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In vitro Callus Induction under NaCl Salt Stress and Subsequent Plant Regeneration in Oats (*Avena sativa* L.)

A. A. Mamun*, M. M. Islam, N. E. K. Alam and S. Noushin

Agrotechnology Discipline, Khulna University, Khulna-9208, Bangladesh

Key words: Oats, Salt stress, Callus induction, Plant regeneration, Survivability

Abstract

An effort was made to identify the *in vitro* responsiveness for callus induction in NaCl (salt stress) and subsequent plant regeneration of oats (*Avena sativa* L.). Callus induction was tested in different concentrations (0, 5, 10, 15 and 20 mg/l) of NaCl salt added in MS. The experiment was laid out in CRD considering 2,4-D growth regulator and NaCl salt as factors. The best response in callus induction was observed on MS containing 3 mg/l 2,4-D and 5 mg/l NaCl where the frequency of callus induction was 68.57%, callus weight was 0.36 g and callus diameter was 7.63 mm. During regeneration, the maximum frequency of regeneration (41.67%), regenerated shoots per callus (14.80), shoot length (12.14 cm), root number (10.90) and root length (3.76 cm) was observed from the callus derived from MS containing 3 mg/l 2,4-D and 5 mg/l NaCl. Plantlets that were regenerated from callus induced in MS with 3 mg/l 2,4-D and 5 mg/l NaCl showed higher rate of *ex vitro* survivability. Therefore, MS with 3 mg/l 2,4-D and 5 mg/l NaCl found better for *in vitro* callus induction and subsequent plant regeneration from mature seeds of oat.

Introduction

Both biotic and abiotic stresses often impose a major threat to agriculture. Salinity is one of the most decisive environmental factors limiting the productivity of crop plants. This abiotic stress causes serious yield losses in all important glycophytic crops (Ashraf and Harris 2004, Hussain et al. 2009, Semiz and Suarez 2015, Nawaz et al. 2016) and reduces average yields by 50 - 80% (Panta et al. 2014). Salt accumulation in soil solution reduces water and nutrient uptake. This leads to osmotic stress, ion toxicity, nutrient imbalances and water-deficit. Excessive concentrations of salt ions also injure photosynthetically active leaves, and may lead to chlorosis and early leaf senescence (Hanin et al. 2016).

*Author for correspondence: <mamungpbat@ku.ac.bd>.

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Salinity can cause crop yield losses even though the effects of salinity may not be obvious. Both salt tolerance and sensitivity of a specific crop depend on its ability to extract water and nutrients from saline soils and to avoid excessive tissue accumulation of salt ions (Ahmad et al. 2017). Plants adapt to stresses using their astonishing plasticity to remodel themselves (Badea and Basu 2010). Worldwide, there are 952 million ha of salt affected land and out of 2.86 million ha of coastal and off-shore lands in Bangladesh about 1.056 million ha of arable lands are affected by varying degrees of salinity (IWM 2014). The growing demands of the expanding population for various biomass products have necessitated an exploitation of these soils. Salinity tolerance would therefore be a highly desirable characteristic to be induced in economically important multipurpose plant species like oats (*Avena sativa* L).

Oats is an important and traditional agricultural cereal crop grown in winter both under irrigated and rain fed conditions. It ranked around sixth in the world cereal production statistics following wheat, maize, rice, barley and sorghum. Oat plantations have a comparatively low input demand of insecticides, fungicides and fertilizers due to disease tolerance and low nourishment requirements. However, it requires sufficient water for growth and grain production. It is mainly used as animal feed, but it is one of the most promising future cereals in the functional food area. Food products include oatmeal, oat flour, natural cereals, meat product extenders, cookies and breads, granolas and baby food. Oat-based foods have unique value in human nutrition as it contains 16 per cent or more protein. Looking into the fast growing demography and quick popularization of dairy industry in our country, cultivation of oats as food and fodder crop is catching up in a big way and has an enormous scope in Bangladesh agriculture.

Development of stress-tolerant plants is of immense importance to increase crop productivity. Plant response to abiotic stress is a complex phenomenon, which could be approached efficiently through *in vitro* culture. It can help in the efforts to produce new cultivars against environmental stress factors. In addition, *in vitro* culture studies permit relatively faster responses, shorter generation time, and regular environmental conditions as compared to classical breeding methods (Elmaghrabi et al. 2013). *In vitro* culture of plant cells and tissue has attracted considerable interest over recent years because it provides the means to study plant physiological and genetic processes in addition to offering the potential to assist in the breeding of improved cultivars by increasing genetic variability (Karp et al. 1987). Plant tissue culture techniques provide a promising and feasible approach to develop salt tolerant plants. *In vitro* selection of salt tolerant cell lines has been reported for several species (Tal 1994). *In vitro* selection relies on the induction of genetic variation among cells and/or tissues in cultured and regenerated plants (Rai et al. 2011). This is based on the *in vitro* culture of plant cells, tissues or organs on a medium supplemented with selective salts and only the regenerated plantlets capable of sustaining such environments are selected to obtain desirable characteristics.

There are a number of environmental issues and problems that are hindering the development of Bangladesh and among them soil salanization is a significant issue, particularly within the coastal regions. Salinity in the country received very little attention in the past. Increased pressure of growing population demand more food. Thus it has become increasingly important to explore the possibilities of increasing the potential of these (saline) lands for increased production of crops. As a solution for soil salinity, efforts to this point are primarily geared toward reducing salt concentrations within the soil. But the procedures used for that area are costly and harm the soil. Introduction and/or development of crops that grow well on salt-affected land can be a better alternative to exploit the saline soils. Through this way, degraded soil may become productive once again and that offers new opportunities for farmers. Hence, the study was undertaken to analyze the effect of NaCl on oats callus induction, and on the subsequent regeneration and survival of regenerated plantlets.

Materials and Methods

Salt responses were tested in callus induction and subsequent shoot regeneration of oat (*Avena sativa* L.at Plant Breeding and Biotechnology Laboratory of Agrotechnology Discipline, Khulna University. Seeds of an Indian oats cultivar (Kent) were collected from Central Cattle Breeding and Dairy Farm (CCBDF), Dhaka. Germination test was carried out on Whatmann No. 1 filter paper soaked in distilled water in 9.0 cm Petri dishes covered with lids to check seed viability and above 90% germination was recorded after 5 days of seed inoculation.

The experiment was laid out (CRD) with 21 treatment combinations and five replications. Different concentrations of 2,4-D (0, 1, 2, 3 and 4 mg/l) were considered as a factor and different concentrations of NaCl (0, 5, 10, 15 and 20 mg/l) were considered as another factor. In this study, de-hulled mature oat seeds were washed thoroughly with tap water in order to remove dust and other particles followed by washing in distilled water added with 2 - 3 drops of Tween-20 for 30 minutes. The seeds were rinsed with distilled water for 3 times. Further sterilization was carried out inside the laminar air flow hood. Seeds were treated with 70% (w/v) ethyl alcohol for 30 seconds followed by 0.5% (w/v) $HgCl_2$ solution for 5 minutes. Sterilized seeds were washed thoroughly with autoclaved distilled water for 3 times to remove the poisonous trace of $HgCl_2$. The sterilized seeds were allowed to remove surface water by keeping on sterilized tissue paper under laminar hood for a few minutes.

The sterilized seeds were aseptically cultured in 18 × 150 mm test tubes containing 30 ml MS basal salts and vitamins supplemented with 30 g/l sucrose, 8 g/l agar, and with different concentrations of 2,4-D and NaCl for callus induction. The pH of the medium was adjusted to 5.6 before autoclaving at 121°C for 20 min at 15 psi pressure. The tubes were incubated in a dark growth chamber maintained at 25 ± 1°C and 70% relative humidity. The induced calli were subjected to four subcultures at 4 weeks interval.

The produced calli were placed in test tubes containing 10 ml freshly prepared half strength MS (half MS) solidified with 8 g/l agar and supplemented with 30 g/l sucrose, 2.0 mg/l Kn and 0.5 mg/l IBA for caulo-rhizogenesis. The cultures were incubated under a photoperiod of 16 hrs light/8 hrs darkness under fluorescent light of 3000 lux illumination intensity at temperature of 25 ± 1°C and 65% relative humidity. The cultures were subjected for two subcultures at 3 weeks interval. At the end of this period, callus with clearly differentiated shoots and roots was scored as regenerating callus.

The well regenerated plants from *in vitro* cultures were transferred to small pot containing garden soil, sand and vermicompost (1 : 1 : 1). The transferring was done after removal of agar attached with the roots and treated with 0.1% bavistin (w/v) fungicide. The plants were watered with rain water and covered with polythene after spraying water inside it to check evapotranspiration. Pots with regenerated plantlets were kept in hardening room for 7 days after removal of polythene and then kept in net house for next 7 days to sustain the growth of plantlets.

The data were obtained on callus induction efficiency measured as (the number of seeds produced calli/total number of seeds tested) × 100; the regeneration efficiency measures as (the number of calli regenerated to plantlets/total number of calli plated) × 100. Colour and structure of the produced calli were scored by visual observation. The number of shoots and roots per plantlet were scored by counting and the callus weights were measured by using a 4-digit electronic balance. The diameter of the calli, and the length of shoot and root were determined by using a digital slide calipers. Data on survivability (%) were recorded as (the number of plants survived after 2 weeks/total number of plants transferred to pots) × 100.

The mean values of the collected data were computed from five replicates with standered error (SE). The data were analyzed by using ANOVA to compare the results with the help of the computer package 'Statistical Tools for Agricultural Research (STAR)' and the mean differences were adjudged with DMRT at 5% levels (Gomez and Gomez 1984).

Results and Discussion

Different physical and chemical biotic stress factors could affect the callus induction frequency. Improvement of cereal and other important crops by applying different abiotic stresses on callus induction were attempted by earlier plant scientists (Bregitzer et al. 1998, El-Meleigy et al. 2004, Redha and Islam 2010). These factors could change cell cycle and different biological systems. In this study, two major factors such as 2,4-D and NaCl salt stress were used for callus induction in oats. It was observed that variations in the concentrations of both 2,4-D and NaCl had remarkable response in callus induction frequency. A distinct impact of salt was evident on callus type and its colour (Table 1). The calli which were formed on salt-free medium became fragile and callus from salt stress condition were compact. This may because of the presence of necrotic cells in the

calli. A huge number of necrotic cells turned the calli brown and deep brown in colour. Through visual observation, it was observed that the higher frequency of necrosis was

Table 1. Effect of varying concentrations of 2,4-D and NaCl on callus induction efficiency and morphological characteristics of produced calli of oat seeds.

Treatments (mg/l)		Callus induction frequency (%)	Callus color	Callus type
2,4-D	NaCl			
0	0	0.00	No callus	-
1	0	20.00	White	Fragile
1	5	31.43	"	Compact
1	10	20.00	Deep brown	"
1	15	17.14	"	"
1	20	0.00	No callus	-
2	0	57.14	White	Fragile
2	5	60.00	"	Compact
2	10	40.00	Brown	"
2	15	31.43	"	"
2	20	25.71	Deep brown	"
3	0	65.71	White	Fragile
3	5	68.57	"	Compact
3	10	48.57	"	"
3	15	25.71	Brown	Compact
3	20	17.14	Deep brown	"
4	0	62.86	White	Fragile
4	5	65.71	Brown	Compact
4	10	45.71	"	"
4	15	17.14	Deep brown	:
4	20	14.29	"	:

appeared with increased salt concentration. This could be due to lower osmotic potentiality of the cells. Maximum callus induction (68.57%) was recorded from the media supplemented with 3 mg/l 2,4-D and 5 mg/l NaCl and that of minimum (14.29%) was noticed from media supplemented with 4 mg/l 2,4-D and 20 mg/l NaCl. No callus was formed in the media with 1 mg/l 2,4-D and 20 mg/l NaCl and in control. In terms of 2,4-D, the callus induction was increased with the increasing concentrations up to 3 mg/l but for 4 mg/l the callus induction was decreased. The results of present study were in concomitant with the findings of Salunke et al. (2017) where the callus induction of oat was increased with increased concentration of 2,4-D. The callus induction was also increased with the decreasing concentration of NaCl. But the callus induction was better in slight salt stress than stress free condition. Benderradji et al. (2011) reported a similar effect of slight salt stress and stress less condition on wheat.

Callus weight and diameter were differed significantly with the concentration of 2,4-D. Both weight and diameter of the produced calli have increased with the increasing concentration of 2,4-D up to 3 mg/l. But for 4 mg/l 2,4-D these decreased a little (Table 2). The best callus weight (0.25 g) and callus diameter (6.29 mm) were derived from the medium supplemented with 3 mg/l of 2,4-D and minimum callus weight (0.14 g) and callus diameter (3.02 mm) were recorded from the medium supplemented with 1 mg/l 2,4-D. MS without 2,4-D produced no callus but germinated the inoculated seeds directly. Benlioglu et al. (2016) had an evidence of the best result for callus weight in MS with 2 mg/l 2,4-D but a decreased callus weight with 4 mg/l.

Table 2. Effect of 2,4-D on callus weight and callus diameter for oat seed culture.

2,4-D (mg/l)	Callus weight (g)	Callus diameter (mm)
0	0.00^{d}	0.00^{c}
1	0.14^{c}	3.02^{b}
2	0.18^{bc}	4.54^{ab}
3	0.25^{a}	6.29^{a}
4	0.21^{ab}	5.53^{a}
Level of significance	**	**
CV (%)	4.63	6.56

**Indicating the mean difference is significant at the 0.01 level. The values followed by different letters in a column are significantly different at $p < 0.05$ according to DMRT.

There was a significant effect of NaCl salt on callus weight and callus diameter. An adverse effect of NaCl was observed on callus weight and callus diameter (Table 3). The best result for callus weight (0.25 g) and callus diameter (6.75 mm) were found for the media supplemented with 5 mg/l NaCl. With the increasing concentration of NaCl from 5 mg/l in the medium callus weight has decreased markedly but no significant sizeable differences were observed at different salt stress levels up to 15 mg/l. The minimum callus weight (0.15 g) and callus diameter (2.56 mm) have recorded in 20 mg/l NaCl. These phenomena might be happened due to the interference of Na^{+} and Cl^{-} ions to uptake nutrition from the medium and also due to the reduction of water availability and loss of turgor pressure in the cells of the calli. As a result, nutritional imbalance might be created and growth of callus was declined.

Mean comparison of the traits measured for weight and diameter of the *in vitro* grown callus under salt stress conditions displayed significant effects of NaCl salt and 2,4-D concentrations interactions (Table 4, Fig. 1D). The maximum callus weight (0.36 g) and callus diameter (7.63 mm) were found when the MS was supplemented with 3 mg/l

2,4-D and 5 mg/l NaCl. The minimum callus weight (0.06 g) was found from the medium supplemented with 4 mg/l 2,4-D and 20 mg/l NaCl, and that of callus diameter (1.83 mm) from the media supplemented with 2 mg/l 2,4-D and 5 mg/l NaCl. A similar response of callus induction and regeneration capacity was also reported by Piwowarczyk et al. (2015). Benlioglu and Özgen (2014) observed the maximum callus weight when 3 mg/l of 2,4-D was used in barley.

Table 3. Effect of NaCl on callus weight and callus diameter for oat seed culture.

NaCl (mg/l)	Callus weight (g)	Callus diameter (mm)
0	0.24^{ab}	6.29^{a}
5	0.25^{a}	6.75^{a}
10	0.19^{bc}	5.2^{a}
15	0.16^{c}	4.83^{a}
20	0.15^{c}	2.56^{b}
Level of significance	**	**
CV (%)	4.63	6.56

**Indicating the mean difference is significant at the 0.01 level. The values followed by different letters in a column are significantly different at $p < 0.05$ according to DMRT.

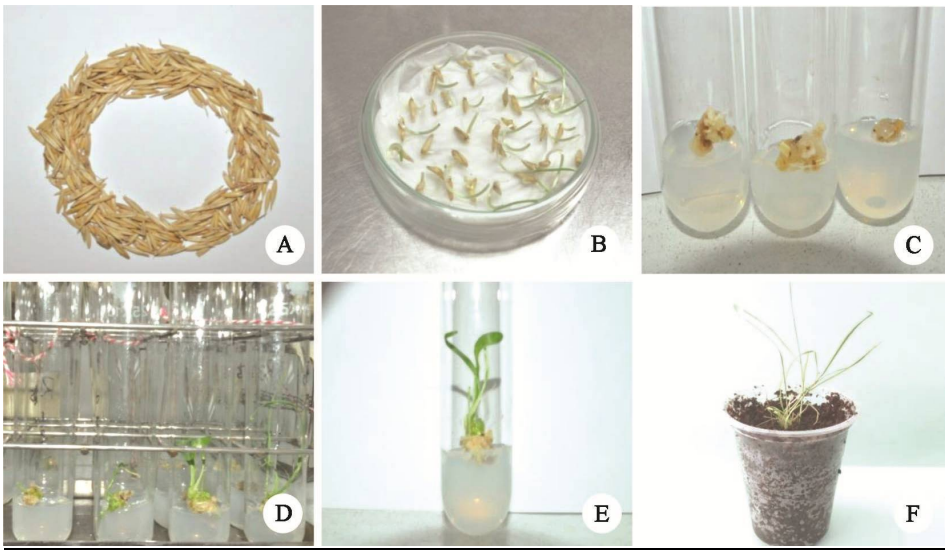

Fig. 1. *In vitro* development of callus and its subsequent regeneration of oats. A- seeds of oats, B- germinated seeds, C- callus formation on MS with 2,4-D and NaCl, D- regenerated plantlets, E- completely regenerated plantlets, F- hardened plants.

Efficient plant regeneration is vital for the establishment of a successful tissue culture system. Inclusion of NaCl during the callus formation and its exclusion during regeneration processes constitutes a convenient way to study the effect of salinity (Saleem et al. 2005). The calli, pre-treated with NaCl salt in callus induction medium, were tested and evaluated for their regeneration potentiality in regeneration medium. In the present study, the regeneration efficiency of *in vitro* produced calli from oat seeds was crucially dependent on the level of NaCl in callus induction medium and was highly

Table 4. Callus weight and callus diameter of seed derived callus exposed to different treatments of 2,4-D and NaCl in oats.

Treatments (mg/l)		Callus weight (g) ± SE	Callus diameter (mm) ± SE
2,4-D	NaCl		
0	0	0.00 ± 0.00^{e}	0.00 ±0.00^{d}
1	0	0.12 ± 0.00^{bcde}	3.95 ± 0.68^{abcd}
1	5	0.23 ± 0.04^{abcd}	2.53 ± 0.59^{bcd}
1	10	0.12 ± 0.00^{bcde}	2.76 ± 0.12^{bcd}
1	15	0.13 ± 0.01^{bcde}	2.82 ± 0.30^{bcd}
1	20	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}
2	0	0.26 ± 0.02^{abcd}	5.70 ± 0.52^{abc}
2	5	0.13 ± 0.02^{bcde}	1.83 ± 0.33^{cd}
2	10	0.19 ± 0.05^{abcde}	4.83 ± 0.74^{abc}
2	15	0.16 ± 0.02^{abcde}	3.99 ± 0.62^{abcd}
2	20	0.10 ± 0.04^{bcde}	2.82 ± 1.17^{bcd}
3	0	0.31 ± 0.08^{ab}	5.14 ± 1.85^{abc}
3	5	0.36 ± 0.14^{a}	7.63 ± 1.42^{a}
3	10	0.16 ± 0.01^{abcde}	6.39 ± 0.46^{abc}
3	15	0.26 ± 0.05^{abcd}	5.61 ± 1.55^{abc}
3	20	0.09 ± 0.04^{cde}	2.63 ± 1.13^{bcd}
4	0	0.31 ± 0.03^{abc}	5.20 ± 0.92^{abc}
4	5	0.25 ± 0.06^{abcd}	2.56 ± 1.12^{bcd}
4	10	0.18 ± 0.03^{abcde}	6.75 ± 0.53^{ab}
4	15	0.20 ± 0.02^{abcde}	4.83 ± 0.19^{abc}
4	20	0.06 ± 0.03^{de}	2.41 ± 1.56^{bcd}
Level of significance		**	**
CV (%)		53.65	50.28

**Indicating the mean difference is significant at the 0.01 level. The values followed by different letters in a column are significantly different at $p < 0.05$ according to DMRT).

inhibited by the adverse effects of high concentration of NaCl (Fig. 2). The calli which were derived from the treatments supplemented with 3 mg/l 2,4-D and no NaCl, and 3 mg/l 2,4-D and 5 mg/l NaCl showed the best response in regeneration (41.67%). The minimum regenerations (8.33%) were observed for the callus obtained from the callus induction medium supplemented with 1 mg/l 2,4-D and 5 mg/l NaCl, 1 mg/l 2,4-D and 10 mg/l NaCl, 2 mg/l 2,4-D and 15 mg/l NaCl, 3 mg/l 2,4-D and 15 mg/l NaCl, and 4 mg/l 2,4-D and 15 mg/l NaCl. No regeneration was observed in the calli that were derived from the media contained more than 15 mg/l NaCl salt stress. This may be due to the preventive effects of salt in the degree of regeneration of the calli. Gless et al. (1998) reported the best response towards regeneration when the calli were derived from the medium containing 2.5 mg/l of 2,4-D. González et al. (2001) mentioned that NaCl inhibited plant regeneration.

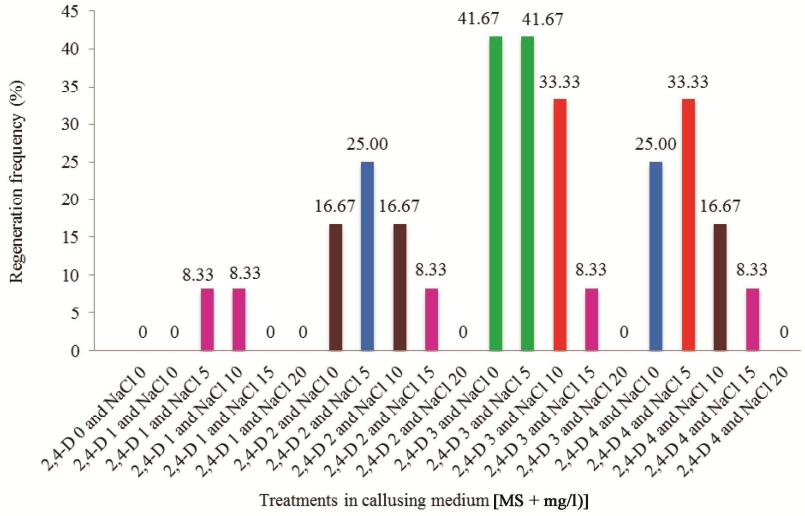

Fig. 2. Regeneration efficiency of callus produced from oat seeds in 2,4-D and NaCl.

A high positive correlation was observed between callus induction percentage and plant regeneration percentage (R^2 = 0.8229, value significant at $p < 0.01$ at degree of freedom = 19, F value = 88.30, Fig. 3). The high correlation indicated that callus induction percentage constitute a good index for callus ability to regenerate later on plantlets. The high value of R^2 clearly indicated that more that 80% variation in regeneration can be explained by the variation in callus induction. The remaining variation may be controlled by other factors.

Various stress pre-treated calli and plants derived from there may show more adaptable characteristics due to some physiological changes of cells. The experimental results showed significant differences in shoot and root characteristics of the regenerated plantlets produced from calli that were induced under different concentrations of NaCl in medium (Table 5). The best results for shoot and root characteristics were observed for

the plantlets regenerated from calli derived from the medium supplemented with 3 mg/l 2,4-D and 5 mg/l NaCl, and the number of regenerated shoot per callus, shoot length, number of roots per plant and root length was 14.80, 12.17, 10.90 and 3.76 cm, respectively. The minimum shoot per callus (0.60), shoot length (0.98 cm), number of roots per plant (0.65) and root length (0.73 cm) was recorded for the regenerated plantlets produced from the calli of medium fortified with 1 mg/l 2,4-D and 5 mg/l NaCl, 1 mg/l

Table 5. Influence of 2,4-D and NaCl concentrations on shoot and root characteristics of *in vitro* regenerated plantlets of oats.

Callusing medium (mg/l)		Shoot(s) per callus ± SE	Shoot length (cm) ± SE	Root number	Root length (cm)
2,4-D	NaCl				
0	0	0.00 ± 0.00^{h}	0.00 ± 0.00^{g}	0.00 ± 0.00^{h}	0.00 ± 0.00^{h}
1	0	0.00 ± 0.00^{h}	0.00 ± 0.00^{g}	0.00 ± 0.00^{h}	0.00 ± 0.00^{h}
1	5	0.60 ± 0.40^{h}	1.09 ± 0.13^{efg}	2.38 ± 0.22^{def}	1.79 ± 0.11^{cdef}
1	10	1.00 ± 0.32^{gh}	1.33 ± 0.22^{ef}	1.12 ± 0.11^{gh}	1.43 ± 0.18^{ef}
1	15	0.00 ± 0.00^{h}	0.00 ± 0.00^{g}	0.00 ± 0.00^{h}	0.00 ± 0.00^{h}
1	20	0.00 ± 0.00^{h}	0.00 ± 0.00^{g}	0.00 ± 0.00^{h}	0.00 ± 0.00^{h}
2	0	3.20 ± 0.66^{fg}	1.16 ± 0.11^{efg}	5.23 ± 0.28^{c}	1.55 ± 0.15^{ef}
2	5	1.40 ± 0.51^{gh}	1.51 ± 0.13^{def}	6.18 ± 0.29^{c}	1.17 ± 0.14^{fg}
2	10	1.40 ± 0.40^{gh}	1.83 ± 0.12^{def}	2.55 ± 0.21^{def}	0.73 ± 0.09^{g}
2	15	1.00 ± 0.32^{gh}	0.98 ± 0.10^{efg}	1.55 ± 0.21^{efg}	1.64 ± 0.11^{def}
2	20	0.00 ± 0.00^{h}	0.00 ± 0.00^{g}	0.00 ± 0.00^{h}	0.00 ± 0.00^{h}
3	0	10.60 ± 0.51^{b}	9.74 ± 0.61^{b}	9.54 ± 0.23^{b}	2.29 ± 0.16^{bc}
3	5	14.80 ± 0.58^{a}	12.17 ± 0.28^{a}	10.90 ± 0.53^{a}	3.76 ± 0.30^{a}
3	10	6.40 ± 0.51^{de}	7.32 ± 0.39^{c}	9.20 ± 0.49^{b}	2.48 ± 0.11^{b}
3	15	6.00 ± 0.89^{ef}	6.17 ± 0.37^{c}	0.00 ± 0.00^{h}	0.00 ± 0.00^{h}
3	20	0.00 ± 0.00^{h}	0.00 ± 0.00^{g}	0.00 ± 0.00^{h}	0.00 ± 0.00^{h}
4	0	7.60 ± 0.68^{cd}	1.13 ± 0.14^{efg}	3.47 ± 0.23^{d}	1.94 ± 0.16^{bcde}
4	5	9.00 ± 0.71^{bc}	2.63 ± 0.29^{d}	2.61 ± 0.19^{de}	2.22 ± 0.14^{bcd}
4	10	6.40 ± 0.51^{de}	2.04 ± 0.15^{de}	1.40 ± 0.18^{fg}	1.68 ± 0.10^{cdef}
4	15	2.20 ± 0.37^{fgh}	1.27 ± 0.09^{ef}	0.65 ± 0.18^{gh}	1.46 ± 0.16^{ef}
4	20	0.00 ± 0.00^{h}	0.00 ± 0.00^{g}	0.00 ± 0.00^{h}	0.00 ± 0.00^{h}
Level of significance		**	**	**	**
CV (%)		32.49	20.36	18.54	24.04

**Indicating the mean difference is significant at the 0.01 level. The values followed by different letters in a column are significantly different at $p < 0.05$ according to DMRT.

2,4-D and 0 mg/l NaCl, 4 mg/l 2,4-D and 15 mg/l NaCl, and 2 mg/l 2,4-D and 10 mg/l NaCl, respectively. In the present work, calli induced in abiotic stress condition with low level of salt (5 mg/l) gave better results than the controls and high levels (10, 15 and 20 mg/l) of NaCl. El-Enany (1997) found that high level of salinity inhibited shoots

regeneration from hypocotyls and cotyledons. On the other hand, Mercado et al. (2000) observed that the presence of NaCl in the media strongly inhibited shoot regeneration. Benderradji et al. (2011) observed the best number of regenerated shoots and roots for slight salt stress condition and decreased root length with increasing concentration of NaCl.

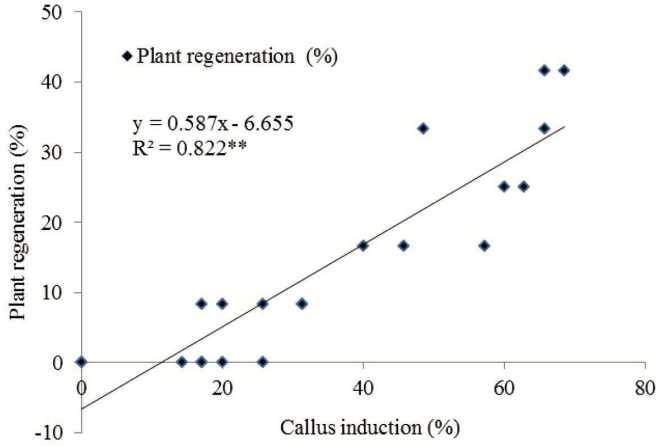

Fig. 3. Relation between callus induction capacity and plant regeneration ability in oat.
**Significant at $p < 0.01$.

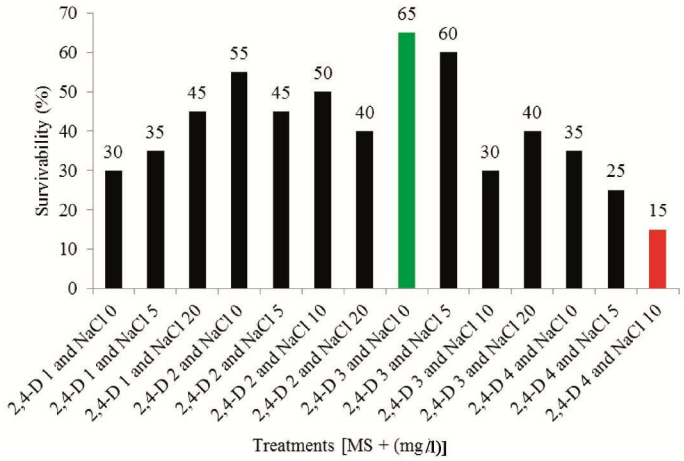

Fig. 4. Diagrammatic representation of survivability rate of *in vitro* grown oat plantlets.

The *in vitro* grown plantlets successfully rooted on the regeneration medium. Furthermore, the regenerated plants responded well to *ex vitro* survivability. The survivability of the regenerated plants varied from 15 to 65% (Fig. 4). The maximum (65%) survivability was recorded for the plantlets that were regenerated from the callus

produced in medium containing 3 mg/l 2,4-D and 5 mg/l NaCl salt and that of minimum (15%) survivability was found for the plantlets obtained from the MS with 4 mg/l 2,4-D and 15 mg/l NaCl.

Conclusion

It is difficult to select salt tolerant plants through traditional methods under field conditions (Richards 1996) because the salinity tolerance is a polygenic trait and there is stress heterogeneity in the field. *In vitro* culture is an effective way to generate salt tolerant plants. *In vitro* culture constitutes a powerful method to improve salinity tolerance via somaclonal variation. From the present investigation, the following generations become apparent: *Avena sativa* L. has a moderate level of tolerance against NaCl salt stress. The different physiological parameters have a great influence on growth of calli and subsequent regeneration to varying levels of salinity. Salt tolerance seemed to be related to the efficiency of a tissue to modulate level of inorganic and organic solutes in response to salt stress. The protocol developed for the *in vitro* selection and production of salt-tolerant plants could be used as an alternative to traditional breeding programs to increase salinity tolerance to oats.

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References

Ahmad R, Jamil S, Shahzad M, Zörb C, Irshad U, Khan N, Younas M and **Khan SA** (2017) Metabolic profiling to elucidate genetic elements due to salt stress. Cl. Soil Air Wat. **45**(12): 1600574.

Ashraf M and **Harris PJC** (2004) Potential biochemical indicators of salinity tolerance in plants. Plant Sci. **166**(1): 3-16.

Badea C and **Basu SK** (2010) Impact of drought on plant proteome and metabolome. *In:* Proceedings of the UGC State Level Seminar on Emerging Trends in Contemporary Education: Implications for 21st Century, Howrah, India, pp. 104-20.

Benderradji L, Brini L, Kellou K, Ykhlef N, Djekoun A, Masmoudi K and **Bouzerzour H** (2011) Callus induction, proliferation, and plantlets regeneration of two bread wheat (*Triticum aestivum* L.) genotypes under saline and heat stress conditions. Int. Scholarly Res. Not. ISRN Agron. 2012, 1-8.

Benlioglu B, Birsin M and **Özgen M** (2014) The effect of different 2,4-D doses on chromosomal structure of regenerants in barley (*Hordeum vulgare* L.) embryo culture. Int. J. Ad. Biotech. Res. **5**(3): 533-538.

Bregitzer P, Dahleen LS and **Campbell RD** (1998) Enhancement of plant regeneration from embryogenic callus of commercial barley cultivars. Plant Cell Rep. **17**(12): 941-945.

El-Enany AE (1997) Shoot regeneration and protein synthesis in tomato tissue cultures. Biol. Plantarum. **39**(2): 303-308.

Elmaghrabi AM, Ochatt S, Rogers HJ and **Francis D** (2013) Enhanced tolerance to salinity following cellular acclimation to increasing NaCl levels in *Medicago truncatula*. Plant Cell Tiss. Org. Cult. **114**(1): 61-70.

El-Meleigy EA, Gabr MF, Mohamed FH and **Ismail MA** (2004) Responses to NaCl salinity of tomato cultivated and breeding lines differing in salt tolerance in callus cultures. Int. J. Agric. Biol. **6**: 19-26.

Gless C, Lorz H and **Jahne-Gartner A** (1998) Establishment of a highly efficient regeneration system from leaf base segments of oat (*Avena sativa* L.). Plant Cell Rep. **17**(6): 441-445.

Gomez KA and **Gomez AA** (1984) Statistical procedures in agricultural research (2nd Ed.). Paperback, New York. p. 680.

González JM, Friero E and Jouve N (2001) Influence of genotype and culture medium on callus formation and plant regeneration from immature embryos of *Triticum turgidum* Desf. cultivars. Plant Breed. **120**(6): 513-517.

Hanin M, Ebel C, Ngom M, Laplaze L and **Masmoudi K** (2016) New insights on plant salt tolerance mechanisms and their potential use for breeding. Fron. in Plant Sci. **7**: 1787.

Hussain K, Majeed A, Nawaz K, Khizar HB and **Nisar MF** (2009) Effect of different levels of salinity on growth and ion contents of black seeds (*Nigella sativa* L.). Curr. Res. J. Biol. Sci. **1**(3): 135-138.

IWM (2014) Salinity in the south west region of Bangladesh. Institute of Water Modeling, Bangladesh.

Karp A, Steel SH, Parmar S, Jones MGK, Shewry PR and **Breiman A** (1987) Relative stability among barley plants regenerated from cultured immature embryos. Genome **29**(3): 405-412.

Mercado JA, Maria AS, Silvia JB, Rosa PQ, Fernando PA and **Miguel AQ** (2000) Assessment of *in vitro* growth of apical stem sections and adventitious organogenesis to evaluate salinity tolerance in cultivated tomato. Plant Cell Tiss. Organ Cult. **62**(2): 101-106.

Nawaz MF, Gul S, Tanvir MA, Akhtar J, Chaudary S and **Ahmad I** (2016) Influence of NaCl-salinity on Pb-uptake behavior and growth of River Red gum tree (*Eucalyptus camaldulensis* Dehnh.). Turk. J. Agric. For. **40**: 425-432.

Panta S, Flowers T, Lane P, Doyle R, Haros G and **Shabala S** (2014) Halophyte agriculture: Success stories. Environ. Expt. Bot. **107**: 71-83.

Piwowarczyk B, Tokarz K and **Kamińska I** (2015) Responses of grass pea seedlings to salinity stress in *in vitro* culture conditions. Plant Cell Tiss. Organ Cult. **124**(2): 227-240.

Rai MK, Kalia RK, Singh R, Gangola MP and **Dhawan AK** (2011) Developing stress tolerant plants through *in vitro* selection– an overview of the recent progress. Environ. Exp. Bot. **71**(1): 89-98.

Redha A and **Islam SMS** (2010) Effect of selected stress factors on androgenesis of wheat (*Triticum aestivum* L.). Kuwait J. Sci. Eng. **37**(1A): 127-138.

Richards RA (1996) Defining selection criteria to improve yield under drought. Plant Growth Regul. **20**(2): 157-166.

Saleem M, Mukhtar Z, Cheema A and **Atta B** (2005) Induced mutation and *in vitro* techniques as a method to induce salt tolerance in Basmati rice (*Oryza sativa* L.). Int. J. Environ. Sci. Technol. **2**(2): 141-145.

Salunke MD, Rathode BU and **Dattagonde NR** (2017) *In vitro* regeneration of oat (*Avena sativa* L.) using different explants. Int. J. Cur. Microbiol. App. Sci. **6**(12): 434-440.

Semiz GD and **Suarez DL** (2015) Tomato salt tolerance: impact of grafting and water composition on yield and ion relations. Turk. J. Agric. For. **39**: 876-886.

Tal M (1994) *In vitro* selection for salt tolerance in crop plants: theoretical and practical considerations. *In vitro* Cell Dev. Biol. **30**(4): 175-180.

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