Effects of salt and heat pre-treatment factors on efficient regeneration in barley (*Hordeum vulgare* L.)

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Abstract An efficient callus induction and plant regeneration system has been developed using salt and heat as pre-treatment factors for three barley genotypes viz. BB-3, BB-6 and BHL-18. Different concentrations of NaCl (1.5, 2.5, 3.5, 4.5, 5.5 and 6.5 g/L) were used and its effects were determined on the basis of the viability of callus (CV), plant regeneration (PR), relative growth rate (RGR) and tolerance index (TI). The BB-6 showed highest performance on tolerance based on CV (14.72%), PR (7.69%), RGR (0.91%) and TI (0.42%) at 6.5 g/L NaCl. Various NaCl concentrations displayed significantly differences at $P < 0.01$ level as compared with the control. Plant regeneration capability was recorded after heat pre-treatment using calli at 30, 35 and 40 °C. In this study, BHL-18 produced highest callus induction (59.71%) after desiccated at 40 °C for BB-6. Highest regeneration was recorded around 41.66% when 4 weeks old calli were pre-treated at 35 °C. Furthermore, heat pre-treatment factors were very effective for enhancing plant regeneration (25–41.66%) which was 1.8–2.14 fold higher compared to the control (13.88–19.44%). Hence, heat treated calli displayed higher tolerance level to survive in NaCl-induced treatment for determining abiotic stress and increased regeneration rate at 35 °C temperature in BB-6 barley genotype.

Keywords Barley · NaCl · Regeneration · Relative growth rate · Tolerance index

Introduction

Plant tissue culture systems have provided prerequisite for an efficient callus induction and plantlet regeneration. Efficient plant regeneration in barley has been reported to be highly genotype dependent and also important for optimization of media and plant growth regulators (Bregitzer et al. 1998; Castillo et al. 1998; Haque and Islam 2014, 2015). In few years, there has been a renewed interest in barley food uses due to its nutritional advantages. Currently, world agriculture is facing a lot of challenges such as producing 70% more food for an additional 2.3 billion people by 2050 (FAO 2009). Plant tissue culture techniques provide a promising and feasible approach to develop various stress tolerant plants (El-Meleigy et al. 2011; Islam and Tuteja 2013). However, the productivity of crops is not increasing in parallel with the food demand in the world. There are some reports on using different explants such as immature embryos (Chang et al. 2003), immature inflorescence (Havrlentova et al. 2001), coleoptile (Sahrawat and Chand 2004), mature embryo (Abumhadi et al. 2005; He and Jia 2008) and seedling explants (Sharma et al. 2004) for callus induction and plant regeneration in barley. Suitable plant regeneration based on mature embryos is reported for other cereal crops, such as rice (Kyungsoon et al. 2002; Khatun et al. 2010), maize (Huang and Wei 2004; Morshed et al. 2014) and wheat (Zale et al. 2004; Saha et al. 2015). Akula et al. (1999) reported the regeneration response using mature embryos in barley, but low regeneration frequencies were observed. Some reports on plant regeneration from mature embryos have been re-evaluated the scope of potential explants used in barley tissue culture by Gurel et al. (2009) and Haque and Islam (2015). Mature embryos are readily available throughout the year and their physiological state is less

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variable and using mature embryos reduces requirement of greenhouse and application via in vitro system shown to be more cost-effective. Salt and other stress pre-treatment factors occur frequently and that can affect most habitats in plants growth. But plants have developed several strategies to cope with these challenges by various adaptation mechanisms, which allow them to survive in any adverse environments to avoid stress conditions (Cano et al. 1996; Rus et al. 2000; Mohamed et al. 2002). To date, there is no report on successful regeneration of Bangladeshi barley genotypes using salts and the evaluation of stress tolerance level. Therefore, the present study has been undertaken to determine the effect of heat pre-treatment and by NaCl to enhance somatic embryogenesis and their subsequent regeneration using three barley genotypes.

Materials and methods

Planting materials and culture conditions

Seeds of three barley (*Hordeum vulgare* L.) genotypes, namely BARI barley-3 (BB-3), BARI barley-6 (BB-6) and BHL-18 were collected for this study from Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. Mature seeds were sterilized with 70% (v/v) ethanol for 1 min and washed three times with sterile water. Then, seeds were sterilized in 0.12% NaOCl for 30 min followed with three washes with sterile distilled water under aseptic condition. After disinfection, mature seeds were cultured for callus induction on MS (Murashige and Skoog 1962) medium supplemented with 2,4-D (4.0 mg/L), L-proline (200 mg/L), casein hydrolysate (300 mg/L) and sucrose (30 g/L). The pH was adjusted at 5.8. Inoculated petri dishes were sealed with parafilm and incubated at 25 ± 2 °C in dark for 2–3 weeks.

Pre-treatment of salt

After 3 weeks of incubation, the induced calli (around 75–100 for each treatment) were individually weighted and placed on MS medium supplemented with various concentrations (0, 1.5, 2.5, 3.5, 4.5, 5.5 and 6.5 g/L) of NaCl. Data were recorded after 4 weeks of culture initiation in salt stress condition. Through visual observation, the viable calli were counted and the efficiency of callus viability was measured by the following formula:

$$\text{Percentage of callus viability (CV)} = \left[\frac{\text{Viable callus}}{\text{Total number of embryos cultured}} \right] \times 100.$$

Salt pre-treated viable calli (39–75 for each treatment) were transferred to the regeneration medium

(MS + 1.0 mg/L BAP + 0.5 mg/L NAA) and incubated them for 3 weeks at 25 ± 1 °C with 16/8 h (light/dark) conditions. Green spotted calli and its regeneration frequency were recorded by the following formula:

$$\text{Percentage of regeneration (PR)} = \left[\frac{\text{No. of callus with green spot}}{\text{Total number of callus}} \right] \times 100.$$

Three weeks old calli with uniform size (approximately 75–100 mg) were placed on MS medium supplemented with different concentration of NaCl (0, 2.5, 4.5 and 6.5 g/L) and plant growth regulators (PGRs). For each treatment, calli were weighted individually which was known as initial fresh weight (FWi) and culture into vessel. After 4 weeks, calli were rinsed with sterile distilled water 4–5 times and moisture was removed by blotting paper and final fresh weight (FWf) was measured. The relative growth rate (RGR) of callus was determined on a fresh weight (FW) using the standard formula of $(FWf - FWi)/FWi$ based on Smith and McComb (1981). The FWi was the initial weight of callus in each treatment and the FWf was the final weight of callus that sub-cultured in medium that contained with various concentration of NaCl. To compare genotype-related responses to stress conditions, tolerance index (TI) based on RGR was calculated according to the formula $TI = RGR_{\text{treatment}}/RGR_{\text{control}}$ (Soheilikhah et al. 2013). The moisture was removed by blotting paper and fresh mass was measured.

Heat pre-treatment factors

Four weeks old calli (around 110–125 mg) were used for heat pre-treatment factors and transferred them to sterile petri dishes that contained with 2–3 pieces of sterile Whatmann 1 filter paper. Calli were incubated at 25 °C for 6 h considered as a control. For three temperature pre-treatment factors, calli were incubated at 30, 35 and 40 °C for 6 h. Callus mass was determined before and after heat treatment. The desiccation percentage was calculated with the following formula:

$$\text{Desiccation percentage} = \left[\frac{\text{Fresh callus weight} - \text{heat treated callus weight}}{\text{Fresh callus weight}} \right] \times 100\%.$$

The heat pre-treated calli (72–108 for each treatment with similar size and shape) were transferred to the regeneration medium containing MS + 1.0 mg/L BAP + 0.5 mg/L NAA and incubated them at 25 ± 1 °C with a 16/8 h (light/dark) photoperiod. After 7 weeks of incubation, the regeneration frequency was calculated by the following formula:

Regeneration frequency (%)

$$= \left[\frac{\text{No. of callus with green spot}}{\text{Total number of callus}} \right] \times 100\%.$$

Plants were transferred to the tubes containing water for hardening after 3–4 weeks and then kept them at room temperature for a week and finally transferred to the pots.

Statistical analysis

The average or mean values were computed from three replicates with standard error (SE) and each experiment was repeated thrice. For each treatment, mean values were compared by least significant difference (LSD) at $\alpha = 0.01$ using SPSS16.0 software.

Results and discussion

Effect of salt on callus viability and regeneration

There are several reports using various abiotic stress pretreatment factors, e.g. physical (heat, starvation, gamma irradiation, hypertonic shock, centrifugal treatment and atmospheric pressure, etc.) and chemical (heavy metal, ethanol, osmotic stress, feminizing agents, ABA, etc.) which could enhance for the induction of somatic and gametic embryogenesis. Previously, plant scientists were attempted improving crops by application of different abiotic stresses on somatic and gametic embryogenesis in cereal and other important crops (Bregitzer et al. 1998; El-Meleigy et al. 2004; Redha and Islam 2010). These factors are co-related to change cell cycle and different biological

systems. In this study, we used two major factors such as heat shock and osmotic stress (salt stress treatment) for embryogenic development in barley and this is the first report in Bangladesh for its improvement. It was observed that the responding genotypes showed well-embryogenic response to induce callus and its subsequent regeneration (Fig. 1a–f). Significant differences were observed among the salt in different concentrations. The results indicated that salinity has preventive effects on callus viability as well as regeneration percentage in these three genotypes. Control treatments showed the maximum callus viability and regeneration frequency. In fact, the lower number of calluses as well as regeneration was obtained under higher salt concentration. Callus viability and regeneration rate of salt treated callus were greatly influenced by the genotypes. Within the genotypes, BB-6 produced highest viability of callus (14.72%) and regeneration (7.69%) with higher NaCl concentrations (6.5 g/L). Data analysis showed callus viability of 14.72, 11.66 and 10.83%, respectively, in BB-6, BHL-18 and BB-3 at the higher NaCl concentrations (Table 1). It was observed that the BB-6 and BHL-18 could be able to regenerate with 6.5 g/L of NaCl except the BB-3 genotype. All the treatments (T_1 – T_6) displayed significantly decreased in callus viability and regeneration in comparison with control (Table 2). These results are in agreement with El-Meleigy et al. (2004). El-Meleigy et al. (2004) noticed that callus weight generally decreased with the increase in NaCl level and mentioned that the highest dry weight of tomato with 2000 ppm and the lowest was at 6000 ppm. Rus et al. (2000) also found that adaptation capacity to salinity varies with the genotype's degree of tolerance. Moreover, frequencies in most of the treatments were significantly different at 0.01 probability levels for

Fig. 1 In vitro development of callus and its subsequent regeneration of barley. **a** Callus initiation after 7 days of culture. **b** Embryogenic callus with green spots, **c** regenerated plantlets without roots, **d** regenerated plants with shoots, **e** regenerated plants, **f** plants with complete well shoot and roots

Table 1 Callus viability and regeneration frequency of seed-derived callus exposed to different salt treatments in three barley genotypes (mean of percentage $\pm$ SE)

Salt treatment (g/L)	Genotypes					
	BB-3		BB-6		BHL-18	
	CV	PR	CV	PR	CV	PR
Cont.	30.33 $\pm$ 2.02	13.33 $\pm$ 3.52	38.66 $\pm$ 2.60	20.00 $\pm$ 2.30	33.66 $\pm$ 1.20	14.66 $\pm$ 1.33
T ₁	25.66 $\pm$ 0.88	12.00 $\pm$ 2.30	34.33 $\pm$ 1.76	17.33 $\pm$ 1.33	28.33 $\pm$ 2.40	13.33 $\pm$ 2.66
T ₂	21.81 $\pm$ 1.38	11.11 $\pm$ 3.20	29.69 $\pm$ 1.32	16.66 $\pm$ 3.20	23.63 $\pm$ 1.04	12.96 $\pm$ 1.85
T ₃	17.87 $\pm$ 1.09	9.25 $\pm$ 1.85	25.15 $\pm$ 1.09	14.81 $\pm$ 1.85	19.69 $\pm$ 1.32	11.11 $\pm$ 3.20
T ₄	15.27 $\pm$ 0.73	7.69 $\pm$ 4.44	20.00 $\pm$ 0.96	12.82 $\pm$ 5.12	15.27 $\pm$ 1.54	10.25 $\pm$ 2.56
T ₅	13.33 $\pm$ 0.48	5.12 $\pm$ 2.56	16.94 $\pm$ 0.73	10.25 $\pm$ 2.56	13.05 $\pm$ 0.73	7.69 $\pm$ 4.44
T ₆	10.83 $\pm$ 0.96	00	14.72 $\pm$ 0.55	7.69 $\pm$ 4.44	11.66 $\pm$ 0.48	5.12 $\pm$ 2.56

Cont. without salt, T₁ 1.5, T₂ 2.5, T₃ 3.5, T₄ 4.5, T₅ 5.5, T₆ 6.5 g/L salt pre-treatment factors, CV callus viability, PR plant regeneration

Table 2 Effect of salt treatment on callus viability and its regeneration efficiency for barley embryo culture (mean of percentage $\pm$ SE)

Salt treatment (g/L)	Callus viability		Regeneration	
	Mean	Difference	Mean	Difference
T ₁	29.44	4.77*	14.22	1.77 ^{NS}
T ₂	25.04	9.17*	13.57	2.42*
T ₃	20.90	13.31*	11.72	4.27*
T ₄	16.84	17.37*	10.25	5.74*
T ₅	14.44	19.77*	7.68	8.31*
T ₆	12.40	21.81*	4.27	11.72*
Control	34.21	–	15.99	–

NS non-significant, T treatment

* Indicating the mean difference is significant at the 0.01 level (LSD test)

callus viability and regeneration from the control except in the frequency of regeneration obtained in T₁ generation. NaCl has been used to simulate salt stress either in vivo or in vitro in barley (Ye et al. 1987). Inclusion of NaCl during the callus formation and regeneration processes constitutes a convenient way to study the effect of salinity and the selective pressure can be applied (Saleem et al. 2005). In the present investigation, significant differences were found within the barley genotypes on viability of calli in NaCl stress. The calli of BB-6 exhibited the highest viability (14.72%) with higher concentrations of NaCl (6.5 g/L). It was observed that 11.66 and 10.83% existing calli were survived in BHL-18 and BB-3 after exposed using 6.5 g/L salt for 4 weeks. The phenomenon was occurred due to the presence of necrotic cells in the calli. A huge number of necrotic cells turned the calli into black and deep brown colour and reduced the percentage survival. Through visual observation, it was observed that the higher frequency of necrosis was appeared when salt concentrations were increased. This could be due to lower osmotic

potentiality of the cells and genotypic effect which created the variability of cell viability within the genotypes. After 4 weeks, most of the calli showed non-viability and expired except BB-6, which was partially adapted to the NaCl stress and showed the highest viability for all the tested salt concentrations. The results are in agreement with the previous findings on Giza 123 genotype which could tolerate at 5 g/L of NaCl (Metwali et al. 2013). Siddique et al. (2014) reported that callus viability decreased in rice as compared to the control at 200 mM NaCl. The present findings confirmed that salt treatment could decrease shoot regeneration in treated calluses derived from these three genotypes. Similarly, T₁–T₆ showed significantly decreased in regeneration percentage in comparison to the control. Only, BB-6 and BHL-18 produced shoot regeneration at 6.5 g/L of NaCl. Therefore, the present investigations are in agreement with the previous report where 47–64% decreased cell viability in NaCl-induced stress for Safflower (*Carthamus tinctorius* L.) varieties and callus growth as well as shoot length both decreased with increasing NaCl concentrations (Soheilikhah et al. 2013). Motohashi et al. (2010) improved various salt tolerance level in rice cultivars by introducing catalyses gene, *katE* even at 200 mM NaCl.

Relative growth rate and tolerance index against NaCl

Relative growth rate (RGR) and tolerance index (TI) were determined and results are shown in Fig. 2a, b. Here, both genotypes showed highly significant results on regeneration. However, 0.91, 0.49 and 0.48 RGR values were recorded at 6.5 g/L NaCl stress in BB-6, BHL-18 and BB-3, respectively. In comparison to the control, RGR was decreased at 57.67, 74.87 and 72.41% for the mentioned genotypes. BB-6 showed the highest RGR when NaCl concentrations used 6.5 g/L. The same genotype carried

Fig. 2 **a** Effect of different concentrations (0, 2.5, 4.5 and 6.5 g/L) of NaCl subjected to relative growth rate (RGR). **b** Effect of different concentrations (0, 2.5, 4.5 and 6.5 g/L) of NaCl subjected to tolerance index (TI)

the highest TI (0.42) which expressed the high capability to grow in salt stress condition developed by NaCl. Comparatively lower TI numbers were investigated in other two genotypes of BHL-18 (0.25) and BB-3 (0.28). Significant differences were found within the genotypes examined based on tolerance index (TI) value at 6.5 g/L NaCl. TI value obtained at 0.42, 0.25 and 0.28 in BB-6, BHL-18 and BB-3, respectively. Within three genotypes, BB-6 exhibited the highest potentiality survived capacity in NaCl-induced abiotic stress with maximum RGR (0.91) and TI (0.42) values. The recorded parameters displayed higher survival capability against the NaCl stress conducting the physiological activities of BB-6. On the other hand, stress sensitivity was found in BHL-18 and BB-3 at lower value. RGR and TI values were decreased when the calli were cultured in higher NaCl level. These phenomena might be happened due to the reduction of water availability and loss of turgor pressure (TP) in the cells of the calli. Such physiological causes were reported in previous investigation for *Oryza sativa* (Siddique et al. 2014), *C. tinctorius* (Soheilikhah et al. 2013), *Triticum aestivum* (Fazeli-Nasab et al. 2012) and *Saccharum* sp. (Errabii et al. 2007). They also reported that this phenomenon occurred due to the interference of Na^+ and Cl^- ions to uptake and

translocation processes. As a result, nutritional imbalance might be created and growth of callus is declined. However, NaCl-treated calli of BB-6 were least affected at the highest dose of salt stress and exhibited high ability in terms of both cellular viability and growth of calli.

Effect of heat pre-treatment factors and regeneration rate

Four weeks old calli of three genotypes were subjected at 25 °C (control), 30, 35 and 40 °C temperature, respectively. It was observed that the percentage of desiccation referred to the percentage loss in callus fresh weight pre and post heat treatment as well as regeneration percentage of heat-treated calli. In case of regeneration, pre-treated calli by air desiccation showed significant differences from both genotypes at 0.05 probability levels (Table 3). Calli of BHL-18 displayed highest desiccation (59.70%) after heated at 40 °C temperature. The performance was around threefold higher than the control (19.71%). However, we found that BB-6 produced highest regeneration rate at 41.66% when the calli were pre-treated by heat (35 °C) and it was around 2.14 folds higher than the control (19.44%). Calli pre-treated by heat (30, 35 and 40 °C) showed significantly higher regeneration than control. But 35 °C was the best treatment for regeneration in barley (Table 3). This treatment induced 25.0–41.66% regeneration over the three genotypes, representing a 1.8–2.14 fold increase compared to the control (13.88–19.44%).

Effect of heat and NaCl on regeneration

Here heat pre-treated calli in the presence of NaCl were tested and evaluated the regeneration potentiality on the studied genotypes as shown in Table 4. Four weeks old calli of the three barley genotypes (BB-3, BB-6 and BHL-18) were pre-treated at 25 °C considered as Control. For treatments, pre-treated calli at 30, 35 and 40 °C temperature were transferred to regeneration medium (MS + 1.0 mg/L BAP + 0.5 mg/L NAA) supplemented with different concentrations of NaCl (0, 150, 300 and 450 mg/L). The results indicated that all the studied genotypes showed well response to regeneration (Table 4). Out of three genotypes, BB-6 showed the highest number of regeneration (46.71%) at 150 mg/L of NaCl when the calli pre-treated at 35 °C heat and the lowest was recorded for BB-3 at 450 mg/L of NaCl when the calli pre-treated at 40 °C heat (1.94%). At 35 °C heat pre-treated calli showed significantly better results for regeneration with the level 150 mg/L of NaCl in the three tested barley genotypes. On the other hand, 40 °C heat pre-treated calli with 450 mg/L NaCl showed lowest regeneration. Furthermore, combination of a low level of NaCl (150 mg/L) with the medium

Table 3 Regeneration frequency of seed-derived callus exposed to different heat treatments in three barley genotypes (mean of percentage $\pm$ SE)

Temp. (°C)	Mean of percentage $\pm$ SE					
	Air desiccation			Plant regeneration		
	BB-3	BB-6	BHL-18	BB-3	BB-6	BHL-18
Cont. (25)	21.07 $\pm$ 0.70	18.48 $\pm$ 2.12	19.71 $\pm$ 1.04	13.88 $\pm$ 1.38	19.44 $\pm$ 1.60	15.62 $\pm$ 1.80
30	35.47 $\pm$ 1.16	32.42 $\pm$ 0.80	37.97 $\pm$ 0.77	18.05 $\pm$ 2.77	28.70 $\pm$ 2.44	23.95 $\pm$ 2.08
35	42.40 $\pm$ 0.92	39.70 $\pm$ 1.09	43.49 $\pm$ 1.50	25.00 $\pm$ 2.40	41.66 $\pm$ 3.20	30.20 $\pm$ 2.75
40	58.67 $\pm$ 1.41	55.75 $\pm$ 0.80	59.70 $\pm$ 2.09	15.27 $\pm$ 1.38	24.07 $\pm$ 2.44	19.79 $\pm$ 1.04

The values (mean of percentage)

Cont. control, Temp. temperature

Table 4 Effect of heat and NaCl on regeneration in three barley genotypes (% $\pm$ SE)

Variety	Temperature (°C)	NaCl (mg/L)			
		Cont.	150	300	450
BB-3	25 (Cont.)	12.56 $\pm$ 1.22a	14.88 $\pm$ 1.38a	6.25 $\pm$ 1.45a	2.41 $\pm$ 0.56a
	30	17.85 $\pm$ 1.96b	21.05 $\pm$ 2.71b	9.97 $\pm$ 1.28ab	3.28 $\pm$ 0.82a
	35	24.60 $\pm$ 2.31c	27.00 $\pm$ 2.54c	13.02 $\pm$ 1.46b	5.60 $\pm$ 1.02ab
	40	13.46 $\pm$ 1.72a	16.27 $\pm$ 1.38a	11.24 $\pm$ 2.08ab	1.94 $\pm$ 0.18a
BB-6	25 (Cont.)	17.71 $\pm$ 1.45b	20.42 $\pm$ 1.92b	9.83 $\pm$ 1.91ab	5.56 $\pm$ 1.08ab
	30	26.45 $\pm$ 2.66c	33.56 $\pm$ 4.17d	16.35 $\pm$ 3.18bc	6.81 $\pm$ 2.56b
	35	38.92 $\pm$ 2.87d	46.71 $\pm$ 5.32e	19.38 $\pm$ 3.44c	8.72 $\pm$ 1.04b
	40	20.56 $\pm$ 3.18b	26.07 $\pm$ 2.21c	11.24 $\pm$ 1.26ab	3.18 $\pm$ 0.46a
BHL-18	25 (Cont.)	13.45 $\pm$ 1.21a	17.62 $\pm$ 1.82a	8.20 $\pm$ 0.91a	3.61 $\pm$ 0.78a
	30	21.60 $\pm$ 2.52b	25.95 $\pm$ 2.36c	13.34 $\pm$ 1.14b	4.26 $\pm$ 0.64ab
	35	28.75 $\pm$ 2.96c	34.20 $\pm$ 2.38d	10.54 $\pm$ 1.82ab	6.54 $\pm$ 1.12b
	40	18.52 $\pm$ 1.32b	20.79 $\pm$ 1.16b	7.31 $\pm$ 1.21a	2.97 $\pm$ 0.28a

The values followed by different letters in a column are significantly different at $P < 0.05$ according to Duncan's multiple range test (DMRT)

for plant regeneration showed better results, which showed significant results compared to the effects of a high level of NaCl (300 and 450 mg/L). Consequently, 150 mg/L NaCl proved to be the most effective for regeneration than control and other NaCl concentrations. Therefore, the data proved that stress pre-treatment factors are important for increasing regeneration in barley.

Plants regeneration is very essential for establishing a successful tissue culture system. Genotype dependency is a great problem for in vitro micropropagation system specially for cereal crops. Sometimes it is difficult to culture and regenerate agronomically important crops (Puhan and Siddiq 2013). The difference in the composition of culture medium and the concentrations of plant growth regulators affects the callus induction and regeneration ability of barley and other plant genotypes (Bregitzer et al. 1998; Haque and Islam 2014; Tariq et al. 2008). Furthermore, genotype variation also plays a vital role in callus initiation, proliferation and even regeneration in barley (Gubišová et al. 2012). Efficient regeneration is vital for the

establishment of a barley tissue culture system. Partial desiccation has been demonstrated as a tool for promoting embryogenesis and plant regeneration in both wheat (Carman 1988) and in indica rice (Tsukahara and Hirokawa 1992; Siddique et al. 2014). Our experimental results showed that heat pre-treatment could increase the shoot regeneration of treated calluses derived from barley genotypes, but the increasing efficiency was crucially dependent on the level of NaCl and degree of temperature.

Remarkably, increased regeneration frequencies were shown after calli cultured on salt stress medium using heat pre-treatment in the present investigation. It could be possible when the cells of calli acquired higher osmotic potential (OP). Due to the reduction of water at a suitable level, OP might be increased, and so that the heat-treated calli survived and able to perform higher regeneration. Siddique et al. (2014) found higher regeneration in rice when they applied various concentrations of salts and air desiccated pre-treated calli were used. Temperatures and drought stress pre-treatment factors are associated with plant

cell dehydration. Dehydration stress factors induce profound cellular response aimed at an elimination of water loss (Kosová et al. 2014). The experimental results showed that desiccation could increase shoot regeneration of treated calluses derived from the tested barley genotypes, but the increasing efficiency was crucially dependent on the degree of temperature. It was observed that calli of BHL-18 performed highest desiccation (59.70%) among the genotypes when it was pre-treated by heat at 40 °C temperature. The performance was around threefold higher than the control (19.71%). The variety BB-6 showed best regeneration (41.66%) when the calli were pre-treated by heat at 35 °C. The regeneration rate was at 2.14 folds higher than the control (19.44%). Similarly, Deng et al. (2009) reported that drought or desiccation increases regeneration in maize. Various stress pre-treated calli (heat and air desiccation) and plants derived from there may be showing more adaptable characteristics due to some physiological changes of cells. Drought has been found to promote regeneration in several plant species (Singh 2014; Haq et al. 2009; Kaur and Gosal 2009; Carman 1988; Rance et al. 1994). The current results inveterate earlier observations that regeneration of somatic embryos was significantly high in desiccated callus as compared to untreated callus. All of the studied genotypes showed water losses as different when calli were incubated by heat stress pre-treatment factors. This implied that the enhancement of shoot regeneration by desiccation is not only dependent on the amount of water loss, but is also related to the degree of temperature. Heat treatment at 35 °C produced higher regeneration percentage compared to the control. The findings from this study were similar in wheat based on Benderradji et al. (2012). They reported the significant effects of thermal stresses on callus induction and shoot regeneration in wheat. The results obtained in this study based on various parameters such as CV, regeneration, RGR, and TI in NaCl-induced pre-treatment confirmed the higher tolerance of BB-6 barley genotype compared to BB-3 and BHL-18. Heat treatment positively influenced the regeneration in all of the barley genotypes tested under this study. Heat pre-treatment also increased the capability of the barley calli to adapt in NaCl stress condition, especially the genotype of BB-6. Abdel-Hamid (1995) studied with various genotypes and found the genotype differences in callus growth and shoot regeneration potentialities. Reisch and Bingham (1980) indicated that the regeneration capacity of plant tissue is genetically controlled and specific for each genotype. The experimental results showed significant differences within the genotypes under different concentrations of NaCl in medium for regeneration. The three tested barley genotypes could be ranked based on regeneration as indicator for their salinity tolerance in the order: BB-6 > BHL-18 > BB-3. This result agreed well with Bregitzer et al. (1998). They reported that improvement of barley

regeneration was genotype specific. They also demonstrated that the development and use of genotype-specific protocols can enhance the plant regeneration.

Plant tissue culture techniques are good tools for salinity studies, particularly for characterization and for obtaining salt tolerance plants (Cano et al. 1998). Mohamed et al. (2011) demonstrated that NaCl stress greatly influenced the in vitro performances of two tomato cultivars and the explants. In the present work, low level of salt (150 mg/L) induced abiotic stress condition; desiccated calli gave higher regeneration than the controls and high level (300, 450 mg/L) of NaCl (Table 4). The results are in agreement with the previous findings concerning the physiological responses of tomato cultures to salt treatments. Here, all the tested tomato genotypes were able to produce callus, shoots and initiate roots under different concentrations of NaCl in callus induction media. However, these capacities were different, depending on genotype and salt level (El-Meleigy et al. 2004). The report of El-Meleigy et al. (2004) also indicated the reduction in tomato callus weight at the highest salinity level (6000 ppm). The increase in callus weight was 43% over at 1000 ppm NaCl, then declined at 2000, 3000, 4000 and 5000 ppm NaCl, respectively. El-Enany (1997) found that high level of salinity inhibited shoots regeneration from hypocotyls and cotyledons, and fresh and dry weight reduced with increased salinity in growth medium. On the other hand, Mercado et al. (2000) observed that the presence of NaCl in the media strongly inhibited shoot regeneration in tomato. From these findings, it may be concluded that salt and heat pre-treatment might be helpful for the regeneration into plantlets in barley.

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Compliance with ethical standards In view of the above, it can be concluded that application of stress treatments like salt and heat can be used to improve plant regeneration in barley. However, further investigation is required in order to understand the actual mechanism of action and factors responsible for this pathway of regeneration.

Conflict of interest The authors declare that they have no competing interests.

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