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Research Article

Pollen morphology, in vitro germination and another culture of Coix lachrymal-jobi L cultivars

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Abstract

To identify the optimal *in vitro* conditions for pollen germination, anther culture and callus proliferation and differentiation of job's tear (*Coix lachrymal-jobi* L) *cv*, features of pollen microspores at different flowering stages were observed, pollen germination conditions identified and anthers cultured to identify callus-forming and differentiating conditions. Pollen vigor and germination rates were determined and compared. Results showed that the late uni-, di- and tri-nucleate pollen grains were coexisting in the early flowering stage; and the pollen grains were homogeneously spherical and the vigor was the highest at the peak flowering stage. Single-tube pollen germination was observed. The optimal conditions for pollen germination was 10~15% sucrose + 0.01% boric solution at 25 for 5~7 hrs. The rates of callus induction from the late uninucleate anthers differed significantly among the *cv*. Calluses were successfully induced in the Murashige-Skoog medium+ 2, 4-dichlorophenoxyacetate1~2 mg/L + Kinetin1.5 mg/L. And some were differentiated into short cluster buds in the medium of Murashige-Skoog medium + indole-3-acetic acid 0.5 mg/L + Kinetin 2 mg/L.

This represents the first report of the morphology, vigor, optimal germination conditions for pollen microspores of *C. lachrymal-jobi* L of this understudied and underutilized yet economically important food and medicine homologous crop.

Keywords: Anther culture; Adlay; Pollen in vitro germination; Pollen grain morphology; Pollen vigor

Introduction

Coix lachrymal-jobi L, an annual or perennial Graminea herb, is an important food and drug homologous plant rich in nutrition and excellent for disease prevention and health care, and it is of extensive application. However, due to its past inadequate research, the cultivation area has been on a sharp drop and its wild resources has been greatly reduced. In recent years, with the increasing of people's awareness for health care, increasing studies have been conducted on its cultivation extension, tissue culture and rapid propagation, germplasm resource protection, and food and drug interaction (Li *et al*. 2016).

Anther culture is a type of tissue culture in which anthers at appropriate development stage are inoculated on artificial medium to induce the differentiation and mitotic division to form cell masses, so as to form undifferentiated calluses, or to differentiate into embryoids, and then induce the calluses to differentiate into complete plantlets. Anther culture is an effective way to obtain homozygote haploid, which can effectively shorten the breeding period, overcome the incompatibility of distant hybridization, and obtain excellent traits. The application of anther culture has been extensive and breakthroughs have been made in tobacco, rice, corn, oilseed rape and other crops (Jiradej *et al*. 2014). A few studies on *in vitro* germination and culture of the anther of *Coix* have been documented. Early *in vitro* anther culture studies of *Coix* by Li *et al* (1981) showed that the anther of *Coix* was most suitable for growing on H medium and regenerating haploid plants. Wang *et al* (1980) studied the induction of sporophyte from *Coix lachrymal* L pollen using N6 culture medium, and obtained callus and embryoid, and found that the plants formed from calluses contain the haploids, diploids and mixoploids, and the plants formed from embryoids showed more stable haploid, but the seedling rate was low. Li *et al* (1997) observed the meiosis process of pollen mother cells of *Coix* and recorded the meiosis process in detail. Dong and Xi (1992) recorded in detail the mega- and micro-sporogenesis and the formation of male and female gametophytes in *Coix lachrymal-jobi* L.

In this research, by using the *Coix cv* GDYY, pollen microspore morphology at different flowering stages were observed, *in vitro* pollen germination conditions were identified, anthers were cultured, and pollen vigor of 7 *Coix cv* were determined, in order to lay a foundation for further research on haploid breeding of *Coix lachrymal-jobi* L by anther culture.

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Materials and Methods

Materials

Coix lachrymal-jobi L cv. Seeds of the 7 *Coix lachrymal-jobi* L *cv* (Table 1) were harvested in Fairy Mountain Town, Wulong District, Chongqing, China.

Methods

Morphological observation on flowers and pollen grains of Coix cv: Judging from the appearance of male flowers, the

Table 1: ***Coix lachrymal-jobi*** **L** ***CV*** **Used in This Study.**

CV	*CV* Code	Source
Guangdong Yi Yi	GDYY	Guangzhou, Guangdong, China
Xing Ren Xiao Bai Ke	XRXBK	Xingyi, Guizhou, China
Guangxi Yi Yi	GXYY	Xilin, Guangxi, China
Fujian Yi Yi	FJYY	Pucheng, Fujian, China
Hebei Yi Yi	HBYY	Anguo, Hebei, China
Zhe Yi Yi Hao	ZYYH	Lishui, Zhejiang, China
Liaoning Bai Ke	LNBK	Jinzhou, Liaoning, China

flowering of *Coix lachrymal-jobi* L occurs in the early July, and the male flowers became full open in about 7~11 days and would last for about 3~7days. To observe the flowering characteristics of *Coix lachrymal-jobi* L, the flowering period is divided into the early, peak and late flowering stages mainly based on the appearance of male inflorescence and the male inflorescence were picked at 9:00-10:00 am.

The characteristics of the male spikes (male inflorescence), male spikelets (flower buds), pollen were observed and compared. Male inflorescence were fixed with Carnoy's fluid fixative for 24h, transferred to 70% ethanol, and stored in 0 ~ 4°C in refrigerator. The mid-region male spikelets of the male inflorescence were placed on the glass slide, cut open with a scalpel, squeezed out the pollen with tweezers, and dyed with I_2-KI. Then microscopic observation were carried out.

Determination of pollen vigor at different flowering stages: Pollen vigor was determined by I_2-KI staining. Anthers of the 3 flowering stages were pick and made into smears, stained for 5min with 1~2 drops of the dye, and microscopically observed. Pollen grains dyed blue were considered vigorous, whereas those dyed yellow or brown were not. Three fields of vision was observed and the averages were the estimates for pollen vigor rates. For each flowering stage 3 replicates were performed.

Determination of Optimum conditions for pollen germination in vitro: Determination of optimum culture medium. Four liquid culture solutions were prepared in 0.01% boric acid, which contain 5%, 10%, 15% and 20% sucrose respectively. 2~3 drops of the solution was added on the slides, and the anthers at the peak flowering stages were added to the solution on the slides, and pollen grains were extruded and made smears. The germination of *Coix* pollen were observed and the germination rates in different culture media were estimated as the average of 3 replicates to identify the optimal culture medium for pollen germination.

Determination of optimum temperature. Pollen from the anthers at the peak flowering stage was added to the optimum culture medium determined, cultured in incubators at 20, 25 and 30°C respectively, and microscopically observed. After 5 hours of incubation, pollen germination rates at different temperatures were determined and compared to identify the optimal temperature for *Coix lachrymal-jobi* L pollen germination.

Determination of optimum time. Pollen of *Coix cv* GDYY were cultured in the above-determined optimal medium and temperature, and the pollen germination rates over time were estimated to determine the optimal germination time.

Observation and identification of microspore development phases: Male inflorescence of *Coix cv* GDYY at the early flowering stage was pick, and the longitudinal lengths of the inflorescence, bud, and anther in the middle were measured. The microscopic characteristics of the microspores were observed after Carnoy fixation of the anthers for about 20min, and then 70% ethanol preservation. Finally smear slides were made and stained by I_2-KI for microscopic determination of the phases of microspore development.

Anther treatment and culture conditions: For there are some correlation between pollen development phase and some external morphological features of the flower bud, it is possible to use these external markers to select the flower buds that is close to the required phase. During the experiment, one anther from each bud should be taken to determine the phase of pollen development through microscopic examination.

According to Li (1981), anther treatment and culture conditions are different from those of the vegetative tissues or organs. The flower buds with microspore development mainly in the uninucleate marginal phase were pick and sterilized by washing with 75% alcohol for 1 min, then rinsing 3 times with sterile water, sterilizing with 0.1% $HgCl_2$ for 5 min, and rinsing 5-8 times with sterile water, and finally dried with sterile dry filter paper. Anthers from the sterilized flower buds were pick and cultured in MS medium with 30 g/L sucrose and 6 g/L agar (pH 5.6-5.8) at 30 °C and in dark for 20 days and then transferred to 25 °C, light 12h (2000lx) for 10 days (Dong *et al*, 2017).

Callus and embryoid induction, multiplication and seedling differentiation: By reference to the report of Gao *et al* (2005), 6 callus induction media were prepared: ① MS + 2, 4-D 1 mg/L + KT 1 mg/L, ② MS + 2, 4-D 1 mg/L + KT 1.5 mg/L, ③MS + 2, 4-D 1mg/L + KT 2 mg/L, ④ MS + 2, 4-D 2 mg/L + KT 1 mg/L, ⑤ MS + 2, 4-D 2 mg/L + KT 1.5 mg/L, and ⑥ MS + 2, 4 - D 2 mg/L + KT 2 mg/L. To each medium 2 mg/L $AgNO_3$ was added. After 5 days of anther culture, the callus induction was observed and the rate of callus formation was estimated, in order to find the optimal medium for callus induction.

After culture for one month, calluses were transferred to multiplication medium ⑦ (MS + 2, 4-D 2 mg/L + KT 1.5 mg/L + GA 1 mg/L). The multiplied calluses was inoculated into the differentiation media ⑧ (MS + IAA 0.5 mg/L + KT 1 mg/L), ⑨ (MS + IAA 0.5 mg/L + KT 2 mg/L), and ⑩ (MS + IAA 0.5 mg/L + KT 2.5 mg/L). Record the seedling differentiation, in hope to find the optimal differentiation medium for seedling differentiation.

Results

Morphological observation of Coix pollen grains at different flowering stages

The male tassels, female panicles and pollen grains of *Coix lachrymal-jobi* L showed different morphological characteristics

in the early, peak and late flowering stages, as exemplified by the GDYY *cv* (Figure 1). In the early flowering stage, the male tassels were green, ca. 18~21 mm in length, male flowers were not yet in bloom, while the stigmas were extending out fresh and tender, clearly indicating the asynchronous development of the male and female flowers, i.e. the female matures ahead of the male flowers. The caryopses were dark green (Figure1A). After I_2-KI staining, it was observed that about 60% of the pollen grains were dyed light yellow or brown and about 40% were dyed blue; and the size of the spherical pollen grains varied. Microspores of uni- and bi-nucleate phase coexisted with one germination pore (Figure 1B, C). These clearly indicated the immature male flowers at this early flowering stage. The male tassels at the peak flowering stage were yellow green, ca. 21~25 mm in length, the stigmas mostly withered, while the male flowers were in full bloom, and the yellow tender anthers fully exposed, ready for pollinating; the caryopses were yellow green (Figure 1D). Almost all the pollen grains at this stage were uniformly spherical, in bigger size and dyed dark blue (Figure 1E, F), indicating the full maturation of the male flowers. At the late flowering stage, tassels were deep yellow, ca. 22~25 mm in length, the male spikes were almost die-out, anthers turned brown, the stigmas completely died-out and fell off, and the caryopses were yellow (Figure 1G). About 60% of the pollen grains were normal in size and shape, and dyed blue, and about 40% irregularly shrunken and dyed yellow (Figure 1 H, I).

Observation of the pollen microspore development stages

Microscopic observation of the microspores in anthers of *Coix lachrymal-jobi* L in the early flowering stage showed continuous development, with the late uni-, bi- and tri-nucleate phases coexist, as shown here in GDYY (Figure2 A-C). But it is not synchronous since different phases of microspores occurred in the same piece of anther (Figure2 D).

In order to identify anthers in the late uninucleate phase for later anther culture, the morphological characteristics of

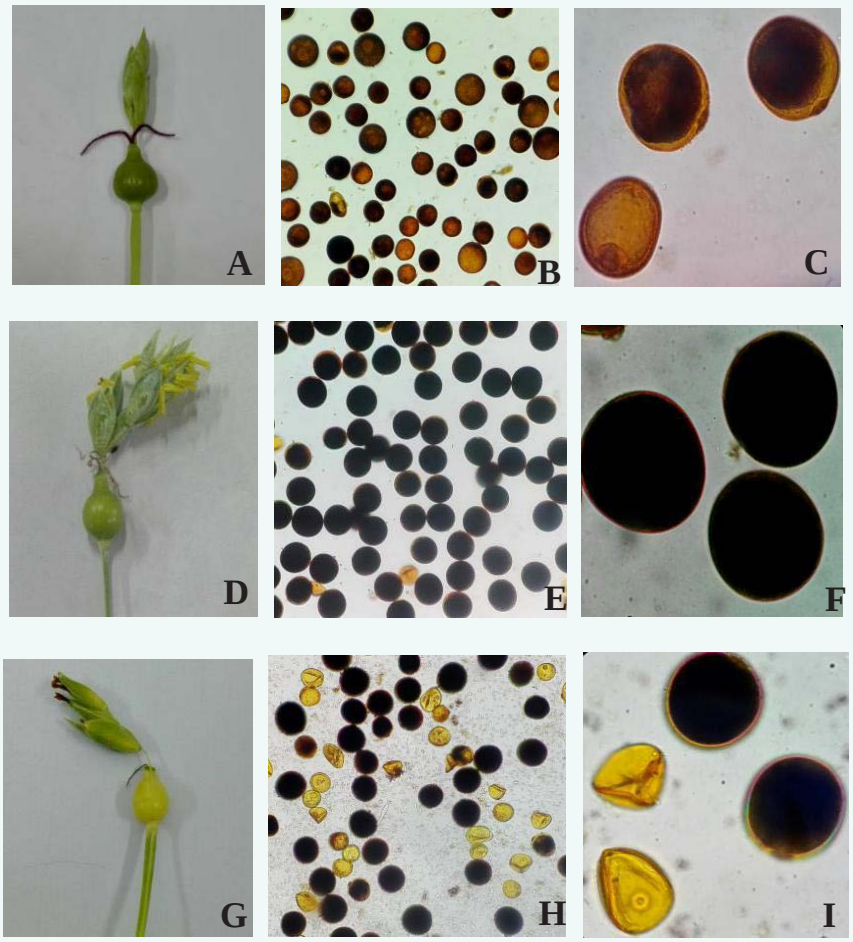

Figure 1 I_2-KI Stained Pollen Grains of Coix Exemplified by CV GDYY at Different Flowering Stages.
A, D and G are the morphology of *Coix lachrymal-jobi* L in the early, peak and late flowering stages respectively; B, E and H are pollen micrographs (20×) from early, peak and late flowering stages respectively; and C, F and I are pollen micrographs (100×) from the early, peak and late flowering stages respectively.

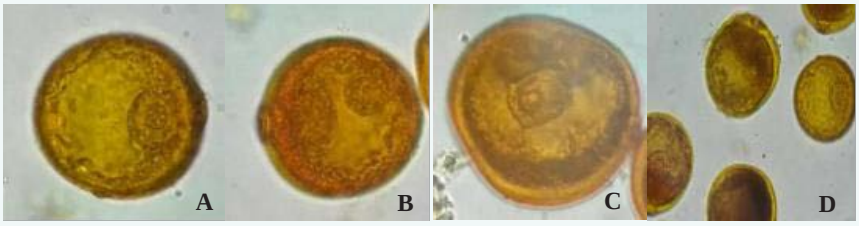

Figure 2 Observation of *Coix* Pollen Microspore Developmental Process (×100).
A: late uninucleate phase; B: binucleate phase; C: trinucleate phase; D: different microspores coexisting in the same anther

flowers when the uninucleate microspores accounted for more than 60% were observed to be in the early flowering stage, at which the male panicle was compact and 1.8-2.1cm in length, the longitudinal length of the flower bud was 0.8-1.0 cm, the anthers were light yellow and 0.4~0.5 cm in length; stigmas were fresh, 1.2-1.6 cm in length, the caryopses were dark green. At this stage the pollen was spherical and only had small vacuoles.

Comparison of pollen vigor of different Coix cv at different flowering stages

Microscopic observation and statistical data showed that pollen vigor varies greatly in different flowering stages and among different *cv* (Table 2). All the 7 *cv* had the greatest vigor in the peak flowering stage, followed by the late flowering stage, while the lowest had been in the early flowering stage due to the less starch accumulation in this immature stage. Pollen vigor of different *cv* was significantly different in the same flowering stage. At the peak flowering stage the pollen vigor of all the *cv* was well-above 85%.

Optimal conditions for pollen germination in vitro

Optimal pollen germination liquid medium: Pollen from different *Coix cv* at the peak flowering stage was cultured in boric acid (0.01%) solution containing 5%, 10%, 15% and 20% sucrose respectively for 5h and the germination rate was estimated. There were significant differences in germination rate and optimal germination medium for the 7 *Coix cv* (Table 3). The germination rates of GDYY, GXYY and HBYY were all above 50% in 15% sucrose +0.01% boric solution, the highest being 53.7% for GDYY, and thus the optimal culture solution for GDYY, GXYY

Table 2: Comparison of Pollen Vigor of Different *Coix CV* at Different Flowering Stages.

Coix cv code	Pollen vigor at different flowering stages (%)		
	Early flowering stage	Peak flowering stage	Late flowering stage
GDYY	29.9±2.7a	94.8±1.5a	79.2±2.5a
XRXBK	17.2±3.4de	85.3±1.6e	47.8±3.6fg
GXYY	13.9±0.8e	93.4±0.7ab	70.0±2.0b
FJYY	26.3±4.1ab	90.2±1.3c	53.4±0.8e
HBYY	29.9±3.6a	91.6±1.3b	55.8±1.8de
ZYYH	24.5±1.5abc	90.7±1.6c	63.8±0.9c
LNBK	15.8±1.7de	91.4±0.6bc	73.9±1.2b

Note: data are shown as Mean ± SD (n=3); data in the same column with different lowercase letters are significantly different (P < 0.05)

Table 3: Effect of Different Concentrations of Sucrose in 0.01% Boric Acid Solution on Pollen Germination Rate of Different *Coix CV.*

Coix cv code	Sucrose concentration (%)			
	5	10	15	20
GDYY	27.4±6.7aC	41.1±0.7abB	53.7±1.1aA	25.9±1.9aC
XRXBK	9.5±0.9efC	35.6±4.0dA	24.7±1.0eB	4.6±1.0eC
GXYY	18.4±1.1bcC	42.5±1.0aB	51.9±1.6aA	17.0±1.0bC
FJYY	15.3±1.9cdBC	41.5±1.4abA	32.1±2.0bB	12.1±1.5cC
HBYY	15.2±1.6cdC	40.7±0.3bcB	49.9±1.2aA	13.1±0.7cC
ZYYH	22.4±2.5abC	38.9±1.0bcA	27.6±0.8dB	13.7±1.5cD
LNBK	13.8±1.3cdeC	39.7±1.5bcA	30.0±1.5cB	7.3±1.2dC

Note: data are shown as Mean ± SD (n=3); data in the same column with different lowercase letters are significantly different (P < 0.05), and data in the same row with different uppercase letters are significantly different (P < 0.01)

and HBYY was 15% sucrose +0.01% boric acid. The germination rates of the other 4 *cv*, XRXBK, FJYY, ZYYH and LNBK were the highest in 10% sucrose +0.01% boric acid, being the optimal medium. The pollen germination rates of the 7 *cv* were the lowest in the 20% sucrose + 0.01% boric acid solution.

Optimal pollen germination temperature: Pollen from GDYY, GXYY and HBYY at the peak flowering stage was cultured in the optimal medium of 15% sucrose + 0.01% boric acid, and XRXBK, FJYY, ZYYH and LNBK in the optimal medium of 10% sucrose +0.01% boric acid at 20, 25 and 30℃ respectively for 5h and pollen germination rates were estimated (Table 4). It is evident that the optimal temperature for pollen germination is 25 ℃ for all the *Coix cv*.

Optimal pollen germination time: Continuous microscopic observation showed that the *Coix* pollen germination occurred slowly. Germination increased over the time of incubation with the 5h germination rate being the highest, around 50%. And after 5h, the pollen germination rate did not increase significantly. The extension of pollen tube also slow, and pollen tubes entangled after 15h when the pollen germination rate was not convenient to estimate. Therefore, 5~7h is the most appropriate time for estimation of pollen germination rate (as exemplified by GDYY in Figure3). Only one circular germination hole and single-tube germination were observed for *Coix* pollen. And the blue-dyed pollen grain germinated readily and their pollen tubes grew faster.

Callus, embryoid induction and differentiation process in Coix anther culture

Anthers in the uninucleate phase at the early flowering stage were freshly pick, surface-sterilized, and inoculated for aseptic culture on MS medium. It was observed that when cultured for 10 days tender-yellow to white lump materials began to outgrow from one end while the other end seemingly dehydrated and somewhat shriveled. The opalescent lump materials were calluses or embryoids (Huang *et al*, 2000), and if the anthers turned brown in culture, it often failed to induce. The calluses or embryoids expanded then and reached maximum after about 30-40 days of culture. Embryoids were different in shape from calluses. Calluses were often loose, amorphorous, crystalline and transparent, and could be further propagated and differentiated

Table 4: *Coix lachrymal-jobi* L Pollen Germination Rates at Different Temperatures.

***Coix cv* code**	**20℃**	**25℃**	**30℃**
GDYY*	18.7±4.9c	44.1±2.4a	33.8±2.0b
XRXBK	3.8±0.7c	24.0±1.2a	18.2±1.7b
GXYY*	14.2±2.7b	44.3±2.3a	43.4±1.8a
FJYY	8.9±0.6c	41.8±1.7a	30.1±1.3b
HBYY*	13.9±1.2c	40.4±3.5a	34.5±0.9b
ZYYH	7.6±0.5b	38.9±1.7a	36.9±2.3a
LNBK	5.9±0.8c	36.2±1.2a	17.9±1.6b

Note: *: determined in optimal medium of 15% sucrose + 0.01% boric acid and the others in 10% sucrose +0.01% boric acid; data are shown as Mean ± SD (n=3); data in the same row with different lowercase letters are significantly different (P < 0.05).

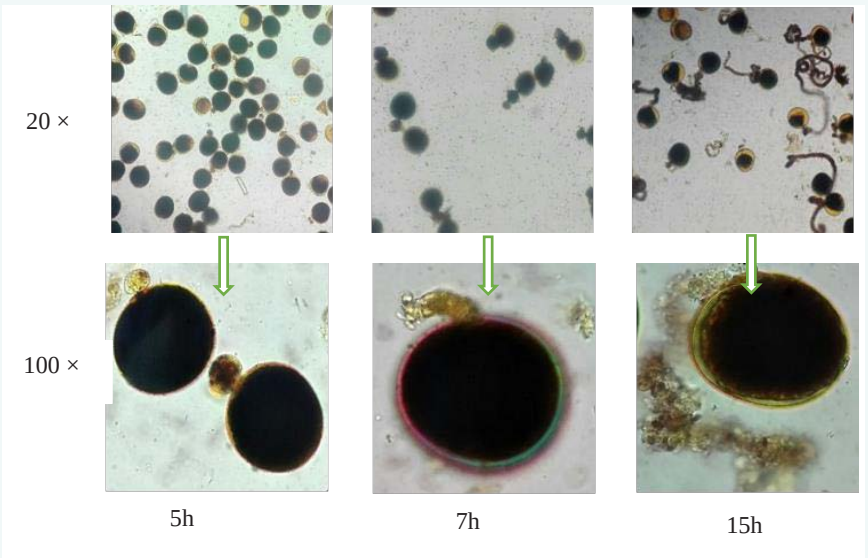

Figure 3 Germination Process of Coix Pollen over Time. Exemplified by GDYY.

into cluster buds on the differentiation medium; embryoids were compact, less transparent, typical shape of zygotic embryos and generally ceased to grow up to a certain size. Usually only one plantlet was differentiated from one embryoid, as exemplified by GDYY (Figure4).

Callus and embryoid induction rate in different media

Effects of different media on callus and embryoids formation from anther culture of different *Coix cv* were studied. The callus and embryoid induction rates of different *cv* were significantly different (Table 5), among which *Coix cv* GDYY, GXYY, HBYY and LNBK had higher induction rates, up to 20%. And the same *cv* had significant difference in induction rate among the 6 media. Relatively speaking, for each *cv*, media ② and ⑤ had the higher induction rates. So the optimal media for induction of callus and embryoid were MS + 2, 4 -D 1~2 mg/L + KT 1.5 mg/L. It was also observed that GDYY, GXYY and HBYY with higher pollen vigor as determined previously (See Table 2) have higher rate of callus and embryoid induction (Table 5).

It was observed that anthers of all the 7 tested *cv* could be effectively induced to form calluses and embryoids in the optimum media of MS + 2, 4 -D 1~2 mg/L + KT 1.5 mg/L. In the differentiation medium ⑨ (MS + IAA 0.5 mg /L + KT 2 mg/L), a few inducers could be differentiated into small clumped buds or seedlings, but the seedling formation rate was very low.

Discussion

In this study, the morphology of flowers and pollen grains at different flowering stages of *Coix cv* were observed for the first time. After I_2-KI staining, most of the pollen grains at the early flowering stage were stained light yellow or brown and were heterogeneous in size, and showed irregular shapes ranging from ellipsoidal or spherical with distinct uninucleate or binucleate phases and single germination pores. At the peak flowering stage, the pollen were mostly dyed blue, and the volumes of pollen grains became larger and regularly spherical. At the terminal flowering stage, about half of the pollen were dyed blue, showing regular spherical shape, and another half were brown, showing irregular empty and shrunken flat shape. The pollen vigor of all the 7 tested *Coix cv* was the highest at the peak flowering stage.

Du *et al* (2011) believed that addition of boric acid to the

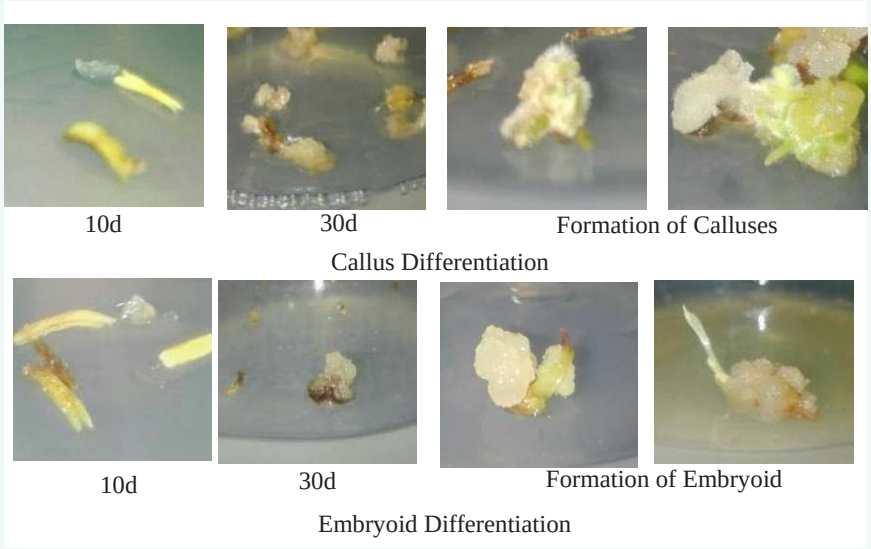

Figure 4 Induction and Differentiation of Calluses and Embryoids from Anther Culture of Coix CV GDYY.

Table 5: Comparison of Callus-inducing and Embryoid-differentiating Rates of *Coix CV* in Different Media.

Coix cv code	Medium					
	①	②	③	④	⑤	⑥
GDYY	5.0±0.6e	20.3±0.9a	7.1±1.2d	15.1±0.8b	18.6±1.8ab	12.5±0.9c
XRXBK	0c	6.4±0.8a	1.7±0.3b	5.4±1.0a	5.9±1.4a	0c
GXYY	8.0±0.7d	14.5±1.2b	8.6±0.6d	16.2±0.7ab	17.3±0.9a	11.1±1.6c
FJYY	0d	7.7±0.8a	2.0±0.4c	4.6±0.5b	6.1±0.9ab	1.9±1.7c
HBYY	1.6±1.7d	16.2±0.6a	6.9±0.7c	14.3±1.5a	16.4±0.8a	9.9±1.2b
ZYYH	3.6±1.2d	9.2±1.1b	0e	7.7±1.5bc	10.2±0.8a	5.9±1.3c
LNBK	0.4±0.6d	10.4±1.4a	1.8±1.0d	9.4±1.0b	12.5±1.7a	6.8±0.9c

Note: data are shown as Mean ± SD (n=3); data in the same row with different lowercase letters are significantly different ($P < 0.05$).

medium could improve the germination rate of pollen. In the present study of nutrient solutions with different concentrations of sucrose and boric acid, it was concluded that the optimal culture medium for pollen *in vitro* culture of different *Coix cv* was somewhat different; the optimum liquid culture medium for pollen culture of GDYY, GXYY and HBYY was 15% sucrose + 0.01% boric acid, and for the rest *cv*, 10% sucrose + 0.01% boric acid. And the optimal temperature and duration were 25 ℃ and 5 ~ 7 h respectively for all the tested *Coix cv*.

As observed, there were considerable disparities between the pollen vigor and the germination rate. The pollen vigor of the 7 *Coix cv* at peak flowering stage was about 85~95% as estimated by I_2-KI staining, while the germination rate determined by liquid medium culture was only about 50%. Similar results were reported by Wang *et al* (2000) and Li *et al* (2009) respectively on *Phyllostachys praecox* and *Phyllostachys nigra* respectively. The reason may be the resolution of I_2-KI staining, which can stain not only the mature pollen with more starch but also some immature and even aborting pollen, and thus make the vigor estimates somewhat higher than the pollen germinating rate, which was based on the growth of pollen tubes in liquid culture.

The pollen grains at the early flowering stage of *Coix* showed that the uni-, bi- and tri-nucleate microspores coexist in different ratios. Lu *et al* (2005) reported that microspores at the late uninucleate phase is more desirable for anther culture and better induction. The microspores at the early flowering stage of *Coix* were spherical with small vacuoles. The male panicle was 1.8~2.1 cm in length, the inflorescence was compact, and the longitudinal diameter of flower bud was 0.8~1.0 cm. The anthers were light yellow, 0.4~0.5 cm in length, and the stigma was fresh and tender, 1.2~1.6 cm in length, and the caryopsis was dark green. The anthers of *Coix* with the above features at this early flowering stage are suitable for use in anther culture.

The coexistence of calluses and embryoids in the same medium were observed during *Coix* anther culture. Yang *et al* (1979) on cabbage also observed that different anther culture inducers (calluses, embryoids, *etc*) coexist; and generally the occurrence is attributed to different hormone levels. It was suggested that higher levels of auxin in medium induce more calluses. By combining the cytological observation of the origin of callus from anther with callus forming process, Chen and Zhang (2011) believed that the yellow-brown dense calluses at the fracture of filaments at about 25 days were diploid calluses induced from anther wall and filaments, and embryoids were readily induced when auxin was low or KT was high. However our results showed that calluses and embryoids from *Coix* anther culture were simultaneously induced in the same culture medium. It seemed there was no clear relationship between the formation of the various inducers and the hormone status in the medium. Further researches are needed to clarify.

Although the callus induction rates from anther culture were in significant difference among different *Coix cv*, calluses could be successfully induced from all the 7 *cv* in the medium of MS+2, 4-D 1~2 mg/L+KT 1.5 mg/L, and some of which could be induced to short cluster buds in the medium of MS+IAA 0.5 mg/L+KT 2 mg/L.

No plantlets were induced from calluses and embryoids from *Coix* anther culture. Part of the reason might be the recalcitrance of the chimeric calluses induced from *Coix* anther culture, as reported by Deng *et al* (2014) on *Jasminum sambac* and/or the difference in genomic DNA methylation level of the regenerated calluses or embryoids as reported by Yao *et al* (2009) on pakchoi. Or proper inducing and differentiating culture conditions are yet to be identified.

This study for the first time observed the microscopic pollen morphology, compared the pollen vigor, identified the optimal *in vitro* pollen germination conditions, and established the anther culture and callus proliferation system of *Coix cv* and laid an excellent basis for induction and breeding of pollen haploid seedlings of this understudied and underutilized food and medicine homologous crop.

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Conflict of interest

The authors declare no conflict of interest. We have no financial and personal relationships with other people or organizations that can inappropriately influence our work. There is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the manuscript entitled.

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