



Assessment of Genetic Variability in Job's Tears (*Coix lacryma-jobi* L.) Germplasm from Indian NEH Region Using Morpho-physiological Traits and SSR Markers

Avinash Pandey^{1,3} · Bharati Lap² · Chubasenla Aochen¹ · Amit Kumar¹ · Wricha Tyagi^{2,4} · Mayank Rai^{2,5}

Received: 12 February 2024 / Accepted: 10 October 2024

© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2024

Abstract

Coix lacryma-jobi L. is a minor millet and an underutilized crop native to Asia that is of paramount importance in food and medicine. In India, the crop is mainly grown in the northeastern hill region for food, medicine, beverage, and fodder purposes. Genetic information on edible Job's tears and wild relatives still needs improvement. Therefore, the study focused on characterizing 65 accessions of Job's tears, including landraces and wild types collected from the northeastern hill region of India, using 20 morphological traits, three biochemical traits, and 16 SSR markers. Based on grain yield data, JTN11 and IC89392 were the most promising accessions. Biochemical analysis recorded IC417053, JTN3, and IC89393 as having the highest phenol content, antioxidant capacity, and flavonoid content, respectively. Principal component analysis showed that traits, namely, total phenol content, leaf length, spikelet/plant, brace root, and seed yield/plant, have significantly contributed to the diversity. In molecular characterization with SSR primers, 62 alleles were generated, with an average of 3.87 alleles per locus. The effective number of alleles detected varied from 1.17 (GBssrJT32) to 4.23 (GBssrJT198). Two alleles were found unique to wild accessions, underscoring the importance of preserving these varieties for future research and breeding. Based on the UPGMA dendrogram, IC416868, IC521338, IC417053, IC419466, IC540173, IC540281, IC89387, IC89393, and JTN3 were identified as diverse accessions. The high level of genetic diversity assessed in this study emphasizes the importance of the Indian NEH region for conserving Job's tears germplasm. The observed pattern of genetic variations may be used to develop the breeding strategy in Job's tears.

Keywords Job's tears · Genetic diversity · Grain yield · Phenol content · Antioxidant · SSR

Introduction

Coix lacryma-jobi L. ($2n = 4X = 20$), also known as Adlay, Jobi, Coix, and Job's tears, belongs to the Poaceae family, which comprises 6–11 species. Coix is a light-sensitive, short-day-long, and kharif-season crop. It is widely cultivated in Southeast Asian countries for food, fodder, and

medicinal purposes. The crop has significant health benefits and provides good medical values with high antioxidant properties. It also has anti-inflammatory, anti-tumour, anti-bacterial, and anti-obesity activities (Diningrat et al. 2021; Ferrer et al. 2021; Igbokwe et al. 2021). Coix seed contains 61–83% starch carbohydrate and protein content of around 12–14 mg/100 mg total weight (Devaraj et al. 2020; Ouyang et al. 2022).

Much research on Coix has been conducted in China, surpassing the efforts made in India. Despite being highly nutritious, resilient to climate variations, less prone to insects and diseases, locally available, and having medicinal importance (Devaraj et al. 2020), the crop is predominantly cultivated as a minor millet in the northeastern hilly regions of India. This region is considered one of the primary centres for cultivating Job's tears (Laxmisha et al. 2022). The limited and sporadic cultivation of Coix's local types and wild relatives in rural areas is now mainly concentrated only for medicinal

Key Message

- Sixty-five Job's tear accessions evaluated using 20 morphological and 3 biochemical traits and 16 SSR markers.
- IC417053, JTN3, and IC89393 with the highest phenol content, antioxidant capacity, and flavonoid content, respectively identified.
- IC416868, IC521338, IC417053, IC419466, IC540173, IC540281, IC89387, IC89393, and JTN3 identified as diverse accessions.

Extended author information available on the last page of the article

and ornamental purposes. Coix is underutilized primarily due to the growing popularity of other cereals in the region, the predominance of local types, and the lack of proper agro-nomic practices, resulting in lower crop yields.

Molecular markers are vital for describing genotypes and providing information about the relationship between markers and traits. Additionally, morphological and biochemical analyses assist in characterizing and classifying accessions. Therefore, it is essential to evaluate the available genetic resources and understand the genetic diversity of Coix germplasm for proper management, utilization, selection, and identification of genotypes for various plant breeding programs. In addition to morphological (Yao et al. 2013; Wang et al. 2013; Kumar et al. 2017; Laxmisha et al. 2022) and biochemical traits (Hore and Rathi 2007; Xi et al. 2016; Laxmisha et al. 2022), molecular markers have been utilized to assess the genetic diversity of Coix genotypes grown in different regions. For example, Li et al. (2001) detected genetic diversity between different Coix genotypes using random amplified polymorphic DNA (RAPD) markers. Similarly, Fu et al. (2019) used amplified fragment length polymorphism (AFLP) markers, Xi et al. (2016) used inter-simple sequence repeat (ISSR) markers, while Ma et al. (2010), Cong et al. (2023), and Kumar et al. (2024) used SSR markers for the same purpose.

Limited research has been conducted on Coix, and the crop remains unfamiliar to many parts of India, except for a few regions in the northeastern states. Most of the research has focused on accessions collected from China and Korea. Further study and research would provide insight into Coix's nutritional richness and high antioxidant properties. This study aims to evaluate the morphological, biochemical, and molecular variations of Job's tears accessions collected from the northeastern part of India. The objective was to gather information to identify the most suitable accessions for use as parents and for better conservation of the germplasm, ensuring their future utilization in other breeding programs.

Materials and Methods

Plant Materials

The study used 65 accessions (Supplementary Table S1) from various locations in India's northeastern hill (NEH) region, gathered from ICAR-NBPGR Regional Station Shillong, including five wild accessions from Meghalaya. The accessions were cultivated in a planned augmented field design at the Plant Breeding farm, ICAR, Umiam, in four 5-m row plots. The planting followed a plant-to-plant spacing of 40 cm and row-to-row distance of 50 cm in a well-ploughed field, with a check (Vhine No.1) planted randomly in each plot.

Morphological Parameters

Observations for twenty-five morphological-biochemical traits were recorded for 65 accessions. Nineteen quantitative traits, including plant height (PH), panicle length (PL), number of tillers/plants, number of nodes/tillers, average internode length/tiller, number of brace roots/tiller (BR), the girth of tiller (cm), number of leaves/tillers (NOL), leaf length (LL), leaf breadth (LB), leaf area Index (LAI), chlorophyll concentration (SPAD value), spikelet/tiller, spike/spikelet, 100 seed wt. (100SW), grain yield, phenol content (PC), anthocyanin content (AC), and flavonoid content (FC), were evaluated (Supplementary Table S2). Plant height was measured from the ground to the top of the spike. NOL, LL, LB, LAI, and SPAD values were measured at the reproducing stage of the crop. Nodes/tiller, internodes/tiller, BR, and girth were recorded at the physiological maturity stage. Spikelets/spike, spike/tiller, GY, and 100SW were determined after crop harvesting. Assessment of six qualitative traits, including stem colour (SC), leaf colour (LC), leaf texture (LT), inflorescence compactness (IC), grain shape (GS), and grain colour (GC), was also recorded (Supplementary Table S2).

DNA Isolation and PCR Amplification

Genomic DNA was extracted from 30-day seedlings using the CTAB method (Saghai-Maroo et al. 1984) with the modification of 2% polyvinylpyrrolidone added in extraction buffer. The relative purity and DNA concentration were estimated using a Thermo Scientific Nanodrop 2000 Spectrophotometer. For molecular characterization, a set of 16 primer pairs (Ma et al. 2006) were used (Supplementary Table S3). Polymerase chain reaction (PCR) amplification was carried out with 40 ng of genomic DNA, 2.5 mM MgCl₂, 1U Taq DNA polymerase, 1X PCR buffer without MgCl₂, 0.25 μM of each of primers, and 200 μM of dNTPs. The volume was made up to 20 μl with PCR-grade water. PCR reactions were carried out in a Veriti™ thermal cycler (Applied Biosystems). The PCR conditions consisted of 94 °C (3 min) initial denaturation followed by 35 cycles of 94 °C (30 s) denaturation, 52–61 °C (45 s) annealing, followed by 72 °C (1 min) extension, and a final extension at 72 °C for 10 min. The amplified products were analyzed by electrophoresis using agarose gel (3%). The gels were visualized in a UV gel documentation system for analysis of DNA bands, and the sizes of the amplified fragments were confirmed by using a 100 bp DNA ladder as the size reference standard.

Biochemical Estimation

The total phenol content of seed extract was estimated using the Folin–Ciocalteu (FC) method (Volluri et al. 2011). Gallic acid was used as a standard, and the total phenols were expressed as mg/g gallic acid equivalent using the standard curve equation: $y = 0.0022x - 0.0155$, $R^2 = 0.999$, where y is the absorbance at 760 nm and x is the total phenolic content in the different extracts of Job's tears expressed in mg/gm (Supplementary Fig. S1).

Total flavonoid content was determined by the aluminium chloride colourimetric method (Chang et al. 2002). The amount of total flavonoid was determined with the Quercetin reagent. Quercetin was used as a standard compound, and the total flavonoid was expressed as mg/g Quercetin equivalent using the standard curve (Supplementary Fig. S2) equation: $y = 0.738x + 0.076$, $R^2 = 0.990$, where y is the absorbance at 510 nm and x is the total flavonoid content in the extracts of Job's tears expressed in mg/gm.

Total antioxidant capacity was estimated by the phosphomolybdenum method (Prieto et al. 1999). Ascorbic acid was used as a standard compound. The total antioxidant content was expressed as mg/g ascorbic acid equivalent using the standard curve (Supplementary Fig. S3) equation: $y = 0.0035x - 0.252$, $R^2 = 0.996$, where y is the absorbance at 695 nm. X is the total antioxidant content in the different extracts of Job's tears expressed in mg/gm ascorbic acid equivalent.

Statistical Analysis

Descriptive statistics of morphological traits, phenotypic correlation coefficient, and principal component analysis (PCA) were computed using XLSTAT software. D^2 and path coefficient analyses based on quantitative traits were done using Windostat 8.5 software (Indostat Services, Hyderabad). Simple sequence repeats (SSR) amplicons obtained were resolved as a single band on the agarose gel system, and the dataset was used to calculate polymorphism information content (PIC), major allele frequency, and pairwise Nei et al. (1983) genetic distance using PowerMarker v3.25 software (Liu and Muse 2005). The nexus tree file generated from PowerMarker v3.25 software was used to create a neighbour-joining dendrogram using MEGA11 software (Tamura et al. 2021). The parameters of genetic diversity Nei's expected heterozygosity, Shannon's information index (I), and Nei's coefficient were calculated using PopGene 1.32 (Yeh et al. 2000). The population structure of Coix accessions was inferred by the Bayesian model-based clustering algorithm of STRUCTURE v.2.3.4 software (Pritchard et al. 2000). The analyses were carried out using the admixture model option. The K value indicating the number of sub-populations was identified by performing

Markov Chain Monte Carlo (MCMC) runs for each value of K from 1 to 10. Each run comprised 50,000 burn-in periods and 100,000 MCMC replicates. The Mantel test was carried out to compare the two distance matrices obtained from the quantitative traits and SSR marker using NTSYS-PC (ver. 2.02j; Exeter Software, NY, Rohlf 1993).

Results

Agro-morphological Trait Analysis

Qualitative traits such as stem colour, leaf colour, leaf texture, panicle compactness, seed shape, and seed colour showed significant differences in the Coix accessions' phenotype. The seed colour diversity ranged from shades of white and brown to black (Fig. 1) and was categorized as cream (29.2%), grey (21.5%), light brown (16.9%), white (15.3%), dark brown (12.3%), and black (4.6%), respectively. Most accessions had distinct stripes, while some had smooth seed surfaces. Among the accessions, 53.8% had ovate seeds, 35.3% had round seeds, and 10.7% had spindle-shaped seeds. Additionally, most accessions exhibited dark green leaf colour (52.3%) and leaf pubescence (53.8%). Compact inflorescence was observed in 15.3% of the accessions, while 84.61% had loose inflorescence. Stem colour also varied, with 80% green and 20% purple. All the quantitative traits showed genetic variation ranging from 12.69 to 72.89% (Table 1). The mean value, standard deviation, and variance of each quantitative trait revealed wide variations among all 65 accessions. The shortest plant height (76.66 cm) among the cultivated accessions was recorded for IC89394 from Meghalaya, indicating its potential inclusion in breeding programs for developing shorter cultivars of Job's tears. Two important morphological traits, 100SW and GY, were highest in accessions IC89383 (14.83 g) and JTN11 (135.81 g), respectively.

Correlation, Path Coefficient, Genetic Divergence, and PCA Analysis

Correlation coefficients were estimated among all the traits, indicating the inherent association between any two variables, which might have occurred due to the pleiotropic action of genes, linkage or, more likely, both. Grain yield per plant was found to be positively and significantly ($P < 0.05$) correlated with the number of tillers/plant (0.76), number of brace root/plant (0.62), and 100 SW (0.53). The 100 seed weight positively and significantly correlated with leaf length, breadth, and tiller number/plant. Phenol content was positively and significantly correlated with flavonoid content. However, phenol and flavonoid contents were negatively and significantly correlated



Fig. 1 Representative picture **a** showing a Job's tears plant in the field during seed set. **b** Variation observed in Job's tears accessions with respect to grain colour, shape, and size. Name of the accession corresponds to Supplementary Table S1

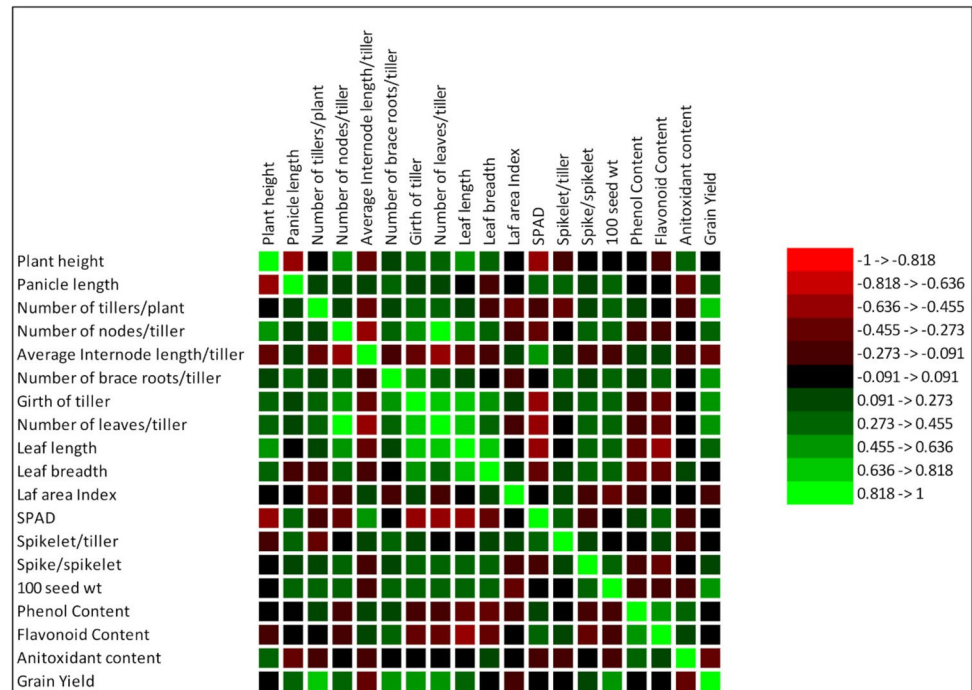
Table 1 Descriptive statistics showing the mean values for agro-morphological and biochemical traits among 65 accessions

| Statistic | Minimum | Maximum | Mean | Variance | Variation coefficient | Standard error of the mean |
|---|---------|---------|--------|----------|-----------------------|----------------------------|
| Plant height (cm) | 76.66 | 179.00 | 113.05 | 544.89 | 0.21 | 2.92 |
| Panicle length (cm) | 17.19 | 166.00 | 85.08 | 574.47 | 0.28 | 3.00 |
| Number of tillers/plants | 1.00 | 28.00 | 4.06 | 19.97 | 1.10 | 0.56 |
| Number of nodes/tillers | 6.00 | 13.00 | 8.51 | 1.85 | 0.16 | 0.17 |
| Average internode length/tiller | 15.40 | 33.78 | 22.93 | 15.94 | 0.17 | 0.50 |
| Number of brace roots/tiller | 1.00 | 22.00 | 5.97 | 11.14 | 0.56 | 0.42 |
| Girth of tiller (cm) | 4.10 | 14.03 | 8.51 | 4.03 | 0.24 | 0.25 |
| Number of leaves/tillers | 6.00 | 14.00 | 9.00 | 1.97 | 0.16 | 0.18 |
| Leaf length (cm) | 39.83 | 77.13 | 51.01 | 59.09 | 0.15 | 0.96 |
| Leaf breadth (cm) | 2.96 | 5.79 | 3.88 | 0.35 | 0.15 | 0.07 |
| Leaf area index | 3.23 | 41.10 | 28.95 | 32.47 | 0.20 | 0.71 |
| SPAD | 24.51 | 65.21 | 42.88 | 83.89 | 0.21 | 1.14 |
| Spikelet/tiller | 9.00 | 125.00 | 52.88 | 642.54 | 0.48 | 3.17 |
| Spike/spikelet | 2.00 | 7.00 | 3.88 | 0.85 | 0.24 | 0.11 |
| 100 seed wt. (g) | 0.00 | 15.12 | 8.91 | 13.17 | 0.41 | 0.45 |
| Phenol content (mg GAE/g sample) | 5.60 | 10.20 | 7.32 | 1.23 | 0.15 | 0.14 |
| Flavonoid content (mg QE/g sample) | 4.60 | 23.60 | 12.84 | 29.32 | 0.42 | 0.68 |
| Antioxidant content (mg AAE/100 g sample) | 20.00 | 72.00 | 40.36 | 137.42 | 0.29 | 1.47 |
| Grain yield | 3.28 | 492.00 | 54.99 | 7910.98 | 1.62 | 11.12 |

with leaf length (LL), leaf breadth (LB), number of leaves/tiller (NOL), number of nodes/plant, and girth of tillers (Fig. 2). Nodes per plant were significantly positively correlated with NOL (0.84), LL (0.59), girth of tillers (0.59),

and LB (0.41). Similarly, the SPAD value positively correlated with internodes (0.46) and spikelet per plant (0.42) but negatively correlated with LL, NOL, number nodes/tiller, and girth/tiller (Fig. 2).

Fig. 2 Correlation matrix for agro-morphological and biochemical characters in 65 Job's tear germplasm. Colour bar indicates the association between two variables where red is for negative and green is for positive correlation ($p=0.05$)



Path coefficient analysis was performed to estimate the direct and indirect contribution of various agro-morphological and biochemical traits to grain yield per plant (Supplementary Table S4). All other characters positively affected grain yield except for plant height, panicle length, number of internodes/tillers, leaf length, leaf area index, phenol, and antioxidant capacity. Tiller per plant exhibited maximum direct effect followed by the number of brace root/tiller, the girth of the tiller, SPAD and 100SW. Except for panicle length, all the characters with a significant positive correlation with grain yield per plant also exerted a high direct positive effect on grain yield. Panicle length had a negative direct effect on grain yield. However, its indirect positive impact through the number of tillers per plant, number of brace root/tiller, tiller girth, and 100 seed weight made its association with grain yield/plant significantly positive. This implies that the increase in grain yield/plant on selection of taller panicle is basically because of the rise in the number of tillers per plant, number of brace root/tiller, tiller girth, and 100 seed weight; the panicle length has the negative direct effect on plant grain yield.

In genetic divergence studies (D^2) based on morphological and biochemical traits, all 65 Job's tear accessions were grouped into 9 clusters (Supplementary Table S5). Cluster I (45) retained the maximum accessions, followed by Cluster III (11). Genotype JTN8 alone formed cluster V and exhibited the least values for all the traits except spikelet/tiller and total antioxidant content, which was the maximum in this cluster. Cluster IX consists of only one

genotype (W1) that exhibited maximum mean values for 100 seed weight (Supplementary Table S6). The maximum contribution towards divergence was phenol content, followed by 100 seed weight and the number of tillers per plant. Grain yield per plant and girth of tillers contributed the least to the total diversity.

Principal component analysis grouped the Job's tears variables into six main components with an eigenvalue greater than 1, accounting for 77.75% of the total variation of agronomic traits. Among the components, PC1 and PC2 exhibited maximum variation of 29.58% and 16.49% for the traits studied with an eigenvalue of 5.62 and 3.13, respectively (Table 2).

Scree plot explained the percentage of variance contributed by each principal component (Fig. 3a).

Leaf length, number of leaves/tillers, girth/tiller, leaf breadth, and number of nodes/tiller were the significant factors contributing to PC1. Meanwhile, panicle length, plant height, SPAD, brace root, number of spikelets/plant, and 100SW contributed the most to PC2 (Table 2). Individuals were also grouped based on their geographical area of collection. Accessions that contributed maximum to two principal components were JTN11, JTN9, JTN3, IC89392, JTN17, JTN10, JTN1, Vhine no.1, JTN16, and JTN4, which were initially collected from Nagaland. The JTN accessions and check variety were grouped together in the PCA biplot. Four out of five wild accessions were also found to be distant from the rest of the accessions (Fig. 3b).

Table 2 Eigen value, variance percentage, and morphological traits that contributed to first five principal components (PC)

| Components | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|--------|--------|--------|--------|--------|
| Plant height (cm) | 0.219 | -0.323 | 0.200 | 0.157 | 0.021 |
| Panicle length (cm) | 0.045 | 0.407 | -0.243 | 0.080 | -0.001 |
| Number of tillers/plants | 0.170 | 0.262 | 0.243 | -0.373 | 0.179 |
| Number of nodes/tillers | 0.330 | 0.024 | 0.042 | 0.167 | -0.064 |
| Average internode length/tiller | -0.246 | 0.095 | -0.261 | 0.124 | -0.119 |
| Number of brace roots/tiller | 0.186 | 0.295 | 0.177 | 0.333 | 0.170 |
| Girth of tiller (cm) | 0.351 | 0.044 | -0.107 | 0.169 | 0.271 |
| Number of leaves/tillers | 0.362 | 0.060 | 0.058 | 0.033 | -0.073 |
| Leaf length (cm) | 0.366 | -0.134 | -0.071 | 0.040 | -0.047 |
| Leaf breadth (cm) | 0.256 | -0.210 | -0.209 | 0.215 | -0.143 |
| Leaf area index | -0.075 | -0.154 | -0.294 | 0.250 | 0.569 |
| SPAD | -0.260 | 0.296 | -0.066 | 0.086 | -0.198 |
| Spikelet/tiller | -0.020 | 0.250 | -0.294 | 0.469 | -0.101 |
| Spike/spikelet | 0.202 | 0.052 | -0.165 | -0.004 | -0.524 |
| 100 seed wt. (g) | 0.221 | 0.244 | -0.047 | -0.143 | -0.231 |
| Phenol content (mg GAE/g sample) | -0.126 | 0.120 | 0.457 | 0.219 | -0.147 |
| Flavonoid content (mg QE/g sample) | -0.178 | 0.189 | 0.352 | 0.365 | 0.077 |
| Antioxidant content (mg AAE/100 g sample) | -0.013 | -0.265 | 0.339 | 0.311 | -0.209 |
| Grain yield | 0.228 | 0.376 | 0.149 | -0.102 | 0.216 |
| Eigenvalue | 5.620 | 3.133 | 2.097 | 1.819 | 1.094 |
| Variance percent | 29.577 | 16.489 | 11.038 | 9.576 | 5.758 |
| Cumulative variance percentage | 29.577 | 46.067 | 57.105 | 66.681 | 72.439 |

Genetic Variation and Cluster Analysis

Sixty-two alleles were detected in 65 of Job's tear accessions using 16 SSR markers. DNA extracted from 65 Job's tears genotypes were used for SSR analysis. Out of 62 alleles, ten alleles were found rare (allele frequency < 5%) (Table 3). Two alleles were found unique to wild accessions used in this study. The number of alleles ranged from 2 (GBssrJT31 and GBssrJT32) to 5 (GBssrJT68, GBssrJT136, GBssrJT170, GBssrJT181, GBssrJT183, and GBssrJT198), with an average of 3.88 alleles per locus. The effective number of alleles detected varied from 1.17 (GBssrJT32) to 4.23 (GBssrJT198). The polymorphism information content (PIC) value was 0.132 (GBssrJT32) to 0.745 (GBssrJT170). The mean of an effective number of alleles was 2.71, with a standard deviation of 1.0. Shannon's Information Index (Lewontin 1972) was the highest (1.52) for GBssrJT198, indicating that this primer was most informative and valuable for analyzing Job's tears landraces. The 62 SSR amplification products obtained from 27 loci were analyzed for the extent of polymorphism and expected heterozygosity (Levene 1949) within Job's tears accessions. The expected heterozygosity ranged from 0.14 to 0.77, with a mean value of 0.58 (Table 3). The proportion of polymorphic loci varied from 100% in cultivars and 68.75% in wild accessions. The low proportion

of polymorphic loci in wild accessions may be due to the smaller number (5) of wild accessions used in this study.

The SSR data was used to calculate the pairwise Nei et al. (1983) genetic distance between the accessions. The pairwise Nei et al. (1983) genetic distance ranged from 0 (IC89394 and IC89385) to 9.4 (JTN7 and IC521339). The average Nei et al. (1983) genetic distance among all pairs of comparisons was 0.57. Accordingly, in the neighbour-joining dendrogram based on Nei et al. (1983) genetic distance, five major clusters were identified (Fig. 4).

Majority of accessions (44) were grouped in cluster III that composed of accessions collected from different parts of Mizoram, Meghalaya, and Arunachal Pradesh. Cluster I consist of only one accession (JTN9), and II had two accessions, JTN7 and JTN8. Clusters IV and V comprised seven and eleven accessions, mainly from Nagaland state. The clustering pattern in the present study implies that the genetic differences observed were not influenced by their geographical location, and accessions collected from the same region can vary due to genetic differences in these accessions.

The two-way mantel test was carried out to compare the distance matrix obtained from the quantitative traits and SSR marker. A moderate matrix correlation value (0.61) was shown. The Mantel test showed that morphological markers and molecular markers were moderately correlated.

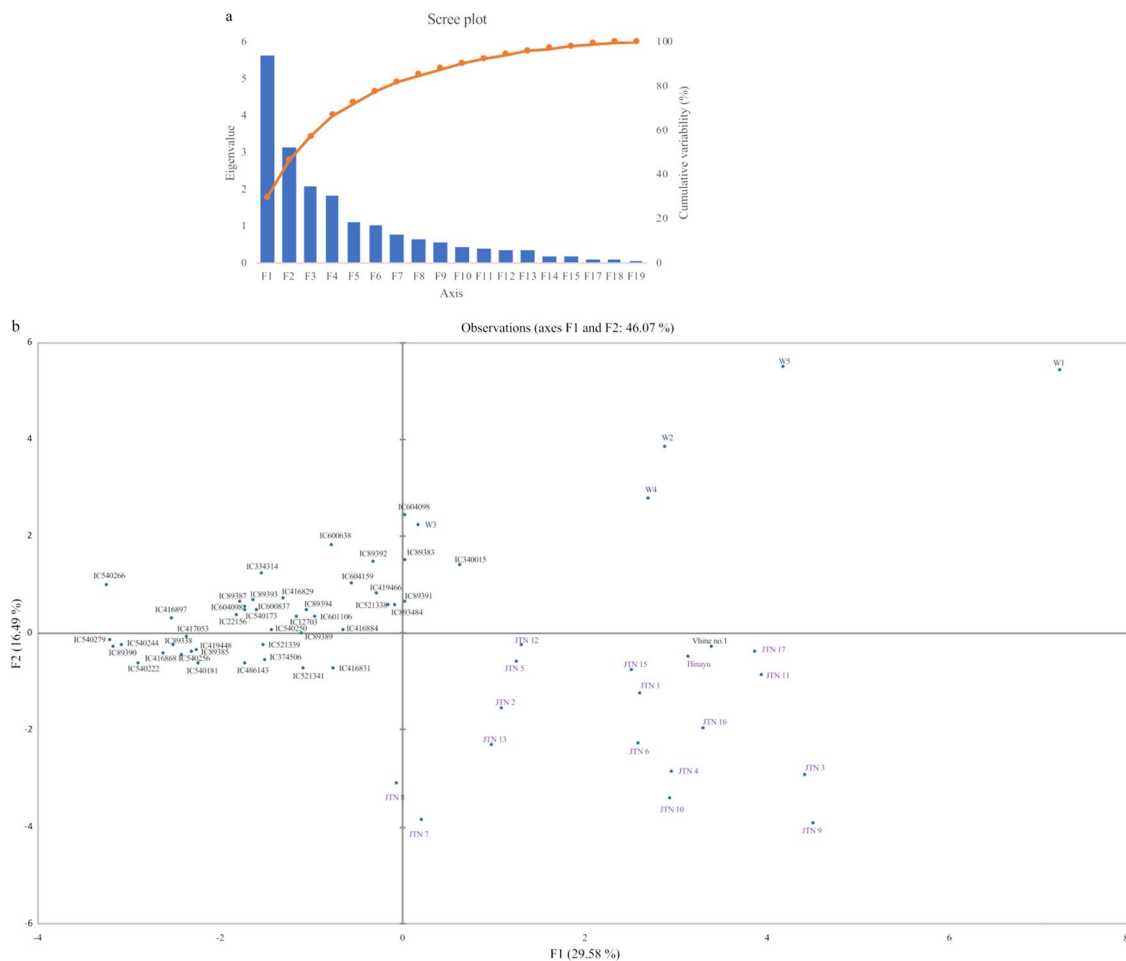


Fig. 3 **a** Screeplot representing principal components (F1 to F18) contributing to phenotypic variation. **b** Distribution of 65 Job's tears accessions across two major principal components F1 (x-axis) and F2

(y-axis). Purple dots indicate cultivated accessions; blue and black dots indicate wild accession and indigenous collection, respectively

The genotypic data analysis using STRUCTURE v.2.3.4 software revealed the maximal ΔK at $K=3$, indicating that at least three distinct groups are present in the present Coix collection (Fig. 5). In the bar-plot with one bar for each accession, we have indicated the subpopulations 1, 2, and 3 with red, green, and blue colours, respectively. The subpopulation-wise distribution of germplasm accessions was as follows: subpopulation-I = 31, subpopulation-II = 17, and subpopulation-III = 17. The number of admixtures were two. The remaining accessions in the collections were pure.

Biochemical Analysis

The phenol content varied from 5.55 to 10.18 mg/g in the Job's tears extract (Table 1). The highest phenolic content was found in the aqueous extract (10.18 mg GAE/g) of the IC416868 genotype. Total flavonoid content varied from 4.6 to 23.56 mg Quercetin/g in the extracts (Table 1). The

highest flavonoid content was found in the aqueous extract (23.6 mg/g) of the IC521341 genotype. Total antioxidant capacity varied from 19.95 to 71.9 mg AAE/g in the extracts (Table 1). The highest antioxidant capacity was found in the aqueous extract (72 mg/g) of the JTN3 genotype from Nagaland.

Discussion

Assessment of genetic diversity is essential for properly characterizing the genotypes for their sustainable use in plant breeding to enhance crop productivity with desirable traits. Incorporating the combined use of morphological and molecular markers has helped in the evaluation of genetic diversity present in many crop species. Hence, the current study analyzed the diversity in 65 Job's tears germplasm and exhibited high variability for growth and yield-related traits.

Table 3 Summary statistics of genetic variation for all 16 SSR loci and annealing temperature

| Locus | na* | ne* | I* | T (°C) | Exp. Hom* | Exp. Het* | Nei** | PIC | MAF |
|------------|------|------|------|--------|-----------|-----------|-------|--------|--------|
| GBssrJT25 | 4 | 1.92 | 0.91 | 59 | 0.52 | 0.48 | 0.48 | 0.4414 | 0.6953 |
| GBssrJT31 | 2 | 1.36 | 0.43 | 59 | 0.73 | 0.27 | 0.26 | 0.2289 | 0.8438 |
| GBssrJT32 | 2 | 1.17 | 0.27 | 59 | 0.86 | 0.14 | 0.14 | 0.1319 | 0.9231 |
| GBssrJT41 | 4 | 2.66 | 1.06 | 52 | 0.37 | 0.63 | 0.62 | 0.5514 | 0.4846 |
| GBssrJT68 | 5 | 2.29 | 1.02 | 53 | 0.43 | 0.57 | 0.56 | 0.4977 | 0.5846 |
| GBssrJT130 | 3 | 2.04 | 0.79 | 57 | 0.49 | 0.51 | 0.51 | 0.4073 | 0.5846 |
| GBssrJT136 | 5 | 4.04 | 1.5 | 57 | 0.24 | 0.76 | 0.75 | 0.7089 | 0.3790 |
| GBssrJT149 | 4 | 2.77 | 1.15 | 58 | 0.36 | 0.64 | 0.64 | 0.5786 | 0.5000 |
| GBssrJT161 | 3 | 2.91 | 1.08 | 58 | 0.34 | 0.66 | 0.66 | 0.5824 | 0.4154 |
| GBssrJT164 | 3 | 1.94 | 0.79 | 59 | 0.51 | 0.49 | 0.48 | 0.4039 | 0.6508 |
| GBssrJT170 | 5 | 4.15 | 1.48 | 60 | 0.21 | 0.71 | 0.73 | 0.7455 | 0.2769 |
| GBssrJT174 | 4 | 2.21 | 0.93 | 59 | 0.45 | 0.55 | 0.55 | 0.4668 | 0.5769 |
| GBssrJT181 | 5 | 3.19 | 1.3 | 57 | 0.31 | 0.69 | 0.69 | 0.6299 | 0.4000 |
| GBssrJT183 | 5 | 3.62 | 1.42 | 57 | 0.27 | 0.73 | 0.72 | 0.6792 | 0.4000 |
| GBssrJT185 | 3 | 2.84 | 1.07 | 59 | 0.35 | 0.65 | 0.65 | 0.5751 | 0.4444 |
| GBssrJT198 | 5 | 4.23 | 1.52 | 61 | 0.23 | 0.77 | 0.76 | 0.7250 | 0.3077 |
| Mean | 3.88 | 2.71 | 1.05 | - | 0.42 | 0.58 | 0.58 | 0.5221 | 0.5292 |
| St. Dev | 1.18 | 1 | 0.37 | - | 0.18 | 0.18 | 0.18 | | |

na* observed number of alleles, ne* effective number of alleles, I* Shannon's information index, Exp. Hom* expected homozygosity*, Exp. Het* expected heterozygosity* computed using Levene (1949) and Nei's** (1973), PIC polymorphism information content, MAF major allele frequency

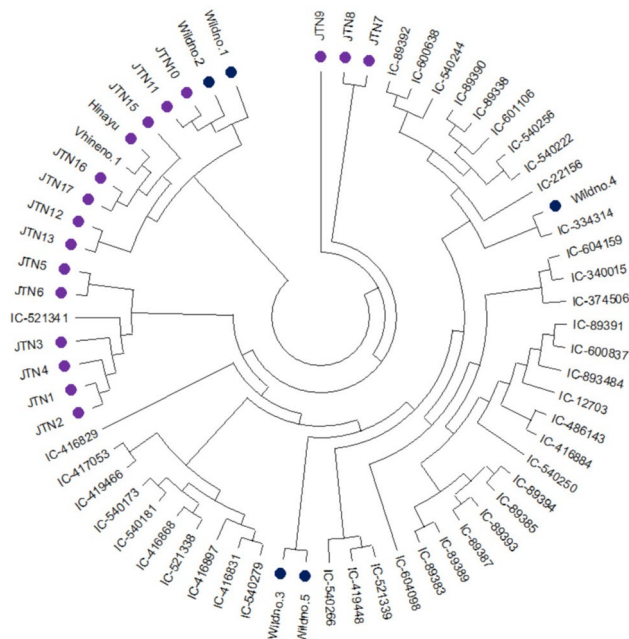


Fig. 4 Neighbour-joining dendrogram showing the relationship among Job's tears accessions based on 16 polymorphic SSR markers. Purple and blue dots indicate cultivated and wild accessions, respectively

Significant variations in various agro-morphological traits were observed among 39 genotypes collected from Mizoram (Soni et al. 2023). A similar study by Shen et al. (2019) reported that 94 Job's tears were accessions collected from different geographical areas of China using 12 morphological traits. The study suggested that characters with a positive association with grain yield per plant also contribute directly to grain yield, and the selection of genotypes could be reliably based on these characters. Arya et al. (2023) further supported this finding and observed a positive correlation between grain yield and 100 seed weight in 34 Job's tears accessions.

Grain yield has a complex quantitative character that is highly influenced by environmental fluctuations. Therefore, before embarking on grain yield improvement, it is necessary to understand the relationships between grain yield and other metric traits of the crop (Eleweanya et al. 2005). Further, the compartmentalization of correlation coefficients into direct and indirect effects discerned the true nature of the association between the observed characters. Tiller per plant exhibited maximum direct effect followed by the number of brace root/tiller, the girth of the tiller, chlorophyll content, and 100 seed weight. In Job's tears germplasm collected from China, Yao et al. (2013) found that yield was determined by 1000 grain weight, grain number, and effective panicles. Earlier studies reported the positive direct effect of tillers per plant and the negative direct effect of plant height in cereals (Efisue et al. 2014; Laxuman et al. 2011). Amini et al. (2013)

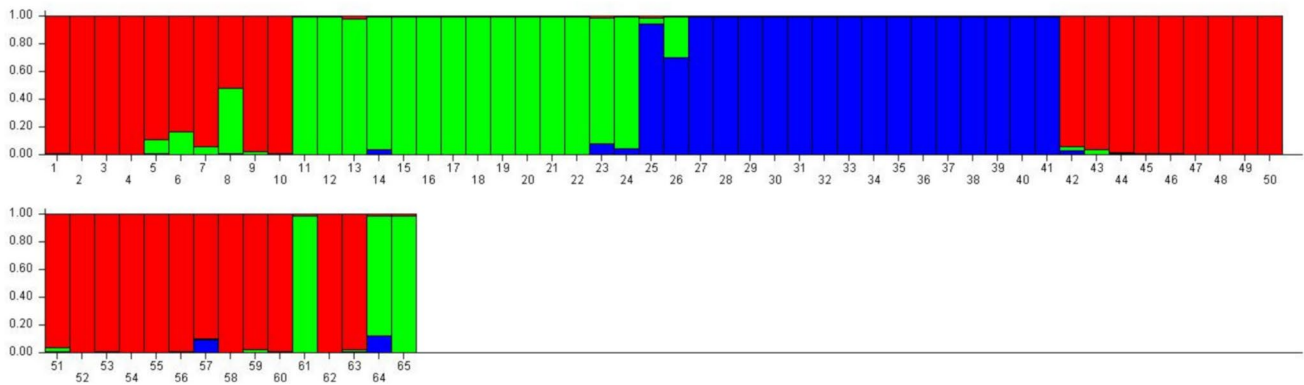


Fig. 5 Population genetic structure based on 16 polymorphic SSR markers in 65 Job's tear accessions. The numbers of x -axis indicate genotypes (Supplementary Table S1) while the y -axis indicates per-

centage ancestry (0–100) when the number of populations (K) is taken as 3. Genotypes with two or more colours indicate admixture

also stressed the significant positive association and high direct positive effect of 100 seed weight with grain yield per plant in maize crops. Traits exhibited a positive direct effect on grain yield. The correlation and path analysis results suggest that all the characters having a positive association with grain yield/plant are also directly contributing to grain yield, and the selection of genotypes may reliably be made based on these characters. They may also be used to develop a selection index to help select desirable parents and segregate them in a breeding program. The maximum response to index selection will be achieved if the correlation between genetic worth and the index is maximized (Baker 1986). Plant breeders have had more success using index selection to increase expected responses than by using direct selection of different traits in different plants (Gravois and McNew 1993; Jannink et al. 2000). Moreover, index selection has been used for increasing expected genetic advance in recurrent selection programs. The results of the D^2 analysis could be utilized to identify the best parental combination for generating variability with respect to various traits under study. The identified accessions from diverse clusters should be subjected to population improvement methods to derive superior inbred lines.

The accessions were grouped into five clusters, and the existence of genetic diversity among the accessions was shown using the SSR markers. Assessment of genetic diversity using 17 microsatellite markers was conducted in 79 Job's tears accessions collected from China and Korea (Ma et al. 2010). Genetic diversity and population structure were analyzed using AFLP markers in 139 *Coix lacrym-jobi* L. genotypes from four geographical regions in Southwest China (Fu et al. 2019). Xi et al. (2016) assessed the genetic diversity of 11 Job's tears accessions collected from different regions of China based on morphological characteristics and ISSR markers. They studied the relationship of the accessions with their chemical composition and the

anti-proliferative effects of the seed oil. The clustering pattern based on SSR markers did not show any relationship between geographical distribution and genotypic diversity, as the genotypes of different geographic origins were grouped in one cluster. A similar result was reported by Ma et al. (2010) and Cong et al. (2023) in Job's tears. Li et al. (2019), Fu et al. (2019), and Shen et al. (2019) reported no geographical affinity among genotypes within the same group when AFLP and ISSR markers were used for a diversity study of the Job's tears germplasm collected from different regions of China. However, the classification of Coix collected from China, Brazil, and Japan based on RAPD data reflected differences in geographic origin (Li et al. 2001). The accessions from Nagaland (JTN) were distant from other accessions in PCA based on the morphological and neighbour-joining dendrogram. Further, they have primarily white colour seeds and soft pericarp. All these suggested local adaptation in these accessions and the area may be further explored during germplasm exploration.

The concept of antioxidant activities, which describes the ability of different food antioxidants to scavenge preformed free radicals, has been suggested as a tool for investigating the health effects of antioxidant-rich foods (Abubakar and Giaeze 2016). The present study's total phenol content varied from 5.55 to 10.18 mg/g in the Job's tears extract. In a previous study conducted by Laxmisha et al. (2022), nutritional profiling of 32 Coix germplasm demonstrated different ranges of total phenol content (0.106 to 1.55%) and antioxidant capacity (0.380 to 0.580%). Chaisiricharenkul et al. (2011) determined total phenol content of Job's tears (7.0–8.0 mg GAE/g) to be higher than those of black rice (3.13 mg/g), brown rice (0.54 mg/g), barley (0.50 mg/g), mungbean (0.45 mg/g), foxtail millet (0.47 mg/g), and prosomillet (0.29 mg/g). The seed's total phenol content and antioxidant capacity were reported to be 20.5 mg GAE/g sample (Chhabra and Gupta 2015). However, in our study, the maximum phenol content was 10.18 mg GAE/g (IC417053),

and the antioxidant capacity was 72 mg AAE/100 g in the sample (JTN3). The phenol and flavonoid content was found to be very low in JTN3. This may be due to its high anthocyanin content (purple stem) and/or other factors which should be explored in future studies. A considerable range of variation was observed for all the biochemical traits under study, indicating enough scope to improve the desired direction.

Conclusions

The genetic diversity of 65 Job's tear germplasm was analyzed from the northeastern hill region of India at both morphological, biochemical, and molecular levels. Traits such as phenol content, SPAD, 100-seed weight, and number of tillers per plant were found to have contributed the most to the diversity. The study identified better-performing genotypes for yield (JTN11, IC89392) and yield-attributing traits to develop selection indices for future breeding programs. Based on SSR genetic diversity and clustering patterns, nine accessions (IC604098, IC416829, IC417053, IC521341, IC334314, JTN7, JTN8, JTN 9, and JTN15) were identified as diverse accessions and suitable as parents for future hybridization. Wild accessions from Meghalaya may be used as ornaments due to the hard pericarp. This study's results provide valuable information for properly utilizing germplasm and its incorporation into future breeding programs, which is essential given the limited efforts in the crop improvement program for Job's tears.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11105-024-01506-w>.

Acknowledgements The authors would like to express their sincere gratitude to ICAR-Research Complex NEH, Umiam, Meghalaya and the College of Post Graduate Studies in Agricultural Sciences, Umiam, Meghalaya, for providing all the field and laboratory facilities for supporting this research. NBPGR, ICAR, Meghalaya and ICAR, Research Complex for NEH Region, Nagaland Centre is acknowledged for providing the seed materials.

Author Contribution AP designed, planned, and executed the experiment. BL carried out the field and lab experiment. Data analyzed by AP, BL and AK. CA helped with biochemical analysis. Thorough editing of the manuscript was done by WT and MR. All the authors read and approved the final manuscript.

Funding This study was supported by the College of Postgraduate Studies in Agricultural Sciences and ICAR RC NEH Region, Umiam, Meghalaya.

Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of Interest The authors declare no competing interests.

References

- Abubakar B, Giaze RT (2016) Effect of sub-critical temperature during water extraction on the yield and total antioxidant properties of germinated brown rice. *J Basic Appl Res* 2(2):90–93
- Amini Z, Khodambashi M, Houshmand S (2013) Correlation and path coefficient analysis of seed yield related traits in maize. *Int J Agri Crop Sci* 5(19):2217–2220
- Arya M, Bhatt KC, Bhardwaj R, Semwal DP, Gupta V, Laxmisha KM (2023) Agro-morphological characterization and digitization of Job's tears (*Coix lacryma-jobi* L.) germplasm: a minor cereal crop of Northeastern India. *IJPGR* 36(03):362–70
- Baker RJ (1986) Selection indices in plant breeding (1st ed.). CRC Press. <https://doi.org/10.1201/9780429280498>
- Chhabra D, Gupta RK (2015) Formulation and phytochemical evaluation of nutritional product containing Job's tears (*Coix lacryma-jobi* L.). *J Pharmacogn Photochem* 4(3):291–298
- Chaisiricharoenkul J, Tongta S, Intarapichet KO (2011) Structure and chemical and physicochemical properties of Job's tears (*Coix lacryma-jobi* L.) kernels and flours. *Suranaree J Sci Technol* 18(2):109–122
- Chang C, Yang M, Wen H, Chern J (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal* 10:178–182
- Cong DV, Anh DT, Huong TTH, Nhung NT, Ha TTT, Khanh TD, Toan VD (2023) Genetic diversity of Job's tear (*Coix lacryma-jobi* L.) germplasms based on the morphological traits and SSR. *Eur Chem Bull* 12(6):42–52
- Devaraj RD, Jeepipalli SP, Xu B (2020) Phytochemistry and health promoting effects of Job's tears (*Coix lacryma-jobi*)-a critical review. *Food Biosci* 1(34):100537
- Diningrat DS, Harahap NS, Risfandi M, Sari AN (2021) Antioxidant and antibacterial activities of *Coix lacryma-jobi* seed and root oil potential for meningitis treatment. *Jordan J Biol Sci* 14(5):881–887
- Efissue AA, Umunna BC, Orluchukwu JA (2014) Effects of yield components on yield potential of some lowland rice (*Oryza sativa* L) in coastal region of southern Nigeria. *J Plant Breed Crop Sci* 6(9):119–127
- Eleweanya NP, Uguru MI, Eneobong EE, Okocha PI (2005) Correlation and path coefficient analysis of grain yield related characters in maize (*Zea mays* L.) under umudike conditions of South Eastern Nigeria. *Agro-Sci* 4(1):24–28
- Ferrer AM, David JR, Taquiqui A, Bautista A, Deocarís CC, Alinsug MV (2021) Pharmacological mechanisms on the anti-breast cancer property of *Coix lacryma-jobi*: a network-based analysis. *bioRxiv*:2021–10 <https://doi.org/10.1101/2021.10.16.464646>
- Fu YH, Yang C, Meng Q, Liu F, Shen G, Zhou M, Ao M (2019) Genetic diversity and structure of *Coix lacryma-jobi* L. from its world secondary diversity center, Southwest China. *Int J Genomics* 9815697 <https://doi.org/10.1155/2019/9815697>
- Gravois KA, McNew RW (1993) Genetic relationships among and selection for rice yield and yield components. *Crop Sci* 33:249–252
- Hore DK, Rathi RS (2007) Characterisation of Job's tears germplasm in North-East India. *Nat Prod Radiance* 6(1):50–54
- Igbokwe CJ, Wei M, Feng Y, Duan Y, Ma H, Zhang H (2021) Coix seed: a review of its physicochemical composition, bioactivity, processing, application, functionality, and safety aspects. *Food Rev Int* 38(sup1):921–939
- Jannink JL, Orf JH, Jordan NR, Shaw RG (2000) Index selection for weed suppressive ability in soybean. *Crop Sci* 40:1087–1094
- Kumar R, Yhokha K, Rajesha G, Deka BC (2017) Performance of Job's tears lines (*Coix lacryma-jobi*) under foothill condition of Nagaland. *Environ Ecol* 35:440–444

- Kumar A, Das SP, Pandey A et al (2024) Genetic diversity and population structure analysis in Job's tears (*Coix lacryma-jobi* L.) collections from northeastern Himalayan region. *Genet Resour Crop Evol.* <https://doi.org/10.1007/s10722-024-01958-9>
- Laxmisha KM, Semwal DP, Gupta V, Katral A, Bisht IS, Mehta PS, Arya M, Bhardwaj R, Bhatt KC (2022) Nutritional profiling and GIS-based grid mapping of Job's tears (*Coix lacryma-jobi* L.) germplasm. *Appl Food Res* 2(2):100169
- Laxuman, Salimath PM, Shashidhar HE, Mohankumar HD, Patil SS, Vamadevaiah HM, Janagoudar BS (2011) Analysis of genetics variability in interspecific backcross inbred lines in rice (*Oryza sativa* L.). *Karnataka J Agri Sci* 24(5):626–628
- Levene H (1949) On a matching problem arising in genetics. *Ann Math Statist* 20:91–94
- Lewontin RC (1972) The apportionment of human diversity. *Evol Biol* 6:381–398
- Li XH, Huang YQ, Li JS, Corke H (2001) Characterization of genetic variation and relationships among *Coix* germplasm accessions using RAPD markers. *Genet Resour Crop Evol* 48:189–194
- Li X, Zhou X, Li Z, Li Q, Yang C, Zhou M, Fu Y (2019) Genetic diversity of *Coix lacryma-jobi* L. germplasm resources from Guizhou and its adjacent provinces based on AFLP markers. *J Southern Agri* 50(9):1931–6
- Liu K, Muse SV (2005) PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* 21(9):2128–2129
- Ma KH, Kim KH, Dixit A, Yu JW, Chung JW, Lee JH, Cho EG, Kim TS, Park YJ (2006) Newly developed polymorphic microsatellite markers in Job's tears (*Coix lacryma-jobi* L.). *Mol Ecol Notes* 6:689–691
- Ma KH, Kim KH, Dixit A, Chung IM, Gwag JG, Kim TS, Park YJ (2010) Assessment of genetic diversity and relationships among *Coix lacryma-jobi* accessions using microsatellite markers. *Biol Plant* 54(2):272–278
- Nei M (1973) Genetic distance between populations. *Am Nat* 106:283–292
- Nei M, Tajima F, Tateno Y (1983) Accuracy of estimated phylogenetic trees from molecular data. *J Mol Evol* 19:153–170. <https://doi.org/10.1007/BF02300753>
- Ouyang Y, Shi H, Zhao Y, Yin Y, Zhu F, Chen Z, Wang L (2022) Analysis of protein fraction content and peptide activity of *Coix lacryma-jobi* L. var. ma-yuen stapf (POACEAE) from different regions of China. *Pak J Bot* 54(5):1871–1878
- Prieto P, Pineda M, Aguilar M (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem* 269(2):337–341
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155(2):945–959
- Rohlf FJ (1993) NTSYS-PC: Numerical taxonomy and multivariate analysis system. Ver.2.02j. Exeter Software, New York
- Saghai-Marouf MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. *PNAS* 81(24):8014–8018
- Shen G, Girdthai T, Liu ZY, Fu YH, Meng QY, Liu FZ (2019) Principal component and morphological diversity analysis of Job's-tears (*Coix lacryma-jobi* L.). *Chil J Agri Res* 79(1):131–143
- Soni JK, Lalramhlimi B, Kumar A, Navik O, Lungmuana SL, Doley S (2023) Coix: an underutilized functional food crop of Mizoram. *Genet Resour Crop Evol* 1:1–7
- Tamura K, Stecher G, Kumar S (2021) MEGA11: molecular evolutionary genetics analysis version 11. *Mol Biol Evol* 38(7):3022–3027
- Volluri SS, Bammidi SR, Chippada SC, Rasayan VM (2011) In-vitro antioxidant activity and estimation of total phenolic content in methanolic extract of *Barcopa monniera*. *RASAYAN J Chem* 4:381–386
- Wang S, Zhang S, He J, Lu G, Yang Z (2013) The principal component analysis and cluster analysis of *Coix* resource characteristics. *J Yunnan Agri Univ* 28:157–162
- Xi XJ, Zhu YG, Tong YP, Yang XL, Tang NN, Ma SM, Li S, Cheng Z (2016) Assessment of the genetic diversity of different Job's tears (*Coix lacryma-jobi* L.) accessions and the active composition and anticancer effect of its seed oil. *PLoS One* 11(4):e0153269
- Yao F, Yin G, Mo G, Luo Y, Yuan J (2013) *Coix lacryma-jobi* L. growth and yield response to different sowing dates in Sichuan China. *J Sci Appl Biomed* 1(1):8–14
- Yeh FC, Yang RC, Boyle TBJ, Ye Zh, Mao JX (2000) POPGENE32, Microsoft windows-based software for population genetic analysis (version 1.32). Molecular Biology and Biotechnology Centre, University of Alberta. Edmonton, Alberta, Canada

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

Authors and Affiliations

Avinash Pandey^{1,3}  · Bharati Lap² · Chubasenla Aochen¹ · Amit Kumar¹ · Wricha Tyagi^{2,4}  · Mayank Rai^{2,5} 

✉ Wricha Tyagi
wtyagi.cau@gmail.com; wricha.tyagi@icrisat.org

¹ ICAR Research Complex for Northeastern Hill Region (ICAR-RC NEH), Umiam, Meghalaya 793103, India

² School of Crop Improvement, College of Post Graduate Studies in Agricultural Sciences (CPGSAS), Central Agricultural University (Imphal), Umiam, Ri-Bhoi District, Meghalaya 793103, India

³ Present Address: Indian Council of Agricultural Research-Indian Institute of Agricultural Biotechnology

(ICAR-IIAB), Garhkhata, Ranchi, Jharkhand 834003, India

⁴ Present Address: International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana 502324, India

⁵ Present Address: Dr. Rajendra Prasad Central Agricultural University (RPCAU), Samastipur, Bihar 848125, India