ISSN 1021-4437, Russian Journal of Plant Physiology, 2006, Vol. 53, No. 6, pp. 756–761. © MAIK "Nauka/Interperiodica" (Russia), 2006.
Original Russian Text © S.V. Veselova, R.G. Farkhutdinov, D.S. Veselov, G.R. Kudoyarova, 2006, published in Fiziologiya Rastenii, 2006, Vol. 53, No. 6, pp. 857–862.

Role of Cytokinins in the Regulation of Stomatal Conductance of Wheat Seedlings under Conditions of Rapidly Changing Local Temperature

S. V. Veselova, R. G. Farkhutdinov, D. S. Veselov, and G. R. Kudoyarova

Institute of Biology, Ufa Research Center, Russian Academy of Sciences, pr. Oktyabrya 69, Ufa, Bashkortostan, 450054 Russia; fax: +7 (3472) 53-3562; e-mail: veselov@anrb.ru

Received March 27, 2006

Abstract—The influence of an air temperature increase by 4°C and nutrient solution cooling down to 5 ± 1°C on stomatal conductance and hormone level of seven-day-old wheat (*Triticum durum* L., cv. Bezenchukskaya 139) seedlings was studied. An elevated air temperature resulted in a rapid rise of stomatal conductance preceded by the increase in the level of cytokinins in leaves. Cooling of the nutrient solution induced gradual stomatal closure along with a decreasing cytokinin level in leaves. Hormone concentration in the xylem sap of wheat seedlings was determined, and the rate of hormone transport from the roots to shoots was calculated. The role of cytokinins in the regulation of stomatal conductance under conditions of local thermal treatments is discussed.

DOI: 10.1134/S1021443706060057

Key words: Triticum durum - stomatal conductance - cytokinins - local stress - hormonal signal

INTRODUCTION

Stomata play an important role in the control of water evaporation and gas exchange in plant leaves. Transpiration and photosynthesis are regulated by changing the size of stomatal pores. The operation of the stomatal apparatus can be influenced by plant environment [1], and therefore stomatal regulation of transpiration is exerted by both internal and external factors. Ambient temperature is one of the most important external factors in the regulation of transpiration. Evidence exists that, in a warm and moist environment, stomata are widely open [2] to enhance cooling of the plant due to more intense transpiration and to maintain photosynthesis, while at low air temperatures, they tend to close [3]. Low soil temperature seems to have the same effect on plants, reducing the transpiration rate and stomatal conductance [4]. However, the exact mechanism of stomatal conductance regulation under conditions of thermal stress is still unclear.

Hormones are known to play an important role in environmental adaptation of the plant [5, 6]. The hormonal regulation mechanism of stomatal conductance is well studied; however, major focus was made on the role of ABA [7, 8]. Very limited data are available about the role of endogenous cytokinins, although the latter were long ago reported to be able to keep stomata open [9] to balance the stomata closure signal from ABA [10].

Abbreviation: BA—benzyladenine.

In a number of studies, it was shown that, when soil was getting dry, stomata closed, the water status of leaves remaining the same [11]. At the same time, the concentration of ABA in the xylem increased [7, 8]. This means that the ABA signal from leaves can regulate stomatal conductance independently of the water status of leaves. The question is whether cytokinins can be such a signal to control the stomatal state under conditions of fast changing ambient temperature. Cytokinins are known to be produced in roots and transported along the xylem to shoots [12, 13]. For this reason, they are sometimes regarded as signals being sent from the roots to shoots to regulate the operation and activity of the above-ground organs of the plant [14].

The objective of this study was to try to understand whether an abruptly changing level of cytokinins in the leaves and xylem sap was related to the regulation of stomatal conductance in response to abruptly changing temperature.

MATERIALS AND METHODS

Plant material and growth conditions. Spring durum wheat (*Triticum durum* L., cv. Bezenchukskaya 139) seven-day-old seedlings cultivated under laboratory conditions were used for the study. Seeds were germinated in darkness in distilled water containing 5×10^{-5} M $CaCl_2$ during 3 days at 24°C. The three-day-old seedlings were transferred to 10% Hoagland–Arnon medium and cultivated at an illuminance of 18 klx and

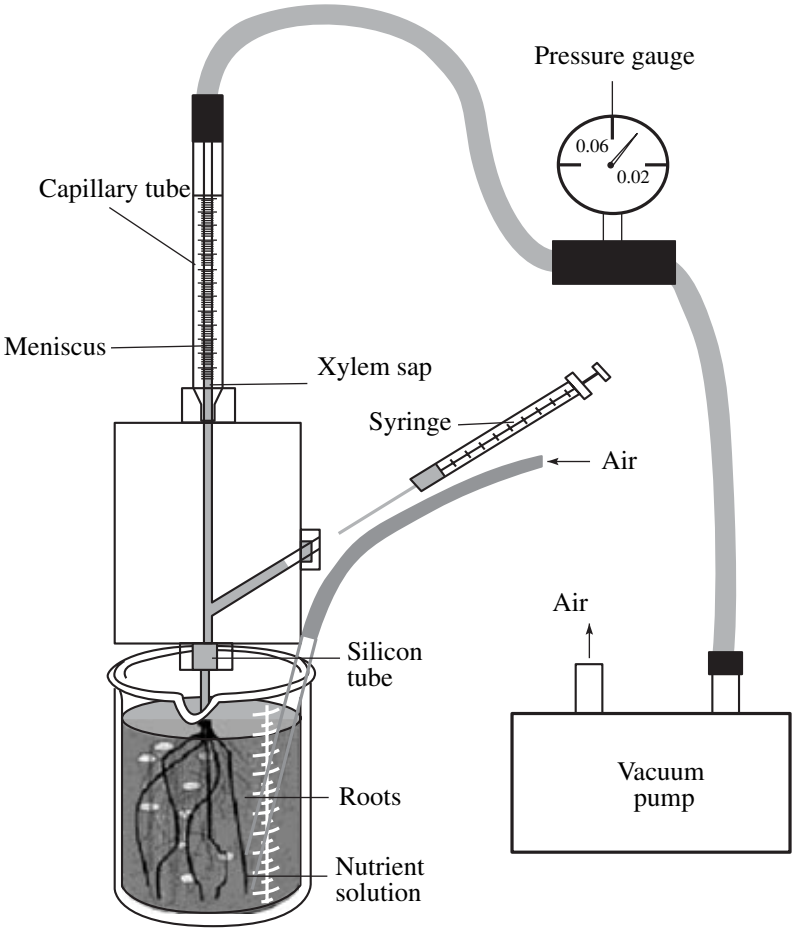

Fig. 1. A device for collection of the xylem sap built at the Prof. W. Hartung Laboratory (University of Würtzburg, Germany).

a 14-h photoperiod. The daytime air temperature was maintained at 20 to 24°C.

Air temperature increase. To imitate the effect of elevated air temperature, a fan heater was used to raise the air temperature in the cultivation chamber by 4 ± 1°C within a few minutes and to maintain it at the new level for 1.5 h. The air movement in plant growth area of the room was insignificant.

Cooling of plant roots. The nutrient medium was rapidly cooled from the initial 24°C to 5 ± 1°C by adding pieces of frozen medium.

Pretreatment with a synthetic cytokinin benzyladenine (BA). This was done by leaf immersion into the 100 μM BA solution with 0.05% Tween 60 added. The control plants were immersed in the 0.05% Tween 60 solution for the same period of time.

Stomatal conductance was determined using the formula:

$$C = 1/r, \quad (1)$$

where C is stomatal conductance (cm/s) and r is leaf resistance to vapor diffusion (s/cm). Stomatal conductance was measured using an MK porometer (Delta T Devices, United Kingdom). This device measures and registers the time during which humidity in the leaf chamber increases by a given value. The stomatal resistance r value was defined by comparison of the above time against measured time values for a set calibration of plates with known diffusion resistance values. Thus obtained value of stomatal conductance in cm/s was converted into that in mol/(m^2 s) using formulae and factors provided in [15].

The cytokinin level in plant material was determined using the immunoassay test carried out according to modified protocol described in [16]. The xylem exudate was collected using the device shown in Fig. 1, which was built in the Prof. Hartung laboratory (Würtzburg University, Germany). The above-ground part of the plant was cut off and the remaining 15- to 20-mm shoot segment together with the root system was connected to the device. In experiments with increasing air temperature, the above-ground parts of plants were cut at different times (15, 30, and 30 min) after the start of the experiment. When analyzing the root cooling effect, the cooling medium was cooled after the plants had been cut off and their underground parts were connected with the device. The vacuum created by a vacuum

Table 1. Stomatal conductance of wheat leaves after treatment with a synthetic cytokinin BA and cooling of the nutrient solution from 24°C down to 5 ± 1°C, mol/(m^2 s)

Treatment	Cooling time, min		
	0	30	60
Control	121 ± 19	109 ± 11	59 ± 10
BA, 100 μM	169 ± 11	148 ± 22	114 ± 17

pump made xylem sap to rise through the capillary tube. Every 15 min, the xylem sap was collected into weighed Eppendorf-type containers for freezing. The cytokinin concentration in the xylem sap [*C*] was determined, and the transport rate of cytokinins from the roots to shoots *D* was calculated as follows:

$$D = [C]Jv, \quad (2)$$

where *D* is the hormone transport rate in ng/h, [*C*] is the concentration of cytokinins in xylem sap (in ng/ml), and *Jv* is the xylem exudate flow rate in ml/h.

Mean values of stomatal conductance and standard errors were calculated in 10 replicates. The phytohormone levels were calculated based on 9 measurements. Standard software was used for the statistic processing of results. In the figures and in tables, mean values and standard errors are shown.

RESULTS

The stomata quickly responded to both elevated air temperature and cooling of the roots. In the case of elevated air temperate, the stomatal conductance remained unchanged for 5 min and then started to increase to reach approximately 3 times the initial level within about 30 min (Fig. 2). Cooling of the roots resulted in a slow decrease in stomatal conductance (see Table 1). The really significant decrease (by a factor of 2) was observed only towards the end of the first hour of cooling. Treatment of wheat leaves with BA under control conditions resulted in a 40% increase in stomatal conductance (Table 1). A 30-min root cooling of BA-pretreated plants resulted in a higher conductance level compared to that in control plants (no BA treatment and no cooling). After one hour of cooling, the conductance was back to the control level (i.e., that before cooling (Table 1)).

Both types of thermal impact on plants led to significant changes in the cytokinin level in leaves. When the air temperature increased, the cytokinin level in leaves rose rapidly (Fig. 3a) to remain at a high level for the rest of the experiment. Conversely, cooling of the roots rapidly decreased the cytokinin level in leaves (Fig. 3b).

The cytokinin level in the xylem was also found to be changing fast (Table 2). In the case of elevating air temperature. the concentration and transport rate of zeatin to shoots rose. Under conditions of cooling of growth medium, the transport rates of zeatin and zeatin riboside dropped (Table 2), their concentrations in the xylem remaining the same.

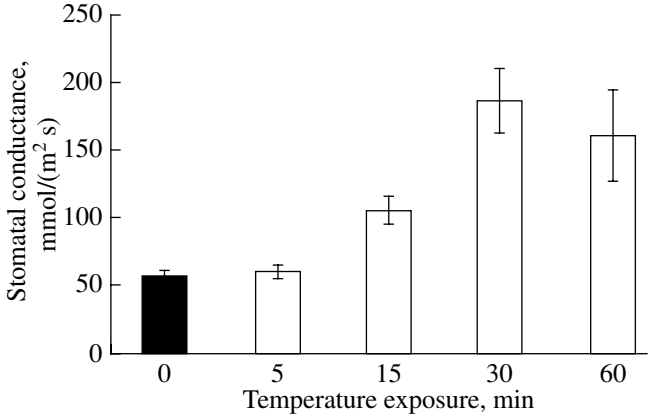

Fig. 2. Changes in stomatal conductance of wheat leaves in response to the elevation of air temperature by 4°C.
n = 10.

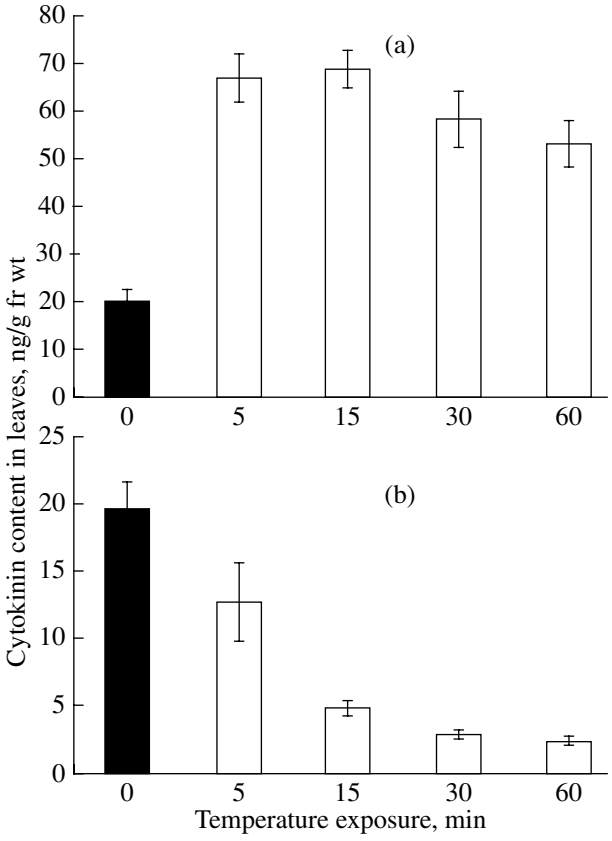

Fig. 3. Changes in cytokinin (zeatin + zeatin riboside) level in leaves of wheat in response to (a) increasing air temperature and (b) cooling of nutrient solution.
n = 9.

Table 2. Effect of temperature on the cytokinin concentration in the xylem sap and their transpiration-driven transport rates

Exposure time, min	Zeatin		Zeatin ribozide	
	[*C*]	*D*	[*C*]	*D*
Air temperature increase from 24°C by 4 ± 1°C				
0	5.6 ± 0.6	0.37 ± 0.06	14.8 ± 1.5	0.98 ± 0.12
15	6.7 ± 0.8	0.68 ± 0.09	12 ± 0.8	1.22 ± 0.21
30	10.7 ± 1.2	0.90 ± 0.08	10 ± 1.3	0.84 ± 0.11
60	9.4 ± 1.3	0.79 ± 0.10	10.4 ± 1	0.87 ± 0.12
Cooling of nutrient solution from 24°C down to 5 ± 1°C				
0	5.4 ± 0.2	0.35 ± 0.01	19 ± 2.4	1.16 ± 0.08
15	4.9 ± 0.7	0.25 ± 0.01	16.3 ± 2	0.85 ± 0.09
30	6.3 ± 0.4	0.15 ± 0.02	17 ± 2.3	0.41 ± 0.05
60	6 ± 0.5	0.10 ± 0.01	20.4 ± 1.8	0.33 ± 0.05

Note: [*C*]—hormone concentration in the xylem sap, ng/ml; *D*—hormone delivery rate, ng/h. $n = 9$.

DISCUSSION

Both elevated air temperature and cooling of nutrient medium influenced the stomatal conductance. The elevated air temperature increased (Fig. 2), while root cooling reduce it (Table 1), and in both cases, some time was required for stomata to open or close. In the case of elevated air temperature, 15 min was required for stomatal conductance to double (Fig. 2). When roots were cooled, the stomatal conductance decreased by half in about 60 min (Table 1). Cytokinins, known to be able to influence the stomatal apparatus, could be signals causing these changes. They are already known to regulate stomatal opening and enhancement of transpiration [9]. Kinetin applied to isolated epidermis was causing stomatal opening [17]. Few studies witness for the correlation between changes in the endogenous cytokinin and stomatal conductance levels [18]. In addition, as was earlier shown by us, the ABA level in shoots fell rather than increased both when roots were cooled [19] or air temperature increased [20]. This means that ABA could neither cause stomatal closure when roots were cooled nor stop them from opening when the air temperature rose.

These data suggest that it is the cytokinin level in leaves to induce changes in stomatal conductance under the conditions of thermal stress. Zeatin is an active form of cytokinin [21] known to influence the activity of the stomatal apparatus [22]. We have found that elevated air temperature leads to the abrupt growth of the zeatin level in leaves (triples within 5 min after heating starts (Fig. 3a)). Cooling of the roots leads to the reduction in the zeatin level in leaves by a factor of 4 after 15 min and almost by a factor of 10 within 60 min. In both cases, changing the zeatin level preceded corresponding changes in stomatal conductance. The suggested role of cytokinins in the regulation of stomatal conductance was confirmed in the course of experiments with BA-treated plants. Thus, BA pretreatment of plants subject to cold stress prevented the stomatal conductance from falling below the control level (i.e., that of plants with no cytokinin treatment and no cooling (Table 1)).

It was very important to find out the exact mechanisms beyond such rapid changes in the cytokinin levels in leaves. Cytokinins are known to be mainly produced in the root tips [23] to be further transported along the xylem to the above-ground organs of the plant. Our findings showed that the accumulation of zeatin in leaves in response to elevated air temperature was a result of its higher export rate from the roots (Table 2).

The cytokinin export from chilled roots decreased less than the cytokinin level in leaves (Table 2). Thus, about one third of the amount of cytokinins found in control plants was transported from roots within the first hour of cooling (Table 2), and their level in shoots dropped almost tenfold (Fig. 3b). These results confirmed our earlier suggestion that a fast decrease in the cytokinin level in leaves was mainly due to their degradation by cytokinin oxidase [24] rather than by the lower export rate from the chilled roots.

Therefore, rapid changes in the hormone levels in response to thermal impact can be caused by changing rates of both transport and metabolism of hormones. The question is what is the initial signal to induce these fast changes in the hormone levels of the wheat leaves in response to thermal impact? Apparently, cold was irritating roots to send a signal to shoots and heat was irritating shoots to send a signal to roots, and this might be a system to activate a fast hormone regulation. No consensus exists as regards the origin of this signaling. It might be an action potential caused by heating or cooling being further transmitted to the other parts of the plant [25]. For example, bioelectric response to root cooling could be observed within 10 to 15 s in leaves [26]. The electric pulse can induce changes in ion trans-

port and consequently in the osmotic pressure [27]. These phenomena are examples of some unspecific effects induced in a part of the plant being caused by environmental factors affecting the other part.

The accumulation of cytokinins in leaves increased the stomatal conductance in response to elevated air temperature while the reduction in the cytokinin level in leaves due to cooling of roots led to a decrease in the stomatal conductance. These are clear examples of active mechanisms involving hormones to help plants quickly adapt to environmental changes.

ACKNOWLEDGMENTS

This study was supported by the President of Russian Federation, grant no MD-2219.2005.4.

REFERENCES

1. Matzner, S. and Comstock, J., The Temperature Dependence of Shoot Hydraulic Resistance: Implications for Stomatal Behaviour and Hydraulic Limitation, *Plant, Cell Environ.*, 2001, vol. 24, pp. 1299–1307.
2. Radin, J.W., Lu, Z., Pichard, R.G., and Zeiger, E., Genetic Variability for Stomatal Conductance in *Pima cotton* and Its Relation to Improvement of Heat Adaptation, *Proc. Natl. Acad. Sci. USA*, 1994, vol. 91, pp. 7217–7221.
3. Wilkinson, S., Clephan, A.L., and Davies, W.J., Rapid Low Temperature-Induced Stomatal Closure Occurs in Cold-Tolerant *Commelina communis* Leaves but Not in Cold-Sensitive Tobacco Leaves, via a Mechanism That Involves Apoplastic Calcium but Not Abscisic Acid, *Plant Physiol.,* 2001, vol. 126, pp. 1566–1578.
4. Ben-Zioni, A. and Dunstone, R.L., Effect of Air and Soil Temperature on Water Balance of Jojoba Growing under Controlled Conditions, *Physiol. Plant.*, 1988, vol. 74, pp. 107–112.
5. Jackson, M., Are Plants Hormones Involved in Root to Shoot Communication?, *Advanced in Botanical Research*, vol. 19, Callow, J.A., Ed., Dordrecht: Academic, 1993, pp. 103–187.
6. Kulaeva, O.N. and Kusnetsov, V.V., Recent Advances and Horizons of the Cytokinin Studying, *Fiziol. Rast.* (Moscow), 2002, vol. 49, pp. 626–640 (*Russ. J. Plant Physiol.,* Engl. Transl., pp. 561–575).
7. Zhang, J. and Davies, W.J., Abscisic Acid Produced in Dehydrating Roots May Enable the Plant to Measure the Water Status of the Soil, *Plant, Cell Environ.*, 1989, vol. 12, pp. 73–81.
8. Hartung, W., Sauter, A., and Hose, E., Abscisic Acid in Xylem: Where Does It Come from, where Does It Go to? *J. Exp. Bot.*, 2002, vol. 53, pp. 27–32.
9. Livne, A. and Vaadia, Y., Stimulation of Transpiration Rate in Barley Leaves by Kinetin and Gibberellic Acid, *Physiol. Plant.*, 1965, vol. 18, p. 658.
10. Blackman, P.G. and Davies, W.J., The Effects of Cytokinins and ABA on Stomatal Behavior of Maize and *Commelina, J. Exp. Bot.*, 1983, vol. 34, pp. 1619–1626.
11. Gollan, T., Passioura, J.B., and Munns, R., Soil Water Status Affects the Stomatal Conductance of Fully Turgid Wheat and Sunflower Leaves, *Aust. J. Plant Physiol.,* 1986, vol. 13, pp. 459–464.
12. Kulaeva, O.N., Root Effects on the Metabolism in Leaves and Kinetin Effects in Leaves, *Fiziol. Rast.* (Moscow), 1962, vol. 9, pp. 229–239 (*Sov. Plant Physiol.*, Engl. Transl.).
13. Sakakibara, H., Nitrate Specific and Cytokinin-Mediated Nitrogen Signaling Pathways in Plants, *J. Plant Res.*, 2003, vol. 116, pp. 253–257.
14. Takei, K., Sukakihara, H., Taniguchi, M., and Sugiyama, T., Nitrogen-Dependent Accumulation of Cytokinins in Root and the Translocation to Leaf: Implication of Cytokinin Species That Induced Gene Expression of Maize Response Regulator, *Plant Cell Physiol.*, 2001, vol. 42, pp. 85–93.
15. Jones, H.G., *Plants and Microclimate: Quantitative Approach to Environmental Plant Physiology*, Cambridge: Cambridge Univ. Press, 1983.
16. Kudoyarova, G.R., Farhutdinov, R.G., Mitrichenko, A.N., Teplova, I.R., Dedov, A.V., Veselov, S.U., and Kulaeva, O.N., Fast Changes in Growth Rate and Cytokinin Content of the Shoot Following Rapid Cooling of Roots of Wheat Seedlings, *Plant Growth Regul.,* 1998, vol. 26, pp. 105–108.
17. Incoll, L.D. and Whitelam, G.C., The Effect of Kinetin on Stomata of the Grass *Anthephora pubescens* Nees, *Planta,* 1977, vol. 137, pp. 243–245.
18. Shashidhar, V.R., Prasad, T.G., and Sudharshan, L., Hormone Signals from Roots to Shoots of Sunflower (*Helianthus annuus* L.). Moderate Soil Drying Increases Delivery of Abscisic Acid and Depresses Delivery of Cytokinins in Xylem Sap, *Ann. Bot.*, 1996, vol. 78, pp. 151–155.
19. Veselov, D.S., Sabirzhanova, I.B., Akhiyarova, G.R., Veselova, S.V., Farkhutdinov, R.G., Mustafina, A.R., Mitrichenko, A.N., Dedov, A.V., Veselov, S.Yu., and Kudoyarova, G.R., The Role of Hormones in Fast Growth Responses of Wheat Plants to Osmotic and Cold Shocks, *Fiziol. Rast.* (Moscow), 2002, vol. 49, pp. 572–576 (*Russ. J. Plant Physiol.*, Engl. Transl., pp. 513–517).
20. Veselova, S.V., Hormonal Regulation of Water Metabolism and Seedling Growth under Temperature Variation, *Cand. Sci. (Biol.) Dissertation,* Ufa: Bashkir. Gos. Univ., 2003.
21. Horgan, R., Present and Future Prospects for Cytokinin Research, *Physiology and Biochemistry of Cytokinins in Plants*, Kaminek, M., Mok, D.W.S., and Zazimalova, E., Eds., The Hague: Academic, 1992, pp. 3–12.
22. Badenoch-Jones, J., Parker, C.W., Letham, D.S., and Singh, S., Effect of Cytokinins Supplied via the Xylem at Multiples of Endogenous Concentrations on Transpiration and Senescence in Derooted Seedlings of Oat and Wheat, *Plant, Cell Environ.,* 1996, vol. 19, pp. 504–516.

23. Miyawaki, K., Matsumoto-Kitano, M., and Kakimoto, T., Expression of Cytokinin Biosynthetic Isopentenyltransferase Genes in Arabidopsis: Tissue Specificity and Regulation by Auxin, Cytokinin and Nitrate, *Plant J.*, 2004, vol. 37, pp. 128–138.

24. Kudoyarova, G., Veselov, D., Symonyan, M., Mustafina, A., Dedov, A., Vysotskaya, L., and Veselov, S., Fast Shoot Responses to Root Treatment. Are Hormones Involved? *Recent Advances Plant Root Structure Functions*, Gasparikova, O., Ciamporova, M., Mistrik, I., and Baluška, F., Eds., Dordrecht: Kluwer, 2001, pp. 135–141.

25. Retivin, V.G., Opritov, V.A., and Fedulina, S.B., Generation of Action Potential Induces Preadaptation of *Cucurbita pepo* L. Stem Tissues to Freezing Injury, *Fiziol. Rast.* (Moscow), 1997, vol. 44, pp. 499–510 (*Russ. J. Plant Physiol.*, Engl. Transl., pp. 432–442).

26. Gunar, I.I. and Panichkin, L.A., Spread of Activation in the Plant and Bioelectric Responses in the Leaf after Root and Petiole Stimulation, *Izv. Timiryazevsk. S-kh. Akad.*, 1967, no. 1, pp. 15–31.

27. Opritov, V.A., Krauz, V.O., and Treushnikov, V.M., The Role of Electric Irritation in the Functional Linkage between Above-Ground Organs and Roots after External Stimulus Effect on the Seedling Apex, *Fiziol. Rast.* (Moscow), 1972, vol. 19, pp. 961–967 (*Sov. Plant Physiol.,* Engl. Transl.).