# Effects of Fertilization Treatments on the Growth of *Fritillaria cirrhosa* D. Don Bulbs

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#### Abstract:

Bulbus *Fritillaria cirrhosa* D. Don (BFC) is a rare wild herbaceous plant of Liliaceae that is commonly found in the Tibetan area of China. The bulb of F. *cirrhosa* is not only used for pharmaceuticals, but also as seeding materials. Two different fertilizers were applied under two different seedling substrates to study the effects of the fertilizers on the growth of F. *cirrhosa* bulbs. Seedlings and one-year-old bulbs of F. *cirrhosa* were two kinds of seedling materials. The results were: 1. The application of these two fertilizers could both enhance the preservation rate of bulbs. 2. When sowing seeds with meida A2 (100% humus soil) and A3 (50% humus soil+50% sandy soil), the application of 60 g•m-2 fertilizer T2 (three-nutrient compound fertilizer, N: P: K=17: 8: 15) could provide the one-year-old bulbs of F. *cirrhosa* with high yield and quality. 3. When sowing one-year-old bulbs with media A1 (100% sandy soil), A2 (100% humus soil), and A3 (50% humus soil+50% sandy soil), the application of F. *cirrhosa* with high yield and quality. From the data, we concluded that A2 (100% humus soil) and A3 (50% humus soil) and A3 (50% humus soil+50% sandy soil) supplemented with fertilizers such as three-nutrient compound fertilizer (N: P: K=17: 8: 15) can serve as excellent growing substrates of F. *cirrhosa* bulbs.

*Keywords*: Fritillaria cirrhosa D. Don Bulbs, One-year-old bulbs, Two-year-old bulbs, Vegetative parameters, Fertilization treatment.

#### I. INTRODUCTION

Wild plant resource conservation is one of the approaches to solve the shortage of high-quality germplasm resources and promote the development of traditional Chinese medicinal materials [1]. Bulbus *Fritillaria cirrhosa* (BFC) is world-renowned for its protected grade and clinical efficacy to relieve cough and eliminate phlegm [2]. After "sprout tumble" of BFC seedlings, the bulbs could be harvested. The bulbs serve as both medicinal and breeding

material [3]. Organic substance of soil provides a reservoir of nutrients for the growth of plants [4].

The natural growth of bulbs, especially one-year-old bulbs and two-year-old bulbs, is extremely slow. The bulbs ripe enough for medical use are usually harvested in 4a or 5a. The Thousan Kernel Weight (TKW) of one-year-old bulbs and two-year-old bulbs are both less than 50 g [5]. Many cultivating substrates of BFC bulb cultivation have been studied, including sandy soil, humus soil, and livestock manure [6-8]. Humus soil supplemented with the excrement of Hepialus *armoricanus* was used as substrate in cultivating high-quality seedlings [9]. Due to low amount of H. *armoricanus* excrement, it is not a suitable substrate for industrial cultivation in factory, and thus not able to provide large number and high-quality seedlings in the near future. Adding sandy soil to substrates or using 100% sandy soil as nursery media could culture BFC bulbs with better shape [10]. Besides, different fertilization methods for cultivating bulbs have been studied [11]. For four-year-old bulbs sown in soil, the optimum fertilization amount of F. *cirrhosa* is nitrogen (N), phosphorus (P) and potassium (K) fertilizer of 330 kg hm-2, 1560 kg hm-2, and 400 kg hm-2, respectively [12]. Sowing two-year-old bulbs in soil supplemented with 200 g m-2 plant ash and 40 g m-2 compound, the seedlings grew well, with the fresh matters to dry matters ratio in bulbs being 2.8 [5].

Based on previous studies, under shed nursery mode at an altitude of 3500 meters, three kinds of media (sandy soil, humus soil, and 50% humus soil +50% sandy soil) and two kinds of fertilizer were studied. Humus soil supplemented with the excrement of H. *armoricanus* were set as control. This study aims to evaluate effects of different treatment on growth of F. *cirrhosa* bulbs. In order to limit production cost and resource utilization, this paper is to provide a research base for the industrial cultivation of Bulbuls *Fritillaria cirrhosa*.

#### **II. MATERIALS AND METHODS**

2.1 Plant Material

Ripe seeds and one-year-old bulbs of cultivated *Fritillaria cirrhosa* D. Don were collected in Xinduqiao Town, Kangding Country, Sichuan Province (Latitude: 101°34' N, longitude: 30°03' E, altitude: 3500 m). Natural stratification was used to break the dormancy of the viable seeds [13]. Seed embryonic development reached normal level. According to Table 1, the size of one-year-old bulbs were measured. The values of longitudinal diameter, transverse dia., and R ranged from 0.180-0.538 cm, 0.200-0.472 cm, 1.00-1.15, respectively.

### TABLE I. Definition of bulbs in F. cirrhosa [7]

Bulbs	Bulbs Definition
Longitudinal dia.	From tip of bulbs to growing points of bulbs' roots
Transverse dia.	Be perpendicular to Longitudinal diameter
R	The ratio of Longitudinal diameter to Transverse dia.
One-year-old bulbs	Sow with seeds and reap bulbs after 'sprout tumble' in the same year
Two year old hulbs	Sow with one-year-old bulbs and reap bulbs after 'sprout tumble' in the
1 wo-year-old builds	same year

### 2.2 Fertilizers

Two kinds of fertilizers were selected. Polyelement composite fertilizer (T1) was purchased from Chengdu Toyota Agriculture Corporation. Three-nutrient compound fertilizer (T2) was purchased from Zhushang fertilizer (Qingdao) Corporation. The nutrients of the fertilizer were showed in Table 2.

#### Nutrients Polyelement composite fertilizer three-nutrient compound fertilizer Organic matter/% 20 Total N/% N : P : K = 17 : 8 : 15Total P/% 25 Total K/% Secondary elements % 10 \_ Humic acid % 5 \_ Amino acid% 6 \_ Micro-element% 0.8 \_

### TABLE II. The nutrients of two kinds of fertilizer

### 2.3 Nursery Substrates

The media chosen was commonly used in cultivation. Sandy soil, humus soil, 50% humus soil+ 50% Sandy soil, and humus soil with the excrement of H. *armoricanus* were selected as cultivation materials. All nursery substrates were collected from Chengdu Enwei Group. The chemical characters of nursery substrates were described in Table 3.

TABLE III.	Chemical	characters	of Nursery	substrates [7]
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Chemical characters	Sandy soil	humus soil	50% humus soil + 50% sandy soil	humus soil with the excrement of <i>H</i> .
рН	6.5	6.3	6.4	5.3
CEC	4.1	71.9	39.2	48.5

Organic matter/%	4.15	38.34	21.46	26.98
Total N/%	0.092	1.530	0.865	1.061
Total P/%	0.027	0.128	0.066	0.177
Total K/%	0.073	1.070	0.538	1.680
Hydrolysable N (mg	74	673	374	599
kg <sup>-1</sup> )				
Available P (mg kg <sup>-1</sup> )	4.4	29.6	14.8	97.6
Available K (mgkg <sup>-1</sup> )	14	309	155	684

Note: CEC=Cation Exchange Capacity.

#### 2.4 Local Conditions

The experiment was carried out in shed nursery in Xinduqiao Town, Kangding Country, Sichuan Province. The climate was plateau continental monsoon. The annual frost-free period was 2525.9 h. The annual frost-free season was 95 days.

#### 2.5 Breeding Experiment

Randomized block design with three replicates was used in the trial. Three nursery media were A1 (100% sandy soil), A2 (100% humus soil), and A3 (50% humus soil + 50% sandy soil). Two nursery fertilizers were polyelement composite fertilizer (T1) and three-nutrient compound fertilizer (T2). The amount of fertilizer application was 60 g m-2. Humus soil supplemented with the excrement of H. *armoricanus* (A4) were set as control. The test numbers of sowing with seeds were marked as SA1, SA2, SA3, ST1A1, ST1A2, ST1A3, ST2A1, ST2A2, ST2A3, and SA4. The test numbers of sowing with one-year-old bulbs were marked as BA1, BA2, BA3, BT1A1, BT1A2, BT1A3, BT2A1, BT2A2, BT2A3, and BA4. Seedbed was split up into plots (size of each plot: 0.25 m2) with bricks. The density of seeds was 10000 per square meter. The density of one-year bulbs was 5000 per square meter (Fig. 1). The plants are watered by drip irrigation system. Breeding tests were performed following the methods of Hu et al. 2013[7]. The growing status of bulbs was recorded until harvest.



Fig 1: Breeding experiment

Note: A: Seedling cultivation experiment. B: Sowing with seeds. C: Sowing with one-year-old bulbs

#### 2.6 Statistical Analyses

Preservation rate, bulb longitudinal diameter (BLD), bulb transverse diameter (BTD), 30-grain fresh weight (GFW), and 30-grain dry weight (GDW) were measured with the methods shown in Table 1. Statistical analyses were performed using SPSS v19 (SPSS Inc., Chicago, USA). One-way ANOVA with Fisher's LSD tests were performed to examine the vegetative parameters of F. *cirrhosa* bulbs under the different treatments.

#### **III. RESULTS**

3.1 Effect of Fertilizers on the Preservation Rate of F. Cirrhosa

According to the data presented in Table 4, sowing seeds with A1 (100% sandy soil), the greatest preservation rate was observed in ST1A1 (51.03%) followed by ST2A1 (50.79%) and SA1 (46.75%). Sowing seeds with A2 (100%humus soil), the greatest preservation rate was observed in ST2A2 (57.83%) followed by ST1A2 (57.59%) and SA1 (53.08%). Sowing seeds with A3 (50% humus soil+50% sandy soil), the greatest preservation rate was observed in ST1A3 (53.07%) followed by ST1A2 (52.37%) and SA1 (48.59%).

Sowing with one-year-old bulbs, the great rate of preservation was observed in BT2A2 (92.27%) followed by BT2A1 (91.71%), BT2A3 (91.41%), BA4 (91.31%), BT1A2 (89.01%), BT1A1 (87.65%), BT1A3 (87.44%), BA3 (85.28%), BA2 (83.36%), and BA1 (83.28%). In A1, the greatest preservation rate was observed in BT2A1 (91.71%) followed by BT1A1 (87.65%) and BA1 (83.28%). In A2, the greatest preservation rate was observed in BT2A2 (92.27%) followed by BT1A2 (89.01%) and BA2 (83.36%). In A3, the greatest preservation rate was observed in BT2A2 (92.27%) followed by BT1A2 (89.01%) and BA2 (83.36%). In A3, the greatest preservation rate was observed in BT2A3 (91.41%) followed by BT1A3 (87.44%) and BA3 (85.28%).

In conclusion, both two kinds of fertilizer could enhance preservation rate of bulbs.

Treatment	Sowing with seeds	Treatment	Sowing with one-year-old bulbs
SA1	46.75±3.05948d	BA1	83.28±9.4735a
ST1A1	51.03±1.55053c	BT1A1	87.65±6.82693a
ST2A1	50.79±1.80751c	BT2A1	91.71±5.57729a
SA2	53.08±2.395c	BA2	83.36±9.11474a
ST1A2	57.59±1.50467b	BT1A2	89.01±3.65936a
ST2A2	57.83±1.42423b	BT2A2	92.27±4.10528a
SA3	48.59±0.71818d	BA3	85.28±13.145581a
ST1A3	53.07±2.56827c	BT1A3	87.44±3.16683a
ST2A3	52.37±1.73853c	BT2A3	91.41±2.9733a
SA4	63.68±2.89902a	BA4	91.31±2.2433a

TABLE IV. Effect of fertilizers on the preservation rate of F. cirrhosa

Note: Different letters in a row are statistically different ( $p \le 0.05$ ).

3.2 Effect of Fertilizers on the Size and Biomass of One-Year-Old Bulbs

Sowing seeds with three kinds of nursery substrates, the addition levels of both polyelement composite fertilizer (T1) and three-nutrient compound fertilizer (T2) were at 60 g m-2. Sowing seeds with A1 (100% sandy soil), A2 (100% humus soil), and A3 (50% humus soil + 50% sandy soil), the values of R ranged from 1.1 to 1.13, 1.25 to 1.27, and 1.23 to 1.27 (Table 5, Table 6, Table 7).

In Table 5, sowing seeds with A1, there were no significant differences in BLD, BTD, R, mean value of BFW, mean value of BDW, and the fresh weight to dry weight ratio (RW).

In Table 6, sowing seeds with A2, there were no significant differences in BLD, BTD, R, and RW between STA2 and SA4.

In Table 7, sowing seeds with A3, there were not significant differences in BLD, BTD, R, mean value of BDW, and RW between STA3and SA4.

# TABLE V. In media A1, effect of fertilizers on the size and biomass of one-year-old bulbs at harvest time

Indox	Treatment					
muex	ST1A1	ST2A1	SA1	SA4		
BTD	0.32±0.019b	0.34±0.00416b	0.34±0.00603b	0.42±0.01443a		
BLD	0.35±0.01115b	0.38±0.01054b	0.38±0.01212b	0.53±0.03205a		
R	1.13±0.04b	1.13±0.01732b	1.1±0.04583b	1.26±0.02887a		
GFW	0.5733±0.08083b	$0.6367 \pm 0.02887b$	0.6233±0.02309b	1.2533±0.09238a		
GDW	0.1337±0.02226b	$0.1475 {\pm} 0.00581 b$	0.1599±0.00771b	0.2923±0.04055a		
RW	23.37±2.48886a	23.18±0.80277a	25.69±1.96001a	23.41±3.87407a		

Note: Different letters in a row are statistically different ( $p \le 0.05$ ).

# TABLE VI. In media A2, effect of fertilizers on the size and biomass of one-year-old bulbs at harvest time

Index	Treatment					
muex	ST1A2	ST2A2	SA2	SA4		
BTD	0.36±0.01701b	0.39±0.01665ab	0.37±0.01952b	0.42±0.01443a		
BLD	0.45±0.02515b	0.49±0.03024ab	0.47±0.01721b	0.53±0.03205a		
R	1.25±0.01732a	1.26±0.04583a	1.27±0.03606a	1.26±0.02887a		
GFW	0.8767±0.06351bc	$1.0167 \pm 0.08083b$	0.8533±0.05774c	1.2533±0.09238a		
GDW	0.2002±0.0117b	0.2433±0.02351b	0.2198±0.01227b	0.2923±0.04055a		
RW	22.86±0.97247a	24.06±3.42015a	25.84±2.31554a	23.42±3.87407a		

Note: Different letters in a row are statistically different ( $p \le 0.05$ ).

# TABLE VII. In media A3, effect of fertilizers on the size and biomass of one-year-old bulbs at harvest time

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	ST1A3	ST2A3	SA3	SA4
BTD	0.37±0.01652b	0.39±0.02303ab	0.38±0.00917b	0.42±0.01443a
BLD	0.47±0.03274a	0.48±0.04029a	0.47±0.03109a	0.53±0.03205a
R	1.27±0.04163a	1.25±0.03606a	1.23±0.04726a	1.26±0.02887a
GFW	0.9333±0.06351b	0.9833±0.06351b	0.9367±0.09815b	1.2533±0.09238a
GDW	0.2262±0.03782b	0.2391±0.03306ab	0.2334±0.01863ab	0.2923±0.04055a
RW	24.14±2.72142a	24.30±2.62519a	25.02±2.23515a	23.42±3.87407a

Note: Different letters in a row are statistically different ( $p \le 0.05$ ).

3.3 Effect of Different Fertilizations on the Size of Two-Year-Old Bulbs

Sowing seeds with three kinds of nursery substrates, the addition levels of both polyelement composite fertilizer (T1) and three-nutrient compound fertilizer (T2) were at 60 g m-2. Sowing one-year-old bulbs with A1 (100% sandy soil), A2 (100% humus soil), and A3 (50% humus soil + 50% sandy soil), the values of R ranged from 1.50 to 1.51, 1.55 to 1.62, and 1.59 to 1.65 (Table 8, Table 9, Table 10).

In Table 8, sowing one-year-old bulbs with A1, there were no significant differences in BLD, BTD, R, GFW, GDW, and RW between STA2 and SA4.

In Table 9, sowing one-year-old bulbs with A2, there were no significant differences in BLD, BTD, R, GDW, and RW between STA2 and SA4.

In Table 10, sowing one-year-old bulbs with A3, there were no significant differences in BLD, BTD, R, mean value of GFW, mean value of GDW, and RW between ST2A3 and SA4.

TABLE VIII. In media A1, effect of fertilizers on the size and biomass of two-year-old bulbs at harvest time

Indox	Treatment					
Index	BT1A1	BT2A1	BA1	BA4		
BTD	0.51±0.02987b	0.57±0.02237ab	0.52±0.06295b	0.60±0.05143a		
BLD	0.77±0.045b	0.88±0.06115ab	0.79±0.0948b	0.98±0.06065a		
R	1.51±0.02249a	1.55±0.10594a	1.52±0.09868a	1.62±0.03824a		
GFW	2.9867±0.50521b	3.9533±0.65592ab	2.59±0.29206b	5.1533±1.29388a		
GDW	0.7572±0.09904b	0.9992±0.19104ab	0.7373±0.11054b	1.3058±0.32907a		
RW	25.48±1.26693b	25.2067±0.60211b	28.3867±1.03732a	25.36±1.05788b		

Note: Different letters in a row are statistically different ( $p \le 0.05$ ).

Index	Treatment					
muex	BT1A2	BT2A2	BA2	BA4		
BTD	0.50±0.01422b	0.56±0.04303ab	0.58±0.02597a	0.60±0.05143a		
BLD	0.77±0.05704b	0.91±0.0412ab	0.93±0.05445a	0.98±0.06065a		
R	1.55±0.0749a	1.6185±0.06723a	1.6119±0.02685a	1.62±0.03824a		
GFW	3.3467±0.65248b	4.03±0.55245ab	4.29±0.59102ab	5.1533±1.29388a		
GDW	0.8224±0.14906b	1.0113±0.15483ab	1.0879±0.1696ab	1.3058±0.32907a		
RW	24.62±0.58287a	25.06±0.58518a	25.32±0.47286a	25.36±1.05788b		

## TABLE IX. In media A2, effect of fertilizers on the size and biomass of two-year-old bulbs at harvest time

Note: Different letters in a row are statistically different ( $p \le 0.05$ ).

# TABLE X. In media A3, effect of fertilizers on the size and biomass of two-year-old bulbs at harvest time

Indox	Treatment				
muex	BFA3	BT2A3	BA3	BA4	
BTD	0.49±0.04244b	0.54±0.03592ab	0.50±0.04093b	0.60±0.05143a	
BLD	0.78±0.11542b	0.87±0.03786ab	$0.82 \pm 0.05879 b$	0.98±0.06065a	
R	1.59±0.08465b	1.65±0.04734ab	1.62±0.02068b	1.62±0.03824a	
GFW	2.7533±0.88115b	3.68±0.64156ab	$2.8833 \pm 0.54848b$	5.1533±1.29388a	
GDW	0.7334±0.23796b	0.9166±0.16443ab	0.7054±0.13367b	1.3058±0.32907a	
RW	26.62±1.37508a	24.89±0.12858a	24.49±1.02198a	25.36±1.05788a	

Note: Different letters in a row are statistically different ( $p \le 0.05$ ).

### **IV. DISCUSSION**

The larger the transverse diameter of the bulb, the better the quality of the bulb. Sowing with seeds, BTD values of ST2A2 and ST2A3 were 0.39 and 0.39, respectively. Both ST2A2 and ST2A3 were not significantly different from SA4. This indicates that sowing seeds with A2 and A3, the application of 60 g m-2 T could provide one-year-old bulbs with same yield and quality as A4 (Humus soil supplemented with the dung of H. *armoricanus*). Sowing with seeds, BTD values of ST2A2, ST2A3, ST1A3, and ST1A2 were 0.39 cm, 0.39 cm, 0.37 cm, and 0.36 cm, respectively. Based on F. *cirrhosa* bulb grading standards shown in Table 11, our observed bulbs were good (Grade i), suggesting successful use of fertilizers in F. *cirrhosa* cultivation with seeds [14, 15]. Thus, judge BTD values, the effect T2 on seedling cultivation is better than T1. In growth period of F. *cirrhosa*, aside of N and P fertilizer, we should also pay attention to the continuous supply of K fertilizer. The nutrient composition of T2 (N: P: K=17: 8: 15) was different from T1 (Total content of N, P, and K, 25%). Sowing seeds with A1(100% sand soil),

there were not significant differences in BLD, BTD, R, mean value of BFW, mean value of BDW, and the fresh weight to dry weight ratio (RW). This indicates that due to lower nutrients level of sandy soil, when sowing seeds with sand soil, the application of 60 g m-2 fertilizers could not provide one-year-old bulbs with yield and quality. In order to reduce substrate cost, humus soil can be added to sand soil as additional cultivation substrate in production, and a certain amount of compound fertilizer can be applied at the same time, so as to obtain high-quality bulbs.

The bulbs of F. *cirrhosa* absorb nutrients from both substrates and the above-ground parts of the plant [7]. Sowing with one-year-old bulbs, the range of preservation rate was [82.28-92.27%]. The greatest preservation rate was observed in BT2A2 (92.27%) followed by BT2A1 (91.71%), BT2A3 (91.41%), BA4 (91.31%), BT1A2 (89.01%), BT1A1 (87.65%), BT1A3 (87.44%), BA3 (85.28%), BA2 (83.36%), and BA1 (83.28%). Therefore, the application of these two kinds of fertilizer could both enhance preservation rate of two-year-old bulbs. Humus soil supplemented with the excrement of H. *armoricanus* was a substrate for cultivating high-quality seedlings [10]. In Table 3, the values of Available P and Available K contents in A4 were 684 mg kg-1 and 97.6 mg kg-1, respectively. Both Available P and Available K contents in A4 were higher than that of A2. Increasing the nutrient content of substrate is one of the effective methods to improve preservation rate of bulbs. In this experiment, the effect of three-nutrient compound fertilizer (T2) on seedling cultivation is better than polyelement composite fertilize (T1).

Standards	One-year-old bulbs		Two-year-old years	
	BLD	BTD	BLD	BTD
Grade i	≥0.36	≥0.35	$\geq 0.80$	≥0.62
Grade ii	≥0.24, <0.36	≥0.23,<0.35	≥0.64,<0.80	≥0.48,<0.62
Grade iii	<0.24	<0.23	≥0.43,<0.64	≥0.37,<0.48

TABLE XI Standards of F. cirrhosa bulb grading [13, 14]

#### **V. CONCLUSION**

The application of these two kinds of fertilizer could enhance preservation rate of bulbs. When sowing seeds with substrates A2 (100% humus soil) and A3 (50% humus soil+50% sandy soil), the application of 60 g·m-2 fertilizer T2 (three-nutrient compound fertilizer, N: P: K=17: 8: 15) could provide one-year-old bulbs of F. *cirrhosa* with high yield and quality. Sowing one-year-old bulbs with substrates A1 (100% sandy soil), A2 (100% humus soil), and A3 (50% humus soil+50% sandy soil), the application of 60 g m-2 T2 could provide two-year-old bulbs of F. *cirrhosa* with high yield and quality. From the data, we concluded that

A2 (100% humus soil) and A3 (50% humus soil+50% sandy soil) supplemented with fertilizers such as three-nutrient compound fertilizer (N: P: K=17: 8: 15) can serve as excellent growing substrates of F. *cirrhosa* bulbs.

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