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INFLUENCE OF HEAT SHOCK ON GERMINATION, Na^+ AND K^+ LEAKAGE AND ELECTRICAL CONDUCTIVITY OF IMBIBED *CALLIGONUM* SEEDS

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Abstract

Relationships between mineral leakage and germination characteristics of five *Calligonum* seed in teat shock treatments were analyzed. The results suggested that heat shock stress for imbibing seeds significantly inhabited the germination and K^+ leakage, and induced more seed transform death. The greater EC induced decreasing of germination of five *Calligonum* species, and seeds with more leakage of K^+ performed greater germinability. Increasing of EC and Na^+ leakage induced more seed dead. Na^+ leakage and EC of germination medium always increased with temperature. K^+ in seeds inhabited germination, Na^+ in seeds determined the vigor of *Calligonum* seeds. Na^+/K^+ and EC both could be considered to be used for indications of seed vigor of Calligonum seeds.

Key words: Calligonum; Germination; Electrical conductivity; Leakage; Imbibition.

Introduction

Heat shock stress was a highly disadvantageous element for seed germination in the stage of plant life cycle by leading to biochemical deterioration and cell membrane system disorganization of imbibing seed (Kim *et al*., 2010; Rahoui *et al*., 2010). The structural disorganization of cell membranes during the initial phase of seed imbibition was critical to the survival and successful germination of seeds. The bankrupt of integrity of cell membrane lead to more permeable, many substances such as sugars, free amino acids, organic acids and various mineral ions including Na^+ and K^+ in seeds leached out into the germination medium. Electrical conductivity and minerals contents in leachates may reflect a general deterioration of seed tissues (Palabiyik and Peksen, 2008; Vieira *et al*., 2008; Beena & Jayaram, 2010; Min & Hong, 2014; Soliman & El-Shaieny, 2014).There was a strong relationship between the mineral ions in germination medium and membrane integrity (Rahoui *et al*., 2010). The electrical conductivity of leachates had been used as a standard method for testing vigor (Anon., 1987; Mavi *et al*., 2014). Seed quality may been indirectly evaluated through the measurement of the electrical conductivity of the imbibition medium. Low conductivities are considered to be an indication of high vigor because it is thought to represent a low level of cell membrane system disorganization. There were significant relationships between the electrical conductivity test and seed vigor (Devi *et al*., 2003; Mirdad *et al*., 2006; Peksen, 2007; Khan *et al.*, 2010; Lazar *et al*., 2014). Because Na^+ and K^+ are small in size and physiologically important, their ratio in imbibitions medium could be a better indicator of seed vigor than the electrical conductivity (Cheng *et al*., 2005).

Calligonum species could survive and reproduce in active sand dunes in conditions of extreme drought. They were dominant perennial shrub in the northern desert of China. They played important role in halting desert encroachment and stabilizing sand dune (Ren, 2001). However, there had been little experimental research dealing with the germination reposes of them in relation to the environmental uncertainty, such as temperature (Ren *et al*., 2005). *Calligonum* seeds were difficult to raise seedlings due to the heat shock stress and low water potentials. The effects of high temperatures on seed vigor of *Calligonum* were variable. In desert zone, rainfall events may be frequent but small, seeds are likely to become partially imbibited under high environment temperature, undergo periodic hydration and minerals leakage (Yasmin *et al*., 1999; Khan *et al*., 2010). Any level of minerals leached from seeds which reduces seed vigor was likely to be critical for successful germination.

The electrical conductivity in imbibition mediums and leaching of Na^+ and K^+ of *Calligonum* seeds had been investigated in the present study, the objectives were to determine the effects of heat shock on the germination and vigor of imbibing seeds, to explore the relationships electrical conductivity in germination medium, Na^+, K^+ leaching and germination characteristics of *Calligonum* species under heat shock stress.

Material and Methods

Plant material and treatment: Seeds of five *Calligonum* species (*C. caput-medusae* Schrenk, *C. arborescens* Litv., *C. alaschanicum* A. Los., *C. potaninii* A. Los. and *C. mongolicum* Turcz.) were collected from at least 10 plants in August, 2013 at Shapotou Desert Research and Experimental Station of the Chinese Academy of Sciences (37°32′ N, 105°02′ E, 1339 m a.s.l.), Ningxia Province, China. The aborted and predated seeds were discarded. The selected seeds were stored in cloth bag under laboratory conditions (18-22°C, 8-12% moisture content) until initiation of any experiments (storage in this manner did not affect the dormancy or viability of the seeds). Intact plump seeds were surface sterilized with Na-hypochlorite prior to any experimental usage, for each experiment, seeds were mixed and then allocated at random.

Seed soaking experiment: Seeds having uniform size, color and shape with intact coats were selected and subjected to imbibitions experiments. Seeds were sterilized with 10% Na-hypochlorite prior to any experimental usage for 10 minutes (Na-hypochlorite have no effect on seed germination), and disinfected in 0.1% mercuric chloride for 3 min, and finally with distilled water rinse several times. For one replicate 5g (about 50 seeds) seeds were soaked in the dark at the high temperature setting (20°C, 25°C, 30°C, 35°C, 40°C) over two sheets of filter paper moistened with 150 mL distilled water for 24 hours. All temperature treatments were consisted of six replicates of 5g seeds.

Seed leachate test: The germination media was analysed for electrolyte and the Na+ and K+ after 24h imbibition of seeds. Special care was taken to remove most of the medium from the filter paper by folding the paper and applying pressure. Small volumes of H_2O were used to wash the medium from filter paper. This operation was repeated three times. The electrical conductivity of germination medium was measured using a conductivity meter (CONSORT Model C533), and expressed on the basis of seeds mass (µS.cm-1g-1). Depending on detection limits, the contents of Na^+ and K^+ in germination medium were measured using the inductively coupled plasma optical emission spectrometry (ICP-AES).

Germination experiment: *Calligonum* Seeds that had undergone 24 hours soaking experiments under heat shock were placed on wet filter paper in glass petri dishes (11.5 cm diameter × 2 cm depth). These were then placed into the temperature-controlled chambers for germination, set at 14 h of daylight at 25°C and 10 h of darkness at 12°C, to approximate general springtime field conditions. To ensure no systematic effects due to position within the chamber, petri dishes were re-arranged at random every 2d. All germination experiments consisted of six replicates of 40 seeds for each temperature treatment. Visible radicle growth was used to define germination. Germination was recorded every 5 d and allowed to proceed for 9 weeks except where specified. Ungerminated seeds were soaked in water at 30°C for 24 hours. Seed coats were cut and the embryo was soaked in 1% tetrazolium chloride for 24 hours at 30°C. Pink embryos were scored as alive. Germination was expressed as percentage of viable seeds germinated.

Statistical analysis: The results of leakage experiments presented are the mean values obtained from 6 replicates. The leakage index (*LI*) was determined using the following equation:

$$LI = \frac{C - C_0}{C_0}$$

where LI is the mineral leakage index, *C* is the electrical conductivity or contents of mineral elements under high temperature treatments (mg/L), C_0 is the electrical conductivity or contents of mineral elements under 20°C treatments (mg/L).

All percent germination data were arcsine-square-root transformed prior to analysis. Arcsine-transformed means and standard errors were backtransformed for graphic presentation. Multiple comparisons of means were made with Duncan's tests at 95%. The following parameters were determined.

$$\text{Germination} = \frac{\text{Number of germinating seeds}}{\text{Number of seeds initiated}} \text{ X } 100\%$$

$$\text{Relative Germination} = \frac{\text{Number of germinating seeds}}{\text{Number of viable seeds initiated}} \text{ X } 100\%$$

$$\text{Dormancy} = \frac{\text{Number of ungerminated but viable seeds}}{\text{Number of seeds initiated}} \text{ X } 100\%$$

$$\text{Mortality} = \frac{\text{Number of inviable seeds}}{\text{Number of seeds initiated}} \text{ X } 100\%$$

Statistical analysis was performed based on STATISTICA. The data were analyzed through one-way analysis of variance (ANOVA) to determine the effect of temperature treatments and species, and Duncan's multiple comparison tests were performed to determine the statistical significance of the differences among means of different temperature treatments and species.

Results

Germination and dormancy: The germination of imbibed seeds was significantly different among heat shock treatments in all five species (one-way ANOVA: $F_{4,15}$=25.48, p<0.001 for *C. caput-medusae*; $F_{4,15}$=38.65, p<0.001 *C. arborescens*; $F_{4,15}$=8.36, p<0.01 for *C. alaschanicum*; $F_{4,15}$=54.63, p<0.001 for *C. potaninii*; $F_{4,15}$=87.47, p<0.001 for *C. mongolicum*). The relative germination also performed significant difference among heat shock treatments in all five species (one-way ANOVA: $F_{4,15}$=5.34, p<0.01 for *C. caput-medusae*; $F_{4,15}$=24.43, p<0.001 *C. arborescens*; $F_{4,15}$=22.36, p<0.001 for *C. alaschanicum*; $F_{4,15}$=44.23, p<0.001 for *C. potaninii*; $F_{4,15}$=52.31, p<0.001 for *C. mongolicum*). For all species, the lowest germination and relative germination both occurred at 40°C treatment and the highest at 20°C treatment, and all heat shock treatments significantly decreased the germination and relative germination of *C. caput-medusae, C. arborescens, C. potaninii* and *C. mongolicum*. Only higher temperature treatments (35°C and 40°C) could significantly decrease the germination and relative germination of *C. alaschanicum* (Figs. 1-2).

There was significant difference in the mortality among different heat shock treatments for five species (one-way ANOVA: $F_{4,15}$=95.34, p<0.001 for *C. caput-medusae*; $F_{4,15}$=46.327, p<0.001 *C. arborescens*; $F_{4,15}$=38.57, p<0.01 for *C. alaschanicum*; $F_{4,15}$=48.56, p<0.001 for *C. potaninii*; $F_{4,15}$=28.66, p<0.001 for *C. mongolicum*). For all species, the higher temperature treatments (35°C and 40°C) always led to the significant increasing of mortality, 25°C and 30°C treatments had no significant influence on mortality comparing with 20°C treatment (Fig. 3). The dormancy did not perform unanimous response on heat shock treatments, and influence of heat shock on dormancy of *Calligonum* seeds was significant difference (one-way ANOVA: $F_{4,15}$=5.24, p<0.05 for *C. caput-medusae*; $F_{4,15}$=13.37, p<0.01 *C. arborescens*; $F_{4,15}$=8.36, p<0.01 for *C. alaschanicum*; $F_{4,15}$=31.27, p<0.001 for *C. potaninii*; $F_{4,15}$=41.68, p<0.001 for *C. mongolicum*). The higher temperature treatments (35°C and 40°C) always brought on more imbibed seeds *C. caput-medusae* and *C. arborescens* to dormancy. The 25°C heat shock treatment significantly induced more seeds of C. *mongolicum* to dormancy (Fig. 4).

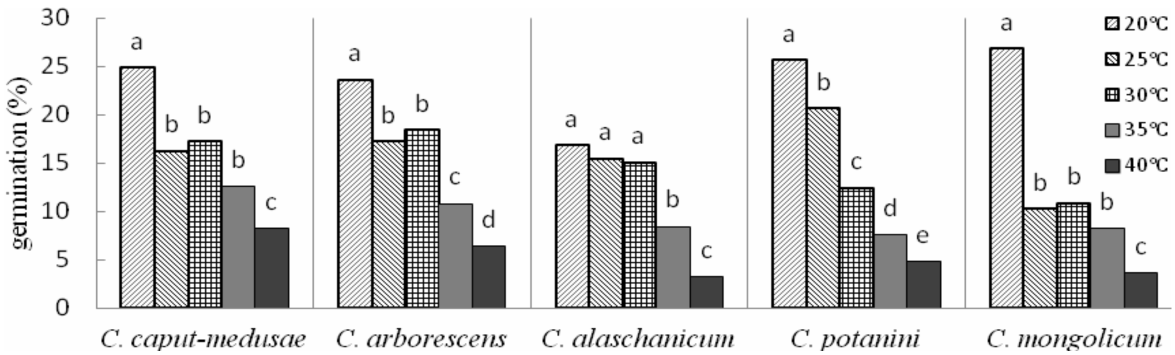

Fig. 1.Germination (%) of imbibed seeds under heat shock treatments for 5 *Calligonum* species. Bars with the same letters are not significantly different at $p<0.05$ according to Duncan's multiple comparison test.

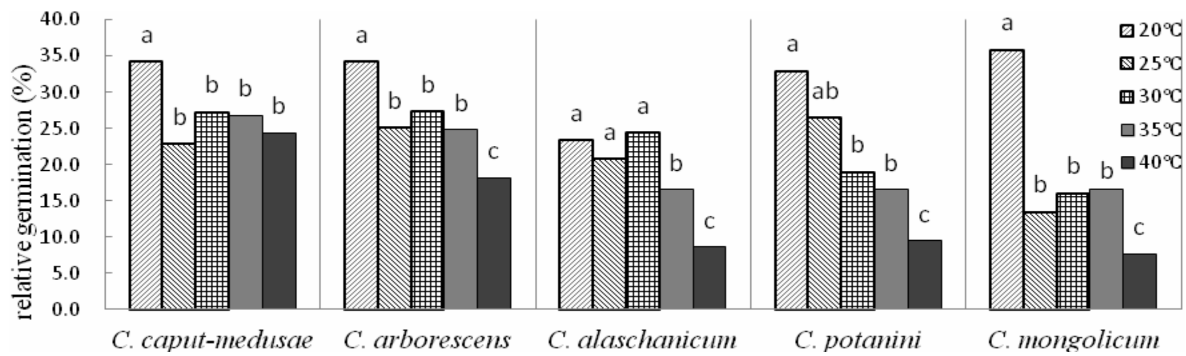

Fig. 2.The relative germination (%) of imbibed seeds under heat shock treatments for 5 *Calligonum* species.

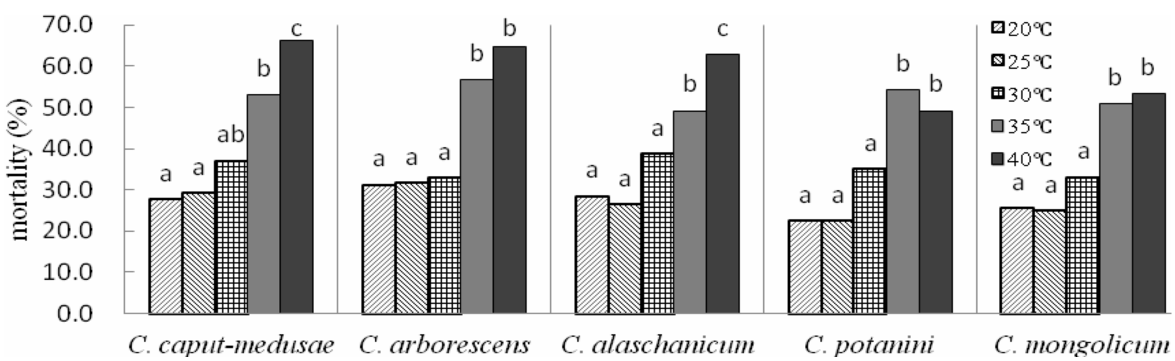

Fig. 3.The mortality (%) of imbibed seeds under heat shock treatments for 5 *Calligonum* species.

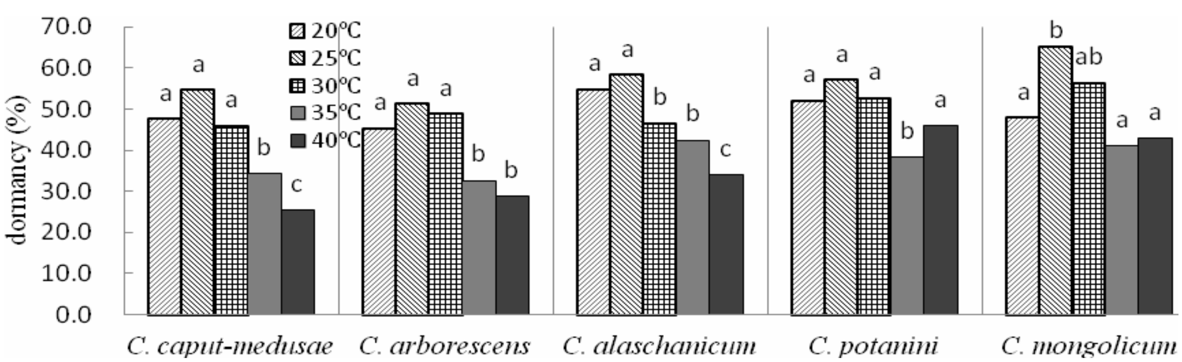

Fig. 4.The dormancy (%) of imbibed seeds under heat shock treatments for 5 *Calligonum* species.

Electrical conductivity: A two-way ANOVA showed that electrical conductivity of germination medium was significantly affected by different heat shock treatments and species, and their interaction (Table 1). The electrical conductivity was significantly different among five heat shock treatments and five species. The harder heat shock treatments (30°C, 35°C and 40°C) significantly accelerated the rise of electrical conductivity of germination medium, and the electrical conductivity always increased with temperature for all five species. Under ambient temperature treatment (20°C), *C. potaninii* and *C. mongolicum* performed lower electrical conductivity comparing other species, but they both dramatically increased with the rise of temperature, and reach significantly the highest electrical conductivity at 35°C and 40°C temperature treatments (Table 2).

Leakage Na^+ and K^+: A two-way ANOVA showed that Na^+ leakage was significantly affected by different temperature treatments and species, and their interaction (Table 1). The Na^+ contents in germination medium were significantly different among heat shock treatments for *C. caput-medusae*, *C. arborescens*, *C. alaschanicum* and *C. potaninii*, the Na^+ leakage always increased with the rise of temperature, and the harder heat shock treatments (35°C and 40°C) significantly induced more Na+ leakage for these four species. The Na+ leakage at 40°C treatment even reached five times as much as the ambient temperature treatment for *C. arborescens* and *C. alaschanicum*. Under ambient (20°C) and lower heat treatments (25°C, 30°C).*C. potaninii* always performed least Na+ leakage comparing with other all species, but under 35°C and 40°C treatments, the Na+ leakages dramatically increased and were significantly higher than lower temperature treatments. Heat shock did not influence on the Na^+ leakage of imbibing seeds of *C. mongolicum* (Table 2).

A two-way ANOVA showed that K^+ leakage was significantly affected by different heat shock treatments and species, and their interaction (Table 1). The K^+ contents in germination medium were significantly different among temperature treatments for all species (Table 2). The K^+ contents always performed significantly differences among all temperature treatments for all species. The K^+ leakage significantly decreased with the rise of temperature. There were significantly difference for K^+ leakage among species under each heat shock treatment, but the differences were not very distinct (Table 2).

A two-way ANOVA showed that the Na^+/K^+ in germination medium was significantly affected by different temperature treatments and species, and their interaction (Table 1). The Na^+/K^+ in germination medium always performed significantly differences among all temperature treatments for each species. The Na^+/K^+ performed same increasing trend with the rise of temperature, like the exhibition of Na^+ leakage (Table 2).

Relationship between leakage and germination: There were significant negative correlations between germination and EC under all temperature treatments, only at 40°C treatment, the germination presented significantly correlations with Na+ leakage and Na+/K+. The relationship between mortality and EC under 40°C treatment was significantly positive and negative under 40°C treatment. Under all heat shock treatments, the mortality always performed significantly correlation with Na^+ leakage and Na^+/K^+ (Table 3).

Table 1. The analysis of variance for the effects of different temperature treatments and imbibitions times, and their interaction on electrical conductivity (EC), and contents and leakage index (*LI*) of Na^+ and K^+ in germination medium of imbibing seeds of *C. arborescens* under high temperature stress.

Traits	Source of variation	df	F-value	P
EC	Temperature treatment	6	1572.913***	<0.0001
	Species	3	398.432***	<0.0001
	Temperature treatment × species	27	347.301***	<0.0001
Na^+	Temperature treatment	6	177.145***	<0.0001
	Species	3	131.035***	<0.0001
	Temperature treatment × species	27	181.209***	<0.0001
K^+	Temperature treatment	6	138.112***	<0.0001
	Species	3	21.665***	<0.0001
	Temperature treatment × species	27	80.893***	<0.0001
Na^+/K^+	Temperature treatment	6	129.602***	<0.0001
	Species	3	63.237***	<0.0001
	Temperature treatment × species	27	98.748***	<0.0001
LI of Na^+	Temperature treatment	3	20.688***	<0.0001
	Species	4	9.861***	<0.0001
	Temperature treatment × species	12	12.325***	<0.0001
LI of K^+	Temperature treatment	3	276.154***	<0.0001
	Species	4	117.296***	<0.0001
	Temperature treatment × species	12	322.310***	<0.0001

Table 2. The electrical conductivity (EC) and content of Na^+ and K^+ in germination medium of imbibing seeds of *Calligonum* under heat shock stress. For each attribute the mean values with the same lowercase letters among temperature in same row were not significantly different at 5% level of probability (Duncan's multiple comparisons test), and with the same capital letters among species in same column are not significantly different.

Traits	Species	20°C	25°C	30°C	35°C	40°C	F-value
EC (μS/cm/g)	*C. caput-medusae*	207.43aA	248.80bA	266.48bA	287.18bA	366.73cA	349.27***
	C. arborescens	275.55aB	251.51aA	317.02bB	350.83cB	336.68cA	53.94***
	C. alaschanicum	298.27aB	283.97aA	376.37bC	391.78bC	485.25cB	214.36***
	C. potanini	120.83aC	284.63bA	354.32cC	423.87dC	498.62eB	995.02***
	C. mongolicum	180.27aA	369.32bB	411.22bD	469.97cD	534.75dB	1235.75***
	F-value	703.776***	488.57***	334.10***	84.00***	373.28***	
Na^+ (ppm)	*C. caput-medusae*	14.47aA	18.93bA	19.91bA	28.16cA	23.79bA	7.76***
	C. arborescens	14.97aA	18.37bA	28.84cB	84.87dB	78.56dB	500.38***
	C. alaschanicum	16.88aA	19.5aA	26.34bB	24.37bA	79.49cB	302.97***
	C. potanini	5.72aB	6.73aB	8.15aC	27.59bA	30.12bA	210.95***
	C. mongolicum	28.49C	26.05C	30.28B	28.26A	29.46A	0.8
	F-value	22.57***	174.82***	48.02***	140.08***	51.60***	
K^+ (ppm)	*C. caput-medusae*	258.88aA	167.07bA	148.65c	127.17dA	103.68eA	426.53***
	C. arborescens	215.85aB	183.37bA	163.08bc	144.63cB	115.92dA	133.47***
	C. alaschanicum	226.82aB	208.25bB	175.18c	156.5cB	125.37dB	235.94***
	C. potanini	232.8aAB	185.82bA	146.78c	165.42cB	137.32cC	181.14***
	C. mongolicum	231.5aAB	201.85bB	187.35bc	163.73cB	133.97dC	20.15***
	F-value	188.58***	291.37***	2.61	40.24***	305.66***	
Na^+/K^+	*C. caput-medusae*	0.053aA	0.109bA	0.151bA	0.218cA	0.231cA	6.485**
	C. arborescens	0.071aA	0.097aA	0.169bA	0.603cB	0.669cB	207.407***
	C. alaschanicum	0.080aA	0.098aA	0.151bA	0.164bC	0.632cB	139.580***
	C. potanini	0.024aB	0.041bB	0.054bB	0.170cC	0.221cA	335.224***
	C. mongolicum	0.118aC	0.132aC	0.158aA	0.168aC	0.216bA	4.150*
	F-value	13.63***	88.12***	115.01***	118.92***	64.18***	

* Means significant difference at 0.05 level, ** Means significant difference at 0.01 level, *** Means significant difference at 0.001 level

Table 3. The correlations between leakage traits and germination characteristics of imbibed seeds under heat shock stress (n=5).

Temperature	Traits	EC	Na^+	K^+	Na^+/K^+
20°C	Germination	-0.77*	0.09	0.27	0.06
	Relative germination	-0.57	0.16	0.21	0.13
	Mortality	0.90***	0.22	-0.30	0.25
	Dormancy	0.02	-0.28	-0.03	-0.27
25°C	Germination	-0.73*	-0.05	-0.48	0.04
	Relative germination	-0.85**	0.08	-0.55	0.20
	Mortality	-0.59	0.47	-0.07	0.53
	Dormancy	0.95**	-0.35	0.55	-0.50
30°C	Germination	-0.80**	-0.23	-0.20	-0.24
	Relative germination	-0.81**	-0.04	-0.17	-0.01
	Mortality	-0.21	0.70*	0.19	0.83**
	Dormancy	0.70*	-0.37	0.04	-0.47
35°C	Germination	-0.92***	0.07	0.02	0.07
	Relative germination	-0.89**	0.30	-0.16	0.31
	Mortality	-0.36	0.69*	-0.57	0.73*
	Dormancy	0.71*	-0.66	0.48	-0.69*
40°C	Germination	-0.84**	-0.76*	0.11	-0.73*
	Relative germination	-0.90***	-0.61	-0.08	-0.55
	Mortality	-0.79**	0.72*	-0.67	0.80*
	Dormancy	0.87**	0.16	0.46	0.06

* Means significant correlation at 0.05 level, ** Means significant correlation at 0.01 level, *** Means significant correlation at 0.001 level

Discussion

Decline in germination and increase in mortality in response to heat shock suggested that *Calligonum* could be sensitive to temperature, may be attributed to altered metabolism of imbibing seeds. heat shock could affect many physiological processes including protein and lipid metabolism and enzyme activity (Madhava Rao *et al*., 2002; Kurganoval *et al*., 1997). The greater Na^+ leakage and smaller K^+ leakage in response to heat shock induced lesser seeds to germinate and more seeds transform death, which are considered that Na^+ and K^+ probably play important roles in germinating seeds of *Calligonum*, the intrinsic-extrinsic balance of K^+ and Na^+ in *Calligonum* seeds could control the germination, dormancy so much as death. it suggested that the Na^+ impacted the viability of seeds seriously, and the K^+ inhabited seed germination (Cheng *et al*., 2005; Rahoui *et al*., 2010), the significantly correlation between Na^+ leakage and mortality under all heat shock treatments indicated that Na^+ leakage may be lethal factor for *Calligonum* seeds. At ambient temperature treatment, strong relationships were found between EC and mortality, the heat shock stress can destroy the integrity and augment the permeability of seed membrane, and motivate more electrolyte leakage, and induced more seeds lost vigor. (Ilbi & Eser, 2006; Mirdad *et al*., 2006; Peksen, 2007; Kim *et al*., 2011), it could be determined that EC was an important test to predict and estimate the germination potential of *Calligonum* seeds (Demir *et al*., 2012).

Conclusion

Heat shock treatments significantly decreased the germination and relative germination, and increased the mortality of imbibed seeds of five *Calligonum*. The greater EC induced decreasing of germination of five *Calligonum* species, and seeds with more leakage of K^+ performed greater germinability. Increasing of EC and Na^+ leakage induced more seed dead. The Na^+ content in germination medium always increased with temperature. The K^+ leakage performed similar changing trends with the germination, relative germination and dormancy with the rise of temperature. The heat shock treatments significantly inhibited the K^+ leakage, and this inhibited effect always induced more seeds dead. The Na^+/K^+ and EC both can be used to reflected seed vigor. EC, leakage of Na^+ and K^+ of *Calligonum* seeds were significantly affected by heat shock treatments. EC of germination medium of imbibing seeds of *Calligonum* performed increasing with temperature corresponding with the mortality. EC could be considered to be an indication of seed vigor lost due to its strong relationship with mortality.

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