Cell. Mol. Biomed. Rep. (ISSN: 2823-2550) **2022, 2(1): 1-9**
https://doi.org/10.55705/cmbr.2022.330292.1019

Review Article

Nitric oxide signaling pathway in medicinal plants

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Article info
Received: 18 July 2021
Revised: 05 Sep 2021
Accepted: 19 Nov 2021

Use your device to scan and read the article online

Keywords:
Aging, Nitric Oxide, Photosynthesis, Respiration, Seed budding

ABSTRACT

Nitrogen monoxide or nitric oxide is a biological active growth regulator that a wide range of studies have recently shown that it acts as a growth regulator (signaling molecule) in plants. As soon as Nitric Oxide (NO) known as a new biological agent in plants and animals, biological branches of sciences like medicine, biochemistry, physiology and genetics have paid special attention to it. NO is a very reactive gas shape free radical which has attracted much attention during recent years. This compound is produced by the plant and it has increased the shelf life of some fruits, vegetables and cut flowers in low concentrations. NO is mostly synthesized from enzymatic and non-enzymatic pathways whose enzymatic biosynthesis pathway is done by reductase nitrate biosynthesis pathway through the cytosol, also it is known as an important and very reactive signaling molecule with short life which is produced by a group of enzymes known as synthesize NO which transforms L-arginine to L-citrulline and NO. It has been revealed that plants use NO as a growth regulator which regulates and modifies antimicrobial defensive responses. Recently, it has been approved that this material plays a vital role in regulating the normal physiological activities of plants such as pores closing, aging, increasing the vase life of cut flowers after harvesting, respiration and photosynthesis, antioxidant enzymes activities and growth.

1. Introduction

1.1. History of Nitric Oxide

Since nitric oxide (NO) has been recognized as a new biological agent in medicinal plants and animals, special attention has been paid to it in the sciences of biological disciplines such as medicine, biochemistry, physiology and genetics [1]. This compound was first reported by Clipper in Soybean (Glycine max) and then further reports on nitric oxide were provided.

1.2. Chemical Properties of Nitric Oxide

Nitric oxide (Figure 1) with the molecular formula HNO_3 has a mass of 63.01 grams, a density of 1.51 grams per cubic centimeter and a boiling point of 121.90 [2].

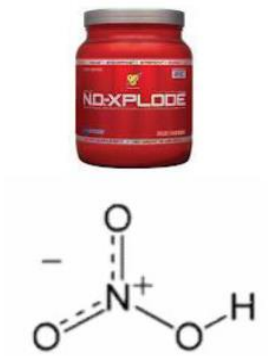

Fig. 1. Chemical composition of nitric oxide (2)

1.3. Nitric Oxide Biosynthetic Pathway

Nitric oxide (Figure 2) is mainly synthesized from enzymatic and non-enzymatic pathways, the enzymatic

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biosynthesis pathway is through the nitrate reductase biosynthesis pathway through the cytosol. But the non-enzymatic pathway is mediated by nitrate dismutation. Today, nitric acid is synthesized from ammonia, and this requires the raw material to be converted to nitrogen oxide by air [3].

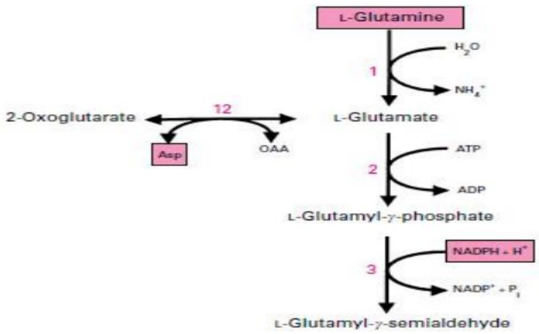

Fig. 2. Nitric oxide biosynthetic pathway in plant cells

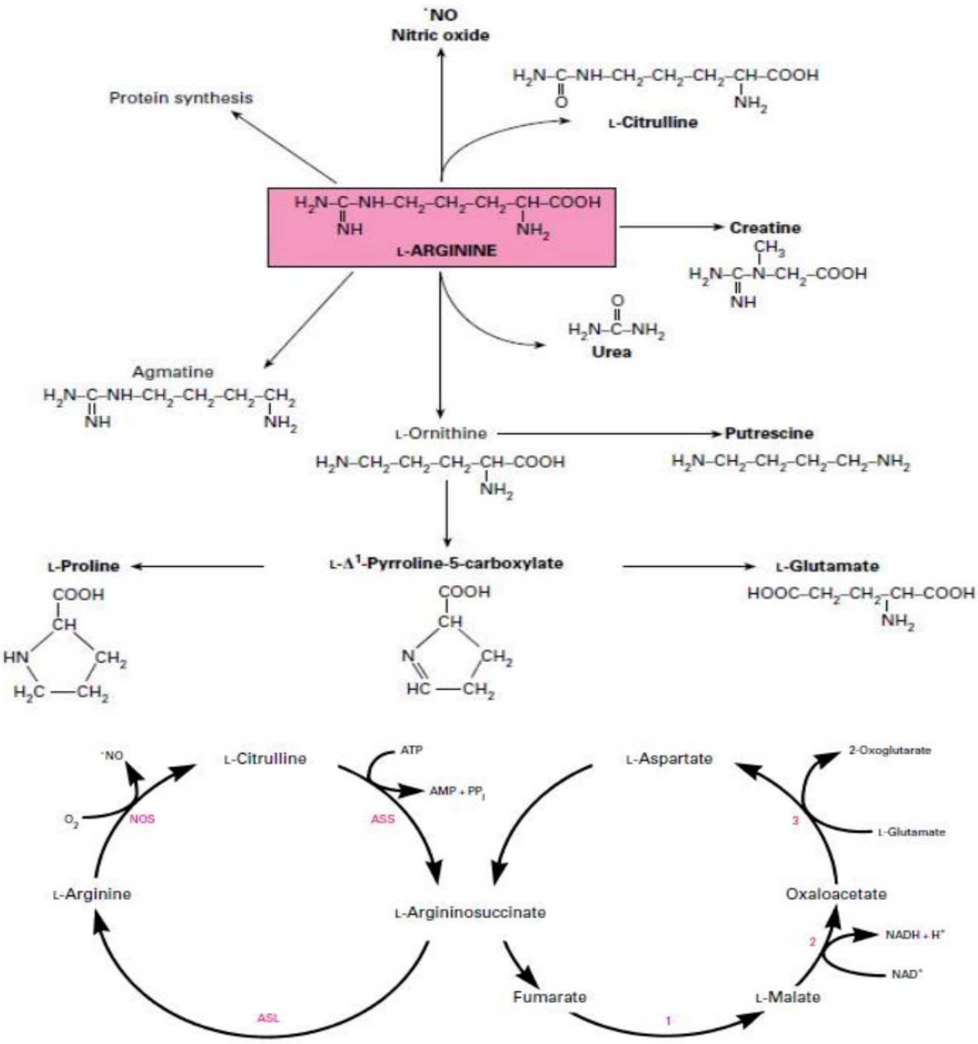

Fig. 2. Continue

2. The Role and Application of Nitric Oxide in Plants

2.1. The Effect of Nitric Oxide on Seed Dormancy

Sodium nitroprusside (SNP) (NO-releasing compound) is one of the compounds that prevent seed stagnation [4]. SNP ability to break seed dormancy has been reported in lettuce (*Lactuca sativa*), Arabidopsis thaliana, barley (*Avena sativa),* soda (*Saueda salsa*) and other plants at concentrations of 0.5 to 2 mM within 18 to 24 hours [5].

2.2. The Effect of Nitric Oxide on Seed Priming

Nitric oxide is one of the materials used as a primer to improve germination. Nitric oxide is an active nitrogen species that are involved in various physiological processes of plants such as growth, germination, maturation and aging, stomatal movement and planned cell death due to its special properties (free radical, small size, short life and high diffusion capacity of biological membranes). Applying to prime accelerates metabolic interactions before the germination process and germination of cultivated seeds is possible under stress with less moisture. The results showed that in the stage of enzyme activation, the priming process is successfully implemented [6].

2.3. The Effect of Nitric Oxide on Plant Growth

Low concentrations of nitric oxide have been reported in leaf development at low concentrations, but high concentrations of nitric oxide are not recommended at all. Concentrations of 0 to 20 mg / L have been reported in lettuce and tomato (*Lycopersicum esculentum*) [1].

2.4. The Effect of Nitric Oxide on Delayed Aging

Aging is a specific process that manifests itself with water loss and drying of plant tissue. Nitric oxide has a positive effect on delayed aging in fruits and vegetables [7]. After silver thiosulfate and 1-methylcyclopropene, this compound is known as 3 ethylene reactants, which has been shown to have a positive effect on various cut flowers, including cloves and eight other cut flowers [8]. Its positive effect has also been proved on fruits such as Longan fruit [9] and strawberry (*Fragaria spp*) [10]. Nitric oxide stimulates host tissue defense responses and may either have a direct effect on pathogen growth or indirectly increase host resistance to the pathogen. It has been reported that in peaches treated with 5 and 10 μmol/L nitric oxide, the activity of AC-C-oxidase, lipoxygenase and ethylene production decreases [7].

Nitric oxide has been shown to delay fruit ripening and improve the postharvest quality of strawberry and avocado fruits when treated at low concentrations [10]. Treatment of strawberry fruits with 5 micromoles per liter of nitric oxide significantly prevented the production of ethylene and also reduced respiration and AC-C synthase activity. As a result, it reduced the content of AC-C but its effect on the activity of AC-oxidase was not significant [11]. External application of nitric oxide has been reported either as direct vapor in an oxygen-free atmosphere or by nitric oxide-releasing chemicals to extend the shelf life of some horticultural products [7].

Weight loss of fruits during storage is due to evaporation of water from the surface of fruits and consumption of fruit reserves as a result of respiration. In this experiment, the lowest weight loss was related to nitric oxide treatment. Nitric oxide treatment prevents the production of ethylene and reduces respiration, reduces cellular metabolism and prevents water loss, thereby preventing weight loss. It has been reported that in peach fruits treated with nitric oxide, the activity of cell walls digestive enzymes such as exo-polygalactronase and endo-polygalactronase is reduced[12].

Nitric oxide treatment also maintains the firmness of fruit tissue. Studies have shown that treatment with nitric oxide solution can prevent the production of ethylene and reduce respiration and thus reduce the rate of breakdown of cell wall polysaccharides and thus lead to a delay in increasing soluble solids. Treatment of strawberry fruits with nitric oxide prevented an increase in soluble solids and a decrease in acidity due to reduced ethylene production, reduced respiration and prevented fruit aging. Nitric oxide treatment reduces cellular metabolic activities, including respiration and ethylene production, and thus preserves membranes and cell walls, which prevents the abnormal increase in soluble solids [12].

2.5. The Effect of Nitric Oxide on Nitrate Reductase Activity

Nitrate reductase activity is due to NO source activity in plant roots. External use of 100 μM SNP has been reported to significantly increase nitrate reductase activity in maize (*Zea mays*) [13].

2.6. The Effect of Nitric Oxide on Respiration

NO affects the mitochondrial structure in plant cells and the respiration of all plant cells is reduced due to the effect on the cytochrome structure [2].

2.7. The Effect of Nitric Oxide on the Movement of Pores

The use of nitric oxide stimulates and increases the chlorophyll content, and this has been reported in potatoes (*Solanum tuberosum*), lettuce and tomatoes [14].

2.8. The Effect of Nitric Oxide on Photosynthesis

Photosynthesis is one of the most important metabolic processes in plants that significantly affect plant growth and yield and resistance to various environmental factors. Therefore, photosynthesis is used as an important indicator in increasing plant growth and tolerance. The effect of nitric oxide on photosynthesis has not yet been fully elucidated, but findings on the effect of S-nitroso-N-acetylpensylamine (SANP), S-nitrosoglutation (GSNO) and SNP on respiration have been confirmed [1].

Research has shown that nitric oxide is involved in increasing photosynthesis by protecting photosynthetic pigments under salinity stress. Simaei et al. [15] Reported in 2011 that salinity stress reduced chlorophyll content in soybeans, and the use of sodium nitroprusside (nitric oxide release) neutralized the negative effect of salinity on pigments and increased chlorophyll content under salinity stress in sodium nitroprusside treatment. Leshem [16] reported that an increase in nitric oxide increased the chlorophyll content in tomatoes and strawberries. Liu et al. [17] showed that nitric oxide increased the amount of chlorophyll a, chlorophyll b and total chlorophyll in cucumber during cold stress.

2.9. The Effect of Nitric Oxide on Antioxidant Systems

Nowadays there is a common belief about the presence of NO as a secondary agent, and its antioxidant effect can be traced back to its concentration and mode of action in the environment. The effect of NO can be due to the maintenance of cellular homeostasis and the regulation and control of ROS toxicity levels [4]. Nitric oxide has also been shown to act as an antioxidant in plant tissues [1]. Levels of superoxide dismutase and hydrogen peroxide have been reported to decrease in nitric oxide-treated kiwi fruit [18]. Under waterlogged stress conditions of Welch onions, reactive oxygen species and hydrogen peroxide increased and their levels decreased in plants treated with putrescine [19].

Nitric oxide is an anti-aging factor and has a positive effect on the response to oxidative stress associated with fruit aging. The dual functions of nitric oxide as a potent oxidizer or antioxidant effects depend on its concentration and position. In systems that have developed free radical toxicity, nitric oxide can neutralize free radicals and prevent damage [20]. Mostofi et al. [21] studies on carnation cut flowers showed that NO increased the activity of antioxidant enzymes catalase, peroxidase, ascorbate peroxidase and superoxide dismutase, and this increase in activity was associated with increased flowering life of cut flowers. Application of sodium nitroprusside (SNP) can increase the life of sage flowers and the activity of antioxidant enzymes (catalase, ascorbate peroxidase, guaiacol peroxidase), reduce ion leakage and water loss in flower branches. It has been reported that application of DETA/NO increased the postharvest life of clove and gerbera cut flowers by 40% and 2.5 times, respectively, compared to the control treatment [22]. Also, the use of NO gas can delay the aging of some fruits, vegetables and cut flowers [23]. It has already been reported that NO is able to react directly with oxygen-free radicals and produce peroxynitrite radicals. Peroxynitrite radicals are much less toxic than oxygen-free radicals, and therefore NO can directly reduce the amount of oxidative damage [24].

The production of NO at low concentrations has been reported to rapidly kill peroxide radicals and reduce their damage by preventing the production of reactive oxygen radicals. NO also protects cells from non-biological stress damage by motivating gene expression and increasing the activity of antioxidant enzymes [5]. Mostofi et al.[21] and Nasibi [25] studies on carnation cut flowers have also shown that exogenous nitric oxide increases the activity of antioxidant enzymes catalase, peroxidase, ascorbate peroxidase and superoxide dismutase, and this increase in activity has been accompanied by the increase of flowering life of cutting branch flowers. The positive effect of NO on quality enhancement after clove harvest [26], lily [27].

Nitric oxide also acts as a neutralizer of the toxic activity of microorganisms and reacts with lipid peroxidase as an antioxidant [39]. Nitric oxide can cause the expression of phenylalanine ammonialyase genes. Phenylalanine ammonialase is a key enzyme in the synthesis of phenylpropanoid and is involved in the synthesis of salicylic acid. Nitric oxide has been shown to act as an antioxidant in plant tissues. There is a good intracellular balance between the production of reactive oxygen species and antioxidants in all cells. On the one hand, free radicals are produced during metabolic processes, and on the other hand, these free radicals must be eliminated by antioxidants [28].

Nitric oxide induces the expression of genes for antioxidant enzymes such as catalase in Arabidopsis cell failure. Treatment of tomato fruits with nitric oxide stimulates the expression of antioxidant enzyme genes which delay fruit aging and increase fruit shelf life. In addition, both salicylic acid and nitric oxide in low concentrations have an antioxidant effect and directly eliminate free radicals ROS in high concentrations causes oxidative damage by lipid peroxidation and protein breakdown, thus leading to cell death and aging. During the ripening process, ethylene and O_2^- are produced, which cause the fruits to ripen. Decreasing O_2^- levels and increasing the activity of antioxidant enzymes prevent cell membrane damage, increase membrane strength, and prevent lipid oxidation [29].

Nitric oxide has a beneficial effect on the oxidation balance and antioxidant capacity of peach fruits and has shown antioxidant activity in rice leaves. Nitric oxide also acts as a neutralizer of the toxic activity of microorganisms and reacts with lipid peroxidase as an antioxidant and prevents the oxidation of fats [39]. Nitric oxide has been reported to inhibit polyphenol oxidase activity and may interact with transport metals such as iron, copper, zinc, and thiols. Nitrogen oxide active species can reduce polyphenol oxidase precursors by reacting with phenolic compounds. They showed that nitric oxide also inhibited polyphenol oxidase and peroxidase activities and kept total phenol levels relatively high during storage. The active site of PPO consists of two copper atoms. It is stated that nitric oxide at low concentrations can react with Cu (B) of PPO to form a copper-nitrosyl (NO - Cu (B) - PPO) complex, which can normalize the structure of the active site of PPO. And thus reduce PPO activity [30].

2.10. The Effect of Nitric Oxide on the Tolerance of Environmental Stress

Metabolism of the essential and non-essential mineral elements in medicinal plants or crops can be affected by various environmental factors and stresses [31]. Today nitric oxide has a special place in botany due to its properties (free radical, small size, no charge, short lifespan, high permeability through biological membranes) and multiple roles in plant growth and regulation of a wide range of plant cellular mechanisms. The role of nitric oxide in the tolerance of plants to non-biological stress has been proven in the last few years. Nitric oxide (NO) is a relatively stable gas radical produced by the enzyme nitric oxide synthetase (NOS) from L-arginine[32].

Two possible mechanisms have been proposed for the role of NO in combating stress: firstly, NO may act as an antioxidant by direct abduction of reactive oxygen species such as superoxide radicals and reduces cell damage by producing radical peroxynitrite which is much less toxic than radicals. Secondly, NO as a signaling molecule can alter the expression of some defense genes. NO also removes this anion from the cell by converting superoxide to hydrogen peroxide and, on the other hand, reduces the toxicity of hydrogen peroxide by stimulating antioxidant enzymes such as ascorbate peroxidase (APX) [32].

Research has shown that some chemical compounds, including nitric oxide, play a role in modulating stresses by interfering with signal pathways. NO is a lipophilic and biologically active free radical that acts as a signal molecule in a variety of physiological processes and is able to regulate many plant responses to biological and abiotic stresses. Nitric oxide reacts with reactive oxygen species, thiol groups, and proteins. The importance of this molecule is in tolerating some environmental stresses. Nitric oxide increases the ability of medicinal plants to adapt to adverse environmental conditions by strengthening the immune system [32].

Various studies have shown that the combination of the NO signal is involved in cell death, defense genes, and reaction with reactive oxygen species (ROS) during plant defense against pathogens. In recent years, researchs have shown that nitric oxide is involved in many vital physiological processes in the plant not only under normal conditions but also under stress conditions (e.g. salinity) [33; 34]. This molecule acts as a secondary agent under osmotic stress. In 2006 Zhu et al. [7] Observed that sodium nitroprusside (as a NO donor) increased the dry and wet weight of maize seedlings under salinity stress.

In 2011, Simaei et al. [15] also reported that salinity reduced the fresh and dry weight of the aerial part in soybeans which of course the presence of sodium nitroprusside (nitric oxide releaser) neutralized the effect of salinity, resulting in wet and dry weight of the aerial part of Glycine. Regarding the effect of nitric oxide protection of nitric oxide against oxidative damage and increased tolerance to salinity stress have been reported by applying a concentration of one micromolar of these substances in reducing the effects of salinity stress in rice seedlings and by applying a concentration of 0.2 mm in wheat seedlings [35].

2.11. The Effect of Nitric Oxide on Programmed Cell Death

There are conflicting and worrying reports of programmed cell death. High concentrations of nitric oxide are able to induce cell death by suspending Arabidopsis cells, and this process is not dependent on reactive oxygen species [36]. In yew species (*Taxus brevifolia*) and a species of Kalanchoe (*Kalanchoe diagremontiana*) sodium nitroprussium has increased the phenomenon of cell death [1].

3. Conclusion

Nitric oxide is a gaseous free radical which is highly reactive. Application of this compound in low concentrations has been reported to increase the shelf life of some fruits and vegetables. It has also been shown to play a vital role in regulating normal plant physiological activities such as stomatal closure and growth. Nitric oxide also directly or indirectly modulates the transcription of specific genes involved in maturation and aging. Plants respond to biotic and abiotic stresses through complex defense mechanisms.

One of these mechanisms is the nitric oxide pathway, which is essential for the development of resistance to pathogens. Many defense responses are stimulated by nitric oxide, and levels of this free radical increase following the onset of pathogens. Nitric oxide, a biologically active molecule, is involved in the regulation of many diverse cellular functions in plants, from root growth to adaptive responses to biological and non-biological stresses.

Conflict of interest

The author declares no conflict of interest.

Consent for publications

The author declares, reads, and approves the final manuscript for publication.

Availability of data and material

The author declares that he embedded all data in the manuscript.

Funding

No company or organization paid for this study.

Ethics approval and consent to participate

The author did not use any human or animal samples for this study

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How to Cite This Article:

Salehi-Sardoei A, khalili h (2022) Nitric oxide signaling pathway in medicinal plants. Cellular, Molecular and Biomedical Reports 2(1):1-9. doi:10.55705/cmbr.2022.330292.1019

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