

**Review Article** 

# Role of nitric oxide in seed biology and seed production: A review

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# Abstract

Nitric oxide (NO) is an important signalling molecule employed by plants to control many physiological aspects. This review summarizes that crosstalk between NO/H<sub>2</sub>O<sub>2</sub>/Ca<sup>2+</sup> signalling pathways that drive pollen tube for sexual reproduction in flowering plants. NO is produced in seeds by both enzymatic and non-enzymatic sources that control many physiological aspects of seeds. The interplay of NO and Reactive oxygen species are likely important players in hormonal crosstalk controlling seed germination and dormancy. Mechanism of seed germination and dormancy is mainly regulated by plant hormones like Abscisic acid (ABA) and Gibberellic acid (GA). Based on mode of action of NO with reference to triggering the germination of crop seeds under abiotic stress condition it is infer that there is a linkage between NO and plant growth regulator production. NO cross-talk with reactive oxygen species (ROS) during abiotic stress condition, modulate the light and hormone depended developmental process in the early stage of plant development. NO action to enhancing abiotic stress tolerance by improving antioxidant enzymes and protection against oxidative damage in many crops are discussed in detail.

Keywords: Nitric oxide, Signalling molecule, Sodium nitroprusside, S-nitroso-N-acetylpenicillamine

# INTRODUCTION

Nitric oxide (NO) could be a small molecule that contains a wide selection of physiological functions in living organisms. Although this gas radical was originally described as an air pollutant, it is involved in signalling activity altogether organisms. Mur *et al.* (2013) witnessed the potential of NO with reference to the regulation of plant physiological process viz., flowering, seed and leaf maturation under stressed envi-

ronmental condition. For the past two decades, exploration work on knowing the mechanism of action of NO in crop plants is in progress. As an outcome past year works, mode of action of NO generated by the plant system either innately or NO generated by the plants with the help of exogenously applied NO donors are coming to limelight. In plants, it was reported that NO regulate the genes connected with hormonal signalling and primary metabolism under environ-

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mental stress condition (Besson-Bard *et al.*, 2009; Grun *et al.*, 2006). NO interacts with the signalling pathways obsessed on 3', 5'-cyclic guanosine monophosphate, Calcium ions, and also with free radicals generated under stress condition (Astier *et al.*, 2010; Mur *et al.*, 2013).

Many researchers observed that NO have a great impact on making the plant to produce many phytohormones as a part of their defence mechanism under not conducive environment for the growth and development of the plants (Freschi, 2013). At protein production and processing level, NO play an important role like S-Nitrosylation of cysteine and even tyrosine residues. In addition to above, they are also able to react with iron centre metalloproteins, that leads to the introduction of conformational changes in the already existing bio-molecules and thus leads to enhance the activity of the concerned bio-molecule (Astier and Lindermayr, 2012).

Mata-Perez et al. (2017) demonstrated the role of NO on nitration of fatty acids in crop plants. An outcome of the NO reaction with sulphur-containing amino acids like glutathione, leads to formation of S-nitrosoglutathione in the plant system with the help of S-nitrosoglutathione reductase enzyme. This process leads to maintain the glutathione content in the plant cell in addition to acting as a reservoir of NO in the plant system (Mur et al., 2013). Several authors observed the role of NO in foliage expansion (Beligni and Lamattina, 1999), lignifications of the cell membrane (Ferrer and Barcelo, 1999), radical development (Pagnussat et al., 2002), multiplication of plants (Grun et al., 2006), transformation of a seed into a seedling (Beligni and Lamattina, 2000; Neill et al., 2003; Zanardo et al., 2005), and overcoming the dormancy related issue of the seeds (Bethke et al., 2006). This review aimed to detailed insight on some of the aspects of NO action in plant reproduction, seed dormancy and germination under abiotic stress condition.

NO sources: The commonly used NO donors are sodium nitroprusside (SNP) and S-nitroso-Nacetylpenicillamine (SNAP), NO scavengers (2-(4carboxyphenyl)-4,4,5,5- tetramethylimidazoline-l-oxyl-3 -oxide [cPTIO]. SNP has been widely used to exploit the diverse NO bioregulatory functions. Positive or negative mode of action of SNP as a signalling compound mainly rely on the system where the experiments are going to be conducted and amount of chemical used for the experiment (Lamattina et al., 2003; Filippou et al., 2012). Metabolites of polyamine and stress amino acid-like proline are properly and timely arranged by SNP at right concentration in view of overcoming the abiotic stress. Further, all the above actions are also carried out on development dependent

#### manners (Filippou et al., 2012).

### NO in pollen germination and pollination

**Stigma pollen-stigma functions:** Initial adhesion of pollen in stigma may depend on biophysical or chemical interactions between stigma surface and exine polymers. Mobilization of pollen coat leads to mixing of lipids and proteins and form "foot" of contact on stigma surface and its later stages proteins and lipids on pollen coat and proteins on stigma surface contribute to adhesion it requires protein-protein interaction. (Luu *et al.*, 1999; Takayama *et al.*, 2000).

Some signals that drive pollen tube toward the micropyle - chemotropic molecules. This process is associated with involvement of more number of tissues, and their associated environmental conditions range from aerobic (stigma are placed) to near an-aerobic condition (inner side of the ovary) and partly an-aerobic during the growth of the pollen tube inside the style (Feijó, 2010). Prevailing conditions among the tissues are totally diverse in nature, and accordingly, some sort of modulation in pollen tube growth take place. Palanivelu and Preuss (2006) observed that pollen tube growth is very much regulated by genes by decoding a different range of chemotropic molecules. They confirmed the interaction between the pistil and pollen tubes were under the control of genes action by conducting mutation studies.

There are two types of chemotropic cues observed are positive chemotropic that grow towards the useful parts, and another one is negative chemotropic that grow away from harmful acids.

In the majority of crops, NO act as positive chemotropic cues. In the process of pollination in flowering plants, the landing of pollen on the stigmatic surface and occurrence of events related to the germination of pollen and pollen tube formation was controlled by NO. Respiratory Burst Oxidase Homologue was expressed very specifically in pollen and made the plasma membrane of pollen to produce important key element H<sub>2</sub>O<sub>2</sub>. Produced excess amount of H<sub>2</sub>O<sub>2</sub> regulates the synthesis of NO through NR and NOA1 or with any one of these. This biochemical event occurrence leads to block respiratory burst oxidase homologue activity by S-nitrosylation, and thus leads to prevention of over and above production of H<sub>2</sub>O<sub>2</sub>. Sometimes NO also produced under acidic pH conditions where in nitrite dismutates convert nitrite to NO and nitrate.

Binding of DGK4 with NO accelerates the formation of phosphatidic acid through phosphorylation of diacylglycerol. Produced phosphatidic acid in-turn made Ca<sup>2+</sup> available from within the cells. Side by side NO also activates protein kinases to enable bonding of Ca<sup>2+</sup> Respiratory burst oxidase homologue H and J and thus leads to triggering the production of ROS.



**Fig. 1.**  $NO/H_2O_2/Ca^{2+}$  Signaling pathways during sexual reproduction in flowering plants (Source: Gutermuth et al., 2013).

Cyclic Guanoshine Mono Phospate also triggers the  $Ca^{2+}$  channels at the plasma membrane of flowering plants. Exogenous compounds like polyamines (acting through ROS), peptides, or secretion of ovules, may also increase NO and cytosolic  $Ca^{2+}$  concentration. It is assumed that a fertilized embryo sac may exhibit an NO burst which will block the further pollen and pollen tube entry into the fertilized ovule and preventing polyspermy (Gutermuth *et al.*, 2013) (Fig 1).

The production of NO by pollen itself was demonstrated by Bright *et al.* (2009). This gas has been co-opted evolutionarily for the task of cell-cell communication during the programming phase of sexual reproduction. A rapid and transient increase in ROS and NO, each showing a distinctive "signature," was recently demonstrated during self-incompatible (SI) fertilization in *Papaver rhoeas* (Wilkins *et al.*, 2011). Both NO and ROS were controlling the key event associated with SI -programmed cell death. This mode of cell death activity was reported to have a strong linkage with an enzyme like DEVDase/caspase-3 and actin punctuate foci produced by the action of SI events occurrence in the flowering plants.

#### Role of NO in germinating seeds

**Non-enzymatic sources:** Nitrite represents a reservoir for the production of reactive nitrogen species, due to the equilibrium of nitrite with its conjugated acid, nitrous acid. Nitrous acid is increased under acidic pH and reductants, but the continuous release of NO and its reactive metabolites occur spontaneously, even in neutral or slightly acidic environments. Due to availability of more amount of nitrite form of nitrogen under field condition and prevalence of low <sub>P</sub>H in the apoplast of seeds resulted in production of more

amount of NO in seed through non-enzymatic reduction reaction is a most important event as for the abiotic stress defence mechanism of seed is concern (Bethke *et al.*, 2004, Miles *et al.*, 1996 and Bethke *et al.*, 2007).

**Enzymatic sources:** The major origin of NO production in plants is through the action of NAD(P)Hdependent nitrate (NR) or nitrite (NiR) reductases (Yamasaki *et al.*, 1999). NR plays a key role with reference to the production of nitrite and NO from nitrate through the reduction process. NO production activity of NR was demonstrated both under in vivo and in vitro conditions by Courtois *et al.*, (2008). NR activity in the aleurone layer of barley is rapidly induced by nitrate treatment, but not by GA treatment (Ferrari *et al.*, 1969 and 1970). NR in the aleurone layer can contribute both to NO production and to the increased availability of nitrite for its non-enzymatic conversion to NO (Bethke *et al.*, 2004).

Role of NO in breaking of seed dormancy and promotion of seed germination: Inorganic nitrites, nitrates, and other nitrogenous compounds have been known to break dormancy and stimulate seed germination in many plant species (Giba *et al.*, 2007 and Bethke *et al.*, 2007). Organic nitrates such as nitroglycerine have also been reported to be efficient stimulators of light-dependent and phytochrome-controlled germination in seeds of *Pauwlonia tomentosa* (Grubisic *et al.*, 1992) and *Stellaria media* (Jovanovic *et al.*, 1997).

Effects of nitrogen oxides on seed germination were obtained during research on the active components of fire smoke, known to significantly induce the germination of dormant seeds (Keeley *et al.*, 1997 and 1998). NO signalling will alter the phytochrome pathways in light-induced germination of *A. thaliana* seeds (Batak *et al.*, 2002). Seed germination associated with phytochrome A was very much affected by exogenous application of both organic and inorganic form of nitrogenous fertilizer and nitric oxide donors, whereas phytochrome B aided germination process is not that much getting affected. Bethke *et al.*, (2004) demonstrated the NO production potential of aleurone layer of barley endogenously.

Inhibitory effects of Abscisic acid could not be overcome by SNP, suggesting that the ABA acted downstream of the NO pathway (Bethke *et al.*, 2004). The enhancement of seed germination by sodium nitroprusside (SNP) and S-nitroso-N-acetylpenicillamine (SNAP) is inhibited by 2-(4-carboxyphenyl)-4,4,5,5tetramethylimidazoline-I-oxyl-3-oxide (cPTIO), a NO inhibitor, and also by inhibition of ethylene biosynthesis. Signorelli *et al.*, (2018) states that NO gates the ABA signalling network at multiple steps, ensuring redundant and effectively irreversible control of germination.

Cross talk of NO, ROS and plant hormones in seed dormancy and germination: Reactive oxygen and nitrogen species are important players in the hormonal cross-talk that controls the seed germination. The interplay of reactive oxygen species, NO and ABA is known from the regulation of stomatal closure (Wilson *et al.*, 2008). Okamoto *et al.*, (2006) conducted a mutation study in view of demonstrating the germination inhibition property of ABA even in imbibed seeds. As an outcome of the above study, they proved that the dormancy period of seeds might be prolonged for a longer period by preventing the catabolic process associated with ABA degradation.

NO signal might increase the activity of ABA degrading enzyme. The rapid NO-induced decrease of ABA in the early stage of *Arabidopsis* seed imbibition is mediated by a rapid increase in *cyp707a2* expression. NO produced in the endosperm layer during imbibition regulates *cyp707a2* expression, preceding the increase of ABA catabolism required for seed germination (Liu *et al.*, 2009).

Signorelli *et al.*, (2018) reported that absence of NO in dry seeds the ABA activities was higher in dry seeds and then binding of ABA to the ABA receptors like PYR/PYL/RCAR, results in the inactivation of type 2C protein phosphatases (PP2C). This inactivation triggers the activity of the SnRK2 kinase, which promotes the activity of the basic leucine zipper transcription factor ABSCISIC ACID INSENSITIVE5 (ABI5). Finkelstein and Lynch, (2000) and Lopez-Molina *et al.*, (2001) reported that ABI5 is thus considered a key repressor of seed germination and post-germination development.

Presence of NO Firstly, RNS (Reactive nitrogen species) can inactivate the PYR/PYL/RCAR receptor by tyrosine-nitration (Castillo *et al.*, 2015), enabling the activity of PP2C, which inactivates SnRK2. Thus, the ABI5 is inactive. Secondly, different SnRK2 proteins (SnRK2.6, SnRK2.2, and SnRK2.3) were shown to be inactivated by S-nitrosylation (by NO), affecting ABA signalling (Wang *et al.*, 2015). Thirdly, NO assists the



Fig. 2. Involvement of RNS in the ABA-Mediated dormancy control (Source : Signorelli et al., 2018).

degradation of ABI5 by the S-nitrosylation (Albertos *et al.*, 2015). Finally, NO promotes the degradation of the Group VII ETHYLENE RESPONSE FACTORS (ERF) which are positive regulators of the transcription of ABI5 (Gibbs *et al.*, 2014) (Fig 2).

Hayat et al., (2014) observed that when the seeds of tomato were soaked in double-distilled water (control) or sodium nitroprusside (SNP; 10<sup>-8</sup>, 10<sup>-7</sup>, 10<sup>-6</sup>, 10<sup>-5</sup>, 10<sup>-</sup> <sup>4</sup>, 10<sup>-3</sup>, 10<sup>-2</sup>, 10<sup>-1</sup> or 1.0 M) a nitric oxide (NO) donor, for 8h. Lower concentrations of SNP (10<sup>-4</sup>, 10<sup>-5</sup>, 10<sup>-6</sup>, 10<sup>-7</sup>, or 10<sup>-8</sup> M) enhanced the germination percent, length of root, shoot, NR, proline and antioxidant activities significantly over the control. Whereas, the higher concentration of SNP (1M) showed an inhibitory effect (Table 1 and 2). It was concluded that nitric oxide acts as a stimulator at low concentrations, whereas at higher concentration, it acts as a stress inducer. The toxic effect of NO in high concentrations has most widely been attributed to its ability to damage membranes and cause DNA fragmentation (Pedroso et al., 2000; Yamasaki, 2000; Romero-Puertas et al., 2004). In addition, high concentrations of NO may cause the arrest of cell division, apoptosis, and ultimately senescence of whole plants. It has also



**Fig. 3.** (*A*) Germination of Desi and Kabuli chickpea at 24, 48, and 72 h post-imbibition. (B) Germination percentage of Desi and Kabuli chickpea at 24, 48, and 72 h post-imbibition (Pandey et al., 2019).

SNP Concentration (M)	Germination (%)	Root length (cm)	Shoot length (cm)
Control (water soaked)	73.30	3.58	2.99
1.0 M	66.65	2.13	2.56
10 <sup>-1</sup> M	66.65	2.99	2.79
10 <sup>-2</sup> M	66.90	3.35	2.96
10 <sup>-3</sup> M	68.30	3.50	3.04
10 <sup>-4</sup> M	86.65	3.66	3.94
10 <sup>-5</sup> M	96.60	4.81	4.23
10 <sup>-6</sup> M	82.12	3.96	3.27
10 <sup>-7</sup> M	80.00	3.82	3.27
10 <sup>-8</sup> M	78.30	3.71	3.10

**Table 1.** Effect of pre-sowing seed soaking in sodium nitroprusside (SNP) on germination (%) and length of root and shoot of tomato.

(Source: Hayat et al., 2014)

(Source: Hayat et al., 2014)

**Table 2.** Effect of pre-sowing seed soaking in sodium nitroprusside (SNP) on POX [ $g^{-1}(F.M.$ )], CAT [n mol H<sub>2</sub>O<sub>2</sub> decomposed  $g^{-1}$  (F.M.)] and SOD (units  $g^{-1}$  FM) activities of tomato (Source: Hayat *et al.*, 2014).

SNP Concentration (M)	POX g <sup>-1</sup> (F.M.)	CAT [n mol H <sub>2</sub> O <sub>2</sub> de- composed g <sup>-1</sup> (F.M.)]	SOD (units g <sup>-1</sup> FM)
Control (water soaked)	7.30	178	35
1.0 M	6.65	145	55
10 <sup>-1</sup> M	6.55	162	59
10 <sup>-2</sup> M	6.20	165	54
10 <sup>-3</sup> M	6.80	169	56
10 <sup>-4</sup> M	8.65	224	66
10 <sup>-5</sup> M	9.60	233	73
10 <sup>-6</sup> M	8.22	216	67
10 <sup>-7</sup> M	8.00	193	65
10 <sup>-8</sup> M	7.83	176	62



**Fig. 4.** Germination response of chickpea seeds in the presence of water, SNAP, SNP, and KNO2. (A) Desi variety; (B) Kabuli variety (Source: Pandey et al., 2019).

been reported that higher concentration of NO inhibits the activity of some potential anti-oxidative enzymes such as catalase leading to higher  $H_2O_2$  and other reactive oxygen species (ROS) (Bethke *et al.*, 2007). Pandey *et al.*, 2019 reported the application of nitric oxide (NO) in two chickpea varieties (Desi and Kabuli) and germination capacity are differing in desi and kabuli varieties. Kabuli has minimum germination and germinates slowly, and Desi has maximum germination capacity. Desi produced more NO than Kabuli and had lower respiratory rates. As a result of the high respiration rates, Kabuli had higher levels of reactive oxygen species (ROS). Treatment with the NO donor S-nitroso-N-acetyl-D, L-penicillamine (SNAP) reduced respiration in Kabuli and decreased ROS levels, resulting in accelerated germination rates. These findings indicate that NO plays a key role in improving germination in Kabuli (Fig. 3 and 4).

Role of NO in response of germinating seeds to stress conditions: Laspina *et al.*, (2005) reported that the unfavourable conditions viz., both soil and air pollutants, soil problem, water deficit conditions of field and more ultraviolet light activity caused serious damage in plant tissues at the molecular level and resulted in the formation of reactive oxygen species. The possible ROS are H2O2, superoxides, singlet oxygen and some other free radicals (Therond *et al.*, 2000). Leshem, (1996) reported that NO induces some sort of stress in the plant system, but most of the author reported that NO is a molecule associated with plants to overcome abiotic stresses, provided it should be made available in the right amount and right time in plants during stress. Some of the plants generate NO either exogenously or innately to combat abiotic stressinduced physiological abnormalities (Garcia-Mata and Lamattina, 2001; Neill *et al.*, 2002), salinity (Zhao *et al.*, 2004 and 2007). Wany *et al.*, (2018) observed the detoxification role of NO with reference to ROS inactivation and making the plant to overcome the abiotic stress.

Nitric oxide being a potential signalling molecule, improves stress tolerance. During seed germination, high metabolic activities are activated, and it consumed high rates of oxygen for their mitochondrial respiration, which creates an oxygen-less environment inside the seed. NO is one of the key regulators for maintaining oxygen consumption inside the seeds (Erusalimsky *et al.*, 2007), which plays a pivotal role in the sensing and

 Table 3. Effect of different concentrations of arginine and SNP on some compatible solutes, phenols, and auxin contents of sunflower plants under salinity stress.

Salinity level	Material	Conc. (mM)	TSS (mg/100g dry wt.)	Proline (mg/g dry wt.)	Free AA (mg/ g dry wt.)	Phenol (µg/g dry wt.)	IAA (µg/g fresh wt.)
	Control	0	1535.10	8.79	124.81	13.91	46.60
	Argining	10	2115.07	12.11	171.96	16.52	50.71
0	Arginine	20	2544.07	14.57	206.84	19.41	56.85
	SND	0.25	1887.00	13.62	155.05	18.70	52.00
	SNP	0.50	2160.50	14.37	175.65	18.34	51.96
5000(mg/L)	Control	0	2611.29	14.95	212.30	16.12	37.57
	Arginine	10	3065.78	17.55	249.25	17.46	40.68
		20	3277.34	18.76	266.45	17.98	43.13
		0.25	2990.35	15.34	217.80	17.10	41.09
	SNP	0.50	3261.29	16.45	232.30	17.62	41.57
LSD at 5%			78.42	0.51	6.14	0.47	1.29

(Source: Ramadan et al., 2019)

**Table 4.** Effect of different concentrations of arginine and SNP on catalase, superoxide dismutase, and peroxidase enzymes (µg/g fresh wt./hour) of sunflower plants under salinity stress.

Salinity level	Material	Conc. (mM)	CAT	SOD	ΡΟΧ
	Control	0	155.05	68.97	143.30
	Arginino	10	175.18	73.87	160.20
0	Arginine	20	186.35	80.60	172.64
	SNP	0.25	176.35	81.81	164.19
		0.50	185.65	90.20	181.59
	Control	0	167.05	80.65	172.65
5000(mg/L)	Arginine	10	188.15	97.75	188.65
		20	193.35	110.48	193.25
	SNP	0.25	195.65	103.72	179.80
		0.50	215.62	114.52	187.93
LSD at 5%			1.448	0.84	0.73

(Source: Ramadan et al., 2019)

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Treatments	Water potential (Ψ <sub>w</sub> , )MPa)	Osmotic potential (Ψ <sub>s</sub> ,)MPa)	Pressure potential (Ψ <sub>p</sub> , MPa)	Relative water con- tents (%)
CK1	0.52	0.98	0.46	85.33
CK2	1.21	1.33	0.12	43.41
SP 50	1.10	1.27	0.17	47.87
SP 100	0.95	1.22	0.27	53.67
SP 150	0.98	1.24	0.26	51.53
FA 50	0.97	1.21	0.24	47.44
FA 100	0.81	1.11	0.30	56.74
FA 150	0.92	1.19	0.27	52.50

Table 5. Effects of Nitric oxide on the plant water relations in rice under drought stress.

balancing of oxygen concentration in plant seeds. NO might be able to integrate oxygen sensing and to balance with the control of respiration, ATP production and the metabolic intensities of the storage pathways (Benamar *et al.*, 2008).

NO application alleviated drought and salinity effects, as revealed from substantially reduced membrane permeability, electrolyte leakage and lowered leaf  $H_2O_2$  and MDA contents. Application of NO seed and foliar will constantly improve the stress tolerance by maintenance of photosynthesis, tissue water status, improved antioxidant activities and reduced ROS injuries. NO application substantially improved leaf water potential, turgor potential and RWC. Exogenous NO application induces stomatal closure and protects cells against oxidative stress. High level of accumulated (Source: Farooq et al., 2009)

proline enabled the plant to maintain turgor and water potential and exogenous NO application promoted the accumulation of free proline (Neill *et al.*, 2008).

**Role of NO under salt stress:** Ramadan *et al.*, (2019) observed that the application of nitric oxide NO donors (arginine and sodium nitroprusside) have protective effects on salinity stress in sunflower plants through improving the antioxidant enzymes and osmotic adjustment which improved plant antioxidative defence system (Table 3 and 4). arginine as a precursor of polyamines which increased vegetative growth, growth promoters, antioxidant enzymes, endogenous amino acids, and their translocation to the produced grains under normal or stress condition.

Salinity stress increases in TSS, proline, and free amino acids and these will improve plant cells tolerance to

 Table 6. Effects of NO application on leaf hydrogen peroxide, leaf malondialdehyde and membrane permeability in rice under drought stress.

Treatments	Leaf H <sub>2</sub> O <sub>2</sub> (µmol g <sup>-1</sup> FW)	MDA (µmol g <sup>-1</sup> FW)	Membrane permeability (%)
CK1	5.3	12.33	10.5
CK2	17.50	25.45	23.33
SP 50	14.50	21.67	14.67
SP 100	12.35	19.74	13.16
SP 150	12.75	20.37	14.33
FA 50	11.69	19.83	13.85
FA 100	10.21	18.33	12.60
FA 150	11.23	19.23	12.88

(Source: Farooq et al., 2009)

Table 7. Effects of NO application on leaf free proline and antioxidants in rice under drought stress.

Treatments	Leaf-free proline content (µmol g <sup>-1</sup> DW)	SOD (unit g <sup>-1</sup> protein) <sup>1</sup>	CAT (µmol min <sup>-1</sup> g <sup>-1</sup> protein)	APX (µmol min⁻ <sup>1</sup> g⁻¹ protein)
CK1	5.8	9.6	7.45	7.21
CK2	7.8	7.75	5.53	6.10
SP 50	9.78	9.32	6.31	6.68
SP 100	10.3	11.63	7.23	7.45
SP 150	9.81	10.67	7.11	6.25
FA 50	9.83	9.37	7.28	6.43
FA 100	12.3	13.00	8.32	8.25
FA 150	10.5	11.60	7.85	7.12
			(5	Source: Farooq <i>et al.,</i> 2009)

salinity stress via increasing osmotic pressure in the cytoplasm moreover relative water contents essential for plant growth (Tawfik *et al.* 2017). Huang *et al.* (2005) observed that phenol considered as an antioxidants defence system, and it will scavenge free radicals from their high reactivity as hydrogen or electron donors. This process leads to stabilize and delocalize the unpaired electron (chain-breaking function) and from their ability to chelate transition metal ions. Khattab (2007) reported that antioxidant enzymes are capable of reducing oxidative damage due to increased production of reactive oxygen species (ROS) resulted from salinity stress. At salinity stress conditions, the harmful effect of hydrogen peroxidase (Dat *et al.* 2000).

Role of NO under drought stress: Faroog et al., (2009) observed the role of NO in improving drought tolerance in fine grain aromatic rice (Oryza sativa L. cv. Basmati 2000) was evaluated. NO donar (Sodium nitroprusside) was used at 50, 100 and 150 µmol 1<sup>-1</sup> both for seed priming and foliar spray. Foliar treatments 100 µmol 1<sup>-1</sup> proved more effective than the seed treatments (Table 5, 6 and 7). Bascon et al., (2017) reported that transgenic barley plants (UHb) low NO production under drought conditions was associated with increased drought tolerance. Transgenic barley plants (UHb) plants having a high number of total polyamines such as spermidine and these correlated with the accumulation of the amino acid precursors of polyamines and with the expression of specific polyamine biosynthesis genes. This suggests a potential interplay between NO and polyamine biosynthesis during drought response.

#### Conclusion

NO is regarded as a poisonous air pollutant- responsible for the formation of photochemical smog, acid rain and destruction of the ozone layer. Nowadays, NO is most appreciated as a molecule essential for innumerable functions in plant and animals. It is a key signalling molecule that controls plant growth and development. NO signals will guide the pollen to reach ovules. The lower concentration of NO will break dormancy and stimulate germination, and at higher concentration, it would be toxic to seeds. The signalling action of NO was evident primarily with enhanced expression of antioxidants, improved cellular membranes stability, photosynthesis and leaf water status by the synthesis of compatible solutes.

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