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**Traditional Chinese medicine —  
*Salvia miltiorrhiza* seeds and  
seedlings**

*Médecine traditionnelle chinoise — Graines et plants de Salvia  
miltiorrhiza*





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## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see [www.iso.org/directives](http://www.iso.org/directives)).

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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

This document was prepared by Technical Committee ISO/TC 249, *Traditional Chinese medicine*.

# Traditional Chinese medicine — *Salvia miltiorrhiza* seeds and seedlings

## 1 Scope

This document specifies the minimum requirements and test method for *Salvia miltiorrhiza* seeds and seedlings. It is suitable for use in quality assurance during the production and management of *Salvia miltiorrhiza* seeds and seedlings.

## 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

International Seed Testing Association (ISTA), *International Rules for Seed Testing*

International Seed Testing Association (ISTA), *Working Sheets on Tetrazolium testing*

## 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at <http://www.electropedia.org/>

— ISO Online browsing platform: available at <http://www.iso.org/obp>

### 3.1

#### **seed lot**

specified quantity of *seed* (3.7) that is physically and uniquely identifiable

[SOURCE: ISTA, International Rules for Seed Testing, 2.2.1]

### 3.2

#### **primary sample**

portion taken from the *seed lot* (3.1) during one single sample action

[SOURCE: ISTA, International Rules for Seed Testing, 2.2.2]

### 3.3

#### **composite sample**

sample formed by combining and mixing all the *primary samples* (3.2) taken from the *seed lot* (3.1)

[SOURCE: ISTA, International Rules for Seed Testing, 2.2.3, modified]

### 3.4

#### **subsample**

portion of a *primary sample* (3.2) obtained by reducing a sample

[SOURCE: ISTA, International Rules for Seed Testing, 2.2.4]

**3.5  
submitted sample**

sample that is to be submitted to the testing laboratory and may comprise either the whole of the *composite sample* (3.3) or a *subsample* (3.4)

Note 1 to entry: The submitted sample may be divided into subsamples packed in different material meeting conditions for specific tests (e.g. moisture or health).

[SOURCE: ISTA, International Rules for Seed Testing, 2.2.5]

**3.6  
working sample**

whole of the *submitted sample* (3.5) or a *subsample* (3.4), to which one of the quality tests described in the ISTA Rules is applied

Note 1 to entry: The working sample is at least the weight prescribed by the ISTA Rules for the particular test.

[SOURCE: ISTA, International Rules for Seed Testing, 2.2.7, modified]

**3.7  
seed**

mature ovule produced by *Salvia miltiorrhiza* Bunge

Note 1 to entry: It consists of three basic parts: embryo, endosperm and the seed coat.

**3.8  
seedling**

young plant of *Salvia miltiorrhiza* Bunge after cultivation for one year, consisting of bud, *tap root* (3.14), lateral root and fibrous root

**3.9  
purity**

weight percentage of pure *seed* (3.7) fraction over the total weight of the *working sample* (3.6)

Note 1 to entry: The pure seed refers to the species stated by the applicant, or found to predominate in the test and includes all botanical varieties and cultivars of that species.

Note 2 to entry: It is expressed in per cent (%).

[SOURCE: ISTA, International Rules for Seed Testing, definition 3.2.1, modified]

**3.10  
viability**

potential ability of a *seed* (3.7) to germinate or the capability of an embryo to live

Note 1 to entry: It is the percentage of stained seeds in the *working sample* (3.6), estimated using the Topographical Tetrazolium Test.

**3.11  
1 000-seed weight**

average weight of every 1 000 pure *seeds* (3.7) of a *working sample* (3.6)

**3.12  
seed moisture content**

loss of weight of *seed* (3.7) after drying over the weight of seed before drying

**3.13  
germination percentage**

percentage of germinated *seed* (3.7) over the *working sample* (3.6)

**3.14  
tap root**

root generated from the one year development of the radical after *seed* (3.7) germination

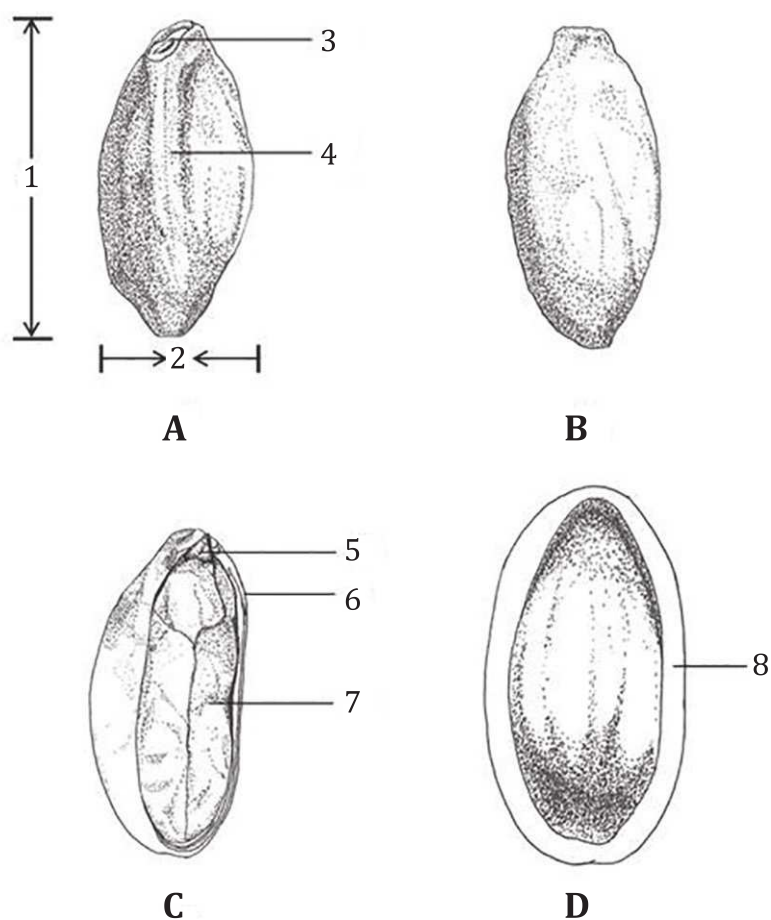
**3.15****seedling length**

largest distance from the lower part to the top

Note 1 to entry: It is expressed in centimetres.

**4 Descriptions**

*Salvia miltiorrhiza* seed is the dried seed of the plant *Salvia miltiorrhiza* Bunge and consists of three basic parts: the embryo, cytoledon and the pericarp (see [Figure 1 C](#)). The epidermal mucilage layer swells to a transparent mucilage membrane surrounding the seed after soaking the seed with warm water ([Figure 1 D](#)).



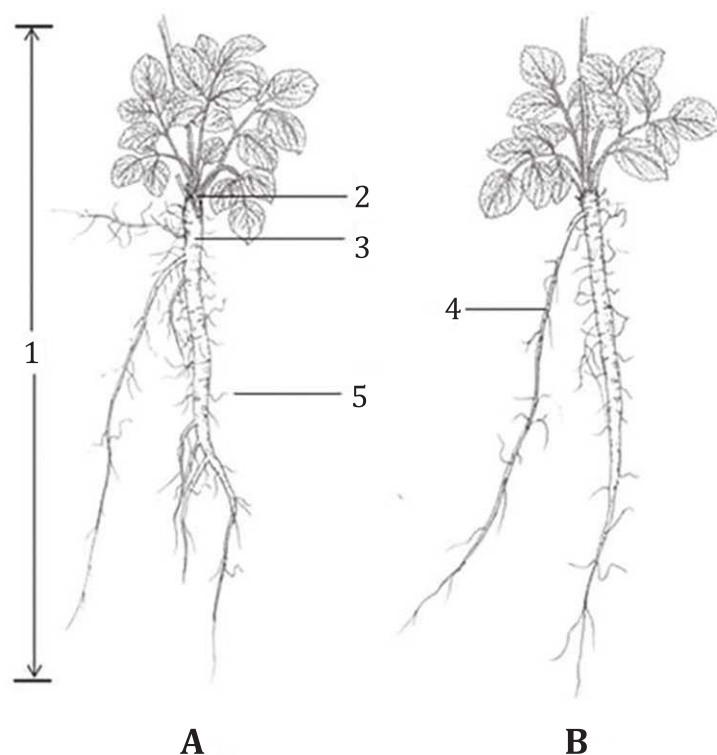
**Key**

- |   |                |   |                                  |
|---|----------------|---|----------------------------------|
| 1 | seed length    | A | facade of the seed               |
| 2 | seed thickness | B | back of the seed                 |
| 3 | hilum          | C | longitudinal section of the seed |
| 4 | raphe          | D | seed after soaking in water      |
| 5 | embryo         |   |                                  |
| 6 | pericarp       |   |                                  |
| 7 | cotyledon      |   |                                  |
| 8 | mucilage layer |   |                                  |

**Figure 1 — Structure of *Salvia miltiorrhiza* seed**

The *Salvia miltiorrhiza* seedling is a one-year seedling, consisting of four parts: bud, tap root, lateral root and fibrous root, as shown in [Figure 2](#).



**Key**

- 1 seedling length
- 2 bud
- 3 tap root
- 4 lateral root
- 5 fibrous root
- A seedling with more lateral roots
- B seedling with fewer lateral roots

**Figure 2 — *Salvia miltiorrhiza* seedling**

## 5 Requirements

### 5.1 General characteristics

The following requirements shall be met before separating the bulk sample into test samples:

- a) *Salvia miltiorrhiza* seedlings shall be healthy and intact;
- b) the presence of living insects, mouldy seeds and external contaminants which are visible to the naked eye shall not be permitted.

### 5.2 *Salvia miltiorrhiza* seeds

**5.2.1** The colour of the seed shall be black or brown. The seed shall have a hard texture, without any peculiar smell and mildew.

**5.2.2** The mass fraction of moisture shall not be greater than 10 %.

5.2.3 Seed purity shall not be less than 70 %.

5.2.4 Viability shall not be less than 90 %.

5.2.5 Maturity shall not be less than 90 %.

5.2.6 The 1 000-seed weight, moisture content, germination percentage and seed purity shall comply with the requirements in [Table 1](#).

**Table 1 — Grading requirements of the *Salvia miltiorrhiza* seeds**

Grade	1 000-seed weight g	Moisture content %	Germination percentage %	Purity %
First	≥1,80	≤6,00	≥60,0	≥90,0
Second	≥1,60	≤8,00	≥50,0	≥80,0
Third	<1,60	≤10	≥40,0	≥70,0

NOTE The establishment of the above requirements is based on the seeds collected from different regions.

### 5.3 Seedlings

5.3.1 The tap root and lateral root of the *Salvia miltiorrhiza* seedling shall be intact with no diseases or insects. The tap root shall be full and substantial.

5.3.2 The bud of the *Salvia miltiorrhiza* seedling shall be intact with no diseases or insects.

5.3.3 Nematode shall not be detected.

## 6 Sampling

### 6.1 Seed sampling

Seed sampling shall be carried out in accordance with ISTA, *International Rules for Seed Testing*, Chapter 2. The maximum weight of the lot and the minimum weight of the sample are specified in [Table 2](#).

**Table 2 — Maximum weight of seed lot and minimum weight of sample**

Maximum weight of seed lot kg	Minimum weight of sample g	
	Submitted sample	Purity analysis
2 000	250	50

NOTE The establishment of the above requirement is based on the seeds collected from different regions and handled with different treatments.

### 6.2 Seedling sampling

The maximum sample lot of seedlings is 300 000 and the minimum samples should not be less than 100.

## 7 Test method

### 7.1 Moisture

Determination of the mass fraction of moisture shall be in accordance with the high constant temperature method specified in ISTA, *International Rules for Seed Testing*.

### 7.2 Maturity

Take duplicate samples of dry seeds using the quartering method, each containing 100 to 150 seeds. The seed shall be cut into two parts along the raphe. The shape of its embryo is determined with a microscope. The seed is considered to be mature if its embryo is pyriform or saddle. [Formula \(1\)](#) is used to express the maturity of a seed lot:

$$M(\%) = \frac{A_m}{A} \times 100 \quad (1)$$

where

$M$  is the maturity (%);

$A_m$  is the number of mature seeds;

$A$  is the number of seeds in the working sample.

If the difference between measured maturities of the duplicate sample is less than 5 %, then the maturity of the seed lot shall be the arithmetic mean of two measured values; otherwise, it shall be re-estimated.

### 7.3 Purity

Determination of the seed purity shall be in accordance with the purity analysis of ISTA, *International Rules for Seed Testing*.

### 7.4 Seed viability

Determination of the seed viability shall be in accordance with ISTA, *International Rules for Seed Testing* and ISTA, *Working Sheets on Tetrazolium Testing*.

### 7.5 1 000-seed weight

Determination of the 1 000-seed weight shall be in accordance with Weight Determination of ISTA, *International Rules for Seed Testing*.

### 7.6 Germination percentage

Germination percentage shall be in accordance with the purity analysis of ISTA, *International Rules for Seed Testing*.

### 7.7 Fungus testing

#### 7.7.1 Preparation of seed

Add 100 seeds and 20 ml sterilized water into a sterilized flask and shake for 8 min. The suspension is then centrifuged at 4 000 rpm for 20 min. The residue is re-suspended with 2 ml of sterilized water. Spot 100 µl of the final suspension on the blotter or filter paper on a plate for incubation. There shall be a total of five spots on the plate.

### **7.7.2 Preparation of seedling**

Add 500 ml sterilized water into a flask. Take 10 to 20 seedlings. Immerge the seedling into the flask and shake for 8 min one by one. The suspension is then centrifuged at 4 000 rpm for 20 min. The residue is re-suspended with 2 ml of sterilized water. Spot 100 micro litter of the final suspension on the blotter or filter paper in a plate for incubation. There shall be a total of five spots on the plate.

### **7.7.3 Test of *Alternaria* spp.**

*Alternaria* spp. testing shall be in accordance with ISTA, *International Rules for Seed Testing*, method 7-001a.

### **7.7.4 Test of *Fusarium* spp.**

*Fusarium* spp. testing shall be in accordance with ISTA, *International Rules for Seed Testing*, method 7-009.

## **7.8 Nematode testing**

Take 100 to 150 seedlings. Test nematode knots or lesions with the naked eye. Check the root knot or root lesion for nematode under microscope.

## **7.9 Seedling diameter**

Take 100 to 150 seedlings and measure the top of the tap root using a Vernier caliper (two digits of the significant figures). The arithmetic mean indicates the average seedling diameter of the seedling lot.

## **7.10 Seedling length**

Take 100 to 150 seedlings and measure from the lower part to the top of the seedling using a ruler (two digits of the significant figures). The arithmetic mean indicates the average seedling length of the seedling lot.

## **8 Test report**

For each test method, the test report shall specify the following:

- a) all information necessary for the complete identification of the sample;
- b) the sampling method used;
- c) the test method used;
- d) the test result(s) obtained;
- e) all operating details not specified in this document, or regarded as optional, together with details of any incidents which may have influenced the test result(s);
- f) any unusual features (anomalies) observed during the test;
- g) date of the test.

## **9 Packaging, storage and transportation**

The packaging shall not transmit any odour or flavour to the product and shall not contain substances that can damage the product or constitute a health risk.

The temperature of seed storage should be not higher than 20 °C.

The temperature of seedling storage should be between 0 °C and 20 °C for long distance delivery.

## 10 Marking

The following information shall be marked or labelled on the packages:

- a) the grade of the product should be in accordance with [5.2](#);
- b) all quality features, indicated in [5.2](#) and [5.3](#), should be determined in accordance with methods specified in [Clause 7](#);
- c) the maximum weight of the lot and that of the samples, as specified in [Table 2](#);
- d) the country and province/state of origin of the seed and seedling;
- e) the expiration date of the seeds;
- f) items required by the regulatory body of the destination country.

## Bibliography

- [1] ISO 78-2, *Chemistry — Layouts for standards — Part 2: Methods of chemical analysis*
- [2] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*
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