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HYDROGEN PEROXIDE AS A SIGNAL UPSTREAM IMPROVING SALINITY TOLERANCE IN ALFALFA PLANTS

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ABSTRACT

Besides many important morphological and physiological facets, an important consequence of abiotic stresses is an increase of reactive oxygen species (H_2O_2) in the cells thereby causing adverse effects upon many metabolic functions. To investigate the effect of H_2O_2 on some important metabolic activities, an experiment was designed under salinized medium (4 mScm^{-1} NaCl) pre-treating the alfalfa seeds in 1,50 and 100 mM H_2O_2 solution for eight hours. The penetration of H_2O_2 in fresh seeds was observed to reduce at all concentrations attaining maximum at 5 hrs. It declined gradually up to water control at 6, 7 and 8 hrs. H_2O_2 treatment brought about an improvement in shoot fresh and dry weight, leaf osmotic potential and crude proteins while mean germination time (MGT) was observed to curtail. It may be suggested that H_2O_2 signaling activates the antioxidants vis-à-vis scavenging of hydrogen in seeds of alfalfa (*Medicago sativa* L.)

KEYWORDS : Hydrogen Peroxide, Alfalfa, Salinity

Soil salinity and sodicity are the measure of the total amount of soluble salts in soil. As the levels of salinity or sodicity increase, plants extract water less easily from soil, aggravating stress of the soil. High soil salinity and sodicity also cause nutrient imbalances, resulting in the accumulation of elements toxic to plants and thereby reducing water infiltration (Wyn Jones and Gorham, 2002)

One amongst the pronounced effects of salinity is an increase in reactive oxygen species (ROS) in the cells, which adversely affect the metabolic functions after conversion to H_2O_2 (Sairam and Tyagi, 2004). There are evidences about the biochemical role of ROS particularly H_2O_2 as a signal molecule in plants (Overmyer et al., 2003), (Hung et al., 2005). Working with berseem and fenugreek plants, (Varshney, 2006) has reported that H_2O_2 functions as a signal upstream of both ethylene and salicylic acid during salinity stress. Thus, H_2O_2 signaling can be considered significant in improving salinity tolerance of plants.

Salt induced production of ROS and their conferring salt tolerance in accomplishing better crop growth and yield is a priority area of research. Better understanding of mechanisms, which enable plants to adapt saline conditions, is necessary to make the best use of salt affected soils. The mechanism of H_2O_2 as seed pre-treatment in improving salt tolerance is still elusive. In this paper, we report some physiological and biochemical changes induced by H_2O_2 during seed pre-treatment and their involvement in improving salt tolerance potential of alfalfa seedlings.

MATERIALS AND METHODS

Alfalfa (*Medicago sativa* L.) var. Pant Ragini seeds obtained from G.B. Pant University of Agriculture and Technology, Pant Nagar (India) were surface sterilized with 0.1% $HgCl_2$ for 2 minutes and then transferred to 1, 50 and 100 mn H_2O_2 solution in capped bottles for 8 hrs. They were washed with distilled water and blot dried. They were sown in 3 sets each of earthenware pots. One set was used as control of only water; second set was given 4 mScm NaCl (salinized control) and pots of third set had H_2O_2 treated seeds with 1, 50 and 100 mM (H_2O_2 controls). Six seeds were sown in each pot. Pots were kept in humidity controlled growth chamber at 27°C± 1°C temperature.

The number of seedlings emerged was recorded daily. On the completion of germination in water control pots, the mean time of germination was computed as per the formula of (Ellis and Roberts, 1981). At the age of 15 days, leaf area, mean germination time, shoot fresh weight, shoot dry weight and leaf osmotic potential (LOP) were determined using standard methods already undertaken in our laboratory. Crude proteins were determined by the method of (Snell and Snell, 1955).

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RESULTS AND DISCUSSION

The data given in table, 1 showed that socking of alfalfa seeds in different concentrations of H_2O_2 accumulates H_2O_2 in seeds up to first 5 hours exhibiting a maximum of 2.2183 mMg^{-1} fresh seeds. The penetration of H_2O_2 reduced at all concentrations approaching water control seeds at 8 hrs. This reveals that H_2O_2 activates antioxidants. Initial absorption and later scavenging of H_2O_2 in alfalfa seeds could induce changes for protecting the seeds against oxidative damage H_2O_2. Thus, H_2O_2 acts as a stress signal in low concentrations (Desikan et al., 2004)

Mean time taken by seeds to germinate was found varying markedly. It declined substantially which became shorter than water controls (Table, 2). H_2O_2 treated seeds demonstrated an increment in almost all the growth contributory attributes of alfalfa seedlings. Fresh and dry weights of shoot revealed more or less identical patterns of variation, being the lowest in salinized controls followed by those given H_2O_2 at 1 and 50 mM levels. The highest fresh and dry weights of shoot were recorded from the seedlings given 100 mM H_2O_2 followed by water control. Likewise, leaf area differed significantly. It was observed highest in water control seedlings and those given 100 mM H_2O_2 treatment. Other treatment of H_2O_2 proved less effective (Table, 2).

It is assumed from the data given in Table, 2 that applied salinity reduced leaf osmotic potential substantially. However, this reduction remained maximum in salinized control seedlings. Seed treatment with H_2O_2 improved this parameter considerably at 1, 50 and 100 mM H_2O_2 levels. Likewise, crude proteins depleted significantly in salinized control seedlings of alfalfa. A well-marked improvement in crude proteins was noticed in H_2O_2 control seedling which was even greater at 100mM level than water controls. In this regard, this concentration was noted most effective.

It may be concluded from the aforesaid results that applied salinity has multifarious effects on the growth and cell metabolism (Agarwal and Pandey, 2004). Treatment of alfalfa seeds with increased H_2O_2 levels did not prove toxic rather it alleviated deleterious effects of salinity stress on seed germination and seedling growth as evidenced by a curtailed mean germination time and minimum reduction in most growth contributory attributes of salt treated seedlings (Table, 2).

Table, 1: Absorption of H_2O_2 in Seeds at different soaking levels

S. No.	H_2O_2 levels	Salinity treatment ($mScm^{-1}$)	Penetration of H_2O_2 (mm) Soaking time (hrs.) of fresh seeds							
			1	2	3	4	5	6	7	8
1	00 (water control)	-	0.983							
2	Salinized control	4	2.395	-	-	-	-	-		2.395
3	1	4		1.732						
4	50	4			2.125	2.197				
5	100	4					2.283	1.852	1.403	
6	00 (water control)	-								0.980

Table 2: Physio-Biochemical Parameters of Alfalfa Seedlings Under Increased H_2O_2 Concentrations Combined With or Without Salinity

S. No.	H_2O_2 levels (mM)	Salinty treatment ($mScm^{-1}$)	Leaf area (cm)	Mean germination time (hrs.)	Shoot fresh weight (gms.)	Shoot dry weight (gms.)	Leaf osmotic potential (atm.)	Crude proteins
1	Water control	-	1.56	9	1.76	0.673	3.25	26.5
2	Salinized control	4.0	1.32	7	1.35	0.546	2.12	24.7
3	H_2O_2 control	4.0	1.49	6	1.48	0.591	2092	25.9
4	50	4.0	1.53	6	1.59	0.612	3.31	26.3
5	100	4.0	1.63	6	1.74	0.670	3.42	26.8

(Values are means of six replicates)

REFERENCES

Agarwal S., and Pandey V., 2004. Antioxidant enzyme responses to NaCl stress in Cassia angustifolia. Biol. Plant., **48**: 555-560.

Desikan R., Cheung M.K., Bright J., Henson D., Hancock J.T. and Neill S.J., 2004. ABA, hydrogen peroxide and nitric oxide signalling in stomatal guard cells. J. Exp. Bot., **55**: 205-212.

Ellis R.H. and Roberts E.H., 1981. The quantification of aging and survival in orthodox seeds. Seed Sci. & Tech., **9**: 373-409.

Hung C.P., Kreiman G., Poggio T. and DiCarlo J. J., 2005. Fast Readout of Object Identity from Macaque Inferior Temporal Cortex. Science, **310**: 863-866.

Overmyer K., Brosche M. and Kangasjarvi J., 2003. Reactive oxygen species and hormonal control of cell death. Trends Plant Sci., **8**: 335-342.

Sairam R.K. and Tyagi A., 2004. Physiology and molecular biology of salinity stress tolerance in plants. Curr. Sci., 86: 407-421.

Snell F.D. and Snell C.T., 1955. Colorimetric methods of analysis Vol. III A. :101.

Varshney S., 2006. Chemical composition and mineral nutrition of Fenugreek (*Trigonella faenumgraecum* L.) and Berseem (*Trifolium alexandrinum* L.) in relation to NaCl salinity. Ph.D. thesis. M.J.P. Rohilkhand Univ., Bareilly, (U.P.) , India.

Wyn Jones R.G. and Gorham J., 2002. Intra-and-inter-cellular compartmentation of ions- a study in specificity and plasticity.Salinity: Environment plants - molecules. Eds. A. Lauchli and U. Luttge, Kluwer, Dordrecht, The Netherlands. :159-180.