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Full Length Research Paper

Emergence and seedling growth of five forage legume species at various burial depth and two light levels

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A field study compared the seedling emergence and structure of five forage legumes (*Trifolium repens* L., *Medicago falcata* L., *Melilotus suaveolens* Ledeb, *Medicago sativa* L. and *Lespedeza davurica* Schindler) at five planting depths (1, 2, 4, 6 and 8 cm) and two light levels (full light and shade) on the 21st day after planting. As expected, increaseing depth lowered and slowed seedling emergence. Maximum emergence occurred at 1 and 2 cm depths for all five forage legumes irrespective of light levels; then decreased as the burial depth increased. Improved seedling emergence was also observed under shade compared to full light. Increased sowing depth diminished seedling size by reducing the plant height, biomass, leaf number and size of all five forage legumes. All the morphological traits and seedling biomass were reduced under shade condition except seedling height and cotyledon size. The optimal planting depth for all the forage legumes in this study was 1 to 2 cm. *M. falcata*, *M. suaveolens* and *M. sativa* had high percentage of emergence and better growth from deeper depth or under shade condition.

Key words: Burial depth, forage legume, optimal planting depth, relative growth rate, seedling emergence, seedling mass, seedling morphology, shade.

INTRODUCTION

Using forage legumes to establish artificial grassland and mixed pasture can increase plant species diversity, enhance forage quality and enhance soil quality by nitrogen fixation, soil structure improvement and water infiltration (Miller and Jastrow, 1996; Sleugh et al., 2000; Frame, 2001). A wide range of legumes are available, with each species having its own agronomic requirements, thus, making it more or less suitable for a particular producer's purpose. Given that seedling establishment and growth can be influence by multiple environmental factors (Bazzaz and Miao, 1993; Walters and Reich, 1997; Benvenuti et al., 2001; Zheng et al., 2005), detailed information on the emergence, growth and development of legume forage seedlings under different environmental factors were required for devising appropriate management practices and choosing the right species to formulate potential seed mixtures for multi-species plantings (Sanderson and Elwinger, 2004).

Depth of sowing was acknowledged in the literature as an important factor that modifies seedling emergence and development (Ries and Hofmann, 1995; Seiwa et al., 2002; Sanderson and Elwinger, 2004). Shallow burial particularly improves the germination of seeds, subsequent emergence and survival of seedlings (Maun, 1998; Forcella et al., 2000; Benvenuti et al., 2001). Deeper burial, on the other hand, usually reduces emergence and vigor of seedlings, frequently leads to poor establishment, despite more deeply sown seeds having a greater chance of accessing soil moisture (Beveridge and Wilsie, 1959; Arnott, 1969; Ries and Hoffman, 1995; Forcella et al., 2000). Therefore, determining optimum planting depth for individual species is critical for establishing productive stands in fields.

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Table 1. Seed characteristics of legume species used in this study.

Species	Cultivar	Collect location	Seed mass (mg)
T. repens	-	Jilin Province	0.58±0.002
M. falcata	-	Inner Mongolia Province	1.52±0.02
M. suaveolens	-	Jilin Province	2.14±0.09
M. sativa	Aohan	Inner Mongolia Province	2.46±0.02
L. davurica	-	Jilin Province	2.58±0.01

The ability of seedlings to emerge from deeper burial depths and establish successfully is related to their morphology or functional type in some extent (Maun and Riach, 1981; Fulbright et al., 1984; Maun and Lapierre, 1986; Redmann and Qi, 1992). Several studies have shown that in herbaceous species, the successful emergence of seedlings is attributed to the effective elongation of the coleoptile, subcoleoptile, first internode or hypocotyl (Maun and Riach, 1981; Maun and Lapierre, 1986; Redmann and Qi, 1992). However, increased planting depth has also been shown to reduce seedling size by reducing seedling height, number and size of leaves and number of tillers in some grasses (Maun and Riach, 1981; Fulbright et al., 1984; Ries and Hofmann, 1995; Sanderson and Elwinger, 2004). Thus, an understanding of these traits is crucial for the successful management of these plants (Ren et al., 2002).

Light is generally known to be one of the ecological factors exerting the greatest effect on germination, seedling emergence and development (Benvenuti et al., 2001). Whether pure sown or in mixture with grasses, competition for light from weeds and grasses will probably limit the establishment of legumes in pastures (Lanini et al., 1991; Simmons et al., 1995). It has been reported that legume inter-seeding was most successful when competition from grasses is reduced through herbicide application (Groya and Sheaffer, 1981; Cuomo et al., 2001) or by cutting swards to low heights before seeding legumes (Taylor and Allinson, 1983). The observation of growth and morphological plasticity of legume seedlings under different light conditions is necessary for the management before seeding.

In promoting any forage species that has not previously been widely used, basic agronomic studies provide a basis for building extension recommendations. Five forage legumes (*Trifolium repens* L., *Medicago falcata* L., *Melilotus suaveolens* Ledeb, *Medicago sativa* L. and *Lespedeza davurica* Schindler) which all have dicotyledon epigeal seedlings were used in this study. They are legume species well adapted to conditions in the grasslands of northern China and may have a big potential for future development, for example, *M. sativa* is the world’s most important perennial forage legume (Michaud et al., 1988) and *M. falcata* tends to have a more decumbent growth habit and more winter hardiness than *M. sativa* (Lesins and Lesins, 1979). The objectives of this study were to examine the effects of depths and light on seedling emergence and seedling structure of these legume forages and provide producers with some useful information about these forage legumes.

MATERIALS AND METHODS

The seeds of *T. repens* L., *M. falcata* L., *M. suaveolens* Ledeb, *M. sativa* L. and *L. davurica* Schindler were collected in 2005 from the grasslands of northern China and were stored at 4°C until used (Table 1). The aborted and predated seeds were discarded and intact plump seeds were selected for this experiment. The seeds of *M. falcata* and *L. davurica* were surface scarified prior to experimental usage.

Experimental procedure

This study was conducted in a nursery, in the experimental station of Northeast Normal University in northeast China (44°38'N, 123°41'E) in August 2006. The maximum and minimum temperatures averaged 14 and 27°C, respectively, during the experimental period. The nursery was divided into six plots, separated from each other by a walking path of 0.5 m wide and each plot was 3 m × 3 m.

A total of five burial depths (1, 2, 4, 6 and 8 cm) and two light regimes (shade: 10% full light and full sunlight) each replicated three times using 50 seeds per replication were used for each species. The pots (20 cm in diameter and 28 cm in height) were first filled with field soil (Aeolian sandy soil) to the specific depth at which seeds were to be placed, and then filled with additional soil. After planting, the pots with soil and seeds were placed in a random arrangement in the plot and sunk in the ground with the soil level in the pots the same as the ground in order to make a more stable environment. Pots were watered every day with tap water. Emerged seedlings (cotyledons visible at the soil surface) were counted twice a day for the first week, and then once a day.

After three weeks, all the seedlings were measured for their height above the soil surface and then they were removed from the pots. The soil was washed from the roots in cold running water. Hypocotyls were divided into two parts (above-ground and below-ground) and the length of each part was measured. The length and width of the cotyledons and the first leaves were measured. The number of leaves for each seedling was counted. Each whole seedling was dried at 65°C for 48 h to determine the dry mass. Mean seedling relative growth rate (RGR) was calculated for the two growing periods. Mean seedling RGR was calculated as $(\ln W_2 - \ln W_1)/(t_2 - t_1)$ where W_2 and W_1 are mean seedling biomass at the end and start of the growth period $(t_2 - t_1)$, respectively. In our study, mean seed mass (without seed coat) per species was used for W_1 values and the growth period $(t_2 - t_1)$ was 20 days.

Table 2. The effects of depth (D), light (L), species (S) and their interaction on germination characteristics, morphological traits and seedlings mass of plants of five species.

Dependent variable	D	L	S	D×L	L×S	D×S	L×D×S
Emergence percentage	***	**	***	NS	NS	***	NS
Time to emergence	***	*	***	*	NS	***	NS
Seedling height	***	***	***	***	***	***	**
Seedling biomass	***	***	***	***	***	NS	NS
Length of above-ground hypocotyls	***	***	***	***	***	***	***
Length of below-ground hypocotyls	***	NS	NS	NS	NS	NS	NS
Length of cotyledons	***	***	***	NS	***	NS	NS
Width of cotyledons	***	***	***	NS	***	***	**
Length of the first leaf	***	***	***	***	***	**	**
Width of the first leaf	***	***	***	**	***	NS	*
Leave number	***	***	***	*	***	NS	NS

$*p < 0.05$; $**p < 0.01$; $***p < 0.001$; NS indicates not significant.

Data analysis

General linear model were used to analyze the effects of burial depth, light, species and their interactions on time of emergence, emergence percentage, seedling mass and seedling height, length of the two parts of the hypocotyls, length and width of the cotyledons and the first leaves and the number of leaves in all the treatments. LSD (least significant difference) and Tukey's test (a = 0.05) were used to compare the mean differences between treatments. To meet assumptions of the ANOVA, data were $\log_{10}$- or arcsin-transformed prior to analysis wherever necessary. Since no seedling emergence occurred at 8 cm depth for any of the species both in shade and full sunlight and only one seedling emerged from 4 cm for *T. repens* and less than 5 seedlings emerged from 6 cm for *M. falcata* and *L. davurica* in full sunlight, these treatments were not included in the analyses. Independent-samples T test was used to statistically test the mean difference between the 1 and 2 cm treatments of *T. repens* under full light condition. All statistical analyses were performed with SPSS (version 11.5, SPSS Inc., Chicago, Illinois, USA. 2002).

RESULTS

Seedling emergence

Seedling emergence was significantly affected by burial depth, light and species (Table 2). And the depth × species interaction was significant for seedling emergence (Table 2). The emergence percentages were 74.8, 69, 43.3 and 12.4% at 1, 2, 4 and 6 cm, respectively combined the light with different species. For all five species, emergence was highest at 1 or 2 cm burial depths and then decreased with increasing burial depth (Figure 1). For *T. repens*, a large percentage of the seedlings emerged only from the 1 and 2 cm burial depths; very few seedlings emerged from the 4 cm burial treatment in full sunlight; no seedlings emerged from the 6 cm depth (Figure 1b). Emergence was highest at the 1 and 2 cm burial depths; with a significant decline in emergence at the 4 and 6 cm burial depths in full sunlight for *M. falcata* and *L. davurica* and the emergence percentage at 6 cm was lower than 3% (Figure 1b). Across all treatments, emergence was usually higher under shade than in full sunlight. For instance, the average proportion of the emerged seedlings for species and burial depth (1 to 6 cm) combinations was 52.3% under shade and 47.5% in full sunlight. Emergence for *M. falcata* at 4 and 6 cm in shade was 54 and 15.3%, respectively, compared with 32 and 2.7% in full sunlight (Figure 1).

The mean number of days to first emergence of seedlings, which reflects the rate of emergence, was significantly affected by burial depth and species (Table 2). For all five species, the fastest seedling emergence occurred at 1 cm burial depth and then the emergence was delayed with increasing burial depth. Seeds buried at 6 cm took significantly longer to emerge than those at the 4, 2 and 1 cm depths (Figure 2). Similarly, seeds buried at 4 cm emerged significantly later than the 2 and 1 cm depths.

Seedling morphology

All morphological traits were significantly affected by burial depths, light level and species except the length of below-ground hypocotyls which was not affected by light level and species (Table 2). Seedling height was usually greatest at shallow burial depths of 1 and 2 cm and then decreased as burial depth increased for all the treatments. Seedling height of *M. sativa* in full sunlight decreased from 2.71 to 1.93 cm as planting depth increased from 1 to 6 cm (Table 3). Seedling height of species with larger seed was usually higher than those of species with smaller

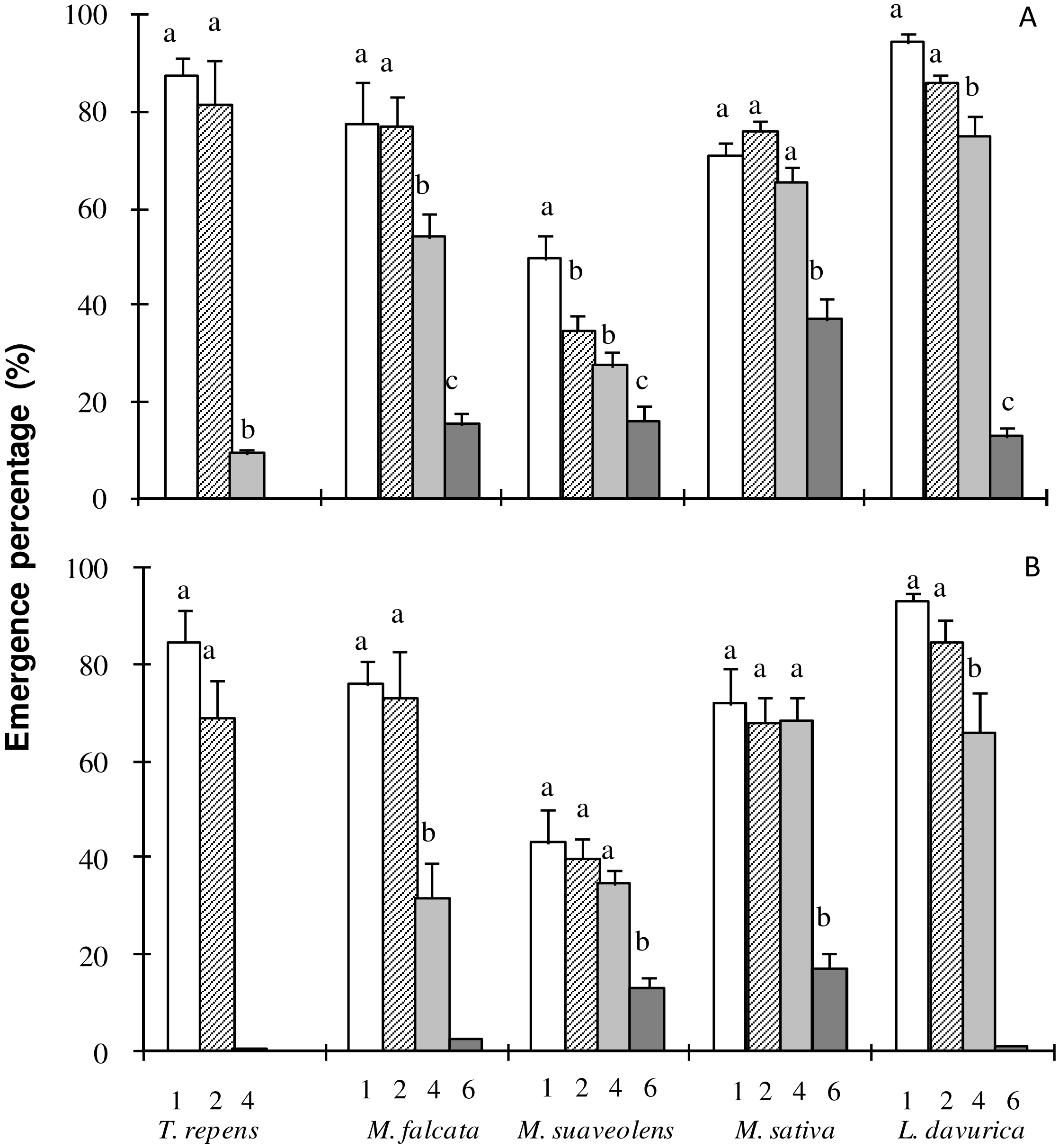

Figure 1. Mean (±SE, N= 3) emergence percentage of seedlings at various burial depths (1, 2, 4 and 6 cm) and light levels (shade A and full sunlight B) for five species. Means with different letters are significantly different at $p < 0.05$ level under various burial depths.

seed. For example, seedling height of the two large seed species *M. sativa* and *L. davurica* was 2.33 and 2.37 cm at 2 cm depth, compared with 0.89 and 1.38 cm of two small seed species *T. repens* and *M. falcata*. Patterns of seedling height in response to light were similar for all five species. Low light significantly increased the seedling height, regardless of burial depth.

As sowing depths increased elongation of the below-ground hypocotyls increased significantly for all five species; light levels had no effect on the below-ground hypocotyls (Table 2). In contrast, the growth of the above-ground hypocotyls in all treatments was significantly favored by shading. Increased sowing depth also reduced seedling size by reducing leaf number and size. The lengths and widths of both the cotyledons and the first leaves and also the total number of leaves declined when seedlings emerged from greater depths for all species. The development of cotyledons length and width was considerably increased under shade condition. For example, the length and width of cotyledons for *M.*

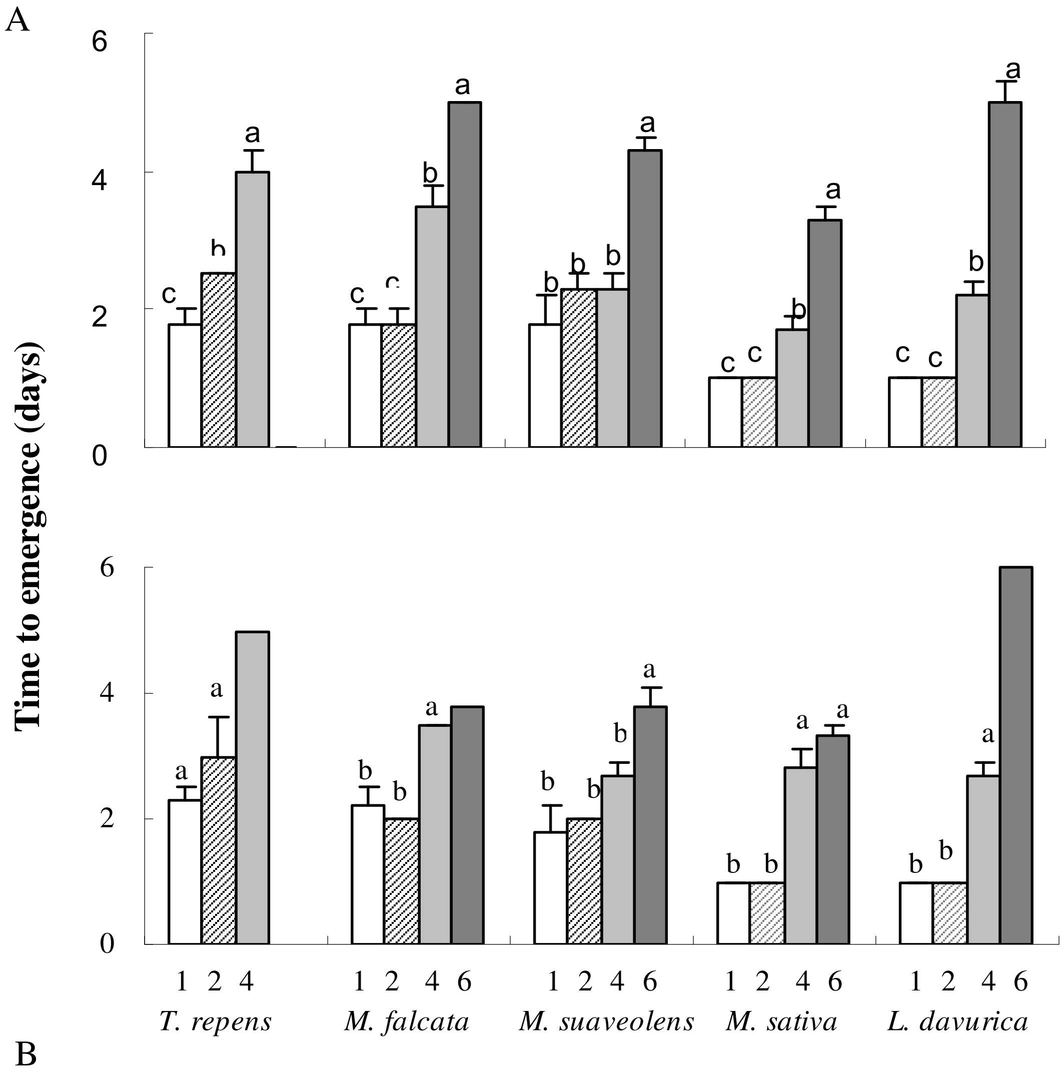

Figure 2. Mean (±SE, N= 3) number of days to the first emergence of seedlings at various burial depths (1, 2, 4 and 6 cm) and light levels (shade A and full sunlight B) for five species. Means with different letters are significantly different at $p < 0.05$ level under various burial depths.

suaveolens at 2 cm in full sunlight was 0.64 and 0.29, respectively, compared with 0.83 and 0.41 in shade (Table 4). However, first leaf size and total leaf number were much greater in full sunlight than in shade. Effects varied amongst species. Seedlings of *M. sativa* had the largest cotyledons; *M. suaveolens* had the biggest first leaves in full sunlight, while leave number was greater in *M. sativa* and *L. davurica* than those in the remaining three species in full sunlight.

Seedling biomass

Seedling biomass was significantly affected by burial depth, light levels and species, but not affected by the interaction between species and burial depth and the interaction among the three factors (Table 2). Seedling biomass was also greatest at shallow burial depth (1 and 2 cm burial depths) and decreased as burial depth increased for all species under the two light levels (Table 5). Large differences between the light treatments occurred with regard to the seedling biomass towards the end of the experiment. The biomass of the legume seedlings was 5 to 7 times smaller under shade than the biomass of those under full sunlight (Table 5). Similarly, the RGR of the seedlings were also significantly lower under deeper depths and shade for all species. For some treatments under shade, like *T. repens M. suaveolens* and *L. davurica* at 4 cm, *M. falcata*, *M. suaveolens* and *L. davurica* at 6 cm, the RGR in shade treatment was even

Table 3. Seedling height and hypocotyl length of seedlings (mean ± SE) at various burial depths (1, 2, 4 and 6 cm) and light conditions (full light and shade) for five species.

Species (cm)	Seedling height (cm)		Length of above-ground hypocotyl (cm)		Length of below-ground hypocotyl (cm)	
	Full light	**Shade**	**Full light**	**Shade**	**Full light**	**Shade**
T. repens						
1	0.93±0.03^{a}	2.58±0.13^{a}	0.23±0.01^{a}	1.26±0.07^{a}	0.85±0.03^{b}	0.86±0.03^{c}
2	0.89±0.04^{a}	2.73±0.12^{a}	0.25±0.02^{a}	0.96±0.05^{b}	1.86±0.02^{a}	1.82±0.02^{b}
4		0.61±0.10^{b}		0.33±0.03^{c}		3.82±0.02^{a}
M. falcata						
1	1.70±0.08^{a}	4.00±0.23^{ab}	0.3 ±0.02^{a}	1.49±0.05^{a}	0.84±0.02^{c}	0.84±0.02^{d}
2	1.38±0.06^{b}	4.41±0.14^{a}	0.24±0.01^{b}	1.26±0.09^{a}	1.84±0.03^{b}	1.84±0.02^{c}
4	1.18±0.07^{b}	3.60±0.18^{b}	0.23±0.01^{b}	0.92±0.07^{b}	3.85±0.03^{a}	3.86±0.03^{b}
6		1.67±0.27^{c}		0.40±0.05^{c}		5.82±0.05^{a}
M. suaveolens						
1	2.23±0.10^{ab}	3.92±0.23^{ab}	0.34±0.01^{a}	2.38±0.14^{a}	0.83±0.03^{d}	0.83±0.02^{d}
2	2.51±0.14^{a}	4.39±0.24^{a}	0.3 ±0.01^{a}	2.39±0.16^{a}	1.85±0.02^{c}	1.85±0.03^{c}
4	1.91±0.08^{bc}	4.18±0.34^{ab}	0.37±0.02^{a}	2.37±0.17^{a}	3.85±0.03^{b}	3.83±0.03^{b}
6	1.51±0.09^{c}	3.25±0.25^{b}	0.32±0.04^{a}	2.10±0.14^{a}	5.79±0.02^{a}	5.82±0.03^{a}
M. sativa						
1	2.71±0.17^{a}	5.55±0.15^{ab}	0.29±0.02^{bc}	2.11±0.09^{a}	0.83±0.02^{d}	0.83±0.02^{d}
2	2.33±0.11^{ab}	5.90±0.19^{a}	0.30±0.02^{b}	1.93±0.10^{ab}	1.84±0.02^{c}	1.85±0.02^{c}
4	2.25±0.10^{b}	5.20±0.15^{b}	0.23±0.01^{c}	1.69±0.07^{b}	3.82±0.03^{b}	3.84±0.03^{b}
6	1.93±0.11^{b}	4.03±0.21^{c}	0.39±0.03^{a}	1.33±0.18^{c}	5.80±0.04^{a}	5.83±0.03^{a}
L. davurica						
1	2.53±0.10^{a}	4.54±0.26^{b}	0.32±0.01^{ab}	0.96±0.04^{b}	0.83±0.03^{c}	0.83±0.02^{d}
2	2.37±0.08^{ab}	5.82±0.15^{a}	0.30±0.01^{b}	1.48±0.07^{a}	1.82±0.03^{b}	1.86±0.02^{c}
4	2.21±0.07^{b}	4.43±0.23^{b}	0.36±0.02^{a}	1.01±0.08^{b}	3.84±0.03^{a}	3.83±0.02^{b}
6		3.00±0.22^{c}		1.13±0.05^{ab}		5.78±0.05^{a}

Means with different letters are significantly different at $p < 0.05$ level under various burial depths.

negative (Table 4).

The differences in biomass and RGR of seedlings between light treatments in seedlings were not the same among the species. For *M. falc*ata, the average seedling biomass across all depths under shade was 21.3% of that in full sunlight, while for *L. davurica*, this was only 15% (Table 5). Seedling biomass was positively related to the seed size of species at all treatments in this study. The exception is that the seedling biomass of *M. stavia* was greater than *L. davurica* at each depth, although the seed mass of the latter was greater. RGR, however, was not correlated with seed mass whether grown under shade or full sunlight. For example, when *T. repens* (smallest seed mass) had a higher RGR at 1 and 2 cm depths under full sunlight than *L. davurica* (largest seed mass) (Table 5).

DISCUSSION

Seedling emergence

In this study, the emergence of seedlings of all species showed a definite pattern: the rate of emergence and emergence percentage within a species decreased with increased sowing depth independently of the light results for other species (Fulbright et al., 1984; Chen and Maun, 1999; Seiwa et al., 2002; Tobe et al., 2005). The highest seedling emergence of Five species in this study was at 1 and 2 cm burial depths, which indicates that seedlings have potential for successful establishment at those depths. However, under full sunlight, the lack of emergence of *T. repens* at 4 and 6 cm depths and the lower emergence percentage at 6 cm depth for the other

Table 4. Leaf traits of seedlings (mean ± SE) at various burial depths (1, 2, 4 and 6 cm) and light conditions (full light and shade) for five species.

Species (cm)	Length of cotyledon (cm)		Width of cotyledon (cm)		Length of the first leaf (cm)		Width of the first leaf (cm)		Leaf number	
	Full Light	Shade	Full light	Shade	Full light	Shade	Full light	Shade	Full light	Shade
T. repens										
1	0.35±0.01^{a}	0.45±0.03^{a}	0.20 ±0.005^{a}	0.26±0.02^{a}	0.42 ±0.01^{a}	0.31±0.02^{a}	0.50 ±0.01^{a}	0.31±0.03^{ab}	1.6 ±0.1^{a}	1.0±0.04^{a}
2	0.35±0.01^{a}	0.40±0.01^{ab}	0.20±0.003^{a}	0.21±0.004^{b}	0.41±0.01^{a}	0.36±0.02^{a}	0.47±0.01^{a}	0.37±0.02^{a}	1.5 ±0.1^{a}	0.9±0.1^{a}
4		0.34±0.02^{b}		0.16±0.10^{c}		0.23±0.02^{b}		0.24±0.02^{b}		0.3±0.1^{b}
M. falcata										
1	0.61±0.01^{a}	0.82±0.02^{a}	0.31±0.01^{a}	0.43±0.01^{a}	0.53±0.02^{a}	0.47±0.02^{a}	0.63±0.02^{a}	0.55±0.02^{b}	2.8 ±0.1^{a}	1.4±0.1^{b}
2	0.61±0.01^{a}	0.83±0.03^{a}	0.28±0.01^{ab}	0.44±0.01^{a}	0.50±0.01^{ab}	0.53±0.02^{a}	0.61±0.01^{ab}	0.69±0.02^{a}	2.7 ±0.1^{a}	1.7±0.1^{a}
4	0.56±0.02^{a}	0.80±0.03^{a}	0.25±0.01^{b}	0.41±0.01^{a}	0.45±0.02^{b}	0.49±0.02^{a}	0.55±0.02^{b}	0.60±0.03^{ab}	2.3 ±0.1^{b}	1.3±0.1^{b}
6		0.62±0.02^{b}		0.34±0.04^{b}		0.33±0.05^{b}		0.45±0.06^{c}		0.7±0.2^{c}
M. suaveolens										
1	0.61±0.01^{a}	0.78±0.03^{a}	0.28±0.01^{ab}	0.43±0.02^{a}	0.84±0.02^{a}	0.40±0.02^{b}	0.99 ±0.03^{a}	0.42±0.03^{b}	1.9 ±0.1^{a}	1.4±0.1^{a}
2	0.64±0.02^{a}	0.83±0.07^{a}	0.29±0.01^{a}	0.41±0.03^{a}	0.86±0.03^{a}	0.54±0.05^{a}	1.01±0.03^{a}	0.54±0.05^{a}	2.0 ±0.1^{a}	1.4±0.1^{a}
4	0.59±0.01^{ab}	0.72±0.02^{a}	0.26±0.01^{bc}	0.40±0.02^{ab}	0.71 ±0.02^{b}	0.44±0.04^{ab}	0.88±0.02^{b}	0.46±0.04^{b}	1.3 ±0.1^{b}	1.2±0.1^{a}
6	0.55±0.02^{b}	0.69±0.03^{a}	0.23±0.01^{c}	0.32±0.02^{b}	0.63±0.03^{b}	0.33±0.04^{c}	0.80±0.04^{b}	0.33±0.04^{b}	1.1±0.1^{b}	0.7±0.1^{b}
M. sativa										
1	0.72±0.02^{a}	1.00±0.03^{a}	0.34±0.01^{a}	0.51±0.02^{a}	0.59±0.02^{a}	0.56±0.02^{b}	0.81±0.02^{a}	0.68±0.02^{bc}	3.1±0.3^{a}	2.0±0.03^{a}
2	0.71±0.02^{a}	1.00±0.03^{a}	0.32±0.01^{a}	0.47±0.01^{ab}	0.60±0.02^{a}	0.62±0.02^{a}	0.79±0.02^{a}	0.79±0.02^{a}	3.1±0.2^{a}	2.1±0.1^{a}
4	0.67±0.02^{a}	0.90±0.03^{ab}	0.30±0.01^{ab}	0.45±0.01^{bc}	0.56±0.02^{a}	0.54±0.02^{b}	0.75±0.02^{ab}	0.71±0.02^{b}	2.7±0.1^{a}	1.8±0.1^{b}
6	0.66±0.01^{a}	0.87±0.03^{b}	0.26±0.01^{b}	0.42±0.01^{c}	0.54±0.02^{a}	0.52±0.02^{b}	0.70±0.02^{b}	0.62±0.03^{c}	2.7 ±0.1^{a}	1.4±0.1^{c}
L. davurica										
1	0.59±0.01^{a}	0.67±0.02^{b}	0.33±0.01^{a}	0.39±0.01^{b}	0.73±0.03^{a}	0.49±0.05^{ab}	0.61±0.01^{b}	0.51±0.07^{a}	3.2±0.1^{a}	1.3±0.2^{b}
2	0.52±0.02^{b}	0.75±0.02^{a}	0.31±0.01^{a}	0.46±0.01^{a}	0.70±0.02^{a}	0.59±0.02^{a}	0.69±0.03^{a}	0.53±0.02^{a}	3.1±0.1^{ab}	1.7±0.1^{a}
4	0.56±0.01^{ab}	0.70±0.02^{ab}	0.30±0.01^{a}	0.39±0.01^{b}	0.71±0.02^{a}	0.43±0.03^{b}	0.66±0.02^{ab}	0.45±0.03^{b}	2.9 ±0.1^{b}	1.3±0.1^{b}
6		0.65±0.01^{b}		0.43±0.03^{ab}		0.37±0.04^{b}		0.31±0.03^{c}		0.7±0.1^{c}

Means with different letters are significantly different at $p < 0.05$ level under various burial depths.

four species were primarily due to the inability of seedlings to grow through that depth of soil. Higher seedling emergence at greater depths was observed for large seeded species compared with small seeded species, probably due to the greater food reserves which can in turn produce longer

Table 5. Seedling biomass (Mean ± SE) and relative growth rate (RGR) at various burial depths (1, 2, 4 and 6 cm) and light conditions (full sunlight and shade) for five species.

Species (cm)	Seedling biomass (mg)		RGR (mg/mg/d)	
	Full sunlight	Shade	Full sunlight	Shade
T. repens				
1	3.79 ± 0.18^{a}	0.56 ± 0.003^{a}	0.11	0.012
2	3.62 ± 0.21^{a}	0.55 ± 0.02^{a}	0.10	0.011
4		0.37 ± 0.01^{b}		-0.009
M. falcata				
1	8.48 ± 0.38^{ab}	1.70 ± 0.12^{a}	0.10	0.017
2	8.97 ± 0.49^{a}	1.79 ± 0.10^{a}	0.10	0.019
4	6.48 ± 0.7^{3b}	1.70 ± 0.07^{a}	0.08	0.017
6		1.00 ± 0.03^{b}		-0.010
M. suaveolens				
1	11.90 ± 0.40^{ab}	1.66 ± 0.06^{ab}	0.10	-0.007
2	13.89 ± 1.53^{a}	1.98 ± 0.02^{a}	0.10	0.007
4	8.30 ± 0.50^{bc}	1.51 ± 0.13^{b}	0.08	-0.002
6	5.74 ± 0.80^{c}	1.45 ± 0.14^{b}	0.06	-0.009
M. sativa				
1	17.73 ± 1.83^{a}	2.80 ± 0.14^{a}	0.11	0.015
2	16.90 ± 1.70^{ab}	2.92 ± 0.12^{a}	0.10	0.017
4	16.16 ± 0.33^{ab}	2.51 ± 0.07^{ab}	0.10	0.009
6	11.33 ± 0.85^{b}	2.29 ± 0.10^{b}	0.08	0.004
L. davurica				
1	16.37 ± 0.59^{a}	2.13 ± 0.06^{b}	0.10	-0.001
2	16.15 ± 0.27^{a}	2.69 ± 0.04^{a}	0.10	0.011
4	14.03 ± 0.38^{b}	2.11 ± 0.09^{b}	0.09	-0.001
6		1.61 ± 0.06^{c}		-0.015

Means with different letters are significantly different at $p < 0.05$ level under various burial depths.

hypocotyls, thus, facilitating their emergence above the surface soil. However, the seedlings emerging from greater depths largely exhausted their food supply before emergence was accomplished.

Improved seedling emergence under shade conditions was found in all legume species we studied, consistent with previous study and indicated that more legume seedlings emerged in the shade than in full sunlight (Gardener et al., 2001). Becker et al. (1988) noted that seeds on mounds under full sunlight may have experienced both heat and moisture stress in the upper layers of the soil. In our results, we also find this promotion in percentage emergence. However, the time to emergence was not affected by the light treatment.

Seedling morphology

Increased planting depth reduced seedling size by reducing seedling height, number and leaf size for all five legume species similar to that reported in some warm-season grasses (Newman and Moser, 1988). The length of below-ground hypocotyls was significantly longer in seedlings that had emerged from seeds sown at greater depths, again consistent with previous studies (Maun and Riach, 1981; Maun and Lapierre, 1986). Such a plastic response in morphology suggests that when required, the seedlings allocate more reserves to the hypocotyls to facilitate emergence from a deeper location.

At low irradiance, species change most in their morphological characteristics because light interception is crucial in a light-limited environment (Poorter and Rose, 2005). As with Kitajima’s (1994) result, in our study, all five legume species exhibited similar degrees of morphological acclimation responses to light. Height growth was promoted significantly by shading similar to that observed in oak (Beon and Bartsch, 2003), and so was the above-ground hypocotyls. The inhibition of hypocotyl elongation by light has also been observed in other species (Vandenbussche et al., 2005). However, compare to seedlings grown under full sunlight, those grown under shade usually had less seedling biomass and leaf number.

It should be pointed out that in this study the hypocotyls were divided into two parts: above-ground and below-ground. Not unexpectedly, the results show that burial depth had little effect on the length of the above-ground hypocotyls, which only changed slightly at different depths. Whereas the length of the below-ground hypocotyls was significantly increased by depth- thus, showing that the plastic response reflected only on the below-ground part of the hypocotyls. In contrast, the shade treatment only significantly affected the above-ground hypocotyls. The increasing growth of the two parts of hypocotyls in shade or more deeply buried demonstrated the survival strategy of the seedlings to an unfavorable environment.

Cotyledon size was larger under the shade in all treatments. Light acclimation responses of leaf photosynthetic traits are crucial for optimization of the net carbon gain of a plant and are normally exhibited by the first seedling leaves (Kitajima, 1994). Cotyledons of epigeal germinating plants are known as the first organ to be photosynthetically active soon after emergence (McWilliam et al., 1970), so enlarged cotyledons under shade enable the seedling to survive in the environment lacking of adequate light.

Seedling biomass and RGR

Seedling biomass decreased with increasing burial depth and from full light to shade. The RGR of the seedlings exhibited the same tendency as the seedling biomass for all five species, that is, it was reduced by low light, similar to the results of Walters et al. (1993). Seedling emergence from deeper burial exhausted a greater fraction of seed reserves by the time the seedling emerged and thus, seedlings had less initial investment for future growth. Furthermore, seedlings grown under shade could not get enough light for photosynthesis during their development. Both of these reasons could also explain the negative RGR under shade in this study. These seedlings may die after longer experimental stage. Walters and Reich (2000) stated that deeply shade resulted frequently in higher seedling mortality.

Seedling biomass was usually greater for large seeded species and smaller for small seeded species in all treatments. Larger seeded species also exhibit superior establishment in resource-limited, competitive habitats, enhanced emergence from deep litter layers and greater tolerance of dense shade and defoliation (Leishman and Westoby, 1994; Westoby et al., 1996; Leishman, 2001). In contrast, smaller seeded species exhibit better establishment in open, disturbed habitats, where they require fewer seed resources for early growth (Salisbury, 1943). However, RGR was not correlated with seed mass whether grown under shade or full sunlight.

Recommendations for producer

Seiwa et al. (2002) indicated that there was an optimal range of burial depth that maximizes seedling emergence and subsequent seedling growth. Knowledge of this optimum planting depth for individual species is critical for establishing productive stands in fields. In our study, maximum emergence and seedling size for all five forage legume species maximum percentage emergence and seedling size occurred at 1 and 2 cm depths. Therefore, we concluded that the five forage legume species in this study established under irrigated conditions would have better performances using more shallow planting depths (1 and 2 cm) than when more deeply buried.

This experiment also showed that deep burial reduced seedling emergence, which was seen normally to be disadvantageous. However, in arid or semi-arid regions, such as some parts of northern China, where these forage legumes may be planted in areas receiving no irrigation, seedlings may germinate then quickly desiccate when planted at shallow depths (Heckman et al., 2002). Sowing more deeply can be advantageous if the seeds have sufficient energy reserves and are capable of sufficient hypocotyl elongation that results in seedling emergence. Therefore in semi-arid regions, it is better choosing species like *M. suaveolens* and *M. sativa*, which have high emergence from greater depths, to establish forage legume grassland. If there is a requirement to over-sow forage legumes to established grassland to create a mixed pasture, then *M. falcata* and *M. sativa* which have more shade tolerance maybe more appropriate. According to the higher emergence percentage of these legume species under shade condition, another approach for this situation could be defoliating after legume grasses emergence is better for seedlings establishment. Especially in some arid climates, the higher solar radiation reaching the soil surface combined with warmed temperatures and even air quality factors tends to increase evaporative losses, lower surface and subsurface moisture (Forcella et al., 2000). Application of the results of this study to any specific situation the field production should be approached with appropriate cautions and further studies may be needed.

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