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SEED GERMINATION, SEEDLING ESTABLISHMENT AND REINTRODUCTION INTO THE WILD OF THE HEMIPARASITIC MEDICINAL PLANT *MONOCHASMA SAVATIERI*

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Abstract: The ecology, seed germination, seedling establishment, and reintroduction of *Monochasma savatieri* Franch. ex Maxim, a traditional Chinese hemiparasitic medicinal herb, were studied by focusing on the distribution, climate, soil type, flowering and seed production, co-occurring vegetation, and pollinators. The distribution range of *M. savatieri* lies between 25°03'-29°12' N to 113°39'-120°27' E, based on our sampling. *M. savatieri* requires vernalization to induce flowering. Seed germination was enhanced by gibberellic acid at an optimum concentration of 500 µM. Other plant growth regulators, including indole-3-acetic acid and 6-benzyladenine, did not enhance seed germination. Low temperature (5 °C) storage was favorable to keep seed viability, which was negatively influenced by increasingly long storage periods. We also examined seedling development and transplantation to the field. Seedlings were interplanted with a moss *Hypnum plumaeforme*, until they developed true leaves. Developed plants were then transplanted into the wild alongside a host, *Gardenia jasminoides*, and 23% of the transplanted plants survived after 8 months.

Key words: distribution, host, light, plant growth regulators, seed viability, transplanting, vernalization.

Introduction

Monochasma savatieri Franch. ex Maxim (Orobanchaceae, Rhinantheae tribe) is a perennial medicinal herb with widely documented medicinal properties [YAHARA & al. 1986; KOHDA & al. 1989; LI & al. 2012; LIU & al. 2013]. Its distribution is limited to a very narrow geographic belt, namely Southeast China and Kyushu, Japan [YAMAZAKI, 1993; HONG & al. 1998]. Phylogenetic studies indicate that *M. savatieri* is likely to have originated in East China and nearby regions [HONG, 1986; BENNETT & MATHEWS, 2006]. The flora of Japan also documents that *M. savatieri* is likely to have been introduced from China [YAMAZAKI, 1993]. Recent years have witnessed the disappearance of wild populations of *M. savatieri* due to overexploitation and habitat destruction, so much so that it has already been listed as an endangered and threatened species in Japan (Environment Agency of Japan, 2000). An additional reason for its sensitivity to ecological changes is that *M. savatieri* is a hemiparasitic plant that requires a suitable host plant [ZHANG & al. 2015]. To better understand why *M. savatieri* is only distributed in a narrow geographic belt, and to better explain its ecological sensitivity, an investigation into ecological aspects, including climate, soil type, flowering and seed production, co-occurring vegetation, and pollinators is required to assess whether these factors influence its natural growth and reproductive traits. To date, only one preliminary study exists on the germination of *M. savatieri* induced by gibberellic acid (GA_3) [YANG, 2009]. However, the conditions required for seed germination

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and seedling development in nature are not known, nor are the influence of light, plant growth regulators and seed storage time on seed germination and seed viability clear. It is also not known whether seeds can be used to establish seedlings for transplantation back into the wild to replenish natural stands. In a bid to shed light on these unknowns, the main objectives of our study were to develop a seed-to-site protocol that would allow for the successful germination of *M. savatieri* seeds, the subsequent growth of seedlings, and the effective transplantation of seedlings back into the wild following sexual propagation. This study, conducted in Southeast China, also aimed to investigate the ecology of existing wild *M. savatieri* resources, and understand its vernalization requirements.

Materials and methods

Distribution of wild resources

Wild populations of *M. savatieri* were investigated between 2010 and 2014. Plants were collected from 14 sites in five provinces of Southeast China (Zhejiang, Jiangxi, Fujian, Hunan and Guangdong) based on the advice and guidance of local elders in these areas (Figure 1). Geographic coordinates were estimated from Google Earth. These sites show relatively abundant populations with a density of 10-50 mature individuals per 100 m^2 (10 m × 10 m). We focused our investigation on Jiangxi province because it is near to South China Botanical Garden (SCBG) (Figure 1O), Guangzhou, in Guangdong province. Data on the climatic conditions and geographic features of the areas studied were obtained from the China Statistical Yearbook (1996-2012). For each collection site, several habitat features, including topography, sunlight and moisture regimes, soil and vegetation type, pollinators, growth and sexual (seed) reproduction were investigated and recorded. A previous study used the same collection sites to confirm the hemiparasitic nature of *M. savatieri* [ZHANG & al. 2015]. Collected plants were identified at SCBG.

Natural growth and vernalization test

To investigate flowering and sexual reproduction, living plants (including hosts) were brought back to SCBG, Guangzhou from Pan'an, Zhejiang province (Figure 1A) on October 25, 2010 and November 5, 2011, from Zherong, Fujian province (Figure 1E) on March 16, 2011, from Shihan, Ganxian county, Jiangxi province (Figure 1H) on December 20, 2012 and from Tengtian, Yongfeng county, Jiangxi province (Figure 1D) on November 29, 2013. All plants (100 individuals/collection) were cultivated separately in plastic pots (10 cm high and 10 cm in diameter) containing loess and placed under a net-covered shelter that reduced natural light incidence by 80%. Southeast China is the natural distribution zone of *M. savatieri* [HONG & al. 1998], and includes a total of five provinces: Jiangsu, Zhejing, Fujian, Jiangxi and Hunan. Among them, Jiangsu, Zhejing, and Fujian belong to a subtropical monsoon climate zone that is influenced by Pacific Ocean circulation. Hunan province belongs to a mainland subtropical monsoon climate, which is affected by both East Asia monsoon circulation and cold and dry air in winter. Jiangxi province is located between Fujiang province and Hunan province, which belong to a subtropical hill and mountainous moist monsoon climate. All these areas belong to a monsoon climate and experience abundant rain in spring and summer [ZHU & al. 2011]. Guangzhou belongs to a subtropical monsoon climate with an average temperature of 21-22 °C and usually no frost in winter and a historically extreme minimum of -2 °C. During our test period, Guangzhou's lowest temperature was always above 0 °C. In Jiangxi province, both Nanchang (Northern Jiangxi) and Ganzhou (Southern Jiangxi) belong to a subtropical hill and mountainous moist monsoon climate. Nanchang's average temperature is 16-17 °C with a historically extreme minimum of -15 °C; Ganzhou's average temperature is 18-19 °C with a historically extreme minimum of -6 °C, and is the southernmost point of *M. savatieri*'s natural distribution in Jiangxi province (Figure 1N). In these areas, in winter, low temperatures (-6 to -10 °C) usually last for one or two months but seldom drop below -15 °C. In summer, high temperatures usually do not exceed 35 °C because *M. savatieri* is distributed in mountain areas.

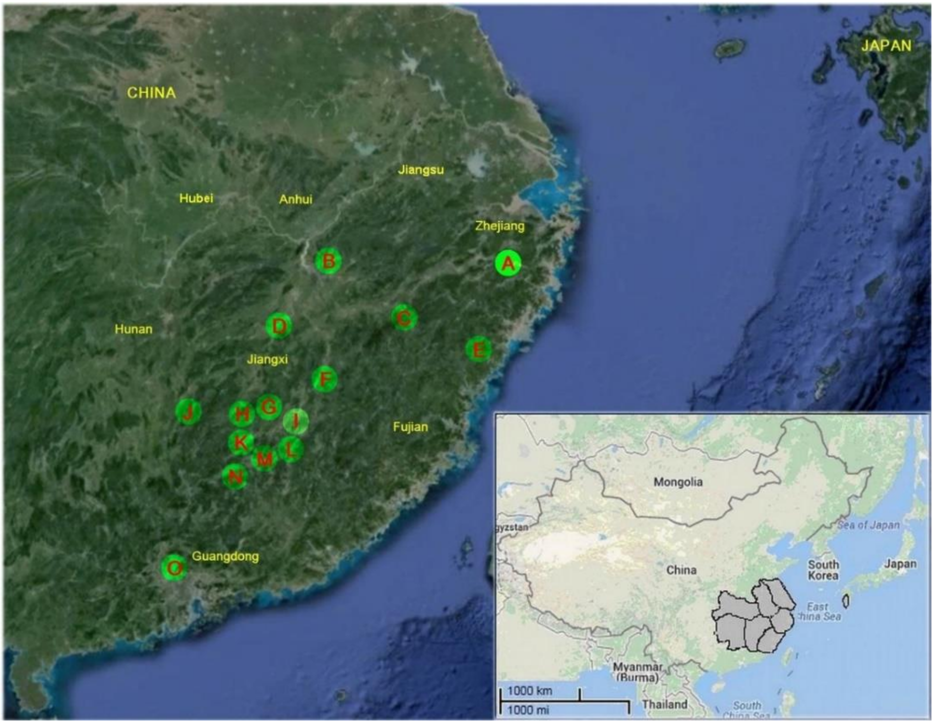

Figure 1. The distribution of *Monochasma savatieri* in East Asia. Areas in grey in the lower right side represent the *M. savatieri* distribution range according to YAMAZAKI (1993) and HONG & al. (1998). Green points with red uppercase letters show the distribution areas of *M. savatieri* in Southeast China based on this study. A: Pan'an (29°40'N 120°27'E); B: Boyang (29°10'N 116°42'E); C: Wuyishan (27°51'N 118°2'E); D: Yongfeng (27°25'N 115°27'E) (transplant location); E: Zherong (27°21'N 119°56'E); F: Shicheng (26°33'N 116°24'E); G: Yudu (25°57'N 115°24'E); H: Ganxian (25°54'N 114°59'E); I: Huichang (25°37'N 115°47'E); J: Rucheng (25°38'N 113°39'E); K: Anyuan (25°14'N 115°25'E); L: Wuping (25°11'N 116°8'E); M: Xunwu (25°30'N 115°38'E); N: Xinfeng (25°05'N 114°56E); O: South China Botanical Garden (23°10'N 113°21'E).

Seed germination tests

M. savatieri seeds for three germination tests were collected from Yongfeng County in Jiangxi province (Figure 1D) in May, 2011-2013. All seeds were surface sterilized with 0.5% mercuric chloride for 3 min then rinsed in distilled water three times. In test 1 (light and PGRs), seeds were stored in a refrigerator (5 °C) for two weeks, and then germinated in Petri dishes on filter paper soaked for 24 h in distilled water, 500 µM GA_3, 500 µM 6-

benzyladenine (BA) or 500 µM indole-3-acetic acid (IAA). Dishes were transferred to a light or dark culture room. In test 2 (storage temperature and duration), seeds were stored in a 25 ± 2 °C culture room or in a refrigerator (5 °C) for one month, six months, one year and two years, and then germinated on Petri dishes with moist filter paper in a culture room at 25 ± 2 °C in the dark by covering with a black cloth.

TTC test for assessing seed viability

The triphenyltetrazolium chloride (TTC) test was conducted to assess seed viability [PETERS, 2000]. Seeds were stored in a refrigerator (5 °C) for one week, one month, one year or two years, and then placed on Petri dishes (100 seeds/dish) with a single sheet of filter paper. Seeds were soaked in 0.5% TTC solution for 24 h and incubated in a growth chamber at 25 °C under a 12–h photoperiod at a photosynthetic photon flux density of 50 µmol m^{-2} s^{-1}. TTC staining was observed under a stereoscope (Olympus SZX16) at 10-20X magnification. Seeds stained red were considered to be viable while white seeds were unviable (Figure 2E). Each treatment was replicated four times in separate Petri dishes.

Statistical analyses

A completely randomized design was applied for seed germination experiments. Germination percentage was calculated as: (number of germinated seeds/total number of seeds) × 100%. Survival percentage was calculated as: (number of surviving plantlets/total number of transplanted seedlings) × 100%. The normal distribution of data was confirmed before performing analysis of variance (ANOVA). Two-way ANOVA was performed using SPSS version 13.0 (SPSS Inc., Chicago, USA) for Microsoft Windows, and means were considered to be significantly different from each other by the Least Significant Difference (LSD) test at $P \leq 0.05$.

Seedling development and field transplantation

A total of 2000 *M. savatieri* seeds were collected from Yongfeng county, Jiangxi province (Figure 1D) in May, 2013, stored in a refrigerator (5 °C), then germinated in December 2013. Seeds were divided into two parts spread evenly on one plate filled with vermiculite and one tray filled with a mixture of peat and sand (1:1, v/v), respectively. Plates and trays were kept in a moist environment in an open greenhouse, and wrapped in 4 m^2 of shade net, which reduced natural sunlight by 95%. After one month, seedlings in the cotyledonary stage about 1 cm tall were transferred to paper cups 4.5 cm high and 4.5 cm in diameter filled with loess. Moss (*Hypnum plumaeforme* Wilson), covering about one quarter of the paper cup's surface area and with the objective of retaining moisture, was placed on the surface of the loess with forceps while 1-4 seedlings were placed gently on top of the moss [ZHANG & al. 2015]. Cups were maintained in a moist and shaded environment (with a black net) for one month and then kept in an open greenhouse for three months at ambient temperature (10-20 °C). In April 2014, after seedlings grew to 2-3 cm in height and developed to the euphyllous stage with 3-4 leaves, 100 cups with about 200 seedlings were transported to Yongfeng county in Jiangxi province (Figure 1D), which is a natural habitat of *M. savatieri*. The four-month-old seedlings, including the loess from the cups, were transferred to small holes (5 cm deep and 5 cm wide) together with a suitable host plant *Gardenia jasminoides* J. Ellis (a good host shrub with an extensive root system) at a distance of 20-40 cm from the host plant stem [ZHANG & al. 2015]. Soil was initially moistened then watered occasionally. After 8 months, seedling growth and survival percentage were assessed.

Results

Distribution of wild resources

Most *M. savatieri* plant populations discovered were sporadically distributed in Jiangxi province but were also distributed in other provinces of Southeast China, albeit in fewer numbers. The existing distribution range of *M. savatieri* is from 25°03'-29°12' N to 113°39'-120°27' E, based on our survey. Growth locations were typified by hills, including in Jiangxi, Fujian, Zhejiang and Hunan. Our assessment indicates that *M. savatieri* is distributed between the northernmost points of Pan'an, Zhejiang province (29°40'N 120°27'E) and Boyang, Jiangxi province (29°10'N 116°42'E) (Figure 1A, 1B) and the southernmost point of Xinfeng County, Jiangxi province (25°05'N 114°56'E) (Figure 1N). The dominant soil types are loess or red earth. Vegetation consists mostly of sparse grasses and shrubs in the center of Jiangxi province (Table 1).

Table 1. Vegetation growing around *Monochasma savatieri*

Species	Family	Habit	Resource
Hypnum plumaeforme Will.	Hypnaceae	moss	relatively abundant
Funaria hygrometrica Sibth.	Funariaceae	moss	relatively abundant
Camellia oleifera Abel.	Theaceae	shrub	abundant
Symplocos chinensis (Lour.) Druce	Symplocaceae	shrub	relatively abundant
Polygala japonica Houtt.	Polygalaceae	forb	abundant
Vitex negundo var. *cannabifolia* Siebold & Zucc.	Verbenaceae	shrub	relatively abundant
Stimpsonia chamaedryoides Wright ex A. Gray	Primulaceae	forb	abundant
Toxicodendron succedaneum (Linn.) O. Kuntze	Anacardiaceae	shrub	infrequent
Berchemia floribunda (Wall.) Brongn.	Rhamnaceae	woody climber	abundant
Wikstroemia indica (L.) C. A. Mey	Thymelaeaceae	small shrub	abundant
Glochidion puberum (L.) Hutch.	Euphorbiaceae	small shrub	abundant
Lagerstroemia indica L.	Lythraceae	shrub	abundant
Lespedeza formosa (Vog.) Koehne	Fabaceae	shrub	relatively abundant
Galactia tenuiflora (Klein ex Willd.) Wight et Arn.	Fabaceae	herbaceous climber	infrequent
Gardenia jasminoides Ellis	Rubiaceae	shrub	abundant
Serissa serissoides (DC.) Druce	Rubiaceae	small shrub	relatively abundant
Hedyotis acutangula Champ. ex Benth.	Rubiaceae	forb	infrequent
Lindera aggregata (Sims) Kosterm.	Lauraceae	shrub	infrequent
Salvia plebeia R. Br.	Lamiaceae	forb	abundant
Scutellaria indica L.	Lamiaceae	forb	infrequent
Ixeridium chinense (Thunb.) Tzvel.	Asteraceae	forb	infrequent
Solidago decurrens Lour.	Asteraceae	forb	infrequent
Eupatorium chinense Linn.	Asteraceae	forb	relatively abundant
Gerbera anandria (Linn.) Sch.-Bip.	Asteraceae	forb	infrequent
Liquidambar formosana Hance	Hamamelidaceae	tree	infrequent
Loropetalum chinense (R. Br.) Oliv.	Hamamelidaceae	shrub	relatively abundant
Smilax glabra Roxb.	Smilacaceae	woody climber	infrequent
Smilax chinense L.	Smilacaceae	woody climber	infrequent
Crataegus cuneata Sieb. et Zucc.	Rosaceae	shrub	infrequent
Rubus parvifolius L.	Rosaceae	woody climber	abundant
Potentilla discolor Bunge	Rosaceae	herbaceous climber	abundant
Rosa cymosa Tratt.	Rosaceae	woody climber	relative abundant
Rhaphiolepis indica (L.) Lindl. ex Ker	Rosaceae	shrub	infrequent
Imperata cylindrica Linn. Beauv.	Poaceae	grass	relatively abundant
Pogonatherum crinitum (Thunb.) Kunth	Poaceae	grass	common

Natural growth and vernalization test

M. savatieri usually grows as a shoot cluster, which may arise from the simultaneous development of multiple seedlings from independent seeds arising from a single capsule, or from one seedling because roots can develop underground adventitious shoots that sprout. These shoots can grow to as high as 20-30 cm, one cluster usually needing 3-5 years to reach this height. In the southernmost point (Figure 1N), *M. savatieri* usually flowers annually in March to April and in the northernmost point (Figure 1A, 1B), it flowers annually in April to May. A single shoot can develop 3-9 flowers and one large shoot cluster can develop several dozen flowers, which flower acropetally on axillary stalks (Figure 2A). Flowers are white-pink and about 3 cm long (Figure 2A) and can be pollinated by both small insects and wind. A single shoot cluster can last for one month during flowering and fruit dehiscence stages. The fruits open upwardly and remain attached to the plant (Figure 2A). Thus, seeds are retained in the capsule, avoiding decay in soil or predation by insects, and tend to be discharged from the capsule after a storm. In normal conditions, capsules drop naturally to the ground usually between May and June. In east China, March to May is usually the wet and rainy season while storms are typical in May to August. These periods provide a wet environment for seed germination and seedling development when seedlings usually grow on moss (Figure 2B), which allows moisture to be retained. Seeds are spindle-shaped and very small, 0.7 mm long and 0.4 mm wide (Figure 2A, 2F), and 10,000-seed weight is only 0.8 g. Seed germination and development of 1-cm high plantlets needs half a year to complete. Seedlings 3-5 cm high growing in the wild in May were derived from the previous year's seed bank (Figure 2B). During the seedling stage, some shrubs and forbs were assumed as host plants (Table 1). The matured plants usually grow rapidly in spring (3-4 cm long each month) and autumn but slowly in summer and winter (0-1 cm long each month). In the south and centre of Jiangxi province, plants can survive year round. However, in winter in northern Jiangxi province, after above-ground shoots die-back while underground roots remain alive, new shoots sprout from its roots in the next spring. Plants that were brought back to Guangzhou (Figure 1O), Guangdong province where temperatures often exceed 35 °C in summer, could grow well in winter and spring, but not in summer. In Guangzhou, *M. savatieri* plants collected from Pan'an on October 25, 2010 and November 5, 2011 could not flower when planted in SCBG, Guangzhou (Figure 2C), while those collected from Zherong on March 16, 2011, from Shihan on December 20, 2012 and from Tengtian on November 29, 2012 were able to flower in February of the next year (Figure 2D).

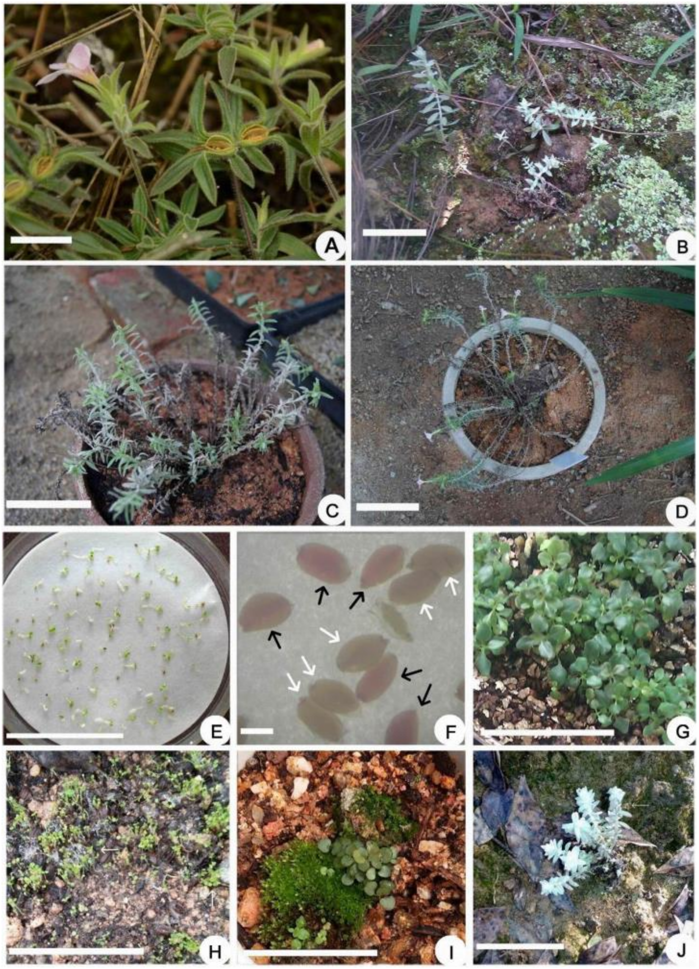

Figure 2. Flowering, fruits, seedling investigation, vernalization test, seed germination tests, seedling cultivation and transplantation of *Monochasma savatieri*. (A) Both flowers grew acropetally where axillary mature fruits gathered on the same plant. Flowering was acropetal or axillary and fruit opened upwardly and remained attached to the plant; bar = 1 cm. (B) One-year-old plantlets found in the wild at Yudou in May 2013 (Figure 1G); bar = 2 cm. (C) A plant brought back from Pan'an (Zhejiang province) on October 25, 2010 and potted, but did not flower in February, 2011 in Guangzhou; bar = 5 cm. (D) A plant brought back from Tengtian (Jiangxi province) in December 20, 2012 and flowered in February, 2013 in Guangzhou; bar = 5 cm. (E) Seed germination test with 500 µM GA_3 in the light after culture on a Petri dish with a single sheet of filter paper for 28 days; bar = 5 cm. (F) Seed viability as assessed by the TTC staining test; black arrows indicate seeds stained red (viable) while white arrows indicate non-stained seeds (unviable); bar = 0.5 mm. (G) Seeds, after pretreatment with 600 µM GA_3, germinated in vermiculite and developed into seedlings at the euphyllous stage after 4 months; bar = 5 cm. (H) Seeds were pretreated with 800 µM GA_3 and germinated and developed into seedlings at the cotyledonary stage (white arrow) on peat and loess (1:1, v/v) after culture for one month; bar = 5 cm. (I) Seedlings at the cotyledonary stage were transferred to paper cups that contained loess and moss (*Hypnum plumaeforme*) after culture for 4 months; bar = 2.5 cm. (J) Seedlings grew well (white arrows) after transplanting with *Gardenia jasminoides*, a shrub, for 8 months; bar = 5 cm.

Effects of light and plant growth regulators on seed germination

When cultured in light, seed germination percentage following exposure to 500 µM GA_3 was about 80% within 28 days (Figure 3). However, after exposure to BA and IAA, seed germination percentage was only about 50% within 28 days. This was the same level as the control but significantly lower than the GA_3 treatment (Figure 3). In the dark, seed germination percentage after treatment with 500 µM GA_3 was only 38%, which was significantly lower than in light culture (80%). After exposure to BA and IAA, seed germination percentage was only 3-4%, which was the same level as the control (Figure 3), but significantly lower than the GA_3 treatment.

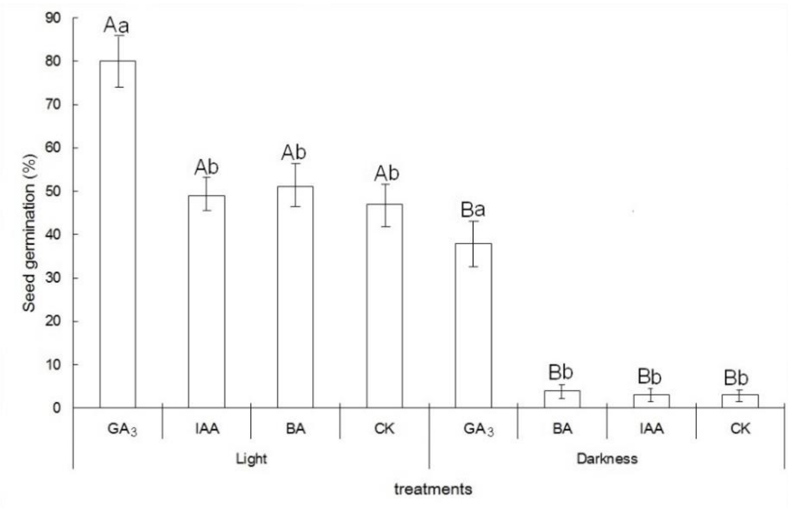

Figure 3. *Monochasma savatieri* seed germination in response to treatments with light and plant growth regulators. The same lowercase letters are not significantly different for the same light (or dark) treatment while the same uppercase letters are not significantly different for the same plant growth regulator treatment (LSD test; $P \leq 0.05$).

Effects of storage temperature and duration on seed germination

Both the germination percentage of *M. savatieri* seeds stored at 25 °C or at 5 °C was about 80%, respectively. The germination percentage of seeds stored for six months decreased to 45% at 25 °C and to 76% at 5 °C. The germination percentage of seeds stored for one year decreased to 18% at 25 °C and to 66% at 5 °C. After two years, seed germination percentage decreased to 1% at 25 °C and to 45% at 5 °C (Figure 4).

Tetrazolium tests to assess seed viability

TTC staining percentage was 86%, 47%, 17% and 3% for seeds stored at 25 °C for one month, six months, one year and two years, and 85%, 74%, 65% (Figure 2F) and 43% (Figure 5), respectively for seed stored at 5 °C. These results almost coincided with the seed germination values.

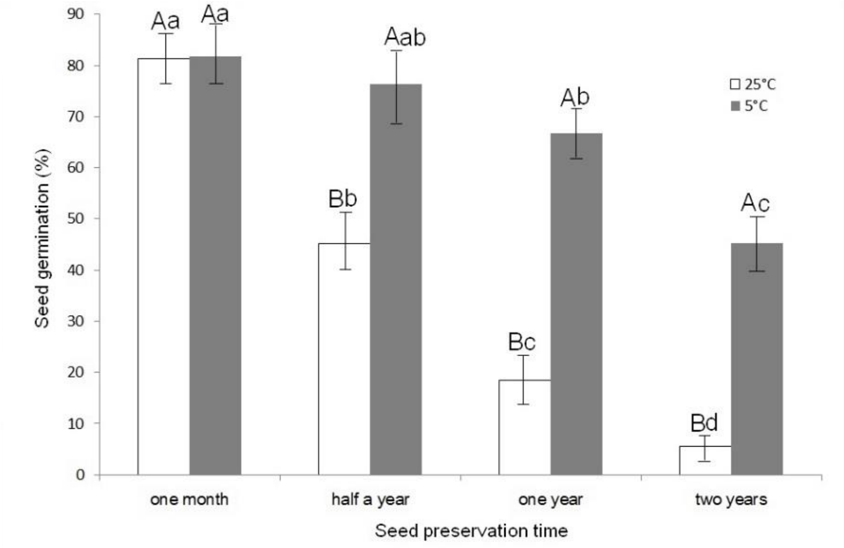

Figure 4. *Monochasma savatieri* seed germination at two temperatures and four storage periods. The same lowercase letters are not significantly different at the same storage temperature (5 °C or 25 °C) and the same uppercase letters are not significantly different at the same seed storage period (LSD test; $P \leq 0.05$).

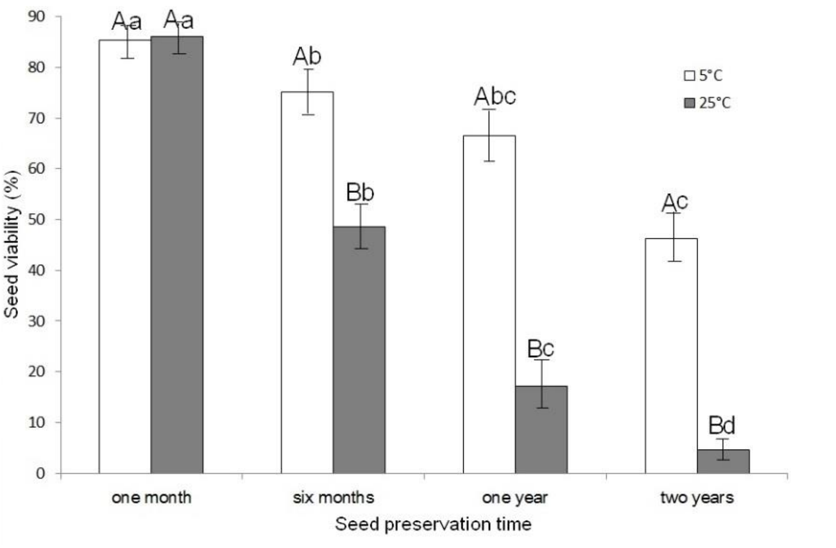

Figure 5. Effects of storage temperature and duration on *Monochasma savatieri* seed viability. The same lowercase letters are not significantly different at the same seed storage temperature and the same uppercase letters are not significantly different at the same storage period (LSD test; $P \leq 0.05$).

Seedling development and field transplantation

Seeds germinated on the surface of vermiculite, peat and loess within two weeks, exceeding 80% (Figure 2G, 2H) after treatment with 600 µM GA_3. It is interesting to note that seedlings without an attached host grew well up until four months of age. Under dim light and wet conditions, seedlings grew slowly and the cotyledonary stage lasted for at least 2-3 months before developing the first true leaf (Figure 2I). During the cotyledonary stage, plantlets grew very slowly, possibly due to the lack of a suitable host and nutrition. After culture for another four months, seedlings could grow to 4-5 cm in height. After interplanting with *G. jasminoides*, 33 *M. savatieri* plantlets survived (23.3%) to 8 months (Figure 2J).

Discussion

Southeast China and Kyushu (Amakusa Islands, Japan) have a subtropical monsoon climate and hilly landform that allows *M. savatieri* to grow. Special biological requirements of *M. savatieri* such as the requirement for low temperature vernalization, moderate temperature for growth or hemiparasitism on host plant roots presumably result in special habitat requirements, which may account for its limited distribution. Since *M. savatieri* is distributed naturally in east China, including Jiangxi, Fujian, and Zhejiang provinces but not in South China (Guangzhou, Guangdong province), we believe that vernalization temperature is likely to be at most -6 °C because the lowest temperature is -6 °C at the southernmost point (Xinfeng County, Jiangxi).

Under natural conditions, a single flowering shoot cluster lasts one month and seeds, which also take one month to mature, also survive for an additional month. In the center of Jiangxi province, *M. savatieri* flowers from April to May and produces seeds in May. The capsules dehisce when they mature, releasing seeds that may germinate if they encounter ideal conditions. During this period, it often rains, which favors seed germination and seedling development. From our results, seed germination was highest within one month but after one year, seed germination decreased to 20%. In the rainy season, the soil is moist and shaded and moss grows easily, providing a suitable environment for seed germination and seedling growth (Figure 1B). As seedlings mature, *M. savatieri* favors a sunny environment and higher terrain that does not easily become waterlogged. The growth conditions in the same area can change between seasons and may face biotic and abiotic stresses, which may explain the low natural sexual reproduction of *M. savatieri*. Artificial seed storage and proper sowing conditions thus need to be considered.

Our studies also showed that light and GA_3 pretreatment enhanced seed germination, which indicates that *M. savatieri* seeds favor light. Moreover, under natural conditions, after seeds drop to the ground and encounter suitable conditions, natural light can enhance germination. Light usually acts synergistically with GA_3 to enhance seed germination [TOYOMASU & al. 1998; YAMAGUCHI & KAMIYA, 2001; SEO & al. 2006]. Sometimes, seed remains dormant due to the presence of abscisic acid (ABA) while GA_3 can counter the effects of ABA [SHINOMURA & al. 1994; LOVEGROVE & HOOLEY, 2000; NAMBARA & MARION-POLL, 2005]. The physiological dormancy of *M. savatieri* seeds might be broken by GA_3 or low temperature [YANG, 2009]. Even in the absence of GA_3 treatment, seed germination was high (> 60%), indicating that seeds broke dormancy in the first two weeks. At this stage, GA_3 and light could enhance seed germination. After one month, most seeds had broken dormancy, and even in the absence of GA_3, seed germination was high (> 80%). Seed germination and seed viability declined after one month. This careful fine-scale understanding of the balance between these two parameters is necessary to accurately time artificial seed germination.

In its natural environment, all *M. savatieri* seeds germinate on moss (*Hypnum plumaeforme* or *Funaria hygrometrica*) [ZHANG & al. 2015]. This moss might not only supply a moist environment and nutrients for seed germination and seedling growth of some species [REN & al. 2010], but might also secrete phytohormones to enhance seed germination [RESKI, 2006; VON SCHWARTZENBERG, 2006].

The wild habitats of *M. savatieri* are shrinking, and are under rapid threat and decline. By understanding the growth habits of this plant in the wild, including its complex hemiparasitism and the need for host plants to develop haustoria [ZHANG & al. 2015], as well as its vernalization and flowering requirements, seed can be effectively produced, allowing for artificial sexual reproduction of *M. savatieri*, thus providing a robust protocol that would buffer the extinction of this species in the face of rapid urbanization and habitat destruction in China. The protocol in this study is thus useful in practical terms and is also socially important. A transplantation test in Guangzhou indicated that *M. savatieri* plants need a period of vernalization, i.e., a period of low winter temperature, to induce floral bud differentiation in order to flower and produce seeds. Thus, this species is not suitably cultivated in South China where winters are short, not allowing plants to flower in spring. Is then culture of the species suitable in North China? Here, the answer is also negative since too low a temperature (< -20 °C) in winter may kill the whole plant including above - and below - ground parts. *M. savatieri* is confined to small parts of Southeast China and Japan [YAMAZAKI, 1993; HONG & al. 1998]. What confined its distribution, and how? This study shows that characteristic *M. savatieri* habitats include sunny slopes of small mountains and hills, rich with grasses and shrubs, that often contain red clay and loess soil. Thus, it can be concluded that *M. savatieri* is sun-loving, grows in acidic soil and prefers to parasitize small herbs and shrubs. Since *M. savatieri* has strict habitat conditions, it can only grow in limited areas of favorable climatic and geographic environments. Besides, this study also shows that the primary mode of reproduction of *M. savatieri* is by seeds, which is not very efficient because of seed dormancy and a requirement for the early establishment of seedling parasitism. After shedding from plants, seeds may take several months to germinate. This may cause a loss in seedling vigour. Seedlings that form successfully also risk untimely parasitism, which is vital to their survival, especially in dry soil. Thus, certain environmental conditions involving water, temperature, or host roots in the vicinity of seedlings must prevail.

Our study indicates that transplanted *M. savatieri* seedlings could survive in the field when planted with a suitable host. Thus, artificial propagation and reintroduction of this medicinal plant back into the wild is possible and serves as a feasible method for the ecorehabilitation of areas that have been depleted of natural stocks, or that lack genetic heterogeneity.

Conclusions

In this study, the distribution, climate, soil type, flowering and seed production, co-occurring vegetation, pollinators, ecology, seed germination, seedling establishment, and reintroduction of the traditional Chinese hemiparasitic medicinal herb, *Monochasma savatieri*, were investigated. Seed germination was enhanced by gibberellic acid and low temperature storage, which benefitted and sustained seed viability. Seedling development and subsequent transplantation to the field resulted in a low survival percentage, indicating that much more work is needed on this species before cultivation.

Acknowledgments and conflicts of interest

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