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Full Length Article

Effect of Seed Ultra-Drying on Growth and Physiology of Alfalfa Seedlings

Shuqing Zhang[1,2] Jianfeng Li[2*] and Pinghui Huo[3]
[1]*Guizhou Provincial Key Laboratory of Geographic State Monitoring of Watershed, Guizhou Education University, Guiyang, 550018, China*
[2]*Institute of Soil and Environment Bioremediation in Karst habitats, Guizhou Education University, Guiyang, 550018, China*
[3]*Institute of Tropical Pratacultural Science, Lingnan Normal University, Zhanjiang, 524048, China*
[*]For correspondence: alexclover@foxmail.com
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Abstract

Ultra-drying is a technique used to reduce seed moisture content to levels ensuring safe long-term storage at ambient temperature. For alfalfa (*Medicago sativa* L.) seeds, ultra-drying to a moisture content of 2 to 5% increases seed survival rate and vigor. Little is known for its effects on subsequent alfalfa growth and yield. This study evaluated physical and physiological indices to detect differences in germination and seedling growth of ultra-dried alfalfa seed at critical seed moisture content (MC). Germination percentage (GP) of alfalfa seed (cv. Longdong) ultra-dried to seed MC of 5.7 to 5% was higher than air-dried control whereas GP was lower than the control at 4.6% seed MC. Seedling derived from seeds with 5.2% MC had higher total biomass than the control due primarily to a higher biomass of thicker roots and higher root dehydrogenase activity. Enhanced growth from ultra-dried seed increased with age of seedlings derived from seeds dried from 5.7 to 5.2% and had more leaf area and height than the control and seeds dried to 5% or less with less leaf area. Leaf malondialdehyde (MDA) content decreased in seedlings harvested from un-ultra-dried seeds with MC from 9.1 to 5.5%, but increased significantly in seedlings from seeds dried to a MC <5.5%. © 2020 Friends Science Publishers

Keywords: Alfalfa; Seed moisture content; Seedling growth; Seed desiccation

Introduction

The longevity of seeds during storage is influenced by traits such as genetics and environment during maturation and harvest, seed moisture content and storage conditions (Cromarty *et al.* 1982; Walters *et al.* 2010). Low moisture content and low storage temperature slow down the chemical reactions of seed aging by retaining cells structure and enzymes in a stable state for long periods (Chang *et al.* 1996; Sun 1997; Bernal-Lugo and Leopold 1998). Sugars are central to this process. As seed moisture is reduced, soluble sugars form a grassy state which serves as a physical stabilizer by suppressing deteriorative reactions (Franks 1994; Walters *et al.* 2010).

Ultra-drying is a technique used to reduce the seed moisture content to acceptable levels suitable for safe long-term storage at ambient temperature (Li *et al.* 2007). Research on ultra-drying (Ellis *et al.* 1990; Huang *et al.* 2002) has focused mainly on its use as a low-cost plant germplasm conservation method (Zheng *et al.* 1998) which is favored in developing countries because it eliminates the need to use refrigerated storage for seeds (Ellis *et al.* 1989; Zheng 1994).

Potential benefits of ultra-drying include improvement of seed longevity and vigour (Koster 1991; Ellis *et al.* 1995; Li *et al.* 2007; Huo *et al.* 2011). Many reports indicate that ultra-drying to a moisture content of 2 to 5% could potentially increase seed life span by 4 to 39 times, depending on the species (Harrington 1972; Ellis *et al.* 1986). More importantly, Ellis *et al.* (1996) and Demir and Ozcoban (2007) showed that the loss in viability is more rapid in dry (5.1–6.8% moisture content) than in ultra-dried (2.0–3.7% moisture content) seeds of carrot, groundnut, lettuce, oilseed rape and onion stored at 20°C for five years. In contrast, some studies have reported that ultra-drying causes a significant reduction in the viability of stored seeds (Vertucci *et al.* 1994; Hu *et al.* 1998; Walters and Engels 1998). This seems to occur when seeds are dried below a critical water content which is species or even cultivar specific (Woodstock *et al.* 1976; Ellis *et al.* 1988). However, Ellis (1992) has shown that real damage usually occurs when seeds imbibed water during germination and not during the storage process. Based on these research findings, it is now a standard recommendation that dry and ultra-dry seeds should be humidified prior to germination in order to raise their moisture contents slowly in the initial stages of

germination to minimise any loss to viability (Powell and Matthews 1979).

The success of ultra-drying is measured in terms of seed survival rate and vigor. The difference between germination rates measured before and after drying seeds to different moisture contents is indicative of deterioration caused primarily through oxidation damage that affects seed survival (Walters *et al.* 2010). Germination tests have been used extensively to show the positive results from ultra-drying seeds (*e.g.* Ellis *et al.* 1993, 1995, 1996; Li *et al*. 2007; Pérez-García *et al.* 2007; Demir and Ozcoban 2007). However, very few previous reports (Li *et al*. 2007; Pérez-García *et al.* 2007, 2008) have examined the effect of ultra-drying on seed vigour which is known to influence significantly plant growth and yield (Ellis 1992). Seed vigour is a complex term which refers to the aspects of seed performance which show variation such as the rate and uniformity of germination, and seedling emergence and growth under variable environmental conditions (Perry 1978). Enhanced tolerance to desiccation stress, resistance to aging processes and improved germination and seedling growth have been noted for seeds subjected to slow drying processes (Adams *et al.* 1983; Vertucci and Farrant 1995) typical of ultra-drying techniques (Li *et al*. 2007). Rosenberg and Rinne (1986) suggested that improved seedling growth may be explained by the dependence of the initiation of cell division in the root and shoot meristems on a drying treatment during seed development.

There is some evidence that ultra-drying seeds of species and cultivar down to specific critical moisture contents may also increase seedling tolerance to other stresses caused by salinity and soil pH. For example, Huo *et al.* (2015) reported preliminary results showing that ultra-drying accelerated germination and seedling emergence of alfalfa subjected to alkali stress. This suggests that ultra-drying may be a useful process to improve establishment in soils considered marginal for planting alfalfa, especially in western China where alfalfa is the most important forage species currently sown to both improve soil quality and support ruminant production (Jiang *et al.* 2007). However, before these claims of stress tolerance can be substantiated, it is essential to gain a better understanding of the effects of seed ultra-drying on the growth and physiology of alfalfa in unstressed conditions. The aim of this study was to investigate whether the ultra-drying of alfalfa seeds and then storing at room temperature would provide better seedling growth and vigour, and to identify possible mechanisms that explain differences in the growth of alfalfa seedlings grown from normal and ultra-dried seed by monitoring key physical and physiological indices in both seed and seedlings.

Materials and Methods

Alfalfa seed source and ultra-drying treatment

Seeds (~1 kg) of *Medicago sativa* L. cv. Longdong were collected from 200 plants in October 2007 from the Lanzhou Forage Station (36°05′26" N, 103°41′57" E) which forms part of Gansu Agricultural University, Gansu Province, China. The initial seed purity at time of collection was 99 and 79% of initial germination percentage (GP), and the initial moisture content (MC) of 9.05%. The seeds were stored at room temperature and humidity in ventilated darkness for 1.5 years until required for the experiments.

Approximately 160 g of stored seed was packaged in porous nylon bags (20 g/bag), placed into a cooled hermetic desiccator and buried in silica gel that diurnally dried at 120°C. The ratio between seeds and silica gel was 1 to 10 (w/w) (Zeng *et al.* 2006). After desiccation for 12, 24, 48, 72, 96, 120, 144 and 216 h, seed MC was reduced to 7.10, 6.94, 6.36, 5.73, 5.47, 5.19, 4.98 and 4.60%, respectively, from an initial MC of 9.0% for the control (Table 1). Seed MC was determined by drying at 105°C for 72 h (James and Don 2005). Ultra-dried and control seeds were sealed in aluminium foil packages and stored in a desiccator fulfilled with dried silica gel at room temperature (24.5°C ± 1.5°C).

Pre-germination treatment of ultra-dried seeds

Prior to use in the following series of experiments and tests, ultra-dried seeds were rehydrated to avoid imbibitional damage (Ellis *et al.* 1990). Seeds were placed into sealed glass desiccator for 24 h (24.5°C ± 1.5°C) containing saturated $CaCl_2$ solution to produce a relative humidity (RH) of 35%, and then hydrated for another 24 h using NH_4Cl (RH = 75%) (Huang and Gao 2000).

Lipid peroxidation and total soluble sugar in ultra-dried seeds

Lipid peroxidation of seeds was measured in terms of malondialdehyde (MDA) content (μmol/g FW) (Loreto and Velikova 2001). For each ultra-dried treatment four replicates each of 0.5 g seeds were homogenized in 2 mL 20% (v/v) trichloroacetic acid containing 0.5% (v/v) thiobarbituric acid. The mixture was heated for 30 min at 100°C to release protein-bound MDA, centrifuged at 10000 × g for 10 min after cooling, and the absorbance of the supernatant read at 532 nm and 600 nm. The absorbance at 600 nm was subtracted from the 532 nm reading, and the concentration of MDA was calculated by the means of extinction coefficient of 155 L mM^{-1} cm^{-1} (James and Don 2005).

Total soluble sugars were determined using the Anthrone reagent following the method of Stieger and Feller (1994). Four replicates of 0.5 g seeds from each treatment were homogenized and the centrifuged filtrate (12000 g 10 min) (Chandel *et al.* 1995) mixed with anthrone reagent (20 mL ethanol, 100 mL 1 *M* H_2SO_4, 200 mg anthrone) and heated for 10 min in a vigorously boiling water bath. Samples were cooled on ice for 10 min to stop the color development. Absorbance was read at 623 nm and soluble sugar determined using a glucose (0–100 μg) calibration (Roulin and Feller 2001).

Seed germination test

The experiment was set up to investigate the effects of ultra-drying treatment on germination of alfalfa. Seed surfaces were sterilized using 10% (v/v) Na-hypochlorite before the germination test and seedling growth. Four replicates of 50 seeds each were then arranged on two layers of filter paper moistened with 5 mL de-ionized distilled water in 6 cm diameter petri dishes. Dishes were placed in an incubator (SPX-100B-Z Biochemical Incubator; Shanghai Boxun Medical Equipment Factory, Shanghai China) with constant temperature (24°C) and complete darkness. Germination was defined when the length of the radicle was 2 mm (Song *et al.* 2005). Germinated seeds were counted daily. Seed vigour and viability were quantified by the germination index (GI) for each ultra-drying treatment which was calculated as the product of the germination percentage and the hypocotyl length after 4 days of germination (Vertucci *et al*. 1994).

Seedling growth responses

Alfalfa seeds were grown in sand cultures to test the effect of ultra-drying on seedlings growth, biochemical and physiology responses. Ten replicates each of 25 germinated seeds for each seed ultra-dried treatment were sown in individual polyvinyl plastics pots (12 cm in diameter and 15 cm high), filled with dry river sand (2 mm sieve mesh, washed with de-ionized water for 3 times and autoclaved for 2h at 121°C). At the sowing time seeds in each pot were irrigated with 25% Hoagland's solution (Hoagland and Arnon 1938). The same solution which was placed in polyvinyl plastic trays to a depth of 1 cm, was topped up to the marked level with distilled water daily and changed weekly. Pots were arranged in the trays in a randomized block design.

The experiment was performed in controlled environment growth chamber at Gansu Agricultural University (36°05′18″, 103°42′8″E); altitude 1520 m). Typical conditions during the 22-day treatment period included were temperature of 24°C to 28°C, relative humidity 45 to 93%, and maximum photon-flux density under 12 h of light 1430 µmol m^{-2} s^{-1}.

Shoot height of 10 seedlings chosen randomly in each treatment was recorded at 5, 10, 15 and 22 days after sowing. Leaf photosynthetic activity was characterized by the number of active PS II reaction centers determined by chlorophyll fluorescence, and was measured using a fluorimeter (FMS-2, Hansatech Instruments Ltd., U.K.) in randomly selected, fully expanded leaves. The optimal quantum yield of PS II (*Fv/Fm*), reflecting the functional activity of PS II reaction centers, was measured on 10 seedling chosen randomly in each treatment 4 h before plants were harvested 22 days after sowing pre-germinated seeds.

Surviving seedlings in each treatment were counted, harvested and the leaves, stems and roots of each plant were separated. Leaf number, root length, and fresh biomass of shoots and roots of each seedling were recorded. The leaf area of 10 fully expanded leaves chosen randomly in each treatment was measured using a CI-203 Leaf Area Meter (CI-203, CID, Inc., USA). Physiological measurements were done as soon as possible after plants were harvested.

Root dehydrogenase activity related to respiration capacity was measured by the triphenyltetrazolium chloride (TTC) reduction technique (Huang and Gao 2000). Roots (0.5 g) of each treatment were placed into test tubes with 60 m*M* Na_2HPO_4–KH_2PO_4 containing 0.6% (w/v) TTC and 0.05% (w/v) Tween 20, incubated the tubes for 20 h at 30°C. All formazens formed from the reduction of TTC by dehydrogenase enzymes in living roots was extracted in 95% (v/v) ethanol for 4 h at 60°C (all temperature control were in incubators). The absorbance of the extractants was read at 480 nm.

The chlorophyll (a + b) content of randomly sampled leaves (0.5 g) was determined spectrophotometrically in dimethyl sulfoxide (DMSO) (Sigma Chem. Co., USA) using the method of Fridgen and Varco (2004). Leaves were placed in vials containing 10 mL of DMSO for approximately 45 min until all the chlorophyll (a + b) was removed and kept on ice in darkness until analysis. Absorption was measured using a Spectrophotometer (S2000, WPA Co., U.K.). The concentrations of chlorophyll a, b, and total chlorophyll were calculated using equations described by Barnes *et al.* (1992).

Lipid peroxidation in leaves was measured in terms of MDA content (Cakmak and Horst 1991). Four replicates of previously frozen alfalfa leaves (0.5 g) from each ultra-drying treatment were homogenized in 2 mL 20% (v/v) trichloroacetic acid containing 0.5% (v/v) thiobarbituric acid and measured as described above.

Buffer-soluble carbohydrates were measured according to Stieger and Feller (1994). The centrifuged filtrate (10–20 μL) was mixed with 1 mL anthrone reagent (20 mL ethanol, 200 mg anthrone, 100 mL H_2SO_4) and heated for 10 min in a vigorously boiling water bath. Colour development was stopped by incubating the sample on ice for 10 min, and the A_{623} was measured. Glucose (0–100 μg) was used for calibration (Roulin and Feller 2001).

Four replicates of seedling leaves (0.5 g) derived from each treatment were homogenized and analyzed for soluble sugars according to the method described above.

Statistical analysis

For each treatment ten replicates were set up in a completely randomized design. Four tissue sample replicates were used for physiology and biomass analyses. Data is presented as mean ± S.E. and differences of variables between treatments were compared using 1-way ANOVA followed by Duncan's method where $P < 0.05$.

Results

Effect of ultra-drying seed biochemistry and germination

Seed biochemistry: Ultra-dry seeds exhibited a higher vigor level than the air-dried control (MC = 9.1%) indicating that the biochemical processes of stored alfalfa seeds are tolerant to dehydration down to about 5% MC. Soluble sugar concentration increased exponentially (R^2 = 0.794) to a maximum at 5% MC relative to the control (P < 0.05) and then declined back to the control concentration at 4.6% MC (Table 1). In contrast, MDA concentration decreased (P < 0.05) to a minimum at 5.2% and increased second only to the control MDA concentration at 4.6% MC (Table 1). This suggested that MC of 5% is near the optimal for alfalfa seed at which the integrity of the biochemical processes are maintained. There was little decrease in seed MC when the drying period was extended beyond 216 h.

Germination: The germination percentage (GP) of ultra-dry alfalfa seeds with MC ranging from 5.7 to 5.0% were higher (P < 0.05) than the air-dried control seed by 8 to 13% (Table 1). However, for the seeds with a MC of 4.6%, GP was significantly (P < 0.05) lower than control which reflects the impact of changes in seed biochemistry at seed MC <5%, particularly total soluble sugars and MDA (Table 1). A fitted curvilinear relation indicated that total soluble sugar content of seed explained 93% of the variation in germination with germination >92.5% at total sugar content >64 mg g^{-1} FW. In contrast, MDA concentration tended to reduce germination with >95% germination when MDA was <8.98 nmol g^{-1} FW. There was no consistent effect of ultra-drying on hypocotyl length measured 4 days after the start of germination.

Effect of ultra-drying seeds on seedling growth and biochemistry

Seedling growth and root dehydrogenase activity: Seedling derived from seeds dried to a MC of 5.5 to 5.2% had significantly higher (P < 0.05) total biomass than the control (Table 2). This was due to a difference in root mass 33 to 64% higher (P < 0.05) than the control because there was no significant difference in shoot biomass between seed treatments (Table 2). The higher production of root mass was not due to a more extensive root system as several other treatments produced significantly (P < 0.05) more root length than control (Table 2), but rather to thicker roots produced in seedlings derived from seeds with MC 5.5 to 5.2%. Root dehydrogenase activity measured in 22-day-old seedlings showed a cyclic pattern with an initial significant (P < 0.05) decline to a minimum for germinating seeds with 6.9% MC, increasing to a maximum at 5.2% and then declined again to the same minimum for germinating seeds with 4.6% MC (Fig. 1).

Although, there was no difference in shoot mass between treatments and significant differences in the dynamics of the above ground biomass such as shoot height, leaf number and leaf area. Differences in shoot height were most pronounced at day 22 of growth with seedlings derived from seeds dried to 5.7% reaching maximum height 25% higher (P < 0.05) than the control (Fig. 2A). Enhanced growth from ultra-dried seed increased with seedling age since there were no differences in seedling height observed at day 5 of growth and only a few differences at day 10 and 15 (Fig. 2A). Seedling height differences were correlated to differences in leaf number and leaf area. For leaf number, only seedlings from seeds dried to 5.5% MC produced significantly (P < 0.05) more leaves than the control (Fig. 2B). However, the level of ultra-drying had broader positive and negative effects on leaf area. Seedlings derived from seeds dried within a range 5.7 to 5.2% had up to 15% more (P < 0.05) leaf area than the control, whereas seedlings from seeds dried to 5% or less had 9% less leaf area than the control measured at day 22 (Fig. 2C).

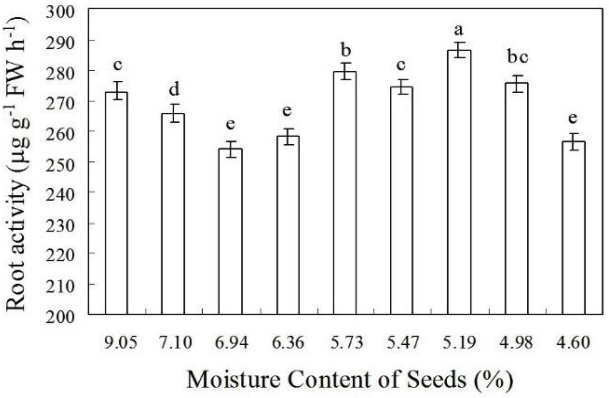

Fig. 1: Root dehydrogenase activity measured in 22-day old seedlings derived from seeds with different MC (%). Columns identified with the same letter are not significantly different at P < 0.05, after applying Duncan test

Chlorophyll content of seedling was not significantly different as a result of pre-germination seed treatment, except for seeds with a MC of 6.9 and 5.0%, where a significant (P < 0.05) reduction and increase in chlorophyll content, respectively was found (Fig. 3A). Irrespective of seed MC, seedlings in this study maintained nearly maximum quantum efficiency of PS II photochemistry (*Fv/Fm*) (Fig. 3B).

Leaf MDA content is a physiological index which reflects the degree of plant's ability to withstand injury or stress. The results showed a pattern of significant (P < 0.05) decline in MDA content of seedlings derived from seeds with MC ranging from 9.1 to 5.5%, and a significant increase in MDA content for seedlings from seeds dried to a MC <5.5% (Fig. 4A). This is consistent with changes of MDA measured in seeds (Table 1). The soluble sugar content of seedling leaves decreased (P < 0.05) in control for seedling derived from seeds within the MC range of 7.1

Table 1: Characteristics of *M. sativa* (*cv.* LongDong) seeds dried to different moisture contents and stored for one year

Ultra dry period (h)	Moisture content (%)	Germination percentage (%)	Hypocotyl length (mm)	MDA content (nmol g^{-1} FW)	Soluble sugar content (mg g^{-1} FW)
0	9.05 ± 0.07 a	85.50 ± 4.43 c	52.38 ± 2.49 cd	11.62 ± 0.22 a	56.99 ± 0.14 e
12	7.10 ± 0.02 b	78.50 ± 2.52 d	69.00 ± 2.58 a	9.49 ± 0.42 b	62.31 ± 0.53 d
24	6.93 ± 0.01 c	72.50 ± 2.52 e	63.13 ± 4.33 b	9.27 ± 0.21 bc	61.23 ± 1.20 d
48	6.36 ± 0.01 d	89.50 ± 4.12 bc	43.25 ± 2.06 e	9.17 ± 0.15 bc	61.81 ± 1.04 d
72	5.73 ± 0.03 e	94.50 ± 3.0 ab	52.13 ± 2.96 cd	8.98 ± 0.37 c	64.04 ± 0.91 c
96	5.47 ± 0.02 f	96.50 ± 3.42 a	53.25 ± 4.94 cd	7.11 ± 0.31 e	66.25 ± 0.95 b
120	5.19 ± 0.05 g	96.00 ± 3.65 a	49.75 ± 3.09 d	6.64 ± 0.36 f	67.73 ± 0.42 a
144	4.98 ± 0.02 h	92.50 ± 3.00 ab	65.25 ± 4.92 ab	8.38 ± 0.37 d	66.44 ± 0.50 b
216	4.60 ± 0.02 i	79.00 ± 3.46 d	57.25 ± 2.06 c	9.50 ± 0.31 b	63.74 ± 0.22 c

Means ± S.E (n =4). Different letters indicate significant difference at $P < 0.05$ (Duncan test)

Table 2: Biomass of roots and shoots, root length for *M. sativa* seedlings derived from seeds with different MC (%) for 22 d

Moisture content (%)	Root length (cm)	Shoot biomass (mg) FW	Root biomass (mg) FW	Total biomass (mg) FW
9.05	6.7 ± 0.3 e	105.3 ± 26.7 bc	84.0 ±14.2 c	189.2 ± 20.6 c
7.10	7.6 ± 0.3 bc	103.0 ± 14.4 bc	110.1 ± 14.4 b	213.1 ± 21.2 bc
6.94	7.1 ± 0.4 cde	105.0 ± 20.0 bc	103.5 ± 21.3 b	208.5 ± 5.1 bc
6.36	7.1 ± 0.3 de	105.9 ± 9.0 bc	111.0 ± 19.2 b	216.9 ± 28.2 bc
5.73	7.0 ± 0.2 de	111.9 ± 12.3 bc	102.0 ± 15.2 b	213.9 ± 16.7 bc
5.47	7.8 ± 0.2 ab	126.4 ± 9 .5 a	112.0 ± 21.3 b	238.4 ± 29.8 ab
5.19	8.1 ± 0.3 a	119.0 ± 15.9bc	138.9 ± 19.5 a	258.0 ± 24.1 a
4.98	7.5 ± 0.4 bcd	102.0 ± 11.6 bc	109.1 ± 14.9 b	211.0 ± 13.8 bc
4.60	7.4 ± 0.5 bcd	98.1 ± 21 .0 c	105.6 ± 10.7 b	203.7 ± 26.1 bc

Values are means ± S.E (n =4). Different letters indicate significant difference at $P < 0.05$ (Duncan test)

to 5.0% (Fig. 4B). However, the soluble sugar content of seedlings increased ($P < 0.05$) by 28% for seedlings derived from seed with MC of 4.6% relative to the control (Fig. 4B).

Discussion

Desiccation tolerance is one of the most fundamental properties of seeds (Leprince *et al.* 1993; Chandel *et al.* 1995; Stéphane *et al.* 2018) and the ability of seeds to remain viable under severe desiccation is dependent on the drying rate and final seed moisture content (Hill *et al.* 2010). Studies indicate that a seed MC of around 5% is optimal for seed storage (Harrington 1972), depending on storage temperature (Ellis and Hong 2006). Our results confirm findings reported by Ellis and Hong (2006) that the low moisture content limit for alfalfa seed is in the range 4.2 to 5.5% with the upper value applicable when seeds are stored at room temperature (24°C) as in this experiment.

The higher ($P < 0.01$) germination observed at seed MC of 5.0 to 5.7% (Table 1) is related to concentrations of total sugars and MDA. The accumulation of sugars appears central to a seed's ability to retain viability under desiccation by protecting membranes and proteins, develop a glassy state for intracellular water and provide a carbon reserve for germination (Hoekstra *et al.* 2001). Although total sugar concentration was increased by ultra-dying to 5% seed MC and explained a high proportion of the variability in germination of alfalfa seed dried to different moisture contents, this correlation most likely reflects an altered carbohydrate composition rather than simply the total sugar concentration *per se*. Tetteroo *et al.* (1994) reported that during slow dehydration of alfalfa seed the carbohydrate content changed with oligosaccharides concentrations increased and both glucose and fructose content decreased. This study measured total sugar content, but the implication of increased total sugar content observed at 5% seed MC together with Tetteroo *et al.* (1994) finding that a glassy state was formed during dehydration with oligosaccharides as the possible stabilizer resulting in less deterioration of ultra-dried seed than the control.

Another positive effect of ultra-drying was a reduction in lipid peroxidation which has been identified as the major cause of reduced viability, vigour and germination percentage of stored seeds (McDonald 1999; Cakmak *et al.* 2010). Determination of MDA provides a convenient method to quantify the extent of lipid peroxidation (Goel *et al.* 2003). The lower level ($P < 0.05$) of MDA measured for alfalfa seed with an MC of 5.5% in present experiment suggests that these seeds had an efficient antioxidant defensive system that limited the degree of lipid peroxidation relative to seeds dried to other moisture contents (Li *et al.* 2007) for *Liminium aureum* (L.) Hill. Zhu and Chen (2007) reported that for moderate ultra-drying of peanut (MC 2.0%) lipid peroxidation and radical emergence where inhibited relative to peanut dried to MC 0.9%, especially after accelerated aging. Seed aging does seem to exert an influence on lipid peroxidation (Huo *et al.* 2011) and ultra-drying treatment of accelerated aged alfalfa seeds to 5.72% seed MC helped to maintain seed vigor due to lower lipid peroxidation.

Changes in seed biochemical composition caused by ultra-drying were also linked to improved seedling performance other than germination. While it is well documented that early seedling growth is correlated directly with the quantity and utilization of seed reserves (Westoby *et al.* 1992), most often expressed as

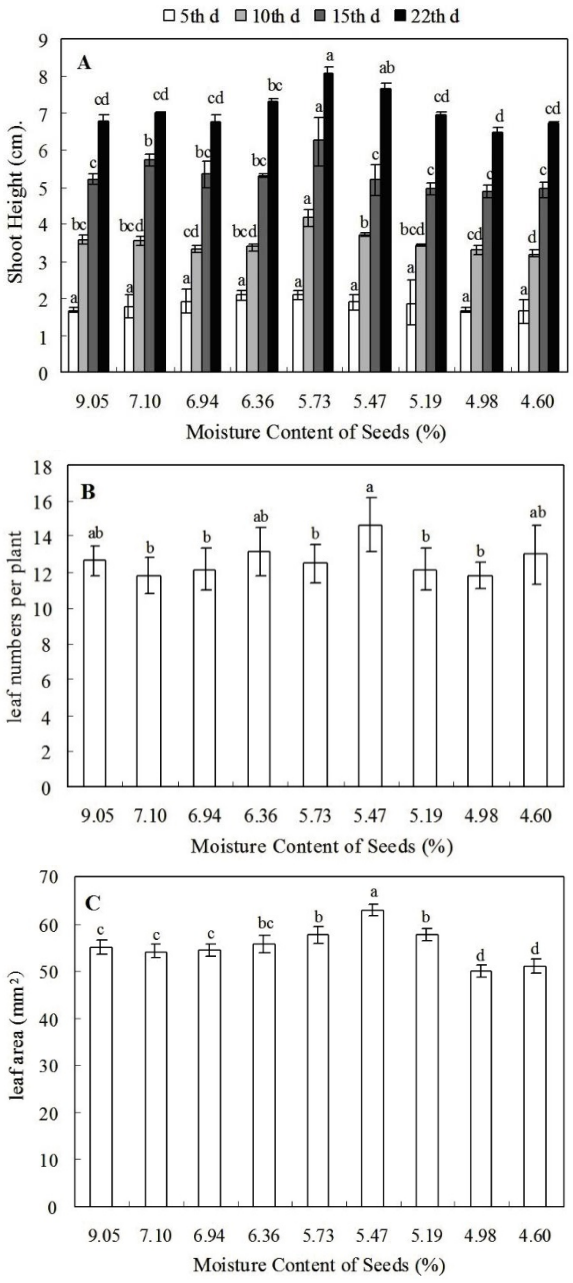

Fig. 2: Growth of *Medicago sativa* seedlings derived from seeds with different MC (%). Number of leaves (**A**) and Leaf area (mm^2) (**B**) at 22 d and Shoot height (**C**) at 5, 10, 15 and 22 d DAT, respectively. Values represent means ± S.E., n = 4. Values sharing same letters differ non-significantly ($P > 0.05$)

differences in seed size (Turnbull *et al*. 2008), there are few reports describing the effects of changes in seed biochemical composition on seedling growth. The study results showed a higher root mass and thicker roots produced in seedlings derived from seeds with MC 5.5 to 5.2% suggesting that relative nutrient allocation to these seedlings was higher than controls. Fits with Lloret *et al.*

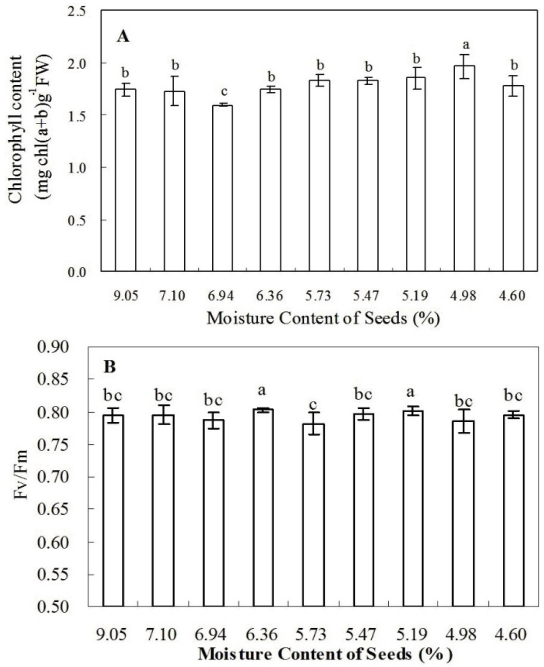

Fig. 3: Chlorophyll content (**A**) and optimal quantum yield of PS II photochemistry (Fv/Fm) (**B**) of seedlings derived from seeds with different MC (%) for 22 d. Optimal quantum yield of PS II photochemistry (Fv/Fm) (B) at midday in randomly selected, fully expanded leaves. Values represent means ± S.E., n=4. Values sharing same letters differ non-significantly ($P > 0.05$)

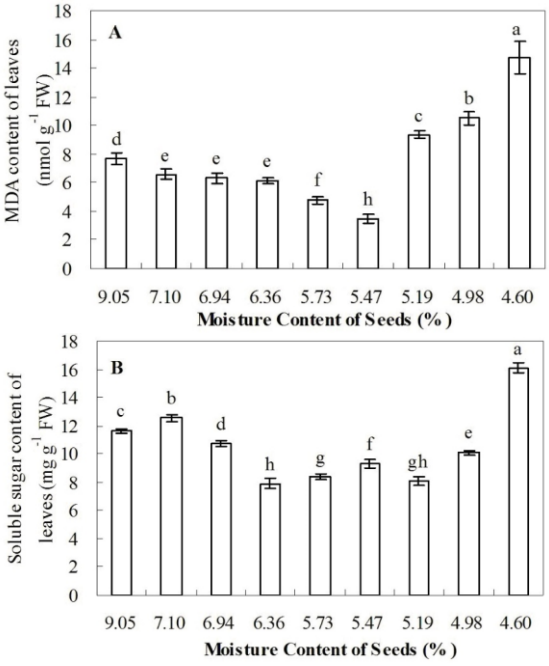

Fig. 4: MDA content (**A**) and soluble sugar content (**B**) of leaves of seedling derived from seeds with different MC (%) at 22 d. Values represent means ± S.E., n=4. Values sharing same letters differ non-significantly ($P > 0.05$)

(1999) observation linking higher relative growth rate with nutrient allocation (especially sugars) to root development.

Conclusion

The results provided an important insight into the possible importance of ultra-drying as a practical means to improve alfalfa establishment. And seedling derived from seeds dried to MC of 5.2% had higher biomass, and higher root dehydrogenase activity. Enhanced growth from ultra-dried seed increased with age with seedlings derived from seeds dried within a range 5.7 to 5.2%. Perhaps more important is the effect of the ultra-drying process on seed biochemistry that promotes tolerance in alfalfa to stress in both saline and alkaline conditions warrant for further studies.

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