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In Vitro Expression of Sodium Chloride Tolerance of Some *Solanum* Species

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Abstract: Fifteen *Solanum* species (13 wild potato forms and two cultivars,Dèsiree and Hansa), representing different degrees of sodium chloride (NaCl) tolerance, were chosen to study the *in vitro* expression of NaCl. Two-node explants from *in vitro* propagated (on a medium of Murashige and Skoog (MS) –salts) plantlets were placed in honey jars containing 50 ml MS supplemented with 0.1mg/l indole acetic acid (IAA), 0.2 mg/l benzylaminopurine (BAP) and either one of four NaCl concentrations (0,40,80 and 120 mM/l). They were placed in a culture room at a temperature of 20°C-23°C, 4000 lux light intensity and 16 hours light period, and arranged in a complete randomized design with four replicates. The results showed that shoot growth in all species was inhibited by increasing NaCl concentration. Analysis of explant shoots and roots showed that Na^+ and Cl^- concentrations increased with increasing NaCl concentration, whereas those of K^+ and Ca^{2+} decreased. There was a tendency of lower Na^+ and Cl^- concentrations in the shoots and higher concentrations in the roots of the NaCl tolerant compared to sensitive species. K^+ concentrations in the shoots and Ca^{2+} concentrations in the roots were higher in NaCl tolerant species than in sensitive ones. Na^+ and Cl^- concentrations in the shoots and explant main stem length were negatively correlated (r=- 0.91 for both). The correlation coefficients between the main stem length and K^+ concentration in shoots and Ca^{2+}concentrations in the roots were positive and highly significant (r= 0.74 and r = 0.90, respectively). However, Na^+ and Cl^- concentrations in the root were not correlated with shoot length. Shoot length could be a good trait for a quick screening of potato genotypes for NaCl tolerance

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using two-node cuttings *in vitro*. However, this approach will be of practical value if a close relationship is proved to exist between the *in vitro* and *in vivo* response of plants growing under salt stress.

INTRODUCTION

Soil salinity is one of the major abiotic stresses of crops affecting their productivity world-wide (Borsani *et al*. 2003). Conventional breeding methods were attempted to improve salinity tolerance of crops with appreciable success, but they need a lot of work and time. Tissue culture was suggested by many researchers for selection and production of new lines with valuable agricultural characters, especially resistance to adverse ecological and climatic conditions such as salinity and drought (McCoy 1987; Van Swaaij *et al*. 1986). Moreover, meristem culture is recommended as a quick screening method of germplasm for salinity tolerance of many crops compared to the labour intensive and costly field assessments using conventional methods (Tal *et al.* 1978; Ashraf 1994; Zhang and Donnelly 1997). The main proplem is to find a suitable growth trait which can be used as indicator of salinity tolerance. Khrais *et al*. (1998) suggested a multivariate cluster analysis based on the relative means of six shoot and root growth parameters of potato cultivars under salt stress *in vitro*. They found clear differences in salinity tolerance using this method; however, assessing six growth parameters at a time is laborious and time consuming.

The importance of specific effect of Na^+ and Cl^- on plant growth under salt stress was reported by many investigators (Wieneke and Laeuchli 1980; Imamul Huq and Larher 1985). Smith and McComb (1981) noted that salinity resistance is frequently judged by the ion content of the tissue or organ analysed. Such studies were difficult to apply to calli due to the nature of the callus which is spongy. Consequently, there will be no control of diffusion of the medium into its intercellular spaces. Therefore, it becomes difficult to differentiate between ions in cell wall or intercellular spaces and those within the cell (Meredith 1978). However, stem segments could be considered as mini plants having anatomical organization and ability to root and grow into a complete plant.

Typical differences among crop species in terms of growth and mineral content of the shoots were found (e.g., Lauechli and Wieneke 1979). The higher salt tolerance of some cultivars of many crops is related to a more effective restriction of shoot transport of both Na^+ and Cl^- (Maas and Hoffman 1977). This study was conducted to find out the degree of NaCl tolerance (as manifested by impaired shoot transport of toxic ions) in potato *in vitro* and whether it is related to shoot growth.

MATERIALS AND METHODS

The plant material used in this study consisted of 15 *Solanum* species (13 wild potato forms and two cultivars: Dèsiree and Hansa) having different NaCl tolerance as shown in a previous unpublished study by the authors. All genotypes were propagated from stem cuttings *in vitro* (Elhag *et al*. 1994) in order to obtain a genetically stable material. Two-node stem cuttings explants were placed in honey jars containing 50 ml of the test medium (Murashige and Skoog–salts 1962) supplemented with 0.1 mg/l indolacetic acid (IAA), 0.2 mg/l benzylaminopurine (BAP) and either one of four NaCl concentrations: 0, 40, 80 and 120 mM/l. The medium pH was adjusted to 5.78. The cultured explants were placed in a growth chamber with a controlled temperature of 20°C-23°C and light intensity of 4000 lux and 16 hours light period at the Institute of Crop Science, Fedral Research Centre, Braunschweig, Germany.

After six weeks, the explants were evaluated for NaCl tolerance using the parameters: shoot length and Na^+, Cl^-, K^+ and Ca^{2+} concentrations in shoots and roots. The dry matter of both explant parts was wet digested (Chapman and Pratt 1961). Na^+ and K^+ were determined with the flame photometer (Eppendorf flame photometer), Ca^{2+} with the atomic adsorption spectrophotometer (Perkin Elmer 430) and Cl^- with an autoanalyser (Zeis-Braun Analyser). The experimental design was complete randomised with four replicates. The data were analysed using the statistical programme SPSS, and the least significant difference was used to separate the means (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

Increasing NaCl concentration inhibited shoot growth in all species under study. The explant main stem length was greatly affected by the addition of NaCl (Table 1), where some of them could not survive the highest NaCl concentration (120 mM/l). Accordingly, the species could be ranked for NaCl tolerance as in Table 1. A general reduction in growth of several potato cultivars under salt stress was also obtained by Ahmad and Abdullah (1982) and Levy *et al*. (1988) using conventional methods. Similar results were also obtained by Zhang and Donnelly (1997) *in vitro*.

Determination of mineral element concentrations in both explant shoots and roots showed that Na^+ (Fig. 1) and Cl^- (Fig. 2) concentrations increased with increasing NaCl concentration. There was a tendency of lower Na^+ and Cl^- concentrations in the shoots but higher concentrations in the roots of the NaCl tolerant compared to sensitive species at the low and middle NaCl concentrations (40 and 80 mM/l); thus, indicating that these higher concentrations of both elements (Na^+ and Cl^-) resulted in great growth depressions of the sensitive species. Meanwhile, higher concentrations of both elements in the roots of NaCl tolerant genotypes indicated their ability to accumulate them in the root tissues and to restrict their transport to the shoot tissues, i.e., toxic ion exclusion. Maas and Hoffman (1977) reported that the high salt tolerance of some cultivars of many crops is related to a more effective restriction of shoot transport of both Na^+ and Cl^-. The importance of the specific effect of Na^+ and Cl^- on plant growth under salt stress was also reported by many investigators (Wieneke and Laeuchli 1980; Imamul Hug and Larher 1985). Smith and Mcomb (1981) noted that salinity resistance is frequently judged by the ion content of the tissue or organ analysed.

Table 1. Effect of NaCl concentrations on explant main stem length (cm) of 15 *Solanum* species *in vitro*

Rank	*Solanum* spp.	Acc. or cv.	NaCl concentration (mM/l)			
			0	40	80	120
1.	*S.chacoense*	16979	0.9	0.8	0.7	0.5
2.	*S.sparsipilum*	24687	1.4	1.2	1.0	0.7
3.	*S.vernei*	15451	1.6	1.4	1.1	0.2
4.	*S.spegazzinii*	18328	1.7	1.4	1.2	0.8
5.	*S.tarijense*	24717	1.6	1.4	1.1	0.7
6.	*S.gourlayi*	16837	1.0	0.8	0.6	0.4
7.	*S.tuberosum* subsp.*tub*.	Dèsiree	1.0	0.8	0.6	0.4
8.	*S.tuberosum* subsp.*andg.*	28043	2.0	1.4	1.2	0.6
9.	*S.tuberosum* subsp. *andg.*	7463	0.9	0.7	0.4	0.2
10.	*S.fendleri*	8088	1.4	1.1	0.7	x
11.	*S.demissum*	53643	0.8	0.6	0.4	x
12.	*S.hawkesianum*	16955	2.3	1.5	0.9	x
13.	*S.alandiae*	31187	1.7	1.1	0.2	x
14.	*S.stenotomum*	18478	2.6	1.8	0.4	x
15.	*S.tuberosum* subsp.*tub*.	Hansa	1.4	0.7	0.4	x
Mean			1.5	1.1	0.7	0.3
LSD at	0.05	0.01				
NaCl concentration	0.1	0.1				
NaCl/genotype	0.2	0.3				

Acc.or cv. = accession or cultivar
x = dead explants

K^+ and Ca^{2+} (Figs. 3 and 4, respectively) concentrations were depressed by NaCl. There were no consistant differences in K^+ and Ca^{2+} concentrations in the shoots or roots of NaCl tolerant or sensitive species at 0.0 NaCl concentration. However, K^+ concentration in the shoots and Ca^{2+} concentrations in the roots were higher in NaCl tolerant species at elevated NaCl levels. In the more salt tolerant barley, Na^+/ K^+ imbalance might be responsible for the adverse effect of high NaCl concentrations on potassium content and protein synthesis (Helal and Mengel 1979). LaHaye and Epstein (1971) showed that sodium toxicity is related to low absolute Ca^{2+} levels in saline substrates or high Ca^{2+}/Na^+ ratios in combination with poor aeration (saline-sodic soils). At low and moderate salinity levels, Na^+ transport to the leaves is restricted. Its exclusion

mechanism relies on relatively high external Ca^{2+} concentrations and or Ca^{2+}/Na^{+} ratios.

Linear correlations of mineral elements concentration in shoots and roots in the presence of NaCl (Fig. 5) over shoot length basically confirmed these observations. Na^{+} and Cl^{-}concentrations in the shoots were negatively correlated with explant main stem length (r = -0.91 for both). K^{+} concentration in the shoots and Ca^{2+}concentration in the roots were highly positively correlated with the explant main stem length (r = 0.74 and r = 0.90, respectively). However, Na^{+} and Cl^{-} concentrations in the roots were not correlated with shoot length, indicating the importance of the last two elements (K^{+} and Ca^{2+}) for growth. Omielan *et al*. (1991) reported a negative correlation beween salt tolerance and Na^{+} and a positive one with K^{+} and K^{+}/Na^{+} ratio, which were strong enough to be exploited as selection criteria in breeding for salt tolerant wheat cultivars. Differences in the capacity for Na^{+} and Cl^{-} exclusion or their restricted shoot transport among cultivars of wheat were also found (Bernal *et al*. 1974). Decreased K^{+} and increased Na^{+}, Cl^{-} and Ca^{2+} in potato and tomato plants under salt stress were also obtained by Potluri and Prasad (1993) and Cano *et al*. (1998), respectively.

It could be suggested that shoot length is a good indicator of NaCl tolerance of potato which could be used for a quick screening for NaCl tolerance, using two-node cuttings *in vitro*. NaCl tolerance of potato *in vitro*, as in other glycophytes, is inversely related to ion uptake, specially Na^{+} and Cl^{-} ,i.e, potato may be a salt excluder. However, this approach will be of practical value if a close relationship is proved to exist between the response of plants under salt stress *in vitro* and *in vivo*.

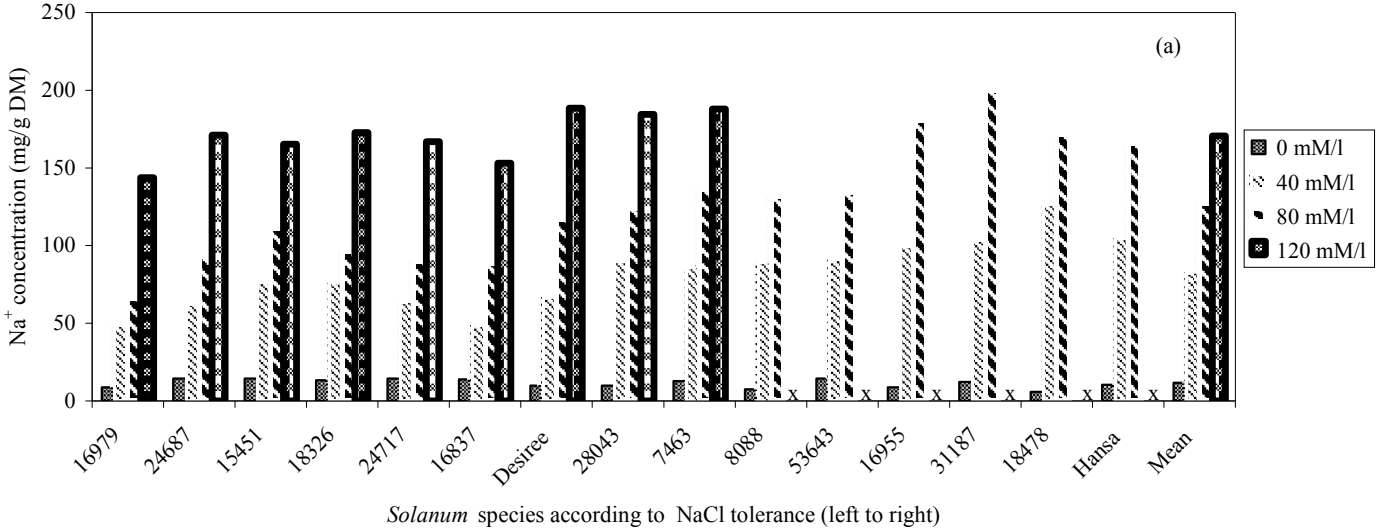

Fig. 1. Effect of NaCl concentrations on Na^+ concentrations in explant (a) shoots and (b) roots of 15 *Solanum* species *in vitro*, where x means dead explants or no roots were formed

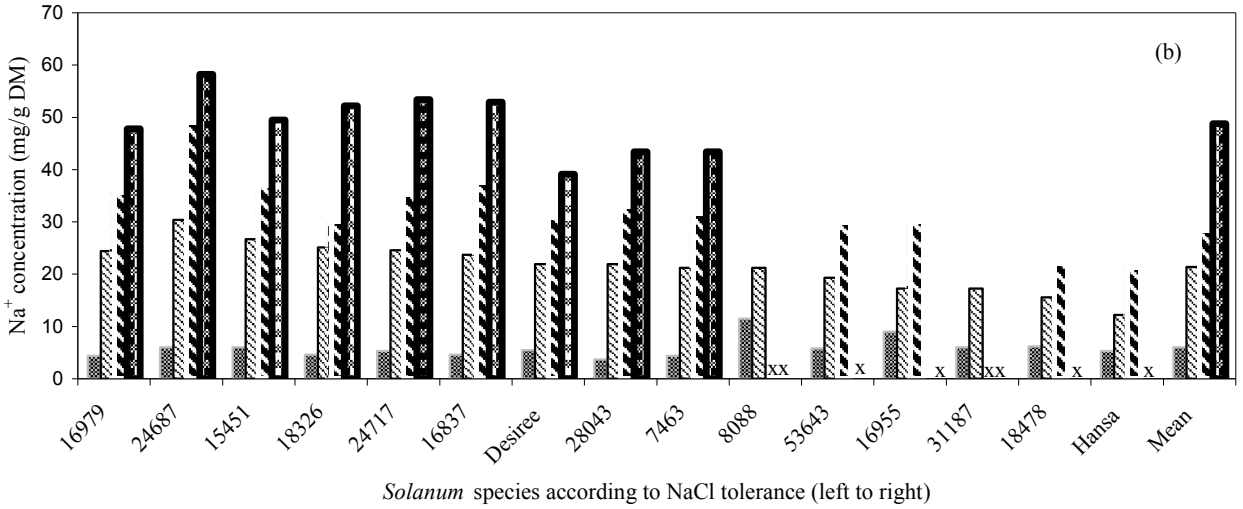

Fig. 1 continued

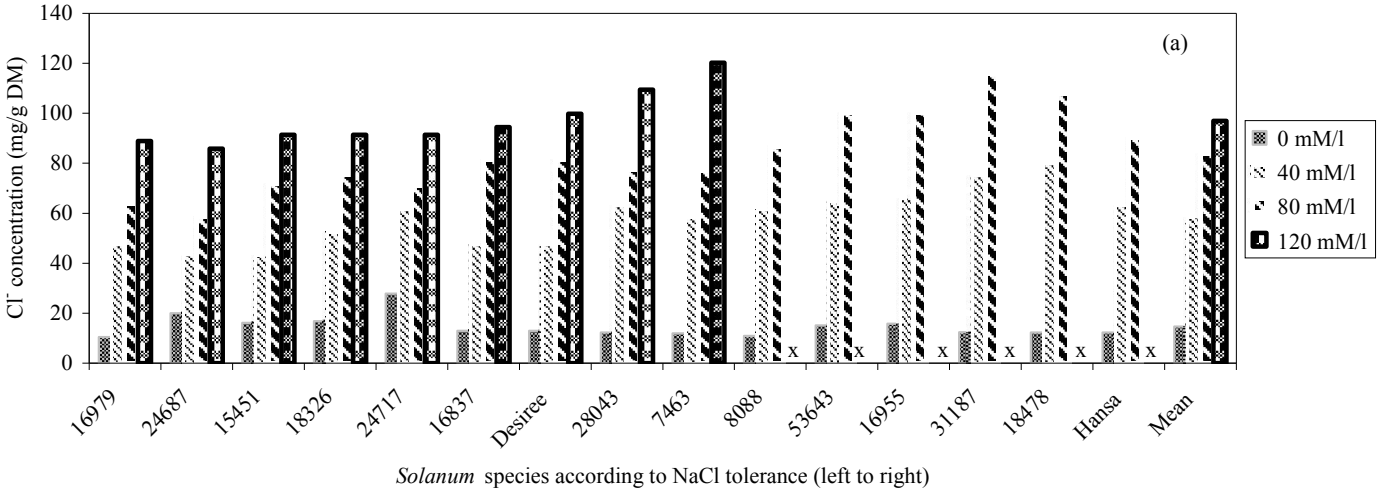

Fig 2. Effect of NaCl concentrations on Cl^- concentrations in explant (a) shoots and (b) roots of 15 *Solanum* species *in vitro*, where x means dead explants or no roots were formed

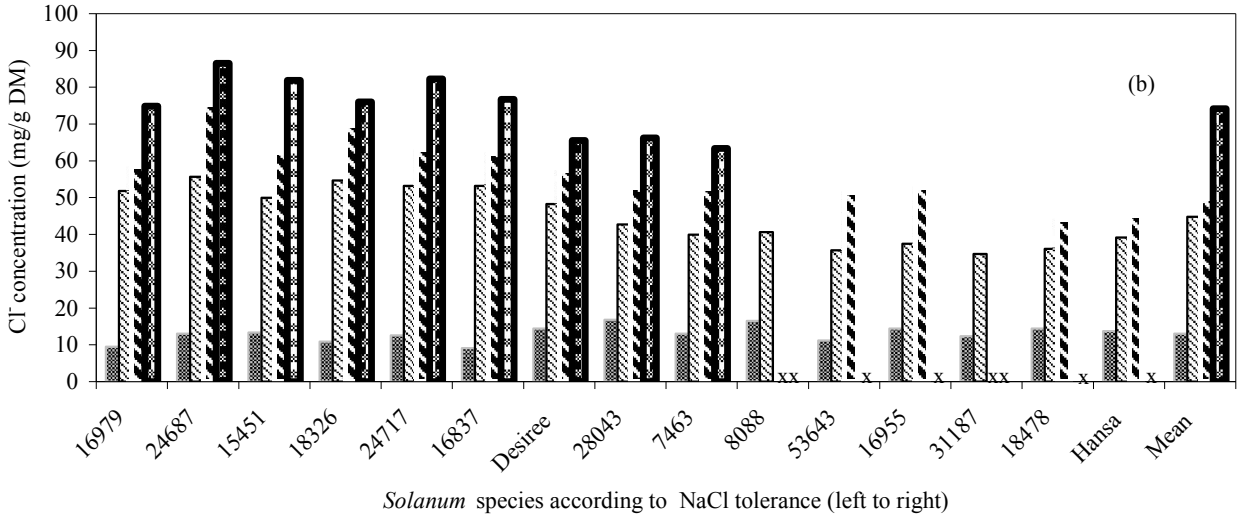

Fig.2 continued

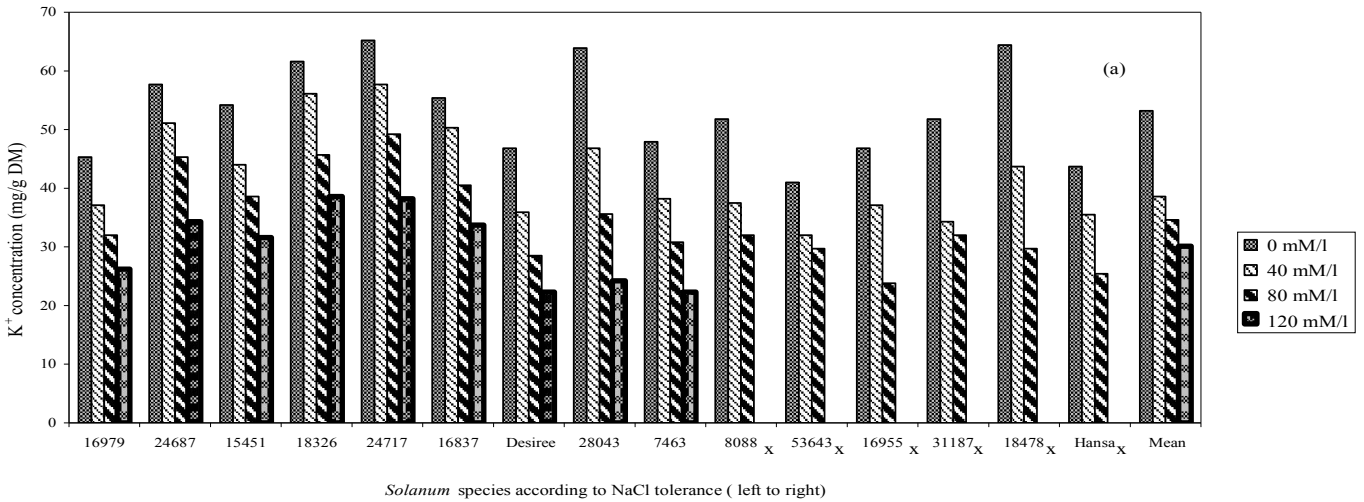

Fig. 3. Effect of NaCl concentration on K^+ concentration in explant (a) shoots and (b) roots of 15 *Solanum* species *in vitro*,where x means dead explants or no roots were formed

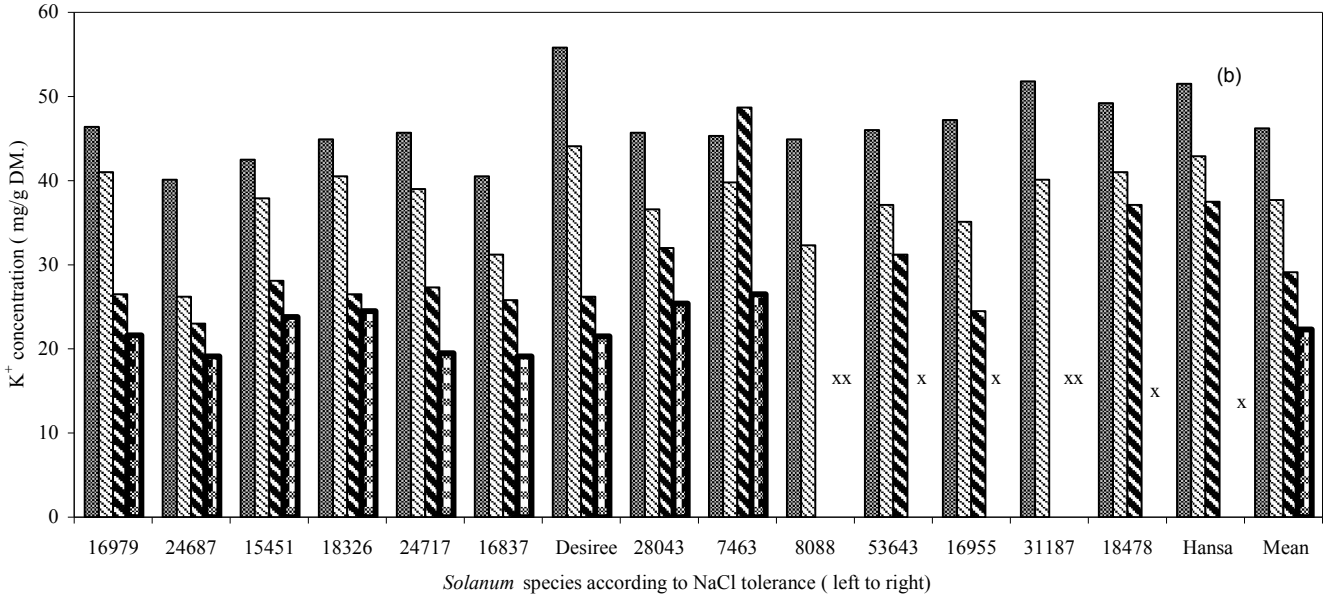

Fig. 3 continued

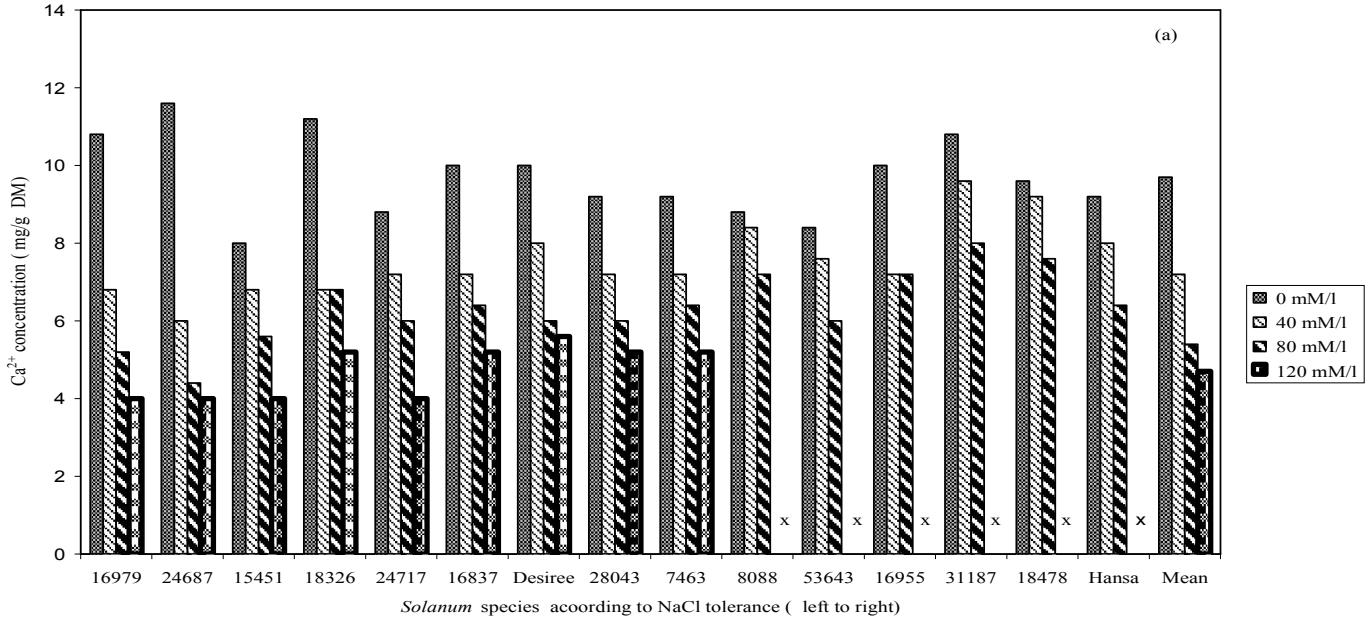

Fig.4. Effect of NaCl concentration on Ca^{2+} concentration in explant (a) shoots and (b) roots of 15 *Solanum* species *in vitro*, where x means dead explants or no roots were formed

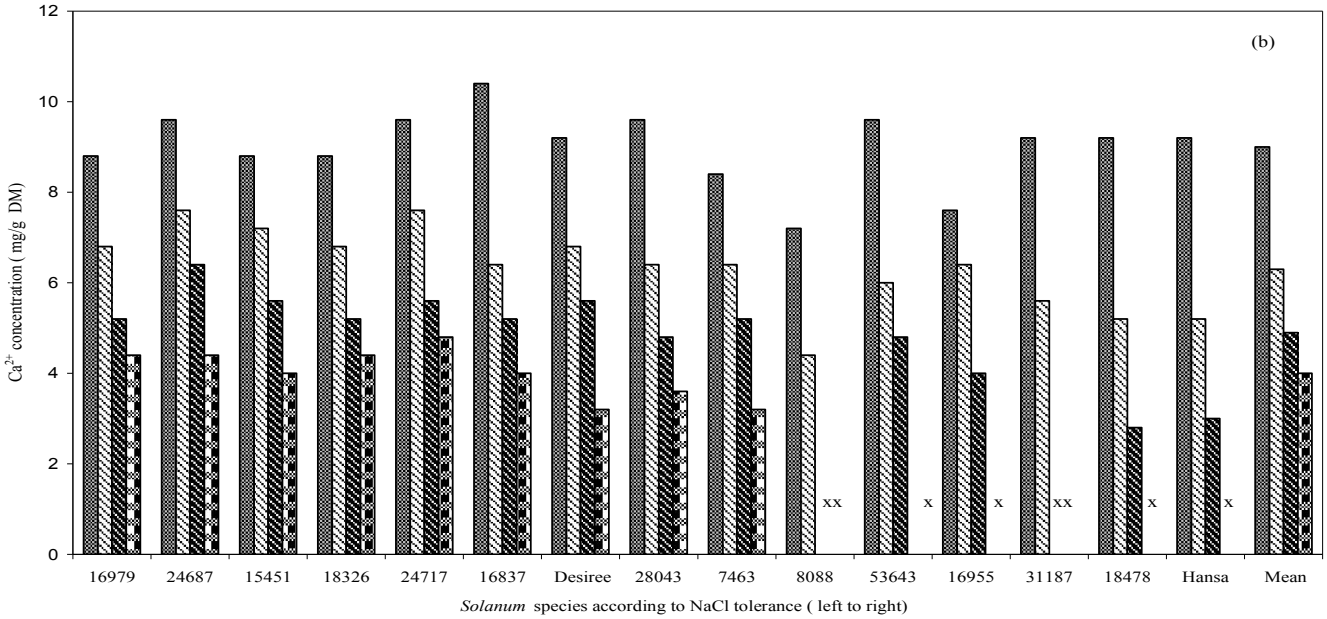

Fig.4. continued

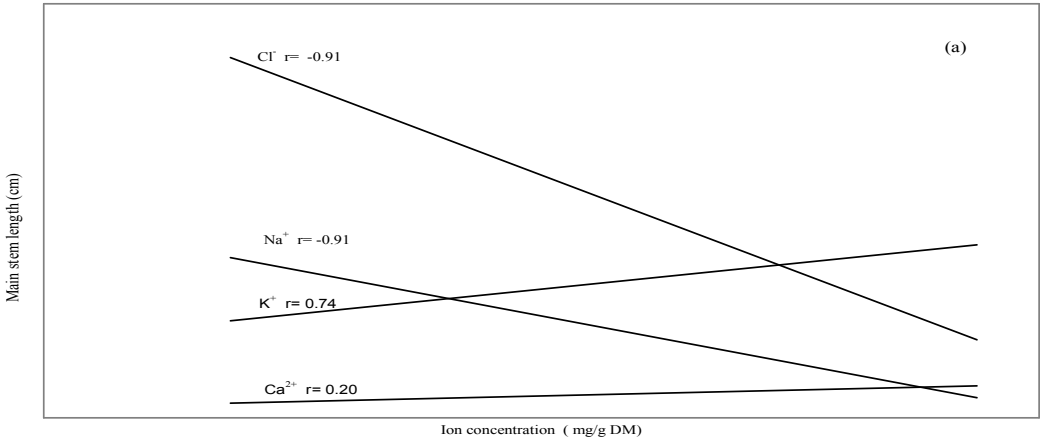

Fig.5. Correlation coeffcients between Na^+, Cl^-, K^+ and Ca^{2+} concentrations in explant (a) shoots and (b) roots and NaCl tolerance (as explant main stem length) of 15 *Solanum* species *in vitro*

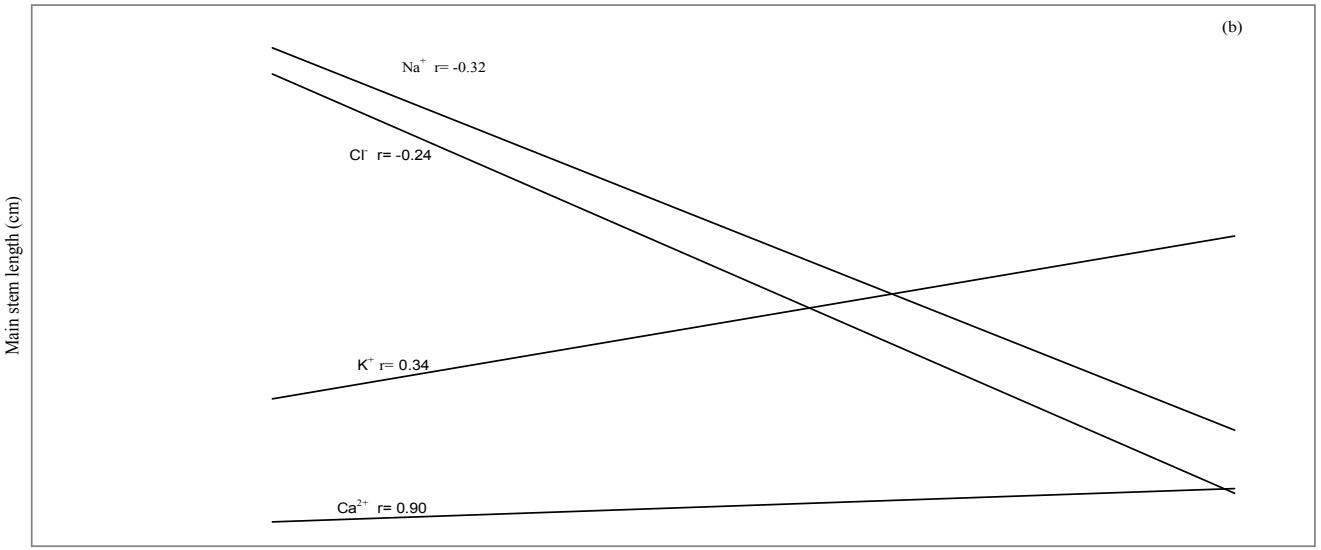

Fig.5. continued

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تعبير طرز البطاطس عن تحملها لكلوريد الصوديوم خارج الجسم الحى

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معهد علوم المحاصيل و المراعى والاعلاف- مركز البحوث الزراعية الفدرالى- برونزويك- المانيا

موجز البحث : تم اختيار 15 طرزاً وراثياً من البطاطس تمثل درجات مختلفة من تحمل الملوحة لدراسة إلى اى مدى يمكن أن تعبر عن تحملها لكلوريد الصوديوم خارج الجسم الحى . وضعت عقل ساقية ذات برعمين لهذه الطرز ، والتى سبق تربيتها فى المختبر (فى وسط موراشجى واسكوج) ، فى قوارير عسل بها 50 مل لتر من وسط موراشيجى واسكوج مضافاً إليه 0.1 ملغرا م/لتر(ملغ/ل) حامض الخليك (IAA) و0.2 ملغ/ل بنزويك امينوبيورين (BAP) وأربعة تراكيز من كلوريد الصوديوم (صفر و40 و80 و 120 ملمول/ل) ، ثم نقلت القوارير إلى غرفة تربية عند درجة حرارة 20°م-23°م و4000 لوكس شدة اضاءة و16 ساعة فترة ضوئية فى تصميم كامل العشوائية باربعة مكررات . بعد ستة اسابيع ، تم تقييم تحمل الملوحة بقياس طول ساق النبيتة الرئيس وتراكيز ايونات الصوديوم والكلور والبوتاسيوم والكالسيوم فى المجموعين الخضرى والجذرى . أدت زيادة تركيز كلوريد الصوديوم إلى تثبيط نمو كل الطرز . ودل تحليل المجموعين الخضرى والجذرى على زيادة تركيز ايونات الصوديوم والكلور بزيادة تركيز كلوريد الصوديوم بينما قل تركيز البوتاسيوم والكالسيوم . كان هناك ميل لانخفاض تركيز الصوديوم والكلور فى السيقان وارتفاعها فى الجذور فى الطرز ذات التحمل لكلوريد الصوديوم مقارنة بالطرز الحساسة . كما أن

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تركيز البوتاسيوم فى السيقان وتركيز الكالسيوم فى الجذور كان عالياً فى الطرز الوراثية ذات التحمل لكلوريد الصوديوم . وقد أكد هذه الملاحظات معامل الارتباط المعنوى الموجب بين طول ساق النبيتة الرئيس وتركيز البوتاسيوم فى سيقان النبيتة والكالسيوم فى جذورها فى وجود كلوريد الصوديوم . الارتباط بين تركيزى الصوديوم والكلور فى المجموع الخضرى وطول ساق النبيتة الرئيس كان سالباً وعالى المعنوية (r =- 0.91 لكل منهما) ، فى حين كان معامل الارتباط بين تركيزى البوتاسيوم فى المجموع الخضرى والكالسيوم فى المجموع الجذرى وطول الساق موجباً وعالى المعنوية (r = 0.74 و r = 0.90 ، على التوالى) . ولا يوجد إرتباط بين طول الساق وتركيز ايونات الصوديوم والكلور فى الجذور . يبدو أن طول ساق النبيتة الرئيس يصلح للاختبار السريع لطرز البطاطس ذات التحمل لكلوريد الصوديوم فى المختبر . تحمل البطاطس خارج الجسم ذا علاقة عكسية بامتصاص الايونات السامة خاصة الصوديوم والكلور . وقد يكون التوجه ذا قيمة عملية إذا امكن اثبات أن هناك علاقة بين استجابة النباتات تحت الاجهاد الملحى داخل وخارج الجسم .