International Journal of Agricultural and Life Sciences- IJALS (2016), Volume 2 (3) pp.43-47
http://doi.org.in/10.9379-sf.ijals-122064-007-0081-x

Available online at http://www.skyfox.co
ISSN: 2454-6127

Research Article

Open Access

Exogenous chemical mediated induction of salt tolerance in Soybean plants

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Received: Sep 2016 / Accepted: Sep 2016/ Published: Sep 2016

ABSTRACT: To evaluate the potential of seed priming tools in soybean salt stress tolerance with improved plant growth and biomass content. Soybean seeds were primed with 5 different priming agents (proline, sodium nitroprusside (SNP), glycienbetaine, hydrogen peroxide & mannitol) then germination under laboratory conditions using 100mM NaCl stress condition was evaluated. Results indicated that SNP (nitric oxide donor) was found most effective agent for growth promotion, while unprimed treatment decreased germination, growth and biomass related parameters. SNP-primed seeds had a higher germination percentage (82%) and seedlings were exhibited increased proline content (105%) as compared to unprimed treatment. The protective mechanism of SNP against oxidative stress was correlated with lower lipid peroxidation (MDA content). Notably, the ability to maintain biomass level (41% reduction) as well as chlorophyll content indicated a role of SNP in alleviation of salt stress and induce tolerance. Altogether, our results highlight that exogenously SNP could be employed to attain better growth and development of soybean and perhaps other legumes under salt stress.
Keywords: Nitric oxide, Priming, Salinity, Soybean, Sodium nitroprusside

INTRODUCTION

Salinity is one of the most brutal environmental constraints, reducing crop productivity and crop expansion worldwide. In the hot and dry regions of the world the soils are frequently saline due to inadequate irrigation management which affects 20% of irrigated land worldwide (Jamil et al., 2011). An area approximate 7 million hectares of land is covered by saline soil in India wherein most of which occurs in indogangetic plane that covers the states of Punjab, Haryana, Uttar Pradesh and Bihar. Arid tracts of Gujarat and Rajasthan and semi-arid tracts of Madhya Pradesh, Maharashtra, Karnataka and Andhra Pradesh are also largely affected by saline lands (Singh et al., 2012). Therefore, the development of salt tolerant crop varieties has become an urgent concern for many crop-breeding programs to ensure global food security. The variation between salt tolerant (halophytes) and salt sensitive (glycophtes) genotypes provide a genetic basis for engineering salt-tolerant crops. Strategies for making tolerant plants to salinity and produce economically valuable species have been extensively studied for decades. Plants have developed many traits that help them to evolve and succeed across the globe under different environmental regimes. Following changes occurred in plants during stress condition like: modification of cell wall, changes in cell cycle and cell division and production of osmolytes etc. Many stress responsive genes are also expressed, that includes the synthesis of osmoprotectants, detoxifying enzymes, and transporters, as well as genes that encode regulatory proteins such as transcription factors, protein kinases, and phosphatases during stress condition. Adaptation to stresses has been suggested to be mediated by pre-existing or "memory" defences that lead to faster and stronger induction of basal tolerance mechanisms upon sub-sequent exposure of stress (Pastor et al., 2013).

The induction of "memory" defenses in plants has been reported through priming with certain chemicals. Plant's perception for exogenous chemicals is able to induce response against abiotic stresses, providing tolerance and so called "Induced systemic tolerance (IST)". These chemicals are natural products produced in plants at very low concentration and when they are synthesized chemically and applied on plants, they start to controlling downstream process by altering gene expression machinery and regulating various biochemical reactions (Tanou et al., 2012). The exogenous treatment of different chemicals such as nitric oxide (NO), hydrogen peroxide (H2O2), sugars, hydrogen sulphide (H2S), proline (Pro), glycinebetaine (GB), β-aminobutyric acid (BABA), jasmonates (JA), salicylic acid (SA) etc. are known to be involved in plant growth and development and play an important role in integrating various stress signals (Ben Rejeb et al., 2013).

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Soybean is a cheaper source of high quality proteins and has potential to reduce malnutrition, a dominant problem in poor sections of society in the country. According to USDA report (2015), soybean contributed about 60% of the total 536 million metric tons of oilseeds produced globally by major oil crops (sunflower, copra, peanut, cotton, palm and rapeseed). Soybean is a salt sensitive crop exclusively grown in Northwest and Central part of India. These parts are regularly affected by high temperature, soil salinity, low pH and metal toxicity which cause a dramatic reduction in soybean yield annually. Thus, the objectives of the current study was to determine the effects of different priming agents on seed germination of soybean under salt stress; fast seed germination and seedling establishment of soybean under salt stress conditions; and find the best priming agent to alleviate the salinity stress during seed germination and seedling growth stage of soybean.

MATERIAL AND METHODS

PLANT MATERIAL, GROWTH CONDITION AND CHEMICAL TREATMENTS

A pot experiment was conducted in plant growth chamber (28+20C and 70% humidity) to confirm the plant growth promoting potential of six different priming agents. Proline, GB and mannitol were used at 1, 5 & 10mM concentration, while H2O2 and sodium nitroprusside (SNP) at 10, 50 & 100μM. The soybean seeds JS9560 were surface sterilized (1-min, 70% ethanol followed by 3-min, 0.1% HgCl2 then rinsed repeatedly in sterile distilled water) and primed with chemical agents for 1 hr and after that sown in pre-sterilized soil amended with 100mM NaCl. Three seeds were maintained in one pot with six replicates. The growth of the seedlings was recorded after 15 days. There were two more groups, one was unprimed (without any priming treatment) and other was control (without salt treatment).

PHYSIOLOGICAL ANALYSIS

Seed germination, root, shoot length and number of lateral roots were recorded as parameters of plant growth. The value of biomass (dry weight) was compared with the respective non-stressed plants and the reduction in biomass was calculated according to method of Harb and Pereira (2011).

DETERMINATION OF PROLINE CONTENT

Total free proline content was estimated according to method of Bates et al. (1973). The absorbance of pink colour developed was measured at 520 nm and the amount of proline was determined in μg/g f.w from a standard curve.

CHLOROPHYLL ESTIMATION

Chlorophyll estimation was performed according to modified method of (Wellburn et al., 1994) and the total chlorophyll content was calculated using following formula (Lichtenthaler et al., 1985). Chlorophyll a (Chl a) = [(ABS662×11.75)–(ABS645×2.35)], Chlorophyll b (Chl b) = [(ABS645×18.61)–(ABS662×3.96)]. Chlorophyll content = Chl a + Chl b. The amount of pigments was expressed as μg/g f.w.

ESTIMATION OF LIPID PEROXIDATION

The level of lipid peroxidation was measured in the form of malondialdehyde (MDA) content according to the method of Hodges et al. (1999). Malondialdehyde is the end product of lipid peroxidation activity. The absorbance was taken at 532 nm and the amount of MDA content was measured by MDA standard curve (0.1 to 10 nmol).

RESULTS

PHYSIOLOGICAL PARAMETERS

After 15 days of incubation period, proline and SNP treatments were found effective to promote seed germination and seedling's growth as compared to other priming agents under salt stress. The un-treated seeds were exhibited lower germination and plant length under salt stress. Proline treatment was found effective to enhanced seed germination by 70% at 5mM concentration and SNP enhanced by 82% at 100 μM concentration. Glycinebetain, mannitol and H2O2 were showed their effective performance on 10mM, 5mM and 50μM concentrations respectively. Sodium nitroprusside treatment was found to showed highest shoot/root length (12.4/8.3cm) and lateral root numbers (38) than other salt affected treatments. Similarly, SNP treated seedlings showed lower (42%) biomass reduction while, untreated seedlings were found 85% biomass reduction during saline condition (Table 1).

BIOCHEMICAL PARAMETERS

We further tested biochemical response in soybean seedlings. Soybean seedlings were tested for proline, lipid peroxidation and chlorophyll content. After 15 days of salt exposure, SNP treated plants were found to be greener with higher chlorophyll content (6.8 mg/g FW) followed by Pro treated seedlings (4.1 mg/g FW) while un-treated seedlings were exhibited lower chlorophyll content (2.1 mg/g FW) than other treatments under salt stress (Fig.1A). Soybean seedlings treated with SNP were exhibited higher proline content in shoot (57 μg/g FW) and root (97 μg/g FW) as compared with other treatments (Fig. 1B). During salinity stress,

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MDA content was found in all treatment, however SNP treated seedlings were found lower (262 nmol/g FW) as compared to other treatments. Un-treated seedlings were showed highest MDA content (810 nmol/g FW) (Fig. 1C).

DISCUSSION

Plants show different response with respect to their environment. Harsh environmental condition, which is harmful for one plant species (sensitive plant), might not be stressful for another plant (tolerant plant). Of the various legume crops, soybean is most sensitive to salt stress (Manchanda; 2008). Therefore, treatment with 100mM NaCl adversely affected soybean seedlings growth and development by impairing various physiological and biochemical processes, including biomass, membrane permeability, osmotic balance and photosynthesis. To evaluate the efficiency of plant growth promotion, a study was conducted on different exogenous chemical application to protect soybean plant against salt-induced damages. (Hasanuzzaman et al., 2013) described the role and uses of exogenous protectants under salt stress condition and suggested that the positive effect of exogenous protectants depends on their dose, duration and method of treatment. In the present study, after 15 days of plant experiment, SNP treatment was found potent to alleviate 100mM NaCl stress from soybean seedlings as compared to other chemical treatments. Sodium nitroprusside is a nitric oxide (NO) donor which releases NO in the form of nitrosonium cation (NO+) on its reaction with thiolic legends (RSH) (Singh et al., 2016). Nitric oxide is a free radical and considered as a phytohormone and signalling molecule in plants. It has attracted much attention because of its involvement in various physiological processes include germination, growth, senescence, photosynthesis and response mechanisms to various environmental stresses (Tufan et al., 2015). Soil salinity develops ion toxicity in plants which leads to increase ROS content inside the cells. These ROS cause a threat to cells by inflicting peroxidation of lipids, oxidation of protein, harm to nucleic acid and decreased essential cellular metabolic activities including photosynthesis and reduced enzyme activities (Munns; 2008). In this study, SNP treated plants showed higher level of chlorophyll and biomass content which suggest that NO relieves negative effects of salt. The effects of NO in plants under environmental stresses has been extensively studied using exogenous application of NO donors and data indicates that NO regulating the generation of ROS or modulating components of antioxidative system in plants during stress conditions [Singh et al., 2016 ;Fu et al., 2015]. Increased salt concentrations cause the water potential of the soil more negative than the root symplast and results in tissue dehydration. Soybean seedlings treated with SNP were exhibited higher proline content which maintains osmotic balance inside the cells during salt stress. A high level of proline protects plants against osmotic stresses not only by adjusting osmotic pressure but also by stabilizing membrane proteins and enzymes, scavenging of ROS and maintaining redox homeostasis (Banu;2009). Extent of lipid peroxidation was also found lower in SNP treated seedlings. Wu et al. reported that SNP treatment increased chlorophyll, leaf relative water and proline content as well as lower lipid peroxidation and electrolyte leakage in comparison with non-treated plants subjected to salt stress.

CONCLUSION

The findings of the present experiment permit us to conclude that application of SNP in the rhizosphere could be an important strategy in improving performance of soybean plants under salt stress for the following reason. The imposition of salt stress in soybean seedlings led to severe oxidative damage as manifested by sharp increase in lipid peroxidation and decrease in chlorophyll content. However, pre-treatment of seedlings with SNP stimulated chlorophyll and proline content associated with a decreased level of lipid peroxidation and biomass reduction as compared to the seedlings subjected salt stress without SNP pre-treatment. However, additional key players involved in SNP induced-salt tolerance needs to be clarified.

ACKNOWLEDGEMENT

Author would like to acknowledge Prof. Ajit Varma, Amity University, Noida for providing lab facilities.

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ISSN: 2454-6127

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Table 1: Effect of different priming agents on plant growth parameters of soybean under 100mM NaCl stress

Treatments	Growth parameters				
	Germination (%)	**Shoot length (cm.)**	**Root length (cm.)**	**Lateral root (number)**	**Biomass reduction (%)**
Proline					
1mM	43±3.1	6.1±1.1	3.4±0.6	11±3.1	53±2
5mM	70±4.7	8.3±1.7	5.2±1	22±2.2	50±1.8
10mM	64±3.3	7.6±1.4	5±1.3	16±2.1	58±1.9
Glycinebetain					
1mM	40±4.2	6.3±1.4	3.6±1	12±2	65±1.8
5mM	51±3.3	6.8±1.2	4±1.2	16±3.1	62±2
10mM	60±3.6	7±1.9	4.2±0.7	19±3.3	56±1.4
Mannitol					
1mM	44±4.6	5.8±1	3.6±0.4	12±4.1	63±2.1
5mM	65±2.7	6.7±1.4	4.1±0.5	18±3.2	66±2.2
10mM	50±2.8	6.5±1.6	4±0.5	19±4	72±1.9
H_2O_2					
10μM	55±2.9	6.3±2.1	3.6±1	12±2.5	62±2
50μM	67±3.2	7.2±1.8	4.5±1.1	23±2.8	60±1.1
100μM	51±3.5	5±1.4	3.1±0.8	15±2	68±1.9
Sodium nitroprusside					
10μM	52±3.3	6.3±1.3	3.6±0.7	12±1.5	50±1.4
50μM	66±3.5	7.8±1.4	4.5±1.2	24±2.3	52±1.6
100μM	82±3.1	12.4±2	8.3±1.2	38±3.2	41±1.7
Un-treated	40±1.5	6.3±1.5	3.6±1.1	12±1.7	83±1.8
Control	98±1	15.1±1.8	10±0.6	52±3.5	--

Values represent the means ± SD, n=6. Control treatment represents without salt stress.

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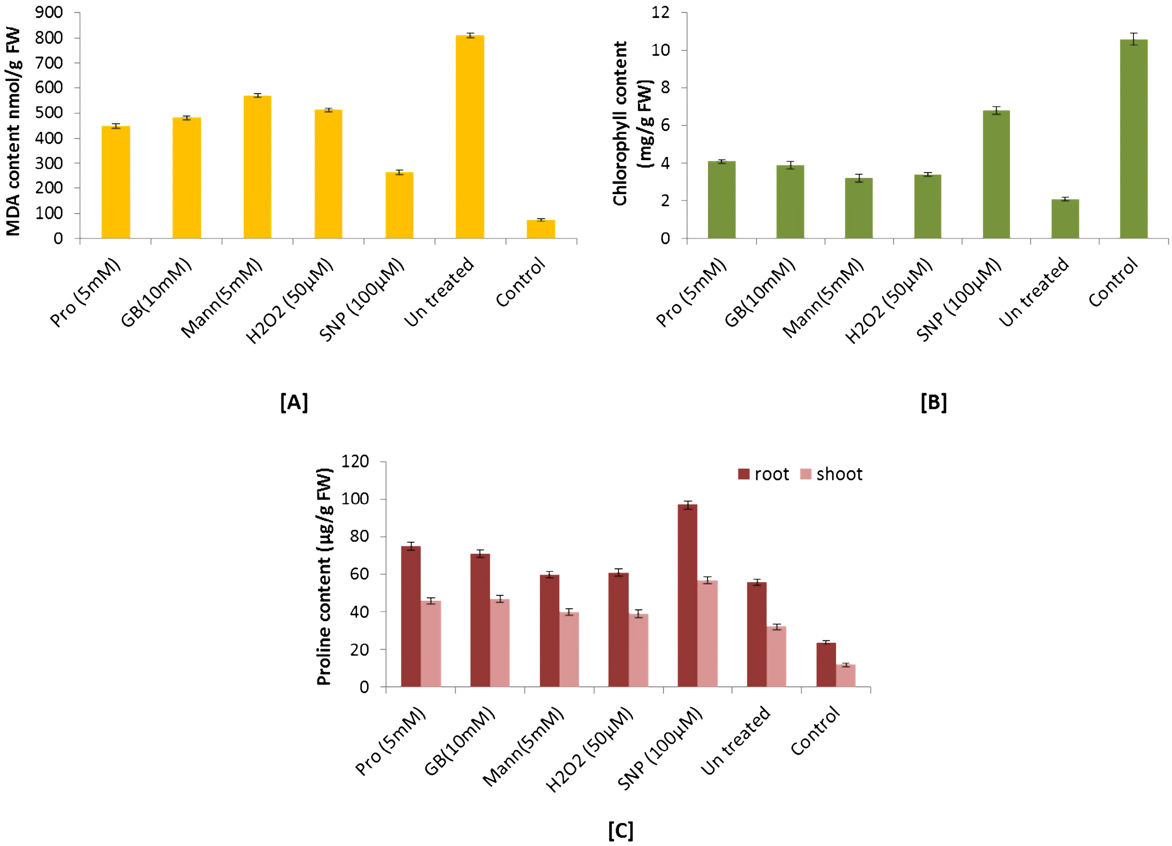
Figure 1: Effect of different priming agents on biochemical parameters of soybean plants under salt stress



The results are shown on effective concentration of each priming agents. Values represent the means ± SD, n=6.

How to cite this article

Anukool, V., Kumari, S., Shekhar, J., Devendra Kumar, C., & Kanti Prakash, S. (2016). Exogenous chemical mediated induction of salt tolerance in soybean plants. *International Journal of Agricultural and Life sciences*, 2(3), 43-47. doi: 10.9379-sf.ijals-122064-007-0081-x

CONFLICTS OF INTEREST

"The authors declare no conflict of interest".