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Factors affecting seed germination and emergence of *Sophora davidii*

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ABSTRACT

Sophora davidii is a popular ecological and feeding plant, but it also is a potential medicinal plant. Its root has high levels of lupin alkaloids, which make a kind of valued matrine-type alkaloids as one of the best alternatives to pentazocine. Very limited agronomic information exists regarding the cultivation of *S. davidii* as a medicinal and industrial crop. The objective of this study was to investigate seed germination and emergence of *S. davidii* in response to temperature, osmotic potential, salinity and depth of seed burial. The effect of temperature, osmotic potential and salinity on seed germination were examined at different temperatures (5–40 °C), while using different polyethylene glycol (PEG)-6000 (0–−1.0 MPa) and different $CaCl_2$ (0–200 mM) solutions. Seedling emergence was also examined for seeds sown at different depths (0–9 cm). Temperature affected germination rates of *S. davidii*; maximum germination (30.67%) was reached at 25 days a constant temperature of 20 °C. Maximum germination rates were obtained under low osmotic potential (0–−0.4 MPa) and low salinity concentrations (20–50 mM $CaCl_2$); increasing osmotic potential or salinity progressively inhibited seed germination. Deep burial significantly decreased seedling emergence rates, seedlings emerged well at depths of 0–3 cm, but no seedlings emerged from seeds buried at depths of ≥9 cm. As a general recommendation to growers *S. davidii* should be seeded no deeper than 3-cm depth, and the suitable environmental conditions (temperatures of 20–30 °C, water stress of 0–−0.4 MPa and salt stress of 20–50 mM $CaCl_2$) may be encountered.

1. Introduction

The karst mountain region of southwest China, spanning an area about 540,000 km^2, is one of the largest continuous karst landscapes in the world (Yang et al., 2012; Qi et al., 2013). Historically, inappropriate agricultural practices and over-exploitation of sloping lands in southwestern China have caused these areas to suffer greatly from desertification, resulting in erosion, and loss of natural vegetation (Wu et al., 2011). *Sophora davidii* is an important leguminous shrub that is widely distributed in karst mountainous region of southeastern China (Fan et al., 2013). In recent years, *S. davidii* with its higher tolerance for dry environments than other shrubs has been widely used for vegetation restoration in karst area (Wu et al., 2012). As a significant species with economic and environmental importance, *S. davidii* is often used as herbal medicines (Ohyama et al., 1996). Its flowers, leaves and fruits usually been used as medicines by local people (Li et al., 2009). A variety of compounds were isolated from the root of *S. davidii*, included the alkaloids that contains (+)-matrine which had an antinociceptive effect identical to that of pentazocine with important medicinal applications (Xiao et al., 1999). As *S. davidii*'s high value but low volume of production, many important best management practices are not well-defined, which could limit its potential. Previous studies about *S. davidii* mainly focused on the morphology and anatomy(Wu et al., 2008; Li et al., 2009; Li and Bao, 2014), high seed dormancy and low seedling establishment (Shao et al., 2010; Li et al., 2009), drought resistance and photosynthesis (Wu et al., 2008; Li et al., 2009; Wu et al., 2012). But, agronomic information involved in seed germination and emergence in *S. davidii* are not well understood.

In this paper, analyses of *S. davidii* have included examining effects of temperature, osmotic potential, and salinity on seed germination as well as the emergence and survival of seedlings when seeds were sown on the surface of karst soils or at different depths in karst soil. We hypothesized that: (1) Seed germination is affected by environmental changes and germination depend on variations

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in temperature, osmotic potential, and salinity; (2) Seedling emergence is affected by burial depths.

2. Materials and methods

2.1. Seed collection and germination tests

Seeds of *S. davidii* were collected in October 2012 from a karst mountain region (W26°21′N, 105°49′E) in Guanling Town, Guizhou, China. The topography is characterized by typical karst mountainous landscape with the altitude ranging from 370 to 1850 m. The climate is mild, with a mean annual temperature of 15.4–16.9 °C (the minimum and maximum monthly mean temperatures were 4 °C in January and 36.1 °C in July, respectively) and an average rainfall range from 1200 to 1650 mm, of which 70% occured between April and August. The mean seed weight of *S. davidii* was 11.9 ± 3.2 mg. After collection, the seeds were dried in a greenhouse for 2 days, bulked, cleaned, and stored in a dry closed cotton bag at 5 °C and 10% relative humidity until used in the experiments. Seed germination experiments were started by placing the seeds in 9-cm-diameter glass Petri dishes containing two layers of filter paper and 5 ml of distilled water or treatment solution. Dishes were placed in an incubator at fluctuating day/night temperature of 30/20 °C in a light/dark regime, unless stated otherwise. The photoperiod was set at 12 h to coincide with the higher temperature interval. Seed germination was assessed 55 days after the start of the experiment as no seeds germinated in a preliminary experiment in the growth chamber after this period. During 55 days the number of germinated seeds were counted and removed every 1 day. A seed was considered to have germinated when the emerging radicle elongated to 2 mm from the seed coat. The examinations of each germination tests were with 4 replications.

2.2. Effects of temperature on germination

To determine the effects of temperature on germination, 50 seeds of *S. davidii* were placed in each of eight incubators (1600 seeds in total) set at eight different temperature regimes (5, 10, 15, 20, 25, 30, 35, 40 °C). These temperature regimes were selected to reflect the temperature variations occurring in southwest China.

2.3. Effects of water stress on germination

The effects of water stress on germination were assessed by incubating 50 seeds of *S. davidii* in each of five Petri dishes with 5 ml solutions having osmotic potentials of −0.2, −0.4, −0.6, −0.8, and −1.0 MPa (1000 seeds in total). The solution concentrations were prepared by dissolving polyethylene glycol 6000 in distilled water.

2.4. Effects of salt stress on germination

The effects of salinity on seed germination were determined by incubating 50 seeds of *S. davidii* in Petri dishes with 5-ml solutions of 0, 20, 50, 100, 150, 200, and 250 mM of calcium chloride (1400 seeds in total).

2.5. Effects of seed burial depth on seedling emergence

This experiment was conducted in a screenhouse with an overhead transparent plastic cover to avoid rain damage. Karst soil, collected from the natural habitat of *S. davidii*, was autoclaved and passed through a 3-mm sieve. Fifty seeds of *S. davidii* were planted in plastic trays (8 cm × 8 cm × 10 cm) by placing them on the karst soil surface or covering them with the same soil to achieve burial depths of 1, 2, 3, 5, 7 and 9 cm (1400 seeds in total). The trays were initially watered with an overhead "mist" sprinkler system and later were irrigated from below.

The pots were monitored every 24 h and the number of seedlings that had emerged and were still surviving counted. A seedling was considered to have emerged when its height exceeded 3 mm above the sand surface, and it was regarded to be dead when it fell over or had turned yellowish or brownish. The percentage of seedling mortality was calculated as: (the number of seedlings that failed to emerge/50) × 100. The experiment had been fallowed for 90 days.

2.6. Statistical analyses

Data were analyzed using SPSS for Windows, version 13.0 (SPSS Inc., Chicago, Illinois, USA). One-way ANOVA tested for the significance of the main effects of temperature, osmotic potential, salinity, and depth of burial on germination, seedling emergence and seedling mortality. A Tukey test (Honestly significant differences, HSD) was used to estimate the significant ($P < 0.05$) differences between individual treatments. A line describing the evolution of final germination percentages was obtained by polynomial regression.

3. Results

3.1. Effects of temperature on germination

At the temperatures tested, the most rapid and highest germination rates occurred at different temperatures (Fig. 1). During the 55 days, seed germination was first observed on the 4th day at 25 °C and 30 °C, the 5th day at 20 °C, the 7th day at 15 °C and 35 °C and 17th day at 10 °C. Seed germination peaked on the 20th day at 30 °C, the 23rd day at 25 °C, the 25th day at 20 °C, the 29th day at 35 °C, the 42nd day at 15 °C and the 45th day at 10 °C. Temperature significantly affected the final germination percentages, and the maximum germination rate (30.67%) of *S. davidii* occurred at a constant temperature of 20 °C. No germination occurred at 5 or 40 °C. The final germination percent at 20 °C was significantly higher than at other tested temperatures.

3.2. Effect of water stress on germination

In response to the tested constant osmotic potential, the most rapid germination and the highest germination rates occurred at different osmotic potential levels (Fig. 2). During the 55 day trial period, seed germination was first observed on the 3rd day at 0 MPa, the 5th day at −0.2 MPa and −0.4 MPa, the 6th day at −0.6 MPa, the 7th day at −0.8 MPa and 8th day at −1.0 MPa. Maximum seed germination was observed on the 34th day at −0.8 MPa, the 38th day at −0.4 MPa and −0.6 MPa, the 39th day at −0.2 MPa, the 40th day at 0 MPa and the 43th day at −1.0 MPa. Osmotic potential significantly affected the final percentages of germination, and the highest germination rates (32.67%) of *S. davidii* occurred at −0.2 MPa. No significant different was observed between 0 MPa and −0.4 MPa. The optimal osmotic potential for germination occurred at osmotic potential 0 MPa, −0.2 MPa and −0.4 MPa, where germination exceeded 30%; however, seed germination could be graphed with a quadratic curve showing increasing germination rates with increases in osmotic potential concentrations.

3.3. Effect of salt stress on germination

Fig. 3 shows the germination percentage and number of days it took *S. davidii* to germinate as a function of a range of $CaCl_2$ concentration. During the 55 days, the first seed germination was observed on the 3rd day at 0 and 20 mM, the 4th day at 50 mM, the 5th day