

Study on Seed Quality Classification Standard of *Vaccariae Semen*

Gao Qin, Yang Taixin* and Liu Xiaoqing

Agronomy College, Hebei Agricultural University/Crop Growth Regulation Laboratory of Hebei, Baoding 071001, China

Abstract

Objective: To establish the seed quality classification standard of *Vaccariae Semen*. Method: 34 batches of *Vaccariae Semen* seeds samples from different producing areas of the whole country were selected. The seed purity, 1 000-grain weight, water content and the germination rate of the seeds samples were measured. K-means analysis was applied for the data analysis. The primary seed quality classification standard of *Vaccariae Semen* was formulated.

Result: Seed purity and seed germination rate were selected as the primary indicators, and the other indicators provide the important reference, the *Vaccariae Semen* seeds were classified into 3 grades.

Conclusion: The quality classification standard of *Vaccariae Semen* seeds were initially established and it can be used as the reference standard.

Keywords: *Vaccariae Semen*; Seed; quality classification standard

Introduction

Vaccariae Semen is the *Vaccaria segetalis* (Neck.) Garcke. dried matured seeds of Caryophyllaceae as the commonly used traditional Chinese medicine, its bitter taste, belong to the liver and stomach channel. It has effect on activating blood to promote menstruation, promoting lactation to reduce swelling, promoting diuresis and relieving stranguria [1]. Seeds mainly contains triterpenoid saponins, flavonoids glycosides, cyclic peptide, lipid and fatty acid, monosaccharide and so on [2], can resist early pregnancy, antitumor, excite uterus, promote the secretion of milk and other pharmacological effects [3].

Vaccariae Semen distributed in North China, East China, China's northeast, northwest and southwest, mainly produce in Hebei, Shandong, Liaoning, Heilongjiang Provinces, the largest origin is Hebei province [4]. In production *Vaccariae Semen* propagated by seeds, but except Gao Xiaojuan was reported the *Vaccariae Semen* seed quality about Guyuan Ningxia, currently at home and abroad the research reports were fewer [5]. This study widely collected 34 different batches of *Vaccariae Semen* seeds samples in Hebei, Shandong, Henan, Jiangsu, Gansu, Anhui 6 provinces, with referencing the requirements and methods of Rules for agricultural seed Testing(GB/T 3543), measured the seed purity, 1 000-grain weight, water content and the germination rate. K-means analysis was applied for the data analysis. The primary seed quality classification standard of *Vaccariae Semen* was established, and provide theoretical basis for seed quality evaluation.

Materials and Methods

Materials

Vaccariae Semen seed samples, were collected from Hebei, Shandong, Henan, Jiangsu, Gansu, Anhui 6 provinces from between 2012 to 2013, and identified by Professor YANG Tai-xin of Hebei Agricultural University. The seeds were divided into 34 batches, 500 g of each batch (Table 1).

Methods

Purity analysis: Take sample referencing the Rules for agricultural seed Testing(GB/T 3543), then divide by quartering until it close to 15 g (contain at least 2 500 seeds) [6,7]. Weighing the sample critically, then placed on a smooth and clean desktop or glass plates, divide pure

seeds, heavy impurities, other plant seeds and impurities individually by tweezers, and weighed critically, calculate the percentages of pure seeds ,repeated 3 times.

Determination of 1 000-grain weight: Mixed pure seeds after purity analysis, divided into 4 parts by quartering, and 250 seeds were selected randomly from each part, each group was composed of 1 000 seeds. Weighed each group by one over one thousands of electronic balance, repeated 3 times, calculate the average.

Determination of water content: Crush the seeds , take the seed pieces through the round hole sieve diameter of 1.0 mm, then load into the grinding reagent bottles after mixing as standby. Low-temperature baking method ($103 \pm 2^\circ\text{C}$) was adopted in this study, preheat drying oven to $110\sim 115^\circ\text{C}$, then take about 5 g sample into the dried and weighted of the sample box in advance then weigh again (accurate to 0.001g); Start timing when box temperature rose to $103 \pm 2^\circ\text{C}$, after 8 h bake out and placed into the dryer to cool to room temperature, then weighed critically and record it , calculate the water content. Repeated 3 times.

water content = $(M_2 - M_3) / (M_2 - M_1) \times 100\%$ (M_1 : weight of the dried sample box; M_2 : the sum weight of the dried sample box and sample before drying; M_3 : the sum weight of the dried sample box and sample after drying).

Determination of germination rate: Random out pure seeds of each Sample after Purity analysis of each 100-grain seeds, repeated 3 times. Immersed the seeds in 0.5% KMnO_4 for 10 minutes, and clean

*Corresponding author: Yang Taixin, Agronomy College, Hebei Agricultural University/Crop Growth Regulation Laboratory of Hebei, Baoding 071001, China, E-mail: yangtaixin@126.com

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Sample No.	Producing area	Collection time
1	Anguo City, Hebei Province	2012
2	Zhangjiakou City, Hebei Province	2012
3	Chengde City, Hebei Province	2012
4	Linyi City, Shandong Province.1	2012
5	Linyi City, Shandong Province.2	2012
6	Anyang City, Henan Province	2012
7	Xuzhou City, Jiangsu Province	2012
8	Chuzhou City, Anhui Province	2012
9	Handan City, Hebei Province	2012
10	Luan City, Anhui Province	2012
11	Baoding City, Hebei Province	2013
12	Xingtai City, Hebei province.1	2013
13	Xingtai City, Hebei province.2	2013
14	Xingtai City, Hebei province.3	2013
15	Xingtai City, Hebei province.4	2013
16	Xingtai City, Hebei province.5	2013
17	Bozhou City, Anhui Province.1	2013
18	Bozhou City, Anhui Province.2	2013
19	Zhangye City, Gansu Province	2013
20	Anguo City, Hebei Province.1	2013
21	Anguo City, Hebei Province.2	2013
22	Anguo City, Hebei Province.3	2013
23	Anguo City, Hebei Province.4	2013
24	Zhangjiakou City, Hebei Province	2013
25	Chengjiawan Village, Neiqiu County	2013
26	Shicuntun Village, Neiqiu County	2013
27	Zhongtainzhuang Village, Neiqiu County	2013
28	Dongjie Village, Neiqiu County	2013
29	Liujiashuang Village, Neiqiu County	2013
30	Xiaoshihe Village, Neiqiu County	2013
31	Xiqiu Village, Neiqiu County	2013
32	Shanao Village, Neiqiu County	2013
33	Nanling Village, Neiqiu County	2013
34	Yonggu Village, Neiqiu County	2013

Table 1: *Vaccariae Semen* seed tested samples.

them several times with distilled water, then placed in petri dishes which covered with two layers moist filter paper, cultured at 20°C, Keep filter paper wet, the germination rate of seeds was counted on the 4th day for the first time, and on the 7th day after sowing for the last time, when the length of hypocotyl that has breakthrough the testa reach to the seed itself counts as a sprout, calculate the germination rate.

Seed quality classification: Through Correlation analysis, Principal component analysis, Systematic cluster analysis and K-means cluster analysis about the seed purity, 1 000-grain weight, water content and the germination rate of the seed samples by SPSS 19.0 software, The *Vaccariae Semen* seeds were classified into 3 grades.

Results and Analysis

Results and analysis of the 4 indicators of *Vaccariae Semen*. seeds samples

34 batches of *Vaccariae Semen* seeds samples from different producing areas were selected, the seed purity, 1 000-grain weight, water content and the germination rate of the seed samples were measured, and results of which were listed in Table 2. The purity of different batches of *Vaccariae Semen* seeds samples were 94.98%~99.61%, at a high standard, on average 97.98%; 1 000-grain weight were 3.605~4.840 g, on average 4.338 g, there are 5 batches samples lower

than 4.0 g, of which four of them are from Neiqiu County Hebei Province, may be related to growing conditions; Water content of these batches were all lower than 11.0%, they were between 9.22% to 10.65%, on average 9.79%, different batches has small differences. Germination rate were 49.67%~99.00%, on average 79.43%, varies big in different batches, there were 19 batches higher than the average, and 3 batches lower than 60%, accounting for 8.8%.

Results and analysis about seed quality classification of *Vaccariae Semen*

Results of correlation analysis: Correlation analysis was applied for the 4 indicators analysis about seed quality classification of *Vaccariae Semen*. and results of which were listed in Table 3. We found only one positive correlation between purity and germination rate, the relations between each other indicators all has negative correlation. The correlation between each indicators has not reached significant level.

Results of principal component analysis: The results about Principal component analysis were listed in Table 4. The cumulative contribution rate has reached 79.949% of the first three eigenvalues, it showed that the first 3 Principal components basically contains

Sample No.	Purity//%	1000-grain weight/g	water content//%	germination rate//%
1	97.63	4.121	9.32	97
2	99.03	4.315	9.37	77.67
3	96.59	4.839	9.83	86
4	99.2	4.375	9.54	99
5	98.52	3.799	9.74	66.33
6	98.63	4.148	10.31	86.33
7	96.55	4.201	10.65	80.33
8	97.14	4.456	10.21	83.67
9	97.55	4.581	9.27	74
10	96.85	4.21	10.12	77.33
11	98.87	4.165	9.58	90
12	96.69	4.394	9.68	85
13	98.44	4.675	10.26	83.67
14	97.68	4.586	9.63	86
15	98.76	4.607	10.23	81.33
16	96.21	4.576	10.37	82
17	97.61	4.499	9.53	70.67
18	94.98	4.35	9.76	62.33
19	98.91	4.598	9.82	93.67
20	99.61	4.438	9.62	98.67
21	98.98	4.816	10.45	53.67
22	99.07	4.667	9.72	96.67
23	97.57	4.734	9.42	62.67
24	97.12	4.84	9.22	57
25	97.88	4.19	9.36	98
26	99.38	3.736	9.7	98.67
27	96.32	3.605	9.75	74.67
28	99.26	4.321	9.22	49.67
29	98.63	3.629	10.29	69.67
30	98.94	4.109	10.56	62
31	98.02	4.485	9.59	72.33
32	98.12	4.518	9.35	70
33	97.61	4.234	9.56	89
34	99.03	3.666	9.82	85.67

Table 2: Results of different batches of *Vaccariae Semen* seeds samples.

Indicators	Average	Standard deviation	Purity	1000-grain weight	Water content	Germination rate
Purity	97.98	1.112	1	-0.129	-0.066	0.188
1000-grain weight	4.3377	0.344	-0.129	1	-0.097	-0.128
Water content	9.79	0.409	-0.066	-0.097	1	-0.051
Germination rate	79.43	13.71	0.188	-0.128	-0.051	1

Table 3: Correlation analysis of 4 indicators about seed quality of *Vaccariae Semen*.

Principal components	Variance contribution	Contribution rate /%	Cumulative contribution rate /%
1	1.3	32.506	32.506
2	1.081	27.033	59.54
3	0.816	20.409	79.949
4	0.802	20.051	100

Table 4: Variance contribution of Principal components.

all the information. Take the first 3 eigenvalues, and calculate the corresponding eigenvectors. The first 3 principal components are:

$$F1=0.699X1 - 0.567X2 - 0.088X3 + 0.694X4$$

$$F2=- 0.184X1 - 0.528X2 + 0.866X3 - 0.137X4$$

$$F3=- 0.343X1 + 0.383X2 + 0.270X3 + 0.692X4$$

In expressions: X1 purity; X2 1 000-grain weight; X3 water content;

Taken together, in the first principal component expression, purity X1 and germination rate X4 had higher Coefficients, these two indicators played a major role in consideration of the first principal component had the highest contribution rate, purity and germination rate were selected as the primary indicators, and the other indicators provide the important reference in this study.

Results of Systematic cluster analysis: Systematic cluster analysis was applied for the data of 4 indicators by SPSS 19.0 software, results are shown in Figure 1. 34 batches of seeds samples were divided into 2 categories by systematic cluster analysis, the *Vaccariae Semen* seeds were classified into 3 grades in this study, of which grade I and II are qualified seeds, grade III are unqualified seeds.

Results of K-means clustering analysis about single indicator: K-means clustering analysis was applied respectively for the data of 4 indicators by SPSS 19.0 software, results are shown in Table 5.

Establishment of quality classification standard of *Vaccariae Semen* seeds: Seed purity and seed germination rate were selected as the primary indicators, and the other indicators provide the important reference, put cluster centers as the reference values of quality classification standard of *Vaccariae Semen* seeds, combined with practices and operability, the quality classification standard of *Vaccariae Semen* seeds were initially established, results are shown in Table 6. The minimum principle was adopted in grading standard: seeds do not meet any indicator of corresponding grade could not come up to the required standard.

Discussions

In the 4 indicators of establishing of quality classification standard of *Vaccariae Semen* seeds, germination rate have a significant impact on seedling rate, determined the value in fields; And germination rate were measured on purity analysis, purity has an important impact on its field sowing; 1 000-grain weight could reflect the maturity degree and the

plumpness of seeds; Water content can affect the seed storage and seed longevity. In this study, seed purity and seed germination rate were selected as the primary indicators, and the other indicators provide the important reference through Principal component analysis.

The grading method that adopted in this study was based on the mathematical principles, because the classification number is known, K-means analysis was applied for the date analysis. This method is widely used in the seed quality classification of Chinese herbal medicine, such as: *Cyathula officinalis* Kuan. *Erigeron breviscapus* (Vant.)Hand.-Mazz. *Leonurus japonicus* Houutt. *Dipsacus asperoides*. *Aucklandia lappa*. *Trollius chinensis* [8-13]. This method were used in this study.

The quality of medicinal plants seeds is the basic guarantee for high quality and high yield of herbal medicine, the research and development of seed standards have great significance in grasping the quality of traditional Chinese medicine herbs and herbal products from the source. To speed up the establishment of seed quality

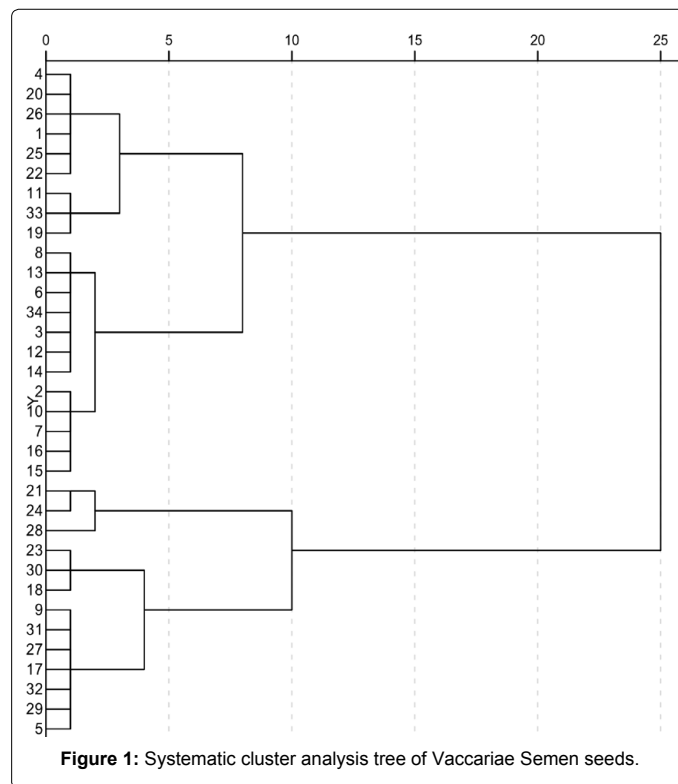


Figure 1: Systematic cluster analysis tree of *Vaccariae Semen* seeds.

Grade	Germination rate//%	1 000-grain weight//g	Purity//%	Water content//%
First grade	≥88.37	≥4.56	≥98.52	≤9.56
Second grade	65.00~88.37	3.99~4.56	96.49~98.52	9.56~10.35
Third grade	≤65.00	≤3.99	≤96.49	10.35~11.00

Table 5: Results of K-means clustering analysis about single indicator.

Grade	Germination rate//%	1 000-grain weight//g	Purity//%	Water content//%
First grade	≥85	≥4.5	≥98.5	≤10.0
Second grade	65~85	4.0~4.5	96.0~98.5	10.0~11.0
Third grade	≤65	≤4.0	≤96.0	≥11.0

Table 6: Quality classification standard of *Vaccariae Semen* seeds.

standards, to standard the quality of Chinese herbal medicine seeds on the market, then achieve the standardization production, ensure the production and quality of herbal medicine. In this study, *Vaccariae Semen* seeds samples of different batches from different producing areas were collected extensively, the quality classification standard of *Vaccariae Semen* seeds were initially established through measurement and statistical analysis, and provide a theoretical basis for the quality evaluation and production of *Vaccariae Semen* seeds.

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