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*Full Length Article*

# Cross Adaptation of *Medicago sativa* Seedlings Germinated from Ultra-dried Seeds to Saline and Alkaline Stresses

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## Abstract

Seed ultra-drying reduces seed moisture content to 5–7% for safe storage. Many experiments have been carried out to study the effect of ultra-drying on seeds, but how germination behaviour from ultra-dried seeds grown under stresses have not been studied. Alfalfa (*Medicago sativa* L.) seeds were temporally (0, 12, 24, 48, 72, 96, 120, 144 and 216 h) ultra-dried with silica gel to study the responses of resulting seedlings to40 mM $L^{-1}$salinity and15 mM $L^{-1}$ alkalinity. The result indicates that seedlings raised from seeds with moisture contents ranged between 5.18% and 5.72% performed better at selected salinity (40 mM $L^{-1}$) and alkalinity (15 mM $L^{-1}$) with higher chlorophyll contents, total soluble sugar and root activity but lower MDA content, and showed neutral and alkaline salt resistant capabilities. In our study, ultra-drying of alfalfa seeds maintains seed viability, by improving the saline and alkaline resistant capabilities of resulting seedlings. © 2015 Friends Science Publishers

**Keywords:** Alfalfa; Moisture content; Seedling growth; Salinity; Alkalinity; Stress

## Introduction

Alfalfa (*Medicago sativa* L.) is a perennial legume that has been sown widely in China and worldwide due to its high production, nutritive value and the ability to fix nitrogen from the atmosphere. Seed production is the major path to preserve and spread alfalfa. Researchers have found that fatty acids account for 9.85% of all the storage substances in alfalfa seed, within which unsaturated fatty acids only take up 40.13% (Yang, 2004). This makes the seed more susceptible to oxidation and therefore results in rancidity and quality degradation. Storage of seeds at -10 to 20°Cis the most commonly used method for germplasm conservation, but this method becomes too costly in many developing countries. Seed ultra-dried storage is a technique which reduces seed moisture content (MC) to 5–7% (Li *et al*., 2007). Studies on *Brassica juncea*, *B. napus* and *Helianthus annuus* have shown that ultra-drying treatment could prevent seed deterioration during storage and improve seed longevity significantly (Ellis and Hong, 1996). Low MC storage has now become a cost-effective technique for germplasm conservation (Zheng *et al*., 1998).

The influence of MC and storage temperature on red clover and alfalfa seeds longevity reported by Ellis and Hong (2006) showed no damage to seeds longevity when the MC was reduced to 5–7% within the temperature ranged from 30°C to 65°C. However, no study has investigated the impact of ultra-drying on the physiological responses of the seedlings produced from ultra-dried alfalfa seeds, especially on their resistance to environmental stresses such as soil salinity and alkalinity.

Globally, salinization has emerged as a major constraint to the sustainable development of irrigation farming with more than 100 countries reporting increases in the occurrence of salt-affected soils due to sodium salts acclamation (Rengasamy, 2006; Barakat *et al.*, 2013). Soil alkalinity (soil caused by alkaline salts) is reported to be causing even greater effects on seedling's survival rate than those of salinity (soil caused by neutral salts) (Peng *et al.*, 2008). Saline and alkaline (sodic) soils ($6.7\times10^{10}m^2$) cover approximately 7% of cultivated lands in China (Deng *et al*., 2006) and is considered to be a serious problem of natural grasslands and farming lands in northwest China.

He *et al*. (2000) suggested that hydration-dehydration treatment may increase seeds germination under salt stress condition. The mechanism for improving seedling tolerance to stress relates to increased enzyme activity and reduced lipid peroxidation together with maintaining the integrity of membrane system due to decreased mass $Na^+$ assimilation and $K^+$ exosmosis (He *et al*., 2000).

This study investigated cross adaptation capability of seedlings derived from differently ultra-dried alfalfa seeds to

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conditions of 40 mM $L^{-1}$ saline stress and 15 mM $L^{-1}$ alkaline stress. These stress concentrations were established from previous research (Huo *et al*., 2011a, b). Pot culture in sand with Hoagland's solution was used in Longdong cultivation, the most widely planted alfalfa cultivar in northwest China.

## Materials and Methods

### Alfalfa Seed Source

Seeds of *Medicago sativa* L. *cv*. Longdong were collected in Oct. 2007 from Lanzhou Forage Station, which forms part of Gansu Agricultural University, China (authority: Gansu Agricultural University). At the time of experiment, the seeds had been stored for 1.5 years at ambient temperature (23–26°C) and humidity (60%) in ventilated darkness with a germination percentage of 85.5%. At the time of collection, the initial seed purity was 98.5%, initial germination percentage (GP) was 78.8% (after damaged seeds been discarded, the initial hard seed rate was approximately 20%), and the initial MC was 9.05%.

### Seed Ultra-drying Treatment

Approximately 160 g seeds were packaged in porous nylon bags (20 g/bag), placed into hermetic desiccator and buried in cooled silica gel that diurnally dried at 120°C, the ratio between seeds and silica gel was 1 to 10 (w/w) (Li *et al*., 2007). After desiccation for 12, 24, 48, 72, 96, 120, 144 and 216 h, seed MC was reduced to 7.09, 6.93, 6.36, 5.72, 5.46, 5.18, 4.97 and 4.59%, respectively, with the initial MC of 9.03% for the control. Ultra-dried and control seeds were sealed in aluminium foil packages and stored in a desiccator fulfilled with dried silica gel at ambient temperature, approximately 23–26°C. Seeds MC was determined by drying seeds at 105°C for 72 h according to the oven-drying method and gravimetric analysis.

### Pre-pot Culture Treatment of Ultra-dried Seeds

Ultra-dried seeds were placed into hermetic glass desiccator at various relative humidities (RH) produced by $CaCl_2$ (RH = 35.0%), $NH_4Cl$ (RH = 75.0%) and distilled water solution (RH = 100.0%) successively for re-hydration, 24 h for each procedure before sowing to avoid imbibition injury (Huang *et al.*, 2002), at ambient temperature of 23–26°C.

### Saline and Alkaline Stress Design

NaCl and $Na_2SO_4$ were mixed (mole ratio: 9 to 1), and $NaHCO_3$ and $Na_2CO_3$ were mixed (mole ratio: 9 to 1) to simulate typical saline and alkaline stress in soil (Liu *et al*., 2008), respectively. The chosen optimum stress concentration for salinity was 40 mM $L^{-1}$, and for alkalinity was 15 mM $L^{-1}$ according to Huo *et al*. (2011a, b).

### Sand Culture Experiment

In May 2009, pot culture with sand was set up to investigate the physiological and biochemical responses of seedlings derived from ultra-dried alfalfa seeds to saline and alkaline stress.

Twenty-five seeds of each treatment (MC based, the control was included) were evenly scattered in polyvinyl plastic pots which was 7 cm in diameter, 7.5 cm in height, and which had already loaded with 480 g dry river sand. Before sowing, the sand was screened with 2 mm sieve, rinsed with distilled water for 3 times and autoclaved at 121°C for 2 h.

Saline stress experiment: all the treatments be covered with 40 g other sand on the top of seeds, placed into polyvinyl plastic trays with half-strength Hoagland's solution containing 40 mM $L^{-1}$ salinity, then the culture solution (the pH value was adjusted to 7.0, with 1 mol $L^{-1}$ NaOH or HCl) infiltrated progressively from the bottom of pots to the top surface, 4 replicates for each treatment.

Alkaline stress experiment: consult the method used above, half-strength Hoagland's solution containing 15 mM $L^{-1}$ alkalinity was used, no need for pH value adjustment.

All treatments were left in trays with relevant culture solutions (40 mM $L^{-1}$saline solution and 15 mM $L^{-1}$ alkaline solution, respectively) to a depth of 1 cm, topped up to the marked level with distilled water daily and changed weekly to maintain the needed stress concentrations. Pots were arranged in the trays in a randomized block design. Typical conditions during the 22 day treatment period were: temperature 24–28°C, relative humidity 45–93% and maximum photon-flux density under 12 h of light 1430 μmol $m^{-2}$ $s^{-1}$. Shoot height of 8 seedlings chosen randomly from each treatment was recorded on day 5, 10, 15 and 22 from emergence.

### Biomass Analyses

The experiment was performed in a controlled environment growth chamber at Gansu Agricultural University (36°05′18″, 103°42′8″E; altitude: 1520 m). On day 22 of seedling emergence, all the sand were completely washed out, leaflet number and leaflet area (the second trifoliate leaf from the top) (with a leaf area meter: CI-203, CID, Inc, USA) were recorded together with the corresponding root length and shoot height with 8 randomly chosen seedlings. Then, underground and aboveground biomasses of the chosen seedlings were separated at the soil line and were oven dried for 48 h at 70°C to determine dry weight (Bertrand *et al*., 2007).

### Seedling Biochemical Indices Detection

Root dehydrogenase activity was measured with triphenyltetrazolium chloride (TTC) reduction technique (Huang and Gao, 2000).

Fresh roots (0.5 g) were placed into test tubes with 0.6% (w/v) TTC in 0.06 mol $L^{-1}$ $Na_2HPO_4$-$KH_2PO_4$ and 0.05% (w/v) Tween 20. The tubes were incubated in a 30°C water bath for 20 h. Water-insoluble red compound formed from the reduction of TTC was extracted in 95% (v/v) ethanol in a 60°C water bath for 4 h. The absorbance of the extractants was recorded at $480_{nm}$ with a spectrophotometer (S2000, WPA Company, UK). TTC reduction quantity was calculated according to standard curve.

Chlorophyll in fully expanded leaves (a randomly chosen mixture for both old and young leaves) from each treatment was extracted using 0.5 g of material in 10 mL of 80% aqueous acetone. 1 mL of the suspension was diluted with another 2 mL of 80% aqueous acetone after filtering. Chlorophyll a (Chl a) and chlorophyll b (Chl b) contents were determined with a spectrophotometer (S2000, WPA Company, UK) using three wavelengths (663, 646 and 470). Concentrations of pigments [mg $g^{-1}$ fresh weight (f. wt)$^{-1}$] were obtained by calculation (Barnes *et al*., 1992).

Leaf lipid peroxidation was estimated according to the formation of malondialdehyde (MDA), an end-product of lipid peroxidation (Heath and Paker, 1968). Lipid peroxides were extracted from 0.5 g fresh leaf samples with 5 mL of 0.1% trichloroacetic acid (TCA). After centrifugation at 10000r/min for 5 min, 1 mL of the supernatant was mixed with 4 mL of 20% TCA containing 0.5% thiobarbituric acid (TBA). The mixture was incubated in boiling water for 30min, then transferred to an ice bath to stop the reaction. The absorbance was read at 532 and 600 for calculation (Cakmak and Horst, 1991).

Four replicates of fresh leaf samples (0.5g) were used in total soluble sugar measurement according to Stieger and Feller (China Forage Plants Volunteer Editors Committee, 1987). The centrifuged filtrates (10–20 µL) were mixed with 1mLanthrone reagent (20 mL ethanol, 200 mg anthrone, 100 mL 1 M $H_2SO_4$) and were heated for 10 min in boiled water bath and then ended by incubating the samples on ice for 10 min, the absorbance value was read at 623. Glucose (0–50 µg) was used for calibration (Roulin and Feller, 2001).

### Statistical Analysis

Statistical analyses were conducted using one-way ANOVA by SNK testat 0.05 probability with SPSS 16.0 (SPSS Inc.). Separate analyses of variance were done on data from seeds with various moisture contents. Standard errors of the mean were calculated for each seed moisture contents.

## Results

### Seedling Height

Seedling height exhibited the same response during the initial stage of emergence (day 5 to 15), with only the 4.97 and 5.15% moisture contents at day 10 significantly performing the control (9.03% treatment). However, by day 22 after emergence, shoot height from seeds of MC 6.93 to 5.18% exceeded the controlat40 mM $L^{-1}$ saline stress. Seed MC and shoot height were binomially related ($R^2$=0.88) with height between 6.93 and 5.18% MC all significantly higher than the control. The peak of unimodal curve was at 5.46% ($P<0.05$) (Fig. 1).

Under 15 mM $L^{-1}$ alkaline stress, shoot heights showed a similar pattern within day 5 and day 10 from emergence with 5.46% MC out-performing the control. On day 15 from emergence, 5.72 and 5.46% MC have shoots significantly higher than control. This pattern continued for 5.46 to 4.97% seed MC to day 22 from emergence, whereas shoot height of seeds with initial MC of 4.59% treatment was reduced ($P<0.05$), indicating that excessive ultra-drying treatment is harmful to seedling growth.

### Individual Leaflet Number

Individual leaflet number increased logarithmically with decreasing seed MC under both saline and alkaline stress condition (Fig. 2). All the treatments except with 7.09% MC had more leaflets compared with the air-dried control ($P<0.05$), indicating that ultra-drying of alfalfa seeds promoted leaf differentiation of seedlings grown under salt stress.

### Individual Leaflet Area

Leaflet area exhibited a unimodal curve for seedlings grown under saline and alkaline stress (Fig. 3). The seeds with 5.72–4.97% and 6.36-5.18% MC performed significantly better than control ($P<0.05$) for saline and alkaline condition, respectively.

### Underground Biomass

There was no significant difference in root biomass under saline stress between control and other ultra-drying treatments except for better root growth ($P<0.05$) in 5.72–5.18% seed MC (Fig. 4). For alkaline stress, root biomass was significantly higher for 6.93–5.46% MC but with time an abrupt decline occurred in 5.18–4.59% treatments (Fig. 4).

### Aboveground Biomass

Aboveground biomass for all ultra-dried treatments was significantly higher ($P<0.05$) compared with the control in both saline and alkaline stresses, respectively (Fig. 5).

### Root Length

Seedlings with 6.36–4.59% MC had a more extensive root system ($P<0.05$) than the air-dried control when grown

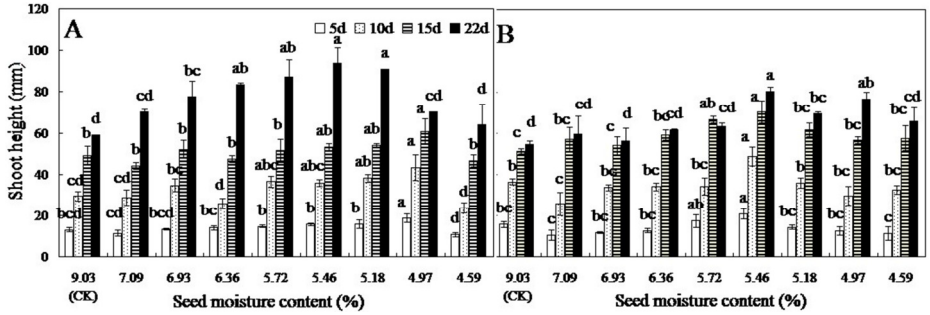


**Fig. 1:** Shoot height of seedlings derived from ultra-dried alfalfa seeds (MC based) in response to 40 mM $L^{-1}$ saline stress (A) and 15 mM $L^{-1}$alkaline stress (B) on 22nd day of emergence. Different lowercase letters indicate treatment means for each seed MC are significantly different from each other (SNK test; $P<0.05$)

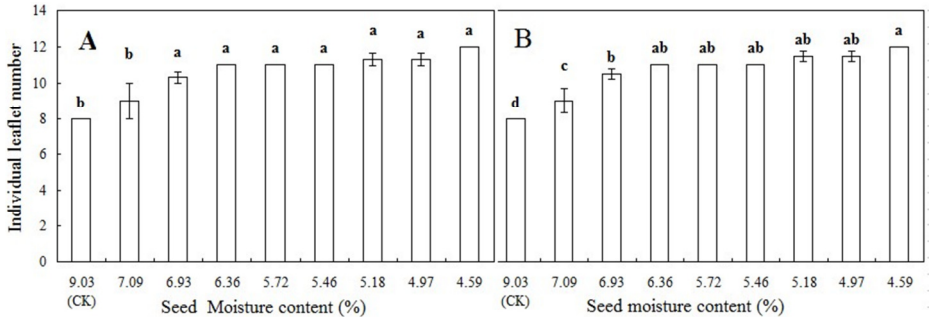


**Fig. 2:** Individual leaflet number of seedlings derived from ultra-dried alfalfa seeds (MC based) in response to 40 mM $L^{-1}$ saline stress (A) and 15 mM $L^{-1}$alkaline stress (B) on 22nd day of emergence. Different lowercase letters indicate treatment means for each seed MC are significantly different from each other (SNK test; $P<0.05$)

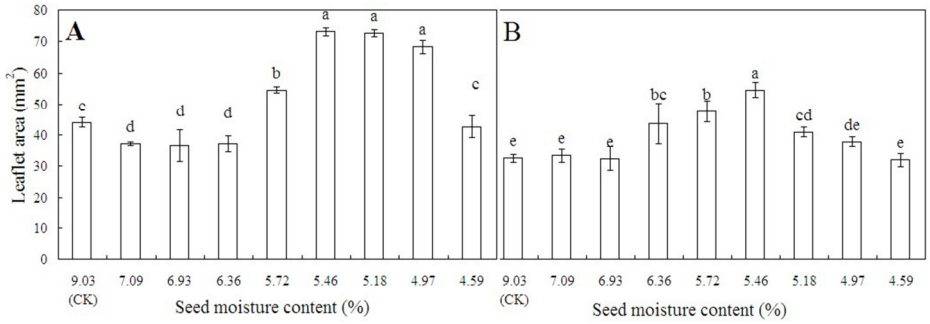


**Fig. 3:** Leaflet area of seedlings derived from ultra-dried alfalfa seeds (MC based) in response to 40 mM $L^{-1}$ saline stress (A) and 15 mM $L^{-1}$ alkaline stress (B) on 22nd day of emergence. Different lowercase letters indicate treatment means for each seed MC are significantly different from each other (SNK test; $P<0.05$)

under saline stress (Fig. 6). For alkaline stress, seed with MC in 5.72–4.97% range produced more root length ($P<0.05$) (Fig. 6).

**Leaf MDA Content**

Leaf MDA contents in alfalfa seedlings on 22 d from emergence (Fig. 7) decreased significantly as seed MC declined to minimum from 6.93–5.18% and 6.93–5.46%, respectively, for saline and alkaline stress indicating that moderate ultra-drying helped to decrease damage to plants grown in salt stress, where as excessive ultra-drying caused un-repairable plant injury.

**Leaf Total Soluble Sugar Content**

Soluble sugar contents first increased and then decreased with decline of seed MC under both saline and alkaline stress conditions (Fig. 8).

A unimodal curve was obversed at seed MC range from 6.36 to 4.59% under saline stress together with a significant binomial correlation between soluble sugar content and seed MC ($R^2=0.80$), the peak of which occurs at 5.46% and is 92.43% times higher than the control and

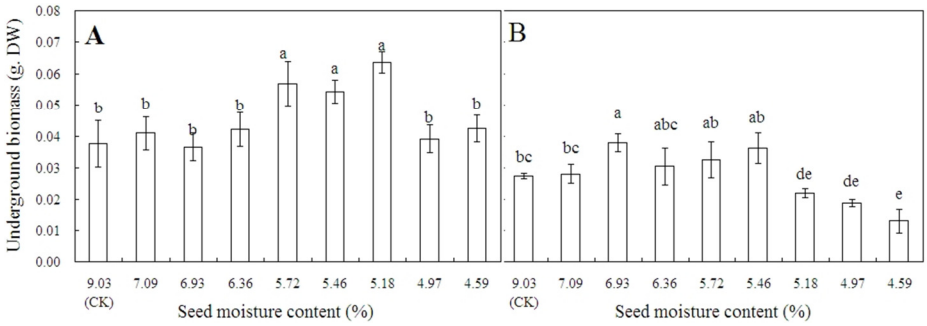


**Fig. 4:** Underground biomass of seedlings derived from ultra-dried alfalfa seeds (MC based) in response to 40 mM $L^{-1}$ saline stress (A) and 15 mM $L^{-1}$ alkaline stress (B) on 22nd day of emergence. Different lowercase letters indicate treatment means for each seed MC are significantly different from each other (SNK test; $P<0.05$)

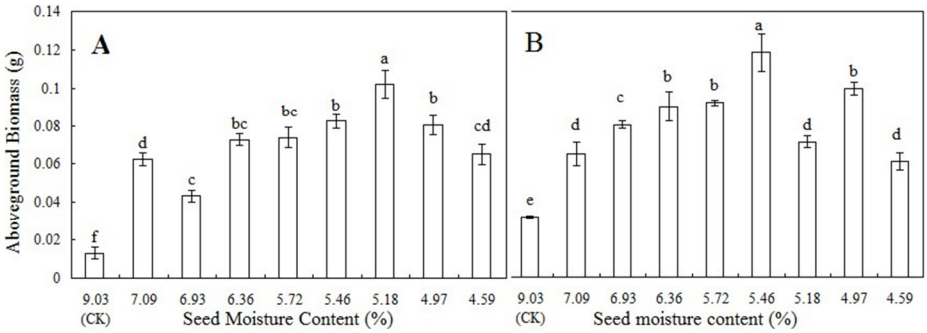


**Fig. 5:** Aboveground biomass of seedlings derived from ultra-dried alfalfa seeds (MC based) in response to 40 mM $L^{-1}$saline stress (A) and 15 mM $L^{-1}$ alkaline stress (B) on 22nd day of emergence. Different lowercase letters indicate treatment means for each seed MC are significantly different from each other (SNK test; $P<0.05$)

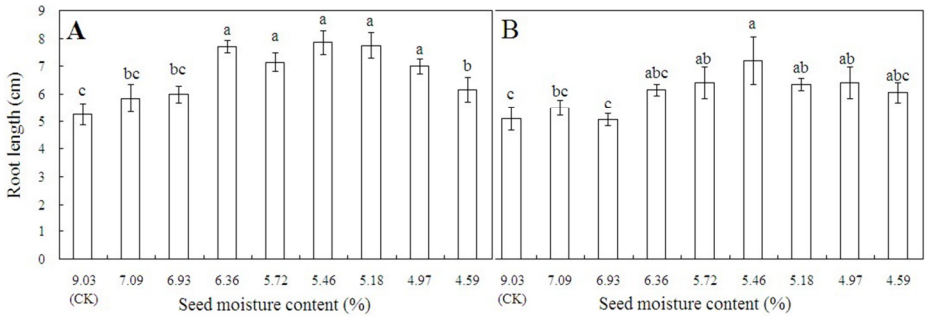


**Fig. 6:** Root length of seedlings derived from ultra-dried alfalfa seeds (MC based) in response to 40 mM $L^{-1}$saline stress (A) and 15 mM $L^{-1}$ alkaline stress (B) on 22nd day of emergence. Different lowercase letters indicate treatment means for each seed MC are significantly different from each other (SNK test; $P<0.05$)

within the seed MC range from5.46 to 4.59%, significant negative correlation occurred ($R^2$=0.93) (Fig. 8).

All the treatments except with MC of 7.09% out-performed the control under alkaline stress, with 6.36% to 5.46% seed MC performed extremely better than other stress treated groups, which is 102.84–135.95% times higher than the control, indicating that ultra-drying treatment effectively promote soluble sugar (Fig. 8).

**Root Dehydrogenase Activity**

Root activity measure the plant's capability for nutrition assimilation and transportation. Root growth and activity directly affects aboveground biomass. Root dehydrogenase activity was measured in seedlings on 22 d of emergence (Fig. 9), values of the treatments increase firstly and then decreased as the decrease of seed MC.

Under saline stress, all but treatment with 7.09% MC performed better than control with the peak at 5.46% MC, which is 155.93% times higher than the control. Within seed MC range from 6.93 to 5.46%, the root dehydrogenase activity correlated with seed MC significantly negatively ($R^2$=0.97).

All the ultra-drying treated groups performed better

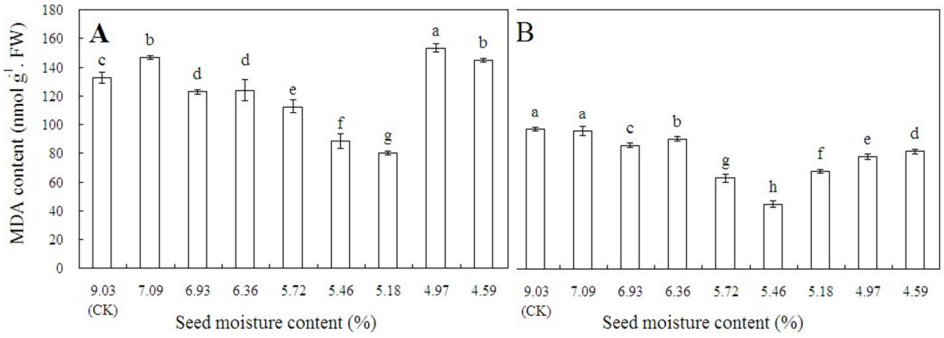


**Fig. 7:** Leaf MDA content of seedlings derived from ultra-dried alfalfa seeds (MC based) in response to 40 mM $L^{-1}$ saline stress (A) and 15 mM $L^{-1}$ alkaline stress (B) on 22nd day of emergence. Different lowercase letters indicate treatment means for each seed MC are significantly different from each other (SNK test; $P<0.05$)

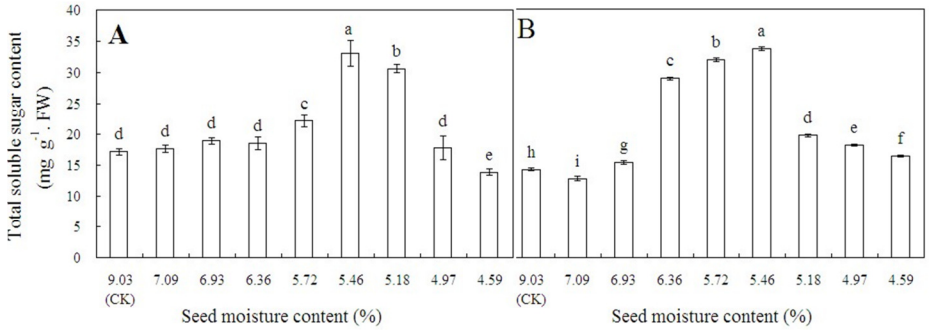


**Fig. 8:** Leaf total soluble sugar content of seedlings derived from ultra-dried alfalfa seeds (MC based) in response to 40 mM $L^{-1}$saline stress (A) and 15 mM $L^{-1}$ alkaline stress (B) on 22nd day of emergence. Different lowercase letters indicate treatment means for each seed MC are significantly different from each other (SNK test; $P<0.05$)

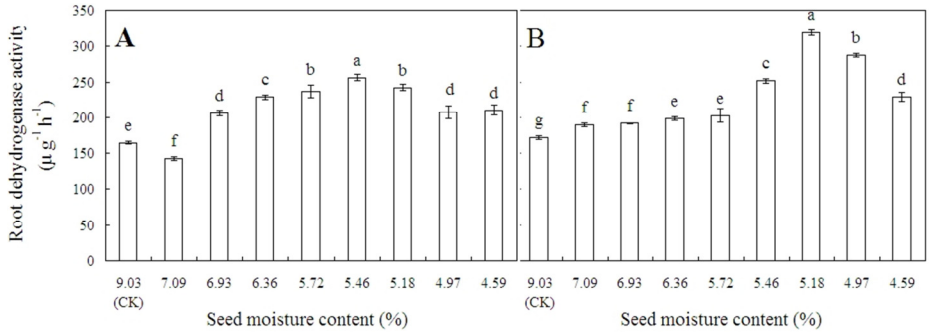


**Fig. 9:** Root dehydrogenase activity of seedlings derived from ultra-dried alfalfa seeds (MC based) in response to 40 mM $L^{-1}$ saline stress (A) and 15 mM $L^{-1}$ alkaline stress (B) on 22nd day of emergence. Different lowercase letters indicate treatment means for each seed MC are significantly different from each other (SNK test; $P<0.05$)

than the air-dried control ($P<0.05$) under alkaline stress (Fig. 9).

### Leaf chlorophyll Content

Chlorophyll contents of all treatments presented a close to or higher value compared with the control under saline stress ($P<0.05$) (Fig. 10), among which, treatments with 5.72 and 5.46% seed MC produced 43.59% and 71.31% times higher chlorophyll contents than the control, respectively. And under alkaline stress, a significant binomial curve exhibited between seed MC and chlorophyll content with the peak at 5.46% seed MC ($P<0.05$).

## Discussion

Seed viability is influenced when ultra-dried to different MC. Woodstock and Simkin (1976) found that ultra-low MC keep onion seeds viable and increase in longevity to 40 times of sesame was found with decrease in MC to 2–5%

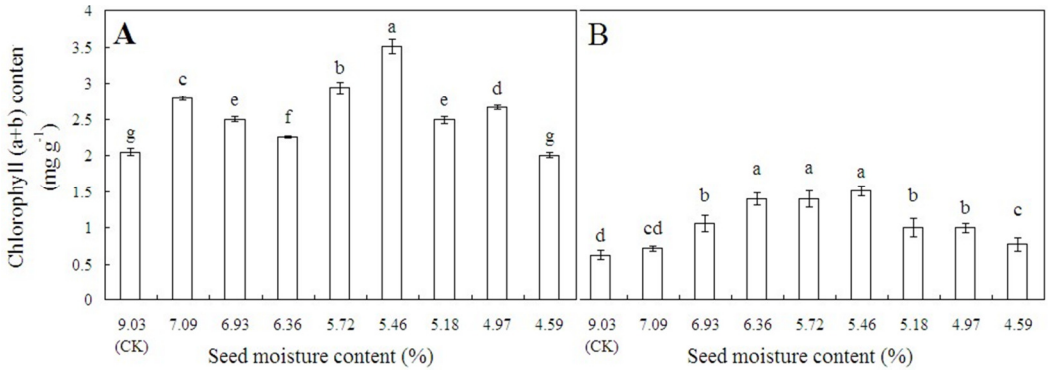


**Fig. 10:** Leaf chlorophyll content of seedlings derived from ultra-dried alfalfa seeds (MC based) in response to 40 mM $L^{-1}$ saline stress (A) and 15 mM $L^{-1}$ alkaline stress (B) on 22$^{nd}$ day of emergence. Different lowercase letters indicate treatment means for each seed MC are significantly different from each other (SNK test; $P<0.05$)

(Ellis *et al*., 1986). This study investigated key physiological and biochemical parameters measured in seedlings derived from seed ultra-dried to different MC when grown under salt stress conditions. Alfalfa seeds with MC between 5.72 and 5.18% significantly improved seedling performance in most physiological parameters than naturally dried control. This indicates that moderate seed ultra-drying could potentially promote growth and tolerance of alfalfa seedlings under salt stress conditions.

Pinhero *et al.* (1998) reported that free radical induced damage plays a key role in seed deterioration during aging. Seed deterioration is always associated with active oxygen accumulation like superoxide radical ($O_2^-$) and hydrogen peroxide ($H_2O_2$), which is inclined to initiate cascade reactions that resulting in the production of hydroxyl radical ($OH^-$) and other destructive substances like lipid peroxides (Noctor and Foyer, 1998). Efficient destruction of $O_2^-$ and $H_2O_2$ requires the synchronous action of different antioxidant enzymes (Song *et al*., 2004). It is believed that seed ultra-drying increased enzymes activity of ascorbate peroxidase, catalase, superoxide dismutase, glutathione reductase and peroxidase, as reflected in our previous study and many other studies (Li *et al.*, 2007; Huo *et al*., 2011c; ), which play important role in scavenging free radicals and peroxides and maintain high vigour of ultra-dried seeds, indicating that changes of antioxidant enzymes activities are closely related to desiccation tolerance and the ultra-drying does not destroy enzymes.

Rosenberg and Rinne (1986) showed that some new polypeptides are synthesized when the soybean seeds harvested 35 d after flowering and treated to lose moisture. This suggests that reduce in MC might be pre-requisite for new proteins synthesis during seeds maturation. For alfalfa, rigidity commonly exist usually 10–20%, and as high as 25–65% for newly harvested seeds (China Forage Plants Volunteer Editors Committee, 1987) and ultra-drying may also accelerate the post maturation process including the synthesis of new substances. It is proposed that the accumulation of osmoregulation substances like soluble sugar formed the biochemical basis which increased alfalfa resistance to osmotic stresses. Saline and alkaline stresses injure plants through osmotic stress which equates to a physiological drought. Hence, for ultra-dried seeds, the desiccation stress enhances osmotic resistance capability, which is retained through the germination and seedling emergence processes.

Re-hydration before sowing may contribute to enhanced alfalfa seed tolerance, during which the cell membrane system is restored (Stieger and Feller, 1994), the storage component metabolized, seed enzymatic activity increased, membrane lipid peroxidation restrained and the synthesis of RNA and proteins(He *et al*., 2000) is accelerated. All these changes which occur during re-hydration are stored and accumulated in seeds. In addition, re-hydration may alleviate cell membrane system damage, bring changes to the inner structure of seeds and the metabolism process, and increase seed vigour (Stieger and Feller, 1994). The re-aligned membrane system during re-hydration promoted the morphological repair, reduced loses of intracellular substances and accelerated seed germination. But this conclusion needs further experiment to better understand the role of these biochemical characteristics in seed germination and seedling adaptation to stress.

Lipid peroxidation commonly occurs when plants senescence or the plant organs are damaged. Lipid peroxidation mediated by free radical and peroxides is one of the most probable reasons for seed viability loss during storage. In our experiment, malondialdehyde (MDA) content was highest in treatments with MC of 4.59 and 4.97% under saline stress and the underground root biomass was lowest in the 4.59% treatment under alkaline stress, indicating that ultra-low MC could decrease seed viability. Stieger and Feller (1994) reported that at ultra-low MC, the membrane system cannot maintain its continuity which leads to exosmosis of intracellular substances during re-hydration and ultimately to decreased seed vigour. The change in intracellular substances may influence the metabolism and ion exchange within seeds (Woodstock and Simkin, 1976; Huang *et al*., 2002), as well as the imbibition injury during germination (Ellis *et al*., 1986).

Based on the performance of seedlings grown under saline and alkaline conditions, we hypothesised that the changes occurred in ultra-dried seeds as reflected in enzymes activity, osmo-regulation substances and integrity of seed membrane system relating to seed viability have been transferred to seedlings and results in resistance of seedlings to stresses, which could be explained by cross adaptation either cross tolerance or cross resistance, and operate at the cellular level for abiotic stresses(Xu *et al*., 1996), and have been proved in many species (Radyukina *et al*., 2012; Volkov *et al*., 2006). In our experiment, ultra-drying as a seed pre-treatment enhanced seedling tolerance to salt stress through cross adaptation.

Saline stress involves osmotic stresses ($Na^+$ and $Cl^-$), but for alkaline stress, high pH value also take effect. Usually alkaline salts cause severe damage to soil physical property, mineral nutrient balance and microbes as well as plant root (Shi and Zhao, 1997). Therefore, the effects of alkalinity on seedling survival rate were more significant than those of salinity (Tang and Turner, 1999), which has been demonstrated in many plant species (Shi and Yin, 1992; Yan *et al*., 2006).The mechanism of salinity tolerance in plants mainly involves osmotic adjustment and toxic ions avoidance(Hamdia *et al*., 2004). However, the mechanism of alkalinity tolerance in plants is still unclear. But according to our experiment, all the treated seedlings except with MC of 4.59% treatment under saline stress and 7.09% treatment under alkaline stress exhibited a better performance than control.

Root is the key structure for plant to contact with soil, and environmental abiotic stresses primarily injure the roots. Alkalinity tolerance of plants is always associated with the maintaining of root tissue structure (Bell *et al*., 1993) and in present study, all the ultra-dried seeds had better performance in root dehydrogenase activity except 7.09% treatment for saline stress.

Osmotic inhibition mediated salinity affects plant growth by restraining water absorption of seeds and causing nutritional imbalance (Launch and Epstein, 1990) of plants. Compared to ultra-dried alfalfa seeds grown in neutral Hoagland's solution (Huo *et al*., 2011b), all ultra-dried seeds grown under stress showed delay in germination. This indicates that salt stress restrained the seedling emergence on the whole, possibly due to lower water potential since germination speed is determined principally by water absorption rate. When cultural solution concentration was lower than 0.4 mM $L^{-1}$ (i.e. equal to 2.3%, saline stress), seeds are capable of absorbing water from the surrounding environment, but relatively at low rate (Zhu *et al*., 2000), leads to germination delay. High salt concentration decreased the quantity and rate of water uptake, resulting in a low free water contents in cell, thereby reducing metabolic rate. Under salt stress, seedlings derived from ultra-dried seeds germinated at a significantly faster rate than the un-ultra-dried control. This may be influenced by the decrease of water potential in seeds due to ultra-drying and the increase in osmoregulation substances. Compared with simultaneous experiment(Huo *et al*., 2011b), stressed seedlings grew relatively weaker on the whole, reflecting a combination of their lower capacity to absorb water, the hardness of membrane reparation, and the damage of membrane structure caused during the imbibition process. These are associated with the leaking of solutes ($K^+$) and its replacement with toxic ions ($Na^+$). The damage of salt stresses to seedlings increased with time and finally affected seed germination and seedling subsistence.

Another important observation is the significant change in seedling chlorophyll contents when grown under different salt conditions. Compared with stress free seedlings, the seedlings grown under saline and alkaline stresses exhibited significantly higher and lower chlorophyll contents, respectively, which reflect the combined effect of nutritional damage, disordered ions balance and metabolic disturbance within the alfalfa seedlings (Liu *et al*., 2008). Generally, neutral salt stress interferes with nutrient cations uptake like $K^+$, $Ca^{2+}$, $Mg^{2+}$( Drenovsky and Richards, 2003; Tuna *et al*., 2007), and alkalinity reduces N availability through the volatilization of mineralized $NH_4^+$ and decreases P, Ca and Mg solubility besides the above effect (Lajtha and Schlesinger, 1988), which ultimately influence chlorophyll synthesis and contents.

## Conclusion

In brief, the above experiment demonstrates that moderate seed ultra-drying is capable of producing the same or better germination of alfalfa compared to naturally dried seeds under stress conditions. Ultra-drying could therefore provide a cost-effective means to improve alfalfa establishment in saline and/or alkaline soils. The optimum storage MC for alfalfa differed from other species indicating that 5% is not a standard as previously thought. Seed ultra-drying technique has great potential in germplasm preservation, but it still has great difficulty in widely application. Nevertheless, it could provide benefits in germination in saline and alkaline soils for alfalfa which makes further study of the effects of ultra-drying on cross adaptation to stress.

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