Chapter 2
Nitric Oxide

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Introduction

Nitric oxide (NO) is a free radical gas, which acts as a multifunctional signaling molecule in plants and animals (Wendehenne et al. 2001). Ya'acov and Haramaty (1996) reported emission of NO from plants; since then NO has been the subject of several studies on agriculture and food technology as an alternative to chemical treatments. At the beginning, NO studies in plants were focused on the phytotoxic properties of the oxides of nitrogen (NO_2, N_2O_3, NO_2^-, NO_3^-) and their effect since considerable amounts of them are produced naturally (Lamattina et al. 2003). Since then, subsequent investigations have linked endogenous NO to several physiological processes including modulation of endogenous ethylene and vegetative stress, water loss, root growth and fruit and flower formation, plant immunity, anthocyanin

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biosynthesis, and chlorophyll production (Ku et al. 2000; Ya'acov and Pinchasov 2000; Del Río et al. 2004; García-Mata and Lamattina 2001).

NO plays an important role in many physiological processes in plants, and it can produce either beneficial or harmful effects, depending on the concentration and location in plant cells. NO present in the plant tissue could alleviate the harmfulness of reactive oxygen species (ROS), and reacts with other target molecules, and regulates the expression of stress-responsive genes under various stress conditions (Qiao and Fan 2008). It also plays important role in control various pathophysiological and developmental processes in plants (Lamattina et al. 2003; Neill et al. 2003). Additionally, several studies demonstrated NO effects on senescence and ripening in several fruits by suppressed respiration rate, ethylene biosynthesis, disease incidence, delayed peel color changes, and reduced enzyme activity (Ku et al. 2000; Duan et al. 2007; Manjunatha et al. 2010).

Postharvest application of NO has been shown to be effective in extending the postharvest life of a range of flowers, fruits, and vegetables when applied as a short-term fumigation treatment at low concentrations. This chapter is focused on the effect that NO application in fresh fruits and vegetables has in its metabolism and how these metabolic responses affect postharvest quality and product safety. However, the gaseous nature of NO is a barrier to its commercial usage. The construction of the infrastructure necessary to undertake large-scale fumigation is substantial and requires a measure of technical operational expertise. Efficient usage is particularly problematical when the site of production is geographically isolated and in developing countries. Therefore, a new alternative has emerged and NO donor technology was developed. In that case, solid compounds that store NO chemically but allow it to be regenerated under appropriate physical conditions are applied (Hou et al. 1999). Several examples of NO application are presented, along with a detail of the application methodology and the compounds used as NO donors.

Biological Factors Involved in the Senescence of Harvested Fruits and Vegetables

The causes of postharvest losses in quality fresh fruits and vegetables are many and depend on the type of product, its morphological structure, composition, developmental stages, and general physiology (Kader 2002). However, the main causes of deterioration are a result of their continuous metabolic activity after harvest, among which respiration, ethylene production, and enzymatic browning are the most important, and it is well established that postharvest quality of fresh fruits and vegetables cannot be improved further but it can be retained till their consumption if the rate of metabolic activities is reduced by applying the appropriate postharvest handling technologies (Kader 2002).

One of the main parameters while determining the metabolic activity of fruits and vegetables is their respiration rate, which is usually associated with the commodity deterioration (Wu 2010). Respiratory rate of produce after harvest is reversely proportional to its storage life, meaning the higher the rate of respiration, the shorter the storability (Kader and Saltveit 2003). Respiration rate of a produce is dependent on a wide range of variables, including commodity, maturity state, and several environmental factors. Among the external factors affecting respiratory rate of fresh fruits and vegetables after harvest, temperature and gas composition surrounding the horticultural produce are two very important variables to be taken into consideration.

Ethylene (C_2H_2) is a gaseous phytohormone produced for all tissues of higher plants and by some microorganisms (Cao et al. 2008) and it regulates many aspects of growth, development, and senescence and is physiologically active in trace amounts (<0.1 ppm). Although ethylene action may have beneficial effects on certain product attributes, as stimulating ripening of climacteric fruit or promoting de-greening of citrus (Saltveit 1999), in most harvested horticultural products, ethylene is associated with degradation, fruit ripening, senescence, and abscission of plant organ (Saltveit 1999; Kader and Saltveit 2003). Ethylene accelerates chlorophyll degradation causing yellowing of green tissues, and induces abscission of leaves and flowers, softening of fruit, and several physiological disorders (Kader and Saltveit 2003). Since exposure to ethylene can be detrimental to most fresh horticultural commodities, ethylene is of major concern to all produce handlers during postharvest storage and handling.

Browning in fresh vegetables, also a detrimental quality, is a result of the oxidation of phenolic compounds (Tomás-Barberán and Espin 2001). Plant cells contain phenolic compounds which, in the presence of oxygen, easily oxidize to quinones by the action of enzymes, mainly polyphenol oxidase (PPO) and peroxidase (POD). Quinones in turn oxidize and polymerize producing brown compounds, which are responsible for superficial and/or deep tissue browning. Therefore, browning in plants is a result of cell injury in their tissues due to lost compartmentalization. When cellular damage occurs, the disruption of cellular compartments and the loss of membrane integrity allow the enzymes (such as PPO) and substrates (such as polyphenols) to mix and hence initiate several browning reactions (Duan et al. 2007; Pristijono et al. 2006).

Chemical Properties of NO

Nitric oxide (NO) is a small, uncharged, scarcely polar molecule; thus it can freely diffuse across membranes from one compartment to the other. Under normal atmosphere conditions, NO is a free radical lipophilic diatomic gas. Its small Stokes' radius and neutral charge allow rapid membrane diffusion (Lamattina et al. 2003). From a chemical point of view it is a free radical; however, it can adopt an energetically more favorable electron structure by gaining or losing an electron, so that NO can exist as three interchangeable species: the radical ($NO^{\bullet}$), the nitrosonium cation (NO^+), and the nitroxyl radical (NO−) (Hasanuzzaman et al. 2010; Neill et al. 2003; Simontacchi et al. 2013) (Fig. 2.1).

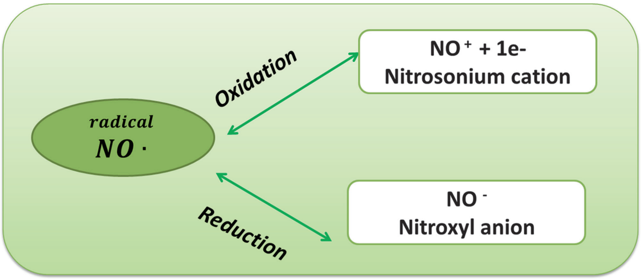

Fig. 2.1 NO forms with biological activity

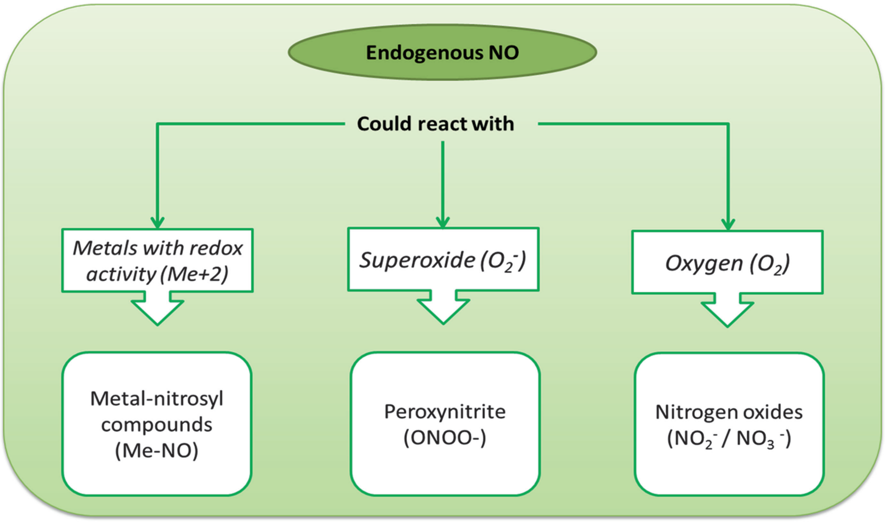

Fig. 2.2 Chemical reactions of endogenous NO and its products

NO reacts with oxygen to produce other nitrogen oxides with different stabilities and decay rates, its reactivity related to its single electron in its 2p-¼ antibonding orbital (Stamler 1994; Lamattina et al. 2003). The un-paired electron is responsible for its elevated reactivity with oxygen (O_2), superoxide ($O_2^{\bullet -}$), other N base compounds, and transition metals (Fig. 2.2). The complex redox chemistry of NO contributes to provide a general mechanism for cell redox homeostasis regulation, which can exert a protective action against oxidative stress (Stamler 1994; Wink et al. 1993).

The Fenton-type reaction between H_2O_2 and redox-active metals produces the hydroxyl radical ($OH^{\bullet}$), which is a powerful oxidant (Stamler 1994). NO can attenuate the Fenton oxidative damage preventing the formation of oxidants by scavenging either iron or superoxide and thus limiting hydroxyl radical formation (Wink et al. 1993). Several studies have also demonstrated that NO can act as a chain-breaking antioxidant arresting lipid peroxidative reactions (Rubbo et al. 1994). On the other hand, NO can also react with superoxide anion ($O_2^{\bullet-}$), thus leading to the formation of the strong oxidant peroxinitrite ($ONOO^-$), which can oxidize thiol residues to sulfenic and sulfonic acids producing a cytotoxic effect (Radi et al. 1991).

Nitric Oxide in Plants

Some years ago, NO was considered as a highly toxic compound due to its free radical nature (Beckman et al. 1990). However, the discovery of NO signaling role in regulation of cardiovascular system has changed the paradigm concerning the cytotoxicity (Loscalzo and Welch 1995; Balligand et al. 1993). Later, the discovery of its biological functions has been elucidated (Ya'acov 1996). Since then, researching the function and metabolism of NO in plants has gained considerable attention in recent years. Some examples of its application are presented in Table 2.1.

NO has shown to play a crucial role in the regulation of several plant physiological processes, including growth and development. NO can act as a key signaling molecule in different intracellular processes in plants (Crawford and Guo 2005; Ya'acov 1996; Wendehenne et al. 2004; Lamattina et al. 2003). Moreover, NO is emitted from plants under stress situations, as well as under normal growth conditions (Arasimowicz and Floryszak-Wieczorek 2007).

The physiological function of NO in plants implicates the induction of different processes, including the expression of defense-related genes against abiotic and biotic stress (Durner et al. 1998; Wendehenne et al. 2004; Klessig et al. 2000) and apoptosis/programmed cell death (Clarke et al. 2000; Beligni et al. 2002), maturation and senescence (Badiyan et al. 2004; Singh et al. 2009), stomatal closure (García-Mata and Lamattina 2001; Neill et al. 2002), seed germination (Beligni and Lamattina 2000; Kopyra and Gwóźdź 2003), root development (Correa-Aragunde et al. 2004; Pagnussat et al. 2002), and so many other examples. However, the effects of NO on different types of cells have been proved to be either protective or toxic, also depending on the concentration and situation (Hasanuzzaman et al. 2010). As a consequence, more research is needed to assess the specific effects of NO in different fruits and vegetables in order to be successfully applied.

As shown in previous studies, endogenously produced NO gas appears to be a natural plant growth regulator in a wide variety of both climacteric and non-climacteric fruits, flowers, vegetables, and legume sprouts (Leshem et al. 2000; Ya'acov et al. 1998; Bredt 1999). In biological systems, NO can be generated enzymatically or nonenzymatically (Wendehenne et al. 2001). The most extensively described NO-producing enzymes have been nitric oxide synthase (NOS) and nitrate reductase (NR) (Hasanuzzaman et al. 2010).

Published data suggest that drought and salinity induce NO generation which activates cellular processes that afford some protection against the oxidative stress associated with these conditions (Neill et al. 2008). Their study suggested an emerging model of stress responses in which ABA has several ameliorative functions. These include the rapid induction of stomatal closure to reduce water

Table 2.1 Effects of NO application in fruits and vegetables

NO donor	Treated produce	Postharvest effect	References
Endogenous NO, induced by chilling stress	Loquat (*E. japonica* Lindl. cv. Luoyangqing)	Alleviated chilling injuries	Xu et al. (2012)
NO gas	Strawberry (*Fragaria ananassa* Duch cv. Pajaro)	50 % extension of shelf life (less mold growth, delayed softening, and color changes)	Wills et al. (2000)
	Cucumber (*Cucumis sativus* L.)	Alleviate chilling injuries system (maintained membrane integrity). Delayed lipid peroxidation, $O_2^{\bullet -}$ and H_2O_2 production. Increased SOD, CAT, APX, and POD activities. Increased DPPH-radical scavenging activity	Yang et al. (2011)
	Chinese Winter jujube (*Zizyphus jujuba* Mill. Cv. Dongzao)	Reduced PPO and PAL activities. Delayed maturation (maintaining low levels of anthocyanins, TF, TSS). Reduced AA oxidation	Zhu et al. (2009)
	Tomato (*Lycopersicum lycopersicum* L. cv. Abunda)	Reduced net photosynthesis	Bruggink et al. (1988)
	Japanese plums (*Prunus salicina* Lindell)	Reduced respiration rate, ethylene production. Delayed ripening by 2–3 days (no color changes or tissue softening). Alleviated chilling injuries symptoms	Singh et al. (2009)
	Peach (*Prunus persica* L. Batsch., cv. Feicheng)	Reduced LOX and ACC oxidase activities and ethylene production	Zhu et al. (2006)
	Mango (*Mangifera indica* L. cv. Kensington Pride)	Alleviated chilling injuries. Suppressed ethylene production. Delayed fruit softening and color development	Zaharah and Singh (2011)
	Mushrooms (*Russula griseocarnosa*)	Increased AOX. Increased TF and flavonoids contents. Increased PAL and chalcome synthase	Dong et al. (2012)
SNP	Litchi (*Litchi chinensis* Sonn.)	Reduced pericarp browning. Increased anthocyanin and TP content. Enhanced AOX. 8 days extension of shelf life	Barman et al. (2014)
	Bamboo shoots (*Phyllostachys violascens*)	Inhibited PPO, POD and PAL activities. Maintained TP content. Delayed external browning and tissue lignification	Yang et al. (2010)
	Logan fruit (*Dimocarpus longan* Lour. cv. Shixia)	In vitro inhibition of PPO and POD activities. Lower pulp breakdown and pericarp browning. Maintained TSS and AA content	Duan et al. (2007)

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