Available online at sjuoz.uoz.edu.krd

journals.uoz.edu.krd

Science Journal of University of Zakho

Vol. 12, No.3, pp.294–298 July-September, 2024

p-ISSN: 2663-628X
e-ISSN: 2663-6298

PROPAGATION AND CALLUS REGENERATION OF POTATO (SOLANUM TUBEROSUM L.) CULTIVAR 'DESIREE' UNDER SALT STRESS CONDITIONS

Atheel Najib Yousef[1*], Gharbia Hrms Danial[2], Diaa Ayoub Ibrahim[3], Sazan Elias Barakat[4]

1 College of Science, Department of Biology, University of Duhok. E-mail: atheel.yousef@uod.ac
2 College of Science, Scientific Research Center, University of Duhok / E-mail: gharbia.danial@uod.ac.
3 College of Science, Scientific Research Center, University of Duhok, diaa.ibrahim@uod.ac
4 College of Science, Scientific Research Center, University of Duhok

Received:23 Nov., 2023/ Accepted: 3 May., 2024 / Published: 17 July., 2024. https://doi.org/10.25271/sjuoz.2024.12.3.1236

ABSTRACT

Under laboratory conditions, segments of potato with single nodes were exposed to varying doses of Sodium Chloride (NaCl) (0 mM, 20 mM, 40 mM, 60 mM, 80 mM and 100 mM), using the Murashige and Skoog growth medium to assess how NaCl salt stress affects micropropagation, callus formation, and regeneration in the 'Desiree' potato plant cultivar, and also seeks to determine the ability of this cultivar to thrive in salt stress conditions. The data were collected after a six-week period for each salt treatment. Remarkably, a significant increase in the mass of green and dry stems and roots was observed specifically under the 40 mM NaCl treatment. Conversely, the length of shoots and branches experienced a reduction as the NaCl concentration increased. The overall impact of the NaCl treatments strongly influenced root weight. Concerning the formation (development) and revitalization of callus, segments of potato microtubers were exposed to the above-mentioned NaCl concentrations, resulting in an intermediate level of salt stress that significantly reduced callus weights as NaCl concentration increased. Furthermore, a gradual decrease in the regeneration rate was noted with increasing concentrations of the plant growth regulator BA (1 to 4 mg/l). The most profound relative regeneration rate occurred at 1 mg/l BA. Notably, the counteractive effect of salt was more apparent with higher NaCl concentrations, with exceptions at 20 mM and 40 mM. These findings propose that the 'Desiree' potato cultivar exhibits moderate tolerance to salt stress and indicates a capacity to endure salinity. Moreover, this valuable variety could potentially be harnessed through genetic manipulation to enhance its salt resilience.

KEYWORDS: Solanum tuberous; Desiree; Murashige and Skoog; NaCl; Microtubers; B

1. INTRODUCTION

Potato plants (*Solanum tuberosum* L.) rank as the fourth most globally consumed crop, following wheat, corn, and rice. These corps serve as significant sources of fiber, minerals, vitamins, and phytochemicals, supplying sufficient energy for various life forms (organisms). Moreover, the need to enhance potato cultivation and production assumes significance to ensure sustained food availability as the world population continues to grow (1,2). Among biotic pressure factors, salinity plays an essential role that significantly impacts plant growth and productivity by disrupting essential physiological processes. The accumulation of Na+ and Cl- ions within plants can lead to highly toxic effects, causing disruption in crucial enzymatic functions (3,4). Soil salinity represents a considerable global challenge that adversely affects plant growth and productivity, contributing to economic losses in agriculture productivity, particularly in aridity and semi aridity regions worldwide. The impacted plants experience disruption due to ionic toxicity, osmotic alterations, diminished photosynthesis, and impaired nutrient transport (5,6).

Despite its inherent ability to tolerate non-biological stress factors, potato cultivation experiences a counterproductive effect, ultimately leading to a reduction in both the growth and harvest yield of potatoes (7,8). Plant tissue culture is a valuable and cost-effective method for enhancing agronomic performance and future plant advancements, such as augmenting crop productivity (9,10,11). In numerous instances, traditional breeding methods have not yielded the intended outcomes. Recently, using *in vitro* plant tissue culture techniques has provided a solution to important challenges related to enhancing crops for sustainable agriculture, particularly in changing climates (12). In Iraq, the main issue with low potato crop productivity is the negative impact of salinity on crop production. It is important to focus on creating resources to develop potato productivity and achieve higher levels of production (13). The aim of this research is to assess how NaCl salt stress affects micropropagation, callus formation, and regeneration in the Desiree potato plant cultivar. It also seeks to determine the ability of this cultivar to thrive in salt stress conditions.

2. MATERIALS AND METHODS

2.1. Plant Micropropagation

Auxiliary buds from *in vitro* 'Desiree' potato plant cultivar were utilized in the experiments. The samples were maintained at a temperature of 25± 2°C with a light duration of 16/8 hours, receiving 45 µMol of LED cool white light. The growth MS growth media (Murashige and Skoog, 1962) was combined with varying concentrations of NaCl (0, 20, 40, 60, 80, and 100 mM). The growth media was supplemented with added casein hydrolysate 100 mg/l, inositol 100 $Mg.l^{-1}$, and 30g/l of sucrose. The pH of the growth medium pH was adjusted at 5.7 of using (NaOH) or (HCl), then 7g/l of agar was added to the culture for solidification and then dispensed in 250 ml Mason jars (25 ml each), The culture media was autoclaved at 121°C and 1.05 kg/cm^2 for twenty minutes and allowed to solidify at room temperature. Segments of plant stems, each consisting of a single node, were placed on the medium surface and covered with non-colored PVP caps secured with rubber bands. Three explants were cultured in each culture jar. The vegetative growth outcomes were observed six weeks after incubation. The fresh and dry weight of both branches and roots were measured during harvest. The dry weight was recorded after 60 hours of oven

* Corresponding author

drying at 60°C.

2.2. Callus Induction and Plant Renewal

Microtubers obtained from *in vitro* cultivated potato plants were cut and placed in the culture medium. Different concentrations of NaCl (0, 20, 40, 60, 80, and 100 mM) were added to the MS growth medium in addition to 3% sucrose and 100 mg/l inositol used for callus promotion. Two-month-old callus was moved to MS medium supplemented with various amounts of BA (1, 2, 3, and 4 mg/l). Subsequently, the percentage of callus regeneration was noted after an 8-week period.

2.3. Experimental Design and Data Analysis

During the experiments, each NaCl concentration was repeated five times, and the design followed a Completely Randomized Design (CRD). To compare the results of all experiments, Duncan's multiple range test ($P < 0.05$) was conducted using a computerized program (SAS).

3. RESULTS AND DISCUSSION

The findings of this study provide clear evidence that the amount of NaCl increases, the growth of non-reproductive plant parts, measured in most of the experiments, decreases (Fig. 1A , Table(1) except the 20 and 40 mM NaCl.

Table 1: Impact of various NaCl concentrations in Potato plant vegetative growth (shoot system) after 6 weeks in culture on MS medium.

NaCl Con. mM.	High shoot length (cm.)	Branch No./explant	Shoot length average (cm.)	Weight of hoot fresh(g.)	Weight of Shoot dry(g.)
0.0	8.798±0.627 A	9.332±1.429 A	4.424±0.657 A	0.336±0.028 B	0.078±0.005 B
20	9.734±0.791 A	7.066±0.826 AB	4.830±0.280 A	0.402±0.052 B	0.086±0.013 B
40	10.070±1.190 A	4.994±0.927 BC	4.640±0.611 A	0.648±0.087 A	0.144±0.021 A
60	6.100±0.208 B	3.734±0.531 C	3.414±0.101 A	0.268±0.031 B	0.070±0.010 B
80	5.232±0.370 B	2.798±0.291 C	3.970±0.417 A	0.272±0.021 B	0.066±0.005 B
100	5.864±0.244 B	3.002±0.279 C	3.902±0.247 A	0.290±0.041 B	0.072±0.008 B

The highest shoot length of 10.07cm was achieved in the 40 mM NaCl treatment, followed by a length of 9.73 cm in the 20 mM NaCl treatment (Fig.1 B, Table 1). These measurements were notably higher compared to the other NaCl treatments, although not surpassing the control treatment (8.79 cm).

This could be due to the plant's adaption to the salty conditions, which has improved its ability to handle the presence of salt in its environment. (14). Furthermore, the number of new shoots varied based on the NaCl concentration treatment. As the concentration of salt (NaCl) increased progressively in the treatments, there was a consistent decrease in the average number of shoots across all NaCl treatments, in contrast to the control treatment with 0 mM NaCl. Meanwhile, there was a decline from 9.33 shoots/plant in the control treatment to 7.06 shoots/plant at the 20 mM NaCl treatment (Fig.1 B, table 1), but this reduction was not statistically significant.

This considerable decrease in the number of shoots was observed, dropping from 9.33 in the control treatment to 4.99, 3.73, 2.79, and 3.00 shoots/plant in the 40, 60, 80, and 100 mM NaCl salt treatments, respectively. (Fig.1 C, Table 1). This significant decrease in the number of shoots due to salinity stress has been observed in various plant varieties. This can primarily be attributed to the effects of osmotic pressure and ion toxicity, which collectively result in reduced plant growth (15,16).

The highest average shoot lengths of 4.83 cm and 4.64 cm were observed in the 20 and 40 mM NaCl treatments, respectively. However, these measurements did not display significant differences when compared to the control treatments, which had an average of 4.42 cm, as well as with all the other treatments.

The selections align with previous research that has demonstrated the negative impact of rising salt concentrations on the growth and development of potato plants (3, 7, 17, 18, 19, 20). This suggests that the addition of NaCl to the growth medium reduces the medium's osmotic capacity, creating salinity stress that adversely affects the growth of potato plants.

The highest fresh weight, averaging 0.648 g, was observed in the 40 mM NaCl concentration, surpassing that of the control treatment. This was followed by the 20 mM NaCl treatment, which had an average weight of 0.402 g. However, this difference was not significant compared to the control treatment, which had a weight of 0.336 g. (Fig Table 1).

The economic advantage primarily lies in the weight of fresh potatoes. The consistent decrease in weight as the NaCl dosage increases demonstrates that this trait is notably affected by high concentrations of salt. These findings are consistent with the observations of numerous researchers (19, 21, 22) who have studied the effects of increased NaCl concentrations on variables such as the fresh weight of potato shoots and roots.

The influence of salinity on shoot dry weight became evident in the MS medium enriched with 40 mM NaCl, which displayed a significant increase to 0.144 g. This was notably higher than the control medium's dry weight of 0.078 g. and higher than the dry weights of other saline media as well.

The findings of this research align with the results of Aghaei et al**.** (2009), who suggested that the white potato variety Desiree shows moderate resistance to salt stress. Recent studies have illuminated a range of adaptive responses to salinity stress at the cellular, molecular, metabolic, and physiological levels (23).

Significant differences were noted in root numbers (Table 2). The data exposed that the highest root count (9.72) was achieved at 40 mM NaCl, closely followed by 9.66 at 20 mM NaCl (Fig. 1, C). However, these increases in root numbers did not show significance when compared to the control (9.40). Moreover, elevated salt concentrations led to a reduction in root numbers. Consistent findings were reported by (3, 24, 25), which observed diminished root development in potato plants subjected to salt stress conditions.

Table 2: The Impact of various NaCl concentrations in Potato plant vegetative growth (root system) after 6 weeks of cultured.

NaCl.Con.	20mM	40mM	60mM	80mM	100mM
Average callus weight (g.)	2.160±0.337 A	1.720±0.154 A	1.018±0.094 B	0.818±0.057 B	0.748±0.075 B

The highest average root length (12.13, 11.23, and 11.11 cm) was recorded by adding NaCl at concentrations of (80, 100, and 60) mM, respectively, into the feed medium. This differed significantly from the control treatment. Additionally, there was a slight increase in average root length at 20 and 40 mM NaCl concentrations compared to the control (Fig.1D,).

The results displayed notable differences in both fresh and dry weight among the applied treatments, as indicated in Table 2. The highest fresh weight (0.342 g) was recorded at NaCl concentration of 40 mM, followed by 0.300 g at 20 mM NaCl concentration. This value was the highest of all NaCl concentrations, including the control at 0.196 g. The lowest fresh weight of 0.100 g was achieved with an 80 mM
NaCl concentration. Conversely, the highest dry weight value of 0.038 g was attained at NaCl concentration of 40 mM, surpassing all other treatments, including the control. The lowest dry weight of 0.014 g was recorded at 100 mM NaCl concentration, representing the lowest value among all treatments. This observation has been consistent in numerous studies, where an increase in salinity levels has been shown to have negatively effects on plant growth and performance. This ultimately leads to a reduction in plant biomass, as reported in various studies (14,19,26,27).

Callus induction and plant renewal

As seen in Table 3, the initiation of callus was affected by NaCl concentrations. With an increase in NaCl levels, there was a distinct decrease in the callus weight.

Table 3: The impact of various NaCl concentrations in Potato microtubers callus weight after 8 weeks of cultured.

NaCl Con. mM.	Root No./ explant	Average of root length (cm.)	The weight of root fresh (g.)	The Weight of root dry (g.)
0.0	**9.402±0.244 A**	**7.266±0.734 C**	**0.196±0.028 B**	**0.022±0.002 BC**
20	**9.664±0.708 A**	**8.764±0.953 BC**	**0.300±0.056 A**	**0.030±0.005 A B**
40	**9.720±0.845 A**	**8.398±0.485 C**	**0.342±0.043 A**	**0.038±0.004 A**
60	**5.800±0.827 BC**	**11.114±0.906 AB**	**0.148±0.026 B**	**0.022±0.003 BC**
80	**4.936±0.323 C**	**12.138±0.600 A**	**0.100±0.012 B**	**0.016±0.002 C**
100	**7.132±0.826 B**	**11.232±1.057 AB**	**0.112±0.020 B**	**0.014±0.002 C**

The highest callus weight was observed in media supplied with 20 mM NaCl, recorded at 2.160 g (Fig.1, E and F). This was followed by 1.720 g in MS media with 40 mM NaCl (Fig. 1, E and F), which significantly differed from other treatments, but the lowest callus weight of 0.748 g was noted at 100 mM NaCl (Fig. 1 H and J). Nevertheless, there were no significant differences observed among the other media containing various levels of NaCl. It can be concluded that the callus weight gradually decreased with increasing NaCl concentrations. These records are in agreement with studied conducted by (28,29,30) which suggest that increased salinity can lead to a reduction in callus growth in potato plants.). The overall outcomes indicate that high salinity can reduce plants' ability to absorb mineral nutrients like N, Ca, P, K, Fe, and Zn ions.

Additionally, the increased level of $Na^{+|}$ and Cl^{-} within plant cells can be toxic. Conversely, the high level of Na^{+} disturbs the absorption of K^{+} ions in the cells, which play a crucial role in maintaining osmotic balance and controlling the opening and closing of stomata. Furthermore, NaCl has the ability to reduce cell permeability to water, resulting in a reduction in the rate of water entering the cells (2,4,31,32).

Table 4 represents that the highest regeneration percentage (66.67%) occurred in MS medium augmented with 1.0 mg^{-1} BA (Fig.1, G and H), whereas a lower regeneration percentage (33.33%) was recorded in MS medium containing 2.0 mg^{-1} BA. These results are in accordance with the research of many scientists who have reported the diverse effects of cytokinin in callus regeneration in potato and many plant species (33,34).

Table 4: The impact of different concentrations of BA in callus renewal after two months of callus in MS medium.

BA concentration	1mg/Liter	2mg/Liter	3mg/Liter	4mg/Liter
Regeneration percentage %	66.67	33.33	46.66	46.67

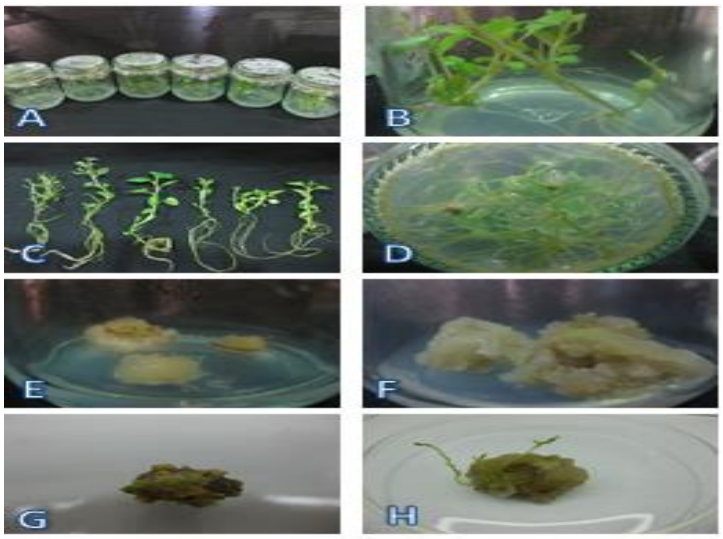

Figure 1: ***In vitro*** **potato response to NaCl salt stress. A.** From left to right, effect of different NaCl concentrations (0,20,40,60,80 and 100 mM) on potato micropropagation 6 weeks after culture. **B.** Potato plants cultured in MS medium with 40 mM NaCl after 6 weeks. **C.** From left to right, rooting of a regenerated shoot in MS medium with increasing NaCl concentration**. D.** Potato root induction in MS medium supplemented with 20 mM NaCl after 6 weeks of culture. **E**. Potato callus induction in MS medium with 20 mM NaCl after 4 weeks of culture. **F.** Potato callus induction in MS medium with 20 mM NaCl after 8 weeks of culture. **G.** Shoots differentiation from callus on MS medium contend 1 mg/l BA after 6 weeks of culture. **H.** Shoots differentiation from callus on MS medium contend 1 mg/l BA after 6 weeks of culture.

REFERENCES

Chandrasekara, A. and Kumar, T. J. 2016. Roots and tuber crops as functional foods: A review on phytochemical constituents and their potential health benefits. International Journal of Food Science: 1-15.

Dahal, K.; Li, X.; Tai, H.; Creelman, A. and Bizimungu, B. 2019. Improving potato stress tolerance and tuber yield under a climate change scenario a current overview. Frontiers in Plant Science, volume 10 article 563.

Ahmed, H. A. A.; Sahin, N. K.; Akdogan, G.; Yaman, C.; Kom, D. and Uranbey, S. 2020. Variability in salinity stress tolerance of potato (*Solanum tuberosum* L.) varieties

using *in vitro* screening. Science and Agrotechnology, 44: e004220, 2020

Chourasia, K. N.; Lai, M. K.; Tiwari, R. K.; Dev, D.; Kardile, H. B.; Patil, V. U.; Kumar, A.; Vanishree, G.; Kumar, D.; Bhardwaj, V.; Meena, J. K.; Mangal, V.; Shelaks, R. M.; Kim, J. Y, and Pramanik, D. 2021. Salinity stress in potato: understanding physiological, biochemical and molecular responses. Life, 11, 545.

Gupta, B. and Huang, B. 2014. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. International Journal of Genomics, Volume 2014, Article ID 701596, 18 pages

Alturki, S.M. 2021. The potential use of $Ca(NO_3)$ to improve salinity tolerance in date palm (*Phoenix dactylifera* L.) Iraqi Journal of Agricultural Sciences, Iraqi Journal of Agricultural Sciences, 52(2):445-453.

Zaman, M. S.; Ali, G. M.; Muhammad, A.; Farooq, K. and Hussain, I. 2015. In *vitro* screening of salt tolerance in potato (*Solanum tuberosum* L.) varieties. Sarhad Journal of Agriculture, 31(2): 106-113.

Farhatulla; Mahmood, R. and Raziuddin. 2002. *In vitro* effect of salt on vigor of potato (*Solanum tuberosm* L.) plantlets. Biotechnology,1(2-4):73-77.

Danial, G. H.; Ibrahim, D. A.; Yousef, A. N. and Elyas, S. B. 2019. Rapid protocol of *Aloe vera in vitro* propagation. Iraqi Journal of Agricultural Sciences 50(5):1377-1382.

Danial, G.H.; Ibrahim, D. A. and Song, G.Q. 2021. *Agrobacterium* mediated transformation of two Tomato cultivars (*Lycopersicone esculentum* Mill.) cv. Sandra and Rocky. Iraqi Journal of Agricultural Sciences, 52 (3):745-755.

Fadladeen, L.H. and Toma, R.S. 2020. Embryo culture and *in vitro* clonal propagation of (*Quercus aegilops* L.) Iraqi Journal of Agricultural Sciences, 51(1):347-355.

Al-Khateeb, S. A.; Al-Khateeb, A. A.; Sattar, M. N. and Mohmand, A. S. 2020. Induced *in vitro* adaptation for salt tolerance in date palm (*Phoenix dactylifera* L.) cultivar Khales. Biological Research, 53:37.

Al-Hachami, I. S. A.; AL-Bahadely, F. H. N. and Jbara,O.K. 2020. Measuring the technical efficiency of potato production and its determinants in Iraq (Baghdad province as case study), Iraqi Journal of Agricultural Sciences,51(6):1634-1643.

Sogoni, A.; Jimoh, M. O.; Kambizi, L. and Laubscher, C. P. 2021. The impact of salt stress on plant growth, mineral composition, and antioxidant activity in (*Tetragonia decumbens* Mill.) an underutilized edible halophyte in South Africa. Horticulturae, 7, 140.

Fageria, N.K.; Stone, L.F. and Santos, A.B. 2012. Dos breeding for salinity tolerance. In Plant Breeding for Abiotic Stress Tolerance; Springer: Berlin/Heidelberg, Germany, pp. 103–122.

Petretto, G.L.; Urgeghe, P.P.; Massa, D. and Melito, S. 2019. Effect of salinity (NaCl)on plant growth, nutrient content, and glucosinolate hydrolysis products trends in rocket genotypes. Plant Physiol. Biochem., 141, 30–39.

Farhatulla; Mahmood, R. and Raziuddin. 2002. ***In vitro*** effect of salt on the vigor of potato (*Solanum tuberosm* L.) plantlets. Biotechnology, 1(2-4):73-77.

Aghaei, K.; Ehsanpour, A. A. and Komatsu, S. 2009. Potato responds to salt stress by increased activity of antioxidant enzymes. Journal of Integrative Plant Biology, 51(12):1095-1103.

Khenifi, M. L.; Boudjeniba, M. and Kameli, A. 2011. Effects of salt stress on micropropagation of potato (*Solanum tuberosum* L.). African Journal of Biotechnology, 10(40):7840-7845.

Sudhersan, C.; Jibi Manuel, S.; Ashkanani, J. and Al-Ajeel. A. 2012. *In vitro* screening of potato cultivars for salinity tolerance. American-Eurasian Journal of Sustainable Agriculture, 6(4):344-348, 2012.

Zhang, Y.; Branult, M.; Chalavi, V. and Donnelly, D.J. 1993. *In vitro* screening for salinity tolerant potato. Congress of Biometeorol. Canada, pp. 491-498.

Sasikala, P.P. and Prazad, P.D. 1994. Salinity effects on *In vitro* performance of some cultivars of potato. J. Fisiol. Veg., 6(1):1-6.

Bhaskar, G. and Bingru, H. 2014. Mechanism of salinity tolerance in plants:physiological, biochemical, and molecular characterization. International Journal Genomics, Volume 2014, Article ID 701596, 18 pages.

Naik, P. S.; Widholm. J. M. 1993. Comparison of tissue

culture and whole plant responses to salinity in potato. Plant Cell, Tissue and Organ Culture, 33:273-280.

Evers, D. and Hausman, J. F. 1999. Salt tolerance *Solanum tuberosum* L. Over expressing a heterologous somatin-like protein. Biologia Plantarum, 42:105-112.

Rahneshan, Z.; Nasibi, F. and Moghadam, A.A. 2018. Effects of salinity stress on some growth physiological, biochemical parameters and nutrient in two pistachio (*Pistacia vera* L.) rootstocks. J. Plant Interact, 13, 73-82.

Orlovsky, N.; Japakova, U.; Zhang, H. and Volis, S. 2016. Effect of salinity on seed germination, growth and ion content in dimorphic seeds of Salicornia europaea L. (Chenopodiaceae). Plant Divers. 2016, 38, 183–189.

Zhang, Y., J.E. Abdulnour, D.J. Donelly and N.N. Barthakur. 2001. Effects of NaCl stress on yield of potato plants derived from

previously saline conditions. Hort. Science, 36: 770-771.

Forooghian, S. and Esfarayeni, S. 2013. An evaluation of effect of salt stress on callus induction in different potato cultivars. American-Eurasian J. Agric. & Environ. Sci., 13 (8): 1135-1140.

Ibrahim, M. A.; Jerry, A. N. and Khalil, A. I. 2017. Effect of plant growth regulators and sodium chloride on indirect organogenesis of three cultivars of potato plant (*Solanum tuberosum* L.) via *in vitro* culture. AAB Bioflux, 9 (3): 103-110.

Tuteja, N. and Mahajan, S. 2005. Cold, salinity and drought stresses:an overview. Arch. Biochem. Biophys., 444:139-158.

James, R.A.; Blake, C. S. and Munns, R. 2011. Major genes for Na^+ exclusion, Nax1 and Nax2(wheat HKT1;4 and HKT1;5), decrease Na^+ accumulation in bread wheat leaves under saline and waterlogged condition. Journal of Experimental Botany, 62(8):2939-2947.

Chai, B. and Mariam, B. 1998. Application of biotechnology in turf grass genetic improvement. Crop Sci., 38:1320-1338.

Khadiga, G. A.; Modawi, R. S. and Khalafalla, M. M. 2009. Effect of plant growth regulators on callus induction and plant regeneration in tuber segment culture of potato (*Solanum tuberosum* L.) cultivar diam te.