

# SNP as an Effective Donor of Nitric Oxide for *in vitro* Plant Cell and Tissue Culture

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The scope of this brief review is about the role that plays a fantastic molecule: Nitric Oxide, and the use of sodium nitroprusside (SNP) as a facilitator of growth and development of *in vitro* plant cell, tissue and organ cultures.

Nitric oxide (NO) is a bioactive inorganic gas, free radical and highly labile of a low molecular weight and a half life of less than six seconds. It contains an unpaired electron in its outer orbital and because this fact, it bears the characteristics of a radical that can win or lose an electron and be converted to one of three different species: the radical (NO<sup>•</sup>), the nitrosonium cation (NO<sup>+</sup>) or nitroxyl anion (NO<sup>-</sup>), which explains its high reactivity and its tendency to bind with reduced heme proteins [1]. These three forms of NO are swappable within the cell and are strongly dependent on the redox state thereof. The radical NO diffuses freely in aqueous solutions and is able to cross lipid membranes, move within cell compartments and from cell to cell [2].

NO has emerged as a new chemical messenger in plant biology after its characterization and functionality in animals, where it acts as an endothelial relaxation factor (EDRF, endothelium-derived relaxing factor). NO is generated from arginine, by the action of the enzyme nitric oxide synthase (NOS) and its three isoforms nNOS, eNOS, iNOS, some of which depend on the concentration of intracellular free calcium [3,4].

In plants the NO is involved in both pathophysiological and developmental processes. Many environmental and hormonal stimuli are transmitted either directly or indirectly by NO signaling cascades. The capacity of NO to act simultaneously in non-related biochemical pathways and its redox homeostatic characteristics, suggest that this may be a molecule for synchronization in plants [4] which can generate NO through enzymatic and non-enzymatic systems. One pathway of NO production in plants is the enzyme nitrate reductase (NR), which is located in the cytosol and catalyzes the reduction of nitrate to nitrite using NADH as electron donor. This enzyme may also catalyze the reduction of nitrite to NO, and NO production depends on the accumulation of nitrite [5]. Other pathway for the NO synthesis in plants is analogous to the animals NO synthesis by the NOS enzyme. There are several studies that asserted the presence of NOS activity in plants. The emergence of NOS activity was reported in the peroxisomes of pea, in the roots, stems and leaves of seedlings of peas besides in olives where induced salinity however studies in an Arabidopsis protein that produces NO in response to hormonal signals shows no similarity with typical animal NOS enzyme but increased synthesis of NO-arginine-dependent. Therefore, it can be postulated that the presence of NOS activity in plants is still mysterious [6].

On the other hand, exogenous NO sources constitute a powerful way to supplement NO to plants or animals with the use of chemical nitric oxide donors which have been studied mainly in cardiovascular medicine for more than two decades. By definition, NO donors refer to any compound that can generate NO in any of the previously mentioned forms (radical, anion or cation) through enzymatic,

chemical, electrochemical and photochemical pathways. Most of these NO donors are organic compounds; however, there is a small group of compounds that form a transition metal NO-complexes such as sodium nitroprusside (SNP) [7]. SNP is the most widely studied compound of the iron nitrosyls family, being its systematic name sodium pentacyanonitrosyl ferrate (II), an inorganic complex where iron is in the ferrous state (Fe<sup>2+</sup>) and nitric oxide is formally bound as NO<sup>+</sup>. In spite of extensive studies of chemical reactivity, particularly with thiols, the mechanism of NO release is still not clearly understood. It is clear, however, that SNP requires either irradiation with light or an electron reduction to release NO. Moreover, sodium nitroprusside in aqueous solution is degraded when exposed to white or blue light but no to red light [8,9].

The addition of SNP to *in vitro* cell, tissue and organ cultures is currently a topic of the highest interest. Ötvös et al. [10] used SNP as nitric oxide donor in combination with auxins for promoting activation of cell division and embryogenic cell formation in *Medicago sativa*. They found that in the absence of exogenous auxin and a concentration of 10 µM SNP did not influence cell division. However, in the presence of both 0.22 and 1 µM 2,4-D and the same SNP concentration, this compound significantly increased the frequency of cell division. A more recent study evaluated the effect of SNP (10 µM) on shoot multiplication and regeneration of *Vanilla planifolia* Andrews [11]. Other studies showed that NO stimulated root organogenesis in *Cucumis sativus*, where the same concentration of SNP (10 µM) was used, and in *Solanum lycopersicum* where the optimum concentration was 200 µM [12,13]. Preliminary results in the micropropagation of the genus *Agave* showed that SNP increased the propagation rate and the size of propagated plants (to be published elsewhere).

Currently, research for the use of Nitric Oxide donors such as Sodium Nitroprusside is of a great interest for basic physiological and biochemical studies as well as for the development of new protocols for plant micropropagation for commercial purposes.

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