EFFECT OF EXOGENOUS APPLICATION OF HYDROGEN PEROXIDE IN AMELIORATION OF SALINITY STRESS IN POTATO

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Abstract

The aim of present study was to determine the effect of exogenous application of H_2O_2 on the growth parameters (root length, shoot length, numbers of roots, numbers of shoot, numbers of leaves, numbers of nodes and fresh weight) and biochemical analysis (protein contents and POD) of potato plants growing in saline conditions under in vitro conditions. The concentrations of NaCl (40, 60 and 80 mM) were given to the MS medium. The method of exogenous application (pretreatment application) and suitable concentrations of H_2O_2 were determined to find the effect on the potato explants. For this, the nodal explants were pretreated for 5 minutes with the stock solutions of H_2O_2 and inoculated into basal MS medium with salt stress. After 30 days, the morphological as well as biochemical parameters were studied. It was observed that at low concentration of salt, the various growth parameters enhanced growth. At lower concentrations of salts, the growth parameters were influenced more effectively. Deleterious effects of NaCl were observed on the morphological growth parameters of the explants; however, exogenous application of H_2O_2 enhanced the growth of plants under salt. The total soluble proteins and POD activity also gradually increased with the concentrations of both NaCl and H_2O_2, when these were applied to the medium. High concentration of salts inhibited the growth of root and a gradual decrease in the fresh weight of the potato plant was observed. Their growth parameters (shoot length/number, numbers of nodes / leaves) were increased at high concentrations of salts with concentrations of H_2O_2. The 15mM H_2O_2 promoted the growth parameters of potato plant supplemented with different salt concentrations. The application of 15mM H_2O_2 will be given to obtain a better yield of crops from the saline areas.

Key words: Exogenous, Explants, Potato, Soluble protein, Saline.

Introduction

Soil salinity is the major defect to many agricultural soils. Soil salinity also affects the yield of many crops which are used as raw materials for the human beings. The soil which is used for cultivation in the entire world is nearly 19.5% and about 2.1% of dry land used in agriculture is affected by soil salinity (FAO, 2,000). Over 6% of the world's land is affected by either salinity or sodicity. The term salt-affected refers to soils that are saline or sodic, and these cover over 400 million hectares, which is over 6% of the world land area. Much of the world's land is not cultivated, but a significant proportion of cultivated land is salt-affected. Of the current 230 million ha of irrigated land, 45 million ha are salt-affected (19.5 percent) and of the 1,500 million ha under dry land agriculture, 32 million are salt-affected to varying degrees (2.1 percent) (FAO, 2008).There are about 3 million hectares soil affected by salinity in Pakistan (govt. of Pakistan). There are about 70% people depend on the crops field in Pakistan. There are arid and semi-arid regions in the Pakistan. The people are needed to improve the yield of different beneficial crops including rice, wheat, cotton, etc. Increase the amount of different fruits and vegetable such as tomato, pea, potato, etc. for their survival. The soil salinity affected our soil. Due to this, our yield becomes low. We need to improve our

soil fertility or grow such crops which cope with the harsh conditions.

Potato (*Solanum tuberosum*) is an important vegetable used in the world. Potato is a member of family Solanaceae. It is the fourth largest crop after rice, wheat and maize used in the world. The world potato production has reached almost 325 million tons in the 2007. Its production increased recent years but its utilization increased day by day. Different factors like pests, diseases and pathogens also affect the yield of this crop. The people belonging to agriculture do not know about the detail study of the crop and soil. This vegetable lacks in the cholesterol and contains antioxidants which resists against the diseases. The potato contains almost 18% starch contents (FAO, 2008). Potato has been considered as the most salt sensitive crop (Grieve, 1999). Potato cannot survive in saline soil due to its shallower root system (Iwama, 2006).

Hydrogen peroxide is unstable and slowly decomposes in the presence of base or a catalyst. Because of its instability, hydrogen peroxide is typically stored with a stabilizer in a weakly acidic solution. Hydrogen peroxide is found in biological systems including the human body. Enzymes that use or decompose hydrogen peroxide are classified as peroxidases. The various modes of application i.e., pre-treatment, foliar application and addition of the H_2O_2 in the medium were assessed. The conditions influencing the establishment of potato plant in vitro and in saline condition were standardized. Considering its significance, emphasis has given on different parameters in the plants. It is also studied the methods of exogenous application of tested of hydrogen per oxide and their effects on growth and biochemical characterization may contribute towards a broader understanding of salinity tolerance in potato. This study includes a better yield of potato cultivation in the saline areas in the future.

Materials and Methods

The germplasm of potato was taken as source of plant material for in vitro study. The germplasm of potato plant (*Solanum tuberosum* L.) was obtained from Plant Developmental and Regenerative Laboratory at the Department of Botany, University of The Punjab, Lahore. The germplasm was already grown in vitro in the laboratory. To study the effect of H_2O_2 on the growth of in vitro potato plants, nodal explants (1.0 cm long) of cultivar was cut and pretreated in the filter sterilized solution of H_2O_2 (5mM and 15 mM concentrations) for 5 minutes in the air laminar flow. The control plant was not pretreated and just inoculated into MS basal medium under salt stress (40, 60, and 80 mM). For each treatment, five replicates were inoculated. Experiment was repeated thrice. After 30 days of inoculation, data were recorded for shoot/root length, numbers of roots/shoots, nodes/leaves and fresh weight of the plant. Total soluble proteins were determined by Biuret method (Racusen and Johnstone, 1961). For the estimation of peroxidases, method of Moral *et al.*, (1977) and David and Murray, (1965) were employed with some modifications.

Statistical Analysis

The data were analyzed statistically by Univariate analysis of variance using SPSS Version 15.0.0. Standard error of the mean value was calculated for each treatment. The values were also compared using Duncan's multiple range tests.

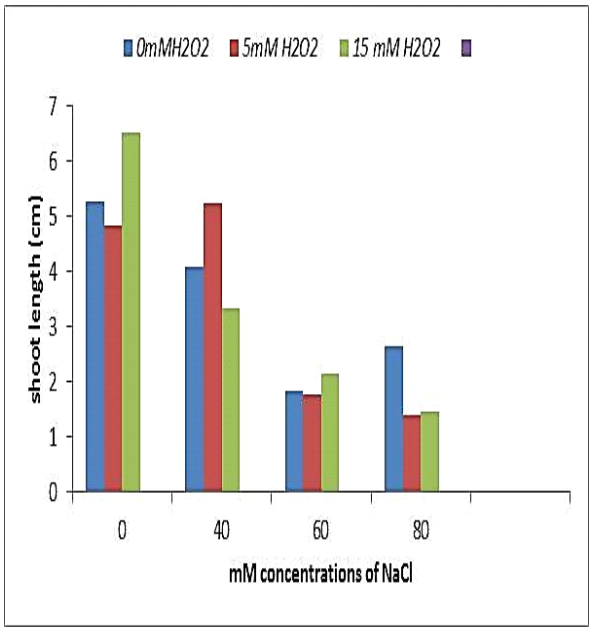
Results

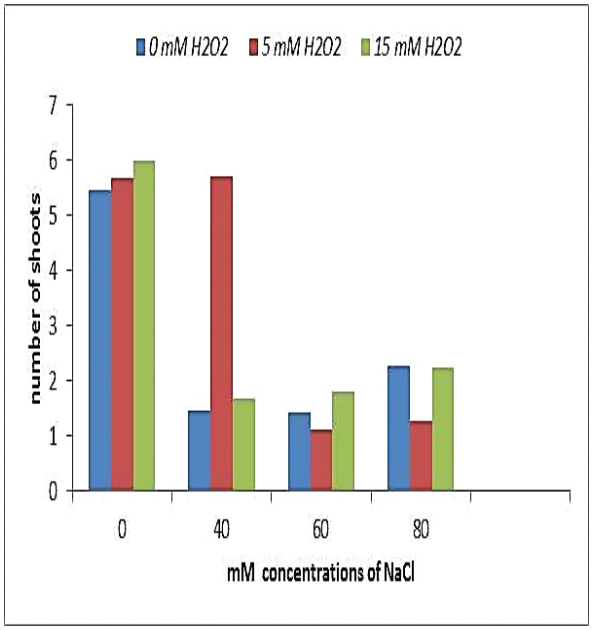
In the present study, MS medium was supplemented with both the NaCl (0, 40, 60 and 80 mM) and H_2O_2 (0, 5 and 15 mM). The growth of all tested parameters were reduced when nodal explants were grown on the saline medium, however, the deleterious effects of salt stress were successfully alleviated with various concentrations of H_2O_2. In potato, reduction in growth overcomes by the exogenous application of H_2O_2. The fresh weights of plants were decreased from 0.18 g to 0.14, 0.11 and 0.065 g at 60 and 80 mM of salt. This reduction in fresh weight was improved by exogenous application of H_2O_2. Total protein contents and POD activity were improved by the addition of hydrogen per oxide.

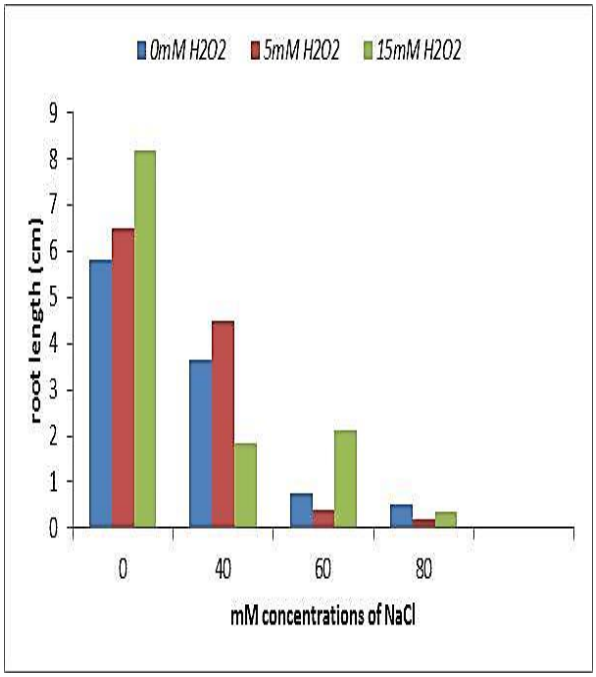
A significant increase in the growth parameters was observed when supplemented with the concentrations of hydrogen per oxide (5 and 15 mM) when compared with control. The numbers of root and length were decreased. The decreased in number of root was observed in 60 and 80 mM NaCl concentrations. The root length was decreased by 0.51, 0.34 and 0.17 cm in 80 mM concentration of salt. The 15 mM H_2O_2 improved all growth parameters of potato plant growing under saline conditions.

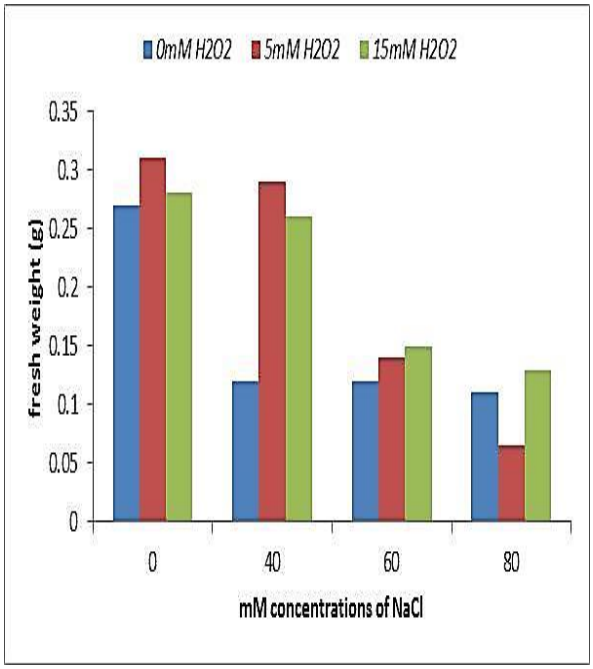
Treatments		Shoot Length (cm)	Root Length (cm)	Shoot (numbers)	Roots (numbers)	Leaves (numbers)	Nodes (numbers)	Fresh Weight (g)	Protein contents (mg/g)	POD (unit /µg of protein)
NaCl (mM)	H_2O_2 (mM)									
0	0	5.26 ± 0.07[a]	5.82 ± 0.13[b]	5.45 ± 0.64[a]	2.73 ± 0.24[b]	17.3 ± 0.56[c]	12.07 ± 0.35[c]	0.27 ± 0.02[b]	0.13 ± 0.0059[b]	0.21 ± 0.0055[b]
	5	4.84 ± 0.14[b]	6.51 ± 0.22[a]	5.67 ± 0.24[a]	4.13 ± 0.18[a]	27.63 ± 0.84[a]	19.60 ± 0.83[a]	0.31 ± 0.01[a]	0.16 ± 0.0109[b]	0.20 ± 0.0068[b]
	15	6.52 ± 0.23[a]	8.19 ± 0.25	6.00 ± 0.36[a]	3.63 ± 0.26[a]	26.07 ± 0.13[d]	18.63 ± 0.26[d]	0.28 ± 0.014[a]	0.12 ± 0.0085[c]	0.2067 ± 0.0033[c]
40	0	4.09 ± 0.06	3.633 ± 0.28[a]	1.45 ± 0.18[c]	2.17 ± 0.12[b]	15.47 ± 0.73[a]	10.27 ± 0.63[a]	0.12 ± 0.006[d]	0.18 ± 0.038[b]	0.22 ± 0.01528[b]
	5	5.24 ± 0.25[a]	4.50 ± 0.41[a]	5.70 ± 0.32[a]	3.47 ± 0.59[a]	24.13 ± 2.90[a]	16.14 ± 2.93[a]	0.29 ± 0.016[a]	0.13 ± 0.0333[b,c]	0.2567 ± 0.024[a]
	15	3.34 ± 0.14[c]	1.83 ± 0.08[e]	1.67 ± 0.13[b]	2.10 ± 0.29[b]	19.73 ± 1.79[b]	14.80 ± 1.40[b]	0.26 ± 0.0082[b]	0.27 ± 0.0633[b,c]	0.22 ± 0.02517[a,b]
60	0	1.83 ± 0.12[e]	0.77 ± 0.09[f]	1.43 ± 0.33[b]	1.00 ± 0.23[c]	18.13 ± 1.04[c]	13.17 ± 1.03[b]	0.12 ± 0.0078[d]	0.11 ± 0.0167[c,d]	0.2017 ± 0.008[c]
	5	1.77 ± 0.09[e]	0.39 ± 0.05[f]	1.13 ± 0.37[c]	0.97 ± 0.12[c]	12.67 ± 0.88[d]	8.73 ± 0.37[e]	0.14 ± 0.029[a]	0.12 ± 0.015[c,d]	0.1827 ± 0.0133[b,c]
	15	2.15 ± 0.16[e]	2.11 ± 0.15[e]	1.80 ± 0.35[b]	0.62 ± 0.26[c]	18.07 ± 1.50[b]	11.17 ± 2.53[a]	0.16 ± 0.0059[c]	0.43 ± 0.073[a,b]	0.1520 ± 0.00404[d]
80	0	2.64 ± 0.18[d]	0.51 ± 0.06[f]	2.27 ± 0.17[b]	0.68 ± 0.06[d]	18.00 ± 0.95[d]	12.57 ± 1.84[a]	0.11 ± 0.010d	0.12 ± 0.0152[c]	0.1683 ± 0.00742[c,d]
	5	1.40 ± 0.12[f]	0.18 ± 0.008[f]	1.27 ± 0.07[d]	0.25 ± 0.07[d]	11.93 ± 1.81[b]	7.88 ± 0.89[f]	0.0657 ± 0.0034[e]	0.49 ± 0.116[d]	0.1683 ± 0.01397[c,d]
	15	1.45 ± 0.09[f]	0.34 ± 0.08[f]	2.23 ± 0.09[d]	0.32 ± 0.04[d]	18.93 ± 3.67[a]	14.47 ± 5.13[a]	0.13 ± 0.0018[c]	0.12 ± 0.01667[c,d]	0.1507 ± 0.00617[d]

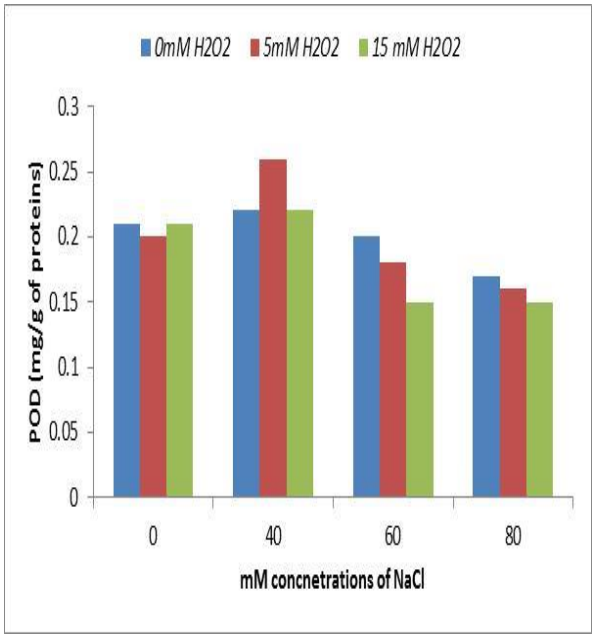
Results on all parameters are mean from fifteen replicate cultures. Comparisons among different treatments are made by analysis of variance (ANOVA). Means within a column followed by the same letter do not differ significantly ($P \leq 0.05$) according to Duncan's one way range test.

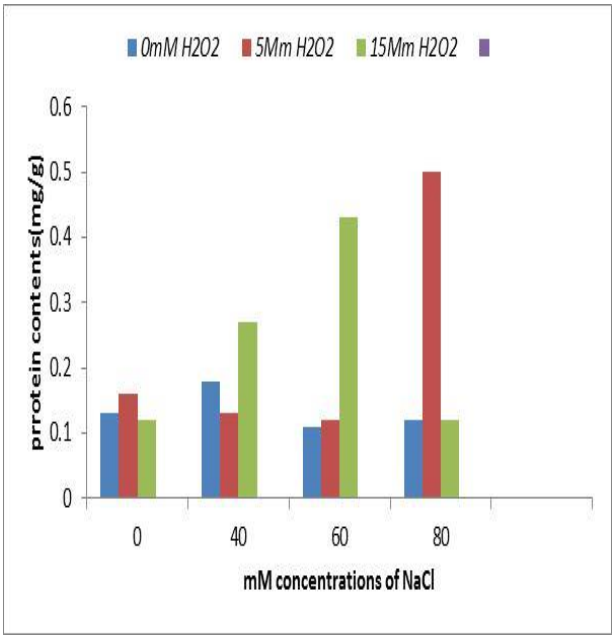












DISCUSSION

This study aimed to evaluate the role of exogenously applied (pretreatment application) H_2O_2 on the morphological and biochemical activities of potato explants grown under non- saline and saline conditions. It is clear from the results that NaCl stress (40, 60 and 80 mM) impeded growth of explants of potato progressively with increasing concentration of salt. The decrease in growth might be due to enhanced accumulation of Na^+ ions in the cells interfering with cell metabolism and inhibiting the cell elongation and cell division in the plant cells as reported by Tester and Davenport, (2003). Although, there was a reduction in all parameters of plant growth, however, at 40 mM NaCl concentration the growth was less affected. This result was shown that at lower concentration of salts, the plant was grown in the in vitro condition well, but at higher concentration, the parameters were reduced. At higher NaCl level (75 mM), the protective role was inefficient and resulted to damaging effects on the plant growth of potato (Benavides *et al*., 2000). The lower concentration of salt promotes the shoot length while higher concentration inhibited it. Hamada, (1995) with his study on maize, *Zea mays* L.Misra*et al*., (2000) with their study on rice seedlings *Oryza sativa* L. vr. Damodar,Dantus*et al*., (2005) in their study on cowpea, *Vignaunguiculata* L., and finally by Memeon*et al*., (2010) in their study on *Brassicacampestris* L. where they indicated that the use of low concentrations of sodium chloride led to increases in plants lengths, whereas higher concentrations inhibits it.

Salinity is a global threat to the agriculture. Salt stress induces several alterations in the growth, cell division and enzymatic activities. On exposure to salt stress, growth retardation is the most profound response to the plants (Zhao, 1993). During present study, various NaCl concentrations (0, 40, 60 and 80) were used to induce salt stress in the in-vitro grown potato plants. A progressive decrease in the morphological parameters (shoot numbers/length, root numbers/length, numbers of nodes/leaves and fresh weight) was observed with a gradual augmentation of salt in the MS medium. The reason behind the reduction of growth was due to lower water potential, stomatal closure and nutrients imbalance and uptake and shoot transport (Marshner, 1995). Potato is considered salt sensitive when compared with other crops because developmental responses in this plant are affected by salinity. Cardinal came up be amongst the moderately salt tolerant species which may be related to some previous studies narrating that that some of the potato genotypes are salt tolerance (Sajid and Aftab, 2009). In vitro studies have been reported as an alternative

to the labor-intensive and costly field trails to investigate the effect of salt stress on potato (Zhang, 1993). In this study, root growth was inhibited at 60 and 80 mM NaCl concentrations, similar effects of these concentrations were observed on the other growth parameters as well. These results are in agreement with some previous studies on potato. Martinez *et al.,* (1996) described inhibition in growth of Andean potatoes on the exposures to NaCl stress (100 and 200 mM). Farhatullah *et al*., (2002) reported a reduction in the growth at high concentration of salt in the potato.

Increase in the total proteins has been observed in the response to exogenous application of either NaCl or H_2O_2 or in combinations. Increase in quantity of proteins has been reported to be the result of salt –induced proteins (Ali *et al*., 1999). The increase in proteins may be due to de novo synthesis of proteins or may be enhanced level of already present proteins in response to salt stress (Singh *et al*., 2007). The damaging effects of salt stress on the crop growth are generally due to ionic and osmotic stress which impairs physiological and biochemicals processes (Munns, 2005). At the primary level, salt stress lowers the water imbalance of plants, whereas at cellular level, it affects the integrity of membranes and proteins (Hopkins, 1995).

Conclusion

The present study illustrates that NaCl stress has negative impacts on the growth and biochemical attributes of potato explants. However, the application of hydrogen peroxide improved the growth parameters of potato by enhancing the activity of enzymatic antioxidant system. Hydrogen peroxide also reduces the uptake of salts by plants. Therefore, it is quite evident that application of hydrogen peroxide can be considered as effective stress ameliorating strategy in potato explants.

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