Research on the mechanism of plant stress resistance assisted by Non-invasive Micro-test Technology

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Abstract: A gene can change the fate of a country, a seed can change the future of a nation, and a new technology will certainly promote the leapfrog development of related disciplines. We are in a good era of doing scientific research. In this era, new disciplines and new technological methods emerge in an endless stream, which will escort us to carry out scientific research. Non-invasive micro-test technology (NMT) can detect the real-time dynamic changes of various ions or molecules in living plants. At present, this technology has reached the international leading level. It has shown great application potential in the fields of plant nutrition, saline-alkali stress and heavy metal stress response, providing an ideal solution for the study of plant response to environmental dynamics. This paper briefly reviewed the development of NMT, and the main teaching contents of relevant experts in the field of plants under the 2022 Advanced Seminar on the Application of Non-injury Micro-measurement Technology. At the same time, combined with my research work, I designed relevant topics, and put forward suggestions for the development of NMT.

Key words: Non-invasive Micro-test Technology, plant stress, salt stress, heavy metal, physiological and biochemical

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

Non-invasive micro-test technology (NMT) was first proposed by American scientist Lionel F. Jaffe, and then gradually applied to solve many key scientific problems through continuous exploration and development (Liu et al., 2022). Professor Xu Yue is a pioneer and leader in the localization of NMT in China. He has guided the development of NMT in China for nearly 20 years, and promoted the application of a vibrant and vigorous technology. At present, NMT technology has reached the international leading level, has obtained a number of patent technologies, and has been widely used in life science, material science, earth science, national economy and people's livelihood and other fields.

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At present, NMT can be used to measure the real-time changes of sodium (Na^{+}), potassium (K^{+}), calcium (Ca^{2+}), magnesium (Mg^{2+}), ammonium (NH_4^{+}), cadmium (Cd^{2+}), lead (Pb^{2+}), copper (Cu^{2+}) and other heavy metal ions between living tissues, cells and the internal/external environment, as well as multiple ion or molecular indicators such as NO_3^-, Cl^-, O_2 and IAA (Liu et al., 2022). These indicators can reflect the dynamic response of living organisms to the external environment, providing strong physiological evidence for revealing the physiological mechanism.

Food security and environmental pollution prevention and control issues related to the national economy and the people's livelihood have aroused widespread public concern. The Zhongguancun NMT Industrial Alliance pays attention to the application and promotion of theory and technology, and actively serves the majority of scientific researchers. In October 2022, the Alliance organized the 2022 Advanced Seminar on the Application of Non-injury Micro-measurement Technology • Plant Field, aiming to cultivate a group of scientific researchers who are familiar with and use NMT, which will certainly set off a research upsurge of NMT technology in the three "pillars" of plants (salt and alkali stress, heavy metals and plant nutrition). This seminar invited 12 experts, scholars and application engineers from front-line scientific research to give lectures to the students. The detailed scientific research data support a rich scientific story. We are deeply impressed by the rigor and difficulty of scientists. Every article we read should be respected and feared. By participating in this seminar, we can have a deeper understanding of the application of NMT in plant nutrition, plant nanobiology, salt stress and heavy metal stress, education and teaching, and seed industry.

II. Application of Non-invasive Micro-test Technology in plant nutrition, nanobiology and stress response research

Root is an important organ for plants to absorb nutrients and water directly. Root configuration, including root length, number of lateral roots, number of root hairs, root orientation or water orientation, is the key factor determining nutrient and water absorption. In the context of climate change, the global arid and semi-arid regions will be further expanded (Huang et al., 2016). Therefore, analyzing the regulation mechanism of plant response to water will help improve and improve the utilization efficiency of water resources and nutrients. Professor Xu Weifeng's team has established a system for studying the hydrophilicity of roots. The research found that the hydrophilicity of plants is characterized by the ability of roots to anchor water and grow towards the soil with water under the stress of drought and water shortage. It also found that brassinolide BR can promote the hydrophilicity of roots by regulating the root proton flow signal (Miao et al., 2018). In this study, the dynamic changes of H^{+}, $Ca^{2+}$and H2O2 were detected by NMT. In addition, the hydrophilicity of root is also closely related to the formation of root sheath. The root sheath is an adaptive structure formed by the mutual cementation and twining of the mucus secreted by the rhizosphere soil particles and roots, the mucus secreted by the rhizosphere microorganisms and the root hairs, which is particularly important for plant drought resistance. The research found that the root sheath is the "highway" for the nutrient and water absorption of crops. The endophytic fungus Pyrosporium indicus under moderate irrigation can promote the construction of rice root sheath, providing important reference value for the efficient use of water by crops (Zhang et al., 2020; Xu et al., 2022).

Nanotechnology has been recognized by the European Commission as one of the six most promising key technologies in the 21st century, and has been widely used in biomedicine, environment, food and agriculture and other fields (Salata, 2004). The negative or positive effects of engineered nanomaterials (EMs) on plant growth and development are attributed to their physical and chemical properties, application concentration, etc. Engineering nanomaterials have great potential in improving plant saline-alkali stress and heavy metal tolerance (Wan et al., 2020; Zhao et al., 2020). Carbon-based nanomaterials, including carbon dots (CDs), single-walled carbon nanotube angles (SWCNHs), single-walled carbon nanotubes (SWCNTs), multi-walled carbon nanotubes (MWCNTs), graphene oxide and fullerene (C60), are new engineering nanomaterials with broad application prospects (Lahiani et al., 2015). Biomass derived carbon dots are typical zero-dimensional carbon-based nanomaterials with good water solubility, biocompatibility and low toxicity. Sun Jian's team found that lectin receptor kinase LecRKs can sense the - OH and COOH functional groups on the surface of the carbon point of Salvia miltiorrhiza, thus promoting the accumulation of cAMP/cGMP and activating CNGCs. In this case, cytoplasmic Ca^{2+}mobilization does not depend on ROS signal. The study also found that under the stress of NaCl, low $K^{+}$and low Fe^{2+}, CDs promote the concentration of Ca2+in the root cytoplasm to increase, and then affect the absorption of K+, Na+and Fe2+, thus improving the adaptability of plants to these stresses (Li et al., 2022). The above research shows that engineering carbon nanomaterials such as SWCNHs and CDs have important application prospects in the fields of sustainable agricultural development and resources and environment.

Saline-alkali stress is one of the main abiotic stresses that endanger plant growth. China's saline-alkali land is more than 500 million mu, and it is estimated that more than 50% of the cultivated land will be saline-alkali by 2050. The red line of 1.8 billion mu of arable land should be maintained, and at the same time, 500 million mu of saline-alkali land should be fully developed and utilized. Therefore, it is necessary to change the traditional conventional crop breeding method to carry out the research on the saline-alkali tolerance mechanism of plants, especially crops. Cultivating saline-alkali tolerant crops will provide important support for China's granary and rice bowl. Saline-alkali tolerant plants adopt different strategies such as salt rejection and salt secretion to reduce salt absorption and thus reduce toxicity. The dynamic detection of Na^{+}/K^{+}ion flow by NMT provides strong support for plants to respond to saline-alkali stress. Professor Zhang Jinlin's team found that the toxicity of Na+was reduced by reducing the inflow of Na+in the root system; Further research found that the salt tolerance of Puccinellia parviflora was affected by PtSOS1 and PtHKT5; 1 and other ion transporters/ channels PutHKT1; 4, PutHKT2; 1 and PutAKT1 (Han et al., 2022). The team also used NMT to screen the salt tolerance of oat varieties, revealing the differential mechanism of AsSOS1 regulating their response to salt stress. Salt tolerant varieties have strong root Na^{+}absorption control ability, root Na^{+} elimination ability and K^{+} retention ability (Zhang et al., 2022).

Heavy metal stress seriously affects the growth and development of plants, especially the crop yield and products, and endangers food security and human health (Zhao et al., 2021). Cd is the primary pollutant of soil pollution in China, and the excess rate of Cd in soil is as high as 7%. The prevention and control of heavy metal pollution is a major strategic demand of China. Physical remediation, chemical remediation, phytoremediation and other technologies have been widely used in the remediation of soil contaminated by heavy metals. Poplar is a fast-growing tree species with developed roots, large aboveground biomass, strong adaptability and wide distribution, and high total Cd accumulation. It has great potential in the application of heavy metal Cd remediation. Professor Luo Zhibin's team has long been committed to the research on the regulation mechanism of poplar Cd enrichment. The hybrid poplar (Poplus tremula x P alba) is a poplar variety with strong Cd accumulation ability selected by his team (He et al., 2013). The research found that leaves and bark are the main parts of poplar Cd accumulation. In addition, the top of root tip (0-900 μ m) It is the main segment of Cd absorption, which may be related to the development degree of root tip tissue Kjeldahl zone. The length of this region is shorter than that of the root elongation region and the mature region. Even though the Cd absorption velocity is fast, the accumulation of Cd in other regions may also play an important role in the total Cd accumulation. Therefore, it is necessary to detect the dynamic changes of Cd uptake in poplar from time and space scales, and explore the different mechanisms of Cd uptake in different regions of poplar roots. They further found that glutathione and ectomycorrhizal fungi were involved in regulating the tolerance of poplar to Cd stress (Ma et al., 2014; Shi et al., 2019). The content of Cd in poplar litter is significantly lower than that in fresh leaves, which suggests that Cd is activated and transferred in poplar leaves, but the specific mechanism remains to be further revealed.

Obviously, the wide recognition and application of NMT cannot be separated from the dutiful researchers and engineers of the Zhongguancun NMT Industry Alliance. Write NMT into the experimental guide of textbooks, let more scientific research institutes and universities understand and use NMT, which plays an important role in promoting NMT, and is also an important measure to implement the national strategy of rejuvenating the country through science and technology. In addition, NMT has also developed a user-friendly guide for visual operation, which provides a convenient channel for new users to learn about NMT. I believe that in the near future, NMT will shine brilliantly in education, scientific research and other fields.

III. Take advantage of NMT to inject new impetus into the research

My research direction is crop nutrition and environmental adaptation, mainly focusing on the physiological and molecular mechanisms of crop response to nutrition and environmental changes, especially the mechanism analysis of upland rice adaptation to terrestrial environment, including low water germination of seeds, emergence of seedlings after germination, response to high or low temperature during growth period, grain filling and endosperm filling, and rice quality. Upland rice, also known as upland rice, is the xerophytic ecological type of Asian cultivated rice. It is planted in an aerated and aerobic dry land environment. The dry direct seeding cultivation of upland rice is faced with complex dry land environment. In production practice, problems such as difficult seedling emergence, uneven seedling emergence, and late lodging are often encountered. Cultivating drought-resistant and water-saving rice varieties and developing upland rice are important development directions for rice production under the situation of water shortage, labor shortage and seasonal drought. Systematic screening and evaluation of upland rice germplasm resources adapted to the terrestrial environment is the key to the development of upland rice.

We will further enrich the content of the research project and improve the quality of the published scientific research results by applying the nondestructive micro-measurement technology. With the help of NMT (SVS-100 system), the vitality of different upland rice germplasm under drought stress was studied using more than 2500 upland rice germplasm resources collected by our research team in the early stage. The main contents include the following aspects: (1) The oxygen consumption during seed germination was detected by simulating different concentration gradients of drought stress, such as 10%, 15%, 20% PEG6000 treatment; (2) Storage of seeds with different responses, including soluble sugar, total protein, etc., and α Amylase and β The physiological and biochemical indexes such as amylase activity were measured to compare the physiological differences of different upland rice germplasm in response to drought stress; (3) Further carry out systematic evaluation of seedling growth indicators to evaluate the reliability and accuracy of NMT early rapid response identification results; (4) Through the construction of genetic population materials, the important candidate genes are precisely located and their regulatory mechanisms are analyzed. Through the above research, we hope to screen excellent upland rice germplasm resources with strong drought-tolerance ability, which can be used to guide subsequent pot or field drought-tolerance identification, analyze the physiological and molecular basis of upland rice to adapt to drought stress, and provide important genetic materials and genetic resources for upland rice molecular design and breeding.

IV.Thinking and Prospect of Non-damage Micro-measurement Technology

After nearly 20 years of development and improvement, the nondestructive micro-measurement technology has become increasingly mature. The craftsman spirit of dedication, excellence, meticulousness and pursuit of excellence can constantly push our work to new heights. A new starting point and a new height. At present, the nondestructive micro-measurement technology has been recognized by the industry and scientific researchers, showing its unique charm and application prospects. Standing at the intersection of historical development, opportunities and challenges coexist. NMT can make more outstanding contributions to the progress of science and technology only by constantly seeking progress and excellence. In view of this, the following thoughts and suggestions are put forward, hoping to provide some reference for the future development of NMT.

First, work hard on the sensitivity of the detector. This requires optimizing and improving the threshold that each detector can detect, which will help reveal the precise mechanism of plant response to changes. As we all know, reactive oxygen species (ROS) is like a "double-edged sword" for plants. ROS with appropriate concentration can be used as signal molecules to activate relevant signal pathways to enhance plant resistance; The excessive accumulation of ROS will cause damage to protein, nucleic acid and other substances and affect the normal growth and development of plants (Li and Kim, 2022; Zhao et al., 2022). The production and elimination of ROS in plants are in a dynamic balance, but due to the specificity of plants, the ability of each plant to maintain ROS balance is different. For specific plants, the threshold of role transformation of ROS as a signal molecule or stress signal has not been clearly revealed. Improving the sensitivity of NMT to detect O_2 will provide an answer to this question by detecting its dynamic changes in space.

The second is to expand the scope of detection of ions or molecules. There is still a lot of room for detecting ions or molecules, especially some multivalent elements and special functional organic small molecule compounds, such as metal elements iron (Liang, 2022), manganese (Zhang et al., 2021), plant hormones ethylene, abscisic acid (Yoshida et al., 2019), etc. These substances are essential for plant growth and development. At present, the indicators that can be detected are far lower than the number of important functional substances that plants respond to environmental changes. If NMT can detect different valence states of the same element, it will provide evidence for revealing the dynamics of the transformation of related elements in plants. At the same time, the interaction between plant hormones plays an important role in the regulation of plant environmental adaptability. It is urgent to carry out the detection of relevant hormones, especially gas hormones, which will help clarify the function of hormone dynamic changes in plant response to environmental changes, and provide a basis for further development of genetic and molecular mechanism analysis.

The third is to develop new compact composite detectors. This kind of detector can be used to detect the dynamic changes of different target ions or molecules simultaneously and multi-dimensionally. Plant system is a complex organism, and its response to environmental change is a systemic and global response. If several ions or molecules of concern can be detected synchronously, the mechanism of its response will be better explained.

Fourth, improve the NMT sharing open platform and provide affordable testing services. Although there are more than 500 laboratories of more than 100 units in China with NMT equipment, the resources are relatively concentrated in developed regions, while the layout of northwest and southwest regions is relatively small. These areas are faced with more serious environmental problems such as saline-alkali stress, drought, heavy metal stress, etc. It is necessary to vigorously carry out research on the mechanism of the response of protobiotic species to these stresses with the help of NMT. In addition, the cost of the current test is relatively high, which limits the space for researchers to use the NMT system to a certain extent. It is hoped that the Zhongguancun NMT Industrial Alliance will be based on the long term and allow this technology to benefit more researchers in scientific research institutes and universities.

Epilogue

The Non-invasive Micro-test Technology provides us with a window to dynamically detect the response of plants to the dynamic changes of internal and external environment, and can accurately grasp the physiological response of plants in real time. It also injects new vitality into the screening and identification of excellent germplasm resources and functional gene analysis. It is hoped that NMT people and NMT colleagues will continue to innovate, pursue excellence, maintain the international leading sustainable power, and let NMT, the key core technology, play an increasingly important role in China's scientific and technological self-reliance.

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