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## In Vitro Culture of Traditional Chinese Medicine Heibai Erchou (Semen Pharbitidis)

**Authors:** Mingyu Lu, Shen Huijin, Zhao Shanshan, Yang Jun, Yang Jun

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### Abstract

To screen superior experimental varieties of morning glory, this study investigated the germination rate and early growth and development of black-and-white morning glory. Seed germination and early seedling length data were observed under both in vitro and greenhouse conditions. The results revealed that among the three tested varieties (A19, G12, and G14), G12 exhibited the highest germination rate, followed by A19, while G14 showed the lowest; G12's early growth vigor was also slightly superior to the other two varieties. This study demonstrates that G12 can rapidly complete generation turnover under laboratory conditions, making it a suitable material for subsequent research on average mutation rates between generations.

### Full Text

## In vitro Cultivation of Traditional Chinese Medicine Heibai Erchou

**Mingyu Lu<sup>1</sup>, Huijin Shen<sup>1</sup>, Shanshan Zhao<sup>2</sup>, Jun Yang<sup>2\*</sup>**

<sup>1</sup> Shanghai Chenshan Botanical Garden, 3888 Chenhua Road, Shanghai 201602, China

<sup>2</sup> Shanghai Chenshan Botanical Garden, 3888 Chenhua Road, Shanghai 201602, China

These authors contributed equally to this work.

\*Corresponding author ([yangjun@csnbgsh.cn](mailto:yangjun@csnbgsh.cn))

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### Abstract

To select superior cultivars of morning glory for experimental research, we investigated the germination rates and early growth and development of Heibai

Erchou. We monitored seed germination and early seedling growth of Heibai Erchou under both in vitro conditions and greenhouse cultivation. Among the three tested cultivars—A19, G12, and G14—G12 exhibited the highest germination rate, followed by A19, while G14 showed the lowest germination rate. Additionally, G12 demonstrated slightly superior early growth vigor compared to the other two cultivars. This study reveals that G12 can rapidly complete a seed-to-seed cycle under laboratory conditions, making it an excellent material for subsequent studies on average mutation rates across generations.

**Keywords:** morning glory, early growth, seed-to-seed cycle

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## Introduction

*Ipomoea nil* (morning glory), a member of the Convolvulaceae family, typically germinates in spring and flowers in summer and autumn. With over 1,500 cultivated varieties, this species displays diverse floral colors including blue, crimson, pink, and purple, often with mixed hues and varied petal margins, making it a common ornamental plant that peaks in summer. Its seeds possess medicinal value [1]. As a traditional Chinese medicine, morning glory seeds were first documented in *Mingyi Bielu* and are also known as Heichou (black), Baichou (white), or collectively as Erchou. Research indicates that morning glory seeds contain chemical constituents such as glycosides, sugars, and alkaloids, while immature seeds also contain gibberellins, proteins, pigments, and fats [2]. The chemical properties of pharbitin are similar to jalapin (C H O ). Pharbitin lipids decompose into pharbitin when encountering bile and intestinal fluid in mammalian intestines [3], stimulating intestinal motility and causing purgation, which may lead to symptoms including vomiting, abdominal pain, and diarrhea [4]. Additional studies have shown that morning glory seeds can accelerate inulin excretion in kidneys, suggesting potential diuretic effects, and exhibit certain anthelmintic properties [5]. Clinically, morning glory seeds have been used to treat conditions such as constipation [6], edema [7], simple obesity [8], psychiatric disorders [9], epilepsy [10], pediatric pneumonia [11], and pediatric high fever [12]. However, research on the growth and development of morning glory seeds remains limited [13]. Therefore, this study examined seed germination and early growth processes in three cultivars (A19, G12, and G14). We found that G12 showed the highest germination rate, followed by A19, while G14 exhibited the lowest germination rate. G12 also demonstrated superior early growth vigor. These findings indicate that G12 can rapidly complete a seed-to-seed cycle under laboratory conditions, making it an ideal material for subsequent studies on average mutation rates between generations.

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## 1. Materials and Methods

**1.1 Plant Materials and Preliminary Preparation** Three different cultivars of *Ipomoea nil* seeds were harvested from the experimental field at Shanghai Chenshan Botanical Garden and designated as A19, G12, and G14. G12 represents Baichou (white seeds), while the others represent Heichou (black seeds). One hundred seeds each of A19 and G12 were selected, along with 200 seeds of G14 as experimental materials. Seeds of A19, G12, and G14 (100 each) were placed in 50 ml centrifuge tubes and soaked in 5 ml deionized water for 30–60 minutes. An additional 100 G14 seeds were placed in a 50 ml centrifuge tube with 5 ml concentrated sulfuric acid. Once the sulfuric acid corroded the dense surface structure—evidenced by the transparent acid turning black—the blackened sulfuric acid was removed to a waste container using a pipette, and 40 ml deionized water was added to rinse residual acid from the seed surfaces. Four 10 cm × 10 cm square petri dishes were prepared, each lined with absorbent paper and labeled A19, G12, G14, and G14-H SO . Eighty seeds from each of the four treated groups were placed in the respective dishes in a 9 × 9 arrangement. The remaining 20 seeds from each group were surface-sterilized in a laminar flow hood by soaking in 8% disinfectant for 5 minutes, followed by three washes with 75% ethanol and 2% NaClO, and final rinsing with sterile water. These were then placed in pre-prepared 60 mm petri dishes (one seed per dish) containing 3% Gelrite solid medium (3 g Gelrite per liter of tap water, autoclaved and dispensed into dishes; Gelrite product number G1910, CAS 71010-52-1, SIGMA).

**1.2 Observation of Growth and Development and Stage Classification** The growth and development of morning glory in square petri dishes were observed regularly, typically between 9:00–10:00 AM daily. Germination was recorded, and root-shoot length was estimated using 5 mm × 5 mm grid paper. Daily growth was photographed. When cotyledons expanded and detached from the seed coat, seedlings were transferred to pots in a phytotron for continued cultivation and daily observation. Seeds in sterile medium were transferred to culture bottles after radicle emergence, and subcultured when leaves became dense. All sterile operations were performed in a laminar flow hood using standard sucrose-containing MS medium [14].

**1.3 Calculation and Comparison of Daily Seedling Growth in Petri Dishes** Based on daily measurements recorded on grid paper, the growth of each seed group within a single day was calculated, and average seedling lengths were compared among groups.

**1.4 Observation and Differentiation of Cotyledon and True Leaf Shapes in Pots** Cotyledons and true leaves from various pots in the phytotron were photographed and observed to describe inter-cultivar differences in leaf shape and size.

**1.5 Observation and Differentiation of Floral Morphology in A19, G12, and G14** Flowers from previously planted A19, G12, and G14 were collected from both the experimental field and greenhouse pots to observe and describe differences in floral color, size, and bud number among cultivars.

**1.6 Capsule Morphology Differences and Average Seed Mass** External differences in capsules among cultivars were observed and compared with previously harvested dried materials to evaluate trait stability. Dried capsules of A19, G12, and G14 were crushed, and 100 seeds from each cultivar were weighed in groups of ten. The ten measurements were averaged for comparison.

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## 2. Results and Analysis

**2.1 Seed Observation and Comparison** As shown in [Figure 1: see original paper], significant differences in seed mass were evident among A19, G12, and G14. G12 seeds were the heaviest, with an average mass approximately 1.64 times that of A19, while G14 seeds were the lightest, with A19 seeds being about 1.40 times heavier than G14.

Visual and microscopic comparisons revealed distinct differences in seeds, pods, leaves, and inflorescences among cultivars. A19 and G14 seeds were dark brown, while G12 seeds were yellowish-white. Microscopic examination revealed that A19 seeds were covered with prominent epidermal hairs, whereas G12 and G14 seed surfaces were relatively smooth. A19 capsules were covered with abundant epidermal hairs, while the other two cultivars showed less conspicuous hair coverage ([Figure 2: see original paper]).

**2.3 Observation and Stage Classification of Growth and Development** Daily seedling length records and data analysis yielded average seedling lengths for A19, G12, and G14 on each day following radicle emergence ([Figure 3: see original paper]). A19 showed a linear increase in seedling length from day 1 to day 4, reaching an average length of 8.5 cm on day 4. G12 exhibited the fastest growth between days 2-3, with growth rate slowing by day 4, though average seedling length exceeded 10 cm. Untreated G14 seeds showed no germination or radicle emergence during the two-week observation period, while G14-H SO (sulfuric acid-treated) grew most rapidly between days 2-3, with growth slowing by day 4 and average seedling length reaching approximately 7.5 cm. Analysis revealed that early growth in all three cultivars could be divided into four stages: Stage 1 involved initial radicle emergence with relatively slow growth; Stage 2, characterized by hypocotyl development, showed the fastest growth rate, consistent with the physiological need for rapid soil emergence; and Stage 3 involved cotyledon expansion with decelerating growth.

**2.4 Observation and Differentiation of Cotyledon and True Leaf Shapes in Pots** Observations revealed similar cotyledon shapes among A19,

G12, and G14 ([Figure 4: see original paper]). A19 stems were darker in color during the same growth period, while G12 stems were light green. G14-H SO cotyledons and stems were dark-colored when first expanded. A19 true leaves had more prominent epidermal hairs than G12 and G14, consistent with the hairy phenotype observed on A19 seeds and capsules. Additionally, A19 true leaves and cotyledons were relatively round, G12 leaves were larger, and G14-H SO cotyledons were slender.

**2.5 Floral Differences Among the Three Cultivars** A19 flowers in the experimental field appeared blue in early morning, blue-purple at noon, and gradually turned purple in the afternoon. Flower buds were located in leaf axils, with multiple buds visible in a single axil and prominent epidermal hairs. G12 flowers were larger and pure white, typically with only one flower per leaf axil. G14 flowers cultivated in the artificial greenhouse were smaller, white, with a single bud per leaf axil and relatively smooth leaves. Field-grown G14 leaves were darker than greenhouse-grown leaves, with substantial anthocyanin accumulation; field-grown G14 was not in the flowering stage ([Figure 5: see original paper]).

**2.6 Capsule Differences Among A19, G12, and G14** [Figure 6: see original paper] shows the average capsule mass for A19, G12, and G14. G12 capsules had the highest average mass, approximately 1.43 times that of A19, while G14 capsules were the lightest, with A19 average mass being 1.28 times that of G14. Each capsule generally contained 4-6 seeds. These results were consistent with seed mass data in [Figure 1: see original paper], where G12 seed mass was about 1.64 times that of A19, and A19 seed mass was about 1.40 times that of G14.

**2.7 Long-term Capsule Storage Method** Using 1.5 ml centrifuge tubes to represent capsules and cotton rope to represent vines, a simple knot-tying method was developed to conveniently record plant architecture, capsule position, and seed number for harvested plants. Silica gel desiccant was placed in centrifuge tubes containing seeds, which were then stored in low-humidity cabinets for long-term preservation. Silica gel color was checked regularly to prevent seed moisture damage.

**2.8 Germination Rate** Each square petri dish contained 80 seeds. After two weeks of observation: - A19: 20 of 80 seeds germinated (25% germination rate) - G12: 7 seeds were pre-germinated and excluded; of the remaining 73 seeds, 27 germinated (approximately 37% germination rate) - G14-H SO : 13 of 80 seeds germinated (16.25% germination rate) - G14: No germination observed within two weeks

Additionally, among sterilized seeds, A19 showed radicle emergence on day 2, G12 on day 3, G14-H SO on day 4, while G14 showed no radicle emergence

within two weeks. After two weeks of observation, G12 exhibited the highest germination rate, followed by A19, with G14 showing the lowest rate. G12 also demonstrated superior early growth vigor, making it most suitable for subsequent experiments.

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### 3. Discussion

**3.1 Seed Pretreatment** Microscopic observation of A19, G12, and G14 seeds revealed epidermal hairs on A19 seeds, consistent with the hairy phenotype observed on its capsules and leaves, indicating that trichomes are a widespread whole-plant trait in A19. G12 seed surfaces appeared uneven but lacked epidermal hairs. G14 seeds had smooth surfaces with a dense layer that likely impeded water absorption and germination. Sulfuric acid corrosion effectively disrupted this surface structure, as evidenced by the germination rate increase from 0% to 16.25%. The optimal duration of sulfuric acid treatment warrants further investigation. Whether in moist petri dishes or on sterilized medium, A19 seeds consistently germinated on day 2, G12 on day 3, G14-H<sub>2</sub>SO<sub>4</sub> on day 4, while untreated G14 failed to germinate throughout the two-week observation period.

**3.2 Discussion on Differences Between Field and Greenhouse G14 Leaves** As shown in [Figure 5: see original paper], field-grown G14 leaves generally had deeper lobes and darker coloration than greenhouse-grown leaves, likely due to accumulation of anthocyanins in response to abiotic stresses such as high light intensity and elevated temperature in the field environment.

**3.3 Future Research Questions** Field-grown A19 flowers appeared blue in early morning, transitioned to blue-purple at noon, and turned purple in the afternoon. Based on the pH-dependent color change property of anthocyanins, we hypothesize that the color-changing mechanism involves intracellular pH fluctuations correlated with light exposure and CO<sub>2</sub> absorption, though this requires further elucidation. Additionally, we observed that potted G14 leaves oriented upward at 8:00 AM and downward at 8:00 PM, and that all four G14 pots exhibited right-hand spiral twining. The biological mechanisms underlying these phenomena warrant deeper investigation in future studies.

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