ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology, Volume 1 Number 2-4: 73-77, 2002

In vitro Effect of Salt on the Vigor of Potato (*Solanum tuberosm* L.) Plantlets

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Abstract: The effects of NaCl stress on *in vitro* induced potato plantlets of variety of potato *Solanum tuberosm* L.) commercial variety cardinal was studied at Potato Research Center, Abbottabad, NWFP, Pakistan. Five NaCl treatments, each consisting of 0, 1, 2, 3 and 4% were used. Viability percentage, fresh weight of plantlet and height of plantlet was decreased by given range levels of NaCl. The effect of all treatments was very drastic on length of roots and number of roots per plantlet where no response was recorded. The treatment of 4% NaCl depressed all the parameters understudy. The results indicate that the variety "Cardinal" is not salt tolerant, even at 1% salt concentration.

Key words: *In vitro*, salt stress, NaCl, potato (*Solanum tuberosm* L.) explants

Introduction

Potato is a widely distributed crop and is grown in about 140 countries, more than hundred of which are located in the topical and subtropical zones. However, most production is still concentrated in the temperate regions in the industrialized countries. Almost one third of the crop is produced in developing countries, mainly the countries in Asia. In more recent years, potato has spread to many countries with warmer and drier climates and has become important vegetable in regions as the plains of India, Bangladesh, Pakistan, Central America and Argentina etc. (Benkema and Vanderzaag, 1990).

In Pakistan, in spite of dramatic increase in the area and production of potato, the present national average yield is still very low as compared to other countries. The main reasons for low productivity of potato in Pakistan may be due to lack of potato varieties suited to different agro-ecological zones non-availability of healthy and certified seed, inadequacy of storage and proper marketing mechanism.

For the improvement of plants, various techniques have been developed. Tissue culture, a biological tool, involves the *in vitro* cultivation of plants organs, tissues, or cells in the test tube or artificial media under aseptic conditions.

Plant tissue culture has an important role to play in the production of agricultural or ornamental plants and in the manipulation of plant for improved agronomic performance. Plant tissue culture research is a multi- dimensional science that offers exciting prospects to future improvements in

crop productivity.

Saline stress induces several alterations on growth, cell division and enzymatic activities, among others. Salinity tolerance by plants depends primarily on the genotype that determines alterations on processes such as uptake and transport of salts by roots, together with metabolic and physiological events occurring at cellular level (Winicov, 1993).

Solanum species possess genetic differences in abiotic stress tolerance is interesting not only for potato breeding to abiotic stress but also provides better material for studying the abiotic stress tolerance mechanisms (Sasikala and Devi Prasad, 1994; Martinez *et al.*, 1996). The wide cultivated species of potato (*Solanum tuberosum* L.) possesses very little abiotic stress tolerance (Martinez, *et al.*, 1996) that is the reason that salinity affects a range of developmental responses in potato (Heur and Nadler, 1998).

A large volume of data has been published on the subject of plant responses to salinity, salt tolerance is a complex phenomenon (Martinez *et al.*, 1996). In this experiment salt tolerance was measured by survival and growth of *in vitro* induced plantlets of potato variety cardinal treated with various concentrations of NaCl.

Materials and Methods

This research was conducted at Tissue Culture Laboratory, Potato Research Centre, Abbottabad, NWFP, Pakistan in 2000-2001 to record the effect of various concentration of NaCl on potato variety Cardinal.

Nodal cuttings of the variety Cardinal were used as explants. The explants were surface sterilized (in 10% chlorox) in aseptic conditions as reported by Zaman *et al.* (2001). The explants were cultured on semi solid MS (Murashige and Skoog, 1962) media containing 30 g sucrose and 8 g agar (pH 5.8) dispensed in test tubes. Test tubes were incubated at 25±2°C in continuous light. Plantlets regenerated were recultured on the same medium after 45 days. The reculturing was repeated for three times. The experiment was replicated thrice. In last culturing the media was treated with NaCl concentration to observe the effects of salt on the plantlets.

Following five treatments were obtained with varying salt concentrations:

T_0: No NaCl was added (untreated control)
T_1: 1% NaCl was added, so each test tube in this treatment contained 0.125 g of NaCl
T_2: 2% NaCl was added, so each test tube in this treatment contained 0.25 g of NaCl
T_3: 3% NaCl was added, so each test tube in this treatment contained 0.375 g of NaCl
T_4: 4% NaCl was added, so each test tube in this treatment contained 0.5 g of NaCl

Data was recorded on the following parameters after 45 days of the last reculturing:

Viability (%age): The viability was recorded by counting the number of test tubes with intact green

plantlets. The data was converted to percentage.

Fresh weight of the plantlets (g): Fresh weight of the plantlets was recorded in grams with the help of an electrical balance in the biochemistry laboratory of Ayub Medical College, Abbottabad.

Height of the plantlets (mm): Height of plantlets was recorded with the help of a graduated scale from the top of the media to the tip of the plantlet.

Length of root (mm): Root length was measured with the help of a graduated scale from the top of the media to the tip of the root.

Number of roots per Plantlets: The number of roots was counted by pulling out every plantlet from the test tube.

Results and Discussion

Viability percentage: There was 95% viability in the controlled (untreated) treatment (T0). In T1, 55% viable plantlets were found. Similarly, in T2 and T3, 35% and 10% viable plantlets were recorded, respectively, showing a gradual decrease with each increment of NaCl treatment (Table 1). However, in T4, no viability was observed at all. The results show that the salt (NaCl) dosage has negatively affected the viability of potato. The response of potato explants to various doses of NaCl clarifies that NaCl damages the cell and restrict the growth activities, which is further supported by the effect of T4 where no activity was observed. Andrade *et al.* (1998) and El-Aref *et al.* (1998) found similar response to salt stress in their experiments.

Fresh weight of the plantlets: The maximum fresh weight with an average of 0.591 g was observed in T0, following by T1, T2 and T3 with an average weight of 0.0412, 0.0391, and 0.0351 g, respectively. In case of T4, no viable plantlets were observed (Table 1). The fresh weight of the potato is the most economical character. The declining trend with the inclined dose clearly suggests that this trait is vulnerable to salinity.

Height of the plantlets (after 30 days): Under normal circumstances, plantlets are transferred to green house after 30 days. The average height of the plantlets of T0 was 101 mm followed by T1 with an average height of 2.5 mm. In case of T2 and T3, the response of the plantlets is similar (1.5 mm). It shows that salt concentration has a variable impact on the height of the plantlets. Evers *et al.* (1998) reported salt stress related inhibition of shoot growth. The highest concentration of salt (T4) has a lethal effect on all parameters because no viability being observed.

Table 1: *In vitro* effect of salt on the vigor of potato plantlets

Parameters	Normal	T1	T2	T3	T4
Viability	95	55	35	10	0
Fresh weight of plantlets (g)	0.591	0.0412	0.0391	0.0351	0
Height of plantlet (mm)	101	2.5	1.5	1.5	0
Length of root (mm)	38	0	0	0	0
No of roots plantlet^{-1}	2-3	0	0	0	0

MS media containing: No NaCl (T_0) untreated control, 1% NaCl (T_1), 2% NaCl (T_2), 3% NaCl (T_3) 4% NaCl (T_4)

According to Zhang *et al.* (1997) salinity stress consistently depressed growth in the single-node cuttings of the sweet potato. Similarly, Martinez *et al.* (1996) reported that growth of *S. andigena* and *S. tuberosum* was inhibited by given range levels of NaCl.

Length of roots (after 30 days): The average length of the roots under control treatment (T0) was 38 mm, while in all other treatments (T1-T4), no considerable root length was observed. These results show that the salt concentration directly interferes in the growth process and prevent the plant from osmosis and diffusion process. Depression in root tip bioassays due to salinity stress also been reported by Zhang *et al.* (1997). Evers *et al.* (1999) reported that salt stress has a great impact on root growth. However, Hausman and Evers (2000) observed slight effect of salinity under low concentration on shoot and root development.

Number of roots plantlet^{-1}: Under control treatment (T0) approximately 2-3 roots were observed, while no roots were obtained in all other treatments. This character was adversely affected by the salt concentration and even not a single root was observed in the lowest stress condition. Evers *et al.* (1998) reported that salt stress had the diverse effect on the rooting and root growth in sweet potato. This could be the main reason that all the character under study responded negatively because rooting plays a major role in the supply of the nutrient to the plantlets. It further suggests that plants require very limited salts for their growth. Acclimation to salinity involves numerous physiological and metabolic changes in carbohydrate content, synthesis of new proteins and accumulation of small molecules. These changes are often associated with resistance/tolerance of plants to numerous stresses (Hausman and Evers, 2000).

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