

Review Article

Nitric oxide and calcium signalling in plants under salinity stress and their crosstalk

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Abstract

Salinity is considered as one of the major factor affecting the crop production throughout the world. The oxidative stress induced by salinity can retard plant growth and yield as major part of energy is wasted on conserving water and improving ionic balance. The free radicals produced during stress are considered to be a major factor for most of the damages as these free radicals attack vital biomolecules such as lipids, protein and carbohydrates which are the basic requirement of almost all physiological and developmental processes. Understanding the mechanism of stress tolerance along with the involvement of important signalling molecules in stress signalling network is essential for crop improvement. Likewise, the two signalling molecules nitric oxide and calcium ion have been reported to be actively involved in upregulation of various stress response mechanisms thus indicating the existence of a possible cross talk among these molecules and other associated pathways. In this review, emphasis was given on the impact of salinity and oxidative stress mediated damages on plant system. Additionally, the role of nitric oxide and calcium ion as signalling molecules in response to stress signals and their implication in mitigation of salinity stress has also been discussed.

Keywords: Calcium ion, Free radicals, Nitric oxide, Salinity, Signalling.

Introduction

Salinity is considered as one of the major factor affecting the crop production throughout the world. Salinity either in water or soil represents one of the major abiotic stresses especially in arid and semi-arid regions, which can severely limit the agricultural production (Shanon, 1998). High concentration of salt creates ionic imbalance and hyper osmotic stress in plant system which consequently leads to oxidative damages. Such drastic changes in plant system cause retardation of growth, molecular damages, membrane disruption and even death. For the plant to be tolerant to salinity stress: their homeostasis must be re-established along with detoxification mechanism must be boosted (Zhu, 2001). Most of the cellular damages caused by salinity are usually associated with ROS mediated oxidative stress (Parida and Das, 2005).

Nitric oxide and calcium both are considered as highly versatile signalling molecules. Various literatures have reported

the significant involvement of both of these molecules in wide range of physiological and developmental processes in plants. Additionally, these molecules have found to mitigate the adverse effect of varied environmental stresses including salinity (Wilson *et al.*, 2008; Sirova *et al.*, 2011; Lecourieux *et al.*, 2006).

Effect of salinity on plant system

The two major consequences of salinity on plant system are osmotic stress and ionic toxicity; these physical conditions affect all other physiological, biochemical and developmental processes in plants (Yadav *et al.*, 2011). High salt content in the substratum creates rise in osmotic pressure of the substratum thus, affecting the water uptake capacity of plants. Furthermore, decrease in the turgor pressure of the plant cells cause closing of stomata which leads to reduced carbon fixation but increase in ROS production. These highly reactive and unstable free radicals disrupt various cellular processes by damaging the major biomolecules like lipids, proteins, and nucleic acids (Parida and Das, 2005). Ionic toxicity is the physiological state

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in which the equilibrium of ions is disturbed which causes perturbation in cellular metabolism and processes. High concentration of sodium ions at the surface of the root disrupts plant nutrition by inhibiting both K^+ uptake and enzymatic activities within the cell (Aslam *et al.*, 2011). Potassium is an important nutrient which regulates huge number of enzymes activities associated with various major pathways (Kader and Lindberg, 2010); on the other hand, sodium ions inhibit the activity of enzymes. Na^+ is a cation almost similar to K^+ , for this reason Na^+ can cross the cell membrane without much disturbance (Parida and Das, 2005). As suggested by Rodriguez-Navarro, (2000) optimum concentration of K^+ required is 100-200mM in the cytosol and the concentration of cytosolic Na^+ excess of 10mM creates stress environment in the system. The oxidative stress induced by salinity can retard plant growth as major part of energy is wasted on conserving water and improving ionic balance (Kader and Lindberg, 2010).

Strategy for prevention of Na^+ toxicity in plants

In order to overcome salt stress, plants have developed different strategies for their survival. For instance, for combating Na^+ toxicity most of the glycophytes depend on restriction of Na^+ intake, but this strategy is successful to some extent only because of the electronegative environment in inner cellular system. Additionally, the cation transporters are fairly permeable to Na^+ , therefore the constant influx of Na^+ along the electrochemical gradient is not terminated completely (Amtmann *et al.*, 1999). But interestingly, halophytes overcome this ion toxicity by coupling the uptake of ions via roots with the compartmentation of ions into cellular vacuoles (Hasegawa *et al.*, 2000; Blumwald *et al.*, 2000).

Concept of free radicals

A free radical is defined as a molecular species which is capable of independent existence and posses an unpaired electron in its outermost atomic orbital. This unpaired electron results in presence of certain common properties that are shared by most of the radicals. These free

radicals are highly unstable as well as highly reactive. They have the capability to either donate an electron or accept an electron from other molecules, therefore altering their native properties (Cheeseman and Slater, 1993; Lobo *et al.*, 2010). The free radicals generated from oxygen are called reactive oxygen species (ROS) and those from nitrogen are termed as reactive nitrogen species (RNS). ROS includes various forms of activated oxygen molecules, such as superoxide ($O_2^{\cdot -}$), hydroxyl ($\cdot OH$) and peroxy (ROO^{\cdot}), as well as non-free radicals hydrogen peroxide (H_2O_2) and singlet oxygen (1O_2). Likewise, RNS includes nitric oxide (NO^{\cdot}) and nitrogen dioxide (NO_2^{\cdot}) and non-free radicals such as nitrous acid (HNO_2) as peroxy nitrite ($ONOO^{\cdot}$) (Halliwell, 1994; Chanda and Dave, 2009). These free radicals are generated under normal physiological conditions but become harmful when not being eliminated from the cellular systems. In fact, such imbalance between the production and elimination of reactive oxygen species in the cell system leads to a condition known as oxidative stress. After excessive accumulation, they attack vital biomolecules leading to cell damage and homeostatic disruption. The major targets of these free radicals are lipids, nucleic acids, proteins and carbohydrates (Aruoma, 1994). The formation of free radical is a consequence of both enzymatic and non-enzymatic reactions which occurs continuously in the cell system. Enzymatic reactions include those phenomena involved in the phagocytosis, respiratory chain, synthesis of prostaglandin also in the cytochrome P450 system (Lui *et al.*, 1999; Lobo *et al.*, 2010). Free radicals can also be produced in non-enzymatic reactions between oxygen and organic compounds as well as those initiated by ionizing reactions.

Importance of Nitric oxide signalling in plant system

Nitric oxide (NO) is an important signalling molecule, which has been known to participate in wide spectrum of regulatory functions in almost all stages of plant development (Wilson *et al.*, 2008; Sirova *et al.*, 2011). In the year 1975 the emission of NO from plants was first observed by Klepper in 1975, in soybean plants treated with herbicides (Klepper, 1979).

Plants not only react to the atmospheric or soil NO, but they are also able to generate NO via reduction of apoplastic nitrite (Bethke *et al.*, 2004) or by carotenoids in presence of light (Cooney *et al.*, 1994). The major production of NO in plants, however, is probably carried through the action of NAD(P)H-dependent nitrate reductase enzyme (Dean and Harper, 1988) which is also considered as an endogenous source of NO in plant system (Yamasaki *et al.*, 1999).

The synthesis of NO in animals is carried out by the enzyme nitric oxide synthase (NOS) via deamination of L-Arginine. But, there are no such genes in plant system including *Arabidopsis thaliana* that allow homology with NOS genes of animals (Gupta *et al.*, 2011). Among the photosynthetic members, only *Ostreococcus tauri*, an unicellular green alga was found to possess a NOS having a homology of only 45% with the human NOS (Foresi *et al.*, 2010). At present several pathways involved in NO synthesis in plant system are known, also some are assumed which are given in fig. 1. The biosynthetic pathway leading to the production of NO in plants might be either oxidative or reductive. The oxidative pathway is carried out by NOS like enzyme which also includes synthesis from polyamines. The reductive pathway is mediated by enzymes such as nitrate reductase (NR) and nitrite-NO reductase (Ni-NOR). Furthermore, this pathway includes xanthine oxidoreductase (XOR) in peroxisomes and cytochrome c oxidase (COX) that synthesizes NO from nitrite in mitochondria (Mamaeva *et al.*, 2015).

The application of exogenous NO to plants or cell cultures has revealed valuable information about the influence of this molecule on various physiological and biochemical processes. The summary of the functions NO associated with various physiological, biochemical and molecular processes is given in fig. 2. The earlier reports suggest that NO can mediate the biological effects of signalling molecules such as phytohormones. The biosynthesis of NO has been found to be induced by cytokinin in different plants and hence the possibility of involvement of NO in the cytokinin-induced programmed cell death process is proposed by

Neill *et al.*, (2003). Likewise, it has been demonstrated that NO synthesis in cucumber roots is induced by auxin (Pagnussat *et al.*, 2003). Additionally, the interaction between both the gaseous molecules NO and ethylene in the maturation and senescence of plant tissues has been reported during plant development (Lamattina *et al.*, 2003).

The identification of the NO synthesis enzymes and the discovery of regulatory role of NO in the activity of specific proteins within sub-cellular compartments provided significant understanding of NO signalling at the molecular level (Hanafy *et al.*, 2001; Kone *et al.*, 2003; Stuehr *et al.*, 2004). Over the past decade, considerable progress has been made in understanding the mechanism of NO signalling in plants. NO modulates the activity of most proteins through nitrosylation and tyrosine nitration mechanism. The post translational modifications via nitrosylation as well as S-nitrosylation have been resulted in regulation of several plant proteins *in vitro* and also *in vivo* to some extent. The proteins which are the targets of NO include haemoglobin, cytochrome c oxidase, metacaspase 9, glyceraldehyde-3- phosphate dehydrogenase, and methionine adenosyltransferase (Besson-Bard *et al.*, 2008). Endogenous NO has been found to function as a calcium ion-mobilizing messenger by inducing the rise in cytosolic Ca²⁺ concentrations. The rise of cytosolic Ca²⁺ concentration further aid NO to modulate the protein kinases and channels involved in the signalling cascade, thus regulates important physiological processes such as stomatal closure, adventitious root formation and also the expression of defense genes (Garcia-Mata *et al.*, 2003; Lamotte *et al.*, 2006). In *Arabidopsis*, it was demonstrated that the production of NO by elicitors such as lipopolysaccharides is regulated by Ca²⁺ influx mediated by the cyclic nucleotide-gated channel (Ali *et al.*, 2007). The interplay of both NO and Ca²⁺ and their involvement in the plant acclimation during salinity stress also preventing the oxidative stress mediated damages and their adverse effects have been elucidated in fig. 4.

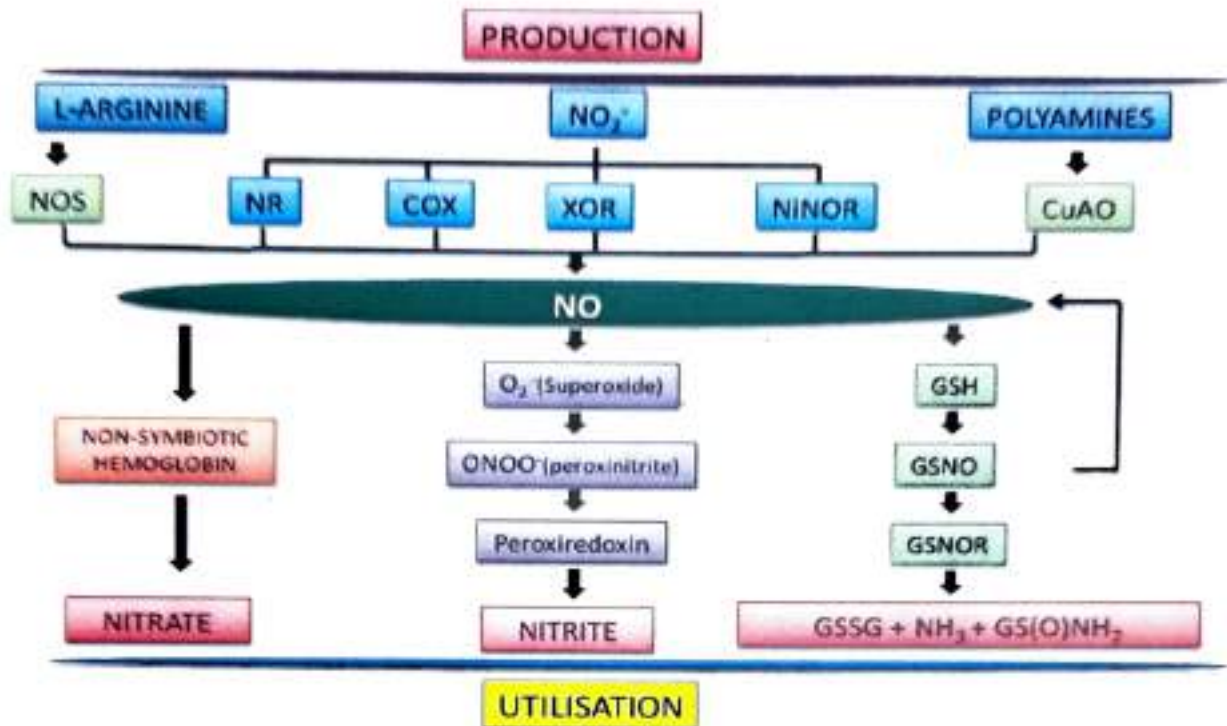


Fig. 1: Pathways involved in synthesis and utilization of NO in plant system (Mamaeva *et al.*, 2015). NOS: Nitric oxide synthase; NR: Nitric reductase; COX: Cytochrome oxidase; XOR: xanthine oxidoreductase; CuAO: Cu-amine oxidase; NINOR: Nitrite-NO reductase; GSH: Reduced glutathione; GSNO: S-nitrosoglutathione; GSNOR: S-nitrosoalutathionereductase; GSSG: oxidized glutathione; GS(O)NH₂: glutathione sulfonamide.

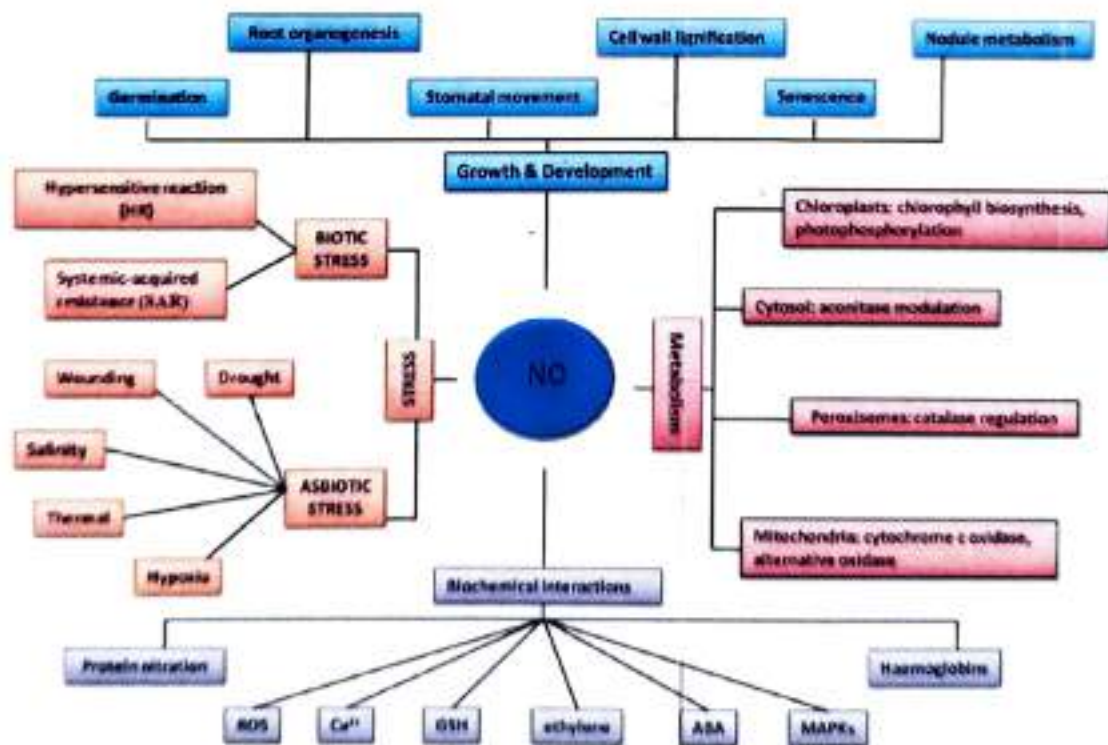


Fig. 2. Functions of Nitric oxide associated with various physiological, biochemical and molecular processes (del Rio *et al.*, 2004).

Role of Calcium signalling in plant system

The calcium ion has been well established as a second messenger in several plant signalling pathways, conveying a wide range of stimuli to appropriate physiological responses. Ca^{2+} signals are considered as a core regulator of cell physiology and cellular responses of plants to the environment. Many extracellular and environmental signals including both abiotic and biotic factors, elicit change in the cellular level of calcium, termed as calcium signatures (Lecourieux *et al.*, 2006). This " Ca^{2+} signatures" represent a central mechanistic principle for stimulus-specific information in the cellular system. The channels, pumps, and carrier proteins serve as the mechanistic basis for generation of Ca^{2+} signals by modulating the flux of calcium ions among the sub-cellular compartments, cell and its extracellular environment (Dodd *et al.*, 2010). The disorders due to Ca-deficiency in plants have been considered to be very much harmful in horticulture sector commercially (Shear, 1975). Some of the diseases caused due to deficiency of calcium in plants are tip burn and brown heart in leafy vegetables, blossom end rot of tomato fruit, empty pod in peanut also structural weakness in cell wall. The Ca-deficiency generally occurs when there is unavailability of sufficient calcium in the developing tissues due to failure of calcium mobilization by phloem. On the other hand, presence of excess calcium in the substratum also creates a cytotoxic environment for plants. The excessive calcium reduces the germination rate of the seeds and also retards the plant growth rates (Shear, 1975; White and Broadley 2003). The other functions of calcium ion in the plant systems are elucidated in fig. 3.

Since the presence of higher calcium ion concentration is cytotoxic, a sub micromolar level of calcium ion is maintained by Ca^{2+} ATPases and $\text{H}^+/\text{Ca}^{2+}$ antiporters in unstimulated cells (Sze *et al.*, 2000; Hirschi, 2001). These proteins maintain this optimum level by fluxing the extra cytosolic Ca^{2+} either to the apoplast or the lumen of vacuole or endoplasmic reticulum (Sanders *et al.*, 2002). There are other class of proteins which change their conformation or catalytic activity

upon binding with the calcium ion and hence regulate the calcium signals. Also it has been reported that specific sensors and signals of calcium ion signatures regulated cellular responses to specific biotic and abiotic stimuli (White, 2000).

The proteins responsible for the perception and decoding of Ca^{2+} signals are present in the cytosol and nucleus of the plant cell. Several calcium sensors with different Ca^{2+} binding characteristics, subcellular localizations and signalling interactions comprises a toolkit that helps in decoding the information within Ca^{2+} signatures in the form of spikes or oscillations (Dodd *et al.*, 2010; Batistic and Kudla, 2012). Further these sensor proteins accordingly carry the processing of this information into respective alterations in cell function. Conceptually, plant Ca^{2+} sensor proteins that are functionally signalling components have been classified into sensor relays and sensor responders (Sanders *et al.*, 2002). The sensor responder proteins which include Ca^{2+} -dependent protein kinases (CDPK) combine both sensing function and responding function, regulated by calcium-binding proteins that often cause conformational changes (e.g., protein kinase activity) within a single protein. Consequently, these kinases mediate the information encoded in Ca^{2+} signals into phosphorylation events of specific target proteins. In contrast, sensor relay proteins such as calmodulin (CaM) and calmodulin like protein (CML) also contain multiple calcium-binding domains and undergo conformational changes with Ca^{2+} signals but lack the enzymatic function. Therefore, these proteins have to interact with other target proteins and regulate their activity for transduction of Ca^{2+} signal, which means they must undergo Ca^{2+} -dependent protein-protein interactions (Luan *et al.*, 2002). The calcineurin B-like (CBL) protein are another family of sensor proteins which lack the enzymatic activity hence belong to sensor relay proteins. However, their specific interaction is with a family of protein kinases designated as CBL-interacting protein kinases (CIPKs), so, CBL-CIPK complexes are considered as bimolecular sensor responders (Hashimoto and Kudla, 2011). CaM is highly conserved in all eukaryotic members, whereas

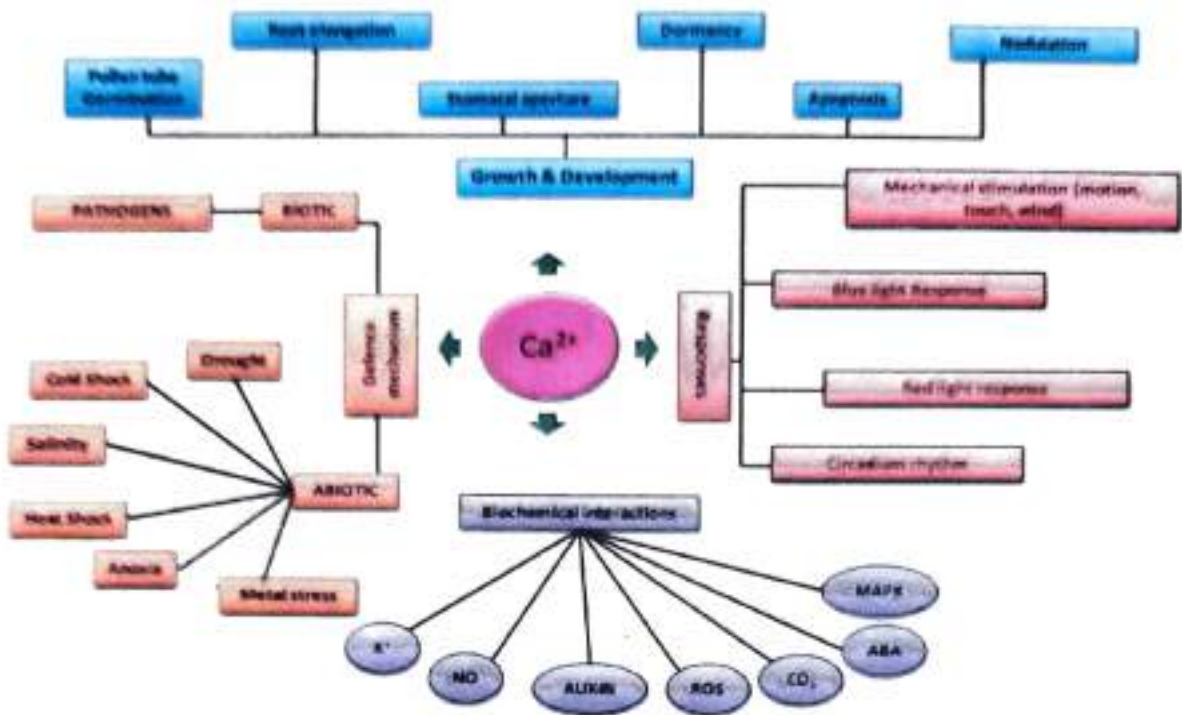


Fig. 3. Involvement of Ca^{2+} signal in various physiological, biochemical and molecular processes in plant system (modified from White and Broadley, 2003; Leucourieux *et al.*, 2006)

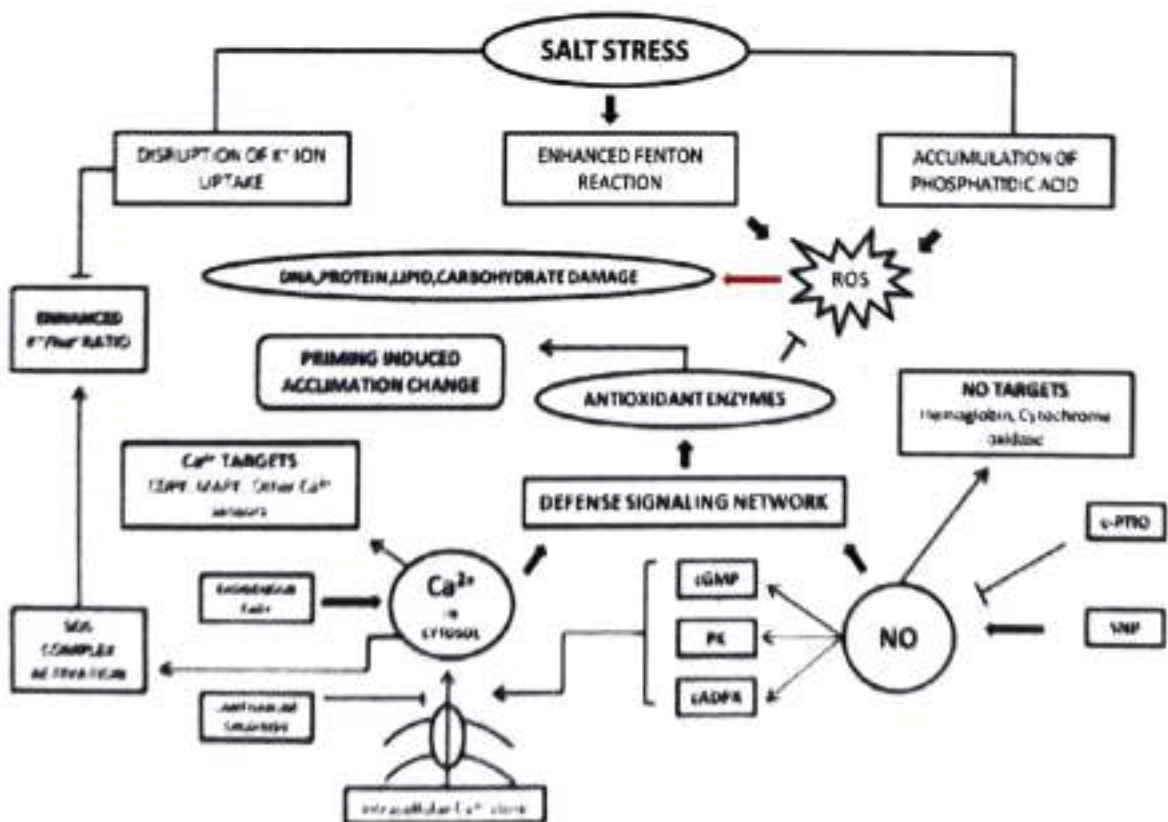


Fig. 4. Interplay of Nitric oxide and Calcium ion and their role in alleviation of oxidative stress mediated damages under salinity stress.

CML, CDPK and CBL proteins have been found to be present only in plant system (Batistic and Kudla, 2009). The specific binding of Ca^{2+} with Calmodulin7 (Cam7) results in direct interaction and regulation, while other calmodulins are likely to mediate gene regulation via interacting with other transcriptional (co)regulators. Metabolic and biosynthetic processes such as brassinosteroid synthesis are important targets of direct Ca^{2+} -dependent modulation (Du and Pooviah, 2005), but on the other hand Ca^{2+} -dependent phosphorylation and gene regulation provides the major cellular currencies for transduction of specific Ca^{2+} signals into targeted downstream responses (Harper and Harmon, 2005).

Implication of NO for maintenance of redox homeostasis during salinity stress

NO is said to possess considerable capacity to regulate oxidative stress mediated damages along with the level and toxicity of ROS.

The properties of NO which makes it capable to exert a protective function against oxidative stress mediated damages as suggested by Yadav (2010) are given below:

- i. It reacts with lipid radicals and stops the propagation of lipid oxidation.
- ii. Scavenging the superoxide anion and formation of peroxynitrite which is toxic for plant are later neutralized by ascorbate and glutathione.
- iii. Involvement in the activation of antioxidant enzymes.

The lupin seeds when subjected to sodium nitroprusside (SNP) treatment showed better germination under saline stress as well as heavy metal stress (lead and cadmium) suggesting involvement of NO in auxin signalling pathway (Kopyra and Gowdz, 2003). Later, Zheng *et al.* (2009) demonstrated that pre-soaking of wheat with SNP for 20h prior to germination resulted in increased germination rate and radicle weight under 300 mM NaCl. Additionally, decrease in Na^+ concentration but increase in K^+ concentration in the seeds were observed thereby indicating role of NO in maintaining a balance between K^+ and Na^+ during germination under salt stress. The pre-treatment of citrus root with exogenous SNP

for a duration of 48h exhibited induction of primary antioxidant responses in the leaves of citrus subjected to salinity stress. The study revealed that SNP pre-treatment enhanced the activity of antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) also prevented the NaCl-dependent protein oxidation (Tanou *et al.*, 2009). Zheng *et al.* (2009) claimed in their study that NO treatment effectively contributed to better accumulation of ferritin, a protein active in chelation of excess of ferrous ion present in the cellular system of barley plant subjected to salt stress. Exogenous application of NO through different modes have reported to be effective in regulating the functioning of photosynthetic pigments (Ruan *et al.*, 2002;), improving salt tolerance by modulating proton pump activity in maize (Zhang *et al.*, 2006), regulating osmotic balance and proline metabolism in tobacco (Ke *et al.*, 2013) and prevention of mitochondrial oxidative damage (Zheng *et al.*, 2009). Furthermore, various evidences have been provided by researchers about the protective effect of NO during other stress conditions besides salinity; alleviating the negative effects of UV radiations in wheat seedlings (Yang *et al.*, 2013); mitigating the oxidative injuries under heavy metal stress in lupin seeds (Kopyra and Gowdz, 2003) and modulating the metabolism of biochemicals during osmotic stress (Ke *et al.*, 2013). Other beneficial effects of NO donor reported are regulation of seed germination in *Senna macranthera* (da Silva *et al.*, 2015); maintenance of optimum Na^+/K^+ ratio in cotton seedlings (Dong *et al.*, 2014); enhancement in the enzymes involved in nitrogen metabolism namely nitrate reductase and nitrite reductase in tomato (Manal *et al.*, 2014) also reduction of lipid peroxidation, hydrogen peroxide and superoxide anions; elevation in the activity of major antioxidant enzymes accompanied with increase in the accumulation of biochemicals such as proline, glutathione and sugars under salinity stress in numerous plant system (Hayat *et al.*, 2012; Dong *et al.*, 2014; da Silva *et al.*, 2015; Hameed *et al.*, 2015; Ahmad *et al.*, 2016).

Multifunctional response of calcium in plant system during salinity stress

Calcium is considered as multifunctional element in plants besides as a nutrient, it is involved in several physiological processes like maintenance of membrane integrity, cell wall structure, increasing the activity of key enzymes and phytohormones interaction (Barker and Pilbeam, 2007). Additionally, it plays vital role in signalling network as a secondary messenger under varied environmental conditions (Tuteja, 2009; Batistic and Kudla, 2012). By virtue of this property it is capable of ameliorating the adverse effects of abiotic stresses including chilling, thermal, drought, heavy metals and salinity (Ma *et al.*, 2005; Shao *et al.*, 2008; Siddiqui *et al.*, 2011; Zehra *et al.*, 2012).

Many authors have suggested the beneficial role of calcium ion in the alleviation of the adverse effects of abiotic stress conditions. Therefore, the maintenance of optimum supply of calcium in saline soil is considered as an important factor in preventing the severity of specific ion toxicities, in those crops which are susceptible during salinity stress injury (Grattan and Grieve, 1999). In their study Hasegawa *et al.*, (2000) suggested that during salt stress, plants are able to tolerate high saline concentration by inducing the signal transduction cascades involving calcium ion. Thus, when exposed to stress conditions including salinity plants increase the cytosolic Ca^{2+} accumulation to combat the oxidative damages. Although the basic mechanism involved has remained unexplained, prevailing models for Ca^{2+} functioning include both membrane stabilisation and signalling significance. Considering the potential role of calcium ion in overcoming the negative impacts of several stresses, it has been implemented in various modes in order to provide stress tolerance to plants. Jaleel *et al.*, (2007) demonstrated that when *Catharanthus roseus* plants were supplemented with calcium chloride under drought condition, calcium ion provided osmoprotection to the plants along with increase in glycine betaine accumulation and indole alkaloid content in the shoot and roots of the plant. Also, a significant enhancement in the activity of antioxidant

enzymes namely superoxide dismutase, catalase and peroxidase was reported in the same plant subjected to salinity stress (Jaleel *et al.*, 2007). According to Khan *et al.*, (2009) when calcium chloride was applied to linseed in combination with gibberellic acid proved more effective in ameliorating the negative effects of NaCl stress. It was found that the electrolyte leakage of membranes was reduced considerably with decrease in the accumulation of lipid peroxides and hydrogen peroxide. Later Sharma and Dhanda, (2015) suggested the protective role of calcium chloride treatment in *Vigna radiata* in which it was found that the presence of calcium ion helped in maintenance of photosynthetic pigments under salt stress. Similarly, calcium was found to maintain the rate of photosynthesis in *Zoysia japonica* under drought stress by reducing the damage of photosynthetic pigment (Xu *et al.*, 2013); increasing the germination rate and growth of forest strand under simulated acid rain (Liu *et al.*, 2011); involved in the enhancement of chilling stress in *Stylosanthes guianensis* by interacting with abscisic acid (Zhou and Ghou, 2009); the application of calcium in the culture medium was found to activate the accumulation of flavonol in *Polygonum hydropiper* (Nakao *et al.*, 1999); also increase in the activity of antioxidant enzymes, regulation of biochemical metabolism and maintenance of membrane integrity by calcium has been reported in plant system under various stress conditions (Jaleel *et al.*, 2007; Khan *et al.*, 2009; Zhou and Ghou, 2009; Xu *et al.*, 2013; Sharma and Dhanda, 2015).

Crosstalk between Nitric Oxide and Ca^{2+}

The complex cross-talk between NO and Ca^{2+} involve components of Ca^{2+} signalling machinery modulated by NO-dependent mechanisms at post-translational and/or transcriptional levels (Besson-Bard *et al.*, 2008). It is considered that NO regulates the overall control of Ca^{2+} homeostasis by regulating almost all types of associated Ca^{2+} channels and transporters. NO controls the Ca^{2+} homeostasis either via S-nitrosylation of the concerned proteins or through other second messengers namely, cGMP and cyclic ADP ribose (cADPR) (Willmott *et al.*, 1996;

Clementi, 1998 ; Stamler *et al.*, 2001; Ahern *et al.*, 2002; Hess *et al.*, 2005) (Fig. 4).

cADPR is a Ca^{2+} mobilizing messenger that induces release of Ca^{2+} from intracellular Ca^{2+} stores into various plant cells by activating the Ca^{2+} permeable channel ryanodine receptors (Allen *et al.*, 1995; Fliegert *et al.*, 2007). The rise in the cytosolic Ca^{2+} concentrations accompanied with an influx of Ca^{2+} from the extracellular space was observed in *Vicia faba* and *Nicotiana plumbaginifolia* when exposed to NO donors (Garcia-Mata *et al.*, 2003; Lamotte *et al.*, 2004). Previous studies suggested ryanodine receptors -like channels act as a main target of NO action and cADPR as key intracellular messenger responsible for mediating NO signals (Willmott *et al.*, 1996; Clementi, 1998). Interestingly, parallel investigation revealed that exogenous NO was unable to trigger any changes in Ca^{2+} concentration in *Nicotiana plumbaginifolia* cells (Lecourieux *et al.*, 2005). Therefore, these findings indicate that the regulation of Ca^{2+} homeostasis by NO might be restricted to specific cellular compartments.

Furthermore, studies on *Vicia faba* guard cells and *Nicotiana plumbaginifolia* cells revealed that protein kinase inhibitors significantly suppressed the NO mediated elevation of $[Ca^{2+}]_{cyt}$ which indicates that besides cADPR involvement of protein kinases is also essential for signalling cascades that relay NO signals to Ca^{2+} channels (Sokolovski *et al.*, 2005; Lamotte *et al.*, 2006).

Interestingly, it has also been suggested that elicitor-induced NO production is enhanced by an upstream influx of extracellular Ca^{2+} (Lamotte *et al.*, 2006; Vandelle *et al.*, 2006). In agreement to above cited literature, a plasma membrane *Arabidopsis* cyclic nucleotide-gated channel (CNGC) member: CNGC2 was identified as a key Ca^{2+} channel which links the rise in Ca^{2+} influx to downstream NOS-like mediated NO production (Ali *et al.*, 2007). Further exploring the complexity of interplay between Ca^{2+} and NO, a study by Vandelle *et al.* 2006 suggested that NO might down regulate its own Ca^{2+} -dependent synthesis by inhibiting elicitor-induced influx of extracellular Ca^{2+} . This negative feedback mechanism is proposed as a strategy to protect the cells from the adverse

effects of excessive accumulation of NO as well as Ca^{2+} .

The evidence summarized above documents the complexity of the interaction between NO and Ca^{2+} , but still a substantial effort is required to understand the mechanisms by which NO modulates fluxes of Ca^{2+} and its cellular homeostasis. Another unresolved issue concerns the impact of interplay between these two signalling molecules on the cell response.

Conclusion

Salinity management strategy is very essential for the production with better yield and quality of agricultural crops. Though some group of plants have developed defence mechanism against the salinity stress, but most of the agricultural crops are found to be susceptible. Therefore, several techniques have been suggested by the scientists for overcoming this problem and the implementation of signalling molecules and other metabolites as potent elicitors has showed considerable success in this field. The signalling molecules NO and Ca^{2+} due to their versatile characteristics are reported to be involved in almost all the processes in plant system. Among which their role in providing salinity tolerance to plants could be of great benefit to agricultural sector. From this review it can be suggested that NO and Ca^{2+} are not only stress signalling molecules but they have active role as intrinsic signal in developmental aspects. Furthermore, extensive study on genetics, proteomics and metabolomics along with additional physiological approaches are essential for better understanding of the mechanistic involvement of both NO and Ca^{2+} in the transduction pathways and their interplay among themselves, other factors and their perception and signal transmission to specific downstream responses.

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