Genetic Diversity of Job's Tear (*Coix lacryma-jobi* L.) Germplasms Based on the Morphological Traits and SSR Markers

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ABSTRACT

Job's tears (*Coix lacryma-jobi* L.) is a nutritious food crop with potential health benefits and has been used for a long time for treating inflammatory diseases. The objective of this study was to evaluate the genetic diversity of 20 Job's tears accessions collected from the Northwest regions in Vietnam using morphological traits and molecular markers. Based on the morphological trait analyses, the 20 genotypes were grouped into two clusters at a Euclidean distance of 3.5. Cluster I included four accessions with hard, thick glume and fruit, and cluster II consisted of 16 accessions with dark gray seeds at full ripeness. Of the 17 SSR markers, seven were found to be polymorphic, generating 28 polymorphic bands over seven SSR loci. The average number of alleles per locus was 4.0, and the PIC value ranged from 0.46 to 0.75, with an average of 0.63. The Dice's similarity coefficient ranged from 0.84 to 1.0. However, the Mantel test revealed a low correlation between the morphological and molecular matrices, indicating differences in the relationships between morphological traits and molecular markers. Both the morphological and molecular approaches provided useful information for breeding programs and germplasm utilization in Vietnam. Overall, this study may contribute to a better understanding of the genetic diversity of Job's tears, which is essential for future crop improvement programs.

Keywords: Genetic diversity, Job's tear, morphology, SSR marker

Introduction

Job's tears (*Coix lachryma-jobi* L.)., commonly called as Adlay, coix millet or adley millet, which belongs to the Coix genus of the family Poaceae. This plant is an important crop for food and medicinal material in many countries Southeast Asian countries (Xi et al., 2016). It is considered to be a good substitute of rice, and grows better in infertile soil in rainfed upland ecosystems as compared to the improved varieties of rice and maize (Lirio et al. 2011). In Vietnam, Job's tear is native to and cultivated mainly in the northern mountainous provinces, including Lao Cai, Ha Giang, Lai Chau, Son La, Dien Bien, Hoa Binh, Cao Bang and Thai Nguyen.

The Job's tear seed contains about 67% starch and 20% protein, the highest protein content among cereal crops. Seed extracts from Job's tear have been reported to display various pharmacological activities, such as anti-inflammatory, anti-cancer, antioxidant, anti-allergic, antidiabetes, anti-

arthritis, and gastroprotective effects (Phạm et al., 2014, Seo et al., 2000). Therefore, Job's tears is considered a nutritious health cereal, with medicinal constituents that are missing in other cereal species. Despite having high economic potential, there has been little work done on its improvement. Most studies have focused mainly on pharmacological effects and chemical components, whereas knowledge on the genetic diversity of Job's tears is still limited. Numerous studies on genetic variation and relationships of Job's tears have been conducted on accessions from China. Shen et al., (2019) characterized 12 morphological traits of 94 Job's tears accessions collected from different geographic areas in China by principal component analysis (Shen et al., 2019). Xi et al. (2016) analyzed the genetic diversity of 11 Job's tears genotypes based on morphological traits and ISSR markers from the main distribution regions in China. By using the AFLP markers, Fu et al. (2019) analyzed the genetic diversity and population structure of 139 Job's tear accessions from four geographical areas in Southwest China. Shen et al. (2021) used 10 ISSR markers to evaluate the population structure and genetic diversity of 8 Job's tears populations in China.

To date, there have been no reports on the evaluation of genetic diversity in Job's tears germplasm accessions in Vietnam. In addition, the evaluation of genetic diversity in Job's tear accessions by using SSR markers is limited. Therefore, in this study, we aim to evaluate the genetic diversity of 20 Job's tears accessions collected from Northwest regions of Vietnam based on morphological characteristics and SSR markers. The results provide valuable information for the establishment of conservation strategies, as well as for the utilization and breeding of Job's tears in this country.

Materials and Methods

Plant materials

Twenty Job's tears accessions comprised of 17 accessions from Son La, 2 accessions from Dien Bien and 1 accession from Lai Chau were studied for phenotypic and molecular analyses (Table 1).

Table 1: The used material samples of Job's tears

Symbol	Location	Symbol	Location
M1	Co Ma 1 - Thuan Chau - Son La	M11	Chieng Khoong - Song Ma - Son La
M2	Ho Thau -Tam Đuong - Lai Chau	M12	Co Ma 2 - Thuan Chau - Son La
M3	Dua Mon - Song Ma - Son La	M13	Chieng Noi 3 - Mai Son - Son La
M4	Chieng Noi 1 - Mai Son - Son La	M14	Chieng Noi 2 - Mai Son - Son La
M5	Long He 1 - Thuan Chau - Son La	M15	Chieng Noi 4 - Mai Son - Son La
M6	Long He 2 - Thuan Chau - Son La	M16	Phieng Pan - Mai Son - Son La
M7	Toa Tinh - Tuan Giao - Đien Bien	M17	Na Ot - Mai Son - Son La
M8	Pa Long - Thuan Chau - Son La	M18	Phinh Giang - Đien Bien Đong - Đien Bien
M9	Phieng Cam - Mai Sơn - Son La	M19	Nam Pam - Song Ma - Son La
M10	Muong E - Thuan Chau - Son La	M20	Chieng Noi 5 - Mai Son - Son La

Morphological traits

The experiment was conducted at the Tay Bac University, during 2021-2022. Thirty seeds from each Job's tears genotype were sown in seedling cups in a randomized complete block design with

three replicates. Plants with 3-4 leaves were moved to the net house. Each replicate had 10 plants with 1 plant per hill. The row and plant spacing were 60 and 40 cm, respectively. For evaluation, all morphological traits were evaluated either by observations or length measurements of selected plants per accession. A total of 33 morphological traits, including quantitative and qualitative traits were assessed in plant, stem, leaf, flower, glume, style, stigma, fruit and seed according to the UPOV guidelines TG/309/1 Adlay 2015-03-25 (UPOV, 2015).

DNA extraction and polymerase chain reaction

Leaf tissue for each accession was harvested from one-month-old seedlings. Genomic DNA was extracted using hexa-decyltrimethyl-ammonium bromide (CTAB) DNA extraction procedure, as described by Doyle and Doyle (1987) with some modifications. The 20 SSR markers developed by Ma et al., 2006 were chosen for polymorphism screening of the 20 accessions (table 4). Each 25 µl reaction mixture contained .5µL 10X PCR buffer, 0.5 µl dNTPs (10 mM dNTPs)), 0.5 µL SSR forward and reverse primer (10 µM) each, 50-100 ng genomic DNA, and 0.5 U Taq DNA polymerase (New England Biolabs). DNA amplification was performed in Thermoblock programmed at 94℃ for 5 min for initial denaturation, followed by 35 cycles at 94℃ for 30 s, 50-58℃ for 45 s, and 72℃ for 30 s, and 72℃ for 7 min for a final extension. PCR products were separated by electrophoresis in at 120V for 1.0 h on 2.5% agarose gels stained with ethidium bromide (0.5 mg ml/1) using 1X TAE buffer. Finally, the ethidium bromide-stained gels were photographed under UV light (Geldoc Bio-Rad).

Table 2: The primer sequences used in the study

No	Name of primer	Primer sequence (5'–3')		Ta (°C)
		Forward	Reverse	
1	DQ205313	GCGCTCTGAAGACACCAC	CCGATGATCACCCTCCTT	58
2	DQ205314	TGGATCCGGAGGAGAACT	TCATATCGATGCTTGGGG	58
3	DQ205315	GATGGATCGAGACAAGCG	TGGAGGTGTGTGCCTACC	58
4	DQ205317	CGCTTGAGGAAGCATCAC	CCTACGTCATCTACGGCG	50
5	DQ205320	GAGGCGCTTTGACACTTG	TCACGGGATGATCCAAGA	50
6	DQ205325	CCACCGTGTCTCTTTCCA	CGCCATGAAACAGCTCTC	56
7	DQ205328	GTGTGTGCCAGTGATCCA	ATTCCCGGAACGACCTT	56
8	DQ205313	AAGCAGAAGAACTCCGCC	GGAATCGATGCAACCAAG	58
9	DQ205314	TGGGGCCAGGAAACTAC	CTCAGGAGCGATCAGACG	58
10	DQ205315	ATTCGTTCGGGGATCAAG	GCTGCATGCACATCACAC	58
11	DQ205317	TTGTTTGCCTTCACCAGG	ACAGTGGAACGGTGGTTG	58
12	DQ205320	CGGACGCCTGATGTGA	CGTCTACGGTATCGGCTG	58
13	DQ205325	CAGCGACAGCAGATCACA	TAGCAGCAGCAGCTCAGG	58
14	DQ205328	TCCACACAGCAACAACCA	CGTGCCAAGATCCAGAAG	56
15	DQ205313	ATAGACAGGCAGCGGACA	GTGGGTGAAGTTCCAGCA	56
16	DQ205314	TCAAGCCAGCCAAAAGAA	AGCCCTAACCCTAGGACG	58
17	DQ205315	ATCTGTCGTCGTTGCTGC	CACGCACCTCCGACTC	58

Data analysis

For the quantitative traits, descriptive statistics were calculated for each accession by SPSS 20.0. The mean, maximum, minimum and standard deviation (SD), were calculated for each quantitative trait. The Euclidean distance between Job's-tears accessions, based on morphological characteristics, was computed in the NTSYSpc software package, version 2.10e.

The presence (1) or absence (0) of the band of each SSR marker in the 20 accessions was scored. The polymorphism information content (PIC) for each SSR was calculated by using PIC-CALC software, according to the formula:

$$PIC = 1- \sum pij^2$$

where, pij is the frequency of the i^{th} allele of the j marker (Liu et al., 2006). Clustering was performed using the UPGMA (unweighted pair group method with arithmetic mean) in the SAHN sub-program. The NTSYS 2.1e was used for statistical analysis. The Mantel test of GenAlEx 6.2 was used to examine the correlation between the morphological matrix and genetic matrix.

Results

Morphological characterization of Job's tears accessions

There were significant genetic variations in all the Job's tear accessions (Table 3). Accessions M6 had the highest values on plant height (271.0 cm), leaf blade length (71.4 cm), ligule length (1.2 mm), lower glume width of male spikelet (3.0 mm), fruit length (11.5 mm), and grain length (10.5 mm). Accession M7 had the highest values on leaf blade width (4.7 cm), leaf sheath length (16.8 cm), ligule length (1.2 cm), style length (20.0 mm), and stigma length (7.0 mm). The minimum values of leaf sheath length (8.7 cm), ligule length (1.1 mm), lower glume length of male spikelet (6.0 mm), lower glume width of male spikelet (2.0 mm), style length (7.5 mm), stigma length (2.5 mm), ovary diameter (0.7 mm), fruit length (6.0 mm), grain length (5.0 mm) were observed in M5. The M8 accession was found to have the minimum values on stem diameter (0.6 cm), ligule length (1.1 mm), glume thickness (0.2 mm), upper glume length of male spikelet (6.0 mm), upper glume width of male spikelet (3.0 mm), lower glume width of male spikelet (2.0 mm), fruit width (4.4 mm).

Qualitative traits of Job's-tears were shown in Table 4. Almost all the accession had similar qualitative morphological traits. The general glume characteristics in 20 accessions were soft, thin, oblong cylinders (85.0% of the total), and the remaining 3 accessions had hard, thick glume (15.0%), in which M2 and M12 were oblong in shape and M5 was a round shape. There were 19 accessions with separate male inflorescence (95.0% of the total) whereas 1 accession had no separate male inflorescence (M5). As for style color and fruit shape, 19 accessions were purple and oblong cylinders, 1 white and round (M5). The trait color of stigma was observed as purple in 18 accessions, 2 white (M5 and M6). Regarding fruit characteristics, except for M2, M5, M12 were hard, thick, and hard to break, and the remaining accessions were thin, soft, and easy to break, with vertical stripes. With respect to grain colour, at watery ripe, there were 14red-purple accessions, 3 green (M2, M5, M6), 2 green with purple-red streaks (M3, M12), 1 dark gray (M8). At fully ripe, M2 was observed in white gray color, M5 turned to light yellow and M12 turned to dark purple colour.

Clustering of accessions

The similarity coefficient of all morphological traits among the 20 accessions ranged from 0.08 to 4.64 by using NTSYS software. As shown in Figure 1, clusters and subclusters were clearly established according to morphological markers. All 20 accessions were grouped into 2 clusters, at a Euclidean distance of 3.5. Four accessions (M2, M3, M5, and M12) were included in cluster I, and cluster II included 16 accessions. Cluster I was characterized by hard, thick glume and fruit characteristics and was composed of 2 subclusters with Euclidean distance of 2.5. Sub-cluster I-1 included 2 accessions (M3 and M12) which were green with purple-red streaks grain at watery ripe and turn to dark purple color at fully ripe. Sub-cluster I-2 included 2 accessions (M2 and M5) that had hard, thick glume, green grain color at watery ripe and hard, thick fruit. Cluster II, the largest cluster, was characterized by grain color at fully ripe of dark gray and was composed of 2 sub-clusters with Euclidean distance of 2.36.

Table 3: Analysis of 19 quantitative morphological traits of 20 Job's tears accessions

Accession	Plant height at flowering (m)	Stem diameter (cm)	Leaf blade length (cm)	Leaf blade width (cm)	Leaf sheath length (cm)	Ligule length (mm)	Glume thickness (mm)	Number of male spikelets per panicle	Upper glume length (mm)	Upper glume width (mm)	Lower glume length (mm)	Lower glume width (mm)	Style length (mm)	Stigma length (mm)	Ovary diameter (mm)	Fruit length (mm)	Fruit width (mm)	Grain length (mm)	Grain width (mm)
M1	1.8	1.6	55.3	3.9	14.2	1.2	0.2	17.0	9.0	4.0	7.0	3.0	13.0	6.0	1.1	7.2	6.1	6.8	5.1
M2	1.6	1.1	54.8	4.6	13.7	1.2	0.7	17.0	8.0	5.0	7.3	2.1	13.0	6.0	1.1	7.1	5.2	6.1	4.2
M3	1.7	1.0	54.5	4.4	13.1	1.1	0.6	20.0	8.0	3.2	7.5	2.0	11.0	6.0	1.0	7.2	5.3	6.3	4.2
M4	1.7	1.6	46.4	3.4	13.9	1.1	0.3	17.5	9.5	4.1	10.2	2.0	15.8	4.0	1.2	8.9	7.8	5.8	4.6
M5	1.6	0.8	54.4	3.9	8.7	1.1	1.0	17.0	6.0	4.0	6.0	2.0	7.5	2.5	0.7	6.0	6.0	5.0	5.0
M6	2.7	1.2	71.4	4.4	12.9	1.2	0.2	17.0	8.0	4.0	8.0	3.0	9.0	6.0	1.0	11.5	7.5	10.5	6.5
M7	1.8	1.2	67.8	4.7	15.8	1.2	0.2	20.0	9.8	3.9	7.5	2.2	20.0	7.0	1.1	10.3	7.2	10.1	7.1
M8	1.8	0.6	51.0	3.8	11.7	1.1	0.2	15.5	6.0	3.0	6.0	2.0	10.0	4.0	1.0	7.7	4.4	7.5	4.5
M9	1.6	0.8	51.9	3.7	13.7	1.1	0.2	16.0	8.0	3.0	7.0	2.0	11.0	6.0	1.0	9.4	6.3	9.3	6.1
M10	2.5	1.0	55.9	4.1	14.1	1.1	0.2	15.5	8.3	3.1	7.2	2.0	10.0	6.0	1.0	9.6	6.4	9.2	6.3
M11	1.9	0.7	44.9	3.3	11.1	1.1	0.2	16.5	6.0	3.0	6.0	2.0	11.0	6.0	1.1	10.5	6.2	9.3	5.8
M12	1.6	1.1	56.2	4.6	13.3	1.2	0.6	20.5	8.2	3.3	7.8	2.0	11.0	6.0	1.0	7.1	5.1	6.3	4.3
M13	1.8	1.5	46.8	3.3	14.2	1.2	0.2	17.0	10.2	3.9	10.0	2.0	16.2	4.0	1.2	8.7	7.5	5.9	4.5
M14	1.7	1.7	46.4	3.4	14.0	1.2	0.3	18.5	9.8	3.9	9.9	2.0	16.0	5.0	1.2	8.6	7.7	5.5	4.8
M15	1.7	1.7	46.8	3.5	14.3	1.2	0.2	18.5	10.0	4.0	10.1	2.0	16.0	4.0	1.2	8.5	7.9	5.8	4.8
M16	1.6	1.2	58.7	3.9	13.5	1.2	0.3	25.0	8.0	3.0	7.0	3.0	18.0	5.0	1.1	10.5	6.5	8.5	5.5
M17	1.7	1.6	52.4	3.6	13.6	1.1	0.2	15.5	9.0	4.0	7.0	3.0	12.5	6.0	1.0	7.1	5.9	6.8	4.7
M18	1.8	1.2	67.6	4.7	15.6	1.2	0.2	18.5	9.0	3.2	7.3	2.1	18.0	7.0	1.1	10.4	7.6	10.2	7.5
M19	1.8	0.9	45.3	3.6	12.3	1.1	0.2	17.0	7.0	3.5	6.7	2.2	11.0	6.0	1.1	10.7	6.4	9.3	5.2
M20	1.8	1.5	46.5	3.4	13.5	1.1	0.3	18.0	9.8	3.7	10.0	2.0	16.4	4.0	1.1	8.7	7.8	5.9	4.9
MIN	1.6	0.6	44.9	3.3	8.7	1.1	0.2	15.5	6.0	3.0	6.0	2.0	7.5	2.5	0.7	6.0	4.4	5.0	4.2
MAX	2.7	1.7	71.4	4.7	15.8	1.2	1.0	25.0	10.2	5.0	10.2	3.0	20.0	7.0	1.2	11.5	7.9	10.5	7.5
MEAN	1.8	1.2	53.8	3.9	13.4	1.2	0.3	17.9	8.4	3.6	7.8	2.2	13.3	5.3	1.1	8.8	6.5	7.5	5.3
SD	0.3	0.3	7.8	0.5	1.6	0.1	0.2	2.2	1.3	0.5	1.4	0.4	3.5	1.2	0.1	1.5	1.0	1.8	1.0

Table 4: Qualitative morphological traits of 20 Job's tears accessions

Characteristics	General glume characteristics	Separate male inflorescence (yes/no)	Male spikelet type (single/double)	Style color	Stigma color	Fruit shape	Grain color at watery ripe	Grain color at fully ripe	Fruit characteristics
M1	Soft, thin, oblong cylinder	Yes	Single	Purple	Purple	Oblong cylinder	Red purple	Dark gray	Thin, soft, easy to break, with vertical stripes
M2	Hard, thick, oblong	Yes	Single	Purple	Purple	Oblong cylinder	Green	White Gray	Hard, thick, hard to break, smooth
M3	Soft, thin, oblong cylinder	Yes	Single	Purple	Purple	Oblong cylinder	Green with purple red streaks	Dark purple	Hard, thick, hard to break, smooth
M4	Soft, thin, oblong cylinder	Yes	Single	Purple	Purple	Oblong cylinder	Red purple	Dark gray	Thin, soft, easy to break, with vertical stripes
M5	Hard, thick, round shape	No	Single	White	White	Round	Green	Light yellow	Hard, thick, hard to break
M6	Soft, thin, oblong cylinder	Yes	Single	Purple	White	Oblong cylinder	Green	Dark gray	Thin, soft, easy to break, with vertical stripes
M7	Soft, thin, oblong cylinder	Yes	Single	Purple	Purple	Oblong cylinder	Red purple	Dark gray	Thin, soft, easy to break, with vertical stripes
M8	Soft, thin, oblong cylinder	Yes	Single	Purple	Purple	Oblong cylinder	Dark gray	Dark gray	Thin, soft, easy to break, with vertical stripes
M9	Soft, thin, oblong cylinder	Yes	Single	Purple	Purple	Oblong cylinder	Red purple	Dark gray	Thin, soft, easy to break, with vertical stripes
M10	Soft, thin, oblong cylinder	Yes	Single	Purple	Purple	Oblong cylinder	Red purple	Dark gray	Thin, soft, easy to break, with vertical stripes
M11	Soft, thin, oblong cylinder	Yes	Single	Purple	Purple	Oblong cylinder	Red purple	Dark gray	Thin, soft, easy to break, with vertical stripes
M12	Hard, thick, oblong	Yes	Single	Purple	Purple	Slightly oblong	Green with purple red streaks	Dark purple	Hard, thick, hard to break, smooth
M13	Soft, thin, oblong cylinder	Yes	Single	Purple	Purple	Oblong cylinder	Red purple	Dark gray	Thin, soft, easy to break, with vertical stripes
M14	Soft, thin, oblong cylinder	Yes	Single	Purple	Purple	Oblong cylinder	Red purple	Dark gray	Thin, soft, easy to break, with vertical stripes
M15	Soft, thin, oblong cylinder	Yes	Single	Purple	Purple	Oblong cylinder	Red purple	Dark gray	Thin, soft, easy to break, with vertical stripes
M16	Soft, thin, oblong cylinder	Yes	Single	Purple	Purple	Oblong cylinder	Red purple	Dark gray	Thin, soft, easy to break, with vertical stripes
M17	Soft, thin, oblong cylinder	Yes	Single	Purple	Purple	Oblong cylinder	Red purple	Dark gray	Thin, soft, easy to break, with vertical stripes
M18	Soft, thin, oblong cylinder	Yes	Single	Purple	Purple	Oblong cylinder	Red purple	Dark gray	Thin, soft, easy to break, with vertical stripes
M19	Soft, thin, oblong cylinder	Yes	Single	Purple	Purple	Oblong cylinder	Red purple	Dark gray	Thin, soft, easy to break, with vertical stripes
M20	Soft, thin, oblong cylinder	Yes	Single	Purple	Purple	Oblong cylinder	Red purple	Dark gray	Thin, soft, easy to break, with vertical stripes

Sub-cluster II-1 included 1 accession (M8) that was dark gray grain at watery ripe and smallest in stem diameter (0.6cm), upper glume length of male spikelet (6.0 mm), upper glume width of male spikelet (3.0 mm) and fruit width (4.4 mm). Sub-cluster II-2 included 15 accessions with red-purple grain at watery ripe.

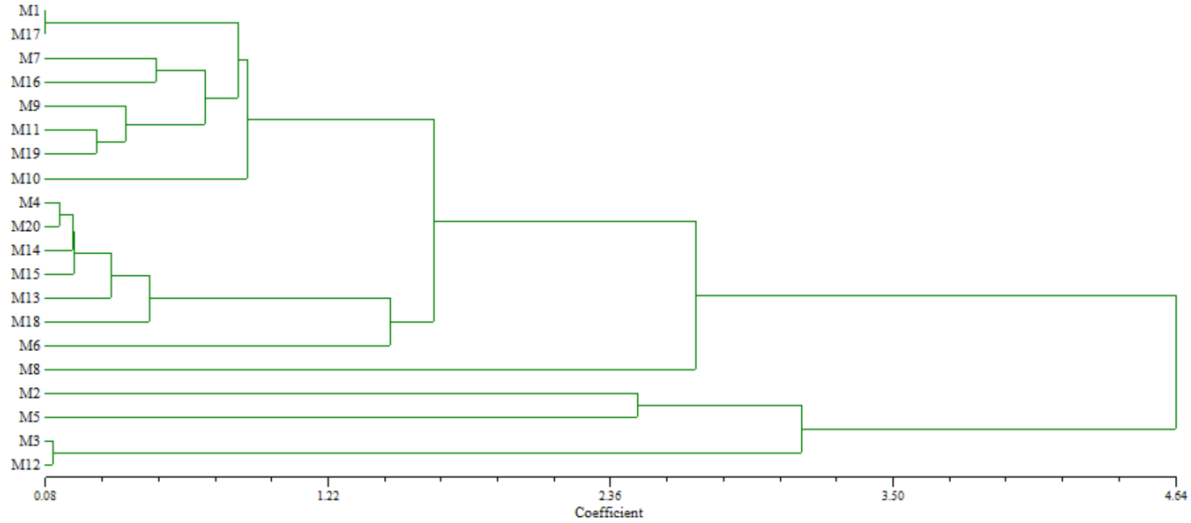

Figure 1: Cluster analysis of morphological markers

Molecular characterization using SSR markers

Allelic polymorphism

The molecular diversity of 20 accessions *C. lacryma-jobi* was assessed using a set of 17 SSR markers. Primers showed a different level of polymorphism among all 20 accessions. Out of 17 primers, the 7 primers created a polymorphic pattern that was repeatable while the 10 monomorphic primers produced a monomorphic pattern. Overall, 28 alleles were generated by polymorphic SSR markers with an average of 4.0 alleles per primer. Out of seven, only 4 primers (DQ205313, DQ205317, DQ205320, DQ205328) gave both monomorphic and polymorphic bands while 3 primers showed only polymorphic band patterns. primers DQ205325 gave the highest number of alleles, while 5 primers (DQ205314, DQ205315, DQ205317, DQ205320, DQ205328) gave a lower number of alleles. The rare alleles (frequency < 0.05) comprised 14.29 %, whereas intermediate (frequency 0.1-0.5) comprised 82.14 % and abundant alleles (frequency > 0.5) comprised 3.57%. Out of 7 polymorphic, only one marker DQ205313 provided the highest number of 3 rare alleles, followed by DQ205325 (Table 5).

Polymorphic Information

The quantity of amplified alleles, the quantity of polymorphic alleles, number of unique alleles, percentage of polymorphic alleles and the polymorphism information content (PIC) were calculated for all primers. Out of the total, 20 alleles showed polymorphism (71.429%) and 8 alleles showed monomorphism (28.571%). The percentage of polymorphic alleles was 69.047%. All primers produced specific, effective, and measurable alleles. The amplified alleles ranged from 110 to 1100 bp. All primers displayed different levels of polymorphism among 20 accessions. Distinction capacities for every single genotype were determined using PIC values. PIC varied from 0.46 to 0.75, with a mean of 0.63 per marker. 6 SSR primers indicated PIC numbers more than 0.5, which means there is a huge genetic variation present among the 20 accessions Job's tear (Table 5).

Table 5: SSR markers with analyzed data

Name of primer	Observed size (bp)	Quantity of amplified alleles	quantity of polymorphic alleles	Number of unique alleles	Percentage of polymorphic alleles	PIC
DQ205313	200-950	6	3	3	50.00	0.72
DQ205314	270-350	3	3	0	100	0.60
DQ205315	250-300	3	3	0	100	0.46
DQ205317	195-1050	3	1	0	33.33	0.65
DQ205320	110-850	3	1	0	33.33	0.66
DQ205325	300-1100	7	7	1	100	0.75
DQ205328	195-1100	3	2	0	66.67	0.54

Cluster analysis among populations of 20 accessions and comparison between morphological molecular traits of Job's tears

Genetic diversity and relationship among the 20 accessions Job's tears were further studied by UPGMA cluster analysis (Figure 2). Genetic similarity indexes based on the 7 SSR markers were calculated by using the Dice coefficient (Dice, 1945; Nei and Li, 1979) and varied from 1 to 0.84. The cluster at the coefficient of 0.85 grouped the 20 accessions into two main clusters. Cluster I consisted of 3 accessions (M1, M2, M5), while cluster II had 14 accessions. An UPGMA dendrogram cluster II was further delineated into three sub-clusters at a level of genetic similarity of 0.88 (Figure 2). The M3 constituted one separated sub-cluster II-1. The M4, M8, M19, M9, M10, M12, M18, M7, M20 clustered into the sub-cluster II-2. The highest genetic similarity value (1.00) corresponded to a pair of M10 and M12. Sub-cluster II-3 comprised seven remaining accessions. The similarity coefficient among this group ranged from 0.89 to 0.98.

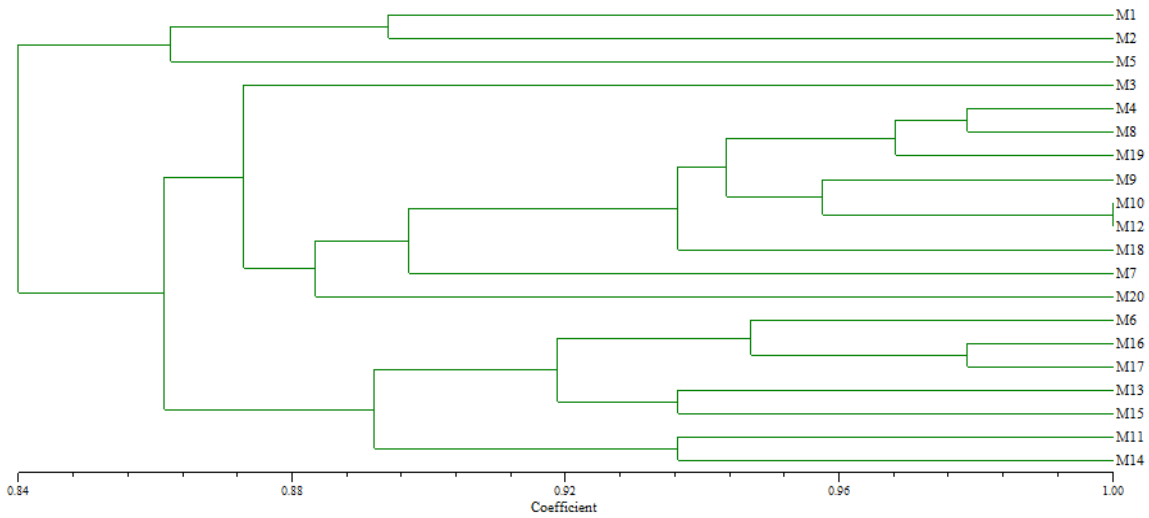

Figure 2. The genetic relationship of 20 *Coix lacryma-jobi* genetic resources based on SSR markers

Mantel tests between the similarity matrix of morphological characterization and the similarity matrix of molecular characterization were analyzed by NTSYS software, and the correlation value was 0.29. The results indicated that there is low uniformity between the relationships assessed based on morphological markers and the genetic relationships among Job's tears accessions revealed by SSR markers.

Discussion

Using a combination of morphological traits and molecular markers has led to more reliable conclusions in assessments of genetic diversity. According to the morphological results presented in the current study, stem diameter, leaf blade, glume, grain colour and fruit character variation are crucial for distinguishing Job's tears. The morphological cluster analysis was effective for classifying Job's tear collected from Northern mountain provinces in this country. Based on morphological traits, the 20 Job'stear genotypes were divided into two clusters with the hard, thick glume and fruit Job's tear gathered in clusters I. However, morphological characteristics can easily be affected by environmental conditions and ecological habitats, some Job's tear accessions can not be discriminated clearly based on morphological characteristics alone.

The PIC value is an important parameter for estimating the genetic diversity of a population; the higher the value, the more diverse the population structure is (Lv et al., 2020). High, medium, and low locus polymorphism is defined as PIC > 0.5, 0.5 > PIC > 0.25, and PIC < 0.25, respectively (Zhou et al., 2015). Therefore, in our case, genomic-SSR markers detected high locus polymorphism among the 20 Job's tears genotypes and other plants (Trung et al., 2013; Huong et al.,2022) indicating SSR markers are of great utility for genetic diversity studies of Job's tears. In this study, the mean PIC of genomic-SSR markers was higher than the mean PIC for AFLP markers (0.628 and 0.202, respectively) which was also observed in 139 job's tears genotypes from four geographical regions in Southwest China (Fu et al., 2019). The distance coefficients of an UPGMA cluster analysis ranged from 0.84 to 1 suggesting that there is a close genetic relationship between 20 accessions Job's tears that group together. Some research on the genetic variation of Job's tears showed relatively high genetic similarity between Job's tear genotypes, ranging from 0.30-0.92 with a mean average is 0.78, 0.48-0.82, and 0.30-0.81.

The Mantel test exposed a low correlation between morphological matrices and molecular matrices of the Job's tear genotypes (r = 0.29). Moreover, clustering based on molecular markers was not coupled with clustering based on morphological characteristics. There were some differences between the relationships of the 20 Job's tears accessions revealed by morphological markers and the relationships revealed by molecular markers. It may reflect the influence of the environment on the performance of the materials. It could be a result of the independent nature of morphological and molecular variations. According to Martínez et al. (2005), the DNA markers and morphological characters will not necessarily gain closely matching results. The reasons for this phenomenon are complex. Yunli et al. (2020) suggested the reasons for the low correlation between DNA markers and morphology: The morphological characteristics are affected by both innate and environmental factors, whereas DNA markers indicate differences in DNA sequence. Furthermore, morphological marker data can be disturbed by anthropogenic activities as well other environmental factors during data collection. SSR marker polymorphisms had low coverage in the complete genomes. Consequently, morphological markers and molecular markers are not exchangeable; otherwise, they are complementary and together assure the comprehensiveness and accuracy of analytical results.

Conclusions

In conclusion, our findings show that morphological and molecular markers revealed low genetic diversity in 20 Job's tear genotypes. Although there was a low correlation between morphological and DNA markers, Both the morphological and molecular approaches provided useful information for breeding programs and germplasm utilization in Vietnam. Our findings may contribute to a better understanding of the genetic diversity of Job's tears, which is essential for future crop improvement programs in this country.

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Conflict of interest

The authors have declared that there is no conflict of interest

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