Journal of Medicinal Plants and By-products (2017) 2: 153-163

Original Article

In vitro Response of *Asparagus breslerianus* to NaCl

Seyyed Javad Mousavizadeh*, Mohammad Reza Hassandokht and Abdolkarim Kashi

Department of Horticultural Sciences, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.

Article History: Received: 21 July 2016 /Accepted in revised form: 09 January 2016
© 2013 Iranian Society of Medicinal Plants. All rights reserve

Abstract

Asparagus breslerianus a wild species in Iran, exhibited tolerance to salt in dry gypsum hills and dry lands. In order to check for salt tolerance threshold via *in vitro* conditions, the *A. breslerianus* callus was subjected to NaCl (sodium chloride) treatments. Six weeks old calli derived from male spear bud, were exposed to 0, 21.88, 43.76, 65.64, 76.58, 87.52, 109.40, 131.28, 153.16 and 175.04 mM NaCl in MS (Murashige and Skoog) basal medium supplemented with 0.88 µM BA (6-Benzylaminopurine) and 1.07 µM NAA (1-Naphthalene acetic acid). According to results, friable and compact type, green and white green calli were obtained up to 87.52 mM NaCl. Soft type, yellow and cream calli were observed with increasing salinity further 109.4 mM NaCl. Calli growth and plantlets regeneration were high in media up to 109.4 mM NaCl. In term of mineral accumulation, sodium content increased with an increase in NaCl levels. It indicates that *A. breslerianus* calli could acclimatize to salt stress by high osmotic adjustment. These suggested that, under *in vitro* salt-induced osmotic stress, *A. breslerianus* is reflected as salt resistant which tolerate NaCl up to 109.4 mM.

Key words: Asparagus, Salt tolerance, Callus type, Callus growth

Introduction

Asparagus L. species (Asparagaceae family) in compare to other vegetable crops, has high capacity to salt tolerance [1], and it is divided into halophyte plants [2]. It was previously stated that *A. officinalis* L. seeds capable to germinate in water salinity up to 9.4 dSm^{-1} [3]. Even applications of NaCl stimulated earliness, total yield, marketable yield and number of spears in *A. officinalis* [4].

In vitro salinity-induced cultures are preferable tool for explore stress mechanism [5-6], which are unconnected to external environment. The investigation on asparagus *in vitro* salt stress was focused on *A. officinalis* [7-8], and *A. maritimus* Pall. [9].

There is a special species in *Asparagus* family named *A. breslerianus* it is infrequent that habits in dry gypsum hills and dry lands [10]. It was mentioned *A. breslerianus* as a rare and saline habitat species, which need preservation [11]. Recently a wild population of *A. breslerianus* has been investigated form Iran and their plants have shown different morphological characters in compare to other *Asparagus* species [12].

In our previous study, octoploid level (2n=8x=80) was identified by flow cytometry in *A. breslerianus* [13]. A distinct polyploid series of diploid (2x), tetraploid (4x), hexaploid (6x) and even dodecaploid (12x) species were previously reported in the *Asparagus* genus [14-15-16-17]. *A. breslerianus* was early reported as diploid [18]. Octoploid level in this species is not occurring very often [13].

Because of both salt tolerance capacity and ploidic level, *A. breslerianus* germplasm should be conservation in gene banks or botanical gardens. This can be more important if we know that they

*Corresponding author: Department of Horticultural Sciences, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran
Email Address: mousavizadeh@ut.ac.ir

are in risk of disappear due to livestock grazing and erosion [12].

The objective of the present study was *in vitro* indices of NaCl using callus culture to broad information about salt tolerance mechanism in this halophytic vegetable.

Material and Methods

Plant Material

Young spears (15-20 cm) of male gender of wild *Asparagus breslerianus* Schult. & Schult.f., emerging in the spring were gathered from natural zone of Iran (36° 01′ N and 56° 02′) as declared in the Flora of Iran [19-10].

Media and Culture Condition

Vessels used for the culture were jam jar with a capacity of 30 ml MS basal medium (Murashige and Skoog). Ten NaCl treatments based on local soil EC (7.2 dSm^{-1}) carry out for salt stress responsive. For this aim, media supplemented with 0, 21.88, 43.76, 65.64, 76.58, 87.52, 109.40, 131.28, 153.16 and 175.04 mM NaCl. Then, uniform size calli (diameter ranking from 8 to 10 mm, derived from spears bud of *A. breslerianus*) were individually weighed and were transferred to media along with 0.88 µM BA and 1.07 µM NAA as used for callus growth and development. The cultured media were incubated in white fluorescent light (illumination at 3000 Lux) in a growth room at 25± 2 ^{o}c. The developed calli were inoculated on the same MS medium to evaluate their response on vegetative bud multiplication potential. Induced shoots were subcultured on the same medium fortified with 3.9 µM Ancymidol and 1.07 µM NAA for rooting [9]. The multiple shoots with root were subcultured for rapid clonal multiplication. The derived plants were acclimated in pots containing cocopeat: perlite: vermiculate (1:1:1) and maintained in growth chamber under controlled conditions with fluorescent light and a photon flux of approximately 3000 Lux, at 25±2 °C, under a 16 hour photoperiod for eight weeks.

Callus Growth, Plantlet Regeneration and Number of Shoot Initiated

The frequency of calli formation per explants was recorded through average samples after four weeks of culture. The plantlet regeneration capacity was determined after six weeks, as the frequency of calli that showed shoot on their surface. Shoots per callus were counted and recorded as number of shoot initiated.

Chlorophyll Content

Chlorophyll a, b and ab content were extracted by acetone 80%, from 1 g of calli. The amount of these pigments were measured by Arnon (1956) method spectrophotometerily (UV/Vis 2100) at 645 and 663 nm and expressed as mgg^{-1} fresh weight [20].

Dry Matter (DM) and Ash Percentage

Fresh weight of calli were determined. The calli were dried in an oven at 70^{o}C for 48 h and dry matter measured. Dry matter and ash content were expressed in percentage of fresh weight and dry matter basis, respectively, according to the following formula:

DM = Dry matter/Fresh weight×100

Ash = Ash weight/Dry matter×100

Callus Relative Growth Rate (RGR)

Callus relative growth rate (RGR) was determined on fresh weight (FW). The RGR of the callus was calculated as:

RGR = [In (final weight) – In (initial weight)]/day [21].

The calli were harvested from saline medium after 54 days of treatments and fresh weight of the callus tissue calculated. The RGR was expressed as mg per fresh weight of callus.

Index of Tolerance (IT)

To compare callus responses to different levels of stress condition, an index of tolerance (IT), based on RGR was calculated according to the following formula:

$IT = RGR_{treatment}/RGR_{control}$ [21].

Relative water content (RWC)

Callus relative water content (RWC) was calculated as a percentage of fresh weight. The callus water content was calculated as:

RWC = (Fresh weight – dry matter) /dry matter × 100 [18].

K^+ and Na^+ analysis

K^+ and Na^+ concentrations were determined by ash at 550 °C for 24 h, dissolving the ash in 10 ml HCl 2 M, and assaying the solution obtained using a flame photometer [22].

Experimental Design and Statistical Analysis

Data were analyzed statistically using analysis of variance in a completely randomized design with 10 treatments (10 levels of NaCl) and three replications (Three jars from each treatment with three explants in each jar). The data of number of shoot initiated were normalized by root square ($\sqrt{X}$). The regression model was fitted to the data using the Proc Reg of SAS. The changes of traits over long-term simulation versus salt condition were describable using polynomial regression model ($y = a + bx + cx^2$). In one case for the K^+ and Na^+ content a simple, linear regression model ($y = a + bx$) was used. Differences among the means were determined for significance at $P<0.05$ using least significant difference (LSD 5%) test and the system program SAS 9.1 software (SAS Institute Inc., Cary, NC, USA).

Results and Discussions

Callus Type

Different callus textures (compact, hard, soft and friable) were evaluated after four weeks. Colors of callus were also noted. Calli without any obvious organ regeneration are typically called friable or compact depending on their tissue characteristics [23]. In this experiment, callus texture was generally observed either compact or friable (Fig. 1, a and b). Independent of the media NaCl, four morphological different callus types could be distinguished: 1) a friable, type with green, white green colored (Fig. 1, a), 2) a compact, hard, friable type, with green or white green colored, 3) a compact, hard type with white green, cream colored (Fig. 1, b), 4) a compact, soft type with cream, yellow colored either surrounded with water (Table 1). Data in Table 1 showed that up to 100% of the calli were formed compact and in media including media with 0 to 87.52 mM NaCl had friable callus. Soft type, yellow and cream colored calli were observed with increasing salinity in more than 109.4 mM (Table 1). These results are in agreement with *Odyssea paucinervis* callus growth in salt condition [24]. Soft, friable and cream colored calli were reported in *Salvadora persica* under NaCl stress condition [25].

Dry Matter (DM), Relative Water Content (RWC) and Ash Percentage

Considerable variations were observed in callus DM, RWC and ash among salinity conditions culture. Results (Table. 2) showed that there was a significant difference among 10 applied NaCl concentrations for DM, RWC ($P<0.01$), and ash percentage ($P<0.05$).

As seen in Fig. 2, the ash was increased until 87.52 mM NaCl. Then, ash decreased with NaCl rising to 175.04 mM. Based on X^2 and X slope, ash was increased rapidly. On the other hand, Aparagus callus ash at different media NaCl was rapidly raised and then slowly reduced than 87.52 mM. As shown in Fig. 3, the highest callus DM% was obtained in 131.28 to 175.04 mM. Below the 87.52 mM by rising DM, ash increased, but up to 87.52 mM, ash decreased by DM decreasing. The remarkable point is that DM has not decreased with increasing salinity. May be Na^+ accumulation at high concentrations of salt, increases the callus density, and increased DM at salt levels. Calli dry matter of *A. officinalis* cv. UC.157 gradually increased as salt concentration increased up to 6000 ppm salt mixture (3 NaCl:1 (3 $MgCl_2$:1 $CaCl_2$)) [8]. The increase in callus dry matter under salinity stress has already been observed in rice [26], tomato [27] and *Salvadora persica* [25].

As seen in Fig. 3, the RWC response towards salt increase was high until 109.4 mM NaCl. Then, RWC decreased with NaCl rising to 175.04 mM. Aparagus callus RWC at different media NaCl was slowly reduced than 109.4 mM. Results of correlation coefficient showed that a significantly negative correlation exists between callus RWC and DM%, and positive correlation exists between callus RWC and callus growth, callus volume, plantlet regeneration, number of shoot initiated, K^+ and RGR (Table 3). RWC at highest salt stress (175.04 mM NaCl) five percent decreased compared to control (Fig. 3). Asparagus with decreasing water potential and osmotic adjustment reduces injuries under drought stress [28]. In another plant for example in sugarcane, callus growth and water content decreased under NaCl stress [29].

Callus Growth, Callus Volume and Plantlet Regeneration

Callus formation was started after one week of culture. Significant differences were recorded (Table. 2) among NaCl concentrations for callus growth percentage ($P<0.05$), callus volume ($P<0.01$) and plantlet regeneration percentage as regeneration capacity of callus ($P<0.01$).

Table 1 Callus form of *Asparagus breslerianus* Schult. & Schult.f. in NaCl treated media.

NaCl concentration (mM)	Callus color	Callus type
0	White green	Friable, Compact
21.88	White green	Friable, Compact
43.76	Green, White green	Friable, Compact
65.64	Green, White green	Compact, Hard, Friable
76.58	White green	Compact, Hard, Friable
87.52	White green	Compact, Hard, Friable
109.4	White green	Compact, Hard
131.28	Yellow, Cream	Compact, Hard
153.16	Yellow, Cream	Compact, Soft
175.04	Cream	Compact, Soft

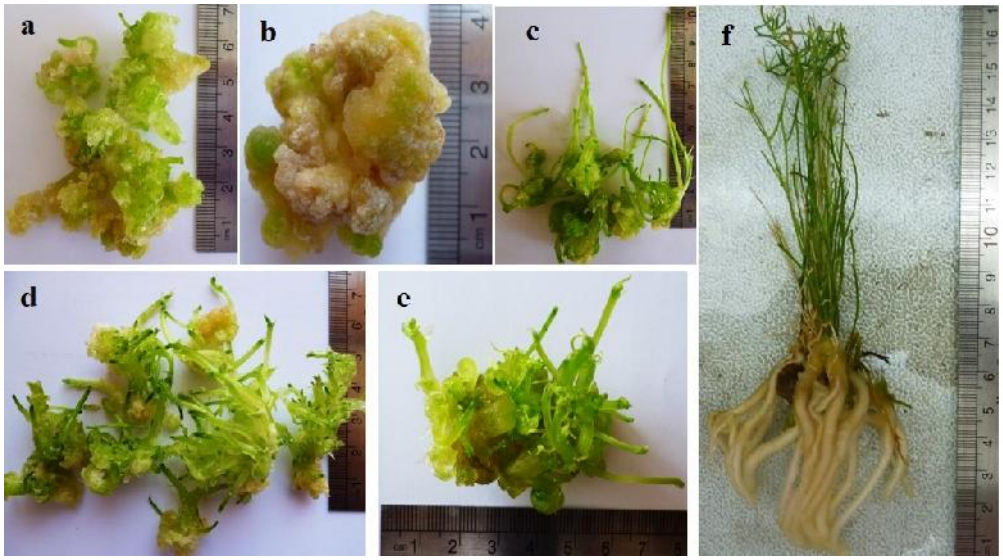

Fig. 1 *Asparagus breslerianus* callus subjected to NaCl concentration after six week of culture in MS medium supplemented with 0.88 µM BA and 1.07 µM NAA.
(a) White green and friable callus derived from medium under 43.76 mM NaCl.
(b) Yellow, cream and compact, hard type callus derived from medium under 131.28 mM NaCl.
(c) Shoots initial and development after eight weeks of culture in medium under 65.64 mM NaCl.
(d) High shoot development after eight weeks of culture in medium under 87.52 mM NaCl.
(e) Shoots initial and development after eight weeks of culture in medium without NaCl (control).
(f) Root development after shoot initiated and subculture in medium containing 76.58 mM NaCl supplement with 3.9 µM Ancymidol and 1.07 µM NAA for eight weeks.

Table 2 Analysis of variance of callus culture of *Asparagus breslerianus* Schult. & Schult.f. in NaCl treated media.

S.O.V	df	Dry matter	Ash	RWC	Callus induction	Callus volume	Bud induction	Number of shoot initiated	Chlorophyll a
Salt	9	10.9**	46.88*	10.9**	242.3*	18.34**	5828.3**	22.91**	1.14**
Error	20	3.01	16.95	3.01	92.33	2.82	192.6	1.13	0.118
CV%		26.36	24.61	1.85	10.17	23.5	21.96	29.76	30.02

** (P<0.01), * (P<0.05).

Continue Table 2

S.O.V	df	Chlorophyll b	Chlorophyll ab	Na^+	K^+	Callus relative growth rate	Index of tolerance	Number of rooted plants
Salt	9	1.56**	1.21**	327.17**	145.3**	90.56**	0.25**	288.6**
Error	20	0.165	0.128	7.17	10.39	5.06	0.01	10.70
CV%		27	27.02	8.72	23.18	13.69	13.69	28.36

** (P<0.01), * (P<0.05).

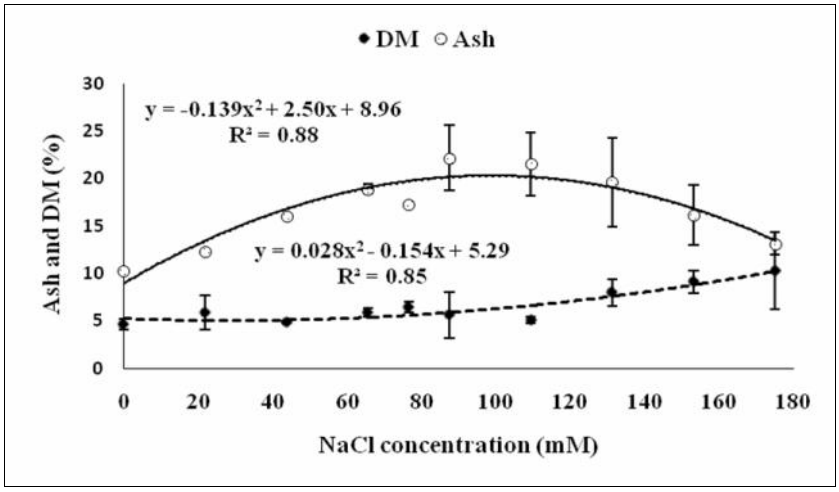

Fig. 2 Ash and dry matter (DM) content of *Asparagus breslerianus* callus subjected to NaCl treated media. Bars represent SD values.

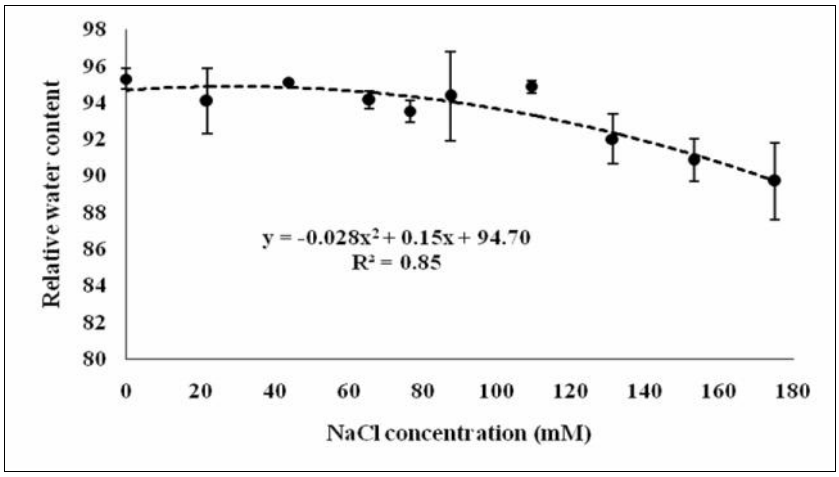

Fig. 3 Relative water content (RWC) of *Asparagus breslerianus* callus subjected to NaCl treated media. Bars represent SD values.

Callus growth and plant regeneration percentage were in maximum level in media with 0 to 109.4 mM NaCl. In opposite media from 131.28 to 175.04 mM NaCl had the lowest callus growth without any plant regeneration percentage (Fig. 4). A very high shoot multiplication rate was attained on media exposed with 0 to 109.4 mM. Shoots and leaves were regenerated into young plantlets (Fig. 1, c). Based on correlation coefficient results, significant correlation observed between callus growth and plantlet regeneration with DM, RWC, callus volume, number of shoot initiated, K^+ and RGR (Table 3). Callus volume in medium without NaCl (0 mM) was high. Callus volume in the media that subjected to NaCl from 21.88 up 175.04 mM, were significantly decreased (Fig. 5). Negative correlation observed between callus volume with DM and Na^+. Also, significant correlation observed between callus volume with RWC, callus growth, plantlet regeneration, number of shoot initiated, K^+ and RGR (Table 3). It was reported that *in vitro* shoot production and growth of *A. officinalis* were inhibited at 2% NaCl [7]. Mahon-Demias, a genotype of bread wheat (*Triticum aestivum*), reacts moderately decrease in the capacity of callus proliferation which reached 25% at 15 $g \cdot L^{-1}$ NaCl treatment [30]. Plantlet regeneration of BRRI dhan genotype of Rice was 80, 20 and 0 % at 0, 100 and 150 mM NaCl, respectively [31].

Number of Shoot Initiated

Number of shoot initiated was affected significantly (P<0.01) with the increasing level of salt in the cultural medium (Table 2). Maximum

shoot initiated was recorded in medium that supplemented with 87.52 mM NaCl (Fig. 1, d). There was not any shoot formation in NaCl from 131.28 to 175.04 mM (Fig. 6). Significant correlation observed between number of shoot initiated with DM, RWC, callus growth, plantlet regeneration, callus volume, K^+ and RGR. Correlation between plantlet regeneration (regeneration capacity of callus) and number of shoot initiated clearly confirms that these characteristics are dependent together (Table 3). The results stated that low content of neutral salt stress was useful for growth of *A. breslerianus* which also was reported previously in asparagus [1]. The highest number shoots in *A. officinalis* proliferated at 2000 ppm salt mixture (3 NaCl:1 (3 MgCl2:1 CaCl2)) in comparison to control cultures [32]. Also, a positive association between photosynthesis and yield under saline conditions has been found in *A. officinalis* [18].

K^+ and Na^+ Content

Considerable variations (P<0.01) were observed in K^+ and Na^+ among salinity condition culture (Table 2). As seen in Fig. 7, the Na^+ content was increased and the K^+ content was decreased by NaCL application. The lowest K^+ content was obtained at 175.04 mM NaCl. The maximum accumulation of Na^+ was observed at 175.04 mM NaCl. Finding results agree with some species were able to substitute K^+ by Na^+ to guarantee the osmotic adjustment which also was reported previously in *Medicago sativa* L., [21] and *Saccharum* sp. [29]. Salinity stress increased the rice callus sodium content (Na^+) while potassium (K^+) content decreased [26]. Both Na^+ and Cl^- increased with salinity treatment in tissues of friable and compact callus cells of asparagus [7]. Potassium has an important role in the plants biochemical and physiological mechanisms [33]. Below common conditions, plant cells require 100 - 200 mM K^+ and less than 1 mM Na^+ to maintain the osmotic balance [34]. Under salt stress, halophytes subjected to high salinity conditions accumulate high content of Na^+ undergo osmotic adjustment [33]. Nutritional imbalance such as Na^+ and Cl^- may cause a reduction in callus growth under salt condition [29].

Chlorophyll Content

Significant differences (P<0.01) were recorded for chlorophyll a, b and ab content in response to NaCl treatments (Table 2). Previous researchers reported that 100 mM NaCl did not affect on chlorophyll content of *in vitro* propagated shoots of *Eucalyptus camaldulensis* [39]. According to Fig.8, Chlorophyll a, b and ab content were obtained a decreasing trend in first levels of NaCl (21.88 and 43.76 mM) and then increased up to 109.4 mM. The chlorophyll was declined with NaCl rising from 131.28 to 175.04 mM. On the other hands, the results demonstrated that chlorophyll was declined slowly by lowest (21.88 and 43.76 mM) and exactly by highest (131.28 to 175.04 mM) levels of NaCl. Significant correlation observed between chlorophyll content with DM, RWC, callus growth, plantlet regeneration, callus volume, number of shoot initiated, K^+ and RGR (Table 3). Correlation between chlorophyll content and K^+ clearly confirms that chlorophyll may have vital character in osmotic adjustment in *A. breslerianus* under salt stress. Close correlation between salt tolerance and chloroplasts has been found. So that chlorophyll content increased in the resistant cultivar [35]. At maximum level of NaCl, breakdown of chlorophyll occurs mainly due to Cl accumulation in tissue [36-37]. Chlorophyll content could be applied to as a sensitive index of cellular that exposed to salt condition; therefore, chlorophyll decrease means toxicity in tissues due to accumulation of Na^+ and Cl^- [38], so, *A. breslerianus* is tolerant to salinity up to 109.4 mM NaCl (Fig. 8).

Relative Growth Rate (RGR)

Based on results (Table 2) and Fig. 9, RGR significantly (P<0.01) decreased with NaCl rising to 109.4 mM. Calli RGR at different media NaCl were reduced over 109.4 mM. A significantly negative correlation was observed between callus RGR and DM%, and positive correlation was obtained between callus RGR and RWC, callus growth, callus volume, plantlet regeneration, number of shoot initiated and K^+ (Table 3). The NaCl influenced the RGR value that is mostly might be due to the selective accumulation of Na^+ ion. With agreement to these results, callus relative growth rate (RGR; fresh) of rice indicated a progressive decrease in salt condition [26]. A significant decrease reported in RGR of *Sesuvium portulacastrum* L. calli in salt levels [33]. Any increase in salinity levels in tomato media was led to decrease of calli RGR [27].

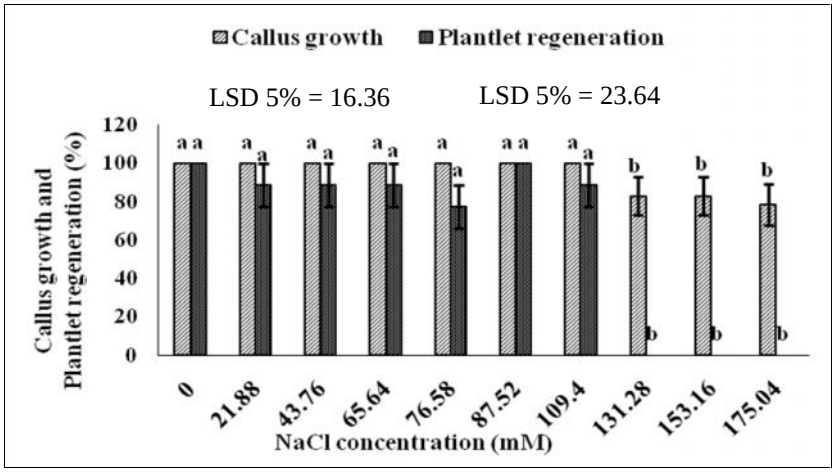

Fig. 4 Callus growth and plant regeneration percentage of *Asparagus breslerianus* subjected to NaCl treated media.

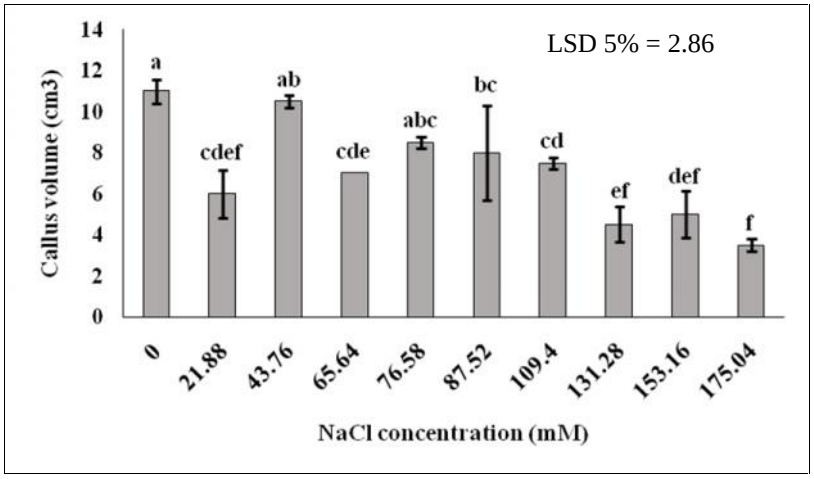

Fig. 5 Callus volume of *Asparagus breslerianus* subjected to NaCl treated media.

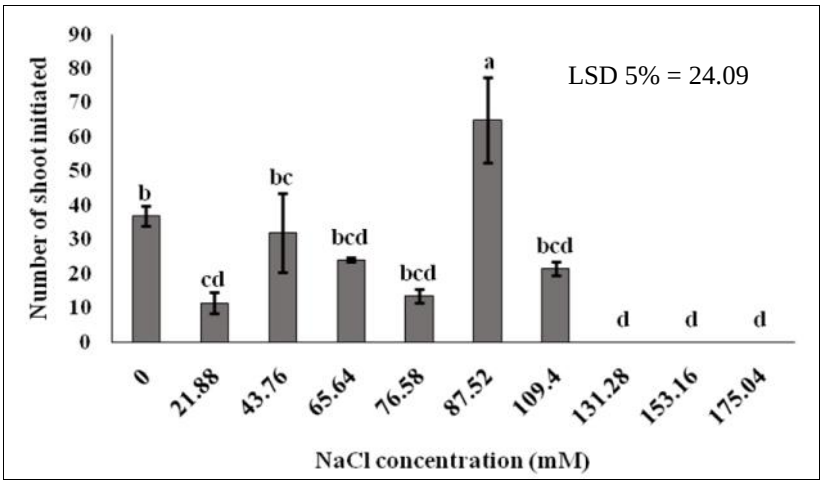

Fig. 6 Number of shoot initiated of *Asparagus breslerianus* callus subjected to NaCl treated media.

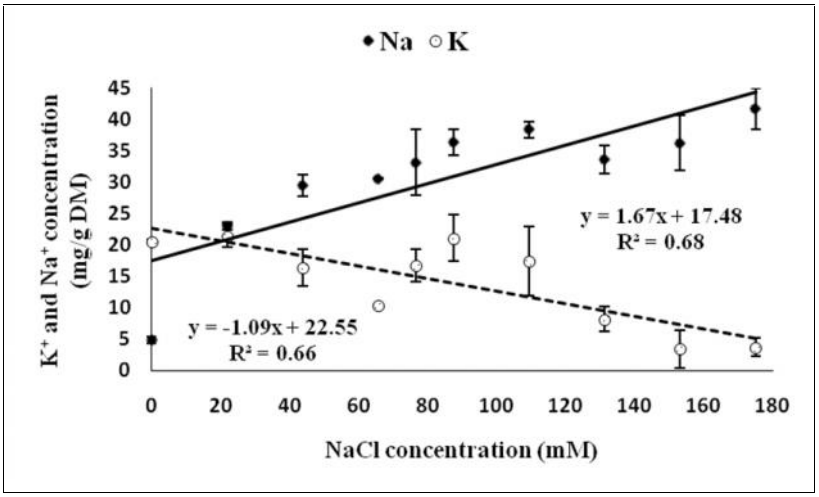

Fig. 7 K^+ and Na^+ content of *Asparagus breslerianus* callus subjected to NaCl treated media. Bars represent SD values.

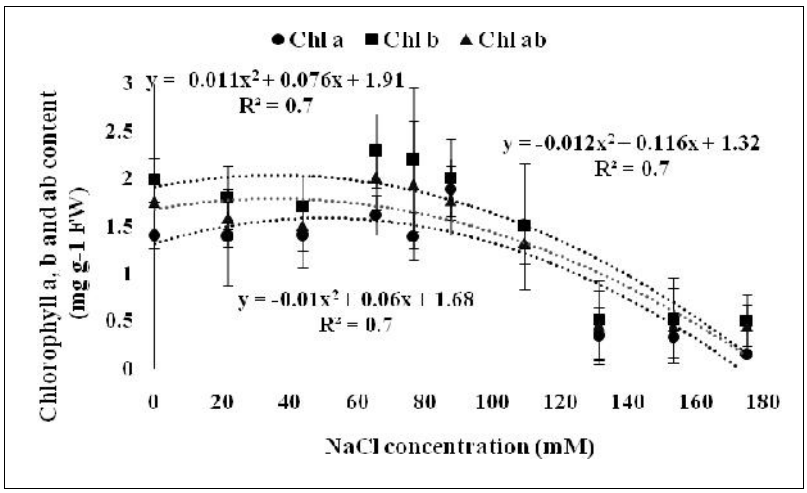

Fig. 8 Chlorophyll a, b and ab content of *Asparagus breslerianus* callus subjected to NaCl treated media. Bars represent SD values.

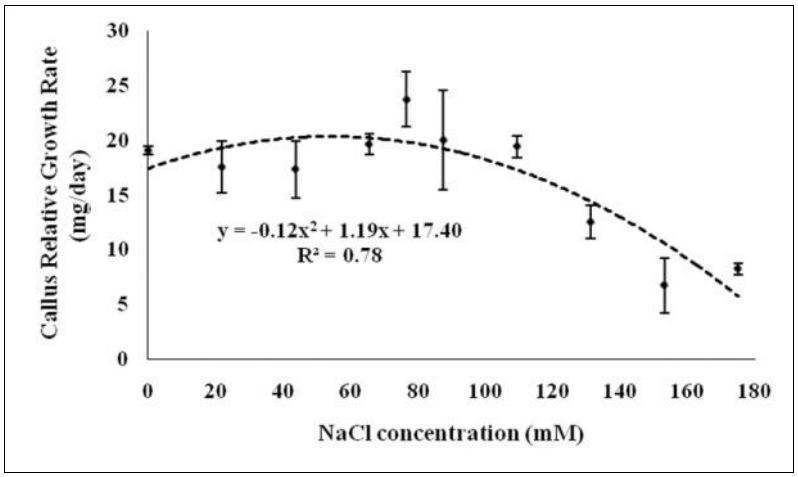

Fig. 9 Relative growth rate (RGR) of *Asparagus breslerianus* callus subjected to NaCl treated media. Bars represent SD values.

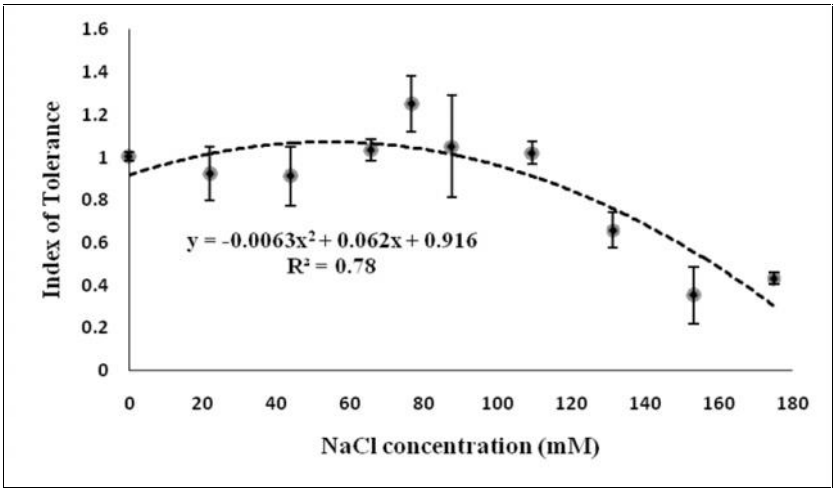

Fig. 10 Index of tolerance (IT) of *Asparagus breslerianus* callus subjected to NaCl treated media. Bars represent SD values.

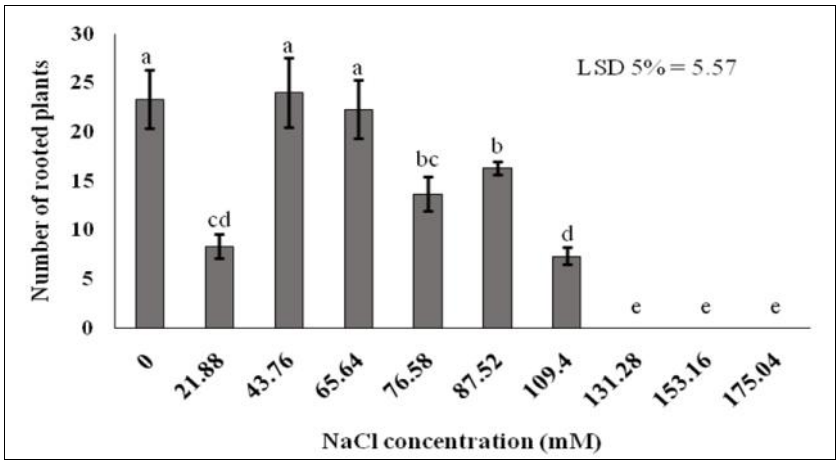

Fig. 11 Number of rooted plants of *Asparagus breslerianus in vitro* shoot initiated subjected to NaCl treated media.

Table 3 Pearson correlation coefficients among traits of *Asparagus breslerianus* callus culture in salinity condition.

Traits	DM	Ash	RWC	Callus induction	Callus volume	Plant regeneration	Number of shoot initiated	Na^+	K^+	RGR	Chl ab
DM	1	-	-	-	-	-	-	-	-	-	-
Ash	-0.09^{ns}	1	-	-	-	-	-	-	-	-	-
RWC	-1^{**}	-0.09^{ns}	1	-	-	-	-	-	-	-	-
Callus induction	-0.38^{*}	-0.01^{ns}	0.38^{*}	1	-	-	-	-	-	-	-
Callus Volume	-0.70^{**}	0.02^{ns}	0.70^{**}	0.64^{**}	1	-	-	-	-	-	-
plant regeneration	-0.72^{**}	0.07^{ns}	0.72^{**}	0.69^{**}	0.68^{**}	1	-	-	-	-	-
number of shoot initiated	-0.60^{**}	0.29^{ns}	0.60^{**}	0.43^{*}	0.71^{**}	0.65^{**}	1	-	-	-	-
Na^+	0.31^{ns}	0.39^{*}	-0.31^{ns}	-0.29^{ns}	-0.45^{*}	-0.34^{ns}	-0.14^{ns}	1	-	-	-
K^+	-0.57^{**}	0.03^{ns}	0.57^{**}	0.68^{**}	0.55^{**}	0.82^{**}	0.46^{**}	-0.42^{*}	1	-	-
RGR	-0.69^{**}	0.31^{ns}	0.69^{**}	0.64^{**}	0.68^{**}	0.80^{**}	0.56^{**}	-0.28^{ns}	0.7^{**}	1	-
Chl ab	-0.53^{**}	-0.02^{ns}	0.53^{**}	0.74^{**}	0.56^{**}	0.82^{**}	0.48^{**}	-0.34^{ns}	0.64^{**}	0.76^{**}	1

** (P<0.01), * (P<0.05), ^{ns} (P>0.05).

Index of Tolerance (IT)

The index of tolerance (IT) revealed significant main effect (P<0.01) for NaCl (Table 2). As seen in Fig. 10, IT decreased with NaCl rising to 109.4 mM. Aparagus callus IT at different media NaCl was reduced over 109.4 mM. Based on results, RGR and IT correlated with K^+ content (Table 3). This clears that salt tolerance in *A. breslerianus* enhanced by K^+ uptake efficiency. IT index is used in order to measure the sensitivity of plants to salt. The less or negative the index indicates the susceptibility of plant to salinity. Index of tolerance (IT) is suitable for comparing responses to stress [21].

Rooted Plants and Acclimation

Root induction was undertaken using 3.9 µM Ancymidol and 1.07 µM NAA. Using of Ancymidol was proposed for rooting of *Asparagus maritimus* [9]. Also, Ancymidol (2 mg 1^{-1}), KIN 0.1 (mg 1^{-1}) and NAA (0.3 mg 1^{-1}) were used for induction of root in explants of asparagus wild species [16]. Initiated shoots from calli were transferred to MS medium containing the same NaCl concentrations suppliment with 3.9 µM Ancymidol and 1.07 µM NAA for rooting (Fig. 1, f). Rooted plants were formed until 109.4 mM NaCl after eight weeks growth periods on media. The acclimatization asparagus rooted plants was better when the micropropagated plantlets were acclimatized after eight weeks incubation [32]. The highest number of rooted plants was recorded at control, 43.76 and 65.64 mM NaCl (Fig. 11). In the media with 131.28, 153.16 and 175.04 mM NaCl were not observed any rooted plants. During the acclimation phase, a high survival rate was achieved after six weeks in the growth chamber. It is deserving to quote that the transfer of rooted plantlets from aseptic growth chamber to an external environment had always to be done carefully. At the end a high multiplication rate can be achieved by this technique.

Conclusion

NaCl induced elevation in callus dry matter (DM), which clarify a cellular tolerance to salinity in *A. breslerianus*. Relative water content (RWC) and relative growth rate (RGR) of callus tissue were found to be inversely correlated with DM and directly correlated with K^+ uptake in salt condition. The highest RGR and RWC decreasing were noticed less than 109.4 mM NaCl. These consequences suggested that RGR restriction may be due to the water availability being made fewer and loss of turgor in cells. Loss of water, but increased dry matter showed high metabolic activity. It is notable that *A. breslerianus* callus at high concentrations of salt is also able to survive via the increase in DM (up to 10.26%), chlorophyll content (up to 2.01 mgg^{-1} FW for chlorophyll ab), preservation of the RWC (up to 89.73%) and K^+ accumulation (41.64 mgg^{-1} DM). Our results recommended that under *in vitro* salt stress condition, the accumulation of Na^+ and keeping of a satisfactory water level can have vital characters in osmotic adjustment in *A. breslerianus* as a halophytic species.

Acknowledgements

This work has been supported by the University of Tehran.

Refrences

1. Shannon MC, Grieve CM. Tolerance of vegetable crops to salinity. Sci Hortic. 1999;78:5-38.
2. Akhani H, Ghorbanli M. A contribution to the halophyte vegetation and flora of Iran. Towards the rational use of high salinity tolerant plants. 1993;1:35-44.
3. Francois LE. Salinity effects on asparagus yield and vegetative growth. J Am Soc Hortic Sci. 1987;112:432-436.
4. Kruistum GV, Poll JT, Meijer J, Lievens M. Effect of NaCl on Asparagus quality, production and mineral leaching. Acta Hort. 2008;776:87-90.
5. Bajji M, Lutts S, Kinet JM. Physiological changes after exposure to and recovery from polyethylene glycol-induced water deficit in callus cultures issued from durum wheat (*Triticum durum* desf.) cultivars differing in drought resistance. J Plant Physiol. 2000;156:75–83.
6. Venkataiah P, Christopher T, Subhash K. Selection and characterization of sodium chloride and mannitol tolerant callus lines of red pepper (*Capsicum annuum L.*). Plant Physiol. 2004;9:158-163.
7. Mills D. Differential response of various tissues of *Asparagus officinalis* to sodium chloride. J Exp Bot. 1989;40:485-491.
8. Bekheet SA, Taha HS, Sawires ES, El-Bahr MK. Salt stress in tissue cultures of *Asparagus officinalis*. Egyp J Hort. 2000;27:275-187. (Abstract).
9. Stajner N, Bohanec B, Jakse M. *In vitro* propagation of *Asparagus maritimus* - A rare Mediterranean salt-resistant species. Plant Cell Tiss Organ Cult. 2002;70:269-274.
10. Rechineer Kh. Flora Iranica. Liliaceae. 1982;15:1-31.

11. Yuritsyna NA. Vegetation of saline habitats on southeastern border of europe. Arid Eco. 2012;2:239-244.
12. Mousavizadeh SJ, Hassandokht MR, Kashi A. Multivariate analysis of edible Asparagus species in Iran by morphological characters. Euphytica. 2015;206:445-457.
13. Mousavizadeh SJ, Hassandokht MR, Kashi A, Gil J, Cabrera A, and Moreno R. Physical mapping of 5S and 45S rDNA genes and ploidy levels of Iranian *Asparagus* species. Sci Hortic. 2016;211:269-276.
14. Castro P, Gil J, Cabrera A, Moreno R. Assessment of genetic diversity and phylogenetic relationships in Asparagus species related to *Asparagus officinalis*. Genet Resour Crop Ev. 2013;60:1275-1288.
15. Moreno R, Espejo JA, Cabrera A, Millan T, Gil J. Ploidic and molecular analysis of 'Morado de Huetor' asparagus (*Asparagus officinalis* L.) population; a Spanish tetraploid landrace, Gen. Res. Crop Evol. 2006;53:729-736.
16. Regalado JJ, Carmona-Martin E, Castro P, Moreno R, Gil J, Encina CL. Micropropagation of wild species of the genus *Asparagus* L. and their interspecific hybrids with cultivated *A. officinalis* L., and verification of genetic stability using EST-SSRs. Plant Cell Tiss Organ Cult. 2015;121:501-510.
17. Sheidai M, Inamdar AC. Polyploidy in the genus *Asparagus* L, Nucleus. Calcutta. 1992;35:93-97.
18. Sun YL, Hong SK. Effects of plant growth regulators and L-glutamic acid on shoot organogenesis in the halophyte *Leymus chinensis* (Trin.). Plant Cell Tiss Organ Cult. 2010;100:317-328.
19. Ghahreman A. Flora of Iran in natural color. Research Institute of Forests and Rangelands, Tehran. 1997; Number 2389. Cod 148.
20. Arnon DI. Photo synthesis by isolated chloroplast. Biochem Biphys. 1956;20:440-461.
21. Shah SH, Wainwright SJ, Merrett MJ. The interaction of sodium and calcium chlorides and light on growth, potassium nutrition, and proline accumulation in callus cultures of *Medicago sativa* L. New Phytol. 1990;116:37-45.
22. Hamada AM, EL-enany AE. Effect of NaCl salinity on growth, pigment and mineral element contents, and gas exchange of broad bean and pea plants. Biol Plant. 1994;36:75-81.
23. Ikeuchi M, Sugimoto K, Iwase A. Plant Callus: Mechanisms of Induction and Repression. The Plant Cell. 2013;25:3159-3173.
24. Naidoo G, Somaru R, Achar P. Morphological and physiological responses of the halophyte, *Odyssea paucinervis* (Staph) (Poaceae), to salinity. Flora. 2008;203:437-447.
25. Sharma V, Ramawat KG. Salinity-induced modulation of growth and antioxidant activity in the callus cultures of miswak (*Salvadora persica*). 3 Biotech. 2013;3:11-17.
26. Ahmad MSA, Javed F, Javed S, Alvi AKh. Relationship between callus growth and mineral nutrients uptake in salt-stressed indica rice callus. J Plant Nutri. 2009;32:382-394.
27. Aazami MA, Torabi M, Shekari F. Response of some tomato cultivars to sodium chloride stress under *in vitro* culture condition. Afri J Agri Res. 2010;5:2589-2592.
28. Yuxia Z, Wei-wei T, Yan-shu W, Jian-hui W. Effect of alkali-salt stress on anti-oxidative enzymes of *Asparagus officinalis*. Journal of Inner Mongolia University for Nationalities (Natural Sciences). 2006;21:165-168.
29. Errabii T, Gandonou ChB, Essalmani H, Abrini J, Idaomar M, Senhaji NS. Effects of NaCl and mannitol induced stress on sugarcane (*Saccharum* sp.) callus cultures. Acta Physiol Plant. 2007;29:95-102.
30. Benderradji L, Brini F, Kellou K, Ykhlef N, Djekoun A, Masmoudi Kh, Bouzerzour H. Callus Induction, Proliferation, and Plantlets Regeneration of Two Bread Wheat (*Triticum aestivum* L.) Genotypes under Saline and Heat Stress Conditions. Agronomy. 2012;367851:1-8.
31. Zinnah KMA, Zobayer N, Sikdar SU, Liza LN, Chowdhury MN, Ashrafuzzaman M. *In vitro* Regeneration and Screening for Salt Tolerance in Rice (*Oryza sativa* L.). Int Res J Biol Sci. 2013;2:29-36.
32. Carmona-Martin E, Regalado JJ, Raghavan R, Encina CL. *In vitro* induction of autooctoploid asparagus genotypes. Plant Cell Tiss Organ Cult. 2014;121:249-254.
33. Lokhande VH, Nikam TD, Penna S. Biochemical, physiological and growth changes in response to salinity in callus cultures of *Sesuvium portulacastrum* L. Plant Cell Tiss Organ Cult. 2010;102:17-25.
34. Tester M, Davenport R. Na^+ tolerance and Na^+ transport in higher plants. Ann Bot. 2003;91:503-527.
35. Madava-Rao KV, Raghavendra AS, Janardhan-Reddy K. Physiology and Molecular Biology of Stress Tolerance in Plants. Springer. 2006;345.
36. Melgar JC, Syvertsen JP, Martinez V, Garcia-sanchez F. Leaf gas exchange, water relations, nutrient content and growth in citrus and olive seedlings under salinity. Biol Plant. 2008;52:385-390.
37. Zekri M. Effect of NaCl on growth and physiology of sour orange and Cleopatra mandarin seedlings. Sci Hortic. 1991;47:305-315.
38. Flowers TJ, Troke PF, Yeo AR. The mechanisms of salt tolerance in halophytes. Annu Rev Plant Physiol. 1977;28:89-121.
39. Woodward AJ, Bennett IJ. The effect of salt stress and abscisic acid on proline production, chlorophyll content and growth of *in vitro* propagated shoots of *Eucalyptus camaldulensis*. Plant Cell Tiss Organ Cult. 2005;82:189-200.