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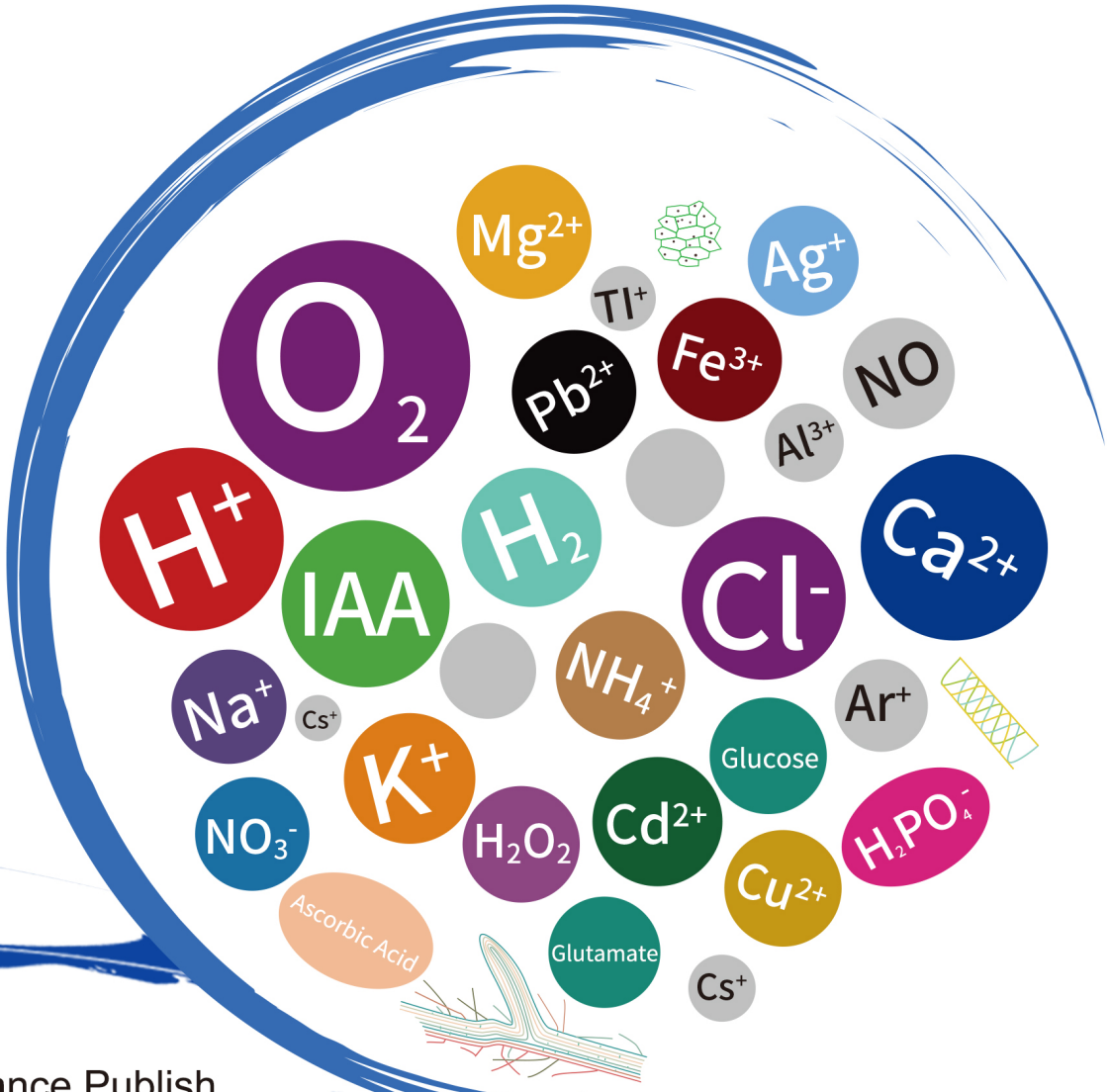

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Invited review

Potential applications of non-invasive micro-test technology in individualized tumor treatments

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Abstract: Non-invasive Micro-test Technology (NMT) is a technology used for studying the physiological functions of living materials. It can detect the three-dimensional flux information of molecules/ions entering and leaving living organisms without damaging the samples. Due to functionalities such as in vivo detection, non-invasive testing, high-resolution imaging, dynamic real-time results, etc., NMT is often used to explore physiological characteristics that are difficult to measure using other techniques. NMT is widely used in medical physiology, botany, zoology and other fields, and has become an essential technology for the study of plant adversity. As an emerging technology in the 21st century, it is becoming more and more refined in the research direction of various disciplines. However, there are still many gaps in NMT research. At present, in the context of the continued high mortality rate of malignant tumors around the world, the achievements of non-invasive micro-test technology in tumor research are increasing each day. This paper introduces the potential application of NMT in the study of tumor physiology, especially in the individualized treatment of tumors in recent years. These include applications related to tumor research such as the nervous system and the digestive system.

Key words: Non-invasive Micro-test Technology; tumor; flux; ion; molecule

1. Non-invasive micro-test technology

1.1. The concept of non-invasive micro-test technology

Non-invasive Micro-test Technology (NMT) is an ultra-high-sensitivity, contactless, flux-based technology that detects the concentration and gradient of ions/molecules outside the sample material.

1.2. Origin of non-invasive micro-test technology

NMT was created based on the theory of American scientist Lionel Jaffe's ion-selective vibrating-microelectrode system. With inspiration and help from Dr. Tingyun Kuang, Dr. Fuyu Yang, and Dr. Kechun Lin, Non-invasive Micro-test Technology (NMT) was named by Professor Yue Xu, a former senior researcher of NASA, and founder of both YoungerUSA, LLC and Xuyue (Beijing) Science &Technology Co.

For more than 20 years, unremitting efforts have been made consistently to improve all aspects of NMT from modularization, automation, specialization, and intelligence to creating standardized technological innovations, commercialized products, industrialization, and localization. Now NMT has independent intellectual property rights for international application innovations and passed the "international leading" review by the Ministry of Science and Technology in 2021.

1.3. Technical characteristics of non-invasive micro-testing

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NMT has many advantages over similar technologies. (1) The living sample is not injured, that is, it can keep the tested sample intact and detections can be made in its real physiological environment; (2) Real-time dynamics, the real-time dynamic data of the sample is obtained over a period of time; (3) High resolution, flux resolution can reach 10^{-12} mol · cm^{-2} · s^{-1}, which is 3-6 orders of magnitude higher than the measured concentration; (4) Wide range of sample sizes, from enriched organelles to single cells, cell layers, tissues, organs, and even whole organisms can be detected; (5) Samples do not need to be labeled, which is convenient, safe and environmentally friendly; (6) There are many ion/molecule detection indicators that can be measured, such as Ca^{2+}, H^+, K^+, Na^+, Cl^-, Mg^{2+}, Cd^{2+}, NH_4^+, NO_3^-, O_2, H_2O_2, indole acetic acid (IAA), glutamic acid (Glu) and other indicators [2, 6] have been commercialized. With development of the technology, the number of ions and molecules that can be measured by NMT are also increasing, which provides an ideal experimental platform for obtaining information on the movement of ions/molecules inside and outside of biological samples. NMT has been widely used in medicine, botany, zoology, agricultural science, pharmacology, environmental science, and other research fields.

2. Research status of individualized cancer therapy

Cancer chemotherapy has progressed significantly in recent years, but there are still obvious differences in chemotherapy sensitivity among different races and even individuals. The effectiveness of current commonly used cancer treatment drugs is less than 70%, and 20%-40% of patients may even receive the wrong treatment [7]. How to identify individual differences between patients and use these differences to guide clinical medication reasonably has become the focus of increasing attention in the medical field. In the study of individualized treatment of tumors, genomics, proteomics, and metabolomics are widely used to promote the optimization and improvement of new target screening, targeted drug development, and chemotherapy regimens [7]. However, the current research is still based on the viewpoint that a "tumor is a molecular disease, and the root cause is genetic alterations" [8], and the research object is tumor cells in vitro. The surrounding environment of tumor cells in the body profoundly affects their proliferation speed, mode, and other characteristics, and cells cultured in vitro proliferate in a special environment, and are very likely to have different phenotypes from orthotopic tumor cells. With the emergence of tissue microstructure theory and stem cell theory [9, 10] in the post-genomic era, researchers have found that tumor cells and tissue microenvironment play a pivotal role in individualized tumor treatment. However, how to accurately measure the material and energy exchange between living tumor cells and tissues and their microenvironment has become an obstacle in this research.

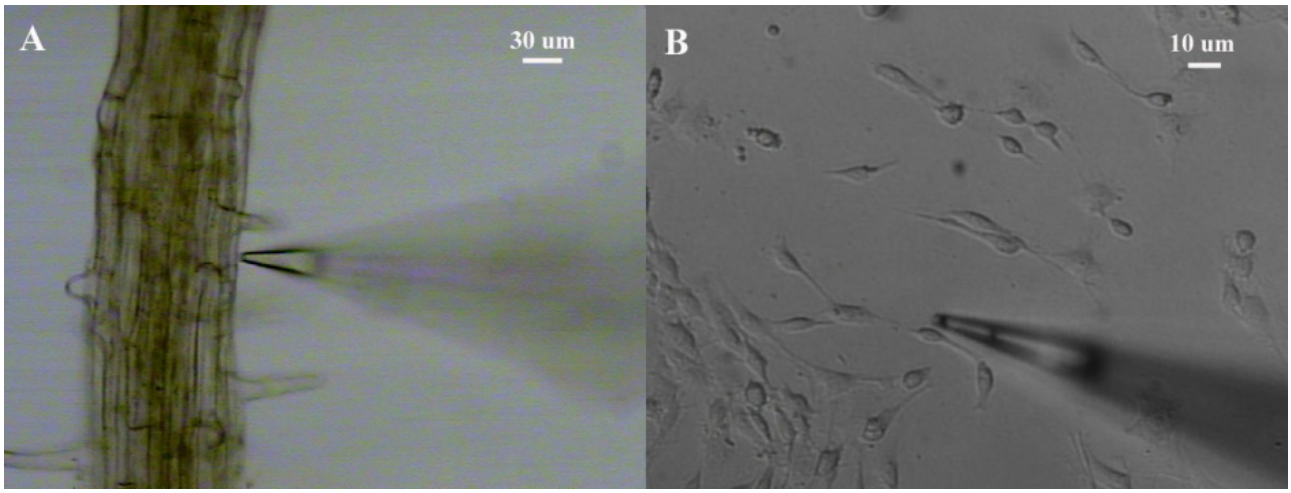

Figure 1. Real-time screenshots detected by non-invasive micro-test technology. A. Arabidopsis root maturation zone, B. Neuron cells

3. Potential application of non-invasive micro-test technology in individualized treatment of tumors

3.1. Potential applications of non-invasive micro-test technology in tumor drug susceptibility testing

3.1.1. Current status of individualization of chemotherapy drugs guided by tumor drug sensitivity testing

Among the many means of malignant tumor treatment, chemotherapy is a systemic tumor treatment method, which can kill tumor cells in the patient's body to the greatest extent. However, in clinical practice, the effect of chemotherapy is often unsatisfactory partly due to the resistance of tumor cells to chemotherapy drugs. This is a key problem that plagues cancer treatment. According to estimates from the American Cancer Society, more than 90% of cancer deaths are affected by drug resistance to varying degrees. With advances in molecular biology and cell biology, two series of in vivo and in vitro have been established, and there are now more than ten kinds of drug susceptibility testing methods.

3.1.2. NMT for cancer drug resistance research

The extracellular acidity of the tumor can effectively block the drug from entering the cell or neutralize the drug, and sequester the drug in the acidic cell vesicles to prevent the drug from reaching the intracellular target, thereby reducing its killing effect on tumor cells. Song Jin et al. [4] established a drug resistance study method (DRSM) using non-invasive micro-test technology, which can be used to study the relationship between organs, tissues, extracellular ions, molecular activities and tumor cell drug resistance. The results showed that the H^+ flux of drug-resistant breast cancer cells was close to zero before adding doxorubicin, while the H^+ flux of sensitive breast cancer cells was obvious. Both the sensitive strain and the drug-resistant strain showed H^+ efflux after adding doxorubicin, but the H^+ efflux of the drug-resistant strain was 5 times that of the sensitive strain (Figure 2). The study provides direct evidence for the correlation between extracellular H^+ activity and tumor drug resistance.

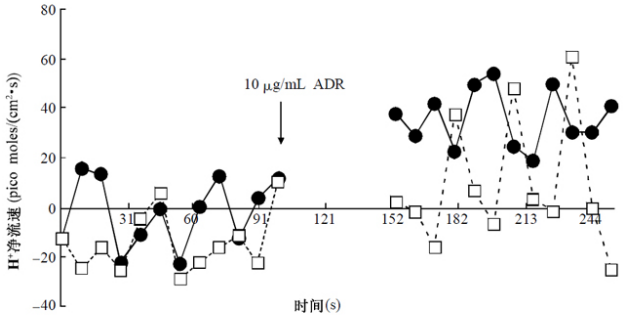

Figure 2. H^+ net flux of breast cancer cells before and after ADR (doxorubicin) treatment. A positive value indicates efflux, a negative value indicates influx.
●Drug-resistant strain MCF-7/R，□ Sensitive strain MCF-7/S7/S

3.1.3. Application of non-invasive micro-test technology in pharmacological research

Among the chemotherapeutic drug sensitivity detection methods, primary tumor cell culture is by far one of the most ideal drug sensitivity testing methods. However, recent studies have shown that changes in tissue structure provide an unfavorable microenvironment for the proliferation and differentiation of cells in the tissue, leading to the occurrence of tumors. From the study of tumor cells to the study of tumor tissues, it can explain the pathogenesis of tumors in essence. Zhu et al. [13] directly showed the detection of Cl^- flux in the colonic mucosa of rats using non-invasive micro-test technology. The results showed that entacapone induced cAMP-dependent Cl^- efflux in the colonic mucosa of rats [11, 12]. In addition, it was also found that a large amount of Cl^- efflux in the colonic mucosa of rats under the action of emodin is closely related to the degranulation of mast cells and the activation of submucosal cholinergic and non-cholinergic neurons.

3.2. Application of non-invasive micro-test technology in tumor metabolomics

3.2.1. Application of metabolomics in individualized tumor treatment

The unique metabolic characteristics of tumor cells make them produce unusual metabolites during growth and proliferation. Using metabolomics technology to analyze these specific metabolites and find new markers has achieved promising results in tumor screening and early diagnosis [14-16]. Metabolomics can detect changes in endogenous metabolites caused by drugs through the study of pharmacokinetics. Changes in endogenous metabolites caused by drugs directly reflects the biochemical process and causes in the body, and illustrates the effects of drug targets or receptors. This guides the individualized treatment of anticancer drugs to evaluate the clinical efficacy and safety.

3.2.2. Application of NMT in the study of tumor cell apoptosis markers

The relative molecular weights of molecules and ions that can be detected by NMT are less than 1000. In a broad sense, the flux detected by NMT can be classified into the category of metabolomics. Due to the in vivo detection advantages of NMT, compared with traditional metabolomics, metabolites can be detected without damaging samples, which can be called "in vivo metabolomics." At present, under the background of chemotherapy, radiotherapy and other unstable curative effects and relatively large side effects, photodynamic therapy (PDT) as a treatment method for micro-injuries has gradually come to people's attention. Song et al. [17] took oral squamous carcinoma cells (OSCC) as the research object, and used non-invasive micro-test technology to detect that after PDT treatment the O_2 influx gradually increased, and the Ca^{2+} efflux rate was also significantly higher than that of untreated cells. At the same time, the apoptosis rate, apoptosis factors and other indicators all increased. The results of this study indicated that changes in O_2 and Ca^{2+} fluxes may be early signals of PDT-induced apoptosis. Hu et al. [18] found that under normal conditions, C6 glioma cells had a small amount of Ca^{2+} efflux and K^+ influx. After PDT intervention, significant Ca^{2+} influx and K^+ efflux occurred, and the cells died. This study further revealed the molecular mechanism of PDT-induced glioma cell death, showing that the death of cancer cells may be related to the transition of Ca^{2+} influx and K^+ efflux.

3.3. Potential application of NMT in the research of targeted individualized anticancer drugs

3.3.1. Research status of targeted individualized anticancer drugs

Molecularly targeted cancer therapy refers to drugs that target specific molecular targets that play a key role in tumorigenesis and development [19]. It has recently become a new method for the treatment of malignant tumors and has received increasing clinical attention. Targeted anti-cancer drugs include targeted epidermal factor blockers, monoclonal antibodies against certain cell markers, drugs against certain oncogenes and cytogenetic markers of cancer, anti-tumor vaccines, and gene therapy etc. [20]. Huang Can et al. [21] found that the expression of p-JNK protein and cell apoptosis rate in tumor cells increased after application of SP600125 inhibitor, and also found that the JNK signal transduction pathway was involved in the multidrug resistance MDR gene and multidrug resistance protein of liver cancer. The expression of P-glycoprotein (P-gp) has a regulatory effect on tumor drug resistance. As a pro-apoptotic factor, JNK is one direction of tumor treatment research. NMT has also achieved results in the research of apoptosis and apoptosis factors.

3.3.2. Application of non-invasive micro-test technology in apoptosis research

Keefe et al. [22] detected the changes in morphology and extracellular K^+ of fertilized eggs under H_2O_2 treatment since experiments have found that apoptosis is activated by substances such as

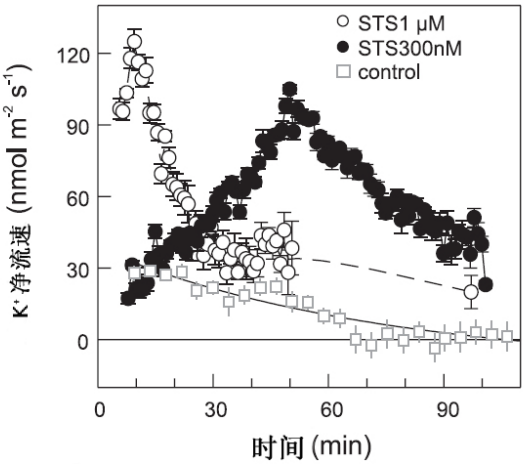

Figure 3. K^+ net flux after STS (staurosporine) treatment of T lymphocytes, positive values indicate efflux, negative values indicate influx. High concentration STS treatment and low-concentration STS treatment show faster K^+ efflux than the control as indicated with the high peaks.

H_2O_2. Figure 3 shows a significant efflux of K^+ ions using non-invasive micro-test technology. This is due to the activation of K^+ channels when cells initiate apoptosis.

Valencia et al. [23] found that after T cells were treated with 1 μM staurosporine, and while they were induced to undergo apoptosis, a rapid K^+ efflux was detected by non-invasive micro-test technique. The K^+ efflux reached a peak in about 15 minutes and then weakened as seen in Figure 3. At the same time, an increase in K^+ channel current was also recorded using the patch clamp technique, accompanied by a sharp decrease in membrane depolarization. Studies suggest that ion flux is an early event of apoptosis; K^+ efflux causes cell contraction, activates caspase, and induces apoptosis. Bcl-x_L promotes the exchange of metabolites between mitochondria and the cytosol and is the major anti-apoptotic protein in the adult brain, and Bcl-x_L overexpression increases the number and size of synapses. Alavian et al. [24] found that Bcl-x_L overexpressed neurons had higher ATP levels, and exogenous Bcl-x_L could reduce or inhibit ATP. The results of neuron O_2 flux detection showed that the O_2 influx of Bcl-x_L overexpressed neurons was relatively small (Figure 4).

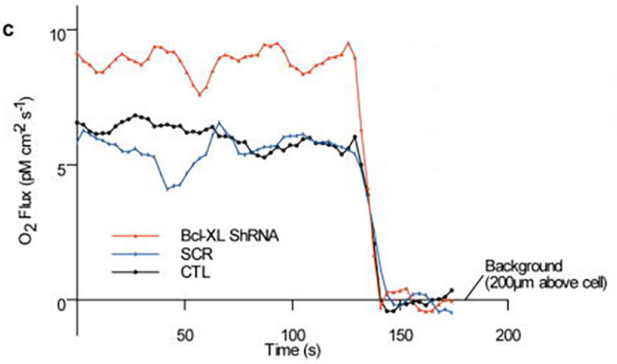

Figure 4. Neuron O_2 net flux, a positive value indicates influx, a negative value indicates efflux. The expression of neuronal Bcl-x_L is proportional to the O_2 influx rate. Ctl: normal control group, Bcl-x_L shRNA: Bcl-x_L expression suppression group, Scr.: Bcl-x_L Overexpression group; background value: measured at a distance of 200 μm from the cell in the vertical direction.

3.4. Application of non-invasive micro-test technology in diseases induced by ion imbalance

Ca^{2+} is an indispensable ion for various physiological activities of the body. It plays an important role in maintaining the biological potential on both sides of the cell membrane, nerve conduction function, normal muscle contraction and relaxation, and the action mechanism of some hormones is also expressed through Ca^{2+}.

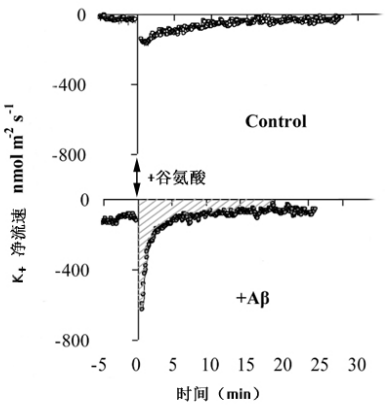

Figure 5. The net flux of K^+ under glutamate stimulation in primary cortical neurons treated with control group and Aβ (amyloid β protein). Positive values indicate influx, negative values indicate efflux. In neurons treated with Aβ, the K^+ efflux was significantly greater than that of the control group under the stimulation of glutamate.

The imbalance of Ca^{2+} can cause diseases such as diabetes, rheumatism, respiratory diseases, neurological diseases, etc., and it is also closely related to the formation of tumors.

Shabala et al. [25] studied the role of β-amyloid (Aβ) in the pathogenesis of Alzheimer's disease and found that after 1 μM Aβ treated primary cortical neurons, the rate of K^+ efflux and Ca^{2+} efflux (not shown) induced by glutamate was significantly greater than that in the control group not treated with β-amyloid protein (Figure 5). Studies have shown that neurons' loss of the ability to maintain Ca^{2+} and K^+ balance may be an indication of the early response of cells to Aβ. Chung et al. [26] found that Zn7MT-2A could prevent Cu(II)Aβ-induced oxidative stress on neurons when studying metal-chelation therapy for Alzheimer's disease. They found that Cu(II)Aβ caused K^+ efflux and Ca^{2+} influx in neurons, and the addition of Zn7MT-2A prevented the change of flux caused by Cu(II) Aβ. Zn7MT-2A maintained K^+ and Ca^{2+} ion flux, reducing the damaging effects of Cu(II)Aβ on neurons.

3.5. Application of non-invasive micro-test technology combined with patch-clamp technique in tumor research

Non-invasive micro-test technology has been combined with laser confocal technology, patch clamp technology, and other technologies to find both internal and external measurement of segregation information exchange. Yang et al. [27] used NMT and patch clamp techniques to find that nasopharyngeal carcinoma cells undergo cell regulatory volume reduction in a hypotonic environment. In the study, the K^+ efflux appears in the initial period. With the efflux of intracellular H^+ to the outer surface of the cell and the closure of K^+ channels on the membrane, the efflux of H^+ replaces the efflux of K^+. This is the first time that K^+ and Cl^- transports are uncoupled during hypotonic-induced cell regulatory volume reduction, which to some extent overturned the conclusions of previous patch clamp studies, and found that H^+ efflux in cells plays an important role in the regulation of cell volume. In the future, non-invasive micro-test technology will play an important role in the study of active transport ion or molecular pumps and co-transport carriers.

4. Outlook

Non-invasive micro-test technology has been widely used in many research fields such as plant stress, photosynthetic mechanism, neurological diseases, signal transduction, etc. Especially in the study of plant stress, a complete set of experimental system methods have been formed, and it has become an essential technology in the research field. However, the individualized treatment of tumors is in the primary stage of applied research, with many blank areas for new research and highly prospective results. As of now, relevant theoretical and practical studies around the world have confirmed the heterogeneity among tumor patients and the heterogeneity within tumors. The former has been widely recognized: the clinical course and treatment plan of any two cancer patients cannot be exactly the same, which has always been an important principle of personalized treatment [28]. The tissue microstructure theory and stem cell theory pointed out that [9, 10] the extracellular matrix component is the primary attack target of various carcinogenic factors, and the abnormal signal exchange between cells caused by it is the main reason for the uncontrolled proliferation of cells. This has opened up a new path for the current research on individualized treatment of tumors, that is, to flexibly select the corresponding treatment methods according to the characteristics of the tissue or cell microenvironment in the individual patient. Non-invasive micro-test technology provides extremely important technical support for this theory with its unique advantages of in-vivo detection, non-invasive, high-resolution, dynamic real-time, etc. Looking at the successful trajectory of non-invasive micro-test technology in the field of plant research, it will surely become an important tool for studying the physiology of living tissue and cell microenvironment.

In addition to the research on individualized treatment of tumors introduced in this article, non-invasive micro-test technology also has

very broad application prospects in other fields of tumor research. For example, in the study of the mechanism of tumorigenesis, there are still relatively few studies on the effects of physical, chemical, biological, genetic, and other carcinogenic factors on the physiological functions of normal living tissues or cells, especially the entry and exit of molecules and ions. The rate and direction (flux) of ions/molecules entering and exiting the cell membrane represent most of the physiological activities of the living tissue or cell in real time. This is of great significance for studying the mechanism of various carcinogenic factors affecting normal living tissue or cells in different parts of the human body. Non-invasive micro-test technology can not only monitor the short-term and long-term effects of carcinogenic factors by detecting one or more ion/molecule fluxes in a specific tissue or cell in real time, but it can also conduct related mechanism research. Moreover, by comparing the ion/molecule flux of normal and cancerous living tissues or cells, the flux spectrum of cancerous tissue and cell segregation can be created, which can be used as a basis to guide clinical research. To sum up, more in-depth research directly related to tumors using NMT is being noticed more and more, and will definitely become a hot spot in future research.

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Opportunities for Innovation

Innovations resulting from NMT functionality improvements: micrometer-scale pH detection of living samples

Contributed by the editorial department of Zhongguancun NMT Industrial Alliance

Editor's note:

Fungal colonization can activate the activity of proton pumps in poplar roots under salt stress, promote the secretion of more H^+ and reduce the pH of the root surface, thereby effectively improving the unfavorable conditions that poplar roots absorb NO_3^- under salt stress. In addition to the dynamic detection of real-time of H^+ fluxes secreted by the root surface during this process, the non-invasive micro-test technology (NMT) can also innovatively observe the fine changes in pH on a micrometer scale on the root surface, which truly shows the "dynamic and static combination" monitoring of H^+.


New Phytologist

Full paper | Open Access

Amelioration of nitrate uptake under salt stress by ectomycorrhiza with and without a Hartig net

Gang Sa, Jun Yao, Chen Deng, Jian Liu, Yinan Zhang, Zhimei Zhu, Yuhong Zhang, Xujun Ma, Rui Zhao, Shanzhi Lin, Cunfu Lu, Andrea Polle, Shaoliang Chen

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Basic Information

Topic: NMT finds that colonizing fungi causes rhizosphere acidification to promote NO_3^- absorption, providing evidence that mycorrhizal promotes salt tolerance in host plants by maintaining nutrient uptake

Journal: New Phytologist

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Title: Amelioration of nitrate uptake under salt stress by ectomycorrhiza with and without a Hartig net

Authors: Gang Sa, Jun Yao, Chen Deng, Jian Liu, Yinan Zhang, Zhimei Zhu, Yuhong Zhang, Xujun Ma, Rui Zhao, Shanzhi Lin, Cunfu Lu, Andrea Polle, Shaoliang Chen

Test sample: gray poplar root

Ion/molecule detection indicators: H^+, NO_3^-

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Editor E-mail: yanhan@nmtia.org.cn

Abstract: Salt stress is an important environmental factor that hinders poplar from absorbing nitrogen nutrients. This article describes the effect of salinity on proton-driven nitrate flux in ectomycorrhizal roots and the importance of a Hartig net for nitrate uptake. Two strains of *Paxillus involutus* were used for root colonization: one MAJ, which forms typical ectomycorrhizal structures (mantle and Hartig net), and one NAU, which colonizes roots with a thin, loose hyphal sheath.

Fungus-colonized and noncolonized *Populus* × *canescens* were exposed to NaCl and used to measure root surface pH, nitrate (NO_3^-) flux and transcription of NO_3^- transporters (NRTs; *PcNRT1.1, -1.2, -2.1*), and plasmalemma proton ATPases (HAs; *PcHA4, -8, -11*).

Paxillus colonization enhanced root NO_3^- uptake, decreased surface pH, and stimulated *NRTs* and *HA4* of the host regardless the presence or absence of a Hartig net. Under salt stress, noncolonized roots exhibited strong net NO_3^- efflux, whereas beneficial effects of fungal colonization on surface pH and HAs prevented NO_3^- loss. Inhibition of HAs abolished NO_3^- influx under all conditions.

It was found that stimulation of HAs was crucial for the beneficial influence of ectomycorrhiza on NO_3^- uptake, whereas the presence of a Hartig net was not required for improved NO_3^- translocation. Mycorrhizas may contribute to host adaptation to salt-affected environments by keeping up NO_3^- nutrition.

Key words: non-invasive micro-test technology, salt stress, mycorrhiza, nitrate, Hartig net, NRT

1. Experimental results of ion/ molecule flux

Since ^{15}N tracing revealed the identity of the transported N compounds, the study used Non-invasive Micro-test Technology (NMT) to determine NO_3^- fluxes in poplar in the presence or absence of fungal colonization and in response to salt stress. Along the root apex (100-2100 μm), the fluxes of NO_3^- were constant in the NO_3^- measuring solution (0.1 mM low NO_3^-, Figure 2a). The magnitude and direction of NO_3^- fluxes were significantly influenced by salt exposure and fungal colonization (Figure 1a). The root tips of non-mycorrhizal (NM) poplar showed moderate NO_3^- uptake, whereas fungus-colonized roots exhibited 7.4- to 11.8-fold higher uptake (Figure 1a). Apparently, a Hartig net was not required for this stimulation because root colonization with either *P. involutus* strain MAJ or NAU resulted in enhanced NO_3^- uptake compared with NM roots (Figure 1a). Furthermore, fungal-colonized roots maintained a net NO_3^- uptake under salt stress, while NM roots showed a net NO_3^- efflux under both short- and long-term salt stress (Figure 1a). Pure fungal mycelia of NAU and MAJ exhibited net NO_3^- influx irrespective of control or salt treatments (Figure 3).

NO_3^- uptake requires H^+ co-transport and is therefore dependent on the pH in the external environment. Here, they determined the pH values at NM and fungus-colonized root surfaces. The pH along NM roots was stable (Figure 2b) with a mean value of 5.41 (Figure 1b). Fungal colonization resulted in a more acidic surface pH, ranging from 5.05 to 5.12 (Figure 1b, Figure 2b). Long-term salinity caused a marked rise of pH in NM plants to about pH 5.8 ($P<0.001$). In fungus-colonized plants, salt exposure also caused pH increments resulting in pH values of *c*. 5.4 at the root surface similar to those of NM control roots (Figure 1b, Figure 2b). The salt-induced increase of pH was due to the decline of H^+ efflux from root surface. The surface pH of NAU did not differ from that of MAJ-colonized roots regardless of control conditions, or short- or long-term salt stress (Figure 1b).

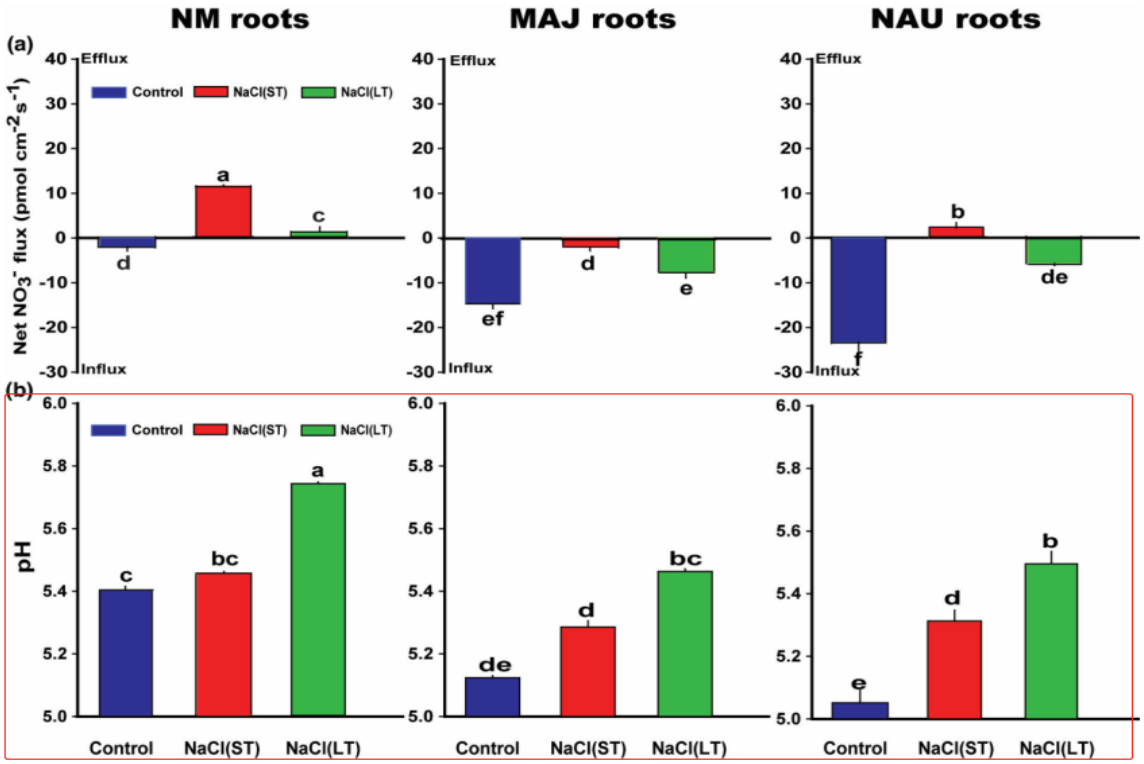


Figure 1. Effects of NaCl on steady-state fluxes of NO_3^- and root surface pH value in *Populus* × *canescens* colonized with or without strains. Positive values represent NO_3^- efflux, negative values represent NO_3^- influx.

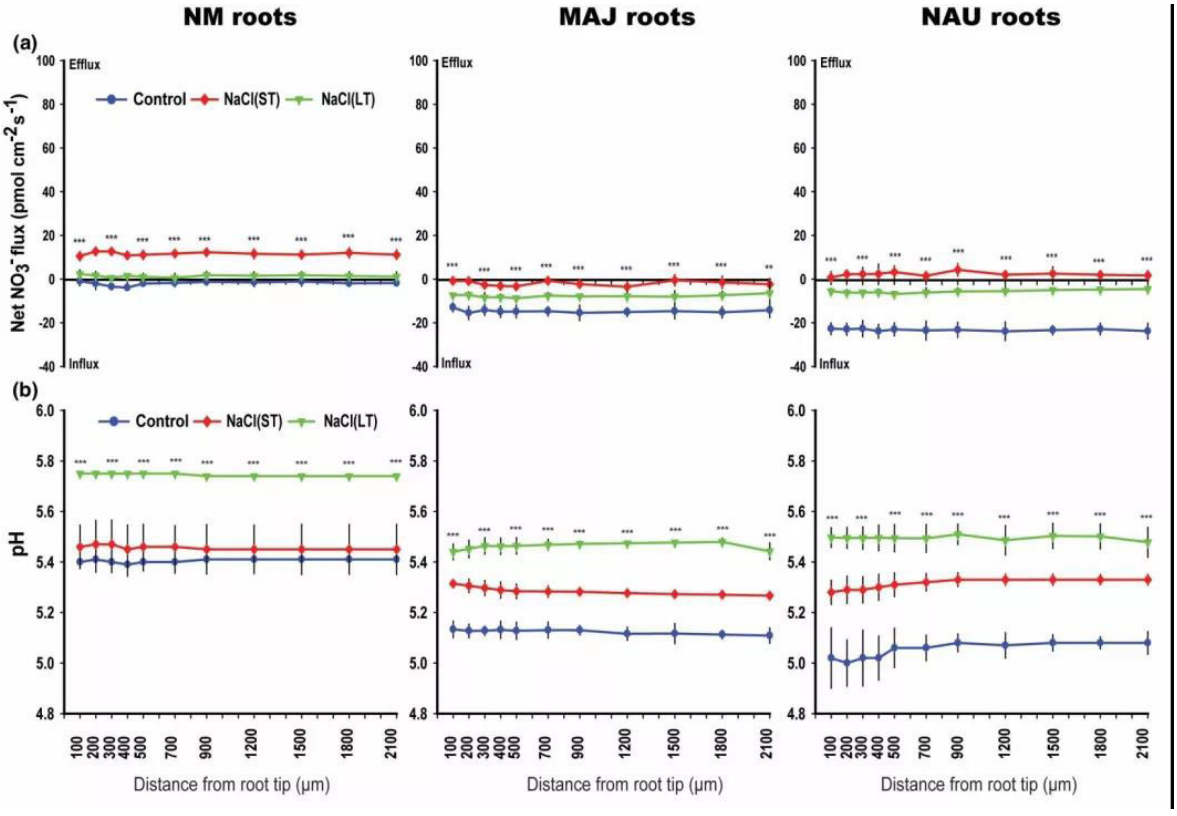


Figure 2. Effect of NaCl on steady-state fluxes of NO_3^- and root surface pH of poplar with and without mycorrhizal inoculation. Positive values represent NO_3^- efflux, negative values represent NO_3^- influx.

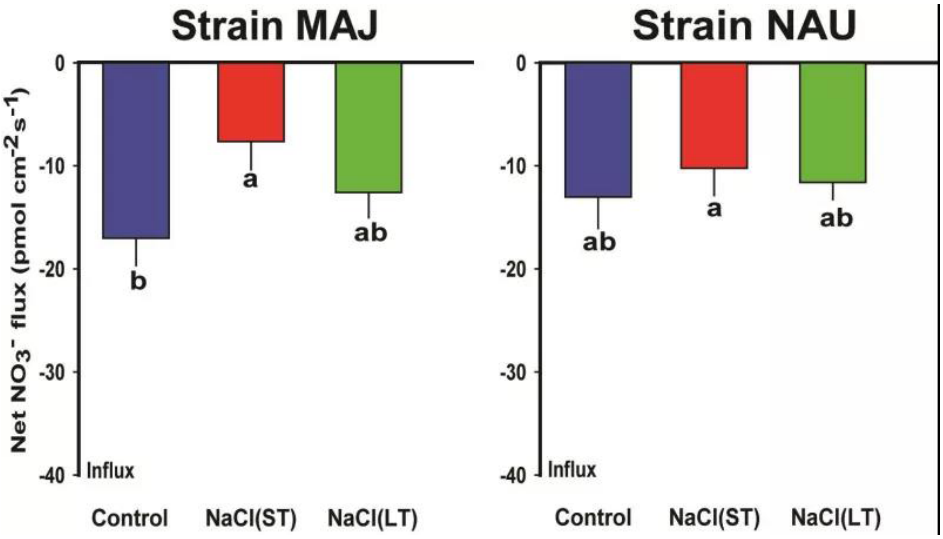


Figure 3. Effect of NaCl on steady-state fluxes of NO_3^- of MAJ and NAU strains. Positive values represent NO_3^- efflux, negative values represent NO_3^- influx.

To test the importance of H^+ gradients for NO_3^- uptake, HAs were inhibited with orthovanadate (plasmalemma HA inhibitor). The inhibitor significantly increased the pH at the surface of NM and fungus-colonized roots (Figure 4b, Figure 5b), indicating that the H^+ pumps were effectively suppressed. Measurements of H^+ fluxes confirmed that the inhibitor orthovanadate shifted net H^+ efflux towards influx under control and saline conditions (Figure 6). This resulted in NO_3^- release regardless of the presence or absence of *Paxillus* or salt stress (Figure 4a, Figure 5a).

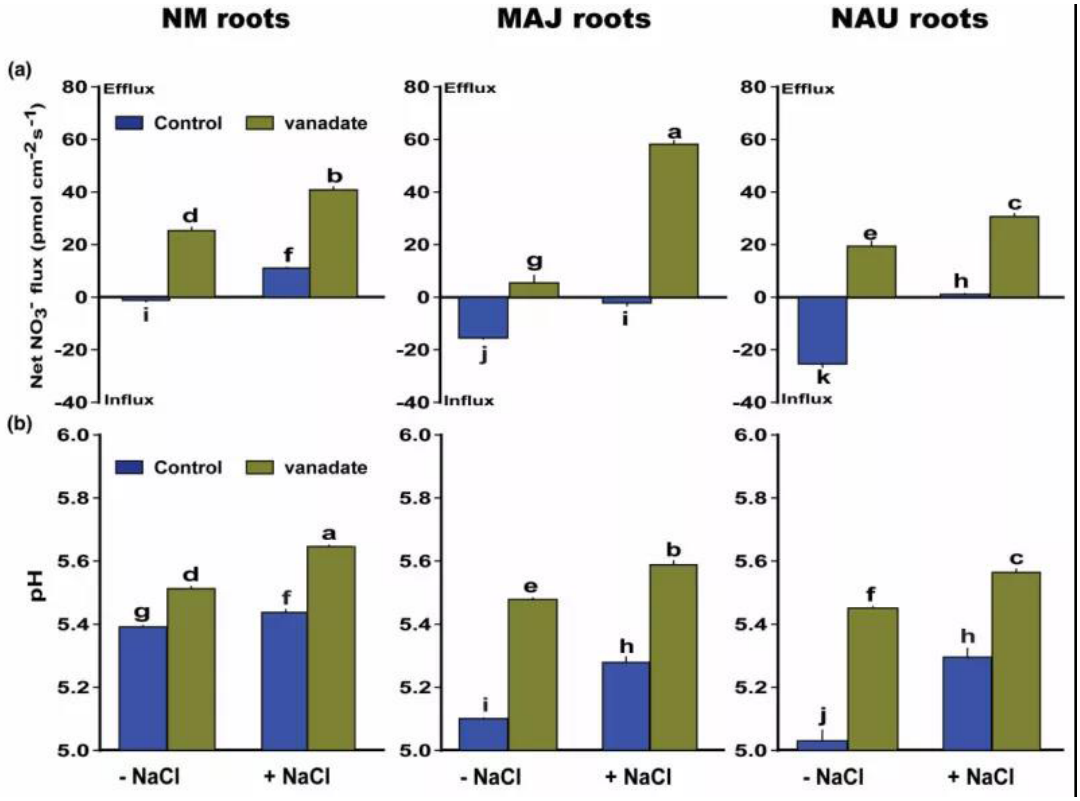


Figure 4. Effect of orthovanadate on steady-state fluxes of NO_3^- and root surface pH in *Populus* × *canescens* with and without mycorrhizal colonization and with or without NaCl. Positive values represent NO_3^- efflux, negative values represent NO_3^- influx.

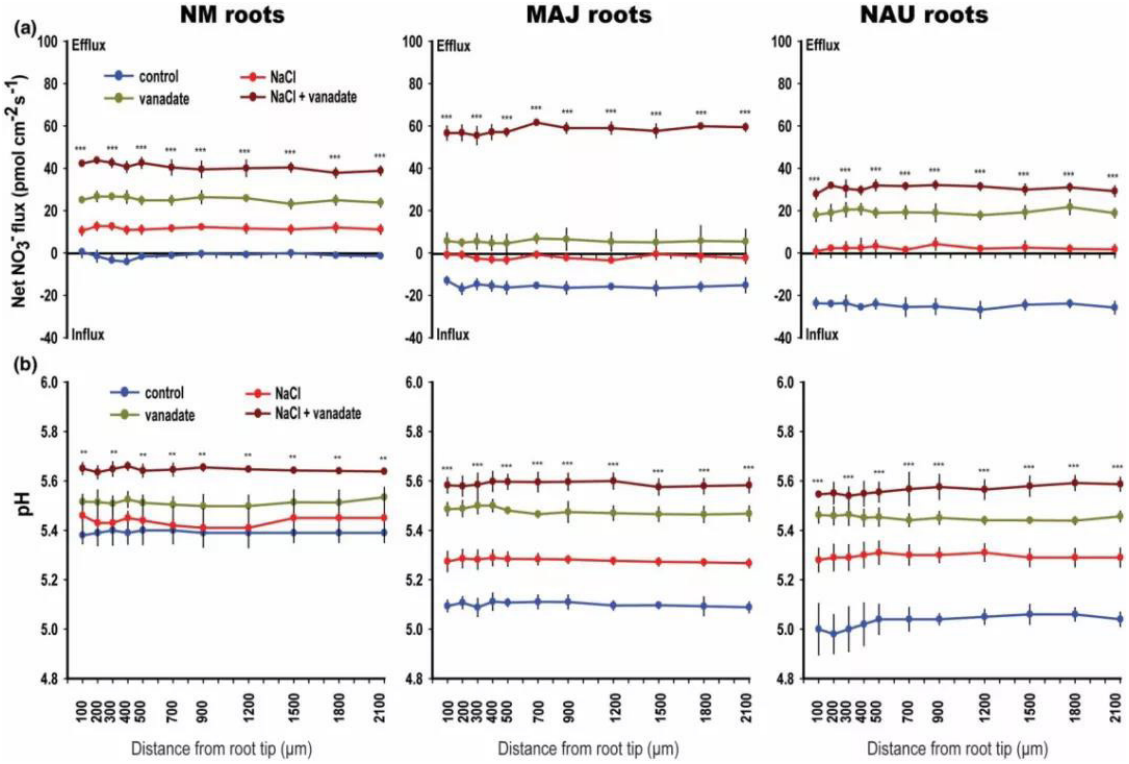


Figure 5. Effect of orthovanadate on steady-state fluxes of NO_3^- and root surface pH in poplars inoculated with and without mycorrhizal roots under NaCl stress. Positive values represent NO_3^- efflux, negative values represent NO_3^- influx.

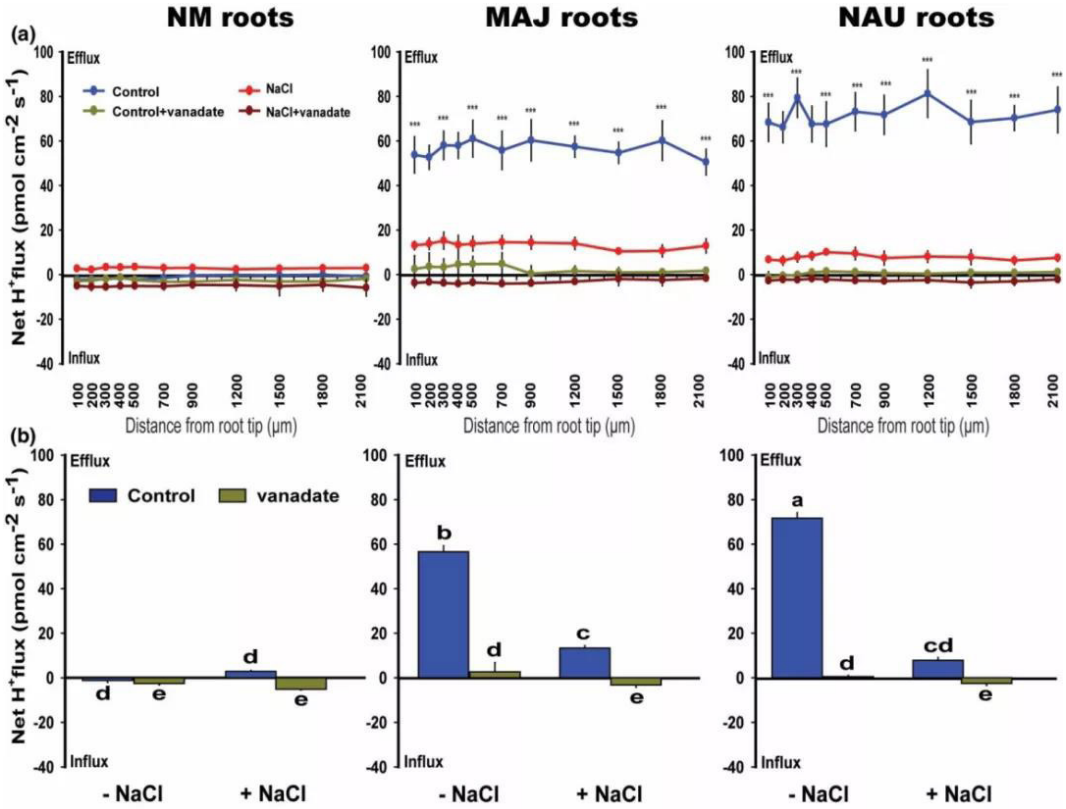


Figure 6. Effect of orthovanadate on steady-state fluxes of H^+ in poplars inoculated with and without mycorrhizae under NaCl stress. Positive values represent H^+ efflux, negative values represent H^+ influx.

2. Ion/molecule flux experimental treatment method

The roots of *P.* × *canescens* were inoculated with and without *P. involutus* strains (MAJ and NAU) for 30 days, and then exposed to 0 or 100 mM NaCl for 24 hours (short term, ST) or 7 days (long term, LT).

3. Other experimental results

1) Compared with NM poplar, the enrichment of ^{15}N in the roots and shoots of poplar colonized by MAJ or NAU was higher.

2) Fungal colonization and salt stress altered the transcript levels of low-affinity and high-affinity nitrate transporters (NRTs) in poplar roots.

3) Under long-term salt stress, the activities of NR (nitrate reductase) and NiR (nitrite reductase) showed a moderate decrease.

4) Despite the salt-induced decrease in the level of PcHA4 transcripts, the transcript levels of PcHA4 were still higher in fungus-colonized than in NM roots under salt stress.

5) *P. involutus*-colonized roots exhibited higher hyperpolarization of the plasma membrane compared with NM roots

6) Roots colonized by MAJ showed higher O_2 uptake at 500–900 μm from the tip.

4. Conclusion

The present study demonstrated that poplar roots colonized with the ectomycorrhizal fungus *P. involutus* exhibited enhanced net NO_3^- uptake and increased expression of several *NRTs* and *HAs*. Without a Hartig net, *P. involutus* strain NAU caused enhanced net NO_3^- fluxes and increases in *NRT* and *HA* transcript levels similar to those in roots colonized with MAJ that form typical ectomycorrhizal structures.

The study concluded that the beneficial effects of fungi on enhancing NO_3^- transport capacities were overruled by salt stress. However, the *Paxillus* strains MAJ and NAU fostered the maintenance of root NO_3^- homeostasis under salt stress due to higher surface acidity of fungus-colonized roots than of NM roots. Salt-stress-induced NO_3^- efflux from NM roots was combatted by mycorrhizal activated H^+-ATPases, which apparently caused an H^+ gradient across the plasma membrane sufficient for NO_3^- retention. The nature of how mycorrhizal fungi can influence H^+-pumping activities and the transcriptional regulation of host *PcNRTs* and *PcHAs* needs to be explored in the future, thus leaving room for more innovative opportunities for scientific researchers. Overall, the results of this study open new insights into the functioning of mycorrhizal symbioses for nutrient uptake and stress amelioration.

(Editor in charge: Xuefei Li)

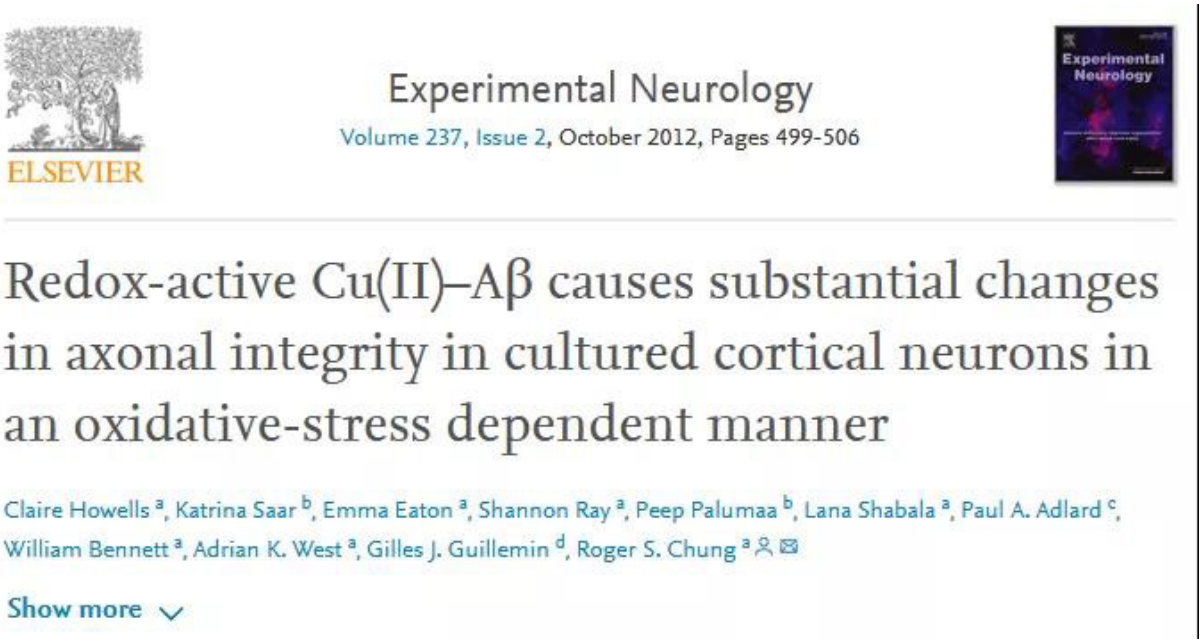
Practical Applications

Medical Applications — Alzheimer's Risk Assessment

Contributed to the editorial department of Zhongguancun NMT Industrial Alliance

The discovery of Cu-induced neuronal K^+ discharge by non-invasive micro-test technology provides evidence for the discovery that Cu and Aβ interact to induce neurodegeneration

Editor's note:
Alzheimer's disease AD, also known as senile dementia, affects about 36.5 million people worldwide, causing economic losses of 250.2 billion US dollars. According to statistics, the number of Alzheimer's patients in China was about 15.07 million in 2020, ranking first in the world. It is estimated that the number of patients will exceed 22 million in 2040, and the younger trend of Alzheimer's disease is obvious. At present, the youngest Alzheimer's patient found in China is only 39 years old. Therefore, it is imminent to carry out large-scale testing and general surveys, provide targeted prevention recommendations, and formulate relevant policies and guidelines. Recently, researchers from the University of Tasmania have made new discoveries in the mechanism of Alzheimer's disease using a non-invasive micro-test technique. The editor hereby shares this document with you.



Date received: 2022-10-10, Date received: 2022-10-10
Editor E-mail: yanhan@nmtia.org.cn

Basic Information

Topic: The discovery of Cu-induced neuronal K^+ discharge by non-invasive micro-test technology provides evidence for the discovery that Cu and Aβ interact to induce neurodegeneration

Journal: Experimental Neurology

Impact factor: 7.299 (2019)

Title: Redox-active Cu(II)-Aβ causes substantial changes in axonal integrity in cultured cortical neurons in an oxidative-stress dependent manner

Authors: Claire Howells, Katrina Saar, Emma Eaton, Shannon Ray, Peep Palumaa, Lana Shabala, Paul A.Adlard, William Bennett, Adrian K.West, Gilles J.Guillemin, Roger S.Chung

Test sample: rat cortical neurons

Ion/molecule detection indicators: K^+

K^+ test liquid composition: 40μM Cu(II)–Aβ real-time processing

K^+ flux experimental treatment method: 150 mM NaCl, 0.5 mM KCl, 0.5 mM $CaCl_2$, 1.5 mM $MgCl_2$, 1.25 mM NaH_2PO_4, 5 mM $NaHCO_3$, 25 mM glucose, pH 7.4

Abstract: The beta-amyloid (Aβ) peptide comprises the amyloid plaques that characterize Alzheimer's disease (AD), and is thought to significantly contribute towards disease pathogenesis. Oxidative stress is elevated in the AD brain, and there is substantial evidence that the interaction between Aβ and redox-active copper is a major contributing factor towards oxidative stress in AD.

The main finding of this study is that redox-active Cu(II)-Aβ causes pronounced axonal pathology in long-term neuronal cultures, including axonal fragmentation and the formation of hyperphosphorylated tau-immunoreactive axonal swellings. Notably, MAP-2 expressing dendritic processes remain largely un-affected by Cu(II)-Aβ treatment. These dystrophic axonal manifestations resemble some of the characteristic neuritic pathology of the AD brain. The present study demonstrates that Cu(II)-Aβ directly causes formation of intra-axonal swellings via the generation of free radicals and subsequent efflux of K^+ out of neurons. In conclusion, this study reports that redox-active Cu(II)-Aβ can induce substantial neurodegenerative changes in mature neurons, and may have an important role to play in the slowly progressing pathogenesis of AD.

Key words: non-invasive micro-test technology; Alzheimer's disease (AD); β-amyloid; neurotoxicity

1. Experimental results of ion/molecule flux

The study used a highly sensitive non-invasive micro-test technique to assess the impact of Cu(II)-$Aβ_{1-40}$ on K^+ ion flux in cultured cortical neurons (Figure 1). It was found that Cu(II)-$Aβ_{1-40}$ caused substantial efflux of K^+ ions (Figure 1). But $Aβ_{1–40}$ alone had no effect on K^+ flux (results not shown). To determine the importance of Cu(II)-Aβ-induced K^+ efflux to cortical neurons, the scientists assessed whether the K^+ channel inhibitor 4-aminopyridine (4-AP) could block Cu(II)-Aβ-induced cytoskeletal disruption. It was found that 4-AP (2 mM) almost completely blocked the Cu(II)-Aβ-induced formation of intra-axonal swellings.

2. Other experimental results

1) Cu(II)–Aβ exerts greater acute neurotoxicity

upon cultured cortical neurons than other biochemical forms of Aβ

2) Chronic exposure to extracellular Cu(II)-$A\beta_{1-40}$ can lead to progressive neurodegeneration in cortical neurons.

3) Chronic exposure to redox-active Cu(II)-$A\beta_{1-40}$ leads to progressive changes in axonal integrity in cortical neurons.

4) Cu(II)-$A\beta_{1-42}$ induced pathological changes in axons of cultured cortical neurons.

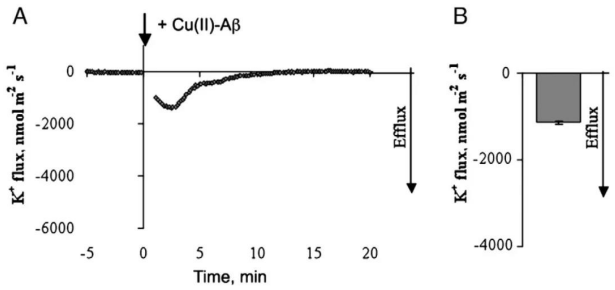


Figure 1. 40 μM Cu(II)-$A\beta_{1-40}$ produced a substantial efflux of K^+ coming out of cortical neurons. Negative values represent K^+ efflux.

3. Conclusion

This study reported that Cu(II)-Aβ can induce substantial neurodegenerative changes in neurons via oxidative stress and K^+ dyshomeostasis pathway, and may play an important role in the slowly progressing pathogenesis of AD.

(Editor in charge: Xuefei Li)

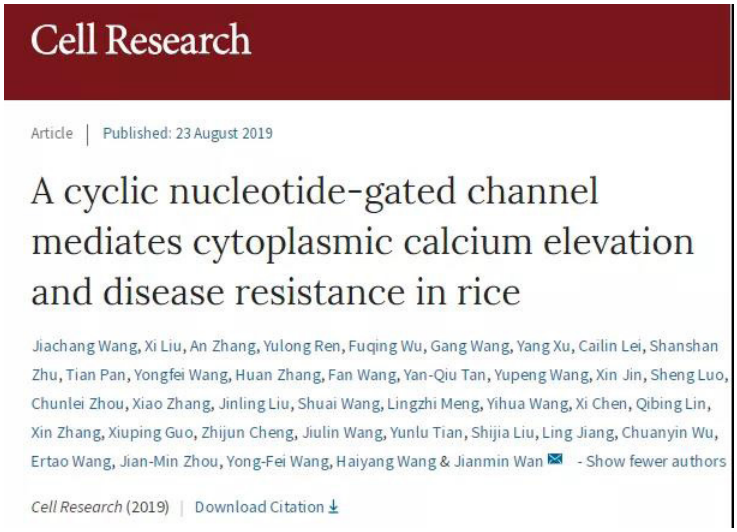
Latest News

People's Daily Online: Non-invasive micro-test technology improves research on rice disease resistance

Contributed by Yunqi Liu, Edited by Xuefei Li

Editor's note:

How to Predict a Rice Blast Ahead of Time? Gene mapping determines the identity of a "disease-resistant hero" in this article that was published on People's Daily Online. "A cyclic nucleotide-gated channel mediates cytoplasmic calcium elevation and disease resistance in rice" was first published by Academician Wan Jianmin of the Chinese Academy of Agricultural Sciences in Cell Research in 2019. There are 6 data graphs in this document, 3 of which are of Ca^{2+} flux data detected by non-invasive micro-test technology (NMT). This proves that non-invasive micro-test technology provided key evidence for the identification of CNGC9, an important calcium channel in the rice disease resistance process. The editor reviews this article here for the benefit of readers.


Cell Research

Article | Published: 23 August 2019

A cyclic nucleotide-gated channel mediates cytoplasmic calcium elevation and disease resistance in rice

Jiachang Wang, Xi Liu, An Zhang, Yulong Ren, Fuqing Wu, Gang Wang, Yang Xu, Cailin Lei, Shanshan Zhu, Tian Pan, Yongfei Wang, Huan Zhang, Fan Wang, Yan-Qiu Tan, Yupeng Wang, Xin Jin, Sheng Luo, Chunlei Zhou, Xiao Zhang, Jinling Liu, Shuai Wang, Lingzhi Meng, Yihua Wang, Xi Chen, Qibing Lin, Xin Zhang, Xiuping Guo, Zhijun Cheng, Jiulin Wang, Yunlu Tian, Shijia Liu, Ling Jiang, Chuanyin Wu, Ertao Wang, Jian-Min Zhou, Yong-Fei Wang, Haiyang Wang & Jianmin Wan - Show fewer authors

Cell Research (2019) | Download Citation

Basic Information

Topic: The molecular mechanism of calcium ions to activate the immune system

Journal: Cell Research

Impact factor: 17.848

Title: A cyclic nucleotide-gated channel mediates cytoplasmic calcium elevation and disease resistance in rice

Authors: Jiachang Wang, Xi Liu, An Zhang, Yulong Ren, Fuqing Wu, Gang Wang, Yang Xu, Cailin Lei, Shanshan Zhu, Tian Pan, Yongfei Wang, Huan Zhang, Fan Wang, Yan-Qiu Tan, Yupeng Wang, Xin Jin, Sheng Luo, Chunlei Zhou, Xiao Zhang, Jinling Liu, Shuai Wang, Lingzhi Meng, Yihua Wang, Xi Chen, Qibing Lin, Xin Zhang, Xiuping Guo, Zhijun Cheng, Jiulin Wang, Yunlu Tian, Shijia Liu, Ling Jiang, Chuanyin Wu, Ertao Wang, Jian-Min Zhou, Yong-Fei Wang, Haiyang Wang, Jianmin Wan

Test sample: rice mesophyll cells

Ion/molecule detection indicators: Ca^{2+}

Ca^{2+} flux experimental treatment method: rice seedlings, 10μM chitin or 10μM flg22 peptide transient flux

Ca^{2+} flux test liquid composition: 0.2mM $CaCl_2$, 0.1mM NaCl, 0.1mM $MgCl_2$ and 0.1mM KCl, pH 5.2

Abstract: The transient elevation of cytoplasmic calcium is essential for pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI). However, the calcium channels responsible for this process remain unknown. The results showed that rice *CDS1* (*CELL DEATH and SUSCEPTIBLE to BLAST 1*) encoding OsCNGC9, a cyclic nucleotide-gated channel protein, positively regulates the resistance to rice blast disease. The results indicated that OsCNGC9 mediates PAMP-induced Ca^{2+} influx and that this event is critical for PAMPs-triggered ROS burst and induction of PTI-related defense gene expression. The study further showed that a PTI-related receptor-like cytoplasmic kinase OsRLCK185 physically interacts with and phosphorylates OsCNGC9 to activate its channel activity. These results suggest a signaling cascade linking pattern recognition to calcium channel activation, which is required for initiation of PTI and disease resistance in rice. The Ca^{2+} influx of rice mesophyll cells under different treatments was detected by non-invasive micro-test technology (NMT). The discovery that OsCNGC9 can mediate Ca^{2+} influx in rice PTI lays the foundation for understanding the role of Ca^{2+} signaling in rice disease resistance.

Key words: non-invasive micro-test technology; calcium ion flux; rice mesophyll cells; transient flux

1. Experimental results of ion/molecule flux

The study used NMT technology to investigate whether OsCNGC9 could mediate Ca^{2+} influx in PTI by measuring the dynamic changes of Ca^{2+} flux in mesophyll cells after treatment with two PAMPs (chitin or flg22). Previous studies have shown that these PAMP elicitors can trigger PTI signaling in plants. Upon chitin or flg22 stimulation, wild type (WT) mesophyll cells (but not rice mutant, *cds1 (cell death and susceptible to blast 1)* mesophyll cells) exhibited robust and fast Ca^{2+} influx (Figure 1a,b). These results suggest that OsCNGC9 can mediate Ca^{2+} influx in rice PTIs, whereas this ability is impaired in the *cds1* mutants.

Ca^{2+} flux analysis showed that Nipponbare mesophyll cells (but not *Osrlck185/55* double mutant mesophyll cells) exhibited fast Ca^{2+} influx upon chitin stimulation (Figure 2e). In addition, after chitin treatment of *Oscerk1* knockout mutants, no obvious Ca^{2+} influx was observed (Figure 2f).

After PAMPs stimulation, mesophyll cells of *OsCNGC9-OE* transgenic plants showed stronger Ca^{2+} influx compared with Kitaake plants (Figure 3f,g).

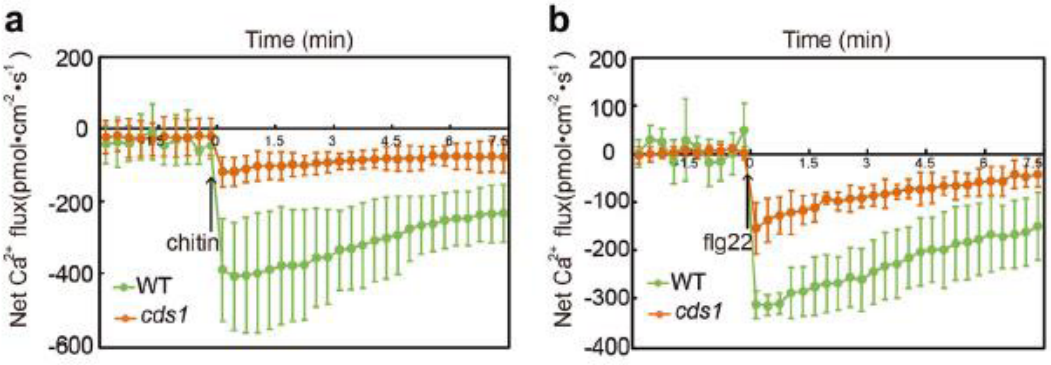


Figure 1. OsCNGC9 is required for Ca^{2+} influx in response to PAMPs. Positive values represent Ca^{2+} efflux, negative values represent Ca^{2+} influx.

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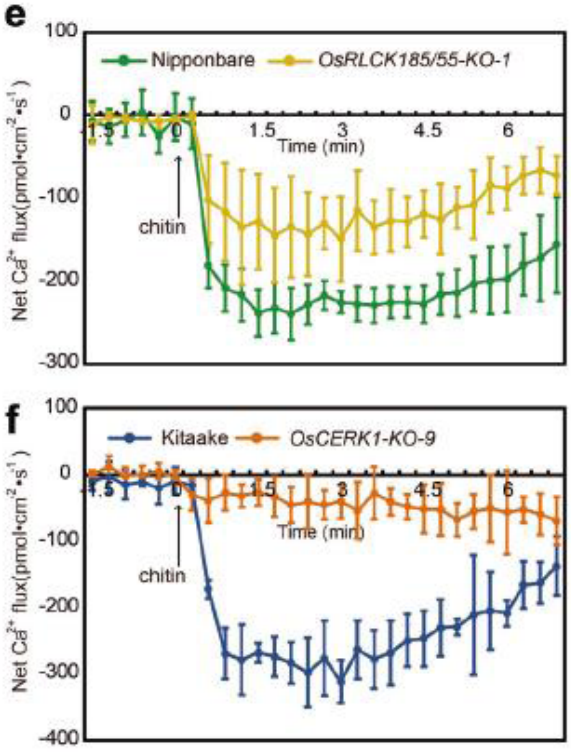


Figure 2. *Osrlck185/55* double mutant plants show reduced chitin-induced Ca^{2+} influx. Positive values represent Ca^{2+} efflux and negative values represent Ca^{2+} influx.

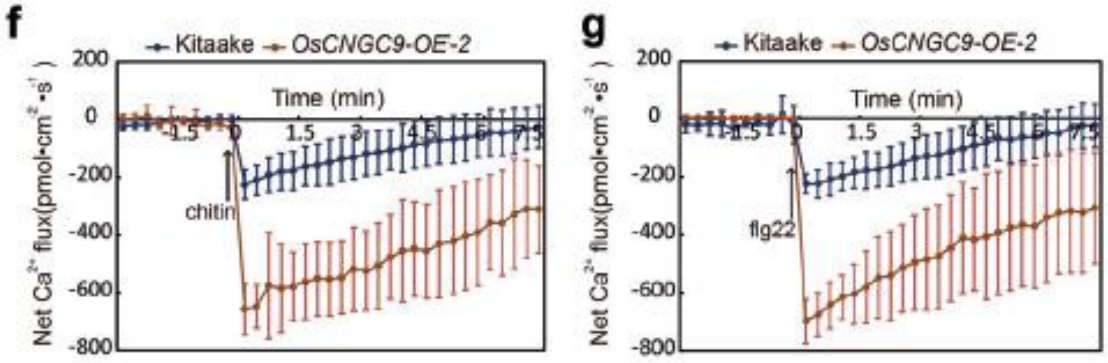


Figure 3. Comparison of Ca^{2+} influx in the mesophyll cells of Kitaake and *OsCNGC9-OE* transgenic plants after chitin or flg22 treatment. Positive values represent Ca^{2+} efflux, negative values represent Ca^{2+} influx.

New to NMT

How to choose a non-invasive micro-test system that suits you?

Weiwei Guo
Xuyue (Beijing) Science & Technology Co., Ltd., 100080

In June 2021, after review by the Ministry of Science and Technology, Chinese scientists were found to be the international leading scientists in the usage of NMT: Non-invasive Micro-test Technology, its applications, products, and imOmics! This achievement has once again verified the general consensus of the scientific community: researching functions of the living body using NMT as the core technology is the developing trend of life science research. It is the tool that life scientists around the world are waiting for! Owning an NMT system has become a necessary choice to keep up with the trend, seize the opportunity, and move towards the international leading level.

As the saying goes: "The one that suits you is the best!" There are seven series of NMT systems of the YoungerUSA and Xuyue Co. brands with dozens of models to choose from.

How to quickly choose a cost-effective system and model most suitable to you?


证书号编号2021112X5478

科学技术成果评价证书

中科评字【2021】第5478号

成果名称：非损伤微测技术及其应用

完成单位：旭月（北京）科技有限公司

主要完成人：许 越、张增凯、叶 斌、刘鹿琦
李 磊、巨尚宇、吴红建、马 跃

成果水平：国际领先

发证日期：2021年06月24日

评价机构：中科合创（北京）科技成果评价中心

签发人：

（盖章）

1) If you are able to spend a large amount of funds, the latest eighth-generation intelligent Non-invasive Micro-test System is a must. This equipment not only has all regular functionalities of an NMT system, but it also integrates cutting-edge intelligent technologies such as image recognition, automatic positioning, automatic sample change, and automatic detection. It also supports high-throughput detection in well-plate containers of various specifications.

Eighth generation intelligent Non-invasive Micro-test System

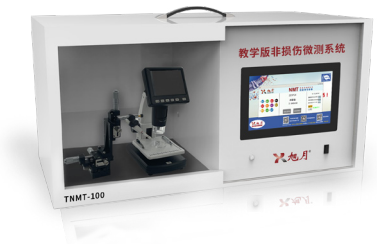
2) If you have sufficient funds, the NMT150 series and NMT100S series are cost-effective choices. NMT150 can detect 9 ions (calcium, hydrogen, potassium, sodium, ammonium, nitrate, cadmium, magnesium, and chlorine), and 3 molecules (oxygen, hydrogen peroxide, and auxin IAA). If we were to call the NMT150 the almighty system type, then NMT100S would be the almighty research and development platform. This device not only has all the same functions of NMT150, but also has a development and upgrade module, which allows you to later add detections for lead, copper, and membrane potential. It also allows for upgrades to any additional ion/molecule detection indicators and functions that may be developed in the future. Additionally, NMT100S includes aSMS, an automatic sensor filling system, which can greatly improve the efficiency and repeatability of experiments.

3) If you don't have the largest budget, then the NMT live workstation could be a good choice. This workstation is equipped with two standard indicators and works with semi-automatic operation. Not only is the price low, but other indicators, functions, and automation features can be upgraded later. In addition, all NMT systems can be imported or domestically selected.

NMT live workstation

4) If you have limited funds and do not want to miss the opportunity to be one step ahead of others, or you have teaching tasks but no supporting teaching equipment, the Teaching Edition of Non-invasive Micro-test System is your best choice. The Teaching Edition of the Non-invasive Micro-test System takes up little space and is easy to install. Working with NMT-related teaching materials, it can be used for teaching non-invasive micro-test technology, experimental processes demonstrating, non-invasive micro-test system experiments, operation training, etc. It can also meet some scientific research needs (such as detecting the ion flux of plant root samples such as rice, wheat, and corn). This system can also be upgraded to add more detection functions later.

I bet you already have a favorite model in mind, want to get the information? Contact us now! 010-8262 2628-ext. 1.



Teaching Edition of Non-invasive Micro-test System

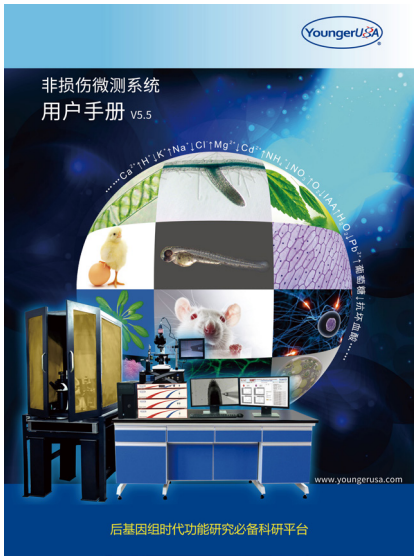
(Editor in charge: Xuefei Li)

Corresponding author E-mail: xuyue_weiwei@126.com
Tel: 18501056730

Equipment Maintenance - Tips for NMT System Maintenance and Repair!

Xiaoyu Ju
Xuyue (Beijing) Science & Technology Co., Ltd., 100080

The best way to prolong the service life of the NMT system is to strictly follow the procedures and practice good habits as directed in the user manual!



Anyone who has purchased a Non-invasive Micro-test System should be familiar with this user manual. As long as the user purchases a system, no matter the model, a copy will be provided with the system (the image shown above is that of the latest version of the Chinese manual).

But how many actually read this manual from cover to cover? I'm guessing not very many. After the system operation training, most people have mastered the basic test operation process, and may never read this manual again, thinking they don't need it.

In fact, this is a big mistake! This manual is the accumulation of more than 10 years worth of experience from YoungerUSA and Xuyue (Beijing) Sci. & Tech. Co., Ltd.'s use and knowledge of the technology. In addition to basic testing operations it also records in detail some important precautions to take during the test and solutions to problems you may encounter. Especially in the process of system operation, there are some operating procedures and usage specifications that you may not pay much attention to on a regular basis, but if you don't pay attention for too long, it may cause great damage to the system hardware, shorten the service life of accessories, and/or cause system failures and other problems.

Therefore, before actually operating the system, carefully reading the user manual is a necessary step, please take it seriously. Strictly follow the procedures and specifications in the manual during operation, and develop good usage habits. This will not only prolong the service life of the system, but also greatly help your experiments. At the same time, new users should choose Xuyue (Beijing) Sci. & Tech. Co., Ltd.'s formal training courses when learning the system operation to ensure the best results from your training.

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Daily use and maintenance of the 3D Manipulator

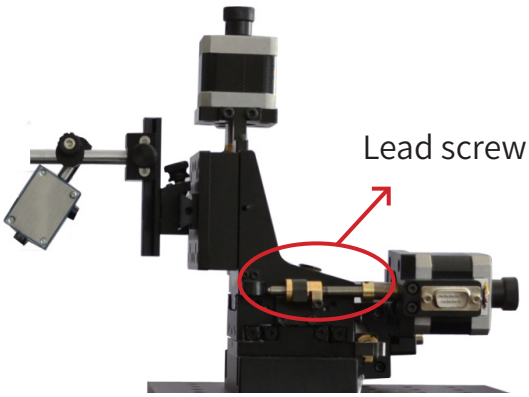
The 3D Manipulator is a very important accessory in the NMT system. It is responsible for the important function of guiding the sensor to automatically move to the designated position and collecting flux data. Therefore, the daily maintenance of the 3D Manipulator is also very important:

1. Pay attention to dust

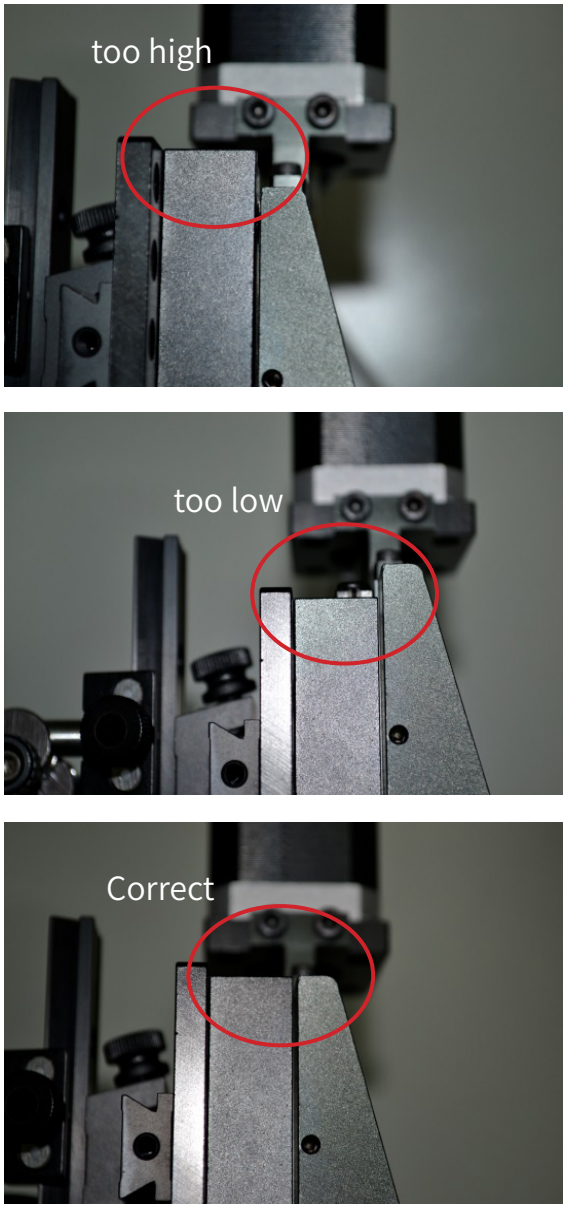
It is necessary to regularly blow off the dust on the 3D Manipulator with an air squeeze blower or an air compression can (purchased separately). This is especially important on the three lead screws.

2. Maintenance of the lead screws

First blow off any dust with a squeeze blower / air compression can, then wipe the entire lead screw thoroughly with a cotton swab dipped in absolute ethanol.



Electric 3D Manipulator



Electric 3D Manipulator

3. Reset the motion translation stages

During normal use, be careful not to let any motion translation stage of X/Y/Z stay close to the range for a long time (you can use the slider to make rough adjustments, and then use the 3D Manipulator knob for fine adjustment). After each use, it is necessary to restore all motion translation stages to their original positions, as shown in the images:

4. Routine maintenance

If you find that the sensor is shaking, isn't going straight, is stuck or the movement distance is inconsistent, it means there is a problem with the electric 3D Manipulator, and you need to repair it yourself (see Chapter 2, Section 3 of the manual) or contact the manufacturer for after-sales maintenance.

5. Routine maintenance

There are some small sliders attached to the 3D manipulator, which may become loose. These sliders have a small hexagon socket screw to adjust the tightness when the slider moves. If the slider is too loose for a long time, it can be improved by tightening the screw using the hexagonal screwdriver provided with the system (Metric 1.3mm), as shown in the picture

Slider and adjusting screw on the 3D Motion slider

6. Motion Controller

Never manually rotate the knob of the electric 3D manipulator when the motion control switch is turned on!

Motion Controller switch turned on

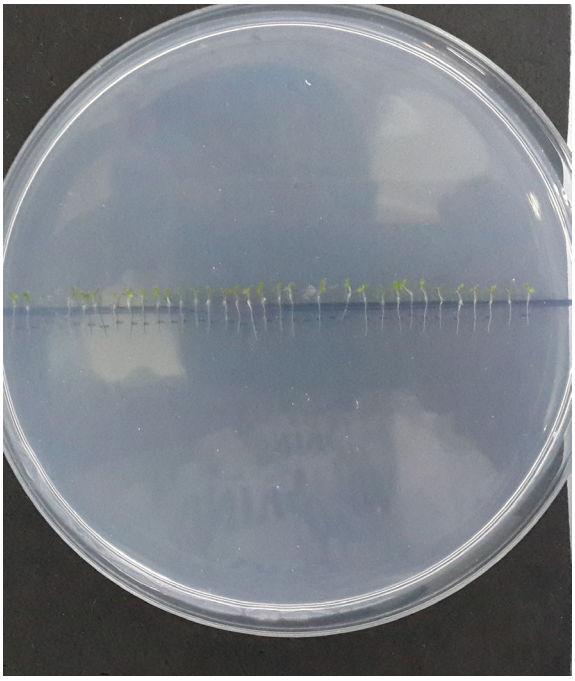
(Editor in charge: Xuefei Li)

Sample preparation for experimental design

Bin Ye
Xuyue (Beijing) Science & Technology Co., Ltd., 100080

Common culture methods include the following: hydroponics (liquid medium), plate culture (solid/semi-solid medium), and soil/sand culture. Hydroponic samples are easiest to work with since non-invasive micro-test systems require the sample to be maintained in a liquid environment during the assay.

Among the detectable sample parts, the detection of roots often involves tissue culture seedlings or soil/sand culture seedlings. If the sample to be tested cannot be hydroponically grown, try soil/sand culture and then hydroponics hair roots. The root of the sample cultivated in this way is intact, so as to avoid affecting the selection of test points. And the substances attached to the surface can also be removed through the test liquid balance process. Thus, the stability and accuracy of the test data are guaranteed. However, if the sample must be taken from the soil or plate before the test, remember to be careful when sampling, so as not to damage the sample due to operational errors. These errors would make it so true and reliable experimental results cannot be obtained. Also, it is recommended that you rinse the roots of the sample you will be testing more carefully and increase the equilibration time of the sample appropriately when sampling.



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Data analysis of experimental design - the meaning of positive and negative values of flux data

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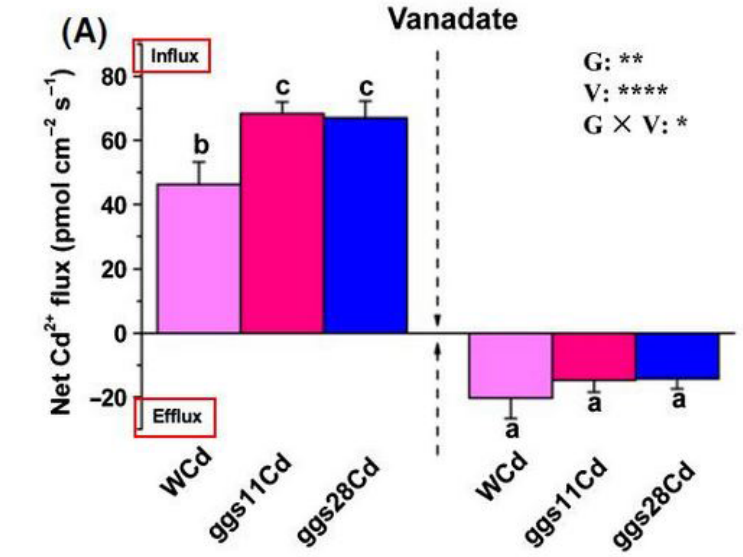
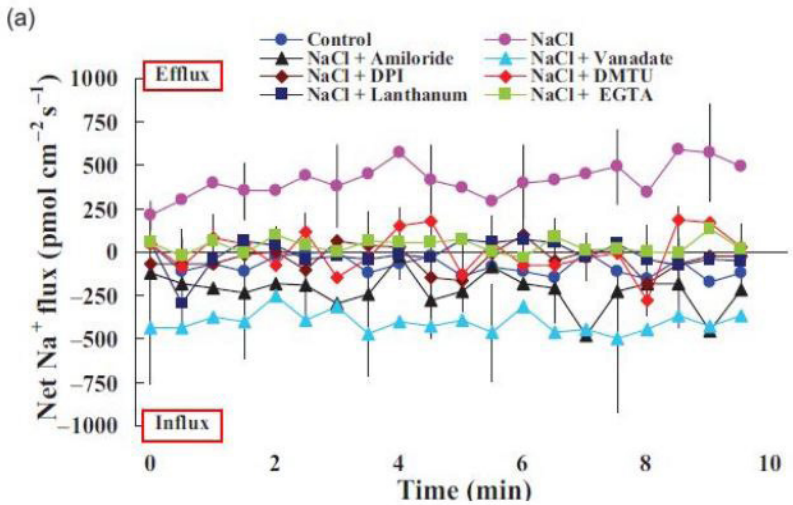
1) When looking at data from a Non-invasive Micro-test System, whether it is experimental results provided to you by the YoungerUSA (Xuyue Beijing) Testing Center, or data obtained using your own NMT system, positive values always indicate efflux (flow from inside to outside) and negative values always represent influx (absorption).

2) However, if you are looking at literature, this is not always the case so you must pay close attention to the labels within the literature. The figures below show two different pieces of literature that label influx and efflux in different ways.





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Tips for Experimental Design

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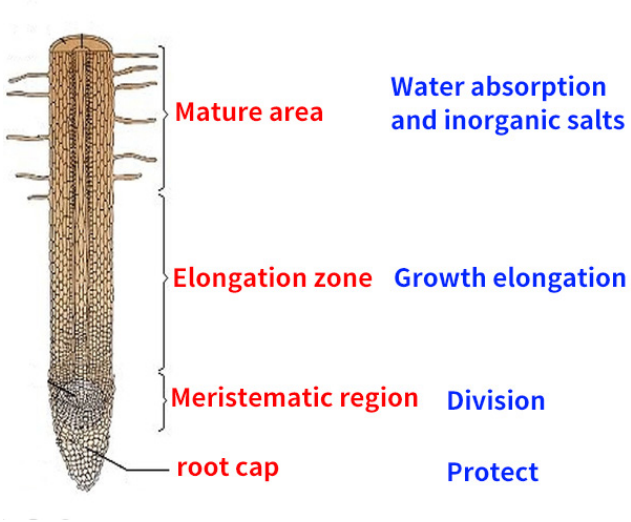
Why use the root maturation zone as the Cd^{2+}/Cu^{2+}/Pb^{2+} detection site



1. The maturation zone is the main site for the stable absorption of heavy metals

2. Why it is not recommended to choose the root zone with the largest absorption rate as the detection site

1) According to the statistics of experimental data, the rate of absorption of heavy metal ions in the meristematic zone and elongation zone is generally greater than that in the maturation zone.

2) Taking rice as an example, on a root, the meristematic zone and elongation zone account for less than 1% of the total root length.

3) If the meristematic area with the highest absorption rate is used as the fixed-point detection site, and the Cd^{2+} absorption rate of the meristematic area of Group A is measured to be greater than that of Group B, can it be said that the rate of Cd^{2+} absorption of Group A is greater than that of Group B? No, because the total length of the meristematic zone is 300-500 μm, no matter how high the absorption rate is, the influence on the Cd^{2+} content of the whole root is minimal.

4) Therefore, the Cd^{2+} uptake rate in the maturation area best represents the Cd^{2+} uptake in the whole root.

3. If the maturation area is measured, at which point should it be set?
According to the statistics of different materials, 99% of the points from 2500 μm away from the apex are typical maturation areas, and all the detected positions greater than 2500 μm away from the apex are in the maturation area. If the total root length is greater than 1cm, it is recommended to choose a position of 5000μm.

4. Is it necessary to scan to detect the absorption rate of Cd^{2+}/Cu^{2+}/Pb^{2+} on the root surface?
According to the above analysis, for the root-surface heavy metal absorption experiment, point sweeping is not necessary.

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