TECHNICAL NOTE:

EFFECT OF HIGH-VOLTAGE ELECTROSTATIC FIELD ON INORGANIC NITROGEN UPTAKE BY CUCUMBER PLANTS

Y. Wu, J. Zhu, Y. Li, M. Li

ABSTRACT. *We investigated the inorganic nitrogen uptake by cucumber (*Cucumis sativus *L.) plants under high-voltage electrostatic field (HVEF). The maximum uptake rates and affinities significantly changed with increasing field intensity. Ammonium uptake slightly increased when the field intensity increased to 1.0 kV cm^{-1} and then decreased between 2.0 and 3.0 kV cm^{-1}. HVEF contributed to the affinity for nitrate in all treatment groups. The affinity of cucumber seedlings for nitrate positively correlated with the intensity of the applied field as the field increased to 1.0 kV cm^{-1}. HVEF enabled the seedlings to opt to consume nitrate when two types of nitrogen were present in the nutrient solution. These results can be attributed to feedback adjustment and electron transportation in nitrogen assimilation.*

Keywords. Cucumis sativus *L., Depolarization, High-voltage electric field, Hyperpolarization, Ion absorption kinetics.*

The biological effects of high-voltage electrostatic field (HVEF) on plants have fascinated researchers for decades. For instance, HVEF increases plant height, dry weight, and leaf surface area (Moon and Chung, 2000; Murr, 1963; Nechitailo and Gordeev, 2001), promotes seed germination (Moon and Chung, 2000; Sidaway and Asprey, 1966), and prolongs food storage (Hsieh and Ko, 2008; Knorr, 1999; Zhao et al., 2011). HVEF also greatly increases the respiration rates (Ma et al., 1991; Palanimuthu et al., 2009), hydrogen output rates, and carbon dioxide output rates of plants (Sidaway and Asprey, 1968). Moreover, HVEF significantly enhances the amount and activity of water-soluble proteins that are important in plant growth and development. Such proteins include plasma membrane enzymes that are crucial for ion absorption (Ma et al., 1991). Hence, HVEF may accelerate the cotransport of several types of ions. Since it has advantages of non-pollution, low cost, and low energy consumption, HVEF is widely employed in seed germination, growth promotion, food storage, and scientific research (He et al., 2014).

Nutrient elements, such as potassium, phosphate, ammonium, and nitrate, are crucial for plant growth, and enhancing the uptake of nutrient elements is important to improve crop yield. Nutrient uptake is mainly influenced by light, temperature, and element content in the soil under natural conditions. Little research has been documented about the effect of HVEF on nutrient uptake. Wu (1989) reported that appropriate HVEF promoted K^+ absorption. However, little is known about the kinetics of HVEF-induced nutrient uptake, particularly for inorganic nitrogen. This type of nitrogen is an essential element for plant growth and can be converted into ammonium or nitrate. As an extension of previous reports, this study investigated the effect and mechanism of HVEF on inorganic nitrogen uptake by plants through the Michaelis-Menten kinetic equation (Saker et al., 1984).

MATERIALS AND METHODS

CUCUMBER PLANT SEEDLING PREPARATION

Cucumber (*Cucumis sativus* L.) seeds were surface sterilized with 0.5% (w/v) aqueous formalin for 30 min, washed, and then imbibed with distilled water for 24 h. After germination, the seedlings were transferred into quartz sand under a 16h/8h light/dark cycle at 25°C ±1°C and an irradiance of 600 μmol m^{-2} s^{-1}. The seedlings were cultured with a regular supply of fresh Hoagland nutrient solution for 30 d. Subsequently, seedlings with consistent appearance were selected, irrigated with 1 mM $CaSO_4$ starvation solution for 2 d, and used for absorption experiments.

REAGENTS AND SOLUTIONS

Graded ammonium dihydrogen phosphate and potassium nitrate were used to prepare three types of nutrient solutions. The compositions of the nutrient solutions are shown in table 1. Ammonium or nitrate concentrations were adjusted to 1 mmol L^{-1}. Double-distilled water was used throughout the analyses.

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Table 1. Compositions of nutrient solutions.

Nutrient Solution	$NH_4H_2PO_4$	KNO_3	pH
1	+	-	6.92
2	-	+	7.49
3	+	+	7.20

HVEF TREATMENT SYSTEM

Experimental Setup

HVEF treatments were performed using a laboratory-scale high-electric field apparatus with a maximum output of 100 kV. As shown in figure 1, the apparatus included a BGG100KV/2MA power generator (Beijing Electrical and Mechanical Institute, Beijing, China), two rectangular 40 cm × 60 cm stainless steel plates as electrodes, a high-voltage controller, a voltmeter, an ammeter, and an electrostatic shield.

Generation of Electrostatic Field

The generator created an electric charge that produced a high voltage between the stainless steel electrodes (fig. 1). The adjustable cathode-anode distance provided different electric field strengths. A parallel plate setup resulted in quasi-uniform interelectrode fields. In this study, the output voltage was variable for an interelectrode distance of 20 cm (Zhao et al., 2011).

The electric field strength was estimated according to the following equation:

$$E = \frac{U}{d} \tag{1}$$

where

E = intensity of HVEF (kV cm^{-1})

U = voltage between positive and negative electrodes (kV)

d = interelectrode distance (m).

ABSORPTION EXPERIMENT

Ten starved seedlings with consistent appearance were placed in 100 mL beakers with the corresponding nutrient solutions (80 mL) as a replication. Five replications were set in one group for the same treatment, and then seedlings of seven groups were exposed to electric field intensities of 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 kV m^{-1}, respectively. The entire experiment was performed in an artificial climate chamber at 25°C ±1°C, relative humidity of 60% to 70%, and photon flux density of 200 µmol m^{-2} s^{-1}. The ion uptake reported at each HVEF level was calculated as the mean of five replicates. Aliquots were collected manually from the nutrient solutions and then placed into PE vessels. Aliquots of each absorption experiment were first taken at 10 min after the beginning of the experiment and then hourly over a 7 h period.

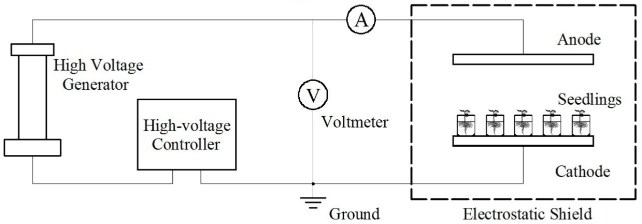

Figure 1. System schematic showing BGG100KV/2MA high-voltage generator and two rectangular 40 cm × 60 cm stainless steel electrodes.

MICHAELIS-MENTEN EQUATION

The kinetics of ammonium and nitrate uptake by the test seedlings were evaluated with a linear fit using the Michaelis-Menten equation (Down and Riggs, 1965):

$$I = \frac{I_{\max} \cdot C}{K_m + C} \tag{2}$$

where

C = ion concentration of the solution on the root surface (mmol L^{-1})

I = uptake rate at concentration C (mmol h^{-1})

$I_{\max}$ = maximum uptake rate (mmol h^{-1})

K_m = half-saturation constant.

Rearrangement of equation 2 yields:

$$\frac{K_m I}{C} + I = I_{\max} \tag{3}$$

Equation 3 was integrated on both sides of equality and then simplified to obtain:

$$C + K_m \ln C = t \cdot I_{\max} + B \tag{4}$$

where t is the absorption time after the beginning of the experiment, and B is the integration constant.

Parameter C was set as C_0 when t was zero. Subsequently, equation 4 was translated as follows:

$$C - C_0 = t \cdot I_{\max} + K_m \ln \frac{C_0}{C} \tag{5}$$

RESULTS

AMMONIUM UPTAKE

The variations in $I_{\max}$ and K_m during HVEF-induced ammonium uptake are shown in figure 2 ($p < 0.05$, Tukey test). The value of $I_{\max}$ slightly increased when the intensity increased to 1.0 kV cm^{-1} and then significantly decreased between 2.0 and 3.0 kV cm^{-1}. The applied field in which the intensity was lower than 1.0 kV cm^{-1} did not remarkably affect K_m. However, K_m sharply increased between 1.0 and 1.5 kV cm^{-1}, decreased before the intensity of HVEF increased to 2.0 kV cm^{-1}, and then significantly increased when the intensity of HVEF increased between 2.0 and 3.0 kV cm^{-1}. The presence of nitrate in the nutrient solution did not affect the overall trends for $I_{\max}$ and K_m. The $I_{\max}$ values were higher with nutrient solution 1 than with nutrient solution 3 when the intensity of the applied field was lower than 1.0 kV cm^{-1} (fig. 2a). Conversely, the $I_{\max}$ values with the two nutrient solutions were basically the same when the field intensity was higher than 2.5 kV cm^{-1}.

NITRATE UPTAKE

An electric field intensity of 1.5 kV m^{-1} increased $I_{\max}$ to a maximum value (fig. 3a) when nitrate was the only nitrogen source (nutrient solution 2) ($p < 0.05$, Tukey test). Sub-

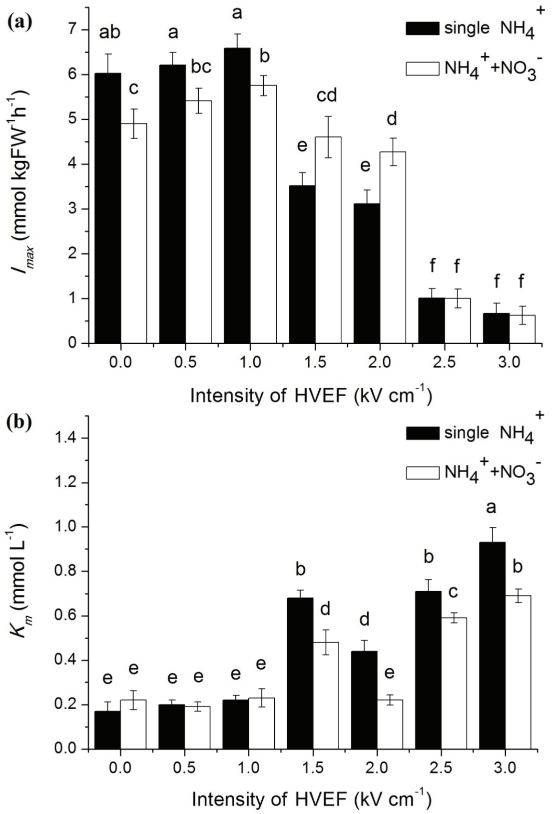

Figure 2. HVEF effects on (a) $I_{\max}$ and (b) K_m for ammonium uptake by cucumber (*Cucumis sativus* L.) seedlings. Seedlings were exposed to electrostatic fields for 7 h at 25°C ±1°C, relative humidity of 60% to 70%, and photon flux density of 200 μmol m^{-2} s^{-1}. The control sample was not subjected to high electrostatic fields. Different letters indicate a statistically significant difference between the kinetics parameters of ammonium absorption for different treatments ($p < 0.05$, Tukey test). Values are means of five replicates.

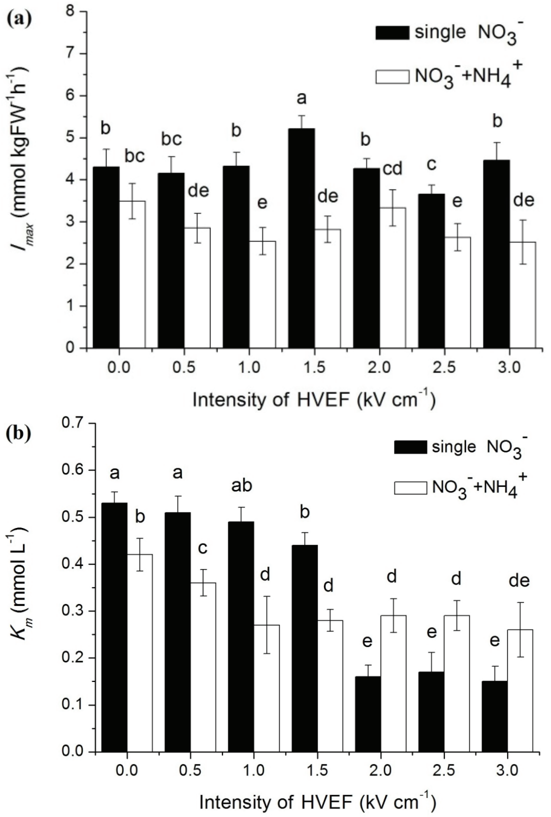

Figure 3. HVEF effects on the kinetics of nitrate uptake for (a) $I_{\max}$ and (b) K_m. Cucumber (*Cucumis sativus* L.) seedlings were exposed to the field for 7 h at 25°C ±1°C, relative humidity of 60% to 70%, and a photon flux density of 200 μmol m^{-2} s^{-1}. The control sample was subjected to high electrostatic fields. Different letters indicate a statistically significant difference between the kinetics parameters of nitrate absorption for different treatments ($p < 0.05$, Tukey test). Values are means of five replicates.

sequently, $I_{\max}$ decreased from this maximum with increasing field intensity and reached a minimum value at 2.5 kV cm^{-1}. The nitrate uptake rate slightly increased when the field intensity increased from 2.5 to 3.0 kV cm^{-1}. The variation in K_m in the absence of ammonium was similar to the variation in the presence of ammonium (fig. 3b). The approximate trend for $I_{\max}$ was similar when ammonium was present (nutrient solution 3) or absent (nutrient solution 2). For nutrient solution 2, the values of K_m slightly decreased when the intensity of the electric field increased from 0 to 1.0 kV cm^{-1}, followed by a remarkable decrease from 1.0 to 2.0 kV cm^{-1}. The K_m value stabilized at a low level when the field intensity was higher than 2.0 kV cm^{-1}. However, in the presence of ammonium (nutrient solution 3), K_m significantly decreased and then stabilized at a low level when the field intensity was higher than 1.0 kV cm^{-1}.

DISCUSSION

AMMONIUM UPTAKE

Ammonium uptake involves the following reaction that limits the rate of ammonium utilization (Joy 1988):

$$\begin{aligned}&2\mathrm{NH_4^+} + 2\mathrm{oxoglutarate} + \mathrm{ATP} + 2(\mathrm{reductant})\\&\rightarrow \mathrm{Glu} + \mathrm{ADP} + \mathrm{Pi} + \mathrm{H^+} + 2(\mathrm{oxidized\ reductant})\end{aligned} \tag{6}$$

In this research, the direction of the applied field was vertically downward. The applied field can contribute in carrying positively charged hydrogen ions, a type of reaction product, to the lower parts of plants that can promote the reaction. Thus, the applied field is conducive to ammonium uptake to some extent. Nevertheless, the applied field cannot accelerate the use of glutamine, which is another type of reaction product. The accumulation of glutamine has feedback inhibition and detrimentally affects the reaction when the reaction is too fast to consume the glutamine in time (Lancien et al., 2000). This effect is dominant when the reaction rate is increased to a critical extent, causing the affinity for ammonium and the ammonium absorption rate to decrease.

An effect called electroporation can increase the cell membrane permeability when the intensity of the applied field increases beyond the critical value for voltage-gating effects (Neumann et al., 1989, Schoenbach et al., 2004; Weaver, 2000; Zimmermann and Neil, 1996). Although not fully understood, pore formation may generate holes that freely transport large molecules into the cell membrane

(Chapel et al., 1984; Glaser et al., 1988; Gowrishankar et al., 1988; Glass et al., 1997; Grubmüller et al., 1996; Puite, 1992; Schoenbach et al., 2004). Under these circumstances, the mechanism of absorption of ammonium changes into a kind of passive transport. Subsequently, the absorption of ammonium that is positively charged decreases as the field intensity increases because of the repelling action of the applied field on positively charged ions.

NITRATE UPTAKE

Compelling evidence shows that the energy sources that contribute to stimulating nitrate uptake are derived from coupling to the proton electrochemical gradient across the plasma membrane. Specifically, two hydrogen ions are required when one nitrate ion is absorbed into the root epidermal cell (Hawkins et al., 2000; McClure et al., 1990). Moreover, the applied field contributes to the concentration of positively charged hydrogen ions surrounding the cucumber root, which benefits the cotransport of nitrate. In addition, only a proportion of the absorbed nitrate is assimilated into the root, and the remaining part is transported upward through the xylem for assimilation into the shoot (Forde, 2000). In this study, the applied field accelerated the transport of negatively charged nitrate and nitrite. The acceleration provides sufficient reactant for glutamate synthesis and promotes nitrate absorption by the plant. Nitrate assimilation is an energy-intensive process that specifically requires the transfer of two electrons per NO_3^- converted to NO_2^-, six electrons per NO_2^- converted to NH_4^+, and two electrons and one ATP per NH_4^+ converted to glutamate (Bloom et al., 1992). Obviously, electrons are required in every process of nitrate uptake. The applied field also provides sufficient electrons to reduce nitrate and assimilate ammonium, and consequently accelerates nitrate absorption. Generally, this study showed that the applied field contributed to nitrate uptake.

CONCLUSIONS

This study investigated the kinetics of ammonium and nitrate uptake by *Cucumis sativus* L. seedlings under HVEF by using the Michaelis-Menten equation. The applied HVEF intensity significantly changed the absorption rate and affinity for ammonium and nitrate. The ammonium uptake slightly increased when the intensity increased to 1.0 kV cm^{-1} and then decreased between 2.0 and 3.0 kV cm^{-1}. HVEF also contributed to the affinity for nitrate in all treatment groups. This effect on affinity positively correlated with the intensity of the applied field as the field increased to 1.0 kV cm^{-1}. Moreover, HVEF enabled the seedlings to opt to uptake nitrate when two types of N were present in the nutrient solution, which demonstrated the importance of HVEF in the nitrate pollution treatment and ammonium tolerance of plants. Feedback adjustment, electron transportation, and electroporation theory were used to explain this effect. However, whether or not this mechanism is suitable for the uptake of other ion species remains ambiguous and requires additional investigation. The biological applications of HVEF consume a minimal amount of energy and may be further investigated to adjust nutrient absorption by plants, which could be used to enhance the uptake of beneficial ions and inhibit the uptake of harmful ions.

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REFERENCE

Bloom, A. J., Sukrapanna, S. S., & Warner, R. L. (1992). Root respiration associated with ammonium and nitrate absorption and assimilation by barley. *Plant Physiol., 99*(4), 1294-1301. http://dx.doi.org/10.1104/pp.99.4.1294.

Chapel, M., Teissie, J., & Alibert, G. (1984). Electrofusion of spermine-treated plant protoplasts. *FEBS Letters, 173*(2), 331-336. http://dx.doi.org/10.1016/0014-5793(84)80800-5.

Down, J. E., & Riggs, D. S. (1965). A comparison of estimates of Michaelis-Menten kinetic constants from various linear transformations. *J. Biol. Chem., 240*(2), 863-869.

Forde, B. G. (2000). Nitrate transporters in plants: Structure, function, and regulation. *BBA-Biomembranes, 1465*(1), 219-235. http://dx.doi.org/10.1016/S0005-2736(00)00140-1.

Glaser, R. W., Leikin, S. L., Chernomordik, L. V., Pastushenkob, V. F., & Sokirkob, A. I. (1988). Reversible electrical breakdown of lipid bilayers: Formation and evolution of pores. *BBA-Biomembranes, 940*(2), 275-287. http://dx.doi.org/10.1016/0005-2736(88)90202-7.

Glass, A. D. M., Erner, Y., Kronzucker, H. J., Schjoerring, J. K., Siddiqi, M. Y., & Wang, M. Y. (1997). Ammonium fluxes into plant roots: Energetics, kinetics, and regulation. *Zeitschrift fürPflanzenernährung und Bodenkunde, 160*(2), 261-268. http://dx.doi.org/10.1002/jpln.19971600220.

Gowrishankar, T. R., Chen, W., & Lee, R. C. (1998). Non-linear microscale alterations in membrane transport by electropermeabilization. *Ann. New York Acad. Sci., 858*(1), 205-216. http://dx.doi.org/10.1111/j.1749-6632.1998.tb10154.x.

Grubmüller, H., Heymann, B., & Tavan, P. (1996). Ligand binding: Molecular mechanics calculation of the streptavidin-biotin rupture force. *Science, 271*(5251), 997-999. http://dx.doi.org/10.1126/science.271.5251.997.

Hawkins, H. J., Johansen, A., & George, E. (2000). Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. *Plant Soil, 226*(2), 275-285. http://dx.doi.org/10.1023/A:1026500810385.

He, X., Liu, R., Tatsumi, E., Nirasawa, S., & Liu, H. (2014). Factors affecting the thawing characteristics and energy consumption of frozen pork tenderloin meat using high-voltage electrostatic field. *Innov. Food Sci. Emerg. Tech., 22*(4), 110-115. http://dx.doi.org/10.1016/j.ifset.2013.12.019.

Hsieh, C. W., & Ko, W. C. (2008). Effect of high-voltage electrostatic field on quality of carrot juice during refrigeration. *LWT - Food Sci. Tech., 41*, 1752-1757. http://dx.doi.org/10.1016/j.lwt.2008.01.009.

Joy, K. W. (1988). Ammonium, glutamine, and asparagine: A carbon-nitrogen interface. *Canadian J. Botany, 66*(10), 2103-2109.

Knorr, D. (1999). Novel approaches in food-processing technology: New technologies for preserving foods and modifying function. *Current Opinion Biotech., 10*(5), 485-491. http://dx.doi.org/10.1016/S0958-1669(99)00015-4.

Lancien, M., Gadal, P., & Hodges, M. (2000). Enzyme redundancy and the importance of 2-oxoglutarate in higher plant ammonium assimilation. *Plant Physiol., 123*(3), 817-824. http://dx.doi.org/10.1104/pp.123.3.817.

Ma, F., Xu, S., Wang, S., Wen, S., & Qu, Z. (1991). Effects of electrostatic field on the photosynthesis and chlorophyll and soluble protein of plant leaves (in Chinese). *J. Northeast Normal Univ., 4*, 53-56.

McClure, P. R., Kochian, L. V., Spanswick, R. M., & Shaff, J. E. (1990). Evidence for cotransport of nitrate and protons in maize roots: II. Measurement of NO_3^- and H^+ fluxes with ion-selective microelectrodes. *Plant Physiol., 93*(1), 290-294. http://dx.doi.org/10.1104/pp.93.1.290.

Moon, J. D., & Chung, H. S. (2000). Acceleration of germination of tomato seed by applying AC electric and magnetic fields. *J. Electrostatics, 48*(2), 103-114. http://dx.doi.org/10.1016/S0304-3886(99)00054-6.

Murr, L. E. (1963). Plant growth response in a simulated electric field-environment. *Nature, 200*(4905), 490-491. http://dx.doi.org/10.1038/200490b0.

Nechitailo, G., & Gordeev, A. (2001). Effect of artificial electric fields on plants grown under microgravity conditions. *Adv. Space Res., 28*(4), 629-631. http://dx.doi.org/10.1016/S0273-1177(01)00370-2.

Neumann, E., Sowers, A. E., & Jordan, C. A. (1989). Dielectrophoresis: Behavior of microorganisms and effect of electric fields on orientation phenomena. In *Electroporation and Electrofusion in Cell Biology* (pp. 59-164). New York, N.Y.: Springer.

Palanimuthu, V., Rajkumar, P., Orsat, V., Gariépy, Y., & Raghavan, G. S. V. (2009). Improving cranberry shelf-life using high voltage electric field treatment. *J. Food Eng., 90*(3), 365-371. http://dx.doi.org/10.1016/j.jfoodeng.2008.07.005.

Puite, K. J. (1992). Progress in plant protoplast research. *Physiol. Plantarum., 85*(2), 403-410. http://dx.doi.org/10.1007/s00425-013-1936-7.

Saker, L. R., Barber, S. A., & Jenkins, W. (1984). Changes in the kinetics of phosphate and potassium absorption in nutrient-deficient barley roots measured by a solution-depletion technique. *Planta, 160*(6), 490-499. http://dx.doi.org/10.1007/BF00411136.

Schoenbach, K. H., Joshi, R. P., Kolb, J. F., Chen, N., Stacey, M., Buescher, E. S., Beebe, S. J. & Blackmore, P. (2004). Ultrashort electrical pulses open a new gateway into biological cells. *Proc. IEEE, 92*(7), 1122-1137. http://dx.doi.org/10.1109/JPROC.2004.829009.

Sidaway, G. H., & Asprey, G. F. (1966). Influence of electrostatic fields on seeds germination. *Nature, 211*(5046), 303. http://dx.doi.org/10.1038/211303a0.

Sidaway, G. H., & Asprey, G. F. (1968). Influence of electrostatic fields on plant respiration. *Int. J. Biometeorol., 12*(4), 321-329. http://dx.doi.org/10.1007/BF01553277.

Weaver, J. C. (2000). Electroporation of cells and tissues. *IEEE Trans. Plasma Sci., 28*(1), 24-33. http://dx.doi.org/10.1109/27.842820.

Wu, Y. (1989). Investigation of potassium absorption of rice plants in high-voltage electrostatic field (in Chinese). *J. Guizhou Agric. Sci., 1*, 39-40.

Zhao, R., Hao, J., Xue, J., Liu, H., & Li, L. (2011). Effect of high-voltage electrostatic field pretreatment on the antioxidant system in stored green mature tomatoes. *J. Sci. Food Agric., 91*(9), 1680-1686. http://dx.doi.org/10.1002/jsfa.4369.

Zimmermann, U., & Neil, G. A. (1996). *Electromanipulation of Cells.* Boca Raton, Fla.: CRC Press.