INT. J. BIOL. BIOTECH., 17 (2): 351-358, 2020.

GERMINATION RESPONSES OF *TYPHA DOMINGENSIS* (PERS.) TO SALINITY, LIGHT AND TEMPERATURE

Rabab Fatima Rizvi[1, 2], Zainul Abideen[1], Saman Ehsen[1], Irfan Aziz[1], Muhammad Zaheer Ahmed[1], Bilquees Gul[1*] and Muhammad Ajmal Khan[1]

[1]*Dr. Muhammad Ajmal Khan Institute of Sustainable Halophyte Utilization, University of Karachi, Karachi-75270, Pakistan*
[2]*Saint Lawrence Government Girls Degree College, Karachi, Pakistan*
*Corresponding author:
Tel. + 0641-99-35313; E-mail address: bilqueesgul@uok.edu.pk

ABSTRACT

Typha domingensis (Pers.) (a potential grass candidate for bioenergy and heavy metal remediation) was selected to monitor the changes in seed germination under various salinity (0, 100, 200, 300, 400, and 500 mM NaCl), temperature (10^o-20^o, 15^o-25^o, 20^o-30^o, and 25^o-35^oC) and light (12:12-h dark: light and 24-h dark) regimes. Optimum seed germination (95%) was observed in non-saline control as well as 100 mM NaCl which decreased with further increase in salinity. Final seed germination was severely inhibited (<10%) above 300 mM NaCl. Rate of germination was similar to the germination percentage. Seeds showed 70 to 95% germination after recovery from saline conditions. Germination was substantially inhibited in the absence of light irrespective of salinity treatment when compared to 12 h photoperiod. Un-germinated seeds in dark showed radicle emergence when transferred to 12 h photoperiod. An improvement in germination was observed at warmer temperatures under saline conditions. All three factors (Salinity, Temperature and light) regulated seed germination in *T. domingensis*. In general, better germination responses in adequate light and moderately saline conditions (<200 mM NaCl) could help in propagation of *T. domingensis* by seeds.

Key words: Abiotic stress, germination rate, photoperiod, recovery, salt marsh, *Typha domingensis* (Pers.)

INTRODUCTION

Seeds in angiosperms act as a bridge between two succeeding generations as they are biologically evolved for the dispersal and propagation of plants (Linkies *et al*., 2010). In order to survive under harsh climatic conditions seeds along with different plant parts have developed a number of stress-resistant structures for sustaining their generations besides allowing long distance dispersal (Dekkers *et al.*, 2013; Fatima *et al*., 2019).

Seed germination is a complex biological process which involves a series of physiological and biochemical events for successful production of crops (Bhattacharjee, 2008). This process is strongly regulated by different abiotic factors like soil salinity, moisture availability, thermoperiod and light (Khan and Gul, 2006; Saeed *et al.*, 2011; Gulzar *et al*., 2013). Hence, for better crop production, it is important to determine the tolerance level of seed during germination in saline environments (Hakim *et al*., 2010). Seed germination studies under different saline conditions reveals that crops may differ in their germination responses with greater variations even if they are of similar nature (Hakim *et al*., 2010).

Pakistan has a diverse climate which is classified under arid to semiarid region due to low precipitation (Khan and Qaiser, 2006). Halophytes are well renowned for their germination and growth in arid conditions and some of the species produce huge biomass (Al Sherif, 2009; Nedjimi, 2009). Halophytes are those plants that can tolerate the salinity during germination or throughout their life cycle. Tolerance quality of halophyte seeds toward salinity may be define is terms of capacity of un-germinated seeds in saline conditions and remains viable or germinate in high saline conditions (Khan, 2002). There is also a variation in temperature and light regimes which may affect seed germination of halophytes (Khan and Gul, 2006). The ability of viable but un-germinated seeds to resist harsh climatic conditions plays a key role during the life cycle of plants (Forbis, 2010). However, in some halophytes (e.g., in brown seeds of *Suaeda moquinii*) germination remains unaltered under different temperature regimes. Temperature doesn't affect the germination of some halophyte like brown seeds of Khan *et al*. (2001). Ahmed and Khan in (2010) reported that light also have great effect on germination of halophyte seed and their unavailability however effect the germination but with the salinity, synergistically repressed seed germination.

Typha domingensis, a tall (2.0 - 2.5 m) marshy grass, inhabits disturbed habitats with- continuous water supply (Abideen *et al.,* 2012; Gul *et al*., 2013). In Karachi, this grass is found to grow near coastal as well as inland areas usually flooded with sewage and rain water. Fluctuating water levels make this grass a good invader (Abideen *et al.*, 2012). *Typha domingensis* is a perennial grass with potential use for bioenergy and heavy metal remediation (Abideen *et al.*, 2014). *T. domingensis* was reported as promising candidate for bioenergy and other possible usages (Abideen *et al*., 2011; Munir *et al.*, 2020). Therefore, a thorough screening of this plant is needed. So the purpose of this study is to determine the effect of light, salinity and temperature on the seed germination of *T. domingensis.*

MATERIALS AND METHODS

Test Species and Seed Collection Site

Mature inflorescences of *Typha domenensis* (Pers.) were collected from populations located near University of Karachi campus, Karachi, Pakistan. Seeds were separated from inflorescence, cleaned manually and then surface sterilized for 1 min with 1% sodium hypochlorite. After sterilization the seeds were thoroughly washed with distilled water and air dried and stored in airtight plastic jars for 4 weeks.

Germination Experiments

Germination experiments were started after 4 weeks of collection by using programmed incubator (Percival Scientific, Boone, Iowa, USA) with 12 h photoperiod under four different temperature regimes 10/20, 15/25, 20/30 and 25/35 °C. For the experiments under dark, aluminum foil was wrapped around each petri dish (50x90 mm) and then covered with black polyethylene bags to ensure complete darkness. Four replicates of 25 seeds containing 5 mL of test solution were used for each treatment. The salinity treatments consisted of six NaCl concentrations (0, 100, 200, 300, 400 and 500 mM NaCl). Mean germination rate of germination velocity was estimated by using modified Timson's Index = $\Sigma G/t$, where G is the percentage of seed germinated at 2-day intervals and t is the total germination period (20 days) (Khan and Ungar, 1997). The maximum value possible for our data with this index is 50 (i.e., 1000/20) and higher values for rate of germination indicate faster germination. Final germination (emergence of the radicle) was recorded after 20 days. Un-germinated seeds exposed to different NaCl solutions and 12 h photoperiod was transferred into DW for a further 20 d to determine recovery from salinity. Un-germinated seeds in complete dark were exposed to a 12 h photoperiod for further 20 d to determine recovery from dark. Seeds which remain un-germinated during recovery were subjected to tetrazolium chloride test to check their viability.

Statistical Analyses

Data were subjected to two ways analyses of variance using SPSS Version 16.0 for windows (SPSS, Chicago, IL, USA). Bonferroni test (multiple range test) was performed to compare the significant differences among means. Means and standard errors were used to construct graphs by Sigma Plot for Windows ver. 10.0 (Systat Software, San Jose, CA, USA).

RESULTS AND DISCUSSION

Seed germination

A three ANOVA showed significant individual effect of salinity, temperature and light as well as their interactions ($P < 0.005$) on seed germination of *Typha domingensis* (Table 1). Optimum seed germination (95%) of *T. domingensis* in 12 h photoperiod was observed under non-saline control (Fig. 1). Although, few seeds germinated at 25/35 C in 500 mM NaCl, salinities higher than 200 mM NaCl inhibited seed germination by <10% in all temperature regimes (Fig.1). Results for decrease in seed germination with increasing salinity are in accordance to the previous studies (Gul *et al.,* 2010; Saeed *et al*., 2011) however, in most of the halophytes < 10% decrease is usually observed in salinities higher than 500 mM NaCl. Salinity induced dormancy in halophytes is inherent as it may provide an advantage to the plants for surviving in harsh climatic conditions for longer period of time (Khan and Gulzar, 2003; Khan and Gul, 2006). However, germination of fewer seeds at 300 mM NaCl even under optimum temperatures (25/35) indicates higher salt sensitivity of *T. domingensis* seeds compared to other halophytes (Khan and Gul, 2002). Seed germination was substantially decreased in complete dark under both non-saline and saline conditions regardless of Salinity and temperature treatments (Fig. 2). Seeds failed to germinate in salinities higher than 200 mM NaCl at all temperature regimes (Fig.2). Requirement of light for seed germination of different halophyte species is highly variable (Baskin and Baskin, 1998). Generally, seed germination is inhibited when they are buried deep in the soil or shaded under canopy cover of nearby plants (Schulz and Rve, 1999; Hroudiva and Zakravski, 2003). Both presence of salt and absence of light imposed twin stresses which may have caused germination in fewer seeds. Germination inhibition in dark could be a strategy of *T. domingensis* seeds to escape from unfavorable conditions (deep burialin soil / water). Rate of seed germination was highest in non-saline conditions. A two way ANOVA showed significant effect of temperature, salinity and their interactions (Table 2). Salinity above 100 mM NaCl and lower temperature (10/20 ^{o}C) significantly delayed rate of seed germination (Fig. 5).

Table 1. Results of Three-way analysis of variance of characteristics by temperature (T), salinity (S), light (L), and their interactions on final germination. All values are significant at the *P* < 0.005 level.

Independent Variables	**SS**	**DF**	**MS**	**F**
Light (L)	40401.0	1	40401.0	1496.3***
Temperature (T)	739.0	3	246.3	9.1***
Salinity (S)	97447.7	5	19489.5	721.8***
Light * Temperature (L x T)	584.3	3	194.8	7.2***
Light * Salinity (L x S)	30231.7	5	6046.3	223.9***
Temperature * Salinity (T x S)	1681.7	15	112.1	4.1***
Light * Temperature * Salinity (L x T x S)	2529.7	15	168.6	6.2***
Error	2592.0	96	27.0	

Table 2. Results of Two-way analysis of variance of characteristics by temperature (T), salinity (S) and their interactions on rate of germination. All values are significant at the P < 0.005 level.

Independent Variables	**SS**	**DF**	**MS**	**F**
Temperature (T)	263.7	3.0	87.9	11.0***
Salinity (S)	19014.2	5.0	3802.8	476.1***
Temperature * Salinity (T x S)	228.4	15.0	15.2	1.9*
Error	383.4	48.0	8.0	

Table 3. Results of two-way analysis of variance of characteristics by temperature (T), salinity (S), and their interactions on recovery from salinity. All values are significant at the P < 0.005 level.

Independent Variables	**SS**	**DF**	**MS**	**F**
Temperature (T)	2472.7	2.0	1236.4	3.3*
Salinity (S)	2551.4	4.0	637.9	1.7
Temperature * Salinity (T x S)	2379.5	8.0	297.4	0.8
Error	11142.0	30.0	371.4	

Table 4. Results of Two-way analysis of variance of characteristics by temperature (T), salinity (S), and their interactions on recovery from Dark. All values are significant at the P < 0.005 level.

Independent Variables	**SS**	**DF**	**MS**	**F**	
Temperature (T)	44.6	2.0	22.3	0.3	0.7
Salinity (S)	92310.6	5.0	18462.1	261.0	3.2***
Temperature * Salinity (T x S)	512.7	10.0	51.3	0.7	0.7
Error	2546.7	36.0	70.7		

Table 5. Results of Two-way analysis of variance of characteristics by temperature (T), salinity (S), and their interactions on Seed viability test. All values are significant at the P < 0.005 level.

Independent Variables	SS	DF	MS	F
Temperature (T)	321.8	2.0	160.9	12.6***
Salinity (S)	408.0	5.0	81.6	6.4***
Temperature * Salinity (T x S)	136.9	10.0	13.7	1.1
Error	458.7	36.0	12.7	

Recovery of germination from salinity and dark:

A Two way ANOVA showed significant effect of temperature, salinity and their interactions on recovery of un-germinated seeds (Table 3). Un-germinated seeds from NaCl treatments showed faster recovery (70-80%) when transferred to distilled water (Fig. 3) which indicates salt induced dormancy. However, recovery of un-germinated seeds in dark was observed up to 200 mM NaCl when transferred to light (Fig. 4 and Table 4). Most of the perennial halophytes show recovery in un-germinated seeds when transferred to distilled water representing conditional dormancy (Table 5) (Shen *et al*., 2003; Khan and Gul, 2006). Seed dormancy of *T. domingensis* in dark indicates a typical strategy of deep seed burial (Bewley and Black, 1994: Pons, 2000; Ren *et al*., 2002). However, the failure in recovery from dark to light in higher salinities (>200 mM NaCl) appear species specific due to combined stress of salinity and dark. Results indicate that *T. domingensis* is drought sensitive with moderate salt resistance as far as their seed germination is concerned.

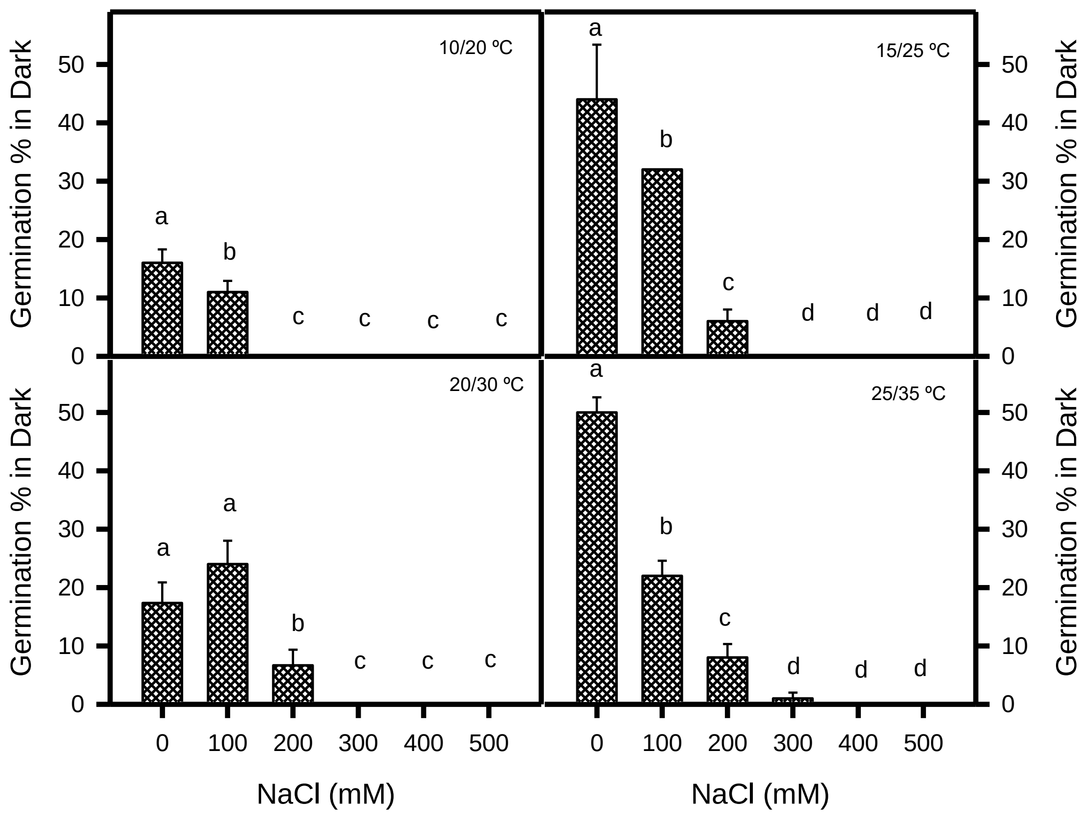

Fig. 1. Effect of light, salinity and temperature on the seed germination of *Typha domingensis*. Similar letters over bars represent mean germination percentages which are not significantly different (P<0.05) from each other at each NaCl concentration, (Bonferroni test.)

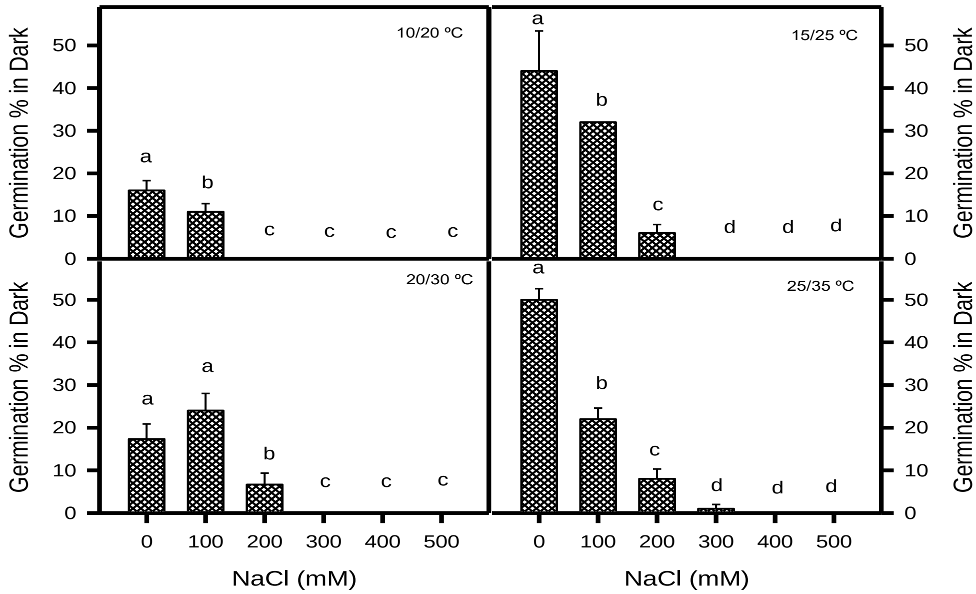

Fig. 2. Effect of Dark, salinity and temperature on the seed germination of *Typha domingensis*. Similar letters over bars represent mean germination percentages which are not significantly different (p<0.05) from each other at each NaCl concentration, (Bonferroni test.)

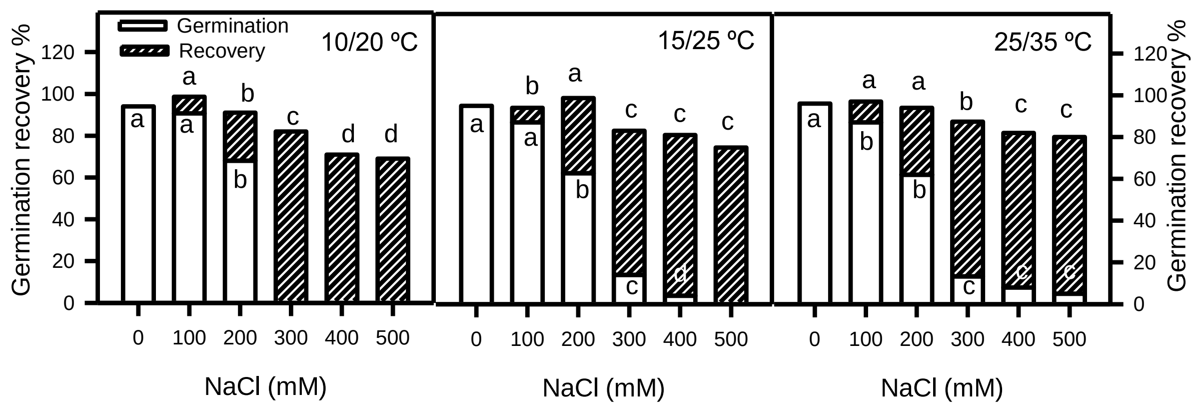

Fig. 3. Seed germination Recovery of *Typha domingensis* from salt (NaCl). Similar letters over bars represent mean germination percentages which are not significantly different (p<0.05) from each other at each NaCl concentration, (Bonferroni test.)

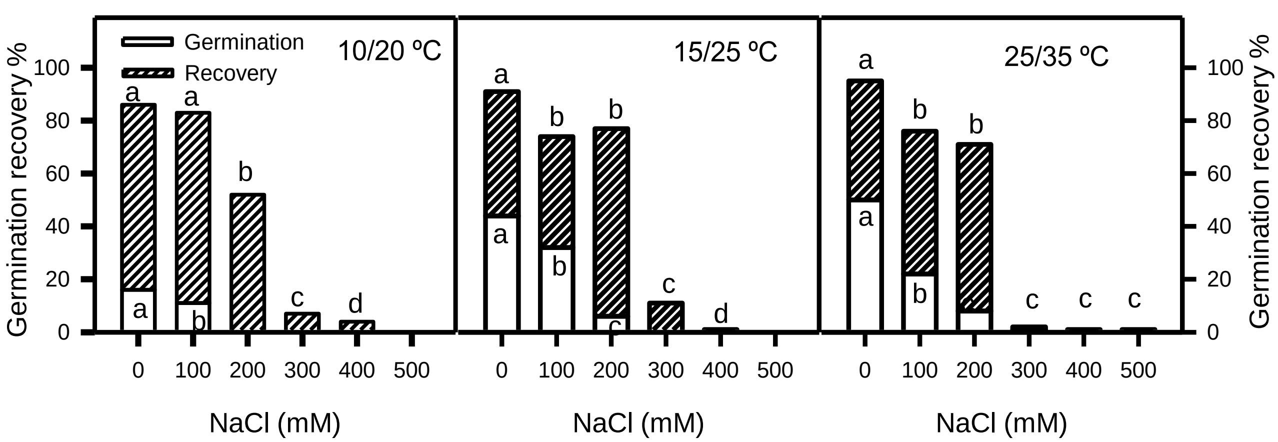

Fig. 4. Seed germination Recovery of *Typha domingensis* from complete dark (24 h Dark). Similar letters over bars represent mean germination percentages which are not significantly different (p<0.05) from each other at each NaCl concentration, (Bonferroni test.)

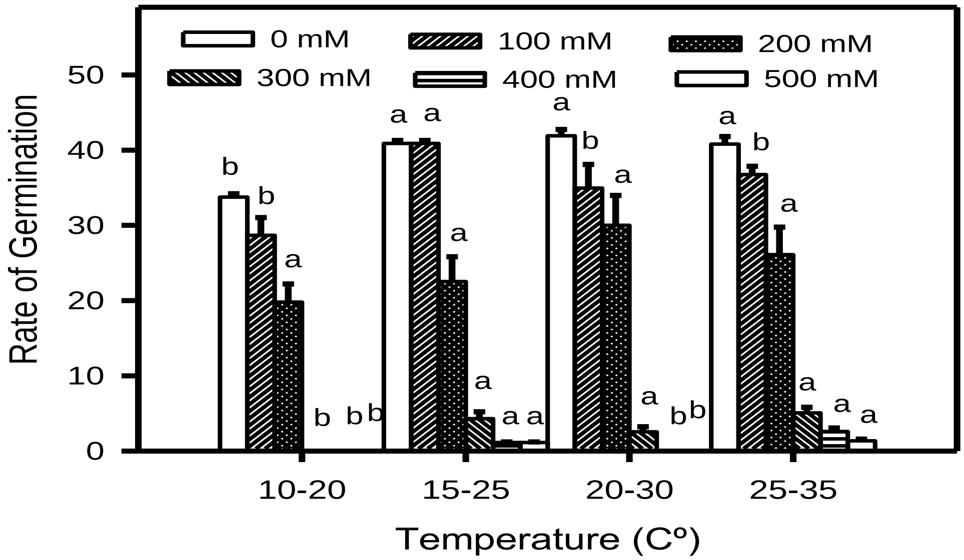

Fig. 5. Percentage of germinated, recovered, alive and dead seeds of *Typha domingensis* in response to salinity (0, 100, 200, 300, 400 and 500 mM NaCl) at all temperature regime in 12-h photoperiod. Different Bonferroni letters ($p < 0.05$) indicate significant differences among rate of germination at different NaCl concentration.

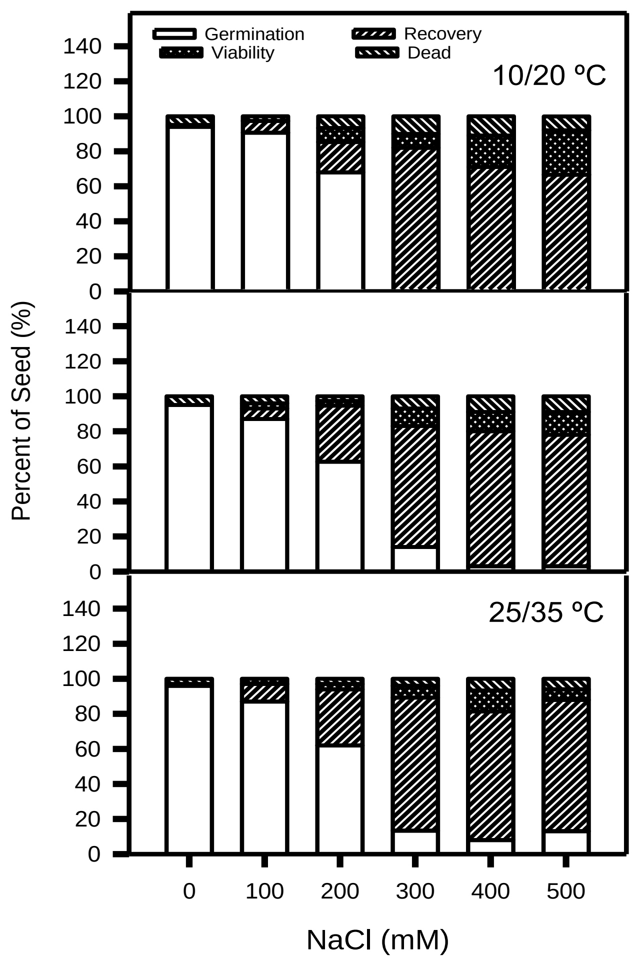

Fig. 6. Percentage of germinated, recovered, alive and dead seeds of *Typha domingensis* in response to salinity (0, 100, 200, 300, 400 and 500 mM NaCl) at all temperature regime in 12-h photoperiod.

Seed viability:

Viability tests showed that about 95% of the un-germinated *T. domingensis* seeds were viable but salt-induced dormant. Around 5% seeds were dead in all treatments (Fig. 6). Similar result was found in another study where seeds of other halophytes like *Halogeton glomeratus*, *Lepidium latifolium* and *Peganum hermala* remained viable under salinity at all temperature regimes (Ahmed and Khan, 2007).

Conclusions

This study suggested that salinity, temperature and light regulate seed germination in *Typha domingensis*. Moderate salt resistance and drought sensitivity at seed germination level indicates that *Typha domingensis* could be propagated by seeds in moderately saline environments with suitable light availability.

REFERENCES

Abideen, Z., R. Ansari and M.A. Khan (2011). Halophytes: Potential source of ligno-cellulosic biomass for ethanol production. *Biomass and Bioenergy*, 35(5): 1818-1822.

Abideen, Z., R. Ansari, B. Gul and M.A. Khan (2012). The place of halophytes in Pakistan's biofuel industry. *Biofuels*, 3(2): 211-220.

Abideen, Z., A. Hameed, H.W. Koyro, B. Gul, R. Ansari and M.A Khan (2014) Sustainable biofuel production from non-food sources-An overview. *Emirates Journal of Food and Agriculture*, 26(12): 1057-1066.

Ahmed, M. Z. and M.A. Khan (2010). Tolerance and recovery responses of playa halophytes to light, salinity and temperature stresses during seed germination. *Flora-Morphology, Distribution, Functional Ecology of Plants*, 205: 764-771.

Al Sherif, E. A. (2009). *Melilotus indicus* (L.) All., a salt-tolerant wild leguminous herb with high potential for use as a forage crop in salt-affected soils. *Flora-Morphology, Distribution, Functional Ecology of Plants*, 204: 737-746.

Baskin, C. C. and J.M. Baskin (2001). *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*. Elsevier.

Baskin, C.C. and J.M. Baskin (1998). *Seeds: ecology, biogeography, and, evolution of dormancy and germination*. Elsevier.

Bewley, J.D and M. Black (1994). *Seeds: Physiology of development and germination*. Second edition. Plenum Press New York and London.

Bhattacharjee, S. (2008). Triadimefon pretreatment protects newly assembled membrane system and causes up-regulation of stress proteins in salinity stressed *Amaranthus lividus* L. during early germination. *Journal of Environmental Biology*, 29: 805-810.

Dekkers, B. J., S. Pearce, R. P. van Bolderen-Veldkamp, A. Marshall, P. Widera, J. Gilbert and A. T. Wood (2013). Transcriptional dynamics of two seed compartments with opposing roles in Arabidopsis seed germination. *Plant Physiology*, 163: 205-215.

Fatima, N., Z. Abideen, M. Qasim, B. Gul and M.A. Khan (2019). Salinity resistance is linked with antioxidant activity, pigmentation pattern and anatomical adjustments in *Phragmites karka* (Retz.) Trin. Ex Steud. *International Journal of Biology and Biotechnology* 16: 631-639.

Forbis, T. A. (2010). Germination phenology of some Great Basin native annual forb species. *Plant Species Biology*, 25: 221-230.

Gul, B., R. Ansari, I. Aziz and M.A. Khan (2010). Salt tolerance of *Kochia scoparia*: a new fodder crop for highly saline arid regions. *Pakistan Journal of Botany*, 42: 2479-2487.

Gul, B., Z. Abideen, R. Ansari and M.A. Khan (2013). Halophytic biofuels revisited. *Biofuels*, 4:6: 575-577.

Gulzar, S. and M.A. Khan (2003). Germination responses of *Sporobolus ioclados*: a potential forage grass. *Journal of Arid Environments*, 53: 387-394.

Gulzar, S., A. Hameed, A.A. Alatar, A. K. Hegazy and M.A. Khan (2013). Seed germination ecology of *Cyperus arenarius*-a sand binder from Karachi coast. *Pakistan Journal of Botany*, 45: 493-496.

Hakim, M. A., A.S. Juraimi, M. Begum, M.M. Hanafi, M.R. Ismail and A. Selamat (2010). Effect of salt stress on germination and early seedling growth of rice (*Oryza sativa* L.). *African Journal of Biotechnology*, 9: 1911-1918.

Hegazy, A. K., N.T. Abdel-Ghani and G.A. El-Chaghaby (2011). Phytoremediation of industrial wastewater potentiality by *Typha domingensis*. *International Journal of Environmental Science and Technology*, 8: 639-648.

Hroudová, Z. and P. Zakravsky (2003). Germination responses of diploid *Butomus umbellatus* to light, temperature and flooding. *Flora-Morphology, Distribution, Functional Ecology of Plants*, 198: 37-44.

Jamil, M., C.C. Lee, S.U. Rehman, D.B. Lee, M. Ashraf and E.S. Rha (2005). Salinity (NaCl) tolerance of Brassica species at germination and early seedling growth. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, 4: 970-976.

Khan, M. A. and B. Gul (2006). Halophyte seed germination. In: *Ecophysiology of high Salinity Tolerant Plants*. Springer Netherlands, pp.11-30.

Khan, M. A. and M. Qaiser (2006). Halophytes of Pakistan: distribution, ecology, and economic importance. *Sabkha Ecosystems*, 2: 129-153.

Khan, M. A. and S. Gulzar (2003). Light, salinity and temperature effects on the seed germination of perennial grasses. *American Journal of Botany*, 90: 131-134.

Khan, M. A., B. Gul and D.J. Weber (2001). Salinity and temperature effects on the germination of dimorphic seeds of *Suaeda moquinii*. *Australian Journal of Botany*, 49: 185-192.

Khan, M.A (2002). Halophyte seed germination: success and pitfalls. In: *International symposium on optimum resource utilization in salt affected ecosystems in arid and semi-arid regions* (pp. 346-358).

Khan, M.A. and B. Gul (2002). *Some ecophysiological aspects of seed germination in halophytes. Halophyte Utilization and Regional Sustainable Development of Agriculture*, pp.56-68.

Khan, M.A. and I.A. Ungar (1997) Effects of thermoperiod on recovery of seed germination of halophytes from saline conditions. *American Journal of Botany*, 84(2): 279-283.

Linkies, A., K. Graeber, C. Knight and G. Leubner-Metzger (2010). The evolution of seeds. *New Phytologist*, 186: 817-831.

Munir, N., Z. Abideen and N. Sharif (2020). Development of halophytes as energy feedstock by applying genetic manipulations. *All Life*, 13(1); 1-10.

Nedjimi, B. (2009). Salt tolerance strategies of *Lygeum spartum* L: a new fodder crop for Algerian saline steppes. *Flora-Morphology, Distribution, Functional Ecology of Plants*, 204: 747-754.

Pons, T. L. (2000). Seed responses to light. In: *Seeds: the ecology of regeneration in plant communities*, *2*. (Eds.): M. Fenner. Second edition, CAB international.

Ren, J., L. Tao and X.M. Liu (2002). Effect of sand burial depth on seed germination and seedling emergence of *Calligonum* L. species. *Journal of Arid Environments*, 51: 603-611.

Saeed, S., B. Gul and M.A. Khan (2011). Comparative effects of NaCl and sea salt on seed germination of *Arthrocnemum indicum*. *Pakistan Journal of Botany*, 43: 2-14.

Schütz, W. and G. Rave (1999). The effect of cold stratification and light on the seed germination of temperate sedges (Carex) from various habitats and implications for regenerative strategies. *Plant Ecology*, 144: 215-230.

Shen, Y. Y., Y. L and S.G. Yan (2003). Effects of salinity on germination of six salt-tolerant forage species and their recovery from saline conditions. *New Zealand Journal of Agricultural Research*, 46: 263-269.

(*Accepted for publication February 2020*)