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Moss is a key nurse plant for reintroduction of the endangered herb, *Primulina tabacum* Hance

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Abstract The rare and endangered plant *Primulina tabacum* is a calciphilous perennial herb found only at the entrances of a small number of karst cave drainages in southern China. In a conservation effort, we identified potentially suitable habitats and reintroduced *P. tabacum* plantlets (propagated in vitro) to one historical and two new cave entrances. The transplanted seedlings survived (10%) at only one new location where a moss, *Gymnostomiella longinervis* Broth, existed. Our field observations indicate that it is probably impossible for this rare plant to naturally recolonize the places where it went extinct because the habitats have changed. Transplanted *P. tabacum* grew slower than wild *P. tabacum*. The transplanted *P. tabacum* performed especially well under the cover of the nursing moss. Positive interactions between species, i.e., nurse plant effects, are important for reintroduction of success. Although light and soil conditions also appeared to be critical for transplantation success, the presence of moss should be considered as a useful and convenient indicator of suitable habitat for *P. tabacum*. This study case suggests that the use of new propagation methods and nurse plants can facilitate the reintroduction of rare and endangered herbs.

Keywords Conservation · Moss · Nurse plant · *Primulina tabacum* · Reintroduction · Survival rate

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Introduction

Primulina tabacum Hance is a calciphilous perennial herb belonging to the family Gesneriaceae. Its distribution is restricted to the entrances of karst cave drainages along the border between northern Guangdong and southern Hunan, China (Flora of China Editorial Committee 1990). *P. tabacum* is on the list of the "First Class Protected Key Wild Plants of China" in 1999 (Peng and Cheng 2002). Its abundance exhibits a bell-shaped curve along an environmental gradient from the entrance of caves to deeper within caves. *P. tabacum* relies on alkaline calciferous groundwater and grows in poor soils (Ren et al. 2003). Because of recent climate change and increasing anthropogenic disturbances, the population size of *P. tabacum* has drastically decreased during the last three decades (He and Li 2005).

Specimens of *P. tabacum* were first collected by Henry Cox, an American churchman, in 1881 at Lianzhou, Guangdong, China. Because of its conservation status, the taxonomy (Hance 1883; Flora of China Editorial Committee 1990), ecological and biological characteristics (Ren et al. 2003), genetic diversity

(Ni et al. 2006; Wang et al. 2008), pollination biology (Li et al. 2006), pollen morphology (Cao et al. 2007), and the close relationship of the species with moss (Ren et al. 2010) have been investigated subsequently.

We began *P. tabacum* conservation efforts and attempted to reintroduce *P. tabacum* to appropriate sites in 2002. We performed seed germination tests at the South China Botanical Garden during 2003–2006 to determine whether seeds could be used for reintroduction but no seeds germinated (unpublished data). However, we were successful in obtaining tissue culture and plantlet regeneration in 2007 (Ma et al. 2007), which led to the reintroduction of in vitro-propagated *P. tabacum* plantlets to the plant's historical and original habitats.

Reintroduction has been regarded as an extinction-prevention strategy for plant species for at least 100 years (Armstrong and Seddon 2007). Successful reintroduction requires knowledge about the taxonomy, reproductive biology, demography, horticulture, and ecology of the reintroduced species (Kleiman 1989; IUCN 1998; SER 2002; Armstrong and Seddon 2007; Godefroid and Vanderborght 2009). Although there are some success stories, most reintroduction attempts have failed, and the causes remain unknown (Griffith et al. 1989; Maschinski and Wright 2006; Maschinski and Duquesnel 2006; Gibbs et al. 2007). In "Global Strategy for Plant Conservation," Target 8 was ex situ conservation and recovery plans for threatened and endangered species to reintroduce about 10% of endangered plants to wild habitats (BGCI 2003). In those reintroductions, reintroduction biology has traditionally focused on factors affecting population establishment and spread to determine whether reintroductions are successful or not. The research, however, has largely concerned the collecting and analyzing of monitoring data rather than the answering of specific questions that might explain successful and unsuccessful reintroductions (Ollero and Bermejo 1979; Sarrazin and Barbault 1996; Vittoz et al. 2006; Armstrong and Seddon 2007).

Bryophytes are important components of ecosystems (Smith 1982; Frego 2007) and could be useful for the reintroduction of *P. tabacum*. Previous studies of bryophytes have mainly focused on taxonomy and have largely ignored their ecological functions (Smith 1982; Ye et al. 2004). Bryophytes have a very strong ability to absorb and retain water and therefore affect water circulation in ecosystems. Bryophytes can also absorb a large quantity of nutrient elements and therefore affect nutrient cycling (Smith 1982; Wu et al. 2003; Frego 2007). Bryophytes can adapt to various habitats and survive in rocky, desert, and other extreme environments (Foote 1966; Rochefort 2000), where they are important pioneers and primary producers (Hein and Van Tooren 1990). These properties of bryophytes can help the growth of other plants in the same communities. For example, a recent study by Groeneveld and Masse (2007) shows that the bryophyte moss, *Polytrichum strictum*, could facilitate the return of *Sphagnum* species or other boreal plants after disturbances. Our previous study also demonstrates strong relationships between mosses and *P. tabacum* in wild communities (Ren et al. 2010).

In this study, we examined the survival and growth of reintroduced populations of *P. tabacum* to determine whether the reintroductions established self-sustaining populations. We focused on the following questions: (1) Are the historical locations appropriate for reintroduction? (2) How do the reintroduced *P. tabacum* survives and grows compared with wild *P. tabacum*? and (3) What factors influence the growth and survival of the reintroduced plants?

Materials and methods

Study area

This study was conducted at three neighboring caves (caves A, B, and C) at Dixiahe (25°1′ N, 112°21′ E), Lianzhou City, Guangdong Province, southern China, which collectively delimited the historical and current distribution area of *P. tabacum* (24°59′–25°58′ N, 111°98′–113°11′ E). The climate is central subtropical monsoon, and the elevation ranges from 130 to 450 m above sea level. The zonal original soil is lateritic, a heavy acid soil. The mean annual temperature is 19.5°C, and the mean annual rainfall is 1,571 mm. The vegetation is dominated by evergreen broad-leaved forests typical of the subtropics. Representative plant families of a climax community include Lauraceae, Euphorbiaceae, and Fagaceae.

Propagation of *P. tabacum* and preliminary experiment and survey

We established an efficient in vitro propagation and plant regeneration system using biotechnology at the

South China Botanical Garden; plantlets were produced with leaf explants (Ma et al. 2007). Before conducting the field experiments, we conducted a preliminary experiment to determine how to increase the survival of transplanted *P. tabacum* plantlets. We transplanted *P. tabacum* plantlets into a planting bed with original soil (from the caves at Dixiahe, Lianzhou City) in the greenhouse of the South China Botanical Garden. We found that survival of plantlets kept in vitro for 8–11 months was increased if the plantlets had a 1-month acclimation period before they were transplanted. During acclimation, the plantlets were grown in a soil mix (sand, vermiculite, limestone, and half original soil) in a wet, shaded environment. A survey of the wild *P. tabacum* population at Dixiahe caves indicated that the density of these wild populations was about 20 individuals/m^2; we used this value to guide the planting density used in the experiments.

Experiment 1

Experiment 1 was conducted in three neighboring caves (A, B, and C) at Dixiahe. Cave B was where *P. tabacum* was historically distributed but was now extinct, and caves A and C had *P. tabacum* during this study. At each cave, we established three 1 m × 1 m quadrats along a transect with distance intervals of 3, 15, and 45 m from the entrance of the cave to deep into the cave (three quadrats per cave). A light gradient existed among the quadrats at each cave because the light exposure declined with distance from the cave entrance. The quadrats at cave A were designated A1, A2, and A3 (3, 15, and 45 m from the cave entrance, respectively). A similar terminology was used to describe the quadrats at caves B and C.

Experiment 1 was performed twice (experiment 1A and 1B). For experiment 1A, we used leaf explants from the *P. tabacum* population at cave C for tissue culture on January 2007 and obtained about 4,000 plantlets in vitro in July. A former genetic diversity study (Wang et al. 2008) suggested that *P. tabacum* populations at caves A and C belonged to two pure but different populations. We acclimated these plantlets at the South China Botanical Garden on 25 September 2007 as described earlier. During the acclimation period, 7.2% of the plantlets died from desiccation. The plantlets were transplanted into the three caves on 26 October 2007 (the dry season). At transplanting, the plantlets were 1.5 ± 0.1 cm in height and 3.0 ± 1.0 cm × 3.5 ± 1.0 cm in crown size. A total of 20 plantlets were evenly spaced in each 1 m × 1 m quadrat. The soil in each quadrat was 50% sterilized soil used for acclimation and 50% original soil. The transplants were watered on day 1 and day 3. The quadrats were not fenced, fertilized, or mulched.

Experiment 1B was identical to experiment 1A except that plantlets were generated with leaf explants from cave A and were transplanted on 16 April 2008 (the wet season). During the acclimation period, 7.9% of the plantlets died from desiccation. The transplants were 3 ± 0.1 cm in height and about 5.0 ± 1.0 cm × 5.0 ± 1.0 cm in crown size.

After transplantation in experiment 1A and 1B, we monitored the survival, and height of all transplants, and inferred the causes of death (i.e., insect defoliation, fungal decay, nutrient deficiency, lack of water, strong radiation). In experiment 1A, data were collected on Dec. 1, 2007; Dec. 31, 2007; Jan. 31, 2008; Feb. 29, 2008; Mar. 10, 2008; May 7, 2008; Aug. 1, 2008; Sept. 1, 2008; Oct. 7, 2008; Nov. 7, 2008; Dec. 7, 2008; and Jan. 17, 2009. In experiment 1B, data were collected on April 16, 2008; May 7, 2008; Sept. 1, 2008; and Jan. 17, 2009.

Experiment 2

To examine the role of mosses on reintroduction, we established six additional 1 m × 1 m quadrats at cave C, three with mosses and no other plants (designated CY1, CY2, CY3) and three without mosses or any other plants (CN1, CN2, CN3). Plantlets were generated with leaf explants from cave C and were transplanted on 26 October 2007. Transplanting procedures were identical to those in experiment 1. As before, quadrat numbers indicated distance from the cave entrance (1, 2, and 3 refer to 3, 15, and 45 m, respectively). We also selected and labeled five similar-sized wild *P. tabacum* individuals close to each of these quadrats; these 15 wild plants were used to compare the growth of wild and transplanted plants. The initial size of labeled wild individuals was similar to that of the transplants. Plants were monitored as described for experiment 1 at all same dates.

Habitat measurements

Because the soils at the cave entrances were very thin, soil samples were randomly collected from five points in each quadrat using a 5-cm diameter soil corer to the soil depth. A composite soil sample of about 0.5 kg from each quadrat was collected. The samples were air-dried and sieved for analysis. We measured soil chemicals including water content (g of water/100 g of dry soil), pH (1:2.5 soil water extracts), N content (decomposed with NaOH, absorbed with boracic acid, titrated with hydrochloric acid), P content (extracted with HCl/NH_4F, analyzed with a spectrophotometer), K content (extracted with CH_3COONH_4, analyzed by atomic absorption spectrophotometry), and soil organic matter (digested with $H_2SO_4/K_2Cr_2O_7$, titrated with $FeSO_4$) (Standford and English 1949; Olsen et al. 1954; Institute of Soil Science CAS 1978; MEWAM 1986).

The photosynthetically active radiation (PAR), CO_2 concentration, air temperature, and relative humidity were measured with a Li-6400 Photosynthesis System (Li-COR Co. Ltd., Lincoln, Nebraska, USA) at each quadrat in each cave in July and Oct. 2007, and Jan. and April of 2008. Measurements were taken between 8:00 and 18:00 and on the same dates when photosynthetic rate and transpiration rate of *P. tabacum* were measured (unpublished data). The average values of each variable were calculated throughout the day with measurements recorded at hourly intervals at each site.

Results

Cave characteristics

Four plant species were present in cave A and five were present in cave C (Table 1), and most were shade-tolerant species. A moss, *Gymnostomiella longinervis* Broth, was broadly distributed at both caves A and C, and the wild *P. tabacum* always grew under the cover of *G. longinervis*. In contrast, cave B contained no mosses and only one vascular plant species (a liane) (Table 1).

The major environmental conditions at the study sites are listed in Table 1. Soil pH ranged from 7.66 to 7.72. The contents of N, P, K, and organic matter in the soils were relatively low. The PAR densities (between 60.3 and 86.3 μmol m^{-2} s^{-1}) were also low. The soil water content was low and relative humidity was high. The temperature was relatively stable. The CO_2 concentration at the sites was relatively high, ranging from 382 to 401 ppm.

Table 1 Plant species and ecological factors (mean ± SD) at the three caves

Factor	Cave A	Cave B	Cave C
Plant species present	*Pilea notata* C. H. Wright *Lindasea orbiculata* Mett *Primulina tabacum* Hance *Gymnostomiella longinervis* Broth	*Pueraria phaseoloide* (Roxb.) Benth	*Pilea notata* C. H. Wright *Lindasea orbiculata* Mett *Primulina tabacum* Hance *Pteris cretica* Linn. *Gymnostomiella longinervis* Broth
Soil water content (%)	9.3 ± 1.5	7.0 ± 3.8	8.1 ± 0.2
pH	7.66 ± 0.12	7.68 ± 0.08	7.72 ± 0.05
P (ppm)	105.93 ± 64.10	77.27 ± 1.08	51.98 ± 2.81
K (ppm)	270.18 ± 63.01	87.15 ± 5.12	341.84 ± 31.89
Soil organic matter (%)	3.77 ± 0.37	1.84 ± 0.63	2.82 ± 0.41
N (%)	0.05 ± 0.03	0.02 ± 0.00	0.02 ± 0.01
PAR (μmol m^{-2} s^{-1})	65.0 ± 40.1	86.3 ± 12.7	60.3 ± 40.1
Relative humidity (%)	96.0 ± 2.0	85.3 ± 1.0	97.0 ± 2.0
Air temperature (°C)	20.0 ± 2.1	21.3 ± 5.0	19.7 ± 2.0
[CO2] (ppm)	398.24 ± 32.21	364.00 ± 8.12	400.36 ± 44.59

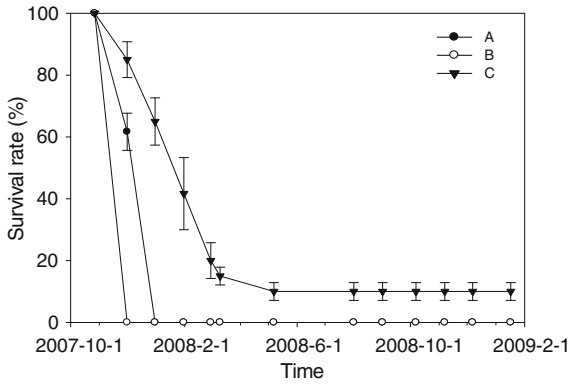

Fig. 1 Survival of transplanted *P. tabacum* at caves A, B, and C (experiment 1A). Values are the means (±SD) of the three quadrats in each cave

Survival of *P. tabacum* plantlets in experiment 1

In experiment 1A, all transplanted *P. tabacum* tranplants had died after 1 month in cave B and after 3 months in cave A (Fig. 1). The death rate of transplants was high at cave C (quadrats C1, C2, and C3) during the first 5 months, and the survival rate was about 10% thereafter (a total of six individuals survived in the three quadrats); the survival rate in cave C was stable after September 2008 (Fig. 1). At cave C, three of the transplanted seedlings bloomed and produced seeds on 1 September 2008, indicating initial reintroduction success.

In experiment 1A, the possible causes of mortality of the transplants differed somewhat among the caves. Fungal disease, nutrient deficiency, and lack of water accounted for 33, 50, and 17%, respectively, of the mortality in cave A. Plantlets cave B died from lack of water; note that cave B had strong radiation, low humidity, and no moss. In cave C, fungal disease and defoliation by a larval Noctuidae accounted for 80 and 20%, respectively, of the mortality of the transplants.

When experiment 1 was repeated (experiment 1B), survival was similar, i.e., all transplants died in caves A and B and about 25% of the transplants survived in cave C (Table 2). The causes of mortality were also similar to those in experiment 1A.

Survival and growth of *P. tabacum* plantlets in experiment 2

In cave C quadrats CN1, CN2, and CN3, which lacked moss, all *P. tabacum* plantlets had died by 31 January 2008 (Fig. 2). In cave C quadrats CY1, CY2, and CY3, which had moss, survival initially declined but then stabilized at about 10% after September 2008 (Fig. 2). In quadrats with moss, survival rate was highest in the middle of the cave (CY2), was lowest deep in the cave (CY3), and was intermediate at the cave entrance (CY1) (Fig. 2). Plant height (Fig. 3) in quadrats with moss, however, decreased from the entrance to locations deeper into the cave; in other words, growth of transplants decreased as light decreased. All transplanted plants grew slower than wild plants (Fig. 3).

Discussion

The success of plant reintroduction is case-specific, and there is no substitute for local knowledge of

Table 2 Numbers of surviving *P. tabacum* in experiment 1B

Cave and location	April 16, 2008	May 7, 2008	September 1, 2008	January 17, 2009
A1	20	12	1	1
A2	20	6	0	0
A3	20	6	0	0
B1	20	10	0	0
B2	20	0	0	0
B3	20	0	0	0
C1	20	17	5	5
C2	20	16	6	6
C3	20	15	4	4

On 16 April 2008, 20 plantlets were transplanted into three locations in caves A, B, and C. The number following the cave letter indicates distance from the cave entrance into the cave (1, 2, and 3 = 3, 15, and 45 m, respectively)

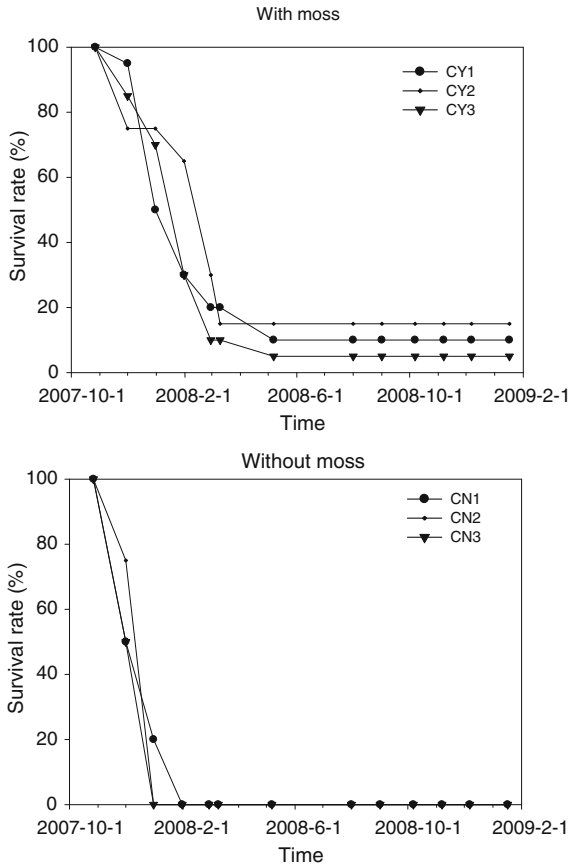

Fig. 2 Survival of transplanted *P. tabacum* as affected by the presence or absence of moss and distance from cave entrance at cave C (experiment 2). CY1, CY2, and CY3 (3, 15, and 45 m from cave entrance, respectively) represent quadrats with moss, and CN1, CN2, and CN3 (3, 15, and 45 m from cave entrance, respectively) represent the quadrats without moss

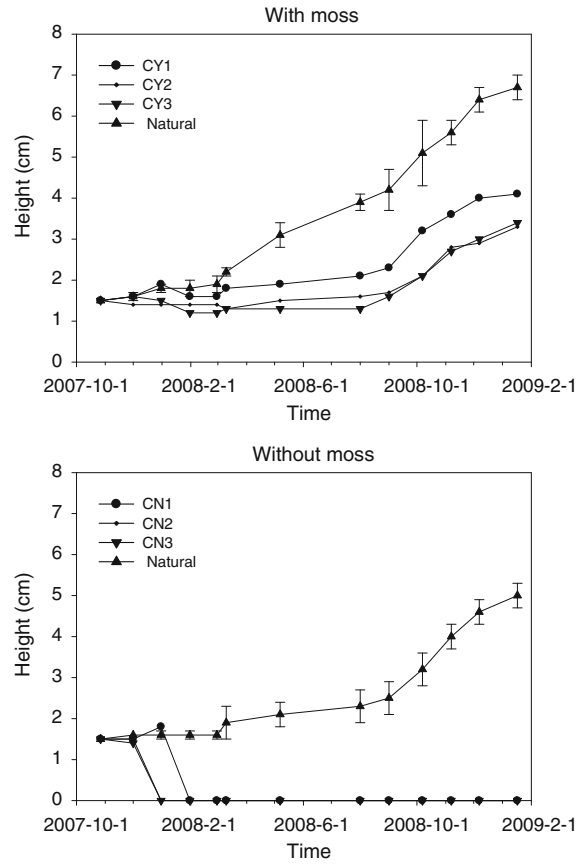

Fig. 3 Height of transplanted *P. tabacum* as affected by the presence or absence of moss and distance from cave entrance at cave C (experiment 2). CY1, CY2, and CY3 (3, 15, and 45 m from cave entrance, respectively) represent quadrats with moss, and CN1, CN2, and CN3 (3, 15, and 45 m from cave entrance, respectively) represent the quadrats without moss. The height of nearby wild *P. tabacum* (five plants per mean) is shown for comparison

species and systems. In this study, we show that moss may positively affect the survival and growth of endangered *P. tabacum,* which highlights the importance of restoring essential components of the community/ecosystem as part of restoration ecology and conservation efforts. Previous study showed that, as a nurse plant of *Sphagnum*, *Polytrichum strictum* could maintain the humidity of peatland fragments (Groeneveld and Masse 2007). As a nurse plant, moss may increase the storage and availability of water and nutrients in the *P. tabacum* community. Nurse plants may be highly useful for the reintroduction of rare and endangered plants. The reintroduction of *P. tabacum* in the karst drainage caves in this study appears to have been partially successful in that; more than three reintroduced individuals bloomed and produced seeds at cave C. The causal mechanisms, however, require further investigation and confirmation.

The environmental conditions at the study sites suggest that *P. tabacum* can only grow in alkali and poor soils. It prefers low light, relatively wet soils, and humid habitats (similar to the habitats at caves A and C). *P. tabacum* might be intolerant of highly fluctuating temperatures but be tolerant of relatively high atmospheric CO_2 levels. Our experiments indicate that it is virtually impossible for this rare and endangered plant to naturally colonize and establish at locations where it went extinct. For example in the case of Cave B, the cave has become dryer and

suffered moss extinction because of ecotourism impacts (Ren et al. 2010), the *P. tabacum* population also suffering local extinction.

In our previous reintroduction studies, plantlet growth was greatly affected by microhabitat factors and insect predators (Ren et al. 2003). The results showed that growth stagnancy and nutrient uptaking may result in unusual growth of the transplanted seedlings. However, wild individuals are apparently adapted to such microenvironments. Our current experiment indicates that the best way to conserve rare and endangered plants is by in situ protection. Because the geographic distributions of rare species are likely to shift under global climate change, reintroduction/translocation of such species to similar habitats might become necessary for effective conservation (Dormann 2007).

Our study emphasizes the importance of experimental approaches and historical distribution in identifying microsites suitable for the species. Some *P. tabacum* reintroduction sites are better than others, and some historical locations are no longer suitable for *P. tabacum.* There is still much room to develop better technologies to reduce the mortality of transplants; mortality could perhaps be reduced by irrigation, fertilization, protection against fungal pathogens and insects, and the identification and use of nurse plants. Periodic introductions of nursery-grown individuals could contribute to population stability (Vandenberghe et al. 2009).

Reintroduction of rare and endangered plants is often costly, and the success rate is low. For future successful reintroductions, we need a better understanding of the microhabitats and biotic conditions required for rare plant seedling establishment. Reintroduction success clearly varies with transplanting location, microhabitat, and the number of plants that are transplanted. Subsequent long-term monitoring is required to assess whether the populations are self-sustaining (Buisson et al. 2008), and multiple reintroductions may be necessary. If done with novel genotypes, this can bolster the genetic diversity (Maschinski and Wright 2006; Armstrong and Seddon 2007). In the future, we intend to introduce experimental populations into different sites to increase the probability that populations of rare species persist.

Although light and soil conditions seemed critical for successful transplantation of *P. tabacum*, the presence of the moss *G. longinervis* should serve as a useful and convenient indicator of suitable habitats for transplantation of this rare herb species. The positive results obtained with *G. longinervis* in this study indicate that nurse plants could be a valuable tool for the reintroduction of rare and endangered herbs. This research will help with the implementation of the "*Globe Plant Conservation Strategy*" in China. In the future, we will determine the physiological mechanisms that underlie successful reintroduction and elucidate why *P. tabacum* tolerates high CO2 concentrations. Our future research will also consider metapopulation dynamics and ecosystem changes associated with successful and failed attempts at the reintroduction of rare and endangered plants.

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