Seed Germination and Early Seedling Growth of *Cynanchum bungei* Decne (Asclepiadaceae) in Response to Photoperiod, Temperature, and Seed Size

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Abstract. *Cynanchum bungei* Decne is a rare, endemic, and important medicinal plant species in China. Seed germination and early seedling growth of large seeds (greater than 7 mm) and small seeds (smaller than 7 mm) were investigated at three temperatures (15, 20, and 25 °C) in both continuous light and alternating light/dark photoperiods to determine seed propagation requirements. Photoperiod significantly affected seed germination and early seedling growth. Germination and seedling growth at a 12:12-h photoperiod performed better than in continuous light. Temperature had a significant effect on germination index (GI), vigor index (VI), germination velocity (GV), mean germination time (MGT), shoot biomass (SB), root biomass (RB), and taproot length (TL), but no significant effect on final germination percentage (FGP). A temperature of 20 °C was the optimum temperature for seed germination and early seedling growth. Average growth height (AGH) and relative growth rate (RGR) of shoots at 15 °C were greater than that at 20 °C. Large seeds had better germination and seedling performance than small seeds. However, small-seeded seedlings had greater biomass allocated to roots (BAR) and root-to-seedling ratio (RSR) than seedlings from large seeds. Small seeds of *C. bungei* could be more competent in unfavorable soil and light conditions than large seeds.

Cynanchum bungei Decne (Baishouwu in China), a member of the Asclepiadaceae family, is a perennial climbing shrub distributed in China. Its root has been used as a tonic medicine or health food for centuries in traditional Chinese medicine. *C. bungei* has useful activities including antitumor and immune regulatory effect and is non-toxic (Gao et al., 2005). As a rare, endemic, and beneficial species, *C. bungei* is becoming more and more precious not only in China, but also in other countries such as Japan and Korea (Song et al., 2006).

C. bungei is grown mainly by cuttings and root propagation and seldom by seed

propagation. With the increasing demand for this endangered wild resources (Zhang et al., 2010), seed propagation can be used to solve supply and demand, protect germplasm resource, and enrich genetic diversity of this species.

Environmental factors such as light and temperature are known to affect seed germination in Asclepiadaceae family. Seeds of *Calotropis procera* and *Leptadenia pyrotechnica* are quick germinating and do not have strict requirements for light and temperature conditions, whereas seeds of *Cryptostegia grandiflora* and *Pergularia daemia* are slow germinating and exhibit optimum germination in dark at ≈30 °C (Sen, 1968). Seeds of *Asclepias tuberosa* and *Asclepias viridiflora* germinate well at 21 °C and light is not an influencing factor (Norman, 1993). Seeds of *Matelea maritima* germinate at an optimum temperature of 25 °C in light, the process being optimized by alternating temperatures of 25/35 °C (Cuzzuol and Lucas, 1999). Also, light requirement for germination can vary with temperature (Smith, 1982).

Seed size is a factor that is associated with seed germination and seedling growth (Farhoudi and Motamedi, 2010). Studies of the relationship between seed size and seed germination and seedling performance have provided various results. A positive relationship

between seed size and seed germination and seedling performance has been found within seed lots in a number of studies (Benard and Toft, 2007). In saline soils, smaller seeds of safflower and chickpea germinate faster and have better seedling performance compared with larger seeds (Farhoudi and Motamedi, 2010; Kaya et al., 2008). In different populations of the dimorphic *Tragopogon pratensis* subsp. *pratensis*, larger seeds yield higher germination percentage, yet no relationship has been found between seed size and seedling growth (Mölken et al., 2005).

Previous studies suggested that most of these factors show diverse effects on seed germination and seedling growth characteristics of different species. Information about *C. bungei* seed germination is lacking. The objectives of the study were to investigate seed germination and early seedling growth responses of *C. bungei* to photoperiod, temperature, and seed size to determine seed propagation requirements, develop standard germination procedures, and provide conservation strategies for *C. bungei*.

Materials and Methods

Seed source. Seeds of *C. bungei* were collected from the north-facing slope of Mount Tai (lat. 36°15'17" N, long. 117°06'15" E) in late fall and winter in 2010. Seeds were air-dried at room temperature and stored in darkness at 4 °C until Mar. 2011.

Experimental procedures. Germination tests were performed from Mar. to May 2011. Seed length was measured manually and separated into two groups, i.e., small (less than 7 mm) and large (greater than 7 mm). Large and small seeds were immersed in 75% ethanol for 5 min and then 5% sodium hypochlorite for 5 min for surface sterilization and then rinsed three times with deionized water and dried on filter paper.

The experiment was designed as a completely randomized factorial of two photoperiods, three temperatures, and two seed size treatments. Each treatment was replicated six times. Forty seeds per treatment were placed in a covered germination box (13 × 19 × 12 cm) containing 1200 g sterilized sand mixed with 187 mL deionized water. Seeds were germinated in incubators (HPG-280BX; Harbin Donglian Electronic Technology Development Ltd., Harbin, China) at 15 (T15), 20 (T20), and 25 °C (T25) in continuous light (Panasonic white fluorescent lamps, 234 μmol·m⁻²·s⁻¹, 580 nm) (L24) and alternating 12-h light/12-h dark (L12) conditions.

The germinated seeds were recorded everyday for 20 d. Seeds were considered to have germinated as soon as cotyledons emerged above the sand. Covers were removed when shoot length reached 5 cm in height. The seedlings were watered regularly. The germination boxes were rearranged daily to avoid effects of potential temperature and light differences or gradients in the incubators.

Germination performance was estimated according to the rate of germinated seeds. FGP is the rate of germinated seeds on the

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20th day. Germination index was $GI = \Sigma(Gt/Tt)$, where Gt is the number of germinated seeds in Day t and Tt is the time corresponding to Gt in days. Vigor index was $VI = GI \times \text{shoot height on the 25th day}$. MGT was calculated according to Hu et al. (2005): $MGT = \Sigma TiNi / \Sigma Ni$, where Ni is the number of the newly germinated seeds in times of Ti . GV was estimated by using a modified Timson's (1965) index of germination through the expression: $GV = \Sigma Gi/Ti$, where i is the number of counting periods at which the germination was evaluated, Gi is the percentage of germinated seeds in the i period, and Ti is the number of days since the initiation of the assay.

Shoot height (SH) was measured every-day for 25 d. Average growth height of shoot: $AGH = (\text{SH on 25th day} - \text{SH on 20th day})/5$; $RGR (\%) = (\text{SH on the 25th day} - \text{SH on the 20th day})/\text{SH on the 20th day} \times 100$. On the 25th day, we carefully uprooted the plant and washed the roots in a sieve with hole size of 1 mm and measured TL. $RSR (\%) = TL / (TL + \text{SH on the 25th day}) \times 100$. The shoot and roots of each seedling were dried at 60 °C for 48 h to quantify SB and RB. The percentage of BAR (%) was $RB/(RB + SB) \times 100$.

Data analysis. FGP and BAR data were arsine transformed before statistical analysis to ensure homogeneity of variance. However, the original percentages are presented in the tables and figure. The germination and seedling growth characteristics were subjected to analysis of variance followed by Duncan's multiple range test using SPSS 11.5 software (SPSS Inc., Chicago, IL).

Results

Germination. Seeds began to germinate earlier and germination performance was better in 12 h light than continuous light (Table 1). The higher the temperature, the earlier seeds began to germinate. At 15, 20, and 25 °C, seeds began to germinate between the 12th to 14th, 10th, and 8th to 9th day period, respectively. Overall, germination was greater at 20 than 15 °C but not well at 25 °C after 14 d (Table 1). Except for the 14th to 17th day period at 15 °C in continuous light, germination performance of large seeds was better than small seeds (Table 1).

Photoperiod and seed size had a significant effect on FGP. FGP was significantly higher in 12 h light than continuous light and

large seeds than small seeds (Table 2). Temperature had no significant effect on FGP (Table 2).

Photoperiod, temperature, and seed size had significant effects on GI, VI, GV, and MGT. GI, VI, and GV were significantly higher in 12 h light than continuous light, high temperature than low temperature, and large seeds than small seeds (Table 2). MGT was significantly shorter in 12 h light than continuous light, high temperature than low temperature, and large seeds than small seeds (Table 2).

Seedling growth. Because the shoots at 25 °C wilted from the 16th day, SH was not determined after 16 d at 25 °C. A high temperature (25 °C) was not favorable for seedling growth and was excluded from the seedling growth analysis. Overall, SH was much higher at 20 °C than 15 °C and in 12 h light than continuous light (Table 3). Except for the 14th to 18th day period at 15 °C in continuous light, SH of large seeds was higher than that of small seeds. Overall, both AGH and RGR of shoot were higher at 15 than 20 °C, in 12 h light than continuous light, and large seeds than small seeds in the early seedling stage.

Table 1. Germination performance of large and small seeds in *C. bungei* at 15 (T15), 20 (T20), and 25 °C (T25) in alternating 12 h light/12 h dark (L12) and continuous light (L24).

			Germination (%)													
Photoperiod	Temperature	Seed size	8 d	9 d	10 d	11 d	12 d	13 d	14 d	15 d	16 d	17 d	18 d	19 d	20 d	
L12	T15	Large	0	0	0	0	1.67	11.67	33.33	56.67	63.33	75.00	78.33	78.33	88.33	
		Small	0	0	0	0	1.67	6.67	16.67	30.00	55.00	61.67	73.33	73.33	81.67	
	T20	Large	0	0	15.00	41.67	71.67	75.00	81.67	83.33	85.00	88.33	88.33	88.33	88.33	
		Small	0	0	6.67	23.33	53.33	61.67	73.33	76.77	83.33	83.33	83.33	85.00	85.00	
	T25	Large	8.33	41.67	65.00	80.00	88.33	90.00	90.00	91.67	91.67	91.67	91.67	91.67	91.67	
		Small	5.00	21.67	41.67	46.67	55.00	61.67	66.67	73.33	73.33	73.33	73.33	73.33	73.33	
L24	T15	Large	0	0	0	0	0	0	6.67	13.33	26.67	48.33	65.00	68.33	73.33	
		Small	0	0	0	0	0	0	13.33	20.00	35.00	50.00	58.33	63.33	66.67	
	T20	Large	0	0	7.50	17.50	42.50	67.50	72.50	72.50	72.50	75.00	75.00	75.00	75.00	
		Small	0	0	2.50	10.00	37.50	42.50	62.50	70.00	70.00	70.00	70.00	72.50	72.50	
	T25	Large	0	18.33	33.33	56.67	68.33	71.67	71.67	71.67	73.33	73.33	73.33	73.33	73.33	
		Small	0	13.33	26.67	46.67	56.67	61.67	65.00	66.67	66.67	66.67	66.67	68.33	68.33	

Table 2. Final germination percentage (FGP), germination index (GI), vigor index (VI), germination velocity (GV), and mean germination time (MGT) of large and small seeds in *C. bungei* at 15 (T15), 20 (T20), and 25 °C (T25) in alternating 12 h light/12 h dark (L12) and continuous light (L24).

Photoperiod	Temperature	FGP (%)		GI		VI		GV		MGT (d)	
		Large	Small	Large	Small	Large	Small	Large	Small	Large	Small
L12	T15	88.33	81.67	1.16	1.03	4.69	3.02	0.28	0.23	15.49	16.14
	T20	88.33	85.00	1.51	1.38	7.81	5.01	0.53	0.46	11.88	12.59
	T25	91.67	73.33	1.88	1.39	—	—	0.72	0.51	9.91	11.01
L24	T15	73.33	66.67	0.88	0.82	1.59	1.39	0.17	0.17	16.89	16.43
	T20	75.00	72.50	1.23	1.13	4.55	3.30	0.42	0.37	12.29	13.00
	T25	73.33	68.33	1.40	1.26	—	—	0.52	0.46	10.63	11.15
Light		**		**		**		**		**	
L12		84.72 a ^z		1.39 a		5.13 a		0.45 a		12.84 b	
L24		71.53 b		1.12 b		2.71 b		0.35 b		13.40 a	
Temperature		NS		**		**		**		**	
T15		77.50 a		0.97 b		2.67 b		0.21 c		16.24 a	
T20		80.21 a		1.31 a		5.17 a		0.44 b		12.44 b	
T25		76.67 a		1.48 a		—		0.55 a		10.67 c	
Seed size		**		*		**		**		**	
Large		81.67 a		1.34 a		4.66 a		0.44 a		12.85 b	
Small		74.58 b		1.17 b		3.18 b		0.37 b		13.39 a	

^zWithin a factor and column, values followed by different letters are significantly different ($P \leq 0.05$).

NS, *, **Nonsignificant or significant at $P \leq 0.05$, or 0.01, respectively.

Table 3. Shoot height (SH), average growth height (AGH), and relative growth rate (RGR) of large and small seeds in *C. bungei* at 15 (T15), 20 (T20), and 25 °C (T25) in alternating 12 h light/12 h dark (L12) and continuous light (L24).

Photoperiod	Temperature	Seed size	SH (cm)																		AGH (cm)	RGR (%)	
			8 d	9 d	10 d	11 d	12 d	13 d	14 d	15 d	16 d	17 d	18 d	19 d	20 d	21 d	22 d	23 d	24 d	25 d			
L12	T15	Large	0	0	0	0	0.01	0.07	0.24	0.36	0.70	1.01	1.33	1.58	2.13	2.52	2.87	3.47	3.62	4.04	0.38	89.67	
		Small	0	0	0	0	0.01	0.04	0.11	0.15	0.43	0.62	0.90	1.08	1.58	1.89	2.20	2.58	2.66	2.93	0.27	85.44	
	T20	Large	0	0	0.12	0.37	0.87	1.35	1.82	2.32	2.75	3.25	3.73	3.96	4.33	4.47	4.73	4.92	5.08	5.16	0.17	19.17	
		Small	0	0	0.06	0.19	0.53	0.85	1.15	1.55	1.90	2.15	2.48	2.65	2.94	3.07	3.25	3.40	3.56	3.64	0.14	23.81	
	T25	Large	0.08	0.49	1.18	1.87	2.53	3.11	3.56	4.04	4.30	—	—	—	—	—	—	—	—	—	—	—	—
		Small	0.02	0.19	0.56	0.82	1.07	1.46	1.72	1.98	2.13	—	—	—	—	—	—	—	—	—	—	—	—
L24	T15	Large	0	0	0	0	0	0	0.04	0.09	0.23	0.45	0.86	1.16	1.37	1.69	1.73	1.85	2.02	2.38	0.20	73.72	
		Small	0	0	0	0	0	0	0.10	0.17	0.33	0.57	0.88	1.05	1.19	1.47	1.52	1.68	1.74	1.99	0.16	67.22	
	T20	Large	0	0	0.02	0.08	0.47	0.97	1.60	1.89	2.08	2.49	2.81	3.04	3.12	3.26	3.35	3.53	3.76	3.89	0.15	24.68	
		Small	0	0	0.02	0.09	0.39	0.65	1.16	1.49	1.63	1.80	2.11	2.42	2.49	2.59	2.65	2.73	2.86	2.91	0.08	16.87	
	T25	Large	0	0.15	0.47	0.92	1.70	2.07	2.53	2.69	2.76	—	—	—	—	—	—	—	—	—	—	—	—
		Small	0	0.09	0.34	0.65	1.13	1.44	1.80	1.98	1.99	—	—	—	—	—	—	—	—	—	—	—	—

Photoperiod, temperature, and seed size had a significant effect on SB, RB, and TL. SB, RB, and TL were significantly greater in 12 h light than continuous light, at 20 than 15 °C, and large seeds than small seeds (Fig. 1A–C). Nevertheless, seedlings derived from small seeds allocated more plant biomass to roots (small seeds = $35.34\% \pm 3.39\%$, large seeds = $31.19\% \pm 5.18\%$; $F = 28.43$, $P < 0.0001$; Fig. 1D) and developed roots with greater RSR (small seeds = $61.65\% \pm 4.22\%$, large seeds = $58.72\% \pm 3.84\%$; $F = 14.44$, $P = 0.00157$) than those germinating from large seeds.

Discussion

Plants vary in their response to light during seed germination (Baskin and Baskin, 1998). This study showed that germination characteristics and seedling growth were better in alternating light/dark than in continuous light at all temperatures tested (Tables 1, 2, and 3; Fig. 1), indicating that continuous light can inhibit seed germination and seedling growth of *C. bungei*. In this sense, presence of dark enhances seed germination and seedling growth of this species. The inhibition of continuous light on germination characteristics and seedling growth of large seeds was greater than that of small seeds, indicating that small seeds are more competent in unfavorable light conditions.

In this study, temperature had a significant effect on GI, VI, GV, and MGT but no significant effect on FGP (Table 2). High temperature enhanced GV, advanced germination initiation time, and shortened MGT, but germination and seedling performance at 25 °C was not satisfactory (Tables 1 and 3). The optimum temperature for seed germination and early seedling growth of *C. bungei* was 20 °C. However, 15 °C treatment had greater AGH and RGR of shoot compared with 20 °C treatment (Table 3). The experiment would have to be repeated with a longer duration of time before we could conclude with confidence whether 15 or 20 °C is the optimal temperature in the whole seedling stage. Temperature shifts may affect a number of processes determining the germinability of seeds, including membrane permeability, activity of membrane-bound

Fig. 1. Shoot biomass (SB) (A); root biomass (RB) (B); taproot length (TL) (C); and the percentage of biomass allocated to root (BAR) (D) of large and small seeds in *C. bungei* at 15 (T15) and 20 °C (T20) in alternating 12 h light/12 h dark (L12) and continuous light (L24). Bars represent $\pm$ SE of means. **Significant at $P \leq 0.01$.

proteins, and cytosol enzymes (Bewley and Black, 1994). In the future, alternating temperature regimes should be considered to further elucidate temperature effects on germination of this species.

Recently, the effects of seed size variation on seed germination and seedling growth have received great attention (Benard and Toft, 2007; Bretagnolle et al., 1995; Mohsen et al., 2011; Moles and Westoby, 2004). There is positive (Mölken et al., 2005), negative (Benard and Toft, 2007; Kaya et al., 2008; Rebecca, 1984; Susko and Lovett-Doust, 2000) or no relationship (Boyle and Hladun, 2005) between seed size and germination performance. In this study, large seeds had higher FGP, GI, VI, GV, SB, RB, TL, and shorter MGT. The author inferred that either embryo size or endosperm content could differ between large and small *C. bungei* seeds. Some authors hypothesized that large seeds enable seedling to allocate proportionally more resources to root development than small seeds (Baker, 1972; Stebbins, 1971).

If this hypothesis stands, then seed size should be positively correlated with the allocation percentage of biomass to root system (Leishman and Westoby, 1994), but evidence to support this hypothesis has been limited (Benard and Toft, 2007). In this study, small-seeded seedlings had a significantly greater BAR and RSR in contrast with other studies that have detected a positive (Benard and Toft, 2007; Leishman and Westoby, 1994) or no relationship (Jurado and Westoby, 1992; Wulff, 1986) between seed size and allocation to root systems. The greater BAR and RSR would be advantageous for small seeds of *C. bungei* to grow in unfavorable soils with high pH, little water, and low nutrients, etc.

Conclusion

Seed propagation is a valuable method for the reproduction and conservation of *C. bungei* and thereby expand genetic variability. We found that alternating light/dark and 20 °C are appropriate requirements for

C. bungei seed germination. Large seeds have a higher germinability than small seeds; however, small seeds could be more competent in unfavorable conditions.

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