Graduation experience

Summary of learning experience and personal experience of non-injury advanced seminar

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Abstract: Non-invasive micro-measurement is an important technology in electrophysiological research. It can obtain real-time physiological and ecological information of samples in a nondestructive manner, and has important biological significance for studying the laws of life activities and environmental impact. After learning the non-invasive technology, I carried out many related experiments, tested a variety of samples, and had some own experience and understanding. It happened that Xuyue Company held an advanced seminar on the application of Non-invasive Micro-test Technology, and invited many famous teachers to explain the application of non-invasive technology. Therefore, this article briefly introduces the Non-invasive Micro-test Technology and its development in China, the learning experience in the seminar and my personal experience in the non-invasive experiment.

Key words: non-injury, electrophysiology, plant adversity

I. Introduction to Non-damage Micro-measurement Technology and Its Development in China

Non-invasive micro-test technology (NMT) was proposed in 1974 by Lionel F. Jaffe, a neuroscientist at the Woods Hole Marine Biology Laboratory in the United States. Non-invasive micro-test technology is composed of scanning ion selective sensor technology (SIET), micro-sensor ionization current technology (MIFE), self-reference polarographic sensor technology (SERP), scanning polarographic sensor technology (SPET), scanning vibration sensor technology (SVET) Self-reference ion selective sensor technology (SERIS) and other technologies. It is a technology to study the physiological function of living materials. It can detect the flow rate (flow rate and direction) of molecules/ions entering and leaving the living body without damaging the sample. The unit is pmol · cm-2 · s-1, which is called flow rate (Flux). It is a non-contact method with ultra-high sensitivity. Because of its characteristics of in vivo detection, non-invasive, high resolution and dynamic real-time,

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it is often used to explore physiological characteristics that are difficult to measure by other technologies. It is one of the best tools for physiological function research at present, and has been widely used in medicine, botany, zoology, microbiology, agricultural science, pharmacy, environmental science and other fields. At the same time, NMT is also the technical basis of dynamic ionics. After years of development, it has become a mature experimental system. He has published a large number of relevant scientific research achievements in the top international magazines Science, Nature, PNAS, Plant Cell, Environmental Science&Technology, etc. The in vivo, dynamic and real-time measurement methods of Non-invasive Micro-test Technology, as well as high resolution and high sensitivity, help human to carry out research in the field of physiological function, and have been applied in many disciplines.

NMT technology has been developed in China for many years. Since Mr. Xu Yue founded the American Younger Company in Massachusetts in 2001, NMT has entered the stage of commercialization. Xuyue (Beijing) Science and Technology Co., Ltd. was established in Zhongguancun, Beijing, in 2005. The academic expert advisory group was composed of Kuang Tingyun, academician of the Institute of Botany of the Chinese Academy of Sciences, Yang Fuyu, academician of the Institute of Biophysics of the Chinese Academy of Sciences, and Lin Kechun, professor of Peking University, and became a strategic partner with the United States Younger Corporation. NMT officially entered China. In 2014, the "Second Non-damage Micro-measurement Technology Seminar" was held in Beijing, and the "NMT International Standardization Committee" was established. In 2015, Xuyue (Beijing) Biological Function Research Institute was established in Beijing. In 2015, the "Zhongguancun Non-injury Micro-measurement Technology Industry Alliance" was established by Xuyue (Beijing) Biological Function Research Institute. The alliance is committed to the industrialization development of NMT. At present, more than 20 scientific research institutions and enterprises have joined the alliance.

II. Learning experience of the seminar

Many teachers in the seminar shared their NMT-related experimental research, which benefited me a lot. The following is my own learning experience:

Professor Zhuo Renying mainly taught the screening and functional analysis of the key genes for cadmium enrichment in Sedum paragonum. 48 genes related to cadmium tolerance were screened by yeast transformation system. It is predicted that these genes may participate in MAPKs-mediated signal transduction, calcium ion signal, reactive oxygen species signal and hormone signal. The SaHsfA4ch gene can improve the cadmium ion tolerance of yeast strain △ ycf1. Compared with the control, the overexpression of SaHsfA4ch was detected by non-invasive micro-measurement. The cadmium ion influx increased, which promoted the uptake of cadmium ion by plants, enhanced the tolerance of Arabidopsis

to cadmium ion, and increased the activity of active oxygen scavenging enzymes. SaHsfA4ch gene was mainly expressed in vascular tissue, flowers and stems, and was significantly induced under cadmium stress. Then we found three downstream genes regulated by SaHsfA4ch gene: SpPDR12, SpPCS2 and SpHsfA4c. Through EMSA and LUC activity detection, it was found that SaHsfA4ch could bind to the HSE element of the SpAPX2 gene promoter. SaHsfA4ch gene significantly improved the cadmium tolerance of transgenic Sedum alfredii. Professor Zhuo's research found the cadmium ion tolerance gene in Sedum, studied its main function, and applied it to the control of heavy metals in combination with practice, which is a very meaningful work.

Professor Sun Jian mainly explained the molecular mechanism of carbon point regulating the stress resistance of sweet potato. Carbon dots have the ability to promote electron transfer, photosynthesis and ROS removal. The carbon dots synthesized by salvia miltiorrhiza powder can induce the hyperpolarization of the root cell membrane of sweet potato and the calcium ion influx independent of ROS, and have the same effect in Arabidopsis, tobacco, rice and other crops. It is found that the calcium ion influx induced by carbon dots is through the hydroxyl and carboxyl functional groups on the surface. The increase of potassium ion and ammonium ion influx in root cells caused by carbon dots proves that carbon dots activate the hyperpolarized non-selective cation channel, which is presumed to be a cyclic nucleotide channel. Then it was detected that carbon point could increase the content of cAMP and cGMP in root cells of sweet potato. After that, it was found that CNGC2/4/19/20 mediated the increase of calcium ion influx and cytoplasmic calcium ion in Arabidopsis root cells induced by carbon dots. The calcium ion influx induced by carbon dots in Arabidopsis depends on the lectin receptor kinase LORE and LERCK-1.8. Applying carbon dots to hydroponic seedlings could enhance the outflow of calcium and sodium ions from root cells of sweet potato under salt stress. The carbon point increases the sodium ion outflow from Arabidopsis roots under salt stress, which depends on CNGC2. Carbon spots can inhibit the absorption of sodium ions and reduce the accumulation of sodium ions at the cell and whole plant level in the root mature zone. The carbon spot enhanced the expression of root calcium signal related genes and the tolerance of sweet potato to nutritional stress. Mr. Sun studied the role of nanomaterials in the resistance of sweet potato to adversity. Nanotechnology, as an emerging technology, has good prospects for development. In the study, NMT was used to detect the ion flow rate of sweet potato after the application of carbon point, and intuitive and clear experimental data were obtained, which can well reflect the treatment effect. The research content is relatively in-depth, and the functional group and action channel of carbon point have been found, which plays an important role in the research of sweet potato stress resistance.

Professor Zhang Jinlin mainly studies the application of NMT in the study of salt tolerance mechanism and salt tolerance germplasm screening of gramineous forage. It was found that the salt vesicles on the stem and leaf surface of Atriplex tetraptera played an

important role in salt resistance and drought resistance. A variety of plants were selected to detect the ratio of sodium and potassium ions in their soil, and it was found that the ratio of sodium and potassium ions in large areas was the highest. By comparing the content of sodium ion in wheat with that in wheat, it was found that the content of potassium ion was significantly lower than that in wheat. This shows that the alkali grass absorbs a lot of potassium ions, but reduces the absorption of sodium ions. The amount of salt secreted on the surface of leaves was measured by washing liquid experiment, and it was found that salt secretion was not the main reason for the salt tolerance of Puccinia arundinacea. After that, using isotope labeling method, it was found that the sodium ion influx rate of the root system of Puccinia arundinacea was lower than that of wheat. In the case of potassium ion starvation and high concentration of sodium ion, sodium ion efflux and hydrogen ion efflux decrease. This indicates that the sodium hydrogen reverse transporter consumes more hydrogen. Then we studied the genes related to sodium ion transport, and found that under potassium starvation, the expression of AKT1 gene was higher, which participated in the absorption of high-affinity potassium ions; Under the condition of high salt and potassium starvation, HKT1:4 and HKT1:5 were expressed in xylem parenchyma cells, and sodium ions were excreted through the plastid pathway. Mr. Zhang studied the salt-tolerance mechanism of saline-tolerant plant and found the related genes of sodium ion transport, and studied their functions in sodium ion transport. As an important function of plant survival under adverse conditions, salt tolerance of plants has important research significance, which can effectively improve the situation of plants that are difficult to survive in high saline-alkali soil.

III. Non-injury personal experience and experimental scheme setting

I gradually learned the convenience of this technology in the process of using the non-invasive system to test the ion flow rate of the sample. At the same time, I also have some personal experience as follows:

1. Solution configuration needs to be accurate. The test solution, calibration solution and filling solution all play a very important role in the experiment process, and the ion concentration of the test solution and calibration solution is very low. Therefore, it is necessary to prepare the mother solution first. The mother solution can be configured with 50 ml, and the weighing powder is greater than 0.1 g, which will be more accurate. During dilution, it can be diluted 10 times or 100 times at a time. If the dilution ratio is too high at a time, the ion concentration will also be inaccurate. Refer to the NISC test solution standard for the configuration of the test solution. The ion concentration can be adjusted appropriately, but some necessary conditions should be met: the test solution should contain the ions to be tested; The ion concentration of the test solution should not be too high or too low, otherwise the detection sensitivity will be affected; The test solution needs to maintain a certain PH value to maintain the stability of the

sample (the cell sample also needs to adjust the osmotic pressure); The high concentration calibration solution and the low concentration calibration solution are only different from the concentration of the ions to be measured, and the rest are consistent with the test solution. The concentration difference of the ions to be measured should be 10 times; When adjusting the PH value, the solution containing the ions to be measured cannot be used, but Tris and MES can be used.

2. There are many kinds of samples that can be tested without damage. The plant samples can be tested by using water culture, soil culture and medium culture, but the most suitable one is water culture, so that the surface is clean. When using soil culture and medium culture, it is better to brush the test part gently with a fine brush to remove the impurities on the surface, and then soak in the balance solution before testing.

3. Production of flow sensor. First, take a sensor and inject 1 cm of filling liquid. The position should not be too far from the tip, otherwise it is not easy to squeeze the filling liquid to the opening. Then connect the sensor to the three-way valve, push the syringe to make the filling liquid rise to the tip and flow out 1 drop, and gently blow off the flow out of the liquid drop with your mouth against the sensor. Dip the LIX holder in the LIX and fix it on the micromanipulation table. Slowly push the pressure adjusting device to make the LIX holder form a convex surface and adjust the focal length to make the picture clear. Then slowly adjust the position of the sensor so that the sensor tip is inserted into the LIX holder convex liquid level to make the LIX flow into the sensor. Gently push the three-way valve back and forth several times, and then adjust the LIX liquid column to the appropriate length to separate the flow sensor from the LIX liquid level.

4. The flow sensor shall be adjusted manually and automatically. During the test, the tip of the velocity sensor should be immersed in the liquid level, not only a little, which will affect the detection effect, but also not too deep, which may touch the bottom of the petri dish and cause the tip to break. Therefore, when adjusting the position of the sensor, it is better to put the sensor down to the liquid level or close to the liquid level, and then rotate the Z-axis knob to make the sensor slowly lower. After the tip is immersed in the liquid level, properly adjust the X-axis and Y-axis to make the sensor tip in the middle of the picture. Finally, turn on the automatic switch, use the automatic adjustment to make the tip clear, and observe whether the tip is complete and not broken. The calibration can be carried out according to the sequence of high concentration calibration solution, low concentration calibration solution and test solution during calibration. The next calibration solution can be replaced after each calibration is stable.

5. Some reasons for the calibration error of the calibration solution: (1) The detection values of the three solutions are very low and there is no difference. This may be because the machine is not turned on. Check whether the switch button is turned on; (2) The detection values of the three solutions are very high, which is much higher than the normal value.

This may be because the circuit is not closed, and whether the reference electrode is put into the liquid level is detected; (3) The measured values of the three solutions are within a reasonable range and the three concentrations have gradient differences, but the slope is incorrect. This may be due to the problem of the calibration solution configuration. You can dilute the high concentration calibration solution by 10 times for testing. If it can be calibrated, it means that the dilution with the mother solution is not accurate. If it can not be calibrated, it means that there are other problems, such as adding too much potassium ion to the detection of sodium ion, which affects the detection.

The design of experimental scheme is an important part of NMT test. The following are some experimental schemes I have done myself:

1. Potassium ion flow in plants under salt stress. Potassium ion is a major element necessary for plant growth, and plays an important role in plant nutrition, turgor regulation and osmotic balance. High salinity and alkalinity will form high osmotic pressure, which will make plants lose water and affect the normal growth of plants. Arabidopsis thaliana is used as the test sample. Take plants with similar growth for 7 days and put them into 100 mM NaCl solution for treatment for 24 hours, and use the solution without NaCl as the control. Soak the treated material in the test solution for 30 min, then replace it with a new test solution, and fix the sample with filter paper strips and resin blocks. The velocity sensor detects the velocity of potassium ion at the root tip.

2. Changes of calcium ion flow rate in plant roots under low temperature treatment. Calcium is the second messenger of plant signal transduction and plays an important role in signal transduction and regulation of plant growth and development. The normal development of rice will be affected by low temperature chilling injury, which will lead to yield reduction. Therefore, it is of great significance to study the changes of calcium ion transport in rice under low temperature for the study of low temperature stimulation. In the experiment, put the rice sample into the test solution for balance for 30 minutes, and then replace it with a new test solution for test for 5 minutes. After the data is stable, add crushed ice into the outer culture dish to create low temperature conditions, and detect the flow of calcium ions during low temperature treatment.

3. Absorption of plant roots after heavy metal treatment. Cadmium is a heavy metal element with strong toxicity and belongs to category 1 carcinogen. If a certain concentration of cadmium is accumulated in plants, it will affect the growth and development of plants. Cadmium stress usually stimulates the growth of plant seedlings at low concentrations and inhibits the growth of seedlings at high concentrations. Arabidopsis thaliana was used as the research material and put into the test solution containing 0.05 mM cadmium to balance for 30 minutes. Replace the test solution with a new one, fix the sample, and measure the cadmium ion flow rate at the root.

After years of development, Non-invasive Micro-test Technology has become an important method in electrophysiological experiments and has irreplaceable advantages. The application of Non-invasive Micro-test Technology has greatly promoted the research progress of ionic molecular flow rate signals. I am deeply impressed by the sense of social responsibility of Xuyue Company in organizing the seminar to promote the Non-invasive Micro-test Technology, and I am also honored to participate in the study of this seminar, which has taught me a lot about NMT. It is hoped that the non-injury technology can be extended to more scientific research institutions in the future to serve the scientific research work.

Reference

1. Yue Xu (2022), Plant Physiology and Ecology NMT Experiment Guide, Zhongguancun NMT Industry Alliance

2. Yue Xu, NMT101 asked, Zhongguancun NMT Industrial Alliance

3. Wang J, Cai C, Geng P, Tan F, Yang Q, Wang R, Shen W(2022). A New Discovery of Argon Functioning in Plants: Regulation of Salinity Tolerance. Antioxidants. .

4. Li Y, Tang Z, Pan Z, Wang R, Wang X, Zhao P, Liu M, Zhu Y, Liu C, Wang W, Liang Q, Gao J, Yu Y, Li Z, Lei B, Sun J(2022). Calcium-Mobilizing Properties of Salvia miltiorrhiza-Derived Carbon Dots Confer Enhanced Environmental Adaptability in Plants. ACS Nano.

5. Fu L, Wu D, Zhang X, Xu Y, Kuang L, Cai S, Zhang G, Shen Q(2015). Vacuolar H+-pyrophosphatase HVP10 enhances salt tolerance via promoting Na+ translocation into root vacuoles. Plant Physiol.

6. Ma Y(2015). COLD1 Confers Chilling Tolerance in Rice. Cell.

7. Wang J , Ren Y , Liu X(2020). Transcriptional Activation and Phosphorylation of OsCNGC9 Confer Enhanced Chilling Tolerance in Rice[J]. Molecular Plant.

8. Yan Yan, Sun Mintao, Ma Si, Feng Qian, Wang Yijia, Di Qinghua, Zhou Mengdi, He Chaoxing, Li Yansu, Gao Lihong, Yu Xianchang(2022), Mechanism of CsGPA1 in regulating cold tolerance of cucumber,Horticulture Research.

9. Li L, Mao D, Sun L, Wang R, Tan L, Zhu Y, Huang H, Peng C, Zhao Y, Wang J, Huang D, Chen C(2022). CF1 reduces grain-Cd levels in rice (Oryza sativa). Plant J.

10. Niu M, Bao C, Zhan J, Yue X, Zou J, Su N, Cui J(2021). Plasma membranelocalized protein BcHIPP16 promotes the uptake of copper and cadmium in planta. Ecotoxicol Environ Saf.

11. Xue Wu, Nana Su, Xiaomeng Yue, Bo Fang, JianwenZou, Yahua Chen, Zhenguo Shen and Jin Cui(2020), IRT1 and ZIP2 were involved inexogenous hydrogen-rich water-reduced cadmium accumulation in Brassicachinensis and Arabidopsis thaliana, Journal of Hazardous Materials .

12. He JL(2015). Overexpression of bacterial gamma-glutamylcysteine synthetase mediates changes in cadmium influx, allocation and detoxification in poplar. New phytologist.

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