Eliška VLACHOVÁ HUTOVÁ[1)], Petr MARCOŇ[2)], Karel BARTUŠEK[3)]

Effect of high voltage on the development of the plant tissue

In our experiment the electrical parameters that affect early somatic embryos (ESEs) were investigated. High voltage was generated by a special high voltage generator. High voltages ranging from 5 to 20 kV and frequency of 1Hz were applied longitudinal and transversal directly on the Petri dish with 2 days old ESEs of *Picea abies* for periods of 3 hours every day. One Petri dish was placed directly on top of the high voltage generator and on the other petri dish were fixed two copper plates for transmission of high voltage. Petri dishes were exposed to high voltage for 14 days. After this time, the influence of high voltage was evaluated. To evaluate the experiment were used biological and chemical methods, which confirmed the changes in the growth of ESEs.

1.Introduction

A current part of our thinking is that matter is comprised of particles or waves which have electromagnetic properties. Therefore it is to be expected that externally applied electrical and magnetic fields will cause perturbations in atoms or subatomic particles. Bonding, enzyme action to associate or dissociate molecules, or to alter their configuration, must all be influenced, however slightly, by electromagnetic stimuli. The question is whether electromagnetic stimuli applied externally (usually artificially) can cause changes in plant hormone location, concentration, or action, which will in turn affect growth. In the past, both significant increases of plant growth and impairments of productivity have been postulated in response to electromagnetic fields. On a cellular level, a wide range of physiological effects can be observed. Although several models have been put forward to explain possible mechanisms [1-4], the observed effects are not easily explained by a single hypothesis. Effects on plant growth, the role of electric field effects on cell polarity [5], on calcium transport [6], and on auxin transport [7] have been observed. The difficulties in reproducing results of earlier experiments have been reported in review papers [8-10]. Experiments about electrostimulation date back to the first quarter of the 20th century, when crops were grown under an electrically charged wire mesh resulting in gradients of 20–40 kV/m [11]. The positive yield effects disappeared or were even reversed when the plants were infested with fungal pathogens [12] or the exposure period was followed by dry, hot weather. A hypothetical explanation refers to the sensing of electrical fields in the atmosphere by electroreceptors, possibly by calcium channels [9]. Increased atmospheric gradients frequently occur shortly before thunderstorms, usually accompanied by precipitation.

[1)] Ing., DTEE FEEC, Brno University of Technology, Technicka 10, 616 00 BRNO, CZECH REPUBLIC, tel. +(420) 54114 6854, fax: +(420) 54114 6276, e-mail: xhutov00@stud.feec.vutbr.cz

[2)] Ph.D., DTEE, FEEC, Brno University of Technology, Technicka 10, 616 00 BRNO, CZECH REPUBLIC, tel. +(420) 54114 6275, e-mail: marcon@feec.vutbr.cz

[3)] Prof., Institute of Scientific Instruments of the ASCR, v. v. i., Královopolska 147, 612 64 BRNO, CZECH REPUBLIC, tel. +(420) 54114 6272, e-mail: bar@isibrno.cz

The increased calcium influx would increase metabolic activity, thus preparing the plants in time for higher soil moisture and for more favorable conditions for enhanced nutrient uptake and growth. Plants with a priori higher metabolic activity would be able to take greater advantage of the better growth conditions than if not prepared. Some other experiments [13, 14] observed increased activity of Hþ-ATPases in electric fields, leading, for example, to enhanced lily pollen tube growth. These results show that the electrical field is effective on the single cell or protoplast level and also suggest that under certain conditions, an intact organism may benefit either partly or as a whole. Positive results about magnetic or electric field effects on seed germination have been reported [15, 16]. Nevertheless, considerable difficulties in performing controlled experiments with reproducible results have been pointed out [17, 18]. Additional uncertainties regarding the mechanisms of how electromagnetic fields are ‘‘perceived’’ by the cells and translated into biological effects, restrained the general use of electromagnetism for plants [19]. Consequently, the possibility of negative field effects should be considered. This question could be important for field crops grown in the vicinity of high voltage transmission lines, as in this case large acreages could be influenced adversely. In the case of significant effects of electromagnetic fields on agricultural productivity, economically relevant gains or losses could be a consequence. This question has been addressed in the past, either involving commercially used transmission lines or high voltage test lines. In the test lines, only electric fields could be studied because of the lack of transmission induced magnetic fields. These investigations either did not reveal significant effects or explanations were found for the results other than electromagnetic fields [20-24]. Some other measurements for different settings of the external magnetic field can prove that the external magnetic field can change the dynamics of the model of matter [25].

2. Special High Voltage Function Generator

The generator was designed for special tests of soft tissues. Possibilities of the soft issue testing are described in literature. In this work the aim is to test the soft tissues in high voltage electric field. Tissues are exposed to defined shape electric field up to 20 kV. Voltage of the output electrode of the realized generator is possible to regulate in range 0 kV to 20 kV. Shape of the output voltage is possible to choose as sine, square or ramp. Frequency capabilities of this voltage source start on 0 Hz and reach 300 Hz. Block diagram of the high voltage part is in Fig. 2. The main part of the HV block is HV transformer TR2 working as a flyback converter. Inside the transformer a rectifier diode is connected. Secondary winding SEC2 is possible to link to next module for multiplying of the output voltage. Resistor 1GΩ and the power resistor are as the HV divider connected between the output electrodes. Exceeding of the 20 kV output voltage value cause on the 1GΩ d between the output electrodes. Exceeding of the 20 kV output voltage value cause on the 1 GΩ resistor voltage drop higher then 1 V and consequently a change of pulse ratio of the PWM signal. Limitation of the output current in case of direct contact is realized by pair of the 10 MΩ load resistors, Fig. 4. Maximal voltage rating of these resistors is 20 kV. Output current is limited to 4 mA. Realized HV generator, in detail ring electrodes, 1 GΩ resistors and flyback transformer you can see in Fig. 1. On the top the high voltage cable is connected. Voltage limit of this cable reach 30 kV [26].

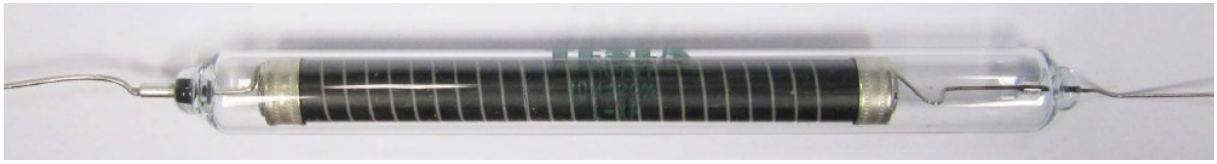


Fig. 1: High voltage load resistor −10 MΩ [26].

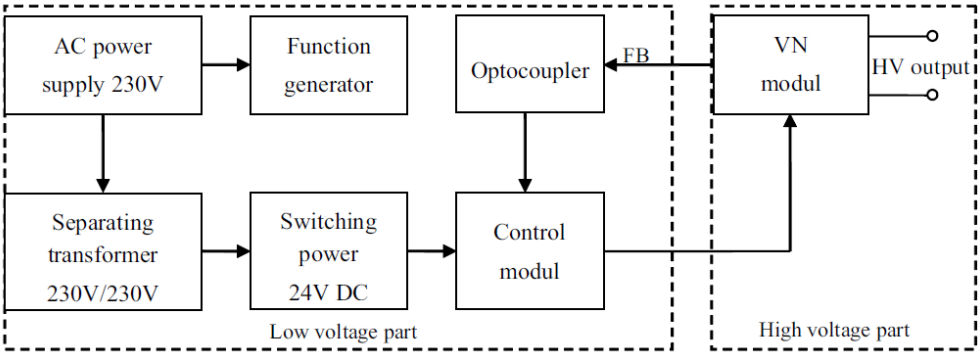


Fig. 2: Block diagram of the high voltage generator [26].

3. Plant Material and Cultivation Conditions

A clone of early somatic embryos of the Norway spruce (Picea abies/L./ Karst.) marked as 2/32 were used in our experiments. The cultures were maintained on a semisolid (Gelrite Gellan Gum, Merck, Germany) half-strength LP medium with modifications. The concentration of 2, 4-dichlorofenoxyacetic acid and N6-benzyladenine was 4.4 and 9 μM, respectively. The pH value was adjusted to 5.7 - 5.8 before autoclaving (121°C, 100 kPa, 20 min). The organic part of the medium, excluding saccharose, was sterilized by filtration through a 0.2 μm polyethylensulfone membrane (Whatman, Puradisc 25 AS). The cultivation was carried out in Petri dishes (diameter 50 mm). The sub-cultivation of stock cultures was carried out in 2-weeks intervals; the stock and experimental cultures were maintained at the temperature of 23±2°C in a cultivation box kept in a dark place. The experiment started with colonies of early somatic embryos which weight was about 3 mg. Only one cluster per one Petri dish was cultivated. Table 1 shows the distribution of the Petri dishes during the experiment.

Table 1: Distribution of Measured Samples

Sample n.	1	2	3
Method	Electrodes attached to the petri dish	Petri dish placed on a HV generator	Control / without external field
Date of measurement	14 days long measurement cycles		

4. Method

Prepared samples of plant tissue (ESEs of the Norway spruce) were exposed to influence of high voltage for 14 days (the time secured growth and development of plant samples). Experiment was conducted under constant conditions. The temperature was held at 23 °C, humidity and pressure in the laboratory had constant value. Distribution of Petri dish is given in Table 1. Position of Petri dishes during the experiment is shown in Fig. 4. To the first Petri dish were fixed copper electrodes (Fig. 4, A). These electrodes had to be fixed to the dish so as to avoid contact of the electrodes. The second Petri dish was placed on top of the generator (Fig. 4, B). The last Petri dish was used as the control and was not exposed to direct effects of high voltage. The size of ESEs was measured before the start of the experiment and after experiments completion. For safety, during the experiment was not tampered with Petri dishes. During the experiment, Petri dishes were regularly exposed to high voltage on each

day for three hours. The mean value of the high voltage during the experiment was approximately 5 to 10 kV, the frequency of the induced voltage was 1Hz.

A. **B.** **C.**

Fig. 3: The distribution of the Petri dishes during the experiment. A: Attaching of electrodes on the Petri dish; B: The location of the second Petri dish on the generator; C: The general view of the location.

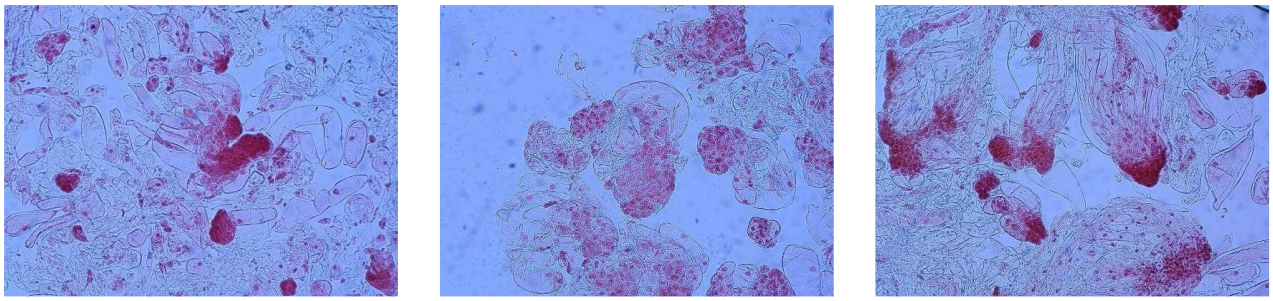
5. Results

Evaluation of the influence of high voltage on the development of ESEs was conducted in two ways. The first method was consisted of scanning the size of ESEs during the experiment. ESEs were photographing at the beginning of the experiment and at the last day of experiment (after 14 days) and then the size was evaluated. Petri dishes were a further 14 days in the same conditions in which the experiment was conducted and then were photographing again. Evaluation of changes in the size is shown in Table 2.

Table 2: The percentage increase in individual samples

Size of tissue [%]	1	2	3
first day of experiment (day 0)	0	0	0
last day of experiment (day 13th)	15	103	164
14 days after end of the experiment	87	205	208

The second method of changes evaluating in development of ESEs was using the microscopic observation in the ESEs using the acetocarmine staining to highlight the embryonic structures. Embryos were observed in a fluorescence microscope Olympus Provis and panned with a digital camera Olympus SP-350, while for subsequent adjustments photos was used software for capturing and editing photos Quick Photo Micro 2.2 (Fig. 4).



A. **B.** **C.**

Fig. 4: Microscopic evaluation of ESEs; A: Embryonic structures from the Petri dish to which electrodes were attached; B: Embryonic structures from the Petri dish which was placed on the high voltage generator; C: Control embryonic structures

Using the microscopic observation technique were evaluated the following conclusions. Fig. 4A shows the embryonic structure of ESEs which were exposed to the influence of high voltage using two electrodes, which were attached to the Petri dish stuck to the Petri dish. Somatic embryos are relatively undeveloped, there are not too significant difference between the embryo and suspensor parts. Fig. 4B shows the embryonic structure of ESEs which were exposed to the influence of high voltage directly on the top part of the high voltage generator. Somatic embryos are usually developed, but there are also embryos at different stages of development, with a large amount of meristematic cells in embryonic heads. Fig. 4C shows the embryonic structure which have been removed from the control ESEs. Somatic embryos are developed, the difference between the embryo and suspensor part is very significant and in the embryonic heads are a large number of meristematic cells.

6. Conclusion

The results of this work show that a high voltage affects the development of ESEs, as has been demonstrated in earlier experiments. Effect of high voltage on these particular ESEs is not a liquidation, and a question for further research can be whether this embryos would showed continued development.

Acknowledgements

This work was financed by a project of the BUT Grant Agency, No. FEKT-S-14-2545.

References

1. Chiabrera A., Bianco B.:*The role of the magnetic field in the EM interaction with the ligand binding*. Mechanistic approaches to interactions of electric and electromagnetic fields with living systems. 1987, 79–95.
2. Liboff A. R., McLeod B. R.: *Kinetics of channelized membrane ions in magnetic fields*. Bioelectromagnetics 9, 1987, 39–51.
3. Smith S. D., McLeod B. R., Liboff A. R., Cooksey K. E.: *Calcium cyclotron resonance and diatom motility*. Bioelectromagnetics 8, 1987, 215–227.
4. Lednev L. L.: *Possible mechanism for the influence of weak magnetic fields on biological systems*. Bioelectromagnetics 12, 1990, 71–75.
5. Schnepf E.: *Cellular polarity*. Ann Rev Plant Physiol 37, 1986, 23– 47
6. Hepler P.K., Wayne R.: *Calcium and plant cell development*. Ann Rev Plant Physiol 38, 1985, 397–439.
7. Rathore K.S., Goldsworthy A.: *Electrical control of shoot regeneration in plant tissue cultures*. Bio/Technol 3, 1985, 1107– 1109.
8. Ellis H., Turner E.R.: *The effects of electricity on plant growth*. Sci Pro Oxf 65, 1978, 395–407.
9. Goldsworthy A.: *Electrostimulation of cells by weak electric currents*. In: Electrical manipulation of cells. New York: Chapman & Hall. 1996, 249–272.

10. Jones D., McLeod B.: *Electromagnetic cell stimulation*. In: Electrical manipulation of cells. New York: Chapman & Hall. 1996, 223–247.

11. Blackman V.H.: *Field experiments in electroculture*. J Agric Sci 14, 1924, 240–267.

12. Blackman V.H., Legg A.T.: *Pot culture experiments with an electric discharge*. J Agric Sci 14, 1924, 268–273.

13. Karcz W., Burdach Z.: *The effects of electric field on the growth of intact seedling and coleoptile segments of Zea mays L*. Biol Plant 37, 1995, 391–397.

14. Plätzer K., Obermeyer G., Bentrup F.W.: *AC fields of low frequency and amplitude stimulate pollen tube growth possibly,* 1996.

15. Davies M.S.: *Effects of 60 Hz electromagnetic fields on early growth in three plant species and a replication of previous results*. Bioelectromagnetics 17, 1996, 154–161.

16. Zhang H., Hashinaga F.: *Effects of high electric fields on the germination and early growth of some vegetable seeds*. J Jpn Soc Hort Sci 66, 1997, 347–352.

17. Phirke P.S., Kubde A.B., Umbarkar S.P.: 1996. *The influence of magnetic field on plant growth*. Seed Sci Technol 24, 1996, 375–392.

18. Potts M.D., Parkinson W.C., Nooden L.D.: *Raphanus sativus and electromagnetic fields*. Bioelectrochem Bioenerg 44, 1997, 131– 140.

19. Vlachova Hutova, E., Bartusek, K., Mikulka, J.: *Study of the Influence of Magnetic Fields on Plants Tissues*. In Proceedings of PIERS 2013 in Taipei. Progress In Electromagnetics, 2013, 57-60.

20. Hodges T.K., Mitchell C.A.: *Influence of High Intensity Electric Fields on Yield of Sweet Corn and Dent Corn—1982*. American Electric Power Service Corporation, North Liberty, Indiana, 1984.

21. Rogers L.E., Beedlow P.A., Carlile D.W., Gano K.A.: *Environmental Studies of a 1100 kV Prototype Transmission Line*. An Annual Report for the 1983 Study Period. Batelle Pacific Northwest Laboratories, Portland, Oregon, 1984.

22. Raleigh R.J.: *Joint HVDC Agricultural Study: Final Report*. Oregon State University, Portland, Oregon, 1988.

23. Conti R., Nicolini P., Cerretelli P., Margonato V., Veicsteinas A., Floris C.: *ENEL's research activity on possible biological effects of 50 Hz electromagnetic fields—Results and plans of a large research programme*. Alta Freq 58, 1989, 395– 402.

24. Müller A.: *Mögliche Auswirkungen elektromagnetischer Felder auf die Vegetation*. Diploma paper, Agricultural University Vienna, 1990.

25. Vlachova Hutova, E., Bartusek, K., Fiala, P.: *The Influence of a Magnetic Field on the Behaviour of the Quantum Mechanical Model of Matter*. In PIERS 2014 Guangzhou Proceedings. Progress In Electromagnetics, 2014, 1847-1851.

26. Marcon, P., Fiala, P., Steinbauer, M., Cap, M.: *Special High Voltage Function Generator*. PIERS ONLINE vol. 7, num. 6., 2011, 547-550.