薏苡臺中一號與臺中三號之DNA條碼

DNA barcode of *Coix lacryma-jobi* varieties Taichung No. 1 and Taichung No. 3

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Abstract

The Job's tears varieties Taichung No. 1 and Taichung No. 3 are main varieties cultured in Taiwan. Extracts of Taichung No. 1 has been employed in medical research, and New Drug Application is ongoing. It is necessary to establish their genetic barcodes for variety identification. In this study, the variety or accessions of Job's tears Taichung No. 1, No. 3, UK accession ABY BS3985 and a bead variety were included in analysis. Genes that recommended as candidates for genetic barcode are cloned, sequenced and aligned to published chloroplast genome of *Coix lacryma-jobi* in NCBI nucleotide database. A total of 20 chloroplast DNA regions were sequenced, which covered 1/10 of chloroplast genome. The results indicate that the genes sequenced were identical between variety Taichung No. 1 and No. 3. Six genes, *trn*H-*psb*A, *rps*16-*trn*Q, atpH-*atp*I, *rps*18-*rps*12, *psb*B Exon, *ccs*A-*ndh*D showed polymorphism when Taichung No.1 is aligned to reference genome, in which 3 indels were detected. The internal transcribed spacer of ribosomal DNA was also cloned and analyzed. There were 4 base transitions compared Taiching No.1 with the reference gene. The rDNA ITS sequence of the 4 accessions investigated in this study have been uploaded to NCBI nucleotide database for variety identification.

Introduction

Genetic resources of *Coix lacryma-jobi* collected by TDARES in Taiwan

Job's tears (*Coix lacryma-jobi* L.) is an andropogonoid grass native to tropical Asia that is widely cultivated and consumed for more than 2000 years in China. It grows adventitiously and sometimes considered invasive (Mito and Uesugi 2004; Mosango et al.2001; Shluker 2003; Townsend and Newell 2006). This crop is regarded nutritious and used as herbal remedy in China (Ruan et al., 2006) and Japan. Extracts from *C. lacryma-jobi,* thought to enhance the effectiveness of chemotherapy in the treatment of cancer, was the first traditional Chinese herbal remedies to be approved for clinical trials in the U.S. (Normile and Yimin, 2003). Recently, the bran extracts of Job's tears seeds is proved to inhibit lung and colon cancer cells growth (Lee, et al., 2008) as well.

Southern China neighboring south-east Asia area is the primary germplasm center of *Coix lacryma-jobi*. There are 2 taxons in the Genus *Coix* in China, viz. *C. aquatic* and C. *lacryma-jobi* L. The species *C. lacryma-jobi* complexes consist of 4 major varieties: (a) var. lacryma-jobi; (b) var. Stenocarpa; (c) var. ma-yuen and (d) var. puellarum (Figure 1) (Jiang *et al*., 2008).

The variety lacryma-jobi (Figure 1a), sometimes named var. major or 'bead' variety, is mainly cultivated in South-East Asia. The features of this land variety are white to grey-brown, smooth shining seed coat, and large seed size. Variety Ma-Yuen (Figure 1c) is the major cultivar widely planted in Taiwan, Japan and China. The variety has brown to dark-brown seed coat, smaller seed size compared with var. lacryma-jobi. The 2 varieties are principle cultivars in Asia. The var. Stenocarpa (Fig. 1b) and the var. Puellarum (Fig. 1d) have slender and flattened seed shape, respectively. The latter 2 varieties are not commercially grown.

Figure 1. Morphology of 4 varieties of the species *Coix lacryma-jobi*. (a) var. lacryma-jobi; (b) var. Stenocarpa; (c) var. ma-yuen and (d) var. Puellarum, bar= 2mm. (Jiang *et al*., 2008)

Germplasm collection, introduction and breeding of Job's tear have been conducted in many Asian countries especially China, Taiwan and Japan. In Taiwan, the Taichung District Agricultural Research and Extension Station (TDARES), affiliated to Council of Agriculture, is in charge of germplasm introduction and breeding programs of *Coix lacryma-jobi* since 1980s. So far there are more than 60 accessions collected by TDARES (Table 1). The germplasm collections in TDARES are exchanged from seed bank of different countries include Japan, UK, German and Brazil. Among the collections, 75% are Japan land varieties (Table 1), which were generous provided by

Agriculture Stations of Japan. The information of each accessions was kept if it is available. A brief map of accessions was sort out as shown in Figure 2.

Table 1. The germplasm collections of *Coix lacryma-jobi* in TDARES

Country	Number of accessions	% of all germplasm collected
Brazil	8	12.5
China	2	3.1
German	1	1.6
India	1	1.6
Indonesia	1	1.6
Japan	48	75.0
Taiwan	2	3.1
UK	1	1.6
Total	64	100.0

Kuroishi x3 黑石, 青森
Akita x2 秋田
Obanazawa x2 尾花澤, 山形
Miyagi x2 宮城
OOU x3 奧羽
Nakazatozairai x3 中里, 新潟
Mukouda x1 向田, 京都
Kyoto x1 京都
Okayama x7 岡山
Ehime x4 愛媛
Tokushima x2 德島,
Tokuda 德田
Yabakei 耶馬溪, 大分
Other Hatomugi x 6 其他薏米

Figure 2. The geographical relation of germplasm collected from Taiwan, China and Japan. The Taichung No.1 is bred by mass selection method from Obanazawa Native population (red circle), Taichung No. 3 is bred by hybridization of OOU3 (blue circle).

There is long history of using Job's tears for medicinal use in Chinese and Japan, their value and mechanisms are gradually disclosed by modern scientific methods. According to literatures, the variety Ma-Yuan is of higher medicinal values than the variety lacryma-jobi (or var. major). Although Job's tears are imported to Taiwan in considerable amount, the researcher and Chinese physicians uses local produced Ma-Yuan variety.

The project, starting from 2012 to 2015, is sponsored to establish the DNA barcode of buckwheat and Job's tears bred by TDARES, and to establish the chemical fingerprint of the 2 crops. The DNA barcode of *Coix lacryma-jobi* varieties cultivated in Taiwan is provided here for investigational new drug (IND) application. The breeding background of Job's tears varieties and present status of cultivation in Taiwan is described in the following sections.

Breeding of *Coix lacryma-jobi* varieties and their cultivation in Taiwan

After the introduction of these germplasm, agronomists of TDARES conducted a series of field observation trials from 1983. The agronomic traits examined include days to heading, days to harvest, plant height, height of lowest spike, tiller number, leaf number, total mid-spike number, non-fertilized seeds, fertilized seeds, grain yield per single plant, 100-grain weight, seed color, seed length, seed width, etc (Tseng and Kao, 1995; Su et al., 2002; Tseng and Chen, 2007, 2009; Su et al., 2009). During a series of field trials since 1983, the land variety 'Obanazawa Native' showed excellent agronomic traits. Therefore 5 elite lines from 'Obanazawa Native' population were selected through 'mass selection' breeding method and put into comparison trials and regional trials subsequently. The Taichung Selection Line No. 5 was later nominated as variety Taichung No. 1 (Tseng and Kao, 1995). As there were 5 elite lines put into regional trials, one of the elite line 'Taichung Selection No. 4' was kept by farmers in

Da-Ya District of Taichung City. The 2 varieties, Taichung Selection No. 4 and Taichung No. 1, are virtually sister lines selected from the same population.

During the breeding of Taichung No. 3 starting from 1999, we used Taichung No. 1 as maternal variety, OOU 3 as paternal variety (Chen et al., 2009; Tseng and Chen, 2009). The elite line TC92-9 derived from this hybridization were put into comparison and regional trials from 2003, and it was nominated as Taichung No. 3 in 2008. It needs to be noted that OOU 3 and Obanazawa are varieties selected from neighboring area. The area of OOU 3 is presented in blue circle and Obanazawa presented in red circle in Figure 1. A linage map for Taichung Selection No. 4, Taichung No. 1 and Taichung No. 3 is presented here (Figure 3).

Figure 3. Stages of the breeding of Taichung No. 1 and Taichung No. 3

Now the major lines or variety cultivated in Taiwan include 1 line, the Taichung Selection No. 4, and 2 varieties, the Taichung No. 1 and No. 3. Although In a broad sense, they resemble in genetic background. The appearance of Job's tears Taichung No. 1, No. 2 and imported variety are shown in Figure 4 to allow comparison. Taichung

Selection No. 4 cannot be distinguished from Taichung No. 1 as they are selected from the same land variety ‘Obanazawa’.

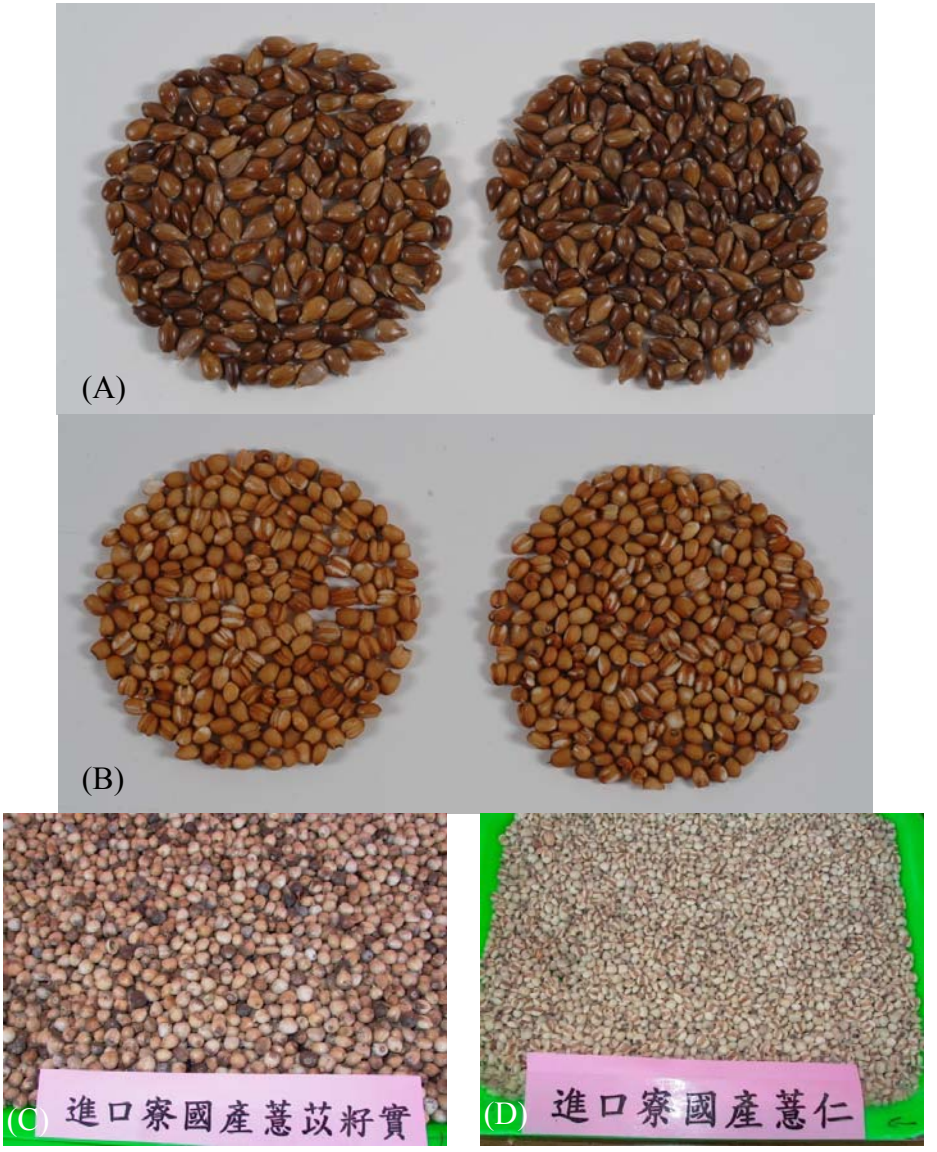

Figure 4. Appearance of Job’s tears Taichung No. 2 and Taichung No. 1. Whole seeds (A) and dehull seeds (B) are shown. In figure (A) and (B), Taichung No. 2 is arranged on the left and Taichung No. 1 on the right. Seeds that imported from Laos is variety ‘lacryma-jobi’, which can be judged by color of seed coat (C). Dehulled seeds imported from Laos also shows different color (D).

DNA barcode of *Coix lacryma-jobi* varieties

The concept of DNA barcoding is simply to find one or few DNA regions that will distinguish among the majority of the world's species (Hollingworth, 2011). In 2009, the "Consortium for the Barcode of Life (CBOL) Plant Working Group" proposed portions of 2 coding regions from the plastid genome –*rbc*L and *mat*K- as a "core barcode" for plants. In addition to the two core barcoding markers, another plastid region, trnH-psbA, and the internal transcribed spacer (ITS) of nuclear ribosomal DNA were also recommended. Each of the gene region had different strength and weakness, for example the *rbc*L region is good for recovery and sequence quality but low species discrimination ability, the species discriminatory power is good for *trn*H-*psb*A and nrDNA ITS. However, for the study of population genetics, more polymorphic markers are needed. In plastid genes, Small Inversions, microsatellites, insertion/deletion (Indel) have been reported in species complexes and at the population level (Scarcelli et al., 2011). However, there has been no report on the diversity of plastids genome of *Coix lacryma-jobi* yet.

In order to study the diversity of plastid DNA among Job's tears population, we decided to follow the list of primers publisheded by Scarcelli *et al*. (2011, PLos One. 6(5):e19954) in their supplemental materials. Considering there are needs to expand the list of primers in the future, we use the same numbering primer pairs to prevent confusing the coding of primers in the future. According the list, we designate numbers to each primer pair sequentially from 1 to 100. Select the sequential number ending with 1 and 6, which are listed below (Table 2), to amplify different lines of *Coix Lacryma-jobi*.

Table 2. List of primers employed in DNA barcoding of different selected lines or varieties of *Coix lacryma-jobi*. (Scarcelli et al., 2011)

Primer coding	Name of gene	Tm	Forward sequence	Reverse sequence
cpDNA 1F-1R	*trn*H-*psb*A	64	CCACTGCCTTRATCCACTTG	TRGCTGCTTGGCCTGTAGT
cpDNA 6F-6R	*rps*16-*trn*Q	62	GTCGCACGTTGCTTTCTACC	GAGGTTCGAATCCTTYCGTC
cpDNA 11F-11R	*trn*G Intron	62	GCGGGTATAGTTTAGTGGTAA	GCTTGGAAGGCTAGGGGTTA
cpDNA 14F-14R	*atp*F-*atp*H	62	AACTCGCACACACTCCCTTT	GGRGTTGGTCAAGGTACTGC
cpDNA 16F-16R	atpH-*atp*I	62	CCAGCAGCAATAACRGAAGC	TTCAAGCTCTTATTTTTGCAACKT
cpDNA 21F-21R	*rpo*C2-*rpo*C1	60	CCGAARTGATCTATTAATCTGCT	GATGGRGATCAAATGGCTGT
cpDNA 26F-26R	*pet*N-*trn*D	58	CTTGGGCTGCTTTAATGGT	CTGTCAAGGCGGAAGCTG
cpDNA 31F-31R	*psb*C Exon	58	GTGGAAACGCTCTTTAATGG	GCCACAAATGDCCCACAA
cpDNA 36F-36R	*ycf*3 Intron1	58	TGACAGATCACGGCCATATT	TTAYAGAGATGGTGCGATTT
cpDNA 41F-41R	*trn*V Intron	62	GAACCGTAGACCTTCTCGGTAA	GTTTACACGYGCGCCAAT
cpDNA 46F-46R	*rbc*L-*acc*D	56	GCTTCWGGKGGTATTCATGT	YATTGTCAATMTCAAAAATCTG
cpDNA 51F-51R	*ycf*4-*pet*A	56	ATGAAYTGGCGATCAGAACA	TGYGCAAAAATGGGATATG
cpDNA 56F-56R	*rps*18-*rps*12	56	ACYTTGAAACAACAACGATTA	TCGAGGAACATGTRCTAGGG
cpDNA 61F-61R	*psb*B Exon	60	GGGTTTRCCTTGGTATCGTG	TCTGGATCAATACCRGCAAA
cpDNA 66F-66R	*pet*D-*rpo*A	56	AAATTCCAAAATCCMTTTCGTC	AATGGAAGTTTAACYCCTAA
cpDNA 71F-71R	*rps*3 Exon	60	CACGYGCAATYTCTTTTC	TCCACTYGGTTTCAGACTTGG
cpDNA 76F7-6R	*ndh*B Exon2	58	TCCTGAGCAATTGCAAGAAT	AAAGTCTCATGCACGGTTTTG
cpDNA 81F-81R	*trn*V-*rrn*16	64	ACCTTGACGTGGTGGAAGTC	TGAGCCAGGATCGAACTCTC
cpDNA 86F-86R	*trn*A Intron	62	TTGGTAGAGCTCCGCTCTTG	GACTCGAACCGCTGACATC
cpDNA 91F-91R	*ndh*A Intron	58	TCYGCTTCTGGTAAATCAAA	AATATCTCTACGTGYGATTCG
cpDNA 96F-96R	*ccs*A-*ndh*D	60	GCAGTRTGGGCTAATGAGG	GGAATGAGYGGTTTTGTTGC
ITS 18SF-26SR	Ribosomal ITS	52	GTCCCTGCCCTTTGTACA	CGCCGTTACTAGGGGAATCCT

Materials and methods

Plant materials

The 2 major varieties cultivated in Taiwan, Taichung Selection No. 4 and Taichung No. 3, are the main subjects to be analysis. A Taiwan ‘Bead’ land variety and a UK accession ABY BS3985 were employed for comparison.

DNA Preparation

Total DNA was isolated from fresh leaf tissue of C. lacryma-jobi using the Tanbead DNeasy Kit (Tanbead Inc., Taoyuan, Taiwan). The amount of DNA used for

PCR reactions was optimized by testing different dilutions with ITS primer pair, which amplified the nuclear DNA ribosomal ITS region (Chen et al., 2001).

PCR Conditions and Sequencing Strategy

Touchdown PCR was performed following "Round I" conditions of Dhingra and Folta (*2005*) with elongation times extended to 40-150 s to insure complete amplification. All amplicons were generated using Pfu Turbo DNA polymerase (Stratagene Inc., Carlsbad, CA), which reduced PCR enzyme generated errors. Automated capillary sequencing was performed by TRI Inc. (Taipei, Taiwan) on each amplicon in both directions giving 2 × coverage within the amplicon. Only sequences with Macrogen QV scores (equivalent to Phred scores) of at least 20 were retained. Sequences were manually inspected in Finch TV vers. 1.4.0 (Geospiza Inc.).

Results and Discussions

During the investigation, we found the DNA regions amplified are identical in Taichung Selection No. 4 and Taichung No. 3. The closed genetic background of the 2 accessions has been revealed in introduction of this report (Fig. 3).

Therefore, gene regions amplified in Taichung No. 3 was used to compare with that of NCBI Reference Sequence NC_013273.1 (Leseberg and Duvall, 2009). Of the 20 universal primer pairs proposed by Scarcelli et al. (2011), 18 of them amplified plastid genes successfully. The amplified regions cover 10.3 % of total chloroplast genome and are thus very representative and reliable (Table 3).

It has been suggested that in plastid genome, gene regions include Intergenic Spacer (IGS), Intron and Exon may more polymorphism than gene regions. Among the 18 gene regions successfully amplified, only 6 regions were polymorphic compared with reference sequence (Table 4), the remaining gene regions are identical. The

polymorphic loci include trnH-psbA (IGS), rps16-trnQ (IGS), atpH-atpI (IGS), rps18-rps12 (IGS+Gene), psbB Exon (exon), ccsA-ndhD (IGS). Among the 6 polymorphic loci, 5 are IGS. The results indicated that for Job's tears population genetic studies, plastid IGS are appropriate candidates.

Table 3. Plastid gene regions as DNA barcode for *Coix lacryma-jobi* variety *Taichung No.3* and *Taichung Selection No. 4* . Plastid genome NCBI Accession FJ 261955.1. was employed as reference sequence for alignment.

Name	Location	Type	Identities (%)	GAP	Sequence region corresponding to reference accession
*trn*H-*psb*A	LSC	IGS	742/743 (99%)	0/743	140315-140745//-312
*rps*16-*trn*Q	LSC	IGS	1501/1502 (99%)	0/1502	5591-7092
*psb*C Exon	LSC	Exon	1194/1194(100%)	0/1194	10551-11744
*trn*G Intron	LSC	Intron	667/667 (100%)	0/667	14428-13762
*rpo*C2-*rpo*C1	LSC	IGS	799/799 (100%)	0/799	27396-26598
atpH-*atp*I	LSC	IGS	831/832 (99%)	1/832	34725-33894
*ycf*3 Intron1	LSC	Intron + Exon	880/880 (100%)	0/830	45418-46300
*trn*V Intron	LSC	Intron	582/582 (100%)	0/582	53435-54016
*ycf*4-*pet*A	LSC	IGS	918/918(100%)	0/918	61041-61958
*rps*18-*rps*12	LSC	IGS + Gene	1463/1465(99%)	2/1465	68189-69652
*psb*B Exon	LSC	Exon	956/958(99%)	2/958	71546-72503
*pet*D-*rpo*A	LSC	IGS	505/505 (100%)	0/505	81697-82201
*atp*F-*atp*H	LSC	IGS	812/812 (100%)	0/812	107719-108530
*ndh*B Exon2	IR	Exon	773/773 (100%)	0/773	89701-90473 133837-133065
*trn*V-*rrn*16	IR	IGS	290/290 (100%)	0/290	95297-95586 128241-127952
*trn*A Intron	IR	Intron	824/824 (100%)	0/824	98488-99311 125050-124227
*ccs*A-*ndh*D	SSC	IGS	746/747 (99%)	0/747	110109-110855
*ndh*A Intron	SSC	Intron + Exon	1071/1071(100%)	0/1071	115169-116239
% of amplification		10.3% of plastid genome (15062 bp sequenced)			

Table 4. Gene regions that shows polymorphism between the variety *Coix lacryma-jobi* L. variety Taichung No. 3 and the reference sequences (Leseberg and Duvall, 2009) registered in NCBI Genebank.

FEATURES

trnH-psbA

GGATTTTCTCTTTTTTCCATTCATTATTATTCTATTTATTCTGACCTCCATACCTCGATCGAGA
TAGTGGACATAGGATGCCACTCTTTAAAATGAAAAAAGGAGTAATCAGCTGTGACACGAAA
AAAAACGAATCCTTTTGTAGCTCGTCATTTATTGGCAAAAATCGAAAAGGTCAATATGAAGG
AGGAGAAAGAAATAATAGTAACATGGTCCCGGGCATCTAGCATTCTACCCGCAATGGTTGG
CCATACAATCGCGATTCATAATGGAAAAGAACATATACCTATTTACATAACAAATCCTATGG
TAGGTCGCAAATTGGGGGAATTCGTGCCTACTCGGCATTTCACGAGTTATGAAAGTACAAGA
AAGGATACTAAATCTCGTCGTTAATTGAATTCAGAATAGAAAGATTCAGAATAAACAAAGA
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CCTATATGTTTAGGAGAAAAACCTTATCCATTAAGAGATGGAACTTCAAGAGCAGCTAGGTC
TAGAGGGAAGTTGTGAGCATTACGTTCGTGCATTACCTCCATACCAAGATTAGCACGGTTGA
TGATATCAGCCCAAGTATTAATAACGCGACCTTGGCTATCAACTACAGATTGGTTGAAATTG
AAACCATTTAGGTTGAATGCCATAGTACTAATACCTAAAGCAGTGAACCAGATCCCTACTA

rps16-trnQ

CCTCTAATTTCATTGCAAAATGGTATCGAGAATTGATCCAATATGGATGGAATCATGAATAG
TCATTGCTTTTGTATACTAATTCAAACCTGCTATCTATGGAGAAAATTGGATAAAAGAAATA
AGTATTTATCGGGGAACGCTCTGCAAAGATACAATTTATTTAAACCCATATTCTATCATATG
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CCTTAACCTTACCGAAATGGATCATTCTGAATATTTAAAGAATCACAATATTTAAAGAATCA
CAGATCGAGATCGTTTTCGCTTAACCAAAGAAAAGAAGGACGCTTCTTTTTTACTAATAATA
CAAGTTTACTAATAATACAAGAAAACAAATAATACAACAAAAATCTATCTCTATCATAAAG
GCATAGATCTCATTTTCTATACAGTGTTTTACCTCATTAAATTTTTTTTTTTTTCAATCAATTC
GAAAGTAGAGTAGACAGAATATATGAATTTATCTCTAATCCTCACATTCCATTGATTTAGAA
ATGGATATTTGCAAATCAGATAATACCTAAAATAGAAAAATAGAGTCCCTATCCGTAGAATG
GAAACCTTTTTCTATTTTTTTCGCGCCGATCTTGGTCAAAAGTTTTAAGGCCTGTGCTGAAAC
TAAAAACATCCTACTCTTTAATCTGGCCATGAAACATAGAATTAGAATACTCCCCCTCTTTTT
TTTACTTACTTTAGTTCTTTAGTTTTGGGAAAATAAATAGGGGGGTACTTCTTTTCTTTCATAT

TGGGTGTCAAATGGATCCTGTAAGAATTCCCACTTCATAGATACGGGGTATAAAGTTTATCC
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ACGAGTTCATAAGAGATAATTATTCTCTTTAATAAACTTTTTTTTGTGTCGTGCAGGGCACAA
TACGATT

atpH-atpI

TCGTGTCAAAAAAAAGAAATGgTtAaGGATACAATCAACCAAGAAATTATTACTTCCAACTTC
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AAAGCCACACAAAAGATGACTGTCTTATAGGCATTAAGGATATAGATCTAACCTGACTCCGC
CCCCCCTGAATGCATATATACTTTACCTCTTCCATAATATAGTCTATTCTCTCTCCTACAACTC
TAGGTTGTATATTCATACGCCTTTCGAACTATTTAGTTTTCCAACTAAGAGGATTATCCGGAA
TCATGCATGAATTGGGCTAAGCTAAAAAAAAAAGACTATTTCAAAAACTAGTTAATTCAATGA
TGACCTTCCATGGAT

rps18-rps12

AACAGGGCTCGTATTTTATCTTTCTTACCATTTCGTAACTATGAGAATGAGAAGCAATTTCAA
GCCCAGGCAATTTCAATAATTACTGGTCCTAGACACAGAAAAAATAGACATATTCCTCAATT
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AATCGAAACTTAAGTTCCGATTGTTGATGTTTTATTCGAAAGGCTCAGACTCATATATAAAG
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GTTCGTCGTCTCCGAGCTATATATCCTCGCGGAACTCTGGTCATTGAATCAAATTAACCTTAA
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TCCGATATATAAAATATTAATTCCAATGGCTTTTGCTACTATAACCTTCCCAACCACGATTTT
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ATTATAGAGTAAATAACTCTTTTCACAATAAGAGTTATTCATACAGTGACGGTATTTAATTAT
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TCTTCCAATCTAGATGATTGGATTTGCACCAAAGGAAACCATAAATTCCATATACCATAGAA
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ATTATTTGTTGAACTCATGATCGA

psbB Exon

TTTGGCTTTGGTGCATTTCATGTAACGGGTTTATATGGCCTGGGATATGGGTGTCCGATCCTT
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tTCGTTCCGGGAGGAATAGCTTCGCATCATATTGCTGCAGGTACTTTGGGCATATTAGCGGGC
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ATGTGGTATGGATCAGCAACTACCCCAATTGAATTATTTGGGCCTACTCGTTATCAGTGGGA
TCAGGGATACTTTCAGCAAGAAATATATCGAAGAGTTAGCGATGGGTTAGCCGAAAATCTTA
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AGTTCTATGGTGGCGAACTTAATGGAGTAAGTTATTCTGATCCTGCTACTGTAAAAAAATAT
GCGCGGCGTGCTCAATTAGGGGAAATTTTTGAATTAGATCGAGCTACTTTGAAATCAGATGG
TGTTTTTCGCAGCAGTCCAAGGGGTTGGTTCACTTTTGGTCATGCTACCTTTGCTTTGCTCTTC
TtTtCGGACACATTTGGCATG

ccsA-ndhD

GGGGGTAATGAGGCATGGGGATCCTATTGGAATTGGGATCCTAAGGAAACTTGGGCATTTAT
TACCTGGACCATATTTGCAATATATTTACATAGTAGAACAAATCCAAATTGGAAGGGTACGA
ATTCCGCACTTGTAGCTTCGATAGGATTTCTTATAATTTGGATCTGCTATTTTGGTATCAATCT
GTTAGGAATAGGTTTACATAGTTACGGTTCATTTACATTACCATCTAAATAATTACATAACAT
AAAATAAAACCCGAAGGTTTTTTCCTTTTTTGTTTGATTTGAGAACCCTTTGAAAAGACGTTC
AAGGGGTTCTCAAAAATGCGAGATAGATCTAATTAGACTTTTTTACTTTTTTCTTAATTTTTT
ATTTTTCCACTCTGGAATATAGAGCGGACTGGTTAAAAAAAGAAAATCCTATTTAGTATAAT
AATTGGATAATAGAACCTCTACCCTATCAACGGATAGAGAGAGAACAAAATCTGGATAAAT
ACCAATTCCTATTACTGGTAAAAAGATACAGATTAAAAGAAAGAGTTCCCGTGGTCCAGAAT
CTACAAAATTTTTGTTTGGAACATTAAATAGCTTGTAGCCATAGAACATCTGGCGTAACATA
GATAATAAATAAATAGGAGTTAATATCATTCCTATTGCCATTACAAAAGTAATTAGCATTTT
TGGCATTAACATAAATTTAGGACTAGTAATTAGTCCAAAAAAGACTACTAATTCTGCAACAA

As ribosomal internal transcribed spacer (ITS) has been shown to reveal higher discrimination power compare to plastid gene regions. The nrDNA ITS gene regions were cloned, sequenced (Table 5) and submitted to NCBI database (accession number KC181916, KC181917, KC181918, KC181919, KC181920 for Taichung No. 3, Taichung selection No. 4, ABY BS3985 and 2 Taiwan native accessions, respectively). Of the accessions we analyzed, there were four C-T transitions observed scattering ITS region. The ITS sequence is indeed more informative than other plastid gene regions in the varieties we tested.

Table 5. DNA barcode of Coix lacryma-jobi variety Taichung No. 3 and Taichung Selection No. 4. Sequence of the partial 18S rRNA, internal transcribed spacer, and partial 26SrRNA are identical between the 2 varieties.

Accession KC181916, KC181917 for Taichung No. 3 and Taichung selection No. 4, respectively

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18S rRNA partial sequence	<1..150>
Internal transcribed spacer	<151..721>
26S rRNA partial sequence	<722..804>

CGATTGAATGGTCCGGTGAAGTGTTCGGATCGCGGCGACGGAGGCGGTTCGCCGCCCCCG
ACGTCGCGAGAAGTCCATTGAACCTTATCATTTAGAGGAAGGAGAAGTCGTAACAAGGTT
TCCGTAGGTGAACCTGCGGAAGGATCATTGTCGTGACCCTTAAACAAAGCAGACCGCGAA
CCCGTCTCTCGTGCCATCGGGCTTCGGCCCGGCCGAAGGCCCCCGAGCTCCGTCCCGGGG
CGGAGGGGCCGCAACAGAACCCACGGCGCCTTAGGCGTCAAGGAACACTAATGCTGCCTT
GCTCGGCGGAGCGGTCGGCCTGCCTTCCGCTCCCCGCGCAGCGATGATATCTTAATACAC
ACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCAAAATGCGA
TACCTGGTGTGAATTGCAGAATCCCGCGAACCATCGAGTTTTTGAACGCAAGTTGCGCCC
GAGGCCTTCTGGCCAAGGGCACGTCTGCCTGGGCGTCACGCCAAAAGACACTCCCAACCC
ACCCCCGGGGAGGGACGTGGTGTCTGGCCCCCCGCGCCGCAAGGCGCGGTGGGCCGAAGT
TGGGGCTGCCGGCGAATCGTGTCGGGCACAGCACGTGGTGGGCGACACCTAGTTGTTCTC
GGTGCAGCGCCCCGGCACGCAGCCAGCACATCGGCCCTAAGGATCCATCGGGCACCGCAG
CGCACCGTCGCTCGGACCGCGACCCCAGGTCAGTCGGGACTACCCGCTGAGTTTAAGCAT
ATAAATAAGCGGAGGAGAAGAAAC

Conclusions

In this article, we reported the breeding process of Coix lacryma-jobi and the genetic background of Taichung No. 3 and Taichung selection No. 4 in Taiwan. For DNA barcoding of these Job's tears varieties, 18 plastid gene regions and nrDNA ITS are successfully amplified and sequenced. At species level, these gene regions can be employed to discriminate Job's tears and other species. However, for discrimination of Job's tears at variety level, we recommend the 6 polymorphic plastid loci and ITS that showed higher resolution. The variety 'lacryma-jobi' and 'ma-yuan' can be distinguished by their seeds characteristics, which are clear indicators. Presently, only the ITS sequences were deposited in NCBI genebank. The plastid genes polymorphism among different accessions of Job's tears collections in TDARES is still under investigation.

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摘　要

本場所育成之薏苡臺中一號及臺中三號爲國內栽培之主要品種，其中臺中一號萃取物已被運用於醫藥研究，並申請進入臨床試驗階段，有必要建立品種基因條碼，作爲基原鑑定之用。本研究以薏苡臺中一號、三號、野生種薏苡UK accession ABY BS3985及小珠薏苡爲材料，參考基因條碼相關文獻所建議之引子對，進行基因選殖與定序，並以發表之薏苡葉綠體基因序列爲基礎，進行選殖序列的比對分析。本研究共選殖20條葉綠體基因條並完成定序比對，分析序列涵蓋1/10之葉綠體基因組，在所分析之基因中，臺中一號和臺中三號的基因序列完全相同，*trn*H-*psb*A、*rps*16-*trn*Q、atpH-*atp*I、*rps*18-*rps*12、*psb*B Exon、*ccs*A-*ndh*D等六個基因和登錄於NCBI 核酸資料庫之薏苡葉綠體參考序列有多型性，其中三個爲插入或刪除，基因條碼變異主要出現在基因間隔區。另選殖核醣體內轉錄間隔區，定序結果發現與參考基因具有更高的多型性，共有四個核酸變異皆爲C-T轉變，結果已登錄於NCBI之核酸資料庫，可作爲基因條碼鑑定之用。