05.

Of particular note is the fact that there is a high concentration of silicon in the leaves of kiwiberry grown in Ukraine. Evidently it may be associated with a drought stress due to the prolonged drought period observed during the growing season of 2018 in Kyiv. In particular, the yearly amount of precipitation in Kyiv was 444 mm, and in April, during the period of active development of plants, the amount was only 8 mm, in August – 22 mm, in October – 17 mm. While in Jiamusi, the total rainfall was more than 580 mm. The evidence that kiwiberry plants grown in Kyiv experienced water deficiency is proved also by anatomical studies of the leaves. The number of stomata per 1 mm² of leaf surface of Chinese plants was within 180 ± 9.5 per 1 mm². By contrast, Ukrainian ones had 152 ± 8.1 stomata per 1 mm² of leaf surface. Besides, leaves of kiwiberry plants grown in Ukraine had additional integumentary formations on the lower surface, which were absent in plants cultivated in China (Fig. 4).

Fig. 4. Influence of soil-climatic conditions on the stomata distribution on the leaf surface: a – Kyiv, b – Jiamusi

Table 3
The content of nutrients in the leaves of different stages of Actinidia plants, macroelements (mg/kg), microelements (%)
Moreover, the leaves of the middle zone are characterized by a higher concentration of nutrients. Similar results were also obtained when studying the distribution of anthocyanins across the mentioned layers (Fig. 5).

**Fig. 5.** Anthocyanins content in leaves of *Actinidia* plants: n = 5; * – P < 0.05

The analysis of the distribution of surface biopotentials in leaves across the mentioned layers as well as content of photosynthetic pigments showed a positive relationship between both these indices, especially for the leaves of the middle layer (Table 4).

### Table 4
Quantitative indicators of photosynthetic pigments in *Actinidia* leaves during the period of active growth

| Stage          | Amplitude of bioelectrical potentials, mV | The content of photosynthetic pigments, mg/g dry mass |
|----------------|-------------------------------------------|-----------------------------------------------------|
|                |                                           | chlorophyll                                         |
|                |                                           | carotenoids                                         |
| Variety 'Sentyabrykskaya' |                                           |                                                     |
| Top            | 13.4 ± 0.45                                | 5.7 ± 0.21                                          |
| Middle         | 14.12 ± 0.31                               | 8.03 ± 0.44*                                        |
| Lower          | 14.03 ± 0.28                               | 7.54 ± 0.41                                         |
| Variety 'Krasunya' |                                           |                                                     |
| Top            | 12.84 ± 0.33                               | 5.01 ± 0.22                                         |
| Middle         | 13.22 ± 0.34                               | 5.62 ± 0.13                                         |
| Lower          | 13.12 ± 0.46                               | 5.22 ± 0.34                                         |
| Variety 'Kievskaya Krumnoplohdnaya' |                                           |                                                     |
| Top            | 12.0 ± 0.36                                | 3.82 ± 0.12                                         |
| Middle         | 13.04 ± 0.31*                              | 5.94 ± 0.34*                                        |
| Lower          | 12.83 ± 0.29*                              | 5.18 ± 0.26*                                        |

**Note:** n = 5; * – P < 0.05.

The obtained results confirm the hypothesis of relative autonomy and the complementary role of different foliar layers in woody vines. Consequently, the described physiological differences in kiwiberry leaves located in different layers suggest also their functional differentiations. In particular, the leaves of the lower layer perform a storage function, while the middle layer is less conservative, and it is characterized by higher sensitivity to environmental factors. We identified it as a synthesis zone. The upper layer is a zone of active growth.

The results of the comparative analysis of the indices of the chloroplasts number per mesophyll cell among the studied kiwiberry cultivars revealed a significant positive correlation with the cultivar productivity (Fig. 6, 7). Therefore, we suggest that the chloroplast index could be used as a diagnostic criterion in predicting productivity of kiwiberry plants.

**Fig. 6.** The number of chloroplasts *Actinidia* plants: n = 5; * – P < 0.05

**Fig. 7.** *Actinidia* plants productivity indicators: n = 5; * – P < 0.05

### Discussion

As a result of a comparative assessment of the content of nutrients in the leaves of kiwiberry plants grown under various soil and climatic conditions, namely in Ukraine and China, the presence of a high concentration of chlorine in the tissues was shown. Similar results were obtained by study of other species of this genus, in particular *Actinidia delicosa* (A. Chev) C. F. Liang et A. R. Ferguson (Smith et al., 1987). It is known that chlorine has a high chemical activity, improves the swelling of the protoplasm and positively affects the water content in tissues, stimulates oxidative phosphorylation. The better moisture supply of kiwiberry plants cultivated in China contributed to an increase in the concentration of chlorine in the leaves by 2.7 times in comparison with kiwiberry plants, cultivated in Ukraine.

Significant differences in the uptake of mineral elements by various cultivars of kiwiberry are also indicated (Stefanian et al., 2017). The results of our studies confirmed the high demand of *A. arguta* plants for fertile soil with a high humus content, which was also shown by other authors (Petricila et al., 2015). The revealed higher silicon content in leaves of kiwiberry grown in Kyiv could be regarded as an adaptation to drought stress. The positive role of silicon in the processes of adaptation of higher plants to the environmental stresses as well as their growth and productivity was shown in our preliminary studies (Zaimenko & Rotiska, 2018; Zaimenko et al., 2018).

Of particular interest are the data obtained in the study of surface bioelectric potentials of leaves along the stem of fruit vines. Evolution of kiwiberry plants leads to the complication and improvement of their structural organization. Adaptation to changing environmental conditions is provided by subordinate systems of organs coordinated in time and space. Periodic processes that determine the functional principle of the constitution of living systems (i.e. the principle of “stable disequilibrium” of Bauer) play an increasingly important role in the evolution of plant organisms, synchronization and coordination in time of various biochemical reactions in cells, and also determine the nature of the interaction of cells and the formation of morphostructures.

The paramount importance that bioelectric processes have in the self-regulation, adaptation and evolution processes proves the importance of the results obtained regarding the various functional roles of organs. The obtained dependence on the reintegration of assimilates turned out to be unified for plants of different ecotype structures.
Conclusions

The results of the given studies showed significant differences in the distribution of assimilate between the leaves of A. arguta depending on the soil and climatic conditions of cultivation. The leaves of kiwiberry plants grown in China were characterized by a higher concentration of nutrients, with the exception of silicon, which is explained by the high fertility of the soil and the intense synthesis of low-molecular-weight organic compounds, in particular hydroxybenzoic, benzoic and triterpenic acids. The analysis on the accumulation of mobile forms of silicon in the foliar tissues of plants grown in Ukraine indicates their stress state due to moisture deficiency in the soil. This is confirmed by xeromorphic anatomical changes, viz. the smaller number of stomata per 1 mm² of leaf surface and formation of additional cover layer on the lower surface of the leaf blade.

The revealed accumulation of chlorine in kiwiberry tissues is consistent with the results obtained by other authors for another species of the same genus A. delicosa. Noteworthy are the results on the accumulation of sodium and aluminum in the leaves of the studied kiwiberry plants, which suggests the necessity for the further studies of the peculiarities of the barrier and barrier-free entry of elements into plant organs.

For the first time, the spatial pattern of bioelectric potentials and photosynthetic activity of the foliar tissues along the stem of a woody fruit vine was analyzed. Three functional layers (the upper, middle and lower) of redistribution of assimilates and mineral elements were distinguished. In particular, the middle part had the highest concentration of macro- and microelements, indices of bioelectric potentials the content of photosynthetic pigments in comparison with the upper and lower parts.

The results obtained suggest the optimization of agricultural practices for kiwiberry cultivation, including foliar feeding. A comparative analysis of the yield of various kiwiberry cultivars made it possible to identify a diagnostic feature for the prediction of their potential productivity, in particular, by counting the number of chloroplasts in the mesophyll cells and determining the content of chlorophyll b.

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