In vitro evaluation of salinity tolerance of potato (Solanum tuberosum var. Spunta)

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Abstract:

In vitro evaluation of salinity (NaCI) effects on potato (Solanum tuberosum L) var. Spunta was investigated with five NaCI levels (0, 20, 40, 60, 80, and100 mM) by using single node explants. Significant differences affects were noticed among the plantlets cultivated in different NaCl levels. Salinity stress gradually depressed plantlets growth and development with increased NaCl concentration in MS media. Growth traits tested of plantlets grown in MS containing 20 mM NaCl exhibited no differences compared to control except plantlet length which was reduced significantly by 36% compared to control. Increasing salinity more than 20 mM showed a progressive reduction in all traits tested. All plantlets survived at high NaCl (100 mM) containing MS media and severely reduced growth rate. Our results estimated the moderate salinity tolerance of variety Spunta.

Keywords: Potato, in vitro, single node, NaCl stress, growth response.

Introduction:

Potato is the most important food crop in the world in terms of its production, which is only exceeded by those of rice, wheat, and maize. The estimated world production of potato in 2013 was as much as 310 million ton [7]. Salinity stress is a critical environmental constraint to crop productivity especially in arid and semiarid regions. The most of the crop plants is intolerable to high salinity conditions resulting in decreased yield. Generally, plants

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are stressed in three ways in saline soils a) low water potential of the root medium leads water deficit, b) the toxic effects of the Na^+ and Cl^-, and C) nutrient imbalance by depression in uptake and/or shoot transport [4]. Toxic accumulation of Na^+ and Cl^- in the leaves has also been correlated with stomatal closure and reduction of total chlorophyll content in leaves both of which limit the amount of photosynthetic production [20]. Cell and tissue culture techniques together with conventional breeding and genetic engineering have been considered as the potential approaches for the development of plants with increased tolerance to environmental stresses in general and for salt stress in particular. The successful selection of mutant lines from cultured cells and the regeneration of whole plants from such cells have stimulated many attempts for the development of salt-tolerant plants. Potato is considered moderately salt sensitive compared with other crops. A range of developmental responses in potato is affected by salinity. A small number of potato genotypes has been reported in salinity tolerance under outdoor, greenhouse or in vitro conditions [3, 12, 16]. Field trials and greenhouse pot trials were used to examine genotype salinity tolerance under NaCl or sodium salt irrigation solutions and were based on either tuber yield [8], relative reduction of foliage dry weight (2) or haulm fresh weight [18]. In vitro evaluations of NaCl or mixed salt stress effects on potato genotypes were proposed as alternatives to the costly, labor intensive and sometimes problematic field based traits. Potential advantages and disadvantages of this method have also been reported [6, 15]. The objective of this study was to find out the effect of different levels of NaCl on *in vitro* growth traits of potato (*Solanum tubersum* var. Spunta) by using single-node cuttings.

Materials and methods:

This experiment was carried out in Biotechnology Research Center (Touisha, 30 Km south of Tripoli- Libya). Potato tubers were obtained from a recognized company for importing seeds and tubers.Tubers were well washed and kept in the dark for few weeks until sprouts reached 1-2 cm in length. Sprouts were surface

sterilized with 70% ethanol for 1 min followed by 2% sodium hypochlorite for 5 min three times, sprouts then washed 3 times by distilled water. Sterilized sprouts 1-2 cm in length were cultured to obtain shoot culture in Murashige and Skoog [14] medium containing 3% sucrose, 0.7% agar. The medium was adjusted to pH 5.7 prior to autoclaving at 120 ^{0}C for 20 min. Cultures were maintained at 25±2 ^{0}C with 16/8 h D/N at 40 µmol m^{-2} s^{-1} photon flux density (cool white fluorescent light). After six weeks of culture plantlets were cut into 1 cm-long single-node with one leaf with axillary bud. Two segments were implanted into 200 mL culture vessel containing 20 mL of MS medium. Twenty replications of each salinity level (0, 20, 40,60, 80, and 100 mM NaCl) were employed. The experiment ended after 4 weeks and the growth response were evaluated on shoot length (SL), leaflets number (LN), shoot fresh mass (SF) and shoot dry mass (SD). Growth data were compared at each salinity level with control. The experimental design used was a completely randomized design. Twenty replicates per treatment were used, and every replicate contained two plantlets. Mean separation was analyzed by Duncan multiple range test at 5% level of significance.

Results and Discussion:

Plantlets growth was not affected by 20 mM NaCl containing MS media and generally it was almost similar to control levels except shoot length which showed a reduction of about 36% compared to control (Table 1). This result suggests that in the potato variety used in the present study, salt stress had more effect on cell division and elongation thus reducing more the shoot height than on dry matter production and accumulation. Moreover; the reduction in the shoot height was compensated by an increase in number of branches shown which was agreed with [10]. In fact, due to the increase in the number of branches (data not shown), total available photosynthetic tissue actually increased, which again may have contributed to dry matter production. To some extent, similar manner was found at higher salt concentrations; for instance, the shoot dry matter showed only 29% reduction at 40

mM NaCl while the decrease in shoot height at the same salt concentration was 66%. Similar pattern to these results of *in-vitro* potato grown under different NaCl concentrations were reported [9, 10]. There is merit in evaluation at a range of salinity levels, since different genes are apparently expressed at different stress levels in vitro as described in vivo [1, 21], which was the motive of choosing 5 NaCl levels in this experiment. Increasing NaCl level more than 20 mM in the media significantly reduced plantlets growth compared with the control. Leaflets number, shoot length, shoot fresh mass, shoot dry mass were gradually decreased with increasing of NaCl level (Table 1), the reduction in percentages, respectively, were 36, 66, 34, and 29 at 40 mm; 37, 72, 47, and 35 at 60 mM; 59, 74, 62, and 71 at 80 mM; 73, 83, 66, and 72 at 100 mM (NaCl). Correlation analysis for the relationship of different growth parameters showed that significant correlation existed among each other in response to different NaCl levels.

Many investigations were performed to examine salinity effect on potato growth in-vivo, all investigations showed harmful effect of salinity on plant growth, the extent to which plant growth is affected may vary among plant species [4, 8, 12, 16, 17]. For example, a suppression on plant emergence was reported as well as a progressive decline in both plant height and weight were recorded along with increase in salinity, the growth of haulms, was also reduced with severe reduction in tuber yield, salinity has also been found to enhance haulm senescence and to delay the flowering in all tested cultivars [4, 8, 12], the onset of leaf senescence and desiccation was found to be progressively advanced with increased salt concentrations in the irrigation water [16, 17]. Screening a large number of genotypes for salinity tolerance in the field is very difficult, due to spatial heterogeneity of soil chemical and physical properties. The *in-vitro* evaluations of NaCl effects on potato genotypes were proposed as alternatives to the costly, labor intensive, and sometimes problematic field traits. Moreover, a correlation among salt stress responses of *in-vitro*, field, and greenhouse potatoes was reported [1, 13,15, 22]. In

view of the significant correlation found between in-vitro growth and field performance, Morpurgo [1991] suggested *in-vitro* screening of potato parental material for tolerance to salinity. Many other studies have proposed the in-vitro screening of potato genotypes for salt stress tolerance as an alternative approach [1, 6, 19]. The successful *in-vitro* screening has stimulated many attempts for the development of salt tolerant plant [5, 9, 19, 22]. The response of several cultivars of potato were investigated, the growth inhibition was found to be varied depending upon the cultivar. and NaCl concentration in the medium [13, 22]. Khrais *et al*., (1998) [11] examined a large number (131) of potato cultivars. for salt tolerance. Nodal cuttings with one axillary bud were used. The average reduction in percentages for all tested cvs. based on seven trails for vegetative traits were recorded. According to their results, all cultivars. studied were ranked to salinity tolerance from 1 to 10, from high to low salinity tolerance. Little information is available on the salt stress tolerance for the variety Spunta, used in this study. According to our results, and to the classification made by Khrais *et al*., (1998) [11], variety Spunta could be classified as moderately tolerant to salinity. This result agrees with previous result obtained by Khenifi *et al.* (2011), [10] where Spunta was classified as moderately salt tolerant based on *in-vitro* screening at different concentrations of NaCl, subsequent field performances of salinity tolerance classification to this variety is still needed.

Table 1. Leaflets number (LN), shoot length (SL), shoot fresh mass (SF), and shoot dry mass (SD) of *in vitro* potato plantlets var. Spunta as affected by NaCl after 4 weeks of incubation.

NaCl (mM)	LN (No.)	SL (cm)	SF (g)	SD (g)
0	8.25 A	5.04 A	0.425 A	0.0488 A
20	8.2 A	3.22 B	0.412 A	0.0456 A
40	5.3 B	1.69 C	0.282 B	0.0347 B
60	5.2 B	1.39 C	0.226 B	0.0317 B
80	3.4 B	1.33 C	0.162 C	0.0139 C
100	2.2 C	0.87 D	0.144 C	0.0136 C

Values with the same letter in each column are not significantly different according to Duncan test at $P<0.05$.

تقييم مقاومة الملوحة للبطاطس .Solanum tuberasum L صنف Spunta باستخدام زراعة الأنسجة النباتية.

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المستخلص:

تم تقييم تأثيرات الملوحة (NaCl) على البطاطس (*Solanum tuberosum* L.) صنف سبونتا باستخدام زراعة الأنسجة النباتية عند 5 مستويات من NaCl وهي (0, 20, 40, 60, 80, 100 ملي مول) باستخدام العقد المفردة. لوحظ وجود تأثيرات معنوية بين النبيتات المزروعة على مستويات مختلفة من NaCl. الإجهاد الملحي أعاق نمو النبيتات وتطورها بصورة تدريجية وذلك بزيادة تركيز NaCl في وسط النمو المستعمل (MS). خصائص النمو التي تم اختبارها للنبيتات النامية في وسط النمو المحتوي على 20 ملي مول من NaCl لم تظهر أي فروق مع الشاهد باستثناء طول النبيتة والذي قل معنوياً بنسبة 36% مقارنة بالشاهد. زيادة الملوحة أكثر من 20 ملي مول أظهرت تناقص مضطرد بكل خصائص النمو المدروسة. كل النبيتات بقيت حية بوسط النمو المحتوي على مستوى عالي من NaCl (100 ملي مول) كما أظهرت انخفاض شديد في معدل النمو. من النتائج المتحصل عليها يمكن تقدير تحمل الملوحة للصنف سبونتا بالمتوسط.

الكلمات الدلالية: البطاطس، زراعة الأنسجة النباتية، العقد المفردة، الإجهاد الملحي، استجابة النمو.

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