After nearly one and half decades of study on nitric oxide (NO) research, we are now clear that NO is one of the major signal molecules that play a role in plant growth development and stress physiology. In order to understand the role of NO, it is very important to precisely quantify NO. Due to a very low half-life and high reactivity, it is very difficult to precisely quantify NO. This problem is further complicated by multiple sources of NO generation in plants. Free radicals play an important role in scavenging NO; therefore cell conditions play a role in the determination of NO.

Nearly a dozen methods are currently available for the quantification of NO. Each method has several advantages and disadvantages. This book will provide a foundation for all nitric oxide researchers to choose the required method for their research work.

For a new researcher who plans to measure NO in plant materials, it may be difficult to select a method from a number of methods. In this book, Yamasaki et al. provide a practical guide on the choosing of a technique for measuring NO from plant materials.

Chemiluminescence is one of the reliable methods in which nitric oxide reacts with ozone and creates chemiluminescence. In this method, one can detect the gaseous form of NO that is released into the atmosphere. Due to high reactivity, NO can be oxidized, and it can be detected by indirect chemiluminescence. The sum of NO released from direct and indirect chemiluminescence can reflect the total amount of NO. The detailed overview of this method is presented by Wany et al. in the second chapter. Chemiluminescence can be used to detect NO from purified enzymes, and it is also possible to detect scavenging of NO by various cell components. Mishra et al. explore these procedures.

The most widely used method for the measurement of NO is the diaminofluorescence method. The excellent advantage of DAF-based dyes is due to its easy applicability by pipette to the tissue of interest and observation of the emitted NO fluorescence using any laboratory fluorescence and confocal microscope. Site-specific NO production (localization) is important in many studies. DAF is the best dye for such studies. Sensitivity of these dyes is in Nano molar range, and no additional fluorescence is observed with several reactive oxygen and nitrogen species such as NO$^-$, NO$_3^-$, H$_2$O$_2$, and ONOO$. Wany and Gupta present a stepwise protocol for localization of NO in roots. Agurla et al. present a method for the detection of NO in guard cells using a DAF-2DA method.

As with every method, DAF has several disadvantages too. In this regard, Ruemer et al. explain that DAF dyes do not only react with nitric oxide but also react with peroxidase enzyme and hydrogen peroxide. Since DAF has several disadvantages, an alternate solution obtained by Jain and Bhatla involves a simple, two-step synthesis, characterization, and application of MNIP-Cu {Copper derivative of [4-methoxy-2-((1H-naphthol [2,3-d] imidazol-2-yl)phenol]} for specific and rapid binding with NO, which can be detected by epifluorescence microscopy and confocal laser scanning microscopy (CLSM). The advantage is that it can detect NO under normoxic and anoxic conditions.

There are also several other methods available. One of the next reliable methods is EPR spectroscopy. In this context, Maia and Moura chapter provide comprehensive information about EPR spectroscopy and of some spin-trapping methodologies to study NO. They also discuss “strengths and weaknesses” of iron-dithiocarbamates utilization, the NO traps, and
also provide a detailed description of the method to quantify the NO formation by molybdoenzymes. Galatro and Susana next provide a stepwise protocol for the measurement of NO from chloroplasts.

Mandon et al. provide extensive information on a laser-based method for the detection of nitric oxide. This works on a principle in which changes in light intensities or polarization occur when the laser light is interacting with NO molecules. This laser-based photoacoustic method is very popular. The molecules absorb the energy from the laser light, and a pressure wave (sound wave) is generated due to their thermal expansion. The amplitude of this sound wave, proportional to the amount of molecules, is detected by a sensitive microphone.

The Griveau et al. chapter describes the principle, the preparation, and the use of a home-made electrode displaying a high specificity for NO detection in plant cell suspensions. This chapter presents an exhaustive introduction regarding NO measurement in plants and the use of electrodes as an alternative method. This allows the reader to understand the problems related to the detection of NO due to its high reactivity.

The Barroso et al. article provides two complementary approaches which can be extensively useful for studying the content and distribution of S-nitrosothiols in different plant tissues and species under various conditions.

The Noelia et al. chapter describes detailed protocols to study the expression and characterization of the enzymatic activity of NOS from *O. tauri*. The authors demonstrate NOS activity using an oxyhemoglobin assay, citrulline assays, and the NADPH oxidation for in vitro analysis, fluorescent probes, and Griess assay for in vivo determination. This chapter further discusses the advantages and drawbacks of each method. Loake et al. describe a procedure for the identification of S-nitrosothiols to study the roles of protein S-nitrosylation in the immune responses of *Arabidopsis thaliana* and other organisms. This technique employs a modified version of the biotin-switch technique, which we termed the sequential cysteine blocking (SCB) technique, encompassing the sequential redox-blocking of recombinant proteins followed by LC-MS/MS analysis.

S-nitrosoglutathione reductase (GSNOR) is considered a key enzyme in the regulation of intracellular levels of S-nitrosoglutathione and protein S-nitrosylation. Kubienová presents optimized protocols to determine GSNOR enzyme activities by spectrophotometry coupled with activity staining after the native polyacrylamide gel electrophoresis. Peroxynitrite is formed in a reaction between nitrite oxide and superoxide, and the detection of this compound is crucial in various situations such as plant pathogen interactions and nitrosative stress. Bellin et al. described a detailed method to measure peroxynitrite.

This methods book contains detailed information about methods in plant nitric oxide research and is very helpful for all researchers working and intending to work on plant nitric oxide research.

Reputed scientists from 11 different counties have contributed to this book to whom I am extremely thankful. I thank Aprajita Kumari for help in the formatting of the manuscripts, and I owe my heartfelt gratitude to John Walker for his support and timely advice during the preparation of this book.

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