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# ALLEVIATION OF TOXIC EFFECTS OF DIFFERENT SALINITY LEVELS ON MEMBRANE INJURY AND CHLOROPHYLL CONTENT BY DIFFERENT NO DONORS IN CHICKPEA LEAVE

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**ABSTRACT**
The present work aims at investigating the role of exogenously supplied nitric oxide (NO) donors in alleviating effects of salinity in chickpea leaves. Chickpea plants (HC-3) were raised in small plastic containers containing 400g of soil saturated with increasing levels of chloride dominated salinity (0, 2, 4, 6 and 8 $dSm^{-1}$). Two NO donors *viz*. Ascorbic acid (100µM) + Sodium Nitrite ($NaNO_2$, 200µm) and SNP (Sodium nitroprusside, 250 and 500µm), were sprayed after 15 days of germination. The plants were sampled 24 and 72 hrs after NO treatment for estimation of relative membrane injury and chlorophyll content. A gradual increase in membrane injury (MI) was observed with increasing levels of salinity. NO donors decreased the MI as compared to salinity treated plants. Among the two NO donors, SNP was more effective in decreasing the MI. The total chlorophyll content decreased with increasing salinity levels. Both the NO donors alleviated the salinity by increasing the chlorophyll content and again SNP was found to be more effective as compared to the other NO donor. In conclusion, the toxic effects of salinity on MI and chlorophyll content could be ameliorated by the use of NO donor SNP.

## INTRODUCTION

A wide range of environmental stresses such temperature, drought, alkalinity, salinity and UV stress are potentially harmful to the plants. Osmotic stress induced by conditions like drought, salinity, water logging and heat etc. impairs plant growth and development by affecting plant physiological processes (Marked *et al*., 2014; Nagar *et al*., 2015; Kumar *et al*., 2015). Out of these salinity is a major threat to crop yields, especially in countries where irrigation is an essential aid to agriculture. Salinity limits the productivity and quality of economically important crops throughout the world (Parvaiz and Satyawati, 2008; Rahdari and Hoseini, 2011). According to the FAO (2008), over 6% of the world's land is affected by either salinity or sodicity. The present investigations were conducted on *Cicer arietinum* L. (HC-3) plants. It is an important pulse crop of arid and semi-arid regions of India which is facing the problem of reduction in crop productivity due to salinity. Several physiological and biochemical processes like photosynthesis, protein synthesis, energy and lipid metabolism are affected by salinity. Salt stress disturbs intracellular ion homeostasis in plants, which leads to membrane dysfunction, attenuation of metabolic activity and other secondary effects that cause growth inhibition and ultimately lead to cell death (Hasegawa *et al*., 2000). Consequence of salinity is that at some stage of exposure it resulted in an increased production of ROS (Wang *et al*., 2004; Kukreja *et al*., 2005). These ROS are highly reactive and cause cellular damage through oxidation of lipids, proteins and DNA injury (Shi *et al*., 2007). To control the level of ROS, plants have evolved an antioxidant defence system comprising of antioxidative enzymes and metabolites which are responsible for scavenging excessively accumulated ROS in plants under stress conditions (Jung *et al*., 2000). Salinity stress also results in alterations in the activities of antioxidative enzymes and metabolites (Shi *et al*., 2007; Abbaspour, 2012).

The plant system under stress could be survived if the free radical production is balanced by free radical scavenger. So by increasing the concentration exogenously applied compounds which is having free radical scavenging properties resulted in detoxification of stress-induced free radical production (Zhang *et al*., 2006; Molassiotis *et al*., 2010). In this study, we have used nitric oxide (NO) as antioxidant compound to reduce salinity toxicity.

NO is a small highly diffusible and ubiquitous bioactive molecule that takes part in many physiological processes in plants (Desikan *et al*., 2004). Its chemical properties makes it a versatile signal that functions through interactions with cellular targets via either redox or additive chemistry (Lamattina *et al*., 2003; Wendehenne *et al*, 2004). In plants enzymes involved in NO biosynthesis are nitric oxide synthase (NOS), nitrate reductase (NR), xanthine oxidase and reductase. Nitric oxide is itself a reactive oxygen species and its dual behaviours (protective or toxic) depends upon both concentrations and tissue where it acts (Hsu and Kao, 2004). Nitric oxide has been suggested to be involved in defence response to biotic or abiotic stresses (Shi *et al*., 2012) and appear to be present

in most of stress reactions (Gould *et al.*, 2003). It has been reported to exert a protective effect in response to drought stress (Wang *et al.,* 2004), osmotic stress (Zhao *et al.*, 2008), salt stress (Sheokand *et al.*, 2008), heavy metal stress (Singh *et al.*, 2008, Kumari *et al.,* 2010) and oxidative stress (Beligni and Lamattina, 2002). However, experimental evidence for NO as a signal molecule under salinity for chickpea is still preliminary. The present work aims at investigating the role of exogenously supplied NO donors in alleviating the effect of salinity on membrane injury and chlorophyll content in chickpea leaves.

## MATERIALS AND METHODS

The present investigation was carried out on chickpea (HC-3) plants. Seeds were procured from Pulses Section, Department of Plant Breeding, CCS Haryana Agricultural University, Hisar. Sodium nitroprusside (SNP) and a mixture of ascorbic acid and sodium nitrite were used as NO donors.

**Raising of plant material**

The surface sterilized seeds (5 seeds per container) were raised in plastic containers containing 400 g of loamy sand. Salinity levels of 0, 2, 4, 6 and 8 $dSm^{-1}$ along with nutrient solution were applied before sowing. Irrigation was given with distilled water. Desired salinity levels were developed by saturating soil with saline water of respective electrical conductivities which were prepared by using the mixture of NaCl, $MgCl_2$, $MgSO_4$ and $CaCl_2$, where N: Ca + Mg (1:1), Ca: Mg (1:3) and Cl:$SO_4$ (7:3) on meq basis *i.e.* chloride dominated salinity was used. After 15 days of germination (DAG) NO donor (i) SNP (250 and 500 µM), (ii) a mixture of ASA (100 µM) and $NaNO_2$ (200 µM) was applied through foliar spray and leaf samples were collected 24 and 72 hrs after NO application. The following estimations were conducted.

**Membrane injury (MI)**

MI was analyzed according to the method of Zhang *et al.* (2006). 250 mg of fully expanded leaves were rinsed with distilled water and immersed in 10 ml de-ionised water in test tubes and incubated at 25ºC for 4 hrs. Electrical conductivity of water in which leaves were kept, was measured. The tissue along with leachate was boiled at 100ºC for 30 min to completely disrupt the cell structure. The solution was brought to 25ºC and EC was measured again. MI (%) was calculated as follows:

$$M.I(\%) = \frac{\text{Intial readings}}{\text{Final readings}} X100$$

**Chlorophyll content**

Leaf discs (0.03 g) were washed blotted dry and dipped in test tubes containing 3 mL of dimethyl sulfoxide (DMSO) overnight as described by Sawhney and Singh (2002). The extracted chlorophyll in DMSO was estimated by recording its absorbance at 663 and 645 nm, and its amount was calculated from the formula:

$$Chl'a' = \frac{12.3\,A_{663} - 0.86\,A_{645}}{ax1000xW} xV$$

Where,

V = Volume of DMSO

a = Path length

W = Weight of tissue taken

**Statistical analysis**

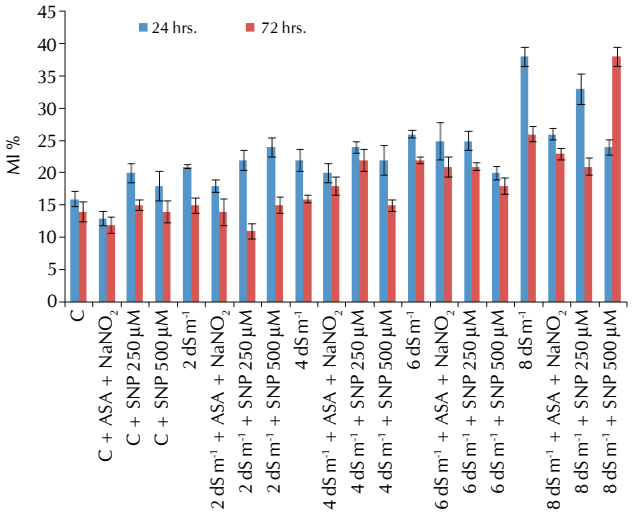
Data was analysed statistically using two factorial CRD

## RESULTS AND DISCUSSION

The objectives of this experiment were to determine the NO donor which is effective in reducing the toxic effects of salinity. The salinity treatments of 0, 2, 4, 6 and 8 $dSm^{-1}$ were given before sowing by saturating the soil with saline solution of desired electrical conductivity and NO donors were applied through foliar spray after 15 of germination. The SNP (250 and 500 µM) and a mixture of ASA (100 µM) + $NaNO_2$ (200 µM) were used as NO donor. The leaf samples were collected after 24 and 72 hrs and were analyzed for membrane injury (%) and Chlorophyll content.

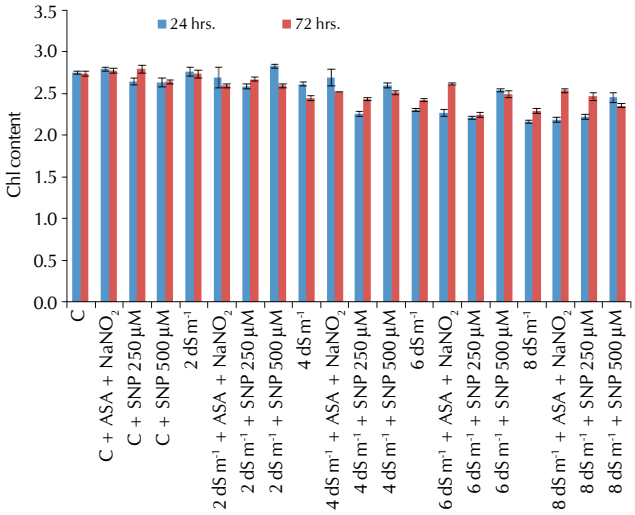
Membrane injury increased with the increase in salinity levels. Salinity induced damage was reflected in terms of increased electrolyte leakage. A concentration dependant increase in electrolyte leakage was observed as the salinity levels were increased from 0 to 8 $dSm^{-1}$ (Fig. 1). Salt stress makes the membrane leaky as evinced by increased electrolyte leakage (Li *et al.*, 2008; Jamil *et al.,* 2012).With 6 $dSm^{-1}$ salinity a 63 to 57 per cent increase in MI (%) was observed. SNP (500 µM) was found to be the effective NO donor in terms of alleviation of toxic effect of salinity on MI (%) as compared to ASA + $NaNO_2$ and SNP 250 µM (Fig. 2). Protective effect of exogenously applied NO donors on membrane damage has been reported under salt stress (Sheokand *et al.,* 2008; Wang *et al.,*2009). NO donors decreased the MI and among NO donors SNP (500 µM) was found to be more effective. It decreased MI (%) by 25 to 29 per cent,

The lower salinity treatment of 2 $dSm^{-1}$ had no significant effect on chlorophyll content after which a gradual decline was observed with 4, 6 and 8 $dSm^{-1}$ treatments. A 16 to 21% decline was observed with 6 and 8 $dSm^{-1}$ salinity treatment. Plant pigments Chlorophyll a, chlorophyll b and carotenoid are main photosynthetic pigments and they play important role in photosynthesis (Sarwat and El-Sherif, 2007). Net photosynthesis and stomatal conductance are significantly affected due to changes in chlorophyll content and chlorophyll fluorescence, damage of photosynthetic apparatus and chloroplast structure (Doganlar *et al.,* 2010). The decrease in chlorophyll content under saline conditions was reported in many plants such as *Zea mays*, *Carthamustinctorius,*Bean and *Paulownia imperiallis* (Rahdari and Hoseini, 2012; Rahdari *et al.*, 2012). Khan *et al.* (2009) also observed a reduction in Chl content under salt stress in wheat genotypes. Sheokand *et al.* (2008) have observed a small decline of 11% in Chl content with short term salt treatment (48 hrs) of 100 mM NaCl. However, with long term salinity a 63 per cent decline in Chl content in wheat leaves was observed




CD at 5% level of significance.; H = 0.895 ;T = 2.831; HxT = 4.004 where H = Hours after treatment (HAT);T = Treatments

**Figure1: Effect of different salinity levels and NO donors on membrane injury (%)**




CD at 5% level of significance; H = 0.025; T = 0.078; HxT = 0.110; where H = Hours after treatment (HAT); T = Treatments

**Figure 2: Effect of different salinity levels and NO donors on chlorophyll content (mg/g FW)**

(Krishnamurthy, 1991).The decline in chlorophyll content is due to increasing of destructive enzymes called chlorophyllase. Pigments system reduction is attributed to a salt induced weakening of protein-pigment-lipid complex or increased chlorophyllase enzyme activity (Turan *et al.,* 2007). However increase in chlorophyll content under salinity has been reported in rice (Doganlar *et al.,* 2010) and that this incrementmay be due to increase in the number of chloroplast in the stressed plant leaves (Chaum and Kirdmanee, 2009).

Among NO donors used SNP was found to be more effective than AsA + $NaNO_2$. Further among the SNP treatment the 500 $\mu$M treatment was found to be more effective in terms of amelioration of toxic effect of salinity by increasing the Chl content by 9 per cent with 6 $dSm^{-1}$ salinity levels (Fig.). Protective effect of exogenously applied NO donor has been reported in wheat seedlings under age induced decline in Chl content (Tu *et al.*, 2003), oxidative stress (Beligni and Lamattina, 1999) and salt stress (Zhang *et al.*, 2006; Sheokand *et al.*, 2008), heavy metal stress (Kumari *et al.,* 2010).

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